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Integrated Approaches to Animal Health and Welfare Across Production and Care Systems

Edited by Arnoldo González-Reyna and Kevin Sargent





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IntechOpen Book Series

Veterinary Medicine and Science

Volume 30

Aims and Scope of the Series

Paralleling similar advances in the medical field, astounding advances occurred in Veterinary Medicine and Science in recent decades. These advances have helped foster better support for animal health, more humane animal production, and a better understanding of the physiology of endangered species to improve the assisted reproductive technologies or the pathogenesis of certain diseases, where animals can be used as models for human diseases (like cancer, degenerative diseases or fertility), and even as a guarantee of public health. Bridging Human, Animal, and Environmental health, the holistic and integrative "One Health" concept intimately associates the developments within those fields, projecting its advancements into practice. This book series aims to tackle various animal-related medicine and sciences fields, providing thematic volumes consisting of high-quality significant research directed to researchers and postgraduates. It aims to give us a glimpse into the new accomplishments in the Veterinary Medicine and Science field. By addressing hot topics in veterinary sciences, we aim to gather authoritative texts within each issue of this series, providing in-depth overviews and analysis for graduates, academics, and practitioners and foreseeing a deeper understanding of the subject. Forthcoming texts, written and edited by experienced researchers from both industry and academia, will also discuss scientific challenges faced today in Veterinary Medicine and Science. In brief, we hope that books in this series will provide accessible references for those interested or working in this field and encourage learning in a range of different topics.

Meet the Series Editor



With more than 35 years of academic experience, Dr. Rita Payan-Carreira is now a Full Professor at the University of Évora (UÉ). She specializes in animal theriogenology, with a particular focus on the determinants of female fertility and fetal-maternal interaction during implantation. Dr. Payan-Carreira is dedicated to promoting a culture of collaboration and knowledge-sharing within the scientific community. In addition to her extensive publication record, she frequently collaborates with various publishing houses on peer-review activities. She further contributes to the scientific advancement of students and professionals by supervising academic theses and offering technical and scientific courses, including scientific communication, critical reasoning and discussion, and the ethical aspects of research.

Meet the Volume Editors



Dr. González graduated from the Autonomous University of Tamaulipas (BSc), Utah State University (MSc), and the University of Saskatchewan (Ph.D., Post-Doc). With academic experience since the early 80s in Canada, he has taught more than 130 academic courses (both graduate and undergraduate), supervised more than 85 theses, and delivered 30+ training courses and workshops. Publications include a variety of formats, such as refereed articles and books. In addition, he conducted several funded research projects. The editorial tasks include serving as a reviewer across several journal platforms, including Elsevier, Archives of the Latin American Assn. of Animal Production, IntechOpen, Journal of Veterinary Research and Studies, and others. Specialty areas include the physiology and endocrinology of reproduction, reproductive biotechnologies, and animal production systems.



Dr. Sargent recently transitioned from higher education to a company that serves agricultural producers by establishing third-party trials to test manufacturer claims at the farm scale. Following his education at the Universities of Missouri-Columbia (B. S., Animal Science) and Nebraska-Lincoln (M. S./Ph.D., Reproductive Physiology), his almost decade in higher education prepared him extensively. He previously taught 40 unique animal science and agribusiness courses. He built two laboratories through donor fundraising, grants, and internal funding requests, blending bench science and on-farm experiential learning/research with classroom material. This required extensive cross-disciplinary collaboration and working with many livestock and companion animal species. He advised hundreds of students academically, with many earning research awards, securing prestigious jobs, and gaining acceptance to veterinary school.

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Preface

Animal care, animal management and handling, good production practices, one-health, and circularity of agricultural bioeconomies are concepts that can be used to summarize the meaning of Animal Welfare in the context of our global world and agricultural production systems, ensuring the production of good-quality foods for human consumption. Currently, animal welfare has been globally placed, according to the World Organization for Animal Health (WOAH), in the forefront as a multi-faceted subject with scientific, ethical, economic, socio-cultural, religious, and political dimensions; as such, it has attracted growing interest from our civil societies and is one of the priorities of WOAH. In 1965. WOAH developed the guiding principles for the work on the welfare of terrestrial animals, and declared the Five Freedoms, which were widely accepted and recognized and describe society's expectations for the conditions under which animals should live and experience while under human control; these five freedoms include:

Freedom from hunger, malnutrition and thirst,
Freedom from fear and stress,
Freedom from heat stress or physical discomfort,
Freedom from pain, injury and disease, and
Freedom to express normal patterns of behavior.

In accordance with these freedoms and to the terrestrial code, WOAH established the current definition and meaning of animal welfare as the physical and mental state of an animal in relation to the conditions in which it lives and dies. Currently, and in line with WOAH's guiding principles and animal welfare as a vital asset for a more sustainable world, a five-domain model was developed as a framework for assessing the welfare needs of all animal species under animal control. These five domains include: nutrition, health, mental state and experiences, environment, and behavioral interactions. Within this vision of animal welfare in WOAH, perhaps the most notorious component is the recognition of some animals as sentient beings capable of perceiving, feeling emotions, and experiencing subjective states of pain, joy, fear, and pleasure. Some degree of scientific consensus has been reached, including the Cambridge Declaration on Consciousness, and in more than 30 countries, where animals as mammals (primates), birds (chickens, parrots, and other species), fish (various species), and some cephalopods (octopuses and crabs) have been included.

Despite, paramount efforts of global non-government organizations (ONG's), including WOAH, FAO World Animal Protection (WAP) and Global Animal Law (GAL), to include animal welfare into the sustainability framework of agriculture activities related to food production and other products, strategies as animal welfare and others have only been considered by less than 40 countries; due perhaps to a diverse variety of reasons. These and other ONGs define sustainable agriculture as ecologically sound, economically viable, socially just and humane, considering environmental impacts, biodiversity and habitat conservation, resource efficiency, economic viability, and

social well-being. Countries such as Sweden, the United Kingdom, Austria, and Switzerland are among the top countries that most protect animals and have strict laws in favor of animal welfare and against animal cruelty.

Finally, on behalf of all authors, my fellow co-editor, Dr. K. Sargent, and I kindly thank and recognize the IntechOpen Editorial Team for the opportunity to work together on this wonderful book project on animal welfare, which I dearly hope will benefit our global scientific community. Last but not least, I also thank and recognize the tremendous work and support provided by Maja Bozicevic, our Publishing Process Manager.

Maja, thank you for your everyday support, input, and great patience.

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Section 1

Animal Production, Health and Welfare

Chapter 1

Animal Welfare Assessment in Exhibition Equines in the Municipality of Técpan de Galeana, Guerrero

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Abstract

This chapter presents the results of a study conducted at the Corraletas, ranch, located in Técpan de Galeana, Guerrero, Mexico, to evaluate animal welfare status in 20 show horses. The methodology used was a mixed approach: qualitative, through direct observation and interaction with the specimens; and quantitative, applying the Quality Welfare protocol, which considers four fundamental principles (good nutrition, good housing, good health, and appropriate behavior) distributed across 12 specific criteria. The analysis of the results showed that the Corraletas, ranch obtained a high category in the principle (Good Housing), and three criteria (comfort during rest, thermal comfort and ease of movement) with 63.30% and excellent category in three principles (Good Nutrition, Good Health and Appropriate Behavior), and nine criteria (Absence of prolonged Hunger, Absence of prolonged Thirst) with 82.50%, (Absence of Injuries, Absence of Diseases, Absence of Pain Induced by Handling) with 83.30%, and Expression of Social Behavior, Expression of other behaviors, Good Human-Animal Relationship, Positive Emotional State) with 81.25%, averaging all principles and criteria: An overall score of 77.59% was obtained, with a high category according to the Animal Welfare scale, with good welfare for the horses of the Las Corraletas ranch.

Keywords: animal welfare, evaluation, horses, welfare quality protocol, shows

1. Introduction

Since the beginning of human and animal coexistence, animals have been not only an inseparable companion but also a basic and indispensable tool in daily life. Until the Industrial Revolution, animals were the fundamental driving force of society, enabling the development of communities, especially in less developed regions, thanks to their role as a means of transportation, support in agricultural work, and a source of food.

However, since they are not considered subjects of legal rights, but rather objects of property, clear regulations were never established regarding the conditions under which they were to perform their various work tasks. This lack of regulation has left significant gaps in the protection of animals, which have been indispensable in various human activities.

European and Spanish legislation regulates aspects such as breeding on livestock farms, transport, slaughter, and the use of animals in laboratories or zoos. However, there are few specific regulations for other equally important functions, such as their participation in security, defense, rescue, transportation, cargo, or therapeutic assistance and guidance.

The concept of animal welfare (AW) has been the subject of debate for the past 30 years, primarily due to its connection with social ethics. This is based on the recognition that animals are sentient beings, capable of experiencing pain, suffering, and stress. Therefore, causing them suffering is not morally acceptable, and it negatively affects the quality and performance of their functions. Animal welfare does not oppose the use of animals by humans, but it does require an ethical commitment to ensuring a good quality of life throughout their life cycle, including humane slaughter when necessary [1].

One of the main objectives of scientific research related to (AW) is to develop methods that allow its assessment in an objective way [2, 3].

The development of a welfare assessment tool for thoroughbred racehorses allows for continuous monitoring of the handling and care these animals require, as in many countries they are excluded from animal protection laws due to their sporting nature. The developed instrument covers the most important life-support components of these animals (health, behavior, management, nutrition, and facilities), with 146 indicators that balance sports medical care, without compromising one or the other.

Validation of the indicators by medical and sports experts allowed the creation of a balanced and comprehensive questionnaire that was valid, reliable and feasible to implement routinely [4].

(AW) assessment is often considered as the set of negative and positive experiences of animals, which should preferably be analyzed directly through behavior and health; or indirectly, in the way in which animals interact and respond to their housing or handling [5, 6].

Although environment-based indicators are easier to measure, they are derived from the characteristics of the production system itself and are therefore not useful for establishing comparative assessments between different management or housing systems or alternatives. Consequently, there is great interest in direct indicators that can be used independently of the system or environment in which the animals are kept [1, 7].

The main cause of the deterioration in animal welfare among draft horses in Mapuche communities in the Araucanía Region is related to poor foot care, which is not performed properly or within the required timeframe.

Less than 50% of the specimens met the hypometric measurements established for draft horses.

The draft horses in these communities are not subject to a health management program, which predisposes them to diseases and parasitic infestations that impair their well-being and reduce their performance.

1.1 Animal welfare

The World Organization for Animal Health states that an animal is in a satisfactory state of well-being when it is healthy, comfortable, well-fed, can express its innate behavior, and is not suffering from pain, fear, or stress [8]. In this context, Equine-Assisted Interventions (EAI) involve an active collaboration between the horse and the client, in a therapeutic space focused on the "here and now." Each situation poses challenges that require not only personal skills, but also empathy and emotional connection. Studies have shown that horses can perceive our emotions through voice and gestures [9].

Developing countries are home to around 60% of the world's equine population [10], with approximately 60 million horses, 55% of which live in the Americas. These animals are primarily used for draft work, supporting family farming [11, 12]. However, many have poor body condition, respiratory, dental and foot problems, and lack timely veterinary care [13, 14], which negatively affects their well-being [15, 16].

Growing concern about these conditions has rekindled the debate on animal welfare, understood as the physical and mental state in harmony with the environment [17, 18]. According to [19], well-being refers to the individual's ability to cope with its environment, relating physiological, behavioral, and emotional aspects. When routines such as eating or resting are altered, well-being is compromised [20].

Welfare assessment can be carried out through direct, animal-centered measures or indirect measures based on available resources such as facilities and management [7]. The latter indicate potential risks rather than the actual condition of the animal [21].

In Colombia, an important milestone was the enactment of Law 1774 in January 2016, which amended the Civil and Criminal Code, recognizing animals as sentient beings. This advance implies that they must be distinguished from material goods, and their protection must be considered throughout the legal system. Colombia thus became the first Latin American country to include this definition in its civil legislation.

In Mexico, there is still no national law that comprehensively regulates animal welfare. Although various initiatives have been introduced since the beginning of the twenty-first century, many remain pending in legislative chambers. It is urgent to establish a legal framework that promotes responsible and sustainable practices, both in animal ownership and production.

Animal welfare is essential for health, conservation, and sustainable production. It fosters practices that meet the biological needs of animals and enables the achievement of objectives such as promoting responsible ownership, improving production systems without harming the environment or society, improving product quality, and conserving biodiversity, including captive wild animals [22].

2. Quality of well-being

The European Welfare Quality Project developed an assessment system for different animal species, based on direct observations focusing on the animal's condition and the quality of its living conditions [23]. This approach seeks to overcome the limitations of the Five Freedoms Principle, which states that animal welfare is guaranteed

when five basic conditions are met: absence of hunger and thirst, physical comfort, health, freedom to express natural behaviors, and absence of fear or distress [23].

Although these freedoms provide a useful basis for studying animal welfare, they present two main problems: their generic nature and the overlap between some of them. In response, the Quality of Welfare project proposed a more precise methodology based on observable and measurable criteria. This project, which began in May 2004 and lasted 5 years, involved more than 40 scientific institutions from 15 European countries. Its objective was to establish an animal welfare assessment system accepted by the European Union, focusing on environmental and behavioral measures.

The Welfare Quality protocols are structured around four principles: feeding, housing, health status, and behavior. Feeding is assessed for the absence of prolonged hunger and thirst. Housing is considered for resting comfort, thermal comfort, and ease of movement. Health status includes the absence of injuries, illnesses, and pain caused by handling practices such as castration or dehorning. Finally, behavior includes the expression of appropriate social behaviors, positive interactions with caregivers, and a balanced emotional state.

In Mexico, the state of Guerrero has made significant progress in this area. On August 9, 2016, Law 491 on Animal Welfare was amended Article 1 establishes the protection of animals, guaranteeing their well-being, care, proper treatment, maintenance, housing, natural development, and health. It also prohibits abuse, cruelty, abandonment, and suffering, recognizing the importance of animal health and public health. The law also defines the powers of state and municipal authorities to enforce these provisions.

In Guerrero, equine livestock (horses, mules, and donkeys) represents a significant portion of the agricultural sector, with 364,155 heads concentrated primarily in the Tierra Caliente and Costa Chica regions, which together account for 39.8% of the state total [24]. In municipalities such as Técpan of Galeana, animal welfare is a growing issue, particularly given the problems arising from the lack of implementation of appropriate measures. It is essential to adopt strategies that prevent mistreatment, reduce stress caused by housing, improper handling, and overwork, thus promoting decent conditions for working animals.

The overall objective of the research was to evaluate animal welfare in exhibition horses using the welfare quality protocol at the Corraletas ranch in Técpan of Galeana, Guerrero.

3. Problem statement

Often the animal welfare of equines is low, since their owners do not have sufficient resources to cover their needs, or lack the appropriate knowledge to care for them, some in work environments, such as the construction industry or adverse environments, may present a particular risk to their welfare, lack of knowledge of Animal Welfare causes atypical behaviors and causes failures in the health status of equines.

To assess the seriousness of the animal welfare problem, the World Animal Health Organization considers that an animal is in a satisfactory state of well-being, healthy, comfortable, and well-fed, can express its innate behavior, and is not suffering from pain, fear, or stress.

Stabling of equines is a management method generally used in equines intended for sport, affecting their behavior directly, [25] states that stabling can have a negative effect on equine welfare. Mainly due to the behavioral modifications they suffer when replacing their natural environment with an artificial one.

4. Material and method

The study was conducted in the municipality of Técpan the Galeana, located in the state of Guerrero, Mexico. It is located on the central coast of Guerrero, at the transition to the Sierra Madre del Sur. Its altitude is 57 meters above sea level, with geographic coordinates of 17°13′21″N and 100°37′56″W.

An observational, prospective, cross-sectional, and analytical study was conducted. The sample was non-probabilistic and consisted of 20 show horses located at the "Las Corraletas" ranch in the municipality of Técpan the Galeana [26].

Procedure:

1. A complete anamnesis was applied to evaluate the physical condition of each equine.

2. A survey of animal welfare owners was conducted.

3. Each equine was observed daily at three times (morning, work, and afternoon) during the months of July and August 2020.

4. The Quality and Well-being protocol adapted for show horses was applied.

5. Daily data were recorded on date, time, location, weather conditions and any observed disturbances.

Indicators evaluated:

1. Facility conditions

2. General physical evaluation of the animal

3. Equine behavior

Instruments used

1. Individual anamnesis

2. Animal welfare survey applied to owners

3. Record tables with animal welfare indicators

The study was carried out during the months of July to August 2020, implementing the welfare quality protocol, developed in Europe, which is based on four principles and 12 specific criteria, recording daily the date, time, place, climatic changes, observation or alteration that each animal may present, as well as the following indicators depending on the facilities, general physical evaluation of the animal and indicators of animal origin and indicators in the environment [27, 28].

5. Results

Once the database was generated, it was analyzed according to the following table of percentages of well-being criteria. To determine the score for each principle, the scores obtained in each criterion are averaged, which are the result of the average completion of each indicator (**Table 1**):

Category	Punctuation	Wellness description
Excellent	≥80	Highest level of well-being
Elevated	≥55	Good Welfare
Acceptable	≥20	Meets minimum requirements
Not classified	<20	Low and unacceptable welfare

Table 1.
Shows the percentages of the criteria and principles. The well-being category was then assigned according to the following scale [29].

1. %CC: Percentage of compliance with the welfare criterion.

2. %CC = [∑% Compliance with the well-being indicator]/(number of indicators evaluated)

3. The data were averaged to calculate the percentages %CP: Percentage of compliance with the principle

4. %CP = [∑% Compliance with the welfare criterion]/(number of criteria evaluated)

Recording of the results of the questionnaires used.

6. First domain good nutrition

6.1 Variable: Absence of thirst

Regarding the first principle of study, "Good Nutrition," two criteria were assessed: absence of prolonged hunger and absence of thirst. Three animal-based indicators and three environmental indicators were evaluated. The body condition indicator was presented in 20 specimens in optimal condition, representing 90% compliance with the indicator. For the other indicators, the level of compliance is 85%, complying with routine inspection processes.

Regarding the criterion of absence of prolonged thirst, the ideal number and management conditions of drinking fountains are met.

In the criterion absence of thirst, 82.50% of the horses gave this result, and only 17.50% presence of thirst (**Table 2**).

Variable	%
Absence	82.50
Presence	17.50
Total	100.0

Table 2.
Thirst.

7. Variable: Absence of hunger

For the hunger indicator, 75% absence and 25% presence were reported (**Table 3**).

Hunger	**%**
Absence	75
Presence	25
Total	100

Table 3.
Variable food-hunger.

8. Second domain good housing

8.1 Variable comfort during rest

The second principle of the study, "Good Housing," analyzed three criteria: sleeping comfort, thermal comfort, and ease of movement. The sleeping comfort criterion considered the type of floor, softness, and sawdust, and ease of movement, where all criteria were met for the 20 specimens, with a rating level of 63.30% for each of these indicators (**Table 4**).

For the stall indicator, fecal cleaning was evaluated, an activity performed routinely twice a day. The indicator showed 20 horses with bedding replacements every 3–6 months in optimal condition. Likewise, for thermal comfort, the temperature indicator was measured with a maximum and minimum thermometer, obtaining average maximum temperatures of 38 degrees and a minimum temperature of 36.7 degrees for horses in stalls. According to A. Sáenz-García [28] the recommended temperature range is 7.2–23.9°C, with an optimal temperature of 12.7°C, considering that horses maintain a body temperature of 37–38°C, and an elevation of more than 3°C can have consequences.

Comfort during rest	**%**
Absence	36.70
Presence	63.30
Total	100.0

Table 4.
Good housing comfort during the rest.

9. Variable: Thermal comfort

Another indicator evaluated for this criterion was thermal shelter, which was determined by the height of the roof and the visual horizon in the mangers. This is recommended due to the type of infrastructure of the hatchery, which is oriented east-west, allowing air currents to enter and exit the manger, aerating the manger.

Thermal comfort	%
Absence	36.70
Presence	63.30
Total	100.0

Table 5.
Good housing/thermal comfort.

It was verified that most mangers have a height of 3.20 meters from the front and 3.40 meters from the back. The average roof height should be 3 meters from the front and 3.75 meters from the back. The rating level for this indicator is 63.30%, indicating adequate facilities (**Table 5**).

10. Third domain good health

10.1 Variable: Absence of lessons

Regarding the third principle of study, "good health," three criteria were assessed: Absence of wounds and injuries, Absence of disease, Absence of handling comfort. Evaluating 16 animal-based indicators and one environmental-based indicator.

The criterion for Absence of Wounds and Injuries was graded using the following indicators (white patches of hair, wounds, whisker length, hoof condition, and absence of lameness). All 20 specimens met the aforementioned indicators, achieving a score of 83.30%. For the Public and Individual Area Safety indicator (environmental indicator), the Breeding Facility infrastructure was inspected to rule out any objects that could cause injury to workers or visitors. The score for the stables and facilities of the 20 specimens studied was 83.30%, and the safety procedures for said indicator were met (**Table 6**).

The criterion of absence of disease was measured with the following indicators: altered breathing, presence of cough, nasal and/or ocular discharge, skin irritation of the extremities, generalized skin problems, scratching of mane and tail, condition of the coat, consistency of feces Where the altered breathing indicator was absent in the 20 specimens representing 100% of compliance with the indicator.

For the cough presence indicator, it was absent in all 20 specimens, representing 100%, meeting an optimal health status for this indicator. For the nasal discharge indicator, it was absent in 71% of the cases. Eye discharge was also evaluated, which was absent in 20 specimens, representing 83.30% of the cases meeting this indicator. The absence of skin irritation on the extremities indicator was valued at 71% in all 20 specimens.

Absence of injuries	%
Absence	83.30
Presence	16.70
Total	100.0

Table 6.
Good health/absence of injuries.

11. Variable absence of diseases

The generalized skin problems indicator, represented by 85, was in optimal condition for 20 specimens meeting this indicator. It should be noted that the specimens that presented any health changes were under the supervision, treatment, and care of the kennel veterinarian at the time of the BA evaluation. For indicators such as tail scratching and mane scratching, scratching of the tail is caused by itching. This can be attributed to various causes, such as parasites, allergies to fly or mosquito bites, or boredom in the stables (**Table 7**).

The incidence is zero and tail scratching is absent in all 20 specimens, thus meeting this indicator. Mane scratching is absent. Evaluating the highest scores indicates compliance with the evaluated indicators. The coat condition indicator is present in all 20 specimens, with 100% compliance, with clean and shiny fur, observing the optimal state of health of the specimen. For the stool consistency indicator, it was evident that 20 specimens had normal bowel movements, equivalent to 100%. The criterion of absence of comfort due to handling was evaluated with the indicators: pain in the back muscles, alterations in the corners of the mouth and/or in the bars, whisker length; where the rating level was 100% for each of these, representing compliance with the indicators for all 20 specimens, for the principle of average good health with a rating of 95%.

Diseases	**%**
Absence	83.30
Presence	16.70
Total	100.0

Table 7.
Good health/absence of disease.

12. Variable absence of induced pain

See **Table 8**.

Absence of pain	**%**
Absence	95.0
Presence	5.0
Total	100.0

Table 8.
Good health/absence of induced pain.

13. Fourth domain appropriate behavior

13.1 Variable: Expression of social behavior

The assessment of the "appropriate behavior" principle measured four criteria: expression of social behavior, expression of species-specific behaviors, "appropriate"

Expression of appropriate social behavior	%
Absence	80.0
Presence	20.0
Total	100.0

Table 9.
Appropriate behavior/expression of social behavior.

expression of social behavior, expression of species-specific behaviors, relationships with people, and positive emotional state. To measure this, indicators based on the animal and indicators based on the environment were evaluated.

The social behavior criterion was measured with two indicators, one based on the animal, approach to a striking object, scored at 80% represented by 20 specimens (**Table 9**).

The criterion for the expression of species behaviors was evaluated using an animal-based indicator. Absence of abnormal behaviors or vices was not present in 20 specimens, representing 88.90% of the indicator fulfillment. Similarly, the criterion for relationship with people was analyzed using the "follow-the-person" test, where 61.76%, representing 20 specimens, walked side by side, yielding favorable results for the study.

14. Variable: Expression of other behaviors

See **Table 10**.

Appropriate expression of other behaviors	%
Absence	88.90
Presence	11.80
Total	100.0

Table 10.
Appropriate behavior/expression of other behaviors.

15. Variable: Good human-animal relationship

Finally, the positive emotional state criterion was assessed by measuring the visual horizon indicator, where the stalls in the exhibition pavilion are open. This represents 100%.

With this design, horses can interact and sniff each other. The average evaluation result for this criterion was 80 (**Table 11**).

Good human-animal relationship	Percentage
Absence	72.0
Presence	28.0
Total	100.0

Table 11.
Appropriate behavior/good human-animal relationship.

16. Variable: Positive emotional state

See **Table 12**.

Positive emotional state	%
Absence	20.0
Presence	80.0
Total	100.0

Table 12.
Appropriate behavior/positive emotional state.

The evaluation of (AW) at the Corrales ranch yielded the following results, considering good welfare in one principle (Good Accommodation) and three criteria (Comfort during rest, thermal comfort and Ease of movement) with 63.30% and finding values higher than 81.20% in three principles (Good Food, Good Bless you and Behavior Appropriate) and nine criteria, (Absence of prolonged sinking, prolonged thirst-free, No injuries, Absence of diseases, Absence of management-induced pain, Expression of social behavior, Expression of other behaviors, Good Human-Animal Relationship and Positive emotional state) as we see in **Table 13**.

Four principles	12 Criteria	Final assessment (BA)
Good	1. Absence of prolonged hunger	82.50%
Nutrition	2. prolonged thirst-free	
Good	3.comfort during rest	63.30%
Housing	4. Thermal Comfort	
	5. Ease of movement	
Good	6. No injuries	83.30%
Health	7. Absence of diseases	
	8. Absence of management-induced pain.	
Appropiate	9.Expression of social behavior	81.25%
	10. Expression of other behaviors	
Behavior	11. Good Human-Animal Relationship	
	12. Positive emotional state	

Table 13.
Results of the welfare quality assessment.

17. Discussion

In this work, animal welfare was evaluated using the Welfare Quality protocol, finding that the 20 horses evaluated demonstrated to be in good Animal Welfare conditions, yielding one principle and three criteria with 63.3% and in three principles

and nine criteria of animal welfare above 81.20% considering this stable with an excellent category.

During this experimental work, it was observed that the animals were in an acceptable and adequate state of well-being, as they met the necessary requirements for good physical and emotional health.

Márquez et al. [25], conducted an evaluation protocol for stable horses, demonstrating that quality structural materials and adequate stable dimensions are essential for high animal well-being, reducing the risk of injuries due to lack of space, and ensuring adequate rest for the animals. This is important so that horses have the opportunity to spend quality time resting after intense work, thereby preventing the development of individual and social behavioral abnormalities due to a lack of space.

The medical management provided to working equines in the state of Guerrero is very deficient, which causes various problems and susceptibility to diseases that affect their performance and quality of life. This is linked to the socioeconomic status of the owner. Reyes et al. [30], refers to the importance of good health management in equines to prevent health problems that can affect the quality of life and behavior of animals, both trophic and social.

The study of breeding equine welfare using an adapted assessment protocol provided initial information, for a specific time of year, on deviations in the breeding population under different management systems. The diverse findings showed that stabled breeding stock presented a more compromised welfare due to deviations from their natural behavior. In the case of mares, the greatest challenges were related to competition for feed and aspects related to their physical condition. Due to the direct impact on individual health, and therefore on their efficiency as breeders, both in this region and in others with the same management system, the application of an animal welfare assessment protocol also allows for the identification of concrete measures for improvement.

The assessment of body condition shows us that animals do not suffer from hunger or thirst, which leads to good performance in the animals' work. The same happens with the human-animal relationship, which is very important to generate trust during training, avoid or reduce the stress that this causes, which can generate feeding problems, and promote the appearance of stereotypical behaviors that put the physical and mental health of horses at risk, as mentioned in this study and in the experiment on breeding mares.

The following results were found at the Villa Maria breeding center, being very similar to those found in the corraletas ranch obtained an excellent rating, since the score in all principles was greater than 55%. Presenting an overall rating of 91.97% for each parameter evaluated: 99.27% good nutrition, 96.08% good housing, 94.29% good health, and 78.23% appropriate behavior. Furthermore, the application of this type of protocol allows monitoring of all breeding processes, thus allowing constant adjustments to be made to the pillars of production (nutrition, health, genetics, and management), the latter of which is of great importance in equine breeding due to the break-in and training processes [31].

18. Conclusions

The analysis of the results showed that Rancho las Corraletas obtained a high category in the principle (Good Housing), and three criteria (comfort during rest, thermal comfort and ease of movement) with 63.30% and excellent category in

three principles (Good Nutrition, Good Health and Appropriate Behavior), and nine criteria (Absence of prolonged Hunger, Absence of prolonged Thirst) with 82.5%, (Absence of Injuries, Absence of Diseases, Absence of Pain Induced by Handling) with 83.30% and Expression of Social Behavior, Expression of other behaviors, Good Human-Animal Relationship, Positive Emotional State) with 81.25%, averaging all the principles and criteria an overall score of 77.59% is obtained with a high category according to the Animal Welfare scale according to Wangerigen.

Furthermore, the application of this type of protocol allows for the monitoring of all dressage and show horses, thus enabling constant adjustments to the foundations of the dressage and show processes.

Finally, with this protocol we observed that the work of these horses does not interfere with the well-being of the animals, with good veterinary medical monitoring, which helps to prevail the health of the animals and a good human-animal relationship, supporting the advice to producers or owners of this type of establishments dedicated to the management and reproduction of horses for exhibition and to workers maintain direct contact with animals so that stress is minimal, animal welfare is optimal and they have a good work pace without affecting their health.

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Chapter 2

Herd Health Management Programme for Smallholder Goat Production

Muhammad Baba Aliyu, Bilkisu Yunusa Kaltungo, Nma Bida Alhaji and Saidu Shehu Na Allah

Abstract

Goats (*Capra aegagrus hircus*) are an integral part of the smallholder farmers all over the world due to their adaptability to different environmental and climatic conditions. They are dispersed, living on every continent except Antarctica. Output from goats in terms of food production, food security, income generation, employments along their value chain, and their role in the agricultural sector development—goats are playing a significant role in livelihoods and the economies of developing countries. Notwithstanding these advantages of goats to the socio-economic livelihood of the smallholder farmers, the management of these farm animals has greatly been challenged, which has posed significant constraints to profitable goat production in developing countries. Clinical management of disease in food animals was mainly on an individual animal bases, but with greater increase in the productivity of food-producing animals and with increase demand for their products, coupled with the growing diseconomy of medical therapy, it has become imperative for farmers and animal health service providers to invest in preventive models within the scope of veterinary herd health management. Provision of veterinary service has gradually shifted substantially to prevention of diseases on the bases of a herd, where maintenance of animal health uses advisory models to protect animals and people from illness, improve animal welfare, protect the environment, and also to mitigate antimicrobial use in food animals. A general proactive management on the bases of the herd in order to achieve healthy farming systems, food safety, and to alleviate poverty on the basis of herd health management is conceived.

Keywords: herd health, management programme, smallholder, goat production, animal welfare

1. Introduction

Goats (*Capra aegagrus hircus*) are versatile and adaptable to diverse climates and terrains, which makes them particularly form an integral part of the small holder

farmers. Due to their adaptability to different environmental and climatic conditions, they are dispersed all over the world, living on every continent except Antarctica and in a quite astonishing range of environments, from humid tropical rain forests to dry hot desert regions and cold, hypoxic high-altitude regions [1, 2]. Globally, there are currently about 1.002 billion goats with astonishingly over 1153 breeds, where they form a vital component of rural economies through the provision of meat, milk, other byproducts like wool, and hides and in cultural and religious benefits [3].

Output from goats in terms of food production, food security, income generation, employments along their value chain, capital asset and their role in input supply to the agricultural sector in the area of manure and biological diversity are playing a significant role in livelihoods and the economies of developing countries [4, 5]. These socio-economic roles are increasing in importance because of increasing demand for animal-based products occasioned by increasing human populations, incomes, and urbanization [6, 7]. More so, resource-poor smallholder farmers are finding goats to be increasingly suitable for diversifying sources of their income and nutrition. Studies have shown that global demand for meat and milk is projected to increase by 57 and 48%, respectively, between 2005 and 2050 [8], and this increase will be in the developing countries [9, 10]. It has also been shown that the market for goat meat has grown from USD 12.41 billion in 2023 to 13.11 billion in 2024. More so, it is expected that this increasing demand will continue to up to 18.34 billion by 2030 [11] Several studies has highlighted the importance of goats to small holder farmers in recent years [12–15].

Notwithstanding these advantages of goats to the socio-economic livelihood of the small holder farmers, the management of these farm animals has greatly been challenged by a number of productions, health, management, and environmental factors. These challenges have posed significant constraints to profitable goat production in developing countries. High lamb mortalities, poor growth rate due to poor quality feeds and feeding regime, endemic diseases, like paste des petit ruminantium (PPR), Contagious echthyma, Foot rot and gastro-intestinal helminthoses often aggravated by poor nutrition and poor effective management, have had dramatic negative impact on the herd or flock productivity [16–18]. More so, poor extension services, climate variability, and unfavourable policy environments coupled with major global factors such as the international trade barriers and associated implications continue to restrict access to market, which have negatively impacted on the growth and development of small holder goat production [19, 20].

Previously, the clinical approach to the management of disease in food-producing animals focused mainly on individual animal medicine with the progressive availability and administration of vaccination, antibiotics, and other therapeutics to manage and control endemic disease. However, with a greater increase in the productivity of food-producing animals and the need to meet up with increased demand for their products and the growing diseconomy of medical therapy, it has become imperative for farmers and animal health service providers to invest in preventive models within the scope of veterinary herd health management.

Recently, provision of veterinary service provision has gradually shifted substantially to prevention of diseases on the bases of a herd, where maintenance of animal health uses advisory veterinary services to protect animals and people from illness, improve animal welfare, protect the environment [21], and also to mitigate antimicrobial use in food animals [22]. Consequently, more than ever before, a general proactive management on the basis of the herds has become paramount over the classical reactive approach to the management of a single animal to achieve healthy farming

systems, food safety, and to alleviate poverty. It is on this basis that herd health management is conceived [21, 23, 24].

2. Definition of herd health management programme

Herd health management is a comprehensive approach to maintaining the well-being and productivity of a group of animals, typically livestock. It involves strategies aimed at preventing, diagnosing, and treating diseases, as well as promoting the overall health and welfare of a group of animals. Effective herd health management is a holistic approach to the management of livestock encompassing nutrition, housing, biosecurity, vaccination programmes, parasite control, reproductive management, and environmental management with the ultimate goal of improving herd productivity [25].

2.1 Importance of herd health management programme

2.1.1 Prevention and control of diseases

Prevention and control of diseases are essential parts of good and profitable livestock management practices by eliminating diseases and management inefficiencies that may negatively impact animal welfare or limit productivity of the operation, thereby ensuring economic viability, food safety, and environmental sustainability [26]. Implementing a herd health management programme helps prevent disease outbreaks and reduces the incidence of infectious diseases among farm animals. This potentially mitigates the impact of diseases on livestock production, which can lead to significant economic losses due to mortality, decreased productivity, and treatment costs [26]. Herd health programme essentially reduces herd illness, increases optimum feed conversion, and improves milk quantity and quality, and, at the same time, increases profitability. Healthy animals have better growth rates, higher reproduction rates, and produce higher-quality products such as milk, meat, or wool [27].

2.1.2 Welfare of animals

There are several management practices and procedures such as adaptive animal environment, mutilating procedures, individual housing conditions of social animals and confinement, lighting, feed and water restriction overcrowding, early maternal separation and premature weaning, which have been shown to have both physical and negative mental effects on the well-being and productivity of farm animals [28]. Animal welfare is an important concept in modern livestock production systems. It entails acceptable care to be provided in terms of adequate space, health care, adequate and nutritious feeding and clean and fresh water [29]. It also entails proper handling, reduced stress, and pain in order to improve the productivity and quality of their products [30]. Concern about livestock production and the quality status of its products has resulted in the introduction of quality standards, codes of practice, and certification programmes aimed at ensuring safe and good quality products from food animals based on acceptable and humane production practices [31]. Ensuring the welfare and well-being of animals is a fundamental component of a herd health management programme.

Healthy animals experience less stress, discomfort, and pain associated with diseases and faulty management practices. Animals with good welfare are able to express normal behaviors, such as grazing and social interactions, which are crucial for their well-being. By providing adequate preventive healthcare and promptly addressing health issues, farmers can promote a higher standard of animal welfare on their farms while at the same time achieving greater profitability in the long run. Perceptions about welfare during production influence consumer attitudes to the acceptability and value of their products [32].

2.1.3 Early detection and treatment of diseases

Regular health monitoring and treatment of disease is an essential component of herd health management programme. These practices facilitate early detection of diseases, which facilitate prompt intervention and treatment. Early diagnosis of health problems improves treatment outcomes, reduces the severity of illnesses, and minimizes the spread of diseases within and between the flocks. Advancements in accurate and rapid diagnostic tests are an essential component of contingency plans to detect, control, and eradicate diseases that pose a serious threat to the productivity of farm animals and the trade of animals and their products [33]. These technologies empower health professionals to make informed decisions quickly in relation to disease detection, care of animals, and treatment and overall control of diseases in farm animals.

2.1.4 Biosecurity and disease management

Biosecurity in animal production systems includes the measures that can be implemented by the producers at the farm level in order to manage the risks of infectious diseases on their premises. It represents the basis of disease control measures against endemic and exotic diseases [34]. A comprehensive herd health management programme includes implementing strict entry protocols for visitors, vehicles, and equipment, enforcing quarantine periods to observe for signs of disease for newly introduced animals, segregating animals based on their age, health status, or production stage, regular cleaning and disinfection of facilities, equipment, feeders, and water sources and managing vectors such as insects, rodents, and wildlife that can carry and transmit diseases [35–38]. These measures reduce the risk of diseases and protect the health of both the current flock and incoming animals. Effective biosecurity practices contribute to sustainable disease management and support long-term economic benefit.

2.1.5 Compliance with regulatory requirements

Farm animal producers must be aware of, and comply with, all legal obligations relevant to livestock production, for example, disease reporting, record keeping, animal identification and carcass disposal methods [39]. An effective herd health management programme ensures that farms meet legal requirements for disease prevention (vaccination protocols, disease surveillance programmes, or biosecurity measures), food safety (antimicrobial use and drug withdrawal period), livestock traceability (origin, health status, and management practices associated with individual animals), and environmental stewardship. By adhering to regulatory standards, farmers uphold the integrity of their operations, maintain good access to the market, and demonstrate their commitment to producing safe and high-quality products for consumers.

2.1.6 Reduced treatment costs

Proactive health management reduces the need for costly treatments and interventions. Preventing diseases through vaccination and good management practices is often more cost-effective than treating sick animals. By implementing effective health management, farmers can significantly reduce the incidence of diseases that would otherwise require costly treatments. Vaccination is a prime example of preventive healthcare measures that contribute to cost savings. Vaccinating livestock against common infectious diseases prevents outbreaks that could necessitate extensive treatment protocols, isolation measures, and potential losses in productivity and even livestock and human deaths where zoonosis is involved. Diseases like Peste de Petits Ruminants (PPR) can be devastating in a flock of goats, for instance, and economically costly to manage once an outbreak has occurred [40, 41]. Similarly, regular deworming and parasite monitoring and control prevent infestations that can lead to poor growth rates, reduced feeding efficiency, and increased susceptibility to other diseases. By maintaining optimal parasite control measures, the need for costly treatments associated with severe parasitic infections will be minimized.

2.1.7 Access to market

Access to market refers to the ability of producers to supply their products to domestic and international markets while meeting regulatory standards, consumer expectations, and industry requirements [42]. Healthy animals are more resilient to diseases, have lower mortality rates, and exhibit better growth performance, which enhances the overall quality and safety of livestock products. Consumers and retailers increasingly prioritize products derived from animals raised under humane conditions and with minimal use of antibiotics or growth-promoting agents. By ensuring compliance with animal welfare standards and ethical practices, producers enhance their reputation and credibility in the marketplace, thereby expanding access to consumers and premium markets. Furthermore, quality and consistency are critical factors that influence market access and consumer preferences in the livestock industry.

2.1.8 Reduced labour costs

Effective herd health management programmes are designed not only to ensure the well-being of the animals but also to streamline operations and reduce costs associated with labour, veterinary care, and losses due to disease outbreaks. By implementing a structured preventive healthcare schedule, the incidence of diseases and health issues that would otherwise require intensive labour to diagnose and effect treatment can significantly be reduced. Preventive healthcare measures not only reduce the need for reactive veterinary interventions but also contribute to overall herd resilience and productivity while at the same time reducing labour costs.

3. The imperative of herd health management programme in small ruminant production

There are many reasons that limit the use of the conventional approach to veterinary medicine in goat production, especially among the small holder farmers. Firstly,

the courses of several infectious diseases in goats are epidemic in nature coccidiosis) can spread very fast and aggressively within a flock (e.g., Pasteurellosis, Peste des Petits Ruminants (PPR), chlamydiosis and pneumococcosis), while some other non-infectious problems like copper poisoning or white muscle disease usually involve more often several animals or even the whole herd [43, 44]. Secondly, diseases in goats are not easily treatable, like enterotoxemia [45, 46]. Furthermore, compared to other livestock species, there is low income per goat unit, and the modest value of the animal itself will make adventuring into treatment not that profitable [47]. In addition, the large number of animals per flock makes conventional case-based therapeutic approaches to health conditions in goat too expensive and too labour intensive as in cases of infectious footrot, peste des petits ruminants, and contagious ecthyma. The lack of registered drugs for use in goat, as such other therapeutic products authorized for use in other food-producing animals like cattle, are prescribed for use in goat [48].

Furthermore, it is a well-known fact that single and multiple antimicrobial resistances have been gradually increasing [49–51]. Goats have increased susceptibility to health challenges as can be seen in some West African breeds of goat in cases of PPR and in Nubian breed of goats and related crosses, which are more susceptible to G6-Sulphate deficiency, a metabolicdefects in which affected goats experience delayed motor development, growth retardation, and early death [52]. Most of the drugs that exist for the treatment of health conditions in goats are actually registered for use in cattle. This means that they either cannot be used in goat or they have a very long milk withdrawal time in these animals, which results in big financial losses and additional labour, because treated animals have to be attended separately in farms. In countries where producers have to comply with the regulations on herd health management, goat producers must comply with regulations and standards related to animal health, welfare, and food safety. By adhering to regulatory standards, farmers uphold the integrity of their operations, maintain market access, and demonstrate their commitment to producing safe and high-quality products for consumers.

3.1 Evolution of herd health management programme in livestock production

Herd health management evolved with the development of the dairy sector and increased demand for dairy products, which changed the management of dairy herds in the area of breeding and breeding policy with more emphasis on cross-breeding with bulls of high genetic potential [53]. This development led to an increased milk yield but also to an increased incidence of production-associated diseases, such as decreased fertility, milk fever, hyperketonaemia, among others [54–56]. As a result of large-scale dairy farming and high-level application of technology in the dairy industry, interest shifted from the treatment of single animals to control of diseases at the herd level [21].

This programme first started in the Netherlands in 1970, where several services are provided by veterinarians through farm visits, consultancy services associated with reproductive problems, diseases, biosafety, nutrition, milk production, and animal welfare [57, 58]. In this programne, the role of the veterinarian is entirely different, as dedicating himself more to the general management of the herds with less attention to the treatment of a sick animal [59]. With these visits, veterinarians are able to see the farms' routines and identify strengths and risk factors that may constrain effective production and productivity in the farms. The aim of the programme is to promote animal health, increase productivity with comparative reduction in production costs, control and prevent diseases, ensure animal welfare, food safety, public health, and sustainable environment [21, 59, 60].

The new dispensation in the development of the livestock industry by using herd health management programme is associated with a new farming approach, where attention on farm operations is presently shifting significantly from therapy to the herd level legal and economic requirements [61–63]. Four stages of developments in preventive veterinary medicine have been described, with phase three involving the application of proactive rather than reactive measures established in the 1960s [64]. In the first stage, the establishment of veterinary medicine in the late nineteenth and early twentieth centuries mainly focused on the eradication of clinical infectious diseases like brucellosis and tuberculosis. This was followed by the second stage, which introduced the era of ambulatory clinics of the 1940s, focusing mainly on individual production animal medicine with the progressive availability of antibiotics and vaccination for the control of endemic disease. In the third stage, a subclinical form of disease as a major limiting factor in the productivity of farms in the mid-1960s was recognized. Both veterinarians and farmers were more proactive, implementing scheduled visits to implant infertility and mastitis control programmes. The coming of the fourth stage 4 saw the development of full-pledged herd health management programmes being introduced, implemented, and consolidated in farms from the late 1980s. These programmes allowed obtaining an overall health status of the herd, improving the animal welfare and rentability of farms.

The highly topical nature of preventive herd management lies in the fact that herd health management has recently become legally binding. Until April 2021, the 2016/429 Regulation (the so-called "EU Animal Health Law") had to be implemented in the European Union (EU) member states. Thus, the law begin to be implemented in the Netherlands [58, 65], Austria [66], Denmark [67], Sweden [59], Ireland [68], and other countries like the United Kingdom [69], and Canada [70]. In these countries, the 'Quality Chain Milk' system specified how milk should be produced and harvested [71]. The programme encouraged non-participating farmers by receiving a milk price seven times lower than those participating in the programme with a view to enrolling in it. This system requires veterinarians to work according to the principles of the Good Veterinary Practice Code. This requirement provides the veterinary surgeon with the opportunity to support farmers in optimizing milk production, preventing diseases, decreasing costs, and supporting fundamental decisions about herd health, welfare, quality, and farm management in general [72].

3.2 Comparison between traditional and modern approaches to herd health management programmes in livestock production

3.2.1 The traditional approach to herd health management

Typically, the traditional approach refers to practices by farmers that have been handed down through generations and are often based on experience, observation, and local knowledge rather than scientific principles. This approach varies widely depending on cultural, geographical, and historical factors, but several common elements can be identified:

3.2.1.1 Use of herbal and natural remedies

Traditional herd health management often relies on herbal treatments and natural remedies derived from plants or minerals. Several studies exist on preparation and utilization of herbal remedies for the treatment and management of livestock

ailments and disease conditions in Nigeria [73–76] and elsewhere in Ghana [77], Ethiopia [78, 79], Algeria [80, 81], South Africa [82], and Pakistan [83]. These remedies are believed to have medicinal properties that can treat various ailments in livestock. However, there is limited efficacy data to support the use of herbal and traditional natural remedies for the management of ailments in livestock [84].

3.2.1.2 Use of observational diagnosis

Traditional herders and livestock keepers rely heavily on visual observations and behavioral cues to diagnose illnesses or health issues in their animals [85]. This type of knowledge is generated over a long sequence of observations and interactions with the environment and transferred from generation to generation orally [86]. This approach is used by pastoralists in Nigeria, for instance, to diagnose trypanosomosis and brucellosis in their cattle [87]. Such practices and practical experience in the diagnosis of diseases, prevention, control, and observations are handed down from generation to generation verbally and maintained by people through generations [88].

3.2.1.3 Rituals and superstitions

Traditional herd health management incorporates supernatural beliefs, rituals, and superstitions believed to ward off diseases or promote animal health by livestock keepers and pastoralists in most African countries. Supernatural beliefs and superstitious practices are common in every society [89]. People in every sphere of life adopt supernatural practices that are associated with their beliefs in a number of ways for different purposes. For example, pastoralists used rituals by way of sticks, thinking such acts would ward away rinderpest in their herds during the rinderpest outbreaks of the 1980s in Africa [87]. A greater share of these practices are constituted by magical, rituals, and occult practices [90]. Superstition is the non-scientific belief in magical connections. Such beliefs include aspects of certain religions and supernatural ideologies such as astrology, omens, witchcraft, and prophecies [91]. There exists a significant number of supernatural agricultural beliefs and practices that people perform for different purposes. However, the majority of these supernatural practices in the field of agriculture are expected to protect the crops and animals from the evil effects of supernatural forces and for increasing the overall production in farming and livestock. A lot of these do not come true as Saidu [92] reported that the pastoralists that used ritual sticks still lost their cattle to rinderpest.

3.2.1.4 Community knowledge sharing

Knowledge about animal health management is often shared within the community or among families who have a history of livestock keeping [93–96]. Indigenous knowledge systems of livestock and health care are the only asset in the hands of the small holder farmers to mitigate the impact of diseases on livestock production. In such cases, knowledge of diseases is passed from parents, friends, and community members in a lot of cases [95, 96].

Ethno-veterinary knowledge is not evenly distributed in a community, as those who have it tend to keep it in the family [96, 97]. Gender, age, and social class are major determinants of the amount of knowledge that one possesses [96, 97]. The aged or community ethno-veterinary attendants have more knowledge of ethno-veterinary practices, which were acquired over the years, making them more specialized, skillful,

and experienced in traditional or indigenous knowledge management. In a Pakistani study, it was found that the old-age population group possessed more ethno-botanical knowledge because of their long association with a typical agro-pastoral lifestyle when compared to younger generations [98, 99]. Older people and professional healers, as custodians of indigenous traditional knowledge and practices, are principally involved in its sharing and preservation of the knowledge in the community, and this is through community practice [100].

3.2.1.5 Reactive approach to animal health

Reactive animal health approach typically involves responding to health issues as they arise rather than actively preventing them. This involves regular checks through behavior by an animal that may be observed and possibly diagnostic tests when issues are suspected. The approach involves administering treatment and intervention when an illness or health problem is identified. Reactive management can sometimes be more costly due to high treatment expenses, reduced productivity during illness, and the potential for large-scale disease outbreak. Reactive management of diseases allows for quick response when health issues arise, potentially preventing worsening conditions or mortality. It is also flexible as it may be able to adapt to various health challenges and the specific needs of individual animals rather than preemptively reacting to the entire herd, thereby reducing the direct resources used to help animals in need. More so, it provides an insight into most prevalent diseases, treatment efficacies, and herd-specific vulnerability through experience and record analysis. A major short coming of the reactive approach to animal health is higher treatment cost due to increased veterinary care and expenses on treatment and medication in addition to reduced productivity of animals during recovery periods. Focusing more on immediate issues may overlook the underlying chronic conditions or systemic health challenges.

4. Modern approach to herd health management

The modern approach to herd health management is grounded on scientific research, technology, and evidence-based practices. It incorporates advancements in veterinary medicine, diagnostics, and management strategies to optimize animal production, health, and productivity. These are approached in a number of ways, which are discussed below.

4.1 Preventive medicine

Goats are susceptible to a variety of infectious and non-infectious diseases that can significantly impact their health and productivity. A well-designed herd health management programme focuses on preventing diseases through vaccination programmes, biosecurity protocol compliance and strict enforcement, parasite control, nutrition management, and regular health screenings to detect and control diseases early. The prevention of diseases reduces the incidence of illnesses such as foot rot, respiratory infections, helminthoses, and reproductive disorders [101, 102]. By minimizing disease risks, farmers can maintain healthier flocks, reduce treatment costs, and prevent economic losses associated with disease morbidity and mortality.

4.2 Diagnostic tools and technologies

Regular health monitoring and early detection of diseases in a herd is an essential component of a herd health management programme [24]. These practices facilitate early detection of diseases or health problems, allowing for prompt intervention and treatment. Early diagnosis also improves treatment outcomes, reduces the severity of illnesses, and minimizes the spread of diseases within the flock. Veterinarians play a key role in conducting health assessments, diagnosing illnesses, and providing recommendations for effective management practices. Advanced diagnostic tools such as blood tests, imaging techniques (like ultrasound and X-rays), and microbiological and molecular assays are also used to diagnose health conditions in livestock.

4.3 Use of pharmaceutical therapeutics

Modern practices include the use of drugs for various purposes in animals, which are developed based on rigorous scientific research and following regulatory approval. Veterinary drugs are used for therapeutics and prophylactic purposes to regulate infection of microbial origin and prevent outbreak of diseases in animals or as growth promoters [103, 104]. These are in addition to other substances like vaccines, disinfectants, and anti-inflammatory substances that are used to protect the animal from microbial infections and the management of pain. Close to 60% of drugs are used for therapeutic purposes, although increasing amounts are administered for prophylaxis to prevent infection. Therapeutic medication refers to the treatment of established infection, whereas prophylaxis is the use of drugs in either individuals or groups to prevent the development of infection. Both therapeutics and prophylaxis use involve administration of the drugs through different routes at recommended dosages for a specified period [103]. The use of antimicrobials as feed supplement can promote the growth of food animals and also enhance feed efficiency [104]. However, there is a growing fear of antimicrobial resistance among food animals in the use of antibiotics as feed supplements [105].

4.4 Data-driven decision making

Data-driven decision making is a fundamental principle of effective herd health management that helps minimize treatment costs in livestock operations. By collecting, analyzing, and interpreting data on animal health, performance metrics, and environmental factors, trends can be identified, health challenges anticipated, and proactive preventive measures implemented. Digital health records and management software streamline data collection and analysis processes, facilitating timely decision making and communication among stakeholders. Real-time access to health records and diagnostic results enables managers to respond swiftly to emerging health issues, implement treatment protocols promptly, and monitor treatment effectiveness. This proactive approach minimizes the duration and severity of illnesses, thereby reducing the overall costs associated with disease management and treatment.

4.5 Professional veterinary care

Access to professional veterinary care provided by trained veterinarians and qualified animal health service providers is a cornerstone of modern herd health

management. Veterinarians provide expertise in disease prevention, treatment, and surgical interventions that enhance animal welfare and productivity. Professional support in herd health management involves the expertise and guidance provided by veterinarians, nutritionists, and other service providers. These professionals play crucial roles in health checks, diagnostic tests, and disease screenings to monitor the health status of the herd. Early detection of health issues allows for prompt intervention and treatment.

4.5.1 Holistic approach in animal health care provision in herds/flocks

A holistic approach to animal health management emphasizes comprehensive care that addresses the physical, mental, emotional, and environmental aspects of animals. It integrates preventive measures, proactive health strategies, and complementary therapies alongside conventional veterinary medicine. This approach aims to optimize animal welfare, enhance production and productivity, and promote sustainable practices across various sectors of animal agriculture, companion animal care, and wildlife management. It optimizes veterinary care, nutritional disease prevention through vaccination, strategic parasite control, and environmental sustainability.

In holistic approach to animal health management there is a stronger collaboration among farmers, veterinarians, industry specialists, and allied health professionals to develop individualized animal care plans and integrated treatment approaches while engaging local communities, regulatory agencies, and industry stakeholders to promote animal welfare standards and sustainable practices.

Some advantages associated with holistic animal health management include the ability to address physical, mental, emotional, and environmental requirements in farms, promoting overall well-being and quality of life of the animal. It has also been shown that these approaches to animal health management reduce the incidence of diseases, injuries, and behavioral problems and enhance the resilience of the animal to stressors, disease outbreaks, and environmental changes through integrated management practices.

4.6 Emerging trends in herd health management

To match up the increase in market demand for livestock products by an ever-growing population, advances in technology have revolutionized livestock production practices, allowing for more precise diagnostics, targeted treatments, and data-driven decision making with the objective of improving the productivity of livestock. Due to the current scale of production, there is increased awareness that monitoring of animals can no longer be done by farmers in the traditional way and therefore requires the adoption of new digital technologies [106].

The application of technology, such as remote monitoring systems, wearable sensors, and data analytics platforms, has provided veterinarians, farmers, and farm managers with real-time insights into the health status of individual animals and the entire herd. This enables early detection of diseases, optimization of feed and nutrition programmes, and identification of management practices that contribute to better animal welfare and performance. Advances in technology are benefiting herd health management system and it is reshaping the future of animal husbandry.

4.7 Some key technologies and their role in herd health

4.7.1 Electronic health monitoring devices

Wearable biosensors are being used as monitoring devices for monitoring and management of animal health and production. These wearable sensors and devices are used to track vital signs, level of animal activities, and behavior patterns, allowing early signs of health issues, particularly in cattle. The types of wearable devices include e-tags, cow collars, and electronic earrings. These wearable gadgets are linked to individual livestock and frequently include data processing systems that can detect diseases early, averts epidemic outbreak among animals. The wearable device is equipped with sensors for monitoring different parameters relevant to animal health. These may include accelerometers for measuring the animal's movement patterns, heart rate monitors, temperature sensors, GPS, and so on. The sensors are carefully incorporated into the wearable gadget to enable precise data collecting [107].

4.7.2 Data analytics and artificial intelligence

Artificial Intelligence (AI) is defined as the ability of a system to correctly interpret external data, to learn from it, and to use that learning to achieve specific goals and tasks through flexible adaptation [108]. It holds a significant place in the application of technology in livestock production. AI have simplified the drudgery associated with animal health, nutrition, and reproduction management by optimizing the potentials of AI in livestock agriculture. Advanced sensors and analysis tools capable of real-time health monitoring facilitate extensive data collection and analysis capabilities, covering everything from animal health to milk production and nutrition to reproductive management. Furthermore, combining milk analysis, genetic data, and environmental information leads to more accurate breeding predictions. AI is being used in robotic milking systems, monitoring animal nutrition and movement, early diagnosis of diseases, and developing treatment methods. Additionally, innovative applications like AI-based virtual fencing systems and systems that analyze animal gaits to detect diseases are explored. These technologies improve farm management, enhance animal health and welfare, and increase efficiency. These programmes not only boost the animals' health and milk output but also reduce feed costs.

4.7.3 Precision livestock farming

Precision Livestock Farming (PLF) is one of the most powerful developments among the livestock farming industry, offering real-time monitoring and management tools for farmers. PLF includes a wide range of technologies that are being applied along with advanced technologies like microfluidics, sound analyzers, image-detection techniques, sweat and salivary sensing, serodiagnosis, and others [109].

Precision technologies can be wearable, incorporated into the milking system, stand-alone, or part of the management software. Precision monitoring uses technologies to measure individual cows' behavioral, production, and physiological variables to improve management and farm performance. Technologies used for PLF range in complexity from daily milk recording to measurement of specific attributes such as temperature or lying time [110]. The PLM technology measurements currently

available to producers include daily milk recording, milk component monitoring, milk conductivity monitoring, feeding behavior, rumination behavior, rumen pH, rumen temperature, body temperature, mounting behavior, lying behavior, animal activity, animal location or positioning, blood component monitoring, and daily body weight measurements [110, 111].

4.7.4 Telemedicine and remote monitoring

According to the American Veterinary Medical Association (AVMA), telemedicine is the use of medical information exchanged from one site to another *via* electronic communications to improve a patient's clinical health status [112]. Telemedicine involves the use of electronic communication and information technologies to provide clinical healthcare remotely. It further includes the provision of veterinary services through the use of video, text, instant messaging or telephone, or by any other remote means [113]. Telemedicine may be as simple as two health professionals discussing a case over the telephone or as complex as using satellite technology and video-conferencing equipment to conduct a real-time consultation between medical specialists in two different countries. Telemedicine generally refers to the use of communications and information technologies for the delivery of clinical care [114].

5. Integrated approach in herd health management programmes

Effective disease management in herd health programmes requires an integrated approach that combines treatment, control, and prevention strategies tailored to the specific needs and challenges of flock operation [115]. Veterinarians, livestock producers, and other stakeholders need to collaborate to develop and implement comprehensive management plans that address disease risks, enhance biosecurity, and promote responsible antimicrobial and other drug use to increase the productivity of operation and ensure food safety and environmental sustainability. By prioritizing disease prevention and adopting integrated approaches to herd health management, stakeholders can enhance resilience against diseases and contribute to the long-term sustainability of livestock farming [115].

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Chapter 3

Intestinal Microbiota and Injurious Behaviors in Laying Hens

Heng-wei Cheng

Abstract

Injurious behaviors in laying hens cause significant health, welfare, and economic challenges to the poultry egg production industry globally. Despite the advance in management, injurious behaviors happen in all the current rearing facilities, including both cage systems (conventional cages and enriched cages) and cage-free systems (aviary system and organic system). Gut microbiota functions as an endocrine organ displaying multiple functions including the regulation of behavioral exhibition *via* the gut-brain axis in humans and various animals including chickens. Based on our earlier success, this chapter outlines the potential of inhibiting injurious behaviors in chickens through remodeling intestinal microbiota composition and function. It provides a novel insight into developing appropriate biotherapeutic strategies for preventing injurious behaviors in chickens.

Keywords: social stress, aggression, injurious behavior, intestinal microbiota, microbiota-gut-brain axis, chicken

1. Introduction

Injurious behaviors (i.e., aggressive pecking, feather pecking, vent pecking, and cannibalistic pecking) in laying hens are a critical unsolved health, welfare, and production issue facing the poultry egg production industry globally. These harmful behaviors cause tissue damage, consequently, increasing morbidity and mortality, reducing productivity, and leading to huge economic losses. Despite the advanced developments for sustainable poultry operations, such as developing alternative housing facilities (i.e., enriched cages, aviaries with single- and multi-tier, and organic system), modifying light intensity and stocking density, balancing diet components, and selecting chickens with kind gentle behaviors [1–6], beak trimming (i.e., removal of 1/3 to 1/2 of the beak) is still the most effective and reliable method to control the injurious behaviors in laying hens [7]. However, beak trimming remains a controversial subject since the avian beak is a multipurpose functional organ playing a vital role in feeding, drinking, preening, and mating as well as defensing predators and parasites [8]. In addition, beak trimming causes tissue damage, subsequently leads to pain (acute, chronic, or both) and functional limitation, and negatively impacts chicken welfare regardless of operation methods (i.e., hot blaze beak trimming or infrared

beak treatment) [9] although infrared beak treatment is a less painful alternative with multiple advanced benefits such as less impacting feed intake, drinking, growth, and engagement in natural behaviors (**Figure 1**) [10]. Omitting beak trimming, however, results in increased incidence of harmful behaviors and related negative consequences for skin damage and survivability with the reduced health and welfare status in chickens [11]. Recent studies also revealed that injurious behaviors still occur in beak trimmed flocks irrespectively rearing facilities, including cage (conventional and enriched cages), cage-free (aviary with single- and multi-tiers), and organic operations [12]. The outcomes from recent studies exploring the solutions to reduce injurious behaviors have proposed that successfully managing injurious behaviors is to increase animal adaptation to and/or reduce management practice-associated stress [13, 14]. In light of these challenges, there is a critical need to explore the active strategies in preventing injurious behaviors in poultry.

Naturally, injurious behaviors (such as aggression and feather pecking) in chickens can be initiated by setting up a dominant order or a ranking system (similar to the nature of social hierarchies or social dominance orientations in humans and other mammals) to determine an individual's status within a flock when they are interacting [15, 16]. A dominance order (also called pecking order) in chickens can be established

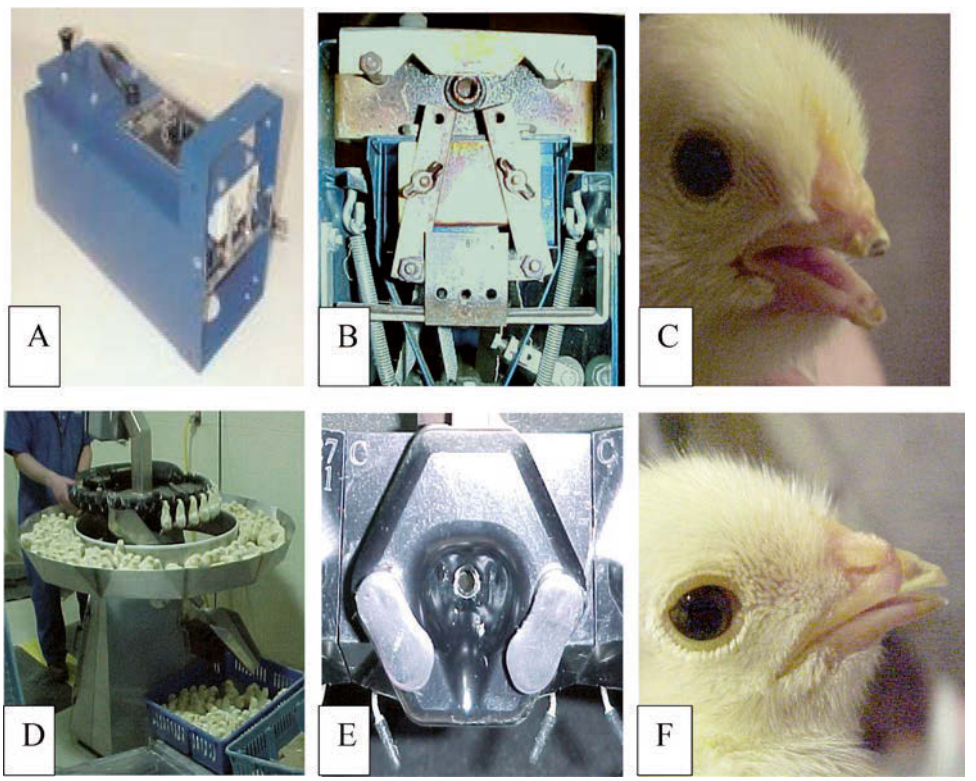


Figure 1.
Traditional equipment used for beak trimming in laying chicks. A. Lyon Super Debeaker used for hot blaze beak trimming. B. There are three holes selected based on a chick's beak size. C. An example of a chick's beak trimmed with a hot blaze debeaker. D. Infrared beak trimmer. E. There is a hole with two push bars to push a chick's beak goes through the hole. F. An example of a chick's beak trimmed with an infrared beak trimmer. Notes: Shrinkage and necrosis of the beak ending of the chick trimmed by an infrared beak trimmer, and inflammatory reaction of the beak ending of the chick trimmed by a hot blade debeaker at day 4 post beak trimming.

at the age of 6 weeks or younger [17, 18]. It has been hypothesized that well-established dominance order with clear boundaries in place among young chickens has benefits as good signs of vigor and reproduction by sharing resources [18, 19], but early experience in pecking can induce severe feather pecking in later life if the dominant individuals constantly show bulling to subordinates or the established pecking order is disrupted during birds moving in a flock with several hundred or even more chickens seen at commercial settings [20, 21]. Recent studies have revealed that early life experience affecting both brain development (programing and reprograming of neural circuits) and gut microbial colonization (microbiota diversity and composition) and the function of the microbiota-gut-brain (MGB) axis. The MGB axis is a crucial bidirectional pathway transferring neural, hormonal, and immune signaling between microorganisms in the gut and cells (neurons and glial cells) in the brain [22–24].

Recent studies have revealed that gut microbiota functions as an endocrine organ releasing various bioactive factors, playing critical roles in early brain programming and later function (sensitivity, adaptability, or plasticity) of the stress responding systems (i.e., the hypothalamic-pituitary-adrenal, HPA axis, and sympathetic-adrenal-medullary, SAM axis) in reacting to various stimulations [25–27]. By contrast, stress-induced intestinal dysbiosis releases various neurotoxic compounds and, consequently, causes neuroinflammation and neural injury, leading to pathophysiogenesis of emotional and behavioral disorders [28–31]. Particularly, adversity during early life blunts intestinal microbial colonization, distribution, and function, influencing the development of brain structure and function through the gut-brain axis, and subsequently causing long lasting adverse outcomes including increased frequency and intensity of aggressive (violent) behaviors across species [32–34]. In addition, the mental health or social wellness of social animals within a group (or a flock) is associated with individuals' differences (phenotypes) in their physical characteristics, behavioral traits, and biological properties including the intestinal microbiota diversity, composition, and function [35–37]. Investigating interventions for reducing aggression (violence) have become a critical topic in humans and various animals including chickens; and targeting gut microbiota using probiotics, synbiotics, and psychobiotics for restoring imbalanced microbiota diversity and composition in early life has become a biotherapeutic strategy in treating neuropsychiatric disorders [38–40]. Despite continuously evolving the outcomes in MGB investigation, there is still a critical need for understanding the regulatory function of the MGB axis in host health and illness, especially in chickens.

2. Social stress and injurious behavior

Stress is a state of an organism's nonspecific biological and/or psychological reactions to a perceived challenge, hindrance, or threat. Chickens as well as other animals endure many stressors raised from their environments on a daily basis. The outcomes of stressful stimulation may cause a positive (eustress) and negative (distress) effect on the animal's health and well-being based on the type of stressor (pleasure vs. unpleasure) as well as its frequency (single vs. repeated), duration (acute, short-term vs. chronic, long-term), and intensity (mildly vs. severe). Repeated stress or chronic stress results in wear and tears on the biological system of animals, leading to physical, mental, and/or emotional changes, especially during the late prenatal and early postnatal developments [41, 42]. An animal stressed during early life impairs its brain architectural development (altering neurogenesis, synaptic formation, the establishment

of neural circuits, and signal transmission), as the critical period of development of both the prefrontal-hypothalamic-amygdala axis and the serotonergic-dopaminergic circuit causes profound long-lasting effects [43]. Dysregulation of the HPA axis (a stress responding system functionally affects mental health) and the central serotonin (5-HT) deficiency (a low brain 5-HT causing an imbalanced neurotransmission) are strongly connected with the variety of mental and/or emotional disorders, including depression, anxiety, and cognitive decline in humans [44, 45]. Both the systems have become an integrated brain center of aggressive investigation (**Figure 2**) [47, 48].

Aggression, as one of injurious behaviors in social animals, is an extraordinarily complex evolutionary conserved behavior during development (i.e., natural, artificial, or both selection processes). Functionally, aggression is an essential component of social behaviors for protecting valuable resources associated with the animal's successful survival, growth, and reproduction [49], while under a stressful condition, aggression (as an unprovoked, instrumental, or reactive behavior) is a symptom of mental or emotional disorders, posing a health and welfare concern, often destructively and maladaptively leading to pain, fear, anxiety, and injury, which can be manifestly throughout the entire lifetime [50]. Particularly, extreme and/or persistent forms of aggression in humans have brought great public alert due to it causing variety damage to society, resulting in great social destruction and economic burden [51]. In addition, current studies have revealed that humans with aggressive behaviors are exhibiting a broader phenotype in patients with mental disorders (i.e., a subset of individuals showing antisocial personality) under the same or similar stressful conditions [52]. Similarly, gene-environmental interaction on behavior has also been revealed in

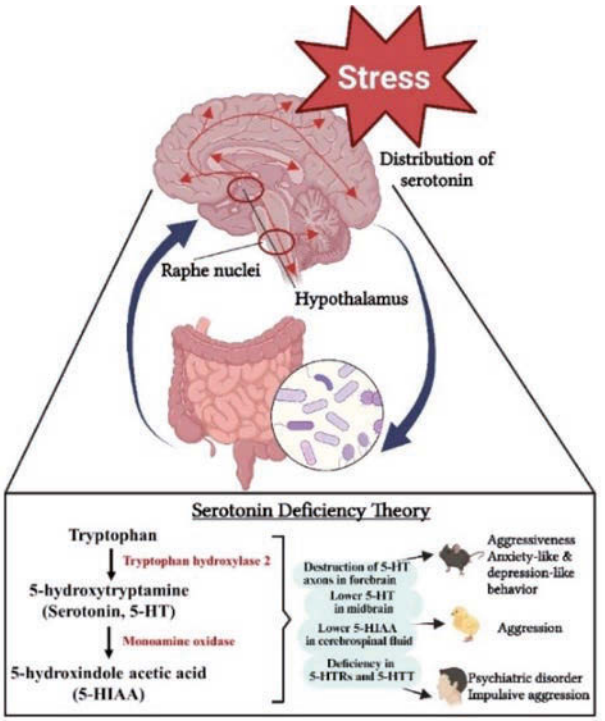


Figure 2.
Injurious behaviors associated with stress-induced dysbiosis and serotonergic deficiency theory (Modified from cited reference [46], with BioRender.com).

chickens. Domestic chickens, like humans and other social animals, exhibit cognition, emotions, and phenotypic personality in responding to stimulations [53].

3. Stress and injurious behaviors in laying hens

Commercial laying hens have been artificially selected for high production performance (egg production and feed efficiency) to meet the demand of animal-derived products for continuously increasing the global population established to reach nearly ten billion by 2050 [54]. The breeding process integrates changes of animals in gene, behavior, morphology, physiology, and psychology to create the suitable traits (domesticated phenotype) coexisting with humans [55, 56]. However, based on the whole organism theory, artificial selection based on individual productivity may affect other physiological and behavioral trials, increasing animal competition and aggression [57, 58]. The outcomes from the studies focusing on injurious behaviors in chickens support the hypothesis that the productivity of an animal is correlated with its competitive ability within a flock [59]. Recent study has reported that the social effects as unintended outcome of selection contribute to half of the heritable variance in mortality due to beak inflicted damage [60]. For instance, beak untrimmed hens of a former commercial Dekalb XL strain had great production performance after more than 20 years of breeding selection, whereas simultaneously, mortality due to aggression and cannibalism had also significantly increased about 10-fold [61]. Like humans and other social animals, aggression in chickens has important functions during interspecific interactions (competition) to protect essential resources for survival and reproduction successfully [62]. However, aggression simultaneously increased the levels of stress, and stress susceptibility has become major health and welfare issues facing the poultry industry [63]. Although the current advances in husbandry practices increase the capability of chickens to combat stress-induced abnormal behaviors, poultry production is still impacted by the environmental stressors associated with the current rearing facilities and practices [64] since chickens naturally have a dominant hierarchy to establish pecking order (dominance rank) within a flock [65]. In addition, as a learning or rewarding behavior, initiated injurious behaviors by one or more chickens can be spread throughout the entire flock. Chickens without adapting to their rearing environments may enter a "pathological state" with atypical responses (hypo- or hyper-reactivity) of the activated stress regulating pathways (both the HPA and SMA axes) and/or the enforced brain award system (the prefrontal cortex-ventral tegmental area-nucleus accumbens-amygdala complex circuit) [66].

Numerous studies have revealed that the neural circuits for regulating social behavior are evolutionarily conserved across the vertebrates [66, 67]. In addition, a "core aggression circuit or social behavioral network" for possessing mental and emotional flexibility presents in the brains, such as the basal forebrain, midbrain, and mesolimbic system, of animals across species [68, 69]. In chickens, these nuclei exert similar cognitive ability with the capability of plasticity in responding to various environmental stimulations as seen in humans and rodents [67, 70]. In addition, the 5-HT deficiency has become the major theory of investigating injurious behaviors in birds [71]. Similar distributions of 5-HT neurons and 5-HT receptors have been identified between birds and mammals [72]. In chickens, increased dietary 5-hydroxy-TRPptophan (26.43 mg/bird), a direct precursor of 5-HT, reduces fearfulness in a tonic immobility test [73]. The test is routinely performed for assessing fear response in birds [74]. In addition, prenatal 5-HT fluctuation *via* injection of exogenous 5-HT

to embryos before incubation (20 μg/fertilized egg) reduces aggression in chickens by increasing the development of serotoninergic neurons and related neural circuits [75, 76]. Injurious behaviors in chickens can also be reduced *via* embryonic exposure to tryptophan (TRP, 500 μg/egg at E12), a precursor of 5-HT [77]. Recently, it has also been discovered that probiotics including *Bacillus subtilis* can prevent aggression in chickens *via* modifying the central serotonergic system through the MGB axis [78].

4. Intestinal microbiota and the microbiota-gut-brain axis in responding to stress

Recent studies have revealed that an imbalanced intestinal microorganism (called gut dysbiosis or dysbacteriosis) during early life alters brain development, affecting neurogenesis, modulating neuronal architecture, and signaling pathway within the neural circuits through the MGB axis [79, 80]. Normally, the intestinal microbiota impacts on a host's health *via* multiple routes, involving in energy metabolism (food digestion and nutrient absorption), bacterial pathogenicity, intestinal barrier integrity, immune function, and synthesis of bioactive factors, such as short-chain fatty acids (SCFAs) and TRP [81]. SCFAs are a family of fatty acids, including butyrate, acetate, and propionate, with the important biological roles. In the gastrointestinal (GIT) tract, SCFAs regulate intestinal energy metabolism, immunity, and microenvironment homeostasis, while in the distant organs, they appear anti-inflammatory and possess immunoregulatory functions [82–84]. TRP, an essential amino acid, is produced within the GIT through the digestion of foods and then enters the brain through the blood-brain barrier for synthesizing 5-HT by the raphe neurons [85, 86]. Chronic stress reduces bacterial TRP synthesis and affects TRP metabolism *via* disrupted the balance between the kynurenine and 5-HT pathways, in turn, lowering brain 5-HT synthesis [87, 88]. Dysregulating tryptophane-derived serotonin-kynurenine balance influences cognition and mood emotion [89] and drives injurious behaviors (aggression and violence) in humans and various animals including chickens [90, 91]. Gut microbiota also serves as a critical behavioral regulator in personality traits, especially during the co-developing stage of the brain neurons and gut microbiota in early life [92–94]. In humans, the differences in gut microbial taxa have become the reason for subsets of individuals are more sensitive to stressful stimulations even under the same or similar living environments [95, 96]. Similarly, chickens with various levels of injurious behaviors have different gut microbiota diversity, composition, and metabolite profiles [97–99]. In addition, laying hens show different fear responses during tonic immobility tests have different gut microbiota composition with the increased inflammatory immunity [100]. Similar to it in humans, modifying or restoring gut microbiomes has become a novel strategy for preventing or inhibiting injurious behaviors in chickens [101, 102]. Taken together, dysfunction of gut microbiota (changes in composition and or synthesis of metabolites) participates in developing neuronal disorders crossing multiple species including chickens. As evidence mounts, the use of psychobiotics (next-generation probiotics) [103, 104] as well as microbiota transplantation (such as fecal microbiota transplantation, FMT) [105, 106] to restore stress-induced dysbiosis has offered novel therapeutic strategies in treating neuropsychiatric patients.

Various probiotics (direct-fed microbials) and synbiotics (mixtures of prebiotics and probiotics) have been used as growth promoters and/or immune stimulators to replace antibiotics in poultry production [107], and several delivery methods have been developed except administered as dietary or drinking water supplements.

To enhance the gut microbial development during early life, *in ovo* injection of living bacteria has been used for improving intestinal health, growth performance, and stress reactions in chickens [108, 109]. In addition, microbiota transplant in chickens, like FMT in humans, has been developed as a novel method for mitigating stress-induced negative effects on health and maintaining optimum production *via* the gut-brain axis [110–112]. In humans, FMT is an approved treatment for *Clostridioides difficile* infection [113, 114]. The aim of the procedure is to transfer fecal bacteria as well as other microbes from a selected healthy individual to a patient (a recipient) with intestinal diseases to replace the pathogenic bacteria with beneficial bacteria for restoring or rebalancing bacterial composition. However, FMT outcomes in chickens as well as in humans are affected by multiple factors, including the practical protocols (such as donor variability, amount of fecal dosage, frequency of infusions, route of delivery, and adjuvant treatments) as well as feed-, geography-, and management-based effects (such as animal feed and rearing system carrying the complex effects on microbial community), donor-recipient interplay, and pathogen transfer with the procedure [115–117]. To further investigate the effects of microbiota on chicken production and health, cecal microbial transplantation (CMT) has been developed with the focus on developing of next-generation probiotics, psychobiotics, and psychobiotic compounds to optimize their efficacy and safety across multiple species [118, 119].

The avian cecum (also named the pyloric ceca) has different structures and biological functions as that of mammals within the digestive system. In mammals, the cecum is relatively small with the major functions in storing and absorbing water and salts [120], while in chickens, there are two ceca, as a multi-purpose organ, playing a critical role in maintenance of overall health [121]. Avian cecum functionally facilitates the fermentation of undigested materials, absorbs substrates, water, and electrolytes, synthesizes bioactive factors, and regulates immune capability [122–124]. Uniquely, the avian cecum releases contents in two directions: the cloaca, excreting as cecal drops, and the ileum, providing bacteria to maintain gut microbiota diversity and composition [125]. Along with the GIT tract, the avian cecum harbors the greatest bacterial biodiversity [126]; and like in mammals [127], chicken cecal microbiome is also sensitive to various stressors with reactions appearing to be the result of gene, environment and/or gene-environment interactions [128, 129]. For instance, chicken lines with different cecal microbiota compositions [130] uniquely respond to environmental stressors (such as heat stress) [131] as well as experimental immune challenges with *Salmonella* or *Eimeria necatrix* [132, 133]. In addition, 1-day-old broiler chicks (recipients) obtained the cecal contents from hens (donors) that have distinctly gut bacterial profiles from day 7 until day 42, which are similar to the donors' cecal microbiota profiles [134]. Newly hatched Ross 308 broiler chicks (within <12-h post-hatch) received cecal samples from adult Roslin broiler breeders (40-week-old) that have greater differences in cecal microbiota composition and diversity compared to control chicks received phosphate-buffered saline (PBS) at day 7 post-hatch [131]. In addition, day-old broiler chicks (recipients) received a single passage of the pooled cecal contents from adult broilers (weeks 5–8) that have a stable gut community with the protective effects against pathogenic challenge (*Salmonella Typhimurium* and a *Campylobacter jejuni* strain) at day 14 compared to PBS controls [135, 136]. Microbial function analysis also shows that day-old chicks received cecal microbiota from healthy old hens (inbred 80-week-old Wannan Yellow Chickens) have different gut microbiota composition and diversity with the improved biological pathways in genetic information processing, cellular processes, and metabolism compared to saline controls [110]. These results indicate that, as in

humans and rodents, the early postnatal period is a vital window for the development of gut microbiome in birds. Similar to FMT in humans, the early-life CMT in chickens profoundly influences the development of intestinal microbial colonization and the brain development with a long-lasting impact on the biological responses to various stressful episodes. CMT offers potential for developing novel management practices to increase productivity and to prevent the physiological and behavioral disorders in chickens [137, 138]. However, it is still unclear how injurious behaviors arise as a consequence of gut dysbiosis (an imbalanced gut bacterial composition) and related dysregulation of the communication between the gut and the brain. In addition, some studies have reported no differences in the cecal microbiota diversity and its major function in chickens when confounding factors, such as breed and diet, were included [139]. A recent study also reported that antagonistic behavior and feather pecking are not linked to gut microbiota composition and predicated function in laying hens [140]. To fill the gaps, we used the diversely selected inbred genetic lines to investigate whether the modulation of gut microbiota during early postnatal time can be an optimal method to prevent injurious behaviors in laying hens.

5. Biological characteristics of the selected chicken lines and cecal microbiota transplantation in injurious behaviors

Two divergently selected inbred chicken lines, 6_3 and 7_2, were used in the multi-year study [141–144]. Chicken line 6_3 and line 7_2 have been selected for resistance or susceptibility to Marek's disease respectively since the late 1960s [145]. As predicted, the selection has resulted in line differences in production with unique neuroendocrine function, immunity, and behavior patterns. Compared to 6_3 chickens, 7_2 chickens have a higher number of CD4 T cells (helper T lymphocytes coordinating the immune response against infection) but a lower number of CD8 T cells (cytotoxic T lymphocytes directly defending against intracellular pathogens and cancer cells) with the suppressed cellular immunity [146]. In responding to Marek's disease virus challenge, 7_2 chickens have higher levels of IL-6 and IL-18 mRNA expressions (pro-inflammatory cytokines), while 6_3 chickens have higher gene expressions of IL-8 (a monocyte-derived neutrophil chemotactic factor and angiogenic cytokine) as well as toll-like-receptors (TLR)-3 and TLR-7 (mediators of inflammatory pathways between the adaptive immunity and the innate immunity) [147, 148]. Furthermore, 7_2 chickens have higher corticosterone levels and heterophil-to-lymphocyte ratio (stress indicators in birds as well as other animals, **Table 1**) with greater exhibiting injurious behaviors (i.e., more aggressive pecking and longer durations of fights) and correlating with the lower levels of brain 5-HT and TRP (**Table 1**) [141, 149]. The selection also causes differences in cecal bacterial diversity. Line 6_3 chickens have enriched levels of several genes including *Ruminococcaceae UCG-005, UCG-008, UCG-009*, while line 7_2 chickens dominate with genes including *Clostridiales vadin BB60* [141]. From the predicated bacterial functional gene analysis, 7_2 chickens have upregulated kynurenine pathway of TRP metabolism. This pathway is the primary route for degradation of TRP to become various metabolites, such as kynurenic acid and quinolinic acid, rather synthesizing 5-HT [89, 150, 151]. In 7_2 chickens, the brain 5-HT levels were negatively correlated with the levels of *Clostridiales vadin BB60* group [141]. These results suggest that social sensitivity and related injurious behaviors of chickens are regulated *via* both the serotonergic system and HPA axis; and the mechanisms underlying injurious behaviors in chickens may be similar to those in humans and rodents [152, 153].

Treatment	5-HT (ng/g)	5-HIAA (ng/g)	5-HT/5-HIAA	TRP (ng/g)
	Brain			
Line 6_3	512.6[a]	151.8	3.2[b]	1183.8[a]
Line 7_2	352.7[b]	168.9	4.9[a]	963.2[b]
SEM	8.2	12.9	0.2	22.4
P-value	**0.01**	0.62	**0.04**	**0.08**
	5-HT (ng/g)	**TRP (ng/g)**	**CORT (ng/mL)**	**H/L ratio**
	Blood			
Line 6_3	61.38	171.52[a]	8.44[b]	0.16[b]
Line 7_2	59.46	121.42[b]	9.75[a]	0.50[a]
SEM	3.79	15.37	1.51	0.04
P-value	0.73	**0.03**	**0.05**	**<0.0001**

Values are least square means ± SEM, n = 10. a, b indicates significant differences ($P \leq 0.05$).
Serotonin, 5-hydroxyindoleacetic acid, and tryptophan in the raphe nucleus were measured using high performance liquid chromatography (HPLC). Blood serotonin and tryptophan levels were detected in duplicate using enzyme-linked immunosorbent assay (ELISA). Plasma corticosterone concentration was measured in duplicate using radioimmunoassay (RIA). The number of peripheral white blood cells were measured for analyzing heterophil/lymphocyte ratios.
Abbreviations: 5-HT, serotonin; 5-HAA, 5-hydroxuindoleacetic acid; CORT, corticosterone; H/L ratio, heterophil/lymphocyte; Line 6_3 and line 7_2, divergently selected inbred chicken lines for resistance or susceptibility to Marek's disease, TRP, tryptophan. Modified from cited reference [144].

Table 1.
The levels of serotonergic metabolism in the raphe nucleus, and the levels of peripheral serotonin, tryptophan, corticosterone, and heterophil/lymphocyte ratios between the two divergently selected inbred chicken lines 6_3 and 7_2.

To examine whether injurious behaviors in chickens can be prevented or reduced *via* remodeling colonization of the intestinal microbiota, early-life CMT was conducted (**Figure 3**). The cecal contents were separately collected from adult hens of

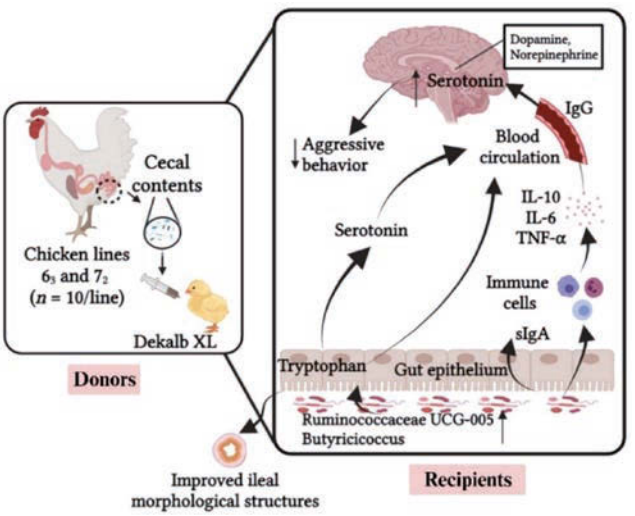


Figure 3.
The experimental design and the proposed mechanisms underlying the effects of cecal microbiota collected from the diversely selected donor lines on health and behavior of recipient chickens (Modified from reference cited [142], with BioRender.com).

the selected lines (6_3 and 7_2 donors, at 60-week-old), and then orally transferred to 1-day-old male chicks of Dekalb XL (recipients), a commercial line. There were three treatments for a 16-week trial: control (0.1 mL saline, control), 6_3-CMT (0.1 mL of line 6_3 cecal content solution), and 7_2-CMT (0.1 mL of line 7_2 cecal content solution). The treatments were daily from postnatal days 1–10, and then boosted from weeks 2–5. The results show that 7_2-CMT recipients showed higher aggression with a lower serotonergic activity (lower concentrations of 5-HT and 5-HIAA in the hypothalamus) compared to 63-CMT recipients, which is similar to the physiological and behavioral characteristics of the donors of line 7_3. In addition, 7_2-CMT chickens have higher phylogenetic microbiome diversity than 6_3-CMT chickens, which could be associated with the lines' different susceptible properties to Marek's disease found between the 6_3 and 7_2 donors. Compared to 7_2-CMT recipients, 6_3-CMT recipients have an abundant ASV belonging to *Ruminococcaceae* UCG-005, a genius that has been reported functionally as SCFAs producers [142–144]. It may directly and indirectly influence the syntheses and metabolism of neurotransmitters (e.g., 5-HT and TRP) and exhibition of aggressive behavior [154, 155]. Gut microbiota-derived SCFAs have been reorganized as major neuromodulators in regulating brain functions *via* the gut-brain axis [156, 157]. Several SCFA-producing bacteria have been used as psychobiotics in treating patients with the neuropsychiatric disorders [158, 159].

The current and previous studies indicate there is a donor-recipient interaction stage during the physiological and behavioral development in chickens. CMT conducted at an early age reduces injurious behaviors in recipients according to the donors' behavioral and biological phenotype *via* modifying the development of the cecal microbiota composition, consequently affecting the hypothalamic serotonergic activity and stress reaction. These findings confirm our working hypothesis that early-life modification of gut bacterial community has long-term effects on health and welfare in chickens. The divergently selected chickens with line's unique biological characteristics and opposite behaviors (63, non-aggression vs. 72, aggression) could be a useful model for preventing injurious behaviors in chickens as well as in humans and other social animals.

6. Conclusion

Gut microbiota has been recognized functionally as a neuroendocrine organ and a second brain, synthesizing and releasing various immunomodulators and bioactive factors, by which it plays a critical role in brain programming and functions in responding to various internal and external stimulations in animals including chickens. The current results show that early-life CMT from the two divergently selected inbred lines uniquely reduces or increases injurious behaviors in recipients through regulating the cecal microbiota composition and hypothalamic serotonergic activity according to each of donors' characteristics. The outcomes could provide new insights into understanding the mechanisms of the gut bacteria in regulating stress-induced abnormal behavior and providing strategies for developing innovative biotherapeutic intervention to reduce injurious behaviors in chickens.

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Conflict of interests

The author declares no conflict of interests.

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Chapter 4

Implication of Poor Body Condition to Reproductive Performance in Mares

Fuziaton Baharudin and Jasni Sabri

Abstract

Body condition score (BSC) on brooding mares plays important roles in their reproductive performance. Mares with poor BSC are commonly observed with perineal anatomical deformity manifested by vulva inclination. These conditions cause stretching of the vulva opening reaching the point of pelvis ischium that compromised vulva's function as the barrier to the reproductive tract protecting it from contaminants. Physical examination was conducted on 116 mares to determine the severity of inclined vulva using Castlick's Index. Mares with the severe vulva inclination were large breeds, had pregnancy several times, and were pregnant before two years old. These mares have a high number of bacterial isolates due to fecal urine backflow entering their reproductive tracts from the compromised protective mechanism of the labia. The contamination of the reproductive tract, was mostly at the fornix and uterus of mares with severe vulva inclination. These mares were observed exhibiting extended standing heat and strong stallion receptiveness, which is coherent with the endocrine dynamics of the affected mares. This behavior matched the situation of lower plasma estrogen concentration during the follicular phase, suggesting a delayed luteinizing hormone surge in facilitating ovulation. The condition causing erratic behavior during estrus creates difficulty in managing reproduction.

Keywords: reproduction physiology, poor body condition, infertility, perineal conformation, uterine bacterial contamination, welfare of breeding horses

1. Introduction

The reproductive performance of mares is influenced by a complex interaction of physiological, environmental, and management factors. Among these, the welfare of the breeding mare is a critical, yet often underestimated, determinant of both fertility and long-term health. Mares that are actively reproducing are expected to have a good body condition score. Mares that score lower than 2 out of 5 are considered poor body condition and is commonly due to lack of nutritional input when there is insufficient feed intake or heavy burden of internal parasites. Other reasons for poor body condition are chronic illnesses such as equine gastric ulcer, kidney or liver diseases, equine infectious anemia, and pituitary pars intermedia dysfunction (PPID) [1].

Considering a financial report that found horse breeding farms will succumb with negative financial returns when 63% or more mares fail to reproduce over seven years of operations due to mares increasing in age, multiple breeders, or producing a low number of foals [2, 3]. Most farms will critically address their reproductive failure issues to ensure the investment of highly priced mares yields a good return [4]. However, in many breeding farms, particularly those driven by high demand for foals, welfare compromises such as poor body condition scores, chronic reproductive stress, and over breeding are not uncommon. In temperate countries, most breeders rarely give their mares a season break. There is a short time frame for the mares to improve their body condition and allocation for parasitic management and sufficient nutrient uptake for the body condition improvement often overlooked.

The body condition score (BCS) of a broodmare is an important indicator of her health, fertility, and reproductive efficiency [5]. Poor body condition in broodmares not only is causing disturbance to the reproductive endocrine mechanism but also induces muscle waste. These results in compromise of perineal anatomical conformation manifested by vulva inclination and commonly observed to demonstrate irregular estrus behavior and a lower pregnancy rate. Pneumovagina commonly manifested by vulva inclination is one of the contributing factors affecting breeding performance in mares [6, 7].

This chapter explains the inter-correlation between the poor body condition of mares and their breeding performance, clarifying the connection between poor physical condition and the development of vulva inclination in mares. The condition implies physiological consequences from the bacterial contamination of the uterus that lead to irregular breeding behavior resulting from plasma estrogen-progesterone concentration variation.

2. Materials and methods

This research is a clinical case study purposive random sampling of mares with unfavorable conditions among the reproductive cases attended by the researcher during equine ambulatory services at Universiti Malaysia Kelantan Veterinary Teaching Hospital. Data were also collected from the breeding record of the selected farms attended.

2.1 Body condition score, degree of inclined vulva, estrus behavior and parameters of mares

About 116 mares (n = 116) of the common breeds including the Thoroughbred, Anglo Arabs, Arabians, Criollo, Quarter Paint, Appaloosa, and the local ponies participated in this study. Their age was between 1 year and 20 years. The average size of the breed was used to classify the mares. These mares' activities included sports, relaxation, and breeding.

2.1.1 Body condition score

The BCS of the mares was assessed using Carol and Huntington's body condition score for horses: BCS 1 as thin or poor, BCS 2.5 to 3 as good, and BCS 4 to 5 as fat [8]. The height of the mares was measured using a commercial nylon measuring tape in

centimeters. The weight of the mares was estimated in kilograms using a specially made nylon measuring tape fitted around its girth.

2.1.2 Degree of inclined vulva

A physical examination of the perineal anatomical conformation was conducted to evaluate the severity of vulva inclination of each mare. **Figure 1** illustrates the method by which the measurement of vulva inclination was made. The angle of inclination (a) from the vulva dorsal commissure against an imaginary vertical line at the brim of the pelvis. A plastic ruler and a protector were used in this measurement protocol. The effective vulva opening (l) from the ischium of the pelvis to the dorsal commissure was measured using a flexible measuring tape. The angle of the vulva inclination and the length of the vulva opening were then multiplied to derive the Caslick's index (CI) [9, 10]. Mares in this study were grouped by the CI. The CI is referred to as the grade of inclined vulva; grade 1: CI < 50 (10°), grade 2: CI 50–100 (10° to 20°), and grade 3: CI > 150 (>30°) [11].

Grade 1 mares are considered mild conditions and do not require any corrective procedures. Their fertility status is usually normal. The grade 2 and grade 3 mares are considered severe conditions [11]. These mares commonly require intervention: the Caslick vulvoplasty procedure, in preventing contamination to the reproductive tract and infertility [5, 9, 10].

2.1.3 Estrus behavior assessment

Each mare's estrus behavior was assessed using teaser stallions individually during on-heat observed in the field. The mares were considered positively on-heat when winking of the vulva and vaginal discharge were observed. The mares that stand firmly with the hind limb abducted while accepting the stallion to mount when teased are interpreted as in estrus and also called standing heat. Mares that do not stand firmly, or refuse to be mounted when teased by the stallion, are in mild estrus.

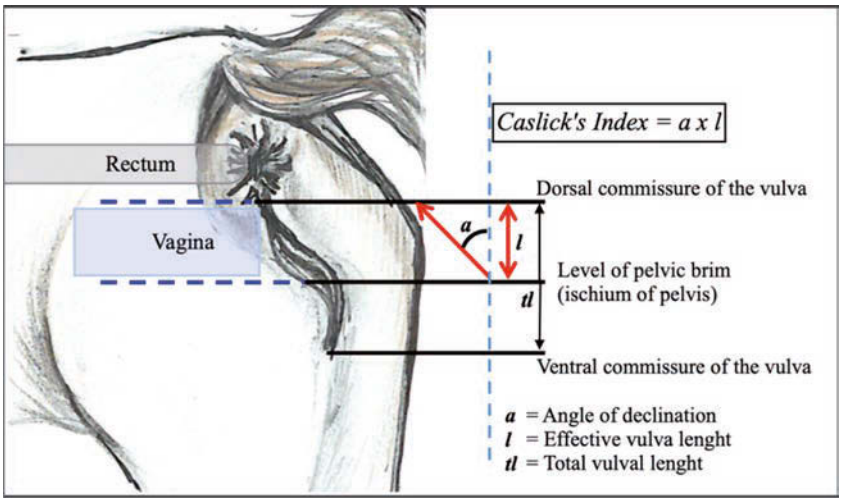


Figure 1.
The vulva inclination angle measured from the brim of the pelvis to the vulva dorsal commissure [9].

The mares showed erratic estrus behavior such as intermittent mild and strong standing heat. At the same time, these mares display irregular standing heat patterns within estrus. The estrus behavior was assessed using the breeding soundness examination in mares' guideline [12].

2.1.4 Other parameters data collection

Information on the mares' population in this study was acquired from the farmer's record and interview using structured questionnaires. The basic parameters including name, age, breed, color, weight, and height were obtained from the farm records and clinical observation. Information on the mares' age when they reached puberty, their first pregnancy, number of pregnancies, eventful pregnancies including fetal death, abortions, and retained placenta. Extended information on the mares' primary and secondary functions and fertility status was obtained from the interview.

2.2 Bacteria samplings

2.2.1 Animal selection for bacteria sampling

About 35 (n = 35) mares with inclined vulva were selected from the 116 mares enrolled in this study. These mares' uterus were examined for endometritis and pyometra using a 5Mhx transrectal probe ultrasound scanner [13, 14]. Endometritis was confirmed when ultrasonography showed more than 8.0 mm echogenic endometrium thickness [15]. Echogenic spots reflected by polymorphonuclear neutrophils (PMNs) floating in the uterine cavity confirm pyometra [16]. The echo density of intrauterine fluid determines the severity of the pyometra [17].

2.2.2 Bacterial swab sampling methods

Bacteria swabs were sequentially sampled along the reproductive tracts starting from the vulva vestibule, middle of the vaginal canal, fornix, and the uterus. The mare's perineal was aseptically prepared to avoid cross-contamination. The bacterial swabs collected were cultured in aerobic and anaerobic environments.

2.2.3 Bacterial isolations and identification

The bacterial identification using the routine identification technique is according to the standard microbiological laboratory procedures [18]. The Citotest Labware Manufacturing Co., Ltd. in Jiangsu, China, supplied all microbiology swabs (Citoswab®) used in this study.

2.2.4 E. coli *phylogenetic using polymerase chain reaction (PCR) protocol*

The *E. coli* isolated from the affected mares was analyzed for their phylogenetic group to confirm the source and route of the bacteria causing uterine infection. Four pairs of primers were used to detect chuA, yjaA, and TspE4C2 genes. Additional two primers (ArpAgpE and TrpAgpC) were to confirm the unknown group C and group E. A quadruplex PCR method was then proceeded using these primers using a Bio-Rad T100 thermal cycler. Forward and reverse sequences of each primer were

conducted to analyze the phylogenetic material of *E. coli* with the primer annealing at 52°C for 30 seconds, then DNA extension at 72°C for 30 seconds. The incubation temperature was at 72°C for 3 minutes and later cooled to 20°C for PCR profile detection using gel electrophoresis.

2.3 Blood samplings for endocrine work-up

2.3.1 Animal selection for endocrine work-up

A total of 20 mares (n = 20) were selected from the same group of animals in subsection 2.2.1. From the total of 20 mares, 15 were the mares of various grades 1, 2, and 3 inclined vulva. These mares were grouped according to severity of inclined vulva, that is, four mares with grade 1 (n = 4), five mares with grade 2 (n = 5), and six mares with grade 3 (n = 6). These mares did not show any estrus behavior for at least six consecutive months. Control group was selected from mares (n = 5) with no vulva inclination and had no records of infertility showing normal estrus behavior during every cycle.

2.3.2 Enzime-linked immunosorbent assay

Blood samples were taken for plasma from each mare on alternate days for 30 consecutive days to determine the plasma concentration of estrogen and progesterone. About 30 days of samplings were conducted with the assumption that the duration shall cover one estrus cycle. Horse estrogen (ES) and Horse progesterone (PROG) enzyme-linked immunosorbent assay kits used in this study were principally a competitive enzyme immunoassay technique of a 1.5 hour solid-phase ELISA, manufactured by BlueGene® Biotech, Shanghai, China.

The samples' optical density (OD) was determined using a microplate reader at 450 nm. All data obtained from the microplate reader were analyzed using the ELISA database (www.elisaanalysis.com) for the actual hormonal concentration. A standard curve was plotted relating the intensity of the color based on the principle of OD to the concentration of standards. The ES and the PROG concentrations in each sample were interpolated from its standard curve.

2.3.3 Data alignment and ovulation day determination

All data were normalized by the highest and lowest value of plasma estrogen and progesterone concentration each mare within the cycle. The median value of hormonal plasma concentration was used to represent the value of each group of mares, and a curved graph was plotted according to the group. Ovulation day was determined by aligning the highest plasma estrogen concentration of each mare within the group and assumed to occur a day after the plasma estrogen concentration reached the maximum level and the plasma progesterone concentration reduced to the minimum level.

2.4 Statistical analysis

The mares' population demographic data were analyzed using chi-square test and correlation coefficient. In the bacteria study, student t-tests were used to determine the presence of bacteria along the reproductive tract at a 95% sensitive level, and the

P-value <0.05 was considered significant. The P-value was determined using Pearson's chi-square test for the significance of the PCR product data. Duncan's test for comparison between the two hormone concentrations and non-linear regression analysis was expressed in Spearman's correlation coefficient at the sensitivity of 95%. All analyses were conducted using IBM SPSS 24.01 for Windows (SPSS).

3. Results

The analyzed results produced the outcomes that reveal the correlation of multiple factors and the incidence of inclined vulva in the affected mares.

3.1 Factors influencing inclined vulva in mares

3.1.1 The correlation between body score and inclined vulva

The mares with poor BCS have severely inclined vulva than those with good body scores, as shown in **Figure 2**. All the mares with BCS 1.5 have inclined vulva: 25% grade 2 and 75% grade 3. About 49% of mares with BCS 2.0 have inclined vulva: 28% grade 1, 13% grade 2, and 8% grade 3. In mares BCS 2.5; 62% of the mares have inclined vulva: 28% grade 1, 13% grade 2, and 21% grade 3. Mares with BCS 3.0; 63% mares have inclined vulva: 21% grade 1, 29% grade 2, and 13% grade 3. All mares with BCS 3.5 do not have inclined vulva. The correlation between mares with severe grades of inclined vulva and body condition was negatively significant ($r^2 = -0.1$; $p = 0.046$).

3.1.2 The correlation between breeds and inclined vulva

The small breed group mares comprised 45 mares among the ponies; 24 (53.3%) of them do not have inclined vulva, 7 (15.5%) have grade 1, 6 (13.3%) have grade 2, and 8 (17.7%) have grade 3. The medium breed mares of 33 standard breed mares;

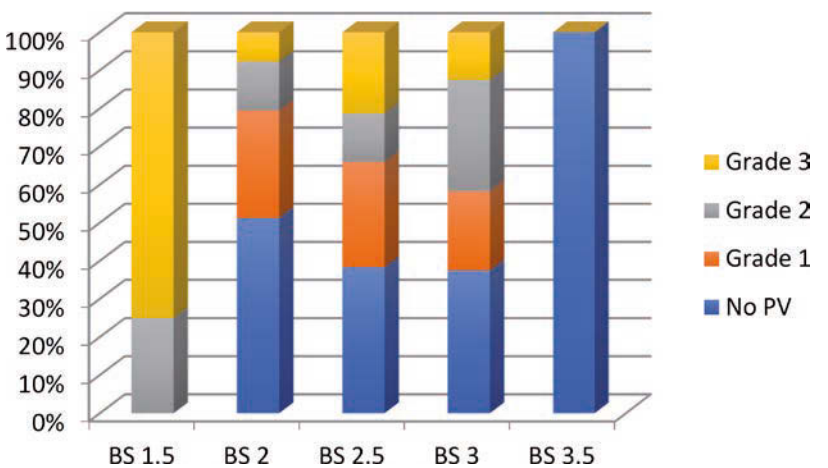


Figure 2.
The percentage of mares with grades of vulva inclination against body score.

10 (30%) of them do not have inclined vulva, 11 (33.3%) have grade 1, 8 (24%) have grade 2, and 4 (12%) have grade 3. About 23 mares were categorized as the larger breeds among the thoroughbred and arabians; 8 (35%) mares do not have inclined vulva, 4 (17%) have grade 1, 4 (17%) have grade 2, and 7 (30%) have grade 3. The correlation between the larger breed mares and severity of vulva inclination was positively correlated ($r^2 = 0.2$; $p = 0.001$).

3.1.3 The correlation between the age of mares with inclined vulva

There was no significant difference between the age of mares and the vulva inclination incident ($r^2 = 0$; $p = 0.082$). The insignificance concludes that age does not implicate the development of vulva inclination in mares. The equal distribution of the mares' age and grades of vulva inclination shows it is a probability of chance and not the influencing factor.

3.1.4 The correlation between mares' activities and inclined vulva

Many mares that worked in low-impact activities such as leisure recreational routines also involved with breeding have inclined vulva. About 22 out of 67 affected mares (32%) were the breeding mares and ran leisure recreational activities. However, lesser mares that work intensely have inclined vulva: those were 21 mares (31%) were polo ponies, 16 (23%) were the racing horses, and 8 (11%) were endurance race horses. These show mares with low-impact activities that have severe inclined vulva ($r^2 = 0.1$; $p = 0.009$).

3.1.5 Mares body condition score and fertility

This study found that mares with higher BCS are more fertile than those with BCS 2.5 and lower. These are shown in **Figure 3** that 50% of mares with BCS below 2.5 were fertile while 59% of mares with BCS 2.5 were 59% fertile. All mares in this study with BCS 3 and 3.5 were found to be fertile. These data show that the fertility of mares is significantly affected by the body score at a probability of 0.008 ($P < 0.05$) [19].

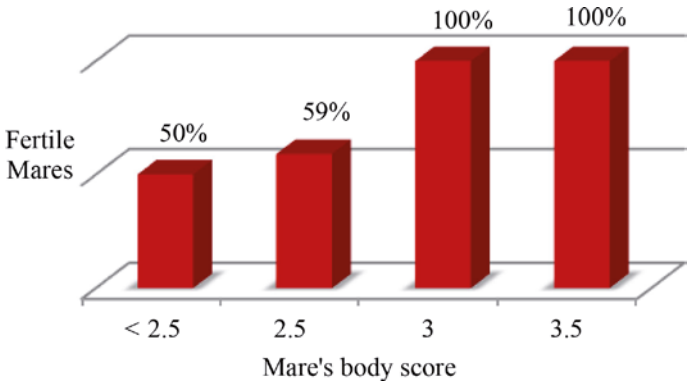


Figure 3.
Body condition score of mares in relation to fertility.

3.1.6 Influence of pregnancies on vulva inclination development

About 60% of the studied mares had at least conceived once in their lifetime, while 40% had never conceived previously. Among the reproductively active mares, 37% of the mares were pregnant at least once, 31.5% were pregnant twice, and 31.5% were pregnant more than three times, also known as multiparous. Forty percent of the mares were pregnant as early as 18 months old, 23% were pregnant between 18 and 36 months old, and 37% were after 48 months old.

Among the mares that got their first pregnancy at early puberty, 68% had grade 2 and grade 3 inclined vulva, 11% had grade 1, and 21% were normal. Those mares that got their first pregnancy at the age between 18 and 36 months: 25% had grade 2 and grade 3, 37.5% had grade 1, and 37.5% were normal. Mares that got their first pregnancy at 48 months old; 11% had grade 2 and grade 3 inclined vulva, 20% had grade 1, and 69% were normal. There is a significant negative correlation between the number of multiparous mares and those pregnant at early puberty age.

3.2 Estrus behavior of the mares with inclined vulva

In this study, 53% of mares with grade 3 inclined vulva showed inconsistent estrus intervals and 47% showed regular estrus intervals. In grade 2 mares, 37% showed inconsistent estrus intervals and in mares with grade 1, 24% showed inconsistent estrus intervals while 76% were normal. In normal cycling mares, only 18% showed inconsistent estrus intervals. These results show that mares with severe grades of inclined vulva have a higher incidence of inconsistent estrus intervals compared to normal mares significantly ($r^2 = -0.32$, $p = 0.049$). Mares with severe inclined vulva: 75% demonstrated intense standing heat with strong stallion reception during estrus, and the standing heat lasts for up to three days. However, grade 1 mares showed nominal intense standing heat and stallion reception that last for a day similar to those of normal mares.

Figure 4 shows the percentage of mares demonstrating erratic estrus intervals and mares' highly receptive behavior towards stallion during standing heat according to

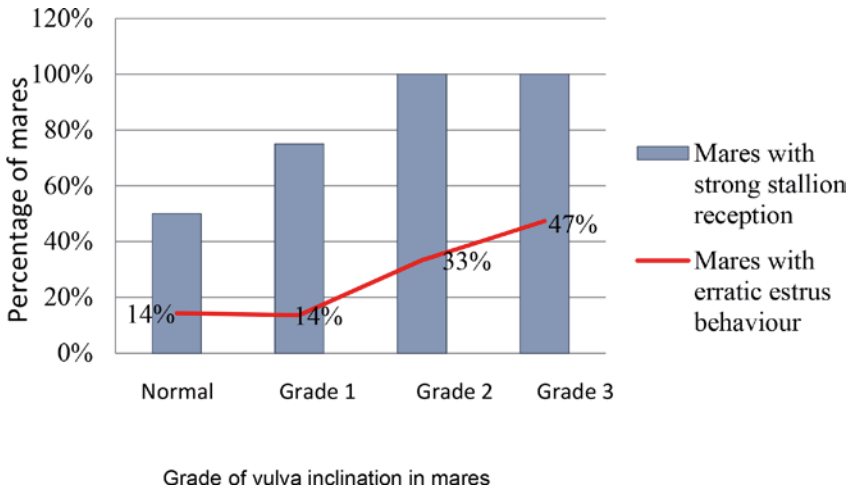


Figure 4.
Mares with severe vulva inclination showing inconsistent (erratic) estrus behavior and strong stallion receptiveness.

the grades of inclined vulva. The graph shows moderate correlation ($r^2 = 0.2$, $p = 0.5$) between mares with erratic estrus behavior and their severity of inclined vulva.

3.3 Uterine bacterial isolates among mares with inclined vulva

The findings of the bacterial samplings along the reproductive tracts of the affected mares are tabulated in **Table 1**.

3.3.1 Bacteria in the reproductive tract of grade-1 inclined vulva mares

Table 1 provides the comprehensive list of bacteria isolated in this study. Twenty-two bacteria of eight species were isolated from the reproductive tract of grade 1 inclined vulva mares. *E. coli* is the most commonly isolated bacteria (n = 13) present in all locations of the reproductive tract, followed by *Staphylococcus aureus* (n = 3). Other bacteria such as *Bacillus cereus* (n = 1), *Bacillus subtilis* (n = 1), *Citrobacter freundii* (n = 1), *Proteus mirabilis* (n = 1), *Serratia spp.* (n = 1) and *Shigella flexneri* (n = 1) were each isolated only once (n = 1) from the mares with grade 1 inclined vulva. Most bacteria were isolated from the vaginal canal (n = 8) and then those isolated from the fornix (n = 10). Only three (23%) bacteria were isolated from the uterus.

3.3.2 Bacteria isolated from the reproductive tracts of grade 2 inclined vulva mares

Table 1 also shows the bacterial isolates from the reproductive tract of the grade 2 inclined vulva mares. The bacteria species most isolated were *Bacillus cereus* (n = 3), *Bacillus subtilis* (n = 1), *E. coli* (n = 7), *Klebsiella aerogenes* (n = 3), *Klebsiella oxytoca* (n = 2), *Proteus vulgaris* (n = 9), *Salmonella enterica* (n = 3), *Shigella flexneri* (n = 1), and *Staphylococcus aureus* (n = 9).

3.3.3 Bacteria isolated from the reproductive tract of grade 3 inclined vulva mares

The reproductive tracts of the grade 3 inclined vulva mares have the most bacterial species and isolates. There were 88 isolates of 13 bacterial species from the reproductive tract of these mares. There was *Bacillus cereus* (n = 1), *Bacteroides spp.* (n = 2), *Corynebacterium spp*. (n = 1), *Klebsiella aerogens* (n = 14), *E. coli* (n = 26), *Klebsiella oxytoca* (n = 3), *Proteus mirabilis* (n = 5), *Proteus vulgaris* (n = 9), *Pseudomonas aeroginos*a (n = 1), *Rhodococcus equi* (n = 3), *Salmonella enterica* (n = 2), *Shigella flexneri* (n = 3), *Staphylococcus aureus* (n = 13), and *Streptococcus spp*. (n = 5).

3.3.4 Phylogenetic group of E. coli *isolates in the reproductive tracts of mares with inclined vulva*

The phylogenetic analysis conducted reveals five phylogenetic groups of *E. coli* were identified as group A, B1, C, D, and Clade 1. Group A and B1 are commensal and non-pathogenic, originated primarily from the environment [20]. The phylogenetic group C is found in mammalian feces and the pasture, whereas the phylogenetic group D from mammalian is pathogenic [21, 22]. Phylogenetic group Clade 1 originated from the environment yet is pathogenic and considered "*the environment E. coli*" [23]. *E. coli* from fecal samples is 40% phylogenetic group B1, and the rest was the unknown phylogenetic group.

Name of bacteria	Grade 1				Grade 2				Grade 3			
	vs	va	fx	ut	vs	va	fx	ut	vs	va	fx	ut
Bacillus cereus	0	1	0	0	0	1	1	1	0	1	0	0
Bacillus subtilis	0	1	0	0	0	0	1	0	0	0	0	0
Bacteroid spp.	0	0	0	0	0	0	0	0	0	1	1	0
Citrobacter freundii	0	0	1	0	0	0	0	0	0	0	0	0
Corynebacterium spp.	0	0	0	0	0	0	0	0	0	1	0	0
Escherichia coli	1	4	5	3	0	2	3	2	4	6	10	6
Klebsiella aerogenes	0	0	0	0	0	1	2	0	2	3	8	1
Klebsiella oxytoca	0	0	0	0	1	1	0	0	0	2	0	1
Proteus mirabilis	0	0	1	0	0	0	0	0	0	2	2	1
Proteus vulgaris	0	0	0	0	2	4	1	2	1	3	2	3
Pseudomonas aeruginosa	0	0	0	0	0	0	0	0	0	1	0	0
Rhodococcus equi	0	0	0	0	0	0	0	0	1	2	0	0
Salmonella enterica	0	0	0	0	0	1	1	1	0	1	1	0
Serratia spp.	0	1	0	0	0	0	0	0	0	0	0	0
Shigella flexneri	0	0	1	0	0	1	0	0	0	1	1	1
Staphylococcus aureus	0	1	2	0	0	3	3	3	1	4	6	2
Streptococcus spp.	0	0	0	0	0	0	0	0	1	2	1	1
Total isolates	1	8	10	3	3	14	12	9	10	30	32	16

Table 1.
List of bacteria isolated from the reproductive tract of grade 1, 2, and 3 inclined vulva mares.

3.4 Plasma estrogen-progesterone concentration variations among mares with inclined vulva

In a normal cycling mare, estrogen is high during the estrus to induce behavioral changes while the progesterone levels will be at the lowest before the luteinizing hormone (LH) surge for ovulation takes place. Progesterone will rise after ovulation to maintain the mare's cycle and suppress estrus. Progesterone remains low (under 1.0 ng/ml) before the LH surge but increases to 2–3 ng/ml on the day of the LH surge and 3–4 ng/ml the day after, suppressing further heat cycles.

The plasma estrogen and progesterone concentration levels of the grade 2 and 3 mares show distinct wave patterns and amplitudes as shown in **Figure 4** and **Figure 5** respectively. The plasma estrogen concentration and wave pattern are lower and inconsistent in the affected mares compared with those in normal mares. The apparent variation can be seen in the grade 3 mares. The variations are obvious with flatter waves throughout the estrus cycle.

3.4.1 Plasma estrogen-progesterone concentration in mares with grade 2 inclined vulva

Figure 5 shows the plasma estrogen and progesterone concentrations in mares with grade 2 inclined vulva. There are differences in the plasma estrogen concentrations between the affected mares and the normal mares. The affected mares show low amplitude of estrogen waves at pre and post ovulation, indicating low plasma concentration. However, there is no significant difference in the plasma progesterone concentration of these mares compared to the normal mares.

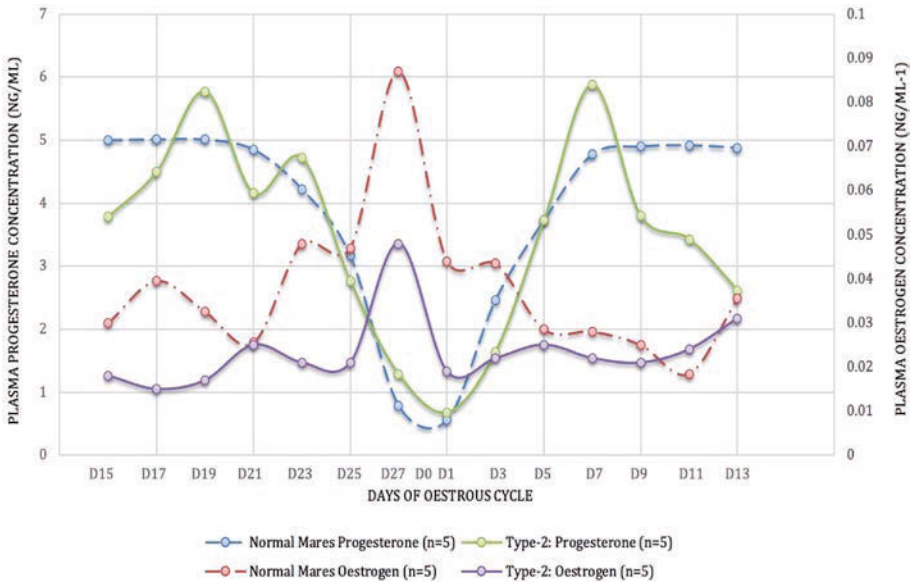


Figure 5.
The level of plasma estrogen and progesterone concentration of mares with grade 2 vulva inclination.

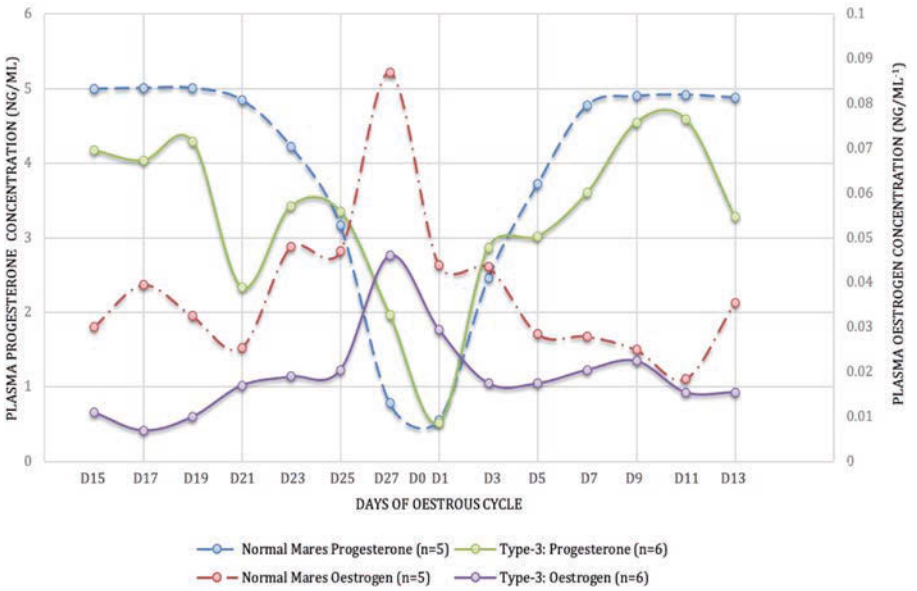


Figure 6.
The level of plasma estrogen and progesterone concentration of mares with grade 3 vulva inclination.

3.4.2 Plasma estrogen-progesteron concentration in mares with grade 3 inclined vulva

Figure 6 shows the plasma estrogen and progesterone concentrations of mares with grade 3 inclined vulva. The graph shows the significant difference between the plasma estrogen and progesterone concentrations of the affected mares and the normal mares. There is low plasma estrogen concentration at pre- and post-ovulation. However, the plasma progesterone concentration shows two smaller waves pattern before it dives to zero level on ovulation day in these mares. The dipping of progesterone and peaking of estrogen in the affected mares occurred on ovulation day similar to those in the normal mare even at low plasma concentration.

4. Discussion

The mare's body condition is often overlooked by farmers with poor husbandry practice. Farmers are keen to get their mares pregnant within the same breeding season and have little time to upkeep the mares' body condition score. This generally happened in most temperate countries when the breeding season starts at the beginning of summer when daylight increases and ends when daylight is reduced in late summer just before autumn. The breeding season lasts for 3.5 months and accommodates only about four estrus cycles in a mare. Mares' gestation period varies according to breeds and size between 320 and 365 days; therefore, if the mares are pregnant at the end of the breeding season, it is hoped they will give birth in early summer of the next season. The mares will then get cleaned in foaling heat and left with two more estrus cycles for the pregnancy to take place before the end of the breeding season [24].

The breeding farm management plays an important role in this issue. When farmers are not willing to miss a season that will cost them a year of non-producing mares,

then mares will be exploited for rapid pregnancy. Lesser time for the farmers to provide their mares the necessary to up-keep the mares' health condition for recovery such as providing enough deworming, and even increasing their body condition that will make the mares' body condition deteriorate and eventually deprive them of reproductive physiology. Added with lactation demand from the newborn will make the body condition worsen. Besides breaching animal welfare, this situation also triggers reproductive performance issues. Therefore, certain levels of body fat must be maintained by brood mares for important reproductive activities, including parturition, lactation, and reproductive endocrine maintenance to keep the mares actively cycling [25].

4.1 Factors contributing to inclined vulva among mares

From this study the poor body condition scores trigger the whole issue of reproductive performance in mares. Mares with less than BCS 2.0 have high potential to succumb with inclined vulva. This incident is higher in larger breed horses due to lesser body fats surrounding the reproductive organ affecting intra-vaginal pressure, which implicates the development of shortening and angulation of the vulva that signifies inclination [26].

Unlike with mares that are involved in high intensity activities such as racing, endurance, and polo, shows fewer incidents of vulva inclination compared to mares that have low impact activities such as light recreational activities and are solely involved in breeding. It has been reported that retired racing and endurance horses turned to breeding mares have a high incidence of inclined vulva [16, 27].

Age of mares does not have any association with the incident of inclined vulva; however, the age of mares at their first pregnancy and number of pregnancies they underwent do implicate the occurrence of this condition in mares. The mares that had their first pregnancy before 18 months old showed a significant high incidence of vulva inclination at later stages of their life. The positive correlation ($r2 = 0.11$) between the age of first pregnancy and types of vulva inclination implicated that early exposure to reproductive hormones caused morphological alteration of the reproductive tract in older mice administered with the hormones during the neonate phase [28].

This study proves that mares with multiple pregnancies have a high incidence of severe vulva inclination compared to those that never had pregnancies before and neither do the mares that have less than four pregnancies. Mares with multiple pregnancies have lesser uterine elasticity that caused changes in the vaginal fibrotic structure [29]. In poor body condition mares have lesser body fat padding around the reproductive tract areas to support the uterus and vagina causing the vulva to incline.

This study reveals that mares with the severe grades inclined vulva exhibited inconsistent estrus intervals with intense standing heat and were very receptive to stallion, whereas normal mares have a standing heat that lasts for three days during estrus [1]. On the other hand, mares with grade 1 inclined vulva have consistent inter ovulation intervals between 14 to 28 days, which is considered within the normal range between 13 to 34 days [27]. Irregularity of the estrus interval was commonly observed in mares with endometritis and pyometra in the study conducted in [5].

4.2 Bacteria in the uterus of mares with inclined vulva

Enterobacteriacea were isolated from all grades of inclined vulva mares' reproductive tract. Those most common were the *E. coli*, *Klebsiella spp.*, *Proteus spp.*,

Salmonella, and Shigella spp. These bacteria are frequently found in the gastrointestinal tract of animals as commensal or pathogens. Thus, in this study, the *Enterbacteriacea* isolated grows in aerobic and anaerobic environments during culture. These facultative anaerobic and flagellated bacteria are the normo-flora of the gut but can turn into pathogenic strains in a stressful environment under the right circumstances [30]. The significant number of facultative anaerobic bacteria isolates was found due to the nature of the vaginal canal and uterus that is always tightly closed.

The facultative anaerobic bacteria that managed their entrance into the reproductive tract will survive by consuming their source of ATP from the fermentation of the organic substrate, including feces to release gases including hydrogen (H_2) and carbon dioxide (CO_2) as a by-product, along with succinic acid, into the surroundings [23]. The survival of the bacteria will increase its population, thus creating more gases and acidic environments to cause inflammation of the reproductive tract including the uterus. Accumulation of gases by a substantial amount of bacteria results in increased intra-vaginal pressure causing the development of pneumovagina.

The flagella on *Enterobacteriacea* enables the bacteria to swarm over the mucosal surface of the stratified squamous cell epithelium and assists in attachment to the host's cell wall of the reproductive tracts [31]. Bacteria trigger the inflammatory secretion of the local cells [32, 33]. The secretion facilitates bacterial motility on the surface of the epithelial cells in the reproductive tract. In this study, the bacteria with flagella: *E. coli, Proteus spp.*, and *Klebsiella spp*. were found in all four sites along the reproductive tract, including the uterus, to cause endometritis [34].

The fornix has most bacterial isolates than the vestibule, vagina and uterus. Mares with vulva inclination had their vaginal canal inclined cranio-ventrally towards the cervix. The inclination is worse when the vaginal canal loses its elasticity due to the absence of visceral fats surrounding the reproductive organ commonly observed in low BCS mares [35]. The slanting vagina downward leads to the accumulation of fluid or urine backflow at the fornix. The tight cervix during diestrus prevents the contaminants of fecal material that come with urine backflow from entering the uterus. This phenomenon proves that the cervix is effective as the third defense mechanism of the reproductive tract.

4.3 The implication of bacterial isolates to the mares with inclined vulva

The bacterial dynamics in the reproductive tract of mares with vulva inclination prove that the vestibule has the lowest bacterial isolates in this study possible due to the location being caudal to the urethra outlet and the vulva [36]. The findings show that the anatomical defect in the inclined vulva can be the portal of entry of zoonotic pathogens causing infertility in mares. Among the zoonotic pathogens isolated were *Salmonella enterica*, *E. coli*, *Shigella flexneri*, *Staphylococcus aureus, Streptococcus spp., Klebsiella spp., Bacteroides spp., Proteus spp., Pseudomonas aeroginosa,* and *Rhodococcus equi*.

There is a higher number of bacterial isolates from the fornix of the cervix than the uterus of the affected mares, especially those with grade 1 and 3 but lesser in grade 2 mares. The vaginal canal has lower bacterial isolates than those in the fornix, indicating that the mobility of the bacteria from the vagina to the fornix has not interfered. The bacteria was not as high in the uterus, likely due to movement restriction by the closed cervix. The lowest bacterial isolates in the vestibule even though it is close to the environment and anal openings [37]. The reason for these is likely due to this area being frequently washed during bathing and/or flushed by the urine stream.

This study concludes that *E. coli* in the reproductive tract of the affected mares has more commensal phylogenetic groups than the pathogenic type. The mares with grade 3 vulva inclination have the commensal phylogenetic *E. coli* more than the pathogenic group. The *E. coli* isolated were 40.6% of those from the environment, and 59.4% were from feces imply an equal chance of bacterial self-contamination from the mares' feces brought in by the urine backflow in the severely affected mares. Other pathogenic bacteria in the reproductive tract likely caused endometritis and hence pyometra. The findings from this study explain that mares with a higher grade of inclined vulva have higher chances of endometritis and pyometra occurrence in the field [36].

4.4 The estrogen and progesterone plasma concentration waves

The difference between the basal and peak level of plasma estrogen concentration of normal mares was seven units to two units in the affected mares. The low amplitude estrogen wave may have resulted in non-exhibition of estrus behavior in affected mares. Estrogen is produced by developing follicles, indirectly dependent on the follicular stimulation hormones (FSH). The FSH plays an essential role in regulating sex hormones that influence estrus behavior during estrus. In normal mares, estrogen was secreted into the circulatory system to reach high concentrations (0.1 to 0.15 ng/mL^{-1}) at 24 to 48 hours before ovulation takes place [38].

The mares with severe inclined vulva showed narrow margin waves amplitude of plasma progesterone concentration at 3.68 units compared to 6.53 units in the normal mares. Even though the progesterone level seems to have shorter cycles, it has a significant basal level that is lower than 1.0 ng/mL, allowing gonadotropin to release estrogen in the blood circulation and LH for ovulation to happen respectively. In the normal mares, at within 24 hours pre-ovulation, the plasma progesterone concentration level must reach the basal level to trigger theca cells in the ovary to secrete estrogen [39]. Unlike in this study, the amplitude of plasma estrogen concentration showed a shallow peak level at 0.04 ng/mL^{-1} compared to the normal mares at 0.08 ng/mL^{-1}. This pattern is mostly due to the progesterone that fails to reduce to the lowest level and could not trigger theca cells to produce estrogen. This condition could happen due to uterine hormone receptors disturbance from contaminants in the uterus [40].

These findings elucidate the inconsistent estrus behavior in mares with severe inclined vulva, manifested by extended standing heat, inconsistent estrus intervals, and even in some mares, they do not display estrus behavior. The short cycle, low plasma estrogen and progesterone concentration variation suggests interference of hormonal receptors located in the endometrium by the presence of causative agents in the uterus disturbing the dynamic regulator of reproductive endocrine [41]. Dynamic regulators are small noncoding RNA molecules called microRNAs (miRNAs) that bind to specific sequence motifs of gene expression in animals' immune system [42]. The miRNAs are believed to regulate all gene expression of all cellular processes, including responding to the immune system [43]. Disturbance to the cellular signals will create irregular responses by the other receptor cells; in this study, the response of the estrogen secretion reacts to the progesterone, LH, and FSH signaling.

4.5 The implication of plasma estrogen: Progesterone concentration variables in the affected mares

In **Figure 5**, the plasma estrogen concentration wave patterns are flatter at pre and post-ovulation, indicating low plasma estrogen concentration at 0.1 ng/mL^{-1}.

The low plasma estrogen concentration may be due to the interference of estrogen receptors due to the overwhelming of inflammatory cells, consequent to the bacterial contamination in the endometrium [13, 44]. In normal cycling mares, the plasma estrogen concentration is higher, which triggers the pituitary gland to secrete a surge of luteinizing hormones (LH) for ovulation to take place [45]. The low plasma estrogen concentration likely has caused a weak follicular stimulating hormone (FSH) release, resulting in a lack of follicular growth [46, 47]. The insignificant follicular growth retards estrogen secretion and simultaneously diminished LH synthesis by the granulosa cells [48]. The low plasma estrogen concentration implicates a reduction of the stimulation of LH receptors in granulosa cells, causing delays in LH surge and resulting in ovulation failure [49, 50].

Even though there were no significant differences among mares on the plasma progesterone concentration, there is a variation of three smaller waves before the major wave in the grade 3 mares as shown in **Figure 5**. The irregular waves of progesterone affect other gonadotropin hormone secretions and cause disturbance in endocrine mechanisms. The interference of the hormones' receptors influencing negative feedback from secondary waves of estrogen levels, which is not sufficient to create negative feedback for PGF2α suppression to send a signal to the pituitary glands for LH surge. Upon luteinizing the follicles, negative feedback is sent to the sensor corpus luteum (CL) for progesterone increment for the following cycle.

5. Conclusions

Mare with poor body condition scores has high potential to develop an inclined vulva. Lack of exercise, pregnant at too young age of below 18 months old, and those that have more than four pregnancies will have high chances of developing the condition worse among large breed mares such as Thoroughbreds.

In the severe grades inclined vulva, the vestibule as the first reproductive tract defense mechanism will be compromised, allowing contaminants of the mares' own feces travel into the vagina to the uterus when the cervix dilated during estrus. The bacterial contamination caused endometritis and later pyometra. These conditions cause disturbance of the uterine environment, giving impact to the endometrial hormonal receptors, causing endocrine dynamic variations.

The affected mares are showing irregular estrus behavior such as extended standing heat, very intense stallion reception over long periods of time, or not showing a definite on-heat behavior. These make determining a precise ovulation day impossible. Therefore, observing mares for standing heat to predict the timing for breeding either by natural covering or artificial insemination will be complicated. In the case of ovum successfully fertilized, a contaminated uterine environment will cause failure in embryonic implantation. The affected mares will eventually become repeat breeders that will cause frustration among farmers as the cost to get these mares to conceive successfully will increase.

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Conflict of interest

The author and co-authors of this article declare that we have no conflict of interest in this study and the production of this book chapter.

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Chapter 5

Parasites and Animal Care

Awotokun Femi

Abstract

Parasitic infections present a major global challenge to animal health and agricultural sustainability, leading to substantial economic losses and zoonotic risks to human health. The economic burden is particularly severe in the livestock sector, with annual losses estimated in billions of US dollars due to reduced productivity from parasites, such as gastrointestinal nematodes and ticks. Parasites affecting livestock and companion animals are categorised as internal (endoparasites), such as helminths (e.g. Nematodes and Cestodes), protozoa (e.g. *Giardia duodenalis* and *Toxoplasma gondii*), and external (ectoparasites), dominated by arthropods, such as fleas, ticks, and mites. These infestations significantly impair animal welfare by causing distress and severe health issues; reducing growth rates through resource competition and physiological stress; and diminishing productivity by negatively affecting weight gain, milk production, and reproductive performance. The economic consequences are further compounded by increased veterinary costs, diminished market value, production losses, and development of drug-resistant parasite strains. To address these multifaceted issues, an Integrated Parasite Management (IPM) approach is essential. IPM involves a holistic combination of sustainable preventative measures (such as strategic grazing, breeding for resistance, and biosecurity), timely diagnostic techniques (such as Faecal Egg Counts and FAMACHA scoring), and targeted treatment options (such as smart drenching and exploring alternative therapies). Adopting a One Health approach, which integrates modern diagnostics, sustainable practices, and traditional knowledge, is crucial for mitigating the global impact of parasitic infections and ensuring long-term health, productivity, and environmental sustainability.

Keywords: parasitic infections, animal health, agricultural production, zoonotic diseases, livestock

1. Introduction

Parasitic infections have a profound impact on animal health and agricultural production worldwide, leading to significant economic losses and posing threats to both human and animal health. These impacts are multifaceted, affecting the health and welfare of livestock, reducing productivity, and challenging agricultural sustainability. Effective parasite control is crucial for improving the quality of life of livestock, reducing zoonotic risk, and ensuring human health [1, 2].

The economic ramifications are particularly notable in the livestock sector, where parasites can significantly reduce profitability. In Brazil, for example, the annual economic loss due to parasitic infections in cattle is estimated to be approximately USD 13.96 billion. Gastrointestinal nematodes alone account for USD 7.11 billion, highlighting their severe impact on cattle productivity. Global production losses due to ticks and tick-borne diseases are estimated to be USD 13.9–18.7 billion annually [3].

Parasitic infections also affect agricultural systems more broadly, particularly through soil-borne nematodes, which lead to the deterioration of crop and animal health. Traditional control strategies, such as chemical pesticides and anthelmintics, often have negative environmental impacts, affecting beneficial soil microbiota and leaving toxic residues in agricultural products. Sustainable alternatives, such as the use of natural nematode enemies, are being explored as environmentally friendly control strategies [4].

Livestock health is intricately linked to human health, particularly in the context of zoonotic diseases. More than two-thirds of emerging infectious diseases in humans are of animal origin, emphasising the critical need for integrated health strategies and an understanding of transmission pathways. Molecular epidemiology and advanced diagnostic methods are vital for managing these challenges, offering tools for the precise identification and control of parasitic diseases across the human-animal-environment interface [5].

Mitigating the effects of parasitic diseases in livestock not only improves productivity, but also enhances animal welfare and reduces the environmental burden of livestock production systems. A One Health approach that considers all aspects of the ecosystem is crucial for this effort, ensuring a comprehensive strategy that benefits both humans and animals [5].

To address these issues effectively, improved parasite control measures that are convenient, effective against drug-resistant parasites, and environmentally sustainable are needed. Moreover, fostering collaboration between traditional healers and scientists in developing countries could enhance the treatment of parasitic infections using ethnoveterinary practices involving plant-based deworming preparations [6].

In summary, parasitic infections significantly affect animal health and agricultural production, necessitating comprehensive control strategies that balance economic interests, animal welfare, and environmental sustainability. Adopting integrated approaches that combine modern diagnostics, sustainable practices, and traditional knowledge could play a vital role in mitigating the effects of these infections globally.

2. Types of parasites affecting livestock and companion animals

Parasites affecting livestock and companion animals can be broadly categorised into internal (endoparasites) and external (ectoparasites).

Internal parasites include *Helminths* and *Protozoa*. Helminths consist of *Nematodes* (roundworms), such as *Toxocara canis*, *Trichuris vulpis*, and *Strongyloides spp.*, which infect various animals including dogs, cats, and livestock [7]. *Cestodes* (tapeworms), like *Dipylidium caninum*, are transmitted to dogs and cats via fleas [8]. *Trematodes* (flatworms) are less common in companion animals but are significant in some livestock[12]. Protozoa include *Giardia duodenalis*, which causes intestinal disorders in a wide range of hosts, including cattle and dogs [9]. *Cryptosporidium spp.* is known to cause cryptosporidiosis, particularly in neonatal ruminants, and can result in varying infection severities in dogs, cats, and livestock [10]. *Toxoplasma gondii*, primarily found in cats, is a concern for human health due to the risk of toxoplasmosis [1, 11]. Lastly, *Cystoisospora spp.* can cause intestinal diseases in dogs [12].

2.1 External parasites

External parasites are dominated by *Arthropods*, which encompass insects, spiders, and crustaceans. These organisms significantly affect animal welfare both through direct interactions with animals and through human-mediated roles, such as their use as biological control agents or in captive breeding. The welfare of captive insects is often a neglected issue due to their general intolerance by humans, yet their biological diversity and low tolerance for suboptimal conditions require specific care standards [13].

Beyond captive and controlled holding, arthropods contribute to animal welfare issues through their interactions with other animals. For instance, arthropods often serve as disease vectors, significantly affecting the health and welfare of livestock and wildlife. This role highlights the need for effective control measures while balancing ecological impacts, emphasising the importance of integrated and informed approaches that address both animal welfare and environmental sustainability [14].

Overall, the welfare of animals affected by arthropods needs careful consideration of multiple factors, including their physical health, ability to live naturally, and psychological well-being. Strategies to improve welfare in these contexts require multidisciplinary approaches and ongoing scientific research to understand and mitigate the impacts effectively [15, 16].

Several insects are particularly important in animal care due to their public health relevance. *Fleas*, common in dogs and cats, cause irritation and can transmit tapeworms [17]. The cat flea, (*Ctenocephalides felis felis)*, is a major pest that threatens cats, dogs, humans, and other warm-blooded animals, potentially leading to dermatological issues and act as vector for infectious agents. Modern insecticides, like imidacloprid, offer therapeutic and preventative solutions. *Ticks* are major ectoparasites that transmit various pathogens globally, including bacteria, parasites, and viruses, and are responsible for diseases like Lyme disease in pets [18, 19]. Their management is crucial due to their severe impact on health and economic productivity [20]. *Mites* cause conditions such as mange in animals and pose significant health challenges[26]. For example, the poultry red mite (*Dermanyssus gallinae*) is a major threat in the animal industry, affecting health, welfare, and productivity in areas like egg production. Mites can also act as disease vectors, worsening veterinary and public health concerns [21, 22]. *Psoroptes cuniculi*, for instance, causes substantial dermatological issues in rabbits [19, 22]. Controlling the source of mites, such as preventing bird roosting near medical settings, is essential in hospital environments where mite infestations have been observed to cause pruritic dermatitis [23]. These parasites all pose *zoonotic risks*, potentially affecting human health through contact or environmental exposure, highlighting the need for effective management and regular deworming programs.

3. Parasitic infestations lead to decreased animal welfare, reduced growth rates, and diminished productivity in various animal species

3.1 How parasitic infestations lead to decreased animal welfare

Parasitic infestations significantly affect animal welfare by affecting their health, productivity, and overall quality of life. Effective control of parasites is essential to improve animal welfare and mitigate the adverse outcomes of these infestations.

Parasitic diseases in animals can lead to severe health issues, compromising their physical condition and productivity. For instance, in livestock, parasitic infestations

can cause considerable economic losses due to decreased productivity and increased mortality. Effective parasite control is vital for maintaining livestock health, thereby enhancing livestock welfare and productivity [3].

Parasitic infections are a concern for livestock and wild animals. These infections can significantly affect the health and fitness of wild animals, with some parasites having zoonotic implications, thereby posing potential health risks to humans [24].

The management of parasitic infestations involves addressing various welfare issues in animal production. For instance, environmental factors such as air quality, temperature, and humidity can influence the transmission of diseases and parasites, thereby affecting the health and comfort of animals. Ensuring access to adequate feed and water and managing diseases and parasites are crucial for maintaining animal welfare.

In many cases, untreated parasitic infections can lead to severe symptoms, causing distress and suffering in animals. This underscores the need for effective treatment and prevention strategies to improve animal welfare in multiple contexts. The development and implementation of environmentally friendly and effective parasite control measures are vital for enhancing animal welfare while lowering the zoonotic risks associated with parasitic diseases [2].

3.2 How parasitic infestations lead to reduced growth rates

Parasitic infestations can significantly affect the growth rate of host organisms through various mechanisms. The relationship between parasites and their hosts often has negative consequences for host growth, survival, and reproductive capacity. There are several ways through which parasitic infestations lead to reduced growth rates.

One of them is resource competition. Parasites often directly compete with their hosts for nutrients. For example, hookworms, after completing their migration to the small intestine, feed by biting deeply into the mucosa and sucking blood, thus causing anaemia in heavy infections. There is strong evidence that infection with *Ascaris* contributes to childhood malnutrition and retards growth. This reduction in nutrient availability for the host leads to diminished growth and productivity.

Parasitic infections also impose physiological stress on hosts by altering normal metabolic functions. Studies on intestinal parasites, such as *Giardia lamblia* and *Enterobius vermicularis*, in children have shown associations with stunting and wasting, both indicators of impaired growth [25]. This physiological stress is often due to the energy and resources spent by the host to counter parasitic infection.

Hosts often mount immune responses against parasites, which is energetically costly and can divert resources away from growth and development. This is evident in situations where hosts experience parasite-induced mortality or reduced reproductive potential due to weakened life-support systems [26].

On a broader scale, the prevalence of parasites affects entire host populations, often leading to reduced growth rates across the population. For example, lungworm infections in cane toads have been shown to reduce growth rates under both laboratory and field conditions, affecting overall population viability.

Addressing these parasitic challenges through management and control strategies is crucial for minimising their impact on host populations.

3.3 How parasitic infestations lead to diminished productivity in various animal species

Parasitic infestations significantly affect the productivity of various animal species, including livestock and wild animals. These infestations lead to substantial economic losses and health challenges in different regions worldwide.

In livestock, particularly cattle, parasitic infestations can lead to substantial economic losses by adversely affecting weight gain, milk production, and overall animal health. For example, in Brazil, parasites such as gastrointestinal nematodes, ticks, and flies have resulted in billions of dollars in annual losses, thereby impacting the profitability of cattle production [3, 27]. In Mexico, similar detrimental effects of parasitism on cattle productivity underscore the importance of sustainable and integrated parasite control strategies to maximise profitability in the livestock industry [28]. Liver fluke infections can significantly impair the health and productivity of ruminant livestock globally. A meta-analysis demonstrated that liver fluke infections lead to reductions in daily weight gain, live weight, and carcass weight in livestock. However, the degree of impact varied, influenced by factors such as the age of the animals and the duration of infection. Wild mammals are also susceptible to parasitic infections, which can threaten their health and fitness. For instance, a study conducted in Greece revealed a high prevalence of parasites in wild mammals, which can have health implications for both wildlife and domestic animals due to their zoonotic potential. Parasitic infections extend beyond the health of individual animals and affect broader ecological systems. Climate change alters host movement and the environment, thereby influencing parasite transmission between wild and domestic animals. This change complicates parasite management strategies and poses additional pressure on livestock productivity [29]. A One Health approach is recommended to better assess and mitigate the impact of parasitic diseases in livestock. This approach emphasises understanding the broader context of parasitic infestations, considering both market-valued resources and those without direct market value. It also highlights the benefits of integrated parasite management for improving productivity, animal welfare, and reducing environmental burden [5]. In conclusion, parasitic infestations pose significant challenges to animal productivity, necessitating comprehensive management strategies that incorporate ecological dynamics and climate change. While parasitic control can greatly enhance animal quality of life and productivity, it is threatened by factors such as drug-resistant parasite strains. Therefore, investment in effective and environmentally friendly parasite control methods is crucial for sustaining productivity and reducing economic losses in various animal species [2].

4. The economic consequences of parasitic infections, such as increased veterinary costs and decreased market value

Parasitic infections in livestock and companion animals have significant economic consequences, primarily due to increased veterinary costs and decreased market values. These impacts can be observed across various aspects of animal husbandry and agriculture.

1. Increased veterinary expenditures: Costs for diagnosing parasitic infections—Expenses for treatment, including antiparasitic medications and supportive

care—Preventive measures like routine deworming programs and vaccinations—Fees for veterinary consultations and follow-up visits.

2. Diminished market value: Reduced weight gain and suboptimal body condition in infected animals—Lower quality of meat, milk, or other animal products—Decreased reproductive performance and fertility rates—Higher mortality rates, especially in juvenile animals.

3. Production losses: Decline in feed conversion efficiency—Reduced milk yield in dairy animals—Lower quality and quantity of wool in sheep—Decreased egg production in poultry

4. Labor costs: Additional time and effort needed for treating and managing infected animals—Increased labor for implementing preventive measures and biosecurity protocols

5. Infrastructure expenditures: Costs for improving housing and sanitation to prevent parasite transmission—Investments in pasture management and rotation systems to reduce parasite loads

6. Market access limitations: Restrictions on animal movement and trade due to parasitic infections—Potential loss of export markets due to concerns about parasitic diseases

7. Indirect economic impacts: Reduced consumer confidence in animal products—Potential human health risks from zoonotic parasites, leading to public health expenditures

8. Research and development costs: Ongoing investments in developing new antiparasitic drugs and control strategies—Expenses for monitoring and managing drug resistance in parasites

9. Environmental costs: Expenses for mitigating the environmental impact of antiparasitic treatments—Costs of implementing sustainable parasite control methods

10. Economic burden on smallholder farmers: Disproportionate impact on smallscale farmers with limited resources for parasite control—Potential contribution to rural poverty in developing countries

5. Parasite control strategies

Addressing these economic consequences requires integrated approaches to parasite management, including improved diagnostics, sustainable control strategies, and targeted interventions to minimise their impact on animal health and productivity.

Integrated parasite management for sustainable animal production.

Integrated parasite management (IPM) is a holistic approach that aims to minimise the reliance on chemical anthelmintics while maximising animal health and productivity. It combines various preventive, diagnostic, and treatment strategies to achieve sustainable parasite control.

5.1 Preventative measures

Strategic grazing management: This involves techniques such as rotational grazing, which involves moving animals between paddocks to allow pasture rest and parasite larval die-off. Adjusting the stocking density and selecting pasture species that are less favourable for parasite development can significantly reduce parasite burdens.

Breeding and selection: Selecting animals with genetic resistance to parasites is a key element of long-term sustainable control. Breeding programs focus using on parasite resistance traits can reduce the need for chemical interventions.

Hygiene and sanitation: Maintaining clean housing and pasture reduces parasite contamination. Regular cleaning and disinfection of animal facilities, proper manure management, and avoidance of overgrazing contribute to parasite control.

Biosecurity: Implementing biosecurity measures, such as quarantine for new animals and limiting contact with other farms, prevents the introduction and spread of parasites in the herd.

Effective control of parasitic insect is crucial in animal care. For instance, effective flea control in animal care is crucial for maintaining the health and well-being of pets, such as dogs and cats. The management of flea infestations and the prevention of associated diseases involve a combination of strategies addressing both the animal host and its environment.

One approach focuses on controlling flea allergy dermatitis in pets, particularly in dogs and cats. It's important to eradicate fleas from the affected animal and its surroundings to manage allergic reactions effectively. Various chemotherapeutic agents are available, each with different biological properties, and these can be applied directly to the animal or its environment.

In recent years, advancements have been made in flea control methods. The introduction of novel topical and oral therapies, such as imidacloprid and fipronil spot-on treatments, has significantly improved flea management. These treatments are designed to break the flea life cycle, preventing reproduction and reducing the impact of diseases related to fleas and ticks [30].

Despite these advancements, adherence to veterinarians' recommendations for flea and tick prevention can be suboptimal. A study in the United States found that even though most veterinary hospitals recommend year-round flea prevention, many dog owners do not follow these guidelines fully, often applying treatments for shorter periods throughout the year. This lack of adherence can lead to persistent flea problems [31].

Another critical aspect of flea control is understanding the biology and ecology of fleas. The cat flea's lifecycle and its interaction with hosts need to be comprehensively understood to develop effective control strategies. The immature stages of fleas are particularly susceptible to environmental factors such as temperature and humidity, which can be exploited using insect growth regulators (IGRs) to disrupt larval development [32].

Overall, the control of fleas in animal care involves integrated approaches combining novel chemotherapeutics, adherence to veterinary recommendations, and a deep understanding of flea biology and ecology. Despite these efforts, challenges such as insecticide resistance and suboptimal owner adherence continue to pose hurdles to achieving effective flea management in pets [33, 34].

Tick is another ectoparasite of complex problem that necessitates diverse control strategies and interdisciplinary efforts.

Traditional tick control has heavily relied on chemical acaricides. However, these are increasingly challenged by the development of resistant tick populations and

environmental concerns associated with chemical use [19]. Biological approaches using natural predators or entomopathogenic fungi, and physical methods like habitat modification, offer sustainable alternatives but need further optimization for effectiveness [19]. Integrated Pest Management (IPM) is emerging as a promising tactic by combining multiple control methods to enhance efficacy and minimise environmental risks [19].

A particularly promising area in tick control is the development of vaccines. Conventional control tactics using acaricides are proving unsustainable due to increased acaricide resistance. Vaccines targeting tick antigens like Bm86 have shown effectiveness by reducing tick infestations and pathogen transmission, highlighted by their use in controlling the cattle tick *Boophilus microplus* [35]. Future vaccine developments are likely to integrate tick antigens and pathogen-derived antigens for comprehensive protection [36].

Early detection and diagnosis of tick-borne diseases are crucial for effective disease management. Modern nucleic acid-based diagnostics, although well-developed for human medicine, are not yet widespread in veterinary applications. Implementing these high-throughput multiplex assays in veterinary medicine could significantly enhance early detection and control of tick-borne pathogens in animals, reducing their transmission to humans [37].

Given the ecological and sociopolitical challenges, tick and tick-borne disease management requires a holistic approach. This includes ecological studies to better understand the interaction between tick populations, their environments, and disease vectors. Enhancing our understanding of these factors will enable more effective area-wide control strategies [38].

Effective tick management is not just about immediate health benefits but is also crucial for economic sustainability, especially in agriculture. Tackling these challenges involves leveraging new technologies, fostering interdisciplinary collaboration, and investing in ongoing research and public health initiatives [19, 39].

Traditional approaches to manage mite infestations in animals have relied heavily on chemical acaricides. However, these have led to significant challenges like resistance, environmental pollution, and residue issues. As a result, there is a growing interest in natural products, such as plant extracts and microbial metabolites, which provide promising acaricidal properties with potentially fewer side effects [20].

Successful prevention of mite infestations involves integrated pest management techniques. These include pretreating clothing with permethrin, using DEET on clothing and skin, and treating infected animals. Strategies must be tailored to address the specific environments and potential mite species involved in the infestation. For example, in the context of hedgehog care, recognition and treatment for ectoparasites like mites are essential due to their high infestation rates and the associated zoonotic risks [20].

There is still a considerable challenge in effectively managing mite infestations due to factors like acaricide resistance and changes in animal husbandry practices. Continued research and adaptation of pest control methodologies, alongside improving awareness and monitoring efforts, are vital to mitigate the impact of mites on animal health and production systems [39, 40].

5.2 Diagnostic techniques

Faecal egg count (FEC): FEC is a common technique used to quantify the number of parasite eggs in animal faeces. Regular FEC monitoring allows for the early detection of infections and guides treatment decisions.

Coprological examination: This involves microscopic examination of faecal samples to identify specific parasite species and assess the intensity of infection.

Blood tests can detect antibodies against specific parasites, providing information on past exposure and infection levels.

Post-mortem examination of animal carcasses can help identify the presence and severity of internal parasite infections.

5.3 Treatment options

Targeted treatment: Instead of treating all animals, only those with high parasite burdens (identified through FEC or other diagnostic methods) receive treatment. This approach reduces the overall use of anthelmintics and minimises the risk of resistance.

Strategic drenching: Careful selection and use of anthelmintics based on parasite identification and susceptibility testing are essential. Using the right drug at the right time and avoiding unnecessary treatments are key to preventing drug resistance in patients with cancer.

Alternative therapies: Exploring alternative therapies, such as herbal remedies and probiotics, may offer additional control options in certain situations, although more research is needed in this area.

5.4 Monitoring and evaluation

Regular monitoring of parasite prevalence and treatment efficacy is essential for assessing the effectiveness of IPM strategies. This involves continuous FEC monitoring, clinical observation and recording of treatment outcomes. Data analysis allows for adjustments to the IPM plan, as needed.

6. Integrated parasite management: A symphony of strategies

Consider a pasture not merely as a field of grass, but as a complex ecosystem rich with both visible and microscopic life forms. Within this dynamic environment, parasites occasionally disrupt the delicate balance. Integrated parasite management (IPM) aims not at eradication but at achieving a sustainable equilibrium. It involves orchestrating a symphony of strategies, each element carefully executed to create a harmonious whole.

1. Preventative measures:

   The first line of defence strategic grazing: Rotational grazing involves the systematic movement of livestock to prevent parasite accumulation in any single area. This practice is akin to crop rotation, which rejuvenates the soil—a natural rhythm that maintains pasture health and mitigates parasite presence. This strategy may also include co-grazing, where different species graze together, thereby reducing the parasite burden on susceptible animals. It represents a harmonious integration of species.

   Pasture management: Maintaining optimal pasture conditions is crucial. A well-managed pasture is less conducive to parasites, similar to maintaining a tidy household to prevent pest infestations. This involves controlling sward height and selecting appropriate forage species.

Breed selection: Certain breeds exhibit natural resistance to specific parasites. Selecting resilient breeds is comparable to choosing robust building materials, ensuring a strong foundation for livestock operations.

2. Diagnostic techniques:

   Monitoring the ecosystem fec (faecal egg counts): Regular serve as a diagnostic tool, akin to taking the pulse of the pasture, providing insights into the parasite burden of the animals. This enables early detection and timely interventions.

3. FAMACHA: FAMACHA scoring assesses anaemia levels in animals, offering a visual indication of parasite load. It is similar to observing an animal's vitality, revealing subtle signs of parasitic infection.

4. Treatment options:

   Targeted interventions smart drenching: The strategic use of anthelmintics, guided by diagnostic testing, minimises resistance and maximises efficacy. This approach is analogous to the responsible use of antibiotics to ensure their future effectiveness.

   Refugia: Leaving some animals untreated helps maintain a reservoir of parasite-resistant animals and prevents widespread resistance. This practice is akin to preserving biodiversity and safeguarding the resilience of the entire system.

The importance of IPM for sustainable animal production: IPM is not merely a collection of techniques; it is a philosophy. It involves viewing the animal, pasture, and parasites as interconnected components of a single ecosystem. By integrating these strategies, we can transition from reactive treatments to proactive management, reduce reliance on chemical interventions, improve animal welfare, and foster a more sustainable and resilient animal production system. It is a holistic approach that ensures the long-term health and productivity of livestock while protecting the environment.

7. Conclusion

Parasitic infections are a critical and complex hurdle for animal care, livestock production, and public health worldwide. Their far-reaching effects, from causing multibillion-dollar economic losses in agriculture to compromising animal welfare and creating significant zoonotic threats, underscore the urgent need for comprehensive and effective control strategies. The adverse effects on animals manifest as decreased health, resource-diverting physiological stress leading to reduced growth rates, and overall diminished productivity in various animal species. The reliance on traditional chemical control is increasingly challenged by acaricide and insecticide resistance, and associated environmental concerns.

Therefore, the future of successful parasite control is the widespread adoption of integrated parasite management (IPM). This holistic philosophy goes beyond simple treatment, integrating a symphony of strategies, including sustainable pasture and grazing management, selection for genetically resistant animals, precise diagnostic

tools, and targeted, responsible use of treatments (smart drenching). Furthermore, leveraging new technologies, such as vaccines for tick control and advanced nucleic acid-based diagnostics, along with fostering collaboration with ethnoveterinary practices, is essential. Ultimately, implementing a One Health approach that views animal, human, and environmental ecosystems as interconnected is vital to transition from reactive treatment to proactive, environmentally sustainable management, securing animal health, improving welfare, and sustaining global food production for the long term.

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Section 2

Animal Care and Welfare

Chapter 6

Diagnostic Techniques for Insulin Dysregulation in Equines

Rodrigo Martins Ribeiro, Débora da Silva Freitas Ribeiro, Alexandre Augusto de Oliveira Gobesso and Rafael Resende Faleiros

Abstract

Insulin dysregulation (ID) is a prevalent metabolic disorder in horses, particularly in those with equine metabolic syndrome, and represents a principal risk factor for endocrinopathic laminitis. ID arises from a combination of insulin resistance, exaggerated pancreatic insulin secretion, and impaired hepatic insulin clearance, with obesity, high intake of nonstructural carbohydrates, physical inactivity, and chronic low-grade inflammation acting as key drivers. Mechanistically, defects in postreceptor insulin signaling, innate immune activation, oxidative stress, and endothelial dysfunction contribute to reduced insulin responsiveness and promote compensatory hyperinsulinemia, which can occur despite only mild hyperglycemia. Sustained hyperinsulinemia and associated metabolic stress are implicated in vascular and tissue changes within the digital laminae, facilitating laminitis development. This manuscript summarizes contemporary concepts of ID pathophysiology and outlines practical diagnostic strategies, emphasizing the strengths and limitations of basal insulin measurements and fasting-derived proxy indices, and highlighting dynamic testing approaches for detecting postprandial hyperinsulinemia in clinical settings. Early recognition and targeted management through dietary modification, weight reduction, structured exercise, and selected pharmacologic interventions are essential to mitigate laminitis risk and improve long-term outcomes.

Keywords: horses, equine metabolic syndrome, hyperinsulinemia, dynamic tests, endocrinopathic laminitis

1. Introduction

Insulin resistance (IR) and broader insulin dysregulation (ID) – which includes peripheral resistance and basal or postprandial hyperinsulinemia – are frequent findings in horses and represent a central pathophysiologic axis of equine metabolic syndrome (EMS) and endocrinopathic laminitis. Population surveys illustrate this relevance: hyperinsulinemia correlated positively with body condition score and crest neck measurements in horses sampled in the United States of America [1], and a

study in Australia reported hyperinsulinemia in 28% of ponies from different breeds [2]. More recent work reinforces that ID is highly prevalent in EMS and closely associated with endocrinopathic laminitis, currently regarded as the most common laminitis phenotype in clinical practice [3–7].

IR may occur as a physiological, adaptive response in specific states, notably pregnancy and stress. In pregnant mares, increases in basal insulin can accompany higher postprandial glucose, enhanced endogenous hepatic glucose production, and greater gluconeogenesis – changes attributed to endocrine shifts (e.g., prolactin, cortisol, glucagon) and counter-regulatory effects related to reproductive hormones and placental mediators, supporting a sustained nutrient supply to the fetus [8–11]. However, contemporary evidence highlights that when pregnancy coexists with obesity, EMS, and hyperinsulinemia, the maternal metabolic milieu may be compromised, with potential repercussions for reproductive function and offspring metabolic programming, emphasizing the need for metabolic monitoring during gestation [12, 13].

Pathological IR/ID, in turn, is strongly linked to obesity, systemic inflammation, hyperadrenocorticism, and acromegaly, reflecting impaired insulin receptor signaling, particularly in muscle and adipose tissue. Reduced insulin effectiveness compromises glucose and nonesterified fatty acid (NEFA) handling and may disturb vascular actions of insulin mediated by nitric oxide and endothelial function [14]. In EMS, chronic low-grade inflammation is a consistent feature, with elevations in acute-phase proteins and circulating inflammatory mediators, especially in obese and hyper insulinemic horses [12, 15, 16]. At the cellular level, multiple mechanisms may contribute, including reduced insulin receptor abundance, defects in insulin receptor activity (InsR-1/InsR-2), impaired phosphoinositide-3-kinase signaling, failure of GLUT4 translocation, and altered intracellular enzymatic activity [17]. Omics-based studies in horses with EMS further implicate dysregulation of pathways related to energy metabolism, oxidative stress, extracellular matrix remodeling, and inflammatory responses, supporting the relevance of postreceptor disturbances and possible epigenetic influences in equine IR [3, 4, 16].

Endocrine and metabolic modulators can amplify these defects. Glucocorticoids (GCs) exert insulin-antagonistic effects in the liver, muscle, and adipose tissue by inhibiting key steps in insulin signaling, including GLUT4 translocation and lipoprotein lipase activity, thereby reducing triglyceride uptake and altering substrate partitioning [18, 19]. Although GC-induced models remain useful experimentally, field phenotypes more often reflect the combined impact of obesity, high nonstructural carbohydrate intake, and sedentary management rather than direct GC exposure [5, 12]. In parallel, adipose-derived mediators (adipokines) and elevated circulating free fatty acids interact with inflammatory pathways and insulin signaling. Horses with metabolic disorders commonly show increased leptin and, in some contexts, increased resistin, particularly when systemic inflammation is present [12, 13, 20]. When insulin-stimulated glucose disposal declines, pancreatic islets may respond with sustained insulin secretion, promoting hyperinsulinemia [21]. Importantly, hyperinsulinemia can further worsen IR through downregulation of insulin receptor availability and reduced receptor affinity and signaling efficiency – forming a self-reinforcing cycle [22]. At the receptor and postreceptor interface, diminished receptor externalization or increased receptor degradation, reduced receptor kinase action, serine phosphorylation of receptor residues, tyrosine dephosphorylation by phosphatases, and inhibitory interactions at the receptor level can all attenuate signal transduction [23].

Inflammatory mediators add another layer of impairment. TNF-α has potent inhibitory effects on insulin signaling in muscle and adipose tissues, while high plasma free fatty acids – common in obesity – can reduce peripheral glucose uptake by limiting phosphoinositide-3-kinase activation; cortisol excess can also inhibit GLUT4 trafficking [24–26]. In EMS, adipose tissue inflammation with macrophage and neutrophil participation and shifts in the adipocyte secretome are increasingly recognized as key links between excess body fat, IR, and laminitis susceptibility [12, 13, 27]. Consistent with this, toll-like receptor (TLR) activation has been associated with IR and impaired GLUT regulation; TLRs coordinate innate immune signaling and cytokine production (e.g., IL-6, TNF-α) and may be activated by free fatty acids and lipopolysaccharides, which rise in insulin-resistant states [28–30]. In obese horses, elevated fetuin-A and activation of the TLR4/NF-κB/MAPK axis with increased pro-inflammatory cytokines have been described, supporting the concept of liver-derived proteins acting as endogenous TLR ligands that promote IR [31].

The clinical expression of equine ID differs from classic diabetes mellitus phenotypes. In many horses with EMS, beta-cell hypersensitivity and prolonged postprandial hyperinsulinemia coexist with reduced peripheral glucose clearance, so hyperglycemia tends to be mild because increased insulin secretion partially compensates for decreased insulin effectiveness [5, 7, 16, 32, 33]. Nevertheless, even modest elevations in circulating glucose can increase exposure of noninsulin-dependent tissues to glucose and contribute to glucotoxicity [34]. Endothelial tissues are particularly vulnerable: glucose-driven formation of advanced glycation end products (AGEs) promotes lipid peroxidation and reactive oxygen species (ROS) generation, amplifying oxidative stress [35–37]. Oxidative stress reduces nitric oxide bioavailability, increases endothelin production, and enhances procoagulant activity, favoring vasospasm, hypercoagulability, and impaired capillary perfusion – mechanisms that align with the vascular-metabolic disturbances observed in endocrinopathy laminitis [30, 38, 39]. In experimental models, prolonged hyperinsulinemia under euglycemic clamp induces laminitis consistently and is associated with proteomic and inflammatory alterations in digital laminae, including markers linked to oxidative processes and tissue injury [4, 40]. Additional hemostatic effects may contribute, as IR has been associated with increases in prothrombotic factors (e.g., plasminogen activator inhibitor-1, von Willebrand factor, fibrinogen, factor VII, thrombin–antithrombin complexes) and reductions in anticoagulant factors (e.g., antithrombin III, protein S, protein C) [41, 42].

Beyond peripheral mechanisms, central regulation of appetite and energy balance may participate in the perpetuation of obesity and IR. Prolonged IR may influence central nervous system pathways; visceral adiposity may activate the hypothalamic–pituitary–adrenal axis through neuroendocrine mechanisms, and insulin's satiety signaling in the ventromedial hypothalamus may be blunted in IR [37]. Leptin normally contributes to satiety, and hypothalamic leptin resistance may further promote weight gain, reinforcing a vicious cycle in which obesity promotes IR and IR favors additional adiposity. While much of the mechanistic framework is extrapolated from other species, equine adipose tissue reviews support roles for leptin, adiponectin, and resistin in both peripheral insulin sensitivity and central energy homeostasis [13, 27, 37].

Given its high prevalence, multifactorial pathogenesis, and strong association with laminitis, early recognition of insulin dysfunction is a clinical priority. Contemporary reviews and guidelines emphasize that prevention and management

of endocrinopathic laminitis depend on identifying ID promptly and implementing targeted dietary strategies (especially reducing nonstructural carbohydrate intake), weight reduction in obese animals, structured exercise when appropriate, and, in selected cases, pharmacologic interventions aimed at lowering hyperinsulinemia, including newer options such as incretin-based approaches and SGLT2 inhibitors [5–7, 43]. This chapter builds a mechanistic foundation for understanding equine IR and dysregulation and frames its clinical consequences – particularly laminitis – while preparing the reader for subsequent sections on diagnosis, monitoring, and evidence-based management.

2. Insulin dysregulation

Currently, the term "insulin dysregulation" has been more widely used to describe changes in metabolism that result in persistent hyperinsulinemia with normal blood glucose values [44]. Horses are considered to have hyperinsulinemia when plasma insulin concentrations are greater than 200 μIU/mL [45, 46]. In the most recent reviews, ID is understood as a spectrum that includes increased pancreatic secretion, reduced hepatic clearance, and variable peripheral resistance, with hyperinsulinemia status – often with blood glucose still within the reference range – being the central component of EMS [47]. Although there is terminological debate between the concepts of insulin "resistance" and "dysregulation" [48], current clinical recommendations remain focused on the identification of hyperinsulinemia at rest or after dynamic tests, using cut-off points generally lower than the 200 μIU/mL initially described and adjusted to the type of assay and protocol employed [49–51].

Previously, it was believed that hyperinsulinemia would be linked only to IR promoted by the decrease in the normal response of tissues to normal levels of circulating insulin, increasing the time that the hormone stays in circulation [52]. However, research in horses has shown that both increased secretion and decreased insulin clearance are determining factors leading to hyperinsulinemia. Recent studies suggest that, in some hyper insulinemic animals, alterations in hepatic clearance – with modulation of proteins such as IDE (insulin-degrading enzyme) and CEACAM-1 – contribute to maintaining high insulin concentrations, even in the absence of marked hepatic steatosis [53]. In parallel, omics approaches have shown that ID in horses with EMS is associated with coordinated changes in proteins linked to inflammation, coagulation, and extracellular matrix remodeling, reinforcing the systemic character of this disorder [4].

It is also known that there are other substances that can be released by the body, interfering with this metabolism. Incretins are a good example. Among these hormones, glucagon-like peptide 1 and glucose-dependent insulinotropic peptide stand out, which are secreted by L and K cells present in the intestinal mucosa and are degraded by the enzyme dipeptidyl peptidase after the ingestion of sugars, amino acids, and fats. Incretins stimulate insulin secretion by pancreatic β cells and slow down stomach emptying, interfering with postprandial hyperglycemia. Thus, hyperinsulinemia in animals with EMS may be due to the stimulation of incretin production or to the failure of dipeptidyl peptidase activity. It has been observed in rats that incretins promote hyperplasia of pancreatic β cells [54, 55]. In horses, the importance of the enteroinsular axis was confirmed by studies that characterized the

response of insulin and incretins to oral glucose and sugar tolerance tests, showing a strong association between the magnitude of the GLP-1 response and postprandial hyperinsulinemia [51, 56]. In addition, experimental administration of a GLP-1 mimetic in horses demonstrated potential to modulate the insulin response to oral sugar testing, mainly by increasing insulin sensitivity, reinforcing the role of incretins as central modulators of ID in this species [57].

Another theory about the increase in plasma insulin values concerns hepatic handling of insulin in animals with EMS. While more than 70% of insulin produced by pancreatic β cells is released into the portal circulation before distribution to the periphery, hepatic lipidosis may interfere with insulin processing and clearance [58]. Recent results indicate that hepatic IDE activity and expression of molecules involved in insulin clearance vary between normoinsulinemic and hyper insulinemic horses, suggesting that hepatic hormone removal capacity may be an additional determinant of the severity of ID, regardless of the presence of overt diffuse liver disease [47, 53].

2.1 Diagnosis

The diagnosis of ID and insulin insensitivity can be made through tests that consider both insulin and glucose, as both constituents exhibit altered dynamics. Several procedures have been developed to perform these measurements. It is necessary to determine appropriate reference ranges for each diagnostic test because multiple factors can influence results, including age, breed, physical fitness, and diet type [59]. Recent reviews emphasize that test interpretation should also consider the analytical method (insulin assay), sampling time relative to the last meal, and the presence of obesity or laminitis; therefore, consensus groups recommend standardizing both the protocol and the laboratory when monitoring the same patient over time [32, 49].

There are two main categories of tests: static tests, based on baseline glucose and insulin values, and dynamic tests, based on the physiological response to administered glucose and/or insulin. Dynamic tests are more complex and involve serial blood sampling, but they are generally considered more reliable because they capture the integrated behavior of insulin and glucose. Although an optimal test has not yet been established, dynamic tests are more sensitive for detecting IR than baseline measurements alone [43]. Among dynamic tests, the oral sugar test (OST), the combined glucose–insulin test (CGIT), and oral glucose/sugar tolerance tests are commonly used in practice and can detect ID even when basal values remain within reference ranges [25, 43, 50]. Recent studies in horses and donkeys further support that standardized oral tests, which better simulate the postprandial state, are superior for identifying postprandial hyperinsulinemia and assessing endocrinopathy laminitis risk, and are therefore recommended as routine screening tools [49, 51, 56].

2.1.1 Measurement of basal insulin concentration

Basal glucose values are rarely elevated in horses with IR; this may happen due to compensated insulin production attempting to reverse IR in tissues [60]. Experimental studies with induced obesity in horses confirm that, in the early phases of dysregulation, fasting glucose remains within the reference range while basal

insulin increases early, characterizing compensatory hyperinsulinemia with normoglycemia [61].

Basal insulin measurement is a useful practice, helping in the diagnosis and monitoring of EMS. Baseline insulin can be used to determine if there is a risk factor for developing laminitis and thus guide when the best time to intervene is [60]. Case series and field studies have shown that horses with persistently elevated basal insulin are more likely to develop endocrinopathic laminitis over time, especially when associated with generalized obesity, regional adiposity, or PPID [50, 62–64].

Before collecting blood samples for insulin measurement, the animal must undergo a minimum period of prior food fasting of at least six hours, and this must be performed in the morning. If repeated collections are to be conducted, it is of great importance that all collections are carried out in a standardized manner [15, 43, 60]. Recent guidelines recommend that fasting be performed with nonstructural, low-carbohydrate hay, avoiding concentrates in the 12 hours prior to collection, and that the same laboratory and immunoassay method be used throughout follow-up in order to reduce biological, circadian, and seasonal variability in insulin concentration [47, 49].

Monitoring basal insulin values may be relevant because compensatory hyperinsulinemia is a common feature in horses with IR. However, there are situations in which this test may fail, for example, when the IR is at the beginning or mild, and the elevation of compensatory insulin has not yet been established, or if the plasma insulin value is still very low. This characteristic can also be evidenced by the fact that the intervals of reference values are very wide. Studies comparing basal insulin with dynamic tests (such as the glucose–insulin combination test and the oral sugar tolerance test) show that the sensitivity of fasting insulin to detect ID is only moderate, with a risk of false negatives, reinforcing the use of dynamic tests when clinical suspicion persists [50, 65].

Depending on the research laboratory, there is great variation in the reference values used to determine if an animal is presenting a condition of hyperinsulinemia. The laboratory at the University of Tennessee considers an animal to be hyper insulinemic when plasma insulin concentration values are above 30 μIU/mL. The Animal Health Diagnostic Center at Michigan State University uses 43 μIU/mL as a reference value [66]. More recently, the Equine Endocrinology Group has proposed interpretation categories for basal insulin (e.g., values < 20 μIU/mL considered normal, 20–50 μIU/mL as a gray area, and > 50 μIU/mL indicative of ID), highlighting that these cut-off points vary according to the assay used and should always be interpreted in conjunction with clinical history and dynamic test results [49, 50].

Although insulin measurement is a viable tool for clinicians working in the field, this procedure is not considered completely reliable due to the existence of many factors that interfere with plasma insulin values. For example, the type of diet provided to these animals. If the diet is rich in concentrates, there may be an increase in basal insulin values. Physiological stress caused by acute exercise or pain can decrease insulin values; this may possibly be caused by an increase in catecholamines in the circulation, which act by inhibiting insulin production by the pancreas [67, 68]. Recent studies also highlight that variables such as season, race, age, sex, and even the analyzer used (differences between immunoassay platforms) can significantly modify basal insulin concentrations, justifying the establishment of specific reference intervals for population, method, and season [18, 47]. Other

factors that can interfere with insulin and glucose concentrations are age, gender, and nutritional status, making it necessary to develop more accurate methods for diagnosing IR [65, 69].

2.1.2 Indirect indicators (proxies) based on baseline glucose and insulin concentrations

Indirect indicators, or "proxies," are values obtained through formulas that use baseline insulin and glucose values to determine whether animals exhibit insulin sensitivity or resistance. They evaluate models of homeostasis by providing standardized estimates, which have been correlated with tests assessing insulin sensitivity. The great advantage of using proxies lies in the fact that they utilize plasma glucose and insulin values, offering a practical way to demonstrate whether the animal is presenting a state of IR [70]. The calculations are straightforward and rely on the baseline values of glucose and insulin for interpretation in "quintiles," where 1 is considered low and 5 high [71].

More recent studies indicate that these indices have good intraindividual reproducibility and are especially useful in epidemiological and group follow-up studies, although they do not always offer a clear advantage over fasting insulin alone for estimating IR at the individual level [61, 72] (**Table 1**).

In the HOMA-IR proxy, the animal is considered to have a positive diagnosis of IR when it has a value greater than 2.71 [74].

Animals are considered healthy when the RISQI proxy values are greater than 0.32; if the values are less than 0.32, it may be indicative of IR, and if the observed value is less than 0.22, it is considered severe IR. In experimental models of induced obesity in horses, it was observed that the G:I ratio and the RISQI change early during

Proxy index	Equation (as used in this chapter)	Inputs required	Notes/unit handling
HOMA	(Glucose – Insulin)/22.5	Glucose, insulin	**Check the equation** (commonly reported as a product-based index in other literature). Ensure glucose/insulin units match the original source.
HOMA-IR	[[Glucose (mg/dL) × 0.0555] × Insulin (μU/mL)]/22.5	Glucose, insulin	Converts glucose from mg/dL to mmol/L by × 0.0555 before calculation.
RISQI	1/√Insulin = Insulin^ – 0.5	Insulin	Uses insulin only; higher RISQI generally indicates greater insulin sensitivity.
G:I ratio	Glucose/Insulin	Glucose, insulin	Ensure consistent baseline sampling conditions (fasting) to compare across animals.
MIRG	(800 – 0.3 × [Insulin – 50]^2)/(Glucose – 30)	Glucose, insulin	Confirm bracket placement, and ensure units follow the original description.
QUICKI	1/log(Glucose × Insulin) = [log(Glucose × Insulin)]^ – 1	Glucose, insulin	Confirm whether the log is base-10 or a natural log in the cited source, and ensure consistency.

All proxies are calculated from baseline (fasting) glucose and insulin values. Ensure units and logarithm base are consistent with the original reference (Treiber [73]) and with the insulin assay used.

Table 1.
Proxy indices based on baseline glucose and insulin values (adapted from Treiber [73]).

weight gain, which suggests good sensitivity of these proxies to detect the progressive worsening of insulin sensitivity [61].

When the MIRG proxy is used and the observed value is less than 5.6, the animal is considered healthy. However, even if the MIRG values and the values of the glucose-to-insulin ratio (G:I) are considered normal, the baseline glucose values should be observed; if they are above 100 mg/dL, it is indicative of IR. Recent data in neonatal foals and adult horses have shown that MIRG and HOMA-BC% differentiate β cell response profiles between age groups, reinforcing the usefulness of these indices as markers of pancreatic function in different equine populations [75].

If the G:I ratio value is less than 4.5, the animal is presenting a severe IR condition; if it is in the range between 4.5 and 10, the animal is considered to have a compensatory IR condition; and if the value is greater than 10, the animal is considered healthy [70, 73]. However, more recent studies show that these cut-off points should be interpreted with caution, as proxies are influenced by diet, fasting protocol, inflammatory state, and dosing method, and are more suitable for use in group studies than as an isolated diagnostic tool in a single individual [72, 76].

Brojer et al. (2015) observed that the proxies QUICKI, RISQI, and HOMA-IR showed a good correlation with the system considered "gold" for the diagnosis of insulin sensitivity, the hyper insulinemic-euglycemic clamp (EHC), but they did not have a good correlation during the period of weight gain in the animals [11]. In a complementary way, Lindåse et al. (2021) confirmed that proxies have good repeatability and correlate with EHC, but concluded that there is no consistent advantage in using them instead of fasting insulin to estimate insulin sensitivity at the individual level [72].

According to Morgan et al. (2015), the most used proxies in horses are RISQI and MIRG. Remembering that these "proxies" use basal values, they are subject to the limitations already observed above, but as they convert nonlinear values to linear values, it is more recommended to use them to monitor animals for long periods. Recent reviews reinforce that these indices are useful for stratifying risk, monitoring response to dietary management, and detecting trends over time, while dynamic tests remain the standard for confirming ID and more accurately assessing the risk of endocrinopathic laminitis [61, 72].

2.1.3 Dynamic tests

2.1.3.1 Hyperinsulinemia euglycemic clamp

Considered a reference standard for determining IR, yet too laborious to be used routinely. This test is performed through a glucose infusion rate in order to maintain the animal's normal blood glucose values during a period of induced hyperinsulinemia, with serial measurements of glucose and insulin being performed. It is started after a 12-hour fasting period. A blood sample is taken five minutes before the test begins for basal glucose and insulin analysis (**Table 2**).

Recent studies reinforce that the hyperinsulinemic-euglycemic clamp (EHC) remains the "gold standard" method for assessing insulin sensitivity in horses, being used mainly as a reference to validate field tests and alternative models for assessing ID [65, 77].

Time (min)	Glucose	Insulin
0		✓
1	✓	
2	✓	✓
3	✓	
4	✓	✓
5	✓	
6	✓	✓
7	✓	
8	✓	✓
9	✓	
10	✓	✓
12	✓	
14	✓	✓
16	✓	
18	✓	✓
20	✓	✓
30	✓	✓
40	✓	
50	✓	
60	✓	✓
70	✓	
80	✓	
90	✓	✓
100	✓	
120	✓	✓
150	✓	✓
180	✓	✓

✓ indicates scheduled sampling time points. Glucose is monitored at short intervals to adjust the glucose infusion rate and maintain euglycemia.

Table 2.
Sampling schedule for glucose and insulin measurements during the hyper insulinemic–euglycemic clamp (minutes).

Insulin is infused through an IV pump at a dose of 40 μU/min/kg body weight. Glucose will be offered concomitantly at a dose of 0.5 g/kg of body weight, IV, in order to keep the animal euglycemic, with a plasma glucose value of 0.2 mmol/L. If there is a variation in this value, it must be corrected empirically. Plasma glucose values should be monitored using a glucometer [78]. In more recent experimental protocols, EHC has been used for prolonged periods (up to 48 h) to induce laminitis-associated hyperinsulinemia (HAL), confirming that sustained exposure to elevated insulin is sufficient to trigger lamellar lesions even in previously healthy animals [79, 80].

The infusion period will be 180 minutes. The first 90 minutes are considered a period of equilibrium, and the final 60 minutes are used to calculate the rate of glucose absorption by the animal's body. As the glucose concentration is not constant, a space correction (SC) calculation is required; the SC will be determined by the following formula: $SC = (G2 - G1) \times (0.19 \times PA)/(T \times PA)$ [81].

The unit of the SC will be mmol/kg/min. G is the concentration of basal glucose, and T is the interval that is being calculated. PA is the weight of the animal. To measure the amount of glucose that has been infused (M), the formula described below is used: $M = GIR - SC$. GIR is the glucose infusion rate (mmol/kg/min), and SC is the correction space. The calculation of insulin (I) concentration is done by averaging the insulin concentrations measured in serum over the final 60 minutes of the test. Insulin sensitivity is then expressed by the M/A ratio.

The determination of the insulin sensitivity index is achieved through the M/A ratio. An animal is considered insulin resistant when the volume of glucose infused is lower compared to animals considered normal [82]. Studies with different breeds (hotbloods, ponies, and saddle horses) have shown wide individual variation in the M index, allowing researchers to discriminate between insulin-sensitive and insulin-resistant animals and correlate this variability with cardiovascular and metabolic changes, as well as the risk of laminitis [77, 83]. In addition, the EHC model has been widely employed to study the pathophysiology of endocrinopathic laminitis: sustained hyperinsulinemia has been shown to increase the expression of inflammatory cytokines and acute-phase proteins in digital tissues [80], and the application of continuous digital cryotherapy reduces inflammation and favorably alters lamellar energy metabolism, mitigating structural injuries [79, 84].

Although it remains a reference test, the EHC is complex, time-consuming, invasive, and requires intensive monitoring, which limits its use to research and a few specialized centers. Thus, in clinical routine, its main relevance is to serve as a standard for validating simpler dynamic tests (such as the oral sugar tolerance test, the glucose–insulin combined test, and the insulin tolerance test), whose performances have been compared to the clamp in recent studies [50, 65].

2.1.3.2 Intravenous glucose tolerance test (IVTT)

The purpose of this diagnostic test is to observe how insulin and glucose concentrations behave after the administration of an intravenous glucose "bolus". After a prior period of food fasting lasting 12–24 hours, the test will begin. A glucose solution with a concentration of 50% should be administered intravenously at a volume of 0.5 g of glucose per kg of body weight over a period of 10 minutes [85, 86]. Venous blood samples will be collected at times 0, 5, 15, 30, 60, 90, 120, 180, 240, 300, and 360 minutes after glucose administration to determine plasma glucose and insulin concentrations. The results will be calculated using measures of the half-life of glucose elimination and fractional turnover rate to provide a picture of IR [87]. Recent studies demonstrate that frequently sampled variations of the IVGTT (frequently sampled IVGTT, FSIGTT) allow the application of minimal model analysis to more accurately quantify insulin sensitivity in horses and donkeys and have been used as a reference method to validate other ID field tests [56, 72, 75].

Healthy animals will show an immediate increase in plasma glucose concentration and a subsequent return to baseline levels within 60 minutes after

glucose administration. An animal with an IR condition has the potential to produce a higher blood glucose spike and a consistent delay in return to baseline of more than two hours [88]. Results from recent studies confirm this pattern, showing that horses with metabolic syndrome or ID have persistently elevated glucose and insulin concentrations after IVTTT/FSIGTT, as well as a larger area under the curve for glucose and insulin when compared to control animals [75, 89, 90].

When analyzing the insulin curve in healthy animals, it should behave in a manner corresponding to the glucose curve, with a peak approximately 30 minutes after glucose administration. If the animal has impaired pancreatic β cell function, a delayed glucose curve will be observed, and the insulin response may be delayed. The IVTT is not a sensitive means of measuring impaired pancreatic response [91]. However, frequently sampled modified versions, associated with minimal model analysis, have been used to separate components of peripheral insulin sensitivity and responsiveness of β cells in different contexts, such as comparison between newborn and adult foals, evaluation of the effect of physical training, or drugs that affect dopaminergic signaling or the inflammatory axis [75, 90, 93, 94].

In addition, the IVTTT/FSIGTT has been used in recent studies to investigate therapeutic interventions aimed at improving insulin sensitivity, such as supplementation with nutraceuticals or essential oils and adjustments in nutritional management, demonstrating that reductions in the area under the glucose and insulin curve after the test are associated with improvements in the ID phenotype [95, 90].

2.1.3.3 Combined glucose-insulin test (GICT)

The combined glucose–insulin test (GICT) is a test that uses an application in "bolus" of glucose intravenously, followed by the infusion of insulin. The volume of glucose (50%) to be administered is 150 mg/kg body weight, and the volume of insulin is 0.10 IU/kg body weight. Insulin should be given immediately after glucose infusion. Blood samples will be collected at times 0, 1, 5, 25, 35, 45, 60, 75, 90, 105, 120, and 135 minutes after drug administration. In recent years, the GICT has established itself as one of the dynamic tests of choice to characterize ID in horses, being widely used in experimental models of IR induction with dexamethasone and in studies comparing different diagnostic approaches, such as the oral sugar tolerance test and insulin sensitivity proxies [76, 92, 93].

Glucose is measured using a glucometer. When the blood glucose value is above the baseline value for a period of 45 minutes, it is indicative of IR. However, if, in the first 45 minutes of the test, glucose values remain close to baseline values and insulin values are greater than 100 μIU/mL, this is also an indication of IR concomitant with an indication of hyperplasia of the β pancreatic cells. This is due to an elevated production of compensatory insulin to try to maintain blood glucose homeostasis. When an animal has high glucose values with low insulin concentrations, this can mean a decompensated IR [81]. Recent studies have confirmed that prolonged glucose permanence in the positive phase of the GIT curve and elevated insulin concentrations between 45 and 75 minutes are associated with poorer insulin sensitivity and increased risk of laminitis, as well as being useful for monitoring response to pharmacological (e.g., bromocriptine) and nutritional (nutraceuticals and essential oil-containing supplements) therapeutic interventions in animals with a history of ID [94–97].

There is a possibility that an episode of hypoglycemia may occur. Thus, it is recommended to keep two 60 ml syringes containing glucose (50%) to be used if the animal presents muscle fasciculations or profound weakness, or if plasma glucose concentrations are less than 40 mg/dL [66, 98]. Although GCT is considered a relatively safe test when performed with adequate monitoring, recent studies highlight the need for clinical surveillance, especially in animals with severe ID or those previously exposed to GCs, in which glycemic and insulinemic dynamics may be more unpredictable [76, 93].

2.1.3.4 Insulin tolerance test (ITT)

The insulin tolerance test (ITT) measures the blood glucose response to insulin "bolus" administration. It is a direct measure of the sensitivity of tissues to an insulin dose. The methodology of this technique should be carried out after a period of 12 hours of prior food fasting. The insulin dose will vary between 0.2 and 0.6 IU/kg of body weight. Samples will be collected at times 0, 5, 10, 15, 20, 30, 40, 50, 60, 90, and 120 minutes, and the area under the curve must be calculated, or only glucose concentrations can be analyzed. In a healthy animal, glucose values should decrease by up to 50% in relation to the baseline value in the period between 20 and 30 minutes, and should return to normal values after 90–120 minutes. In an animal with IR, plasma glucose levels do not decrease to such drastic levels compared to baseline and will return to baseline values more quickly [87, 99]. The glycemic insulin response test is the simplified form of this test [100].

In recent decades, the ITT has been progressively standardized into "short-protocol" (two-step insulin-tolerance/response test) versions, which are widely used in research and recommended as one of the main dynamic tests for assessing peripheral insulin sensitivity in horses with suspected ID, in addition to oral tests [49]. In these abbreviated protocols, 0.1 IU/kg of regular insulin is typically administered IV after basal glucose collection, and blood glucose is reassessed at 30 minutes, maintaining the criterion of a drop ≥ 50% from baseline to classify the animal as insulin sensitive [100, 101].

Recent studies have shown that ITT is sensitive for detecting reduced insulin sensitivity associated with weight gain and pasture intake. In native British ponies, a serial ITT protocol showed a progressive decrease in results (indicating worsening insulin sensitivity) as the animals became obese in a pasture regime, and these values correlated with post-OST hyperinsulinemia and adiponectin alterations [102]. These findings reinforce the usefulness of the ITT as a functional measure of insulin sensitivity in longitudinal studies and in the monitoring of nutritional management and weight loss programs in animals at risk of laminitis. From a practical point of view, there have also been advances in the choice of the type of insulin used. Grob et al. (2024) compared a standard ITT with recombinant regular human insulin (0.1 IU/kg IV) to a ITT performed with porcine zinc insulin (PZI) at the same dose, in a crossover design, and observed a strong correlation between the tests and the absence of relevant adverse effects. However, when using the classic criterion of a 50% reduction in blood glucose at 30 minutes, more animals were classified as resistant when PZI was used; a diagnostic performance analysis indicated that, for PZI, a 40% glucose reduction cutoff at 30 minutes improves test accuracy [101]. These data are important for clinicians who use insulin exclusively for veterinary purposes.

Despite these contributions, recent reviews emphasize that the ITT should not be interpreted in isolation but in conjunction with other dynamic tests based on oral stimuli, which capture, in addition to peripheral sensitivity, components of pancreatic secretion and enteroinsular response. Field studies have shown that oral tests (such as the OST and OGTT variations) detect a higher proportion of animals with ID than simple baseline assessments, which justifies the combination of oral tests and ITT in the phenotypic characterization of EMS [50, 102].

2.1.3.5 Glycemic insulin response test

A simplification of the ITT, this test is performed after the application of intravenous insulin to observe the glucose absorption index. To be performed, a prior fasting period of 12 hours must be respected. After this fast, a basal blood sample will be collected, considered time 0, to analyze glucose through a glucometer device. Immediately after baseline blood collection, regular recombinant human insulin (Humulin, Eli Lilly and Company, Indianapolis, IN) will be administered intravenously at a dose of 0.1 IU/kg body weight. After 30 minutes of insulin administration, a second venous blood sample will be collected for glucose analysis using the glucometer [100, 103]. In recent years, this "two-sample" protocol has been widely described as a two-point insulin tolerance test or abbreviated insulin-response test and is considered one of the main dynamic field tests for evaluating peripheral insulin sensitivity in horses, as it requires little time, low sample volume, and minimal manipulation of the animal [50, 65, 101].

To determine whether the horse is considered insulin resistant, the plasma glucose concentration of the sample collected 30 minutes after insulin application must be greater than 50% of the glucose value of the sample collected at time 0 [100, 103]. Recent studies with variations of the two-point test, using both regular human insulin and PZI, have confirmed that a reduction of approximately 50% in glucose at 30 minutes is compatible with preserved insulin sensitivity, while smaller reductions indicate tissue resistance. However, small cut-off point adjustments (e.g., 40% when using PZI) may improve accuracy in certain protocols [101, 104]. These studies also showed good agreement between abbreviated TRGI and longer dynamic tests in the classification of animals with ID [65].

If the animal has a value less than or equal to 20 mg/dL of glucose, 150 mg/kg of body weight of glucose at 50% should be administered. If the animals show clinical signs of hypoglycemia, such as sweating, muscle fasciculations, or ataxia, carbohydrate-rich feeding should be offered; animals should be observed until the end of these hypoglycemic symptoms [100]. Although the occurrence of symptomatic hypoglycemia is uncommon when using the 0.1 IU/kg dose in appropriately selected animals, careful monitoring is recommended during and after testing, especially in horses with concomitant systemic disease or those recently undergoing corticosteroid therapy, as these conditions may alter glycemic dynamics and test response [65, 76].

2.1.3.6 Oral glucose tolerance test (OGTT)

Currently, it is considered the most practical and complete test, as it evaluates glucose absorption by the small intestine, hepatic glucose uptake, pancreatic endocrine function, and IR. To carry out the test, the animal must fast for 12 hours

prior to feeding. In this test, glucose should be delivered orally at a dose of 1 g/kg using a nasogastric tube; blood samples are collected at intervals of 0, 30, 60, 90, 120, 180, 240, 300, and 360 minutes after glucose administration to analyze plasma glucose and insulin values [59]. However, the protocol can be simplified with collections of up to 120 minutes, provided that the moment of the glucose/insulin peak and the return to baseline are adequately considered [61, 105].

In the last five years, several studies have reinforced the usefulness of TTGO/OGT in the investigation of ID. In horses with induced obesity, a low-dose GO (0.25 g/kg) was able to detect early postprandial hyperinsulinemia and progressive changes in glucose–insulin dynamics during weight gain, characterizing the EMS phenotype [61]. Metabolically, the OGTT has also been used to describe changes in amino acid, lipid, and organic acid pathways in response to oral glucose loading, broadening the understanding of the pathophysiology of EMS [105, 106].

A maximal increase in plasma glucose values is observed between the periods of 90–120 minutes after glucose administration. In healthy animals, glucose concentrations should return to baseline within four to six hours of oral glucose administration. When we observe an increase in glucose concentration and the serum insulin value is greater than 60 μIU/mL, it is suggestive of reduced pancreatic function or IR [107]. The most recent recommendations of consensus groups indicate that both the oral glucose test (OGT) and the OST are dynamic tests of choice to evaluate postprandial hyperinsulinemia in horses, and specific cut-off points should be established for each protocol and assay method used [49, 108].

The test can be affected by the fasting period endured by the animal, as well as by factors such as age, diet, and stress [109–111]. Recent studies on OST/OGT in horses have shown that fasting duration, prior access to roughage, and the dose of sugar or glucose administered significantly alter insulin response and test sensitivity, necessitating strict protocol standardization [108, 112]. Furthermore, seasonal variations, transportation stress, and differences in breed and body condition influence the response to OST/TTGO, increasing the likelihood of being diagnosed with ID during certain times of the year or in stressful situations [15, 113, 114].

In donkeys, the detailed characterization of the OGTT and the oral sugar tolerance test demonstrated that the enteroinsular axis (incretins) plays an important role in the postprandial response, and that these protocols can be adapted to specific species and breeds, respecting the physiological differences between horses and donkeys [56, 115]. These data reinforce that the OGTT variant, in its different variations, continues to be a central tool for the diagnosis and monitoring of ID in horses.

2.1.3.7 Oral glucose low dose test (TBDGO)

This test can demonstrate the ability of insulin secretion, tissue sensitivity to insulin action, and the ability of the intestine to absorb an equine's glucose challenge. TBDGO is delineated by providing glucose at a dose of 0.25 g/kg orally, through the use of a syringe mixed with water or apple juice. Venous blood samples will be collected at times 0, 30, 60, 90, 120, 150, and 180 minutes after glucose administration. In recent years, low-dose TBDGO has been widely used in models of induced obesity and in the characterization of EMS, allowing early detection of postprandial hyper insulinemic responses and monitoring the progression of ID in animals subjected to high-calorie diets [61, 116–118].

Glucose and plasma insulin will be analyzed. In healthy animals, glucose will peak at 90–120 mg/dL between the collections performed at 60 and 90 minutes, and insulin should be below 20 μIU/mL after the samples collected at 60 and 90 minutes [110]. In recent studies, the area under the insulin curve and the magnitude of the insulinemic peak obtained in TBDGO showed a positive correlation with serum concentrations of cholesterol, lipoproteins, and fructosamine, as well as with mean arterial pressure, reinforcing that more prolonged and wide-ranging responses to low-dose oral glucose reflect a more severe metabolic phenotype of ID [117, 118].

Responses with insulin and glucose values far below normal may indicate a decrease in gastric emptying or impaired glucose absorption, and responses with high or prolonged glucose and insulin values may be observed in animals with pituitary dysfunction, obesity, advanced age, genetic predisposition to IR or animals with IR conditions [45, 110, 119–121]. In addition, dynamic analyses with TBDGO in horses with induced obesity demonstrated that animals with higher regional adiposity, dyslipidemia, and lamellar oxidative stress markers exhibit more intense and prolonged insulinemic responses, and these findings are consistent with a higher risk of developing endocrinopathy laminitis associated with EMS [61, 116–118].

3. Conclusions

ID in horses should be understood as a multifactorial syndrome in which postprandial hyperinsulinemia – often with normoglycemia – emerges from variable combinations of increased pancreatic secretion, reduced hepatic clearance, and impaired peripheral insulin sensitivity. This metabolic phenotype is clinically relevant because it is tightly linked to EMS and constitutes the principal endocrine driver of endocrinopathic laminitis, with vascular, oxidative, inflammatory, and hemostatic disturbances contributing to lamellar injury. From a diagnostic standpoint, no single test fully captures all components of the disorder; therefore, interpretation must integrate clinical context (obesity, regional adiposity, laminitis history, PPID risk), assay-specific considerations, and strict protocol standardization. Basal insulin and proxy indices can support screening and longitudinal monitoring, particularly in population or group settings – but their limitations at the individual level justify the use of dynamic tests whenever suspicion persists. Although the hyper insulinemic euglycemic clamp remains the reference standard for quantifying insulin sensitivity, its complexity confines it largely to research, reinforcing the practical value of field-adapted dynamic approaches such as oral glucose/sugar-based challenges and abbreviated insulin-response protocols. Ultimately, early recognition of ID, coupled with consistent monitoring and timely nutritional and management interventions, remains the most effective strategy to reduce hyperinsulinemia, mitigate cardiometabolic consequences, and prevent laminitis in at-risk equids.

Conflict of Interest

The authors declare no conflicts of interest.

Nomenclature

ACTH	Adrenocorticotropic hormone
AGEs	Advanced glycation end products
AUC	Area under the curve
β-cell	Pancreatic beta cell
BCS	Body condition score
CEACAM-1	Carcinoembryonic antigen-related cell adhesion molecule 1
CGIT/GICT	Combined glucose–insulin test
CNS	Central nervous system
EHC	Hyperinsulinemic euglycemic clamp
EMS	Equine metabolic syndrome
FSIGTT	Frequently sampled intravenous glucose tolerance test
G:I	Glucose-to-insulin ratio
GCs	Glucocorticoids
GLP-1	Glucagon-like peptide 1
GIP	Glucose-dependent insulinotropic peptide
GLUT4	Glucose transporter type 4
HOMA-IR	Homeostasis model assessment of insulin resistance
HAL	Hyperinsulinemia-associated laminitis
ID	Insulin dysregulation
IDE	Insulin-degrading enzyme
IL-6/IL-17A	Interleukins 6 and 17A
InsR-1/InsR-2	Insulin receptor substrates 1 and 2
IR	Insulin resistance
IVGTT/IVTT	Intravenous glucose tolerance test
LPL	Lipoprotein lipase
MAPK	Mitogen-activated protein kinase
MIRG	Modified insulin-to-glucose ratio
NEFA	Nonesterified fatty acids
NF-κB	Nuclear factor kappa B
OGT/OGTT/TTGO	Oral glucose tolerance test
OST	Oral sugar test
PAI-1	Plasminogen activator inhibitor-1
PZI	Porcine zinc insulin
QUICKI	Quantitative insulin sensitivity check index
RISQI	Reciprocal insulin square root index
ROS	Reactive oxygen species
SGLT2	Sodium–glucose cotransporter 2
TBDGO	Low-dose oral glucose test
TLR/TLR4	Toll-like receptor/Toll-like receptor 4
TNF-α	Tumor necrosis factor alpha
TRGI	Glycemic insulin response test/abbreviated insulin-response test
TTI/ITT	Insulin tolerance test
vWF	von Willebrand factor

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Appendices

Appendix A. Summary of dynamic tests used for insulin dysregulation (ID) diagnosis in horses.

- **Hyperinsulinemic euglycemic clamp (EHC)**: A reference method for quantifying insulin sensitivity; it is primarily used to validate other tests due to its complexity and intensive monitoring requirements.

- **Intravenous glucose tolerance test (IVGTT/FSIGTT)**: Evaluates glucose–insulin dynamics after IV glucose bolus; frequently sampled protocols allow minimal model analysis and provide robust quantification of insulin sensitivity and β-cell responsiveness.

- **Combined glucose–insulin test (CGIT/GICT)**: IV glucose bolus followed immediately by IV insulin; evaluates the duration of the "positive phase" (time glucose remains above baseline) and insulinemic responses to infer insulin sensitivity and dysregulation severity.

- **Insulin tolerance test (ITT/TTI)**: Measures the glucose fall after an IV insulin bolus as a direct indicator of tissue insulin sensitivity; includes full and abbreviated ("two-sample") versions.

- **Two-step/two-point insulin-response test (TRGI/abbreviated ITT)**: A practical field test requiring minimal sampling; it evaluates the percent decrease in glucose 30 minutes after insulin administration.

- **Oral glucose tolerance test (OGTT/TTGO)**: Oral glucose challenge via nasogastric tube (or protocol variations); assesses intestinal absorption, pancreatic response, hepatic uptake, and peripheral handling; useful for postprandial hyperinsulinemia characterization.

- **Low-dose oral glucose test (TBDGO)**: Oral glucose at 0.25 g/kg; sensitive to early postprandial hyperinsulinemia, particularly in obesity-induced models and EMS monitoring.

Appendix B. Proxy indices derived from fasting glucose and insulin (screening/monitoring tools)

- **HOMA-IR**: Index of insulin resistance, using fasting glucose and insulin.

- **RISQI**: Reciprocal of the square root of insulin; lower values suggest reduced insulin sensitivity.

- **G:I ratio**: Glucose-to-insulin ratio; low values may indicate insulin resistance.

- **MIRG**: Proxy of pancreatic β-cell responsiveness.

- **QUICKI**: Inverse log index using glucose and insulin.

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Chapter 7

Stress in Domestic Animals: The Roles of the Adrenal Chromaffin and Cortical Tissue

Colin G. Scanes and Krystyna Pierzchała-Koziec

Abstract

The adrenal glands play a critical response to stressors or danger in livestock, horses, dogs, cats, poultry, and other amniote animals. In response to stressors, the adrenal cortical cells produce glucocorticoids (cortisol in livestock, horses, dogs, and cats and corticosterone in poultry), modifying metabolism and other physiological processes. The chromaffin cells produce either epinephrine (EP) or norepinephrine (NE) in response to external and internal threats. These hormones affect effects on metabolism and blood flow. The EP- and NE-chromaffin cells are located in the adrenal medulla in mammals but distributed in poultry. Moreover, there are multiple intra-adrenal peptides, each with different roles related to stress and other physiological functions. These peptides originate from both chromaffin and cortical cells and exert local autocrine and paracrine effects. The release of Met-enkephalin is also provoked by stressors.

Keywords: adrenal, cortical cells, glucocorticoids, cortisol, corticosterone, catecholamines, epinephrine, neuropeptide Y

1. Introduction

The adrenal glands are the major peripheral organ responding to stresses and dangers. In response to stresses, the cortical cells produce and release glucocorticoids, cortisol in livestock and corticosterone in poultry. The chromaffin cells in the adrenal medulla produce and release epinephrine (EP) and norepinephrine (NE) in response to dangers. In addition, these cells release a series of other biologically active chemicals including adenosine triphosphate and neuropeptide Y (NPY).

The "classical" viewpoint is that cortical responses of the adrenal gland to stress include release of glucocorticoid hormones from cortical cells, namely, cortisol in livestock and corticosterone in poultry. This is induced by the release of corticotropin releasing hormone (CRH) and vasopressin/vasotocin from the hypothalamus stimulating the pituitary gland to release adrenocorticotropic hormone (ACTH), and this, in turn, stimulates the release of glucocorticoid hormones. Medullary chromaffin cells release the catecholamines, norepinephrine (NE) and epinephrine (EP), in response to stressors, this being controlled by nerves that pass into the adrenal glands.

Walter Bradford Cannon (1871–1945) and János Hugo Bruno "Hans" Selye (1907–1982) were two pioneers who played a pivotal role in the development of concepts of stress and adrenal functioning. Cannon developed the concepts of homeostasis and the physiological changes induced by epinephrine from the sympathetic nervous system and the adrenal medulla in "*flight or fight*" situations [1] (reviewed [2]). Selye not only provided the framework for the physiological response to external stressors in the general adaptation syndrome but also provided the role of the hypothalamo-pituitary-adrenocortical (HPA) axis in the response [3].

Functioning of the adrenal glands is more complex with cross-talk between adrenal cortical and chromaffin cells. Moreover, there are multiple intra-adrenal peptides, each with different roles related to stress and other physiological functions. These peptides originate from both chromaffin and cortical cells and exert local autocrine and paracrine effects. This chapter considers the nature, the synthesis, the actions, and the control of the medullary hormones and cortical glucocorticoid hormones. In addition, the hypothalamic control of ACTH (a.k.a. corticotropin) is discussed.

2. Overview

The adrenal gland is the primary peripheral organ responding to stresses and dangers. **Figure 1** summarizes the HPA responding stresses and dangers.

In mammals, including livestock, the adrenal glands are composed of two principal component structures:

- Medulla (middle, of ectodermal origin) producing epinephrine (EP) and norepinephrine (NE).
- Cortex (surrounding the medulla and of mesodermal origin) producing androgens, glucocorticoids, and aldosterone, with the cortex composed of three layers:
  - Zona reticularis producing the androgens, dehydroepiandrosterone (DHEA), and DHEA sulfate (DHEA-S).

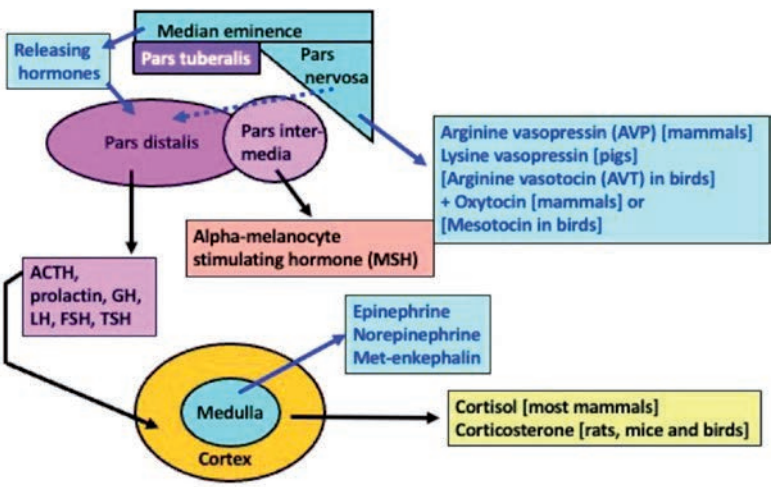


Figure 1.
Summary of the HPA responding to stresses and danger.

- Zona fasciculata producing the glucocorticoids: cortisol in cattle, sheep, pigs, guinea pigs, and humans; corticosterone in chickens and other birds together with rats and mice.

- Zona glomerulosa producing the mineralocorticoid, aldosterone.

In birds, there is intermingling of steroid-secreting cells (adrenocortical cells) and chromaffin cells.

The adrenal gland is composed primarily of two major cell types:

- Adrenal medullary cells producing EP and NE
- Adrenocortical cells producing steroid hormones.

In addition, there are other cells in the adrenal glands, namely the following:

- Endothelial cells
- Immune cells
- Sympathetic neurons.

(reviewed: [4]).

3. Adrenal medulla

3.1 Medullary secretory vesicles

Secretory vesicles in chromaffin cells contain EP and NE together with other neurotransmitters:

- EP
- NE
- Serotonin
- Dopamine
- Gamma-aminobutyric acid (GABA)
- Adenosine triphosphate (ATP)

(reviewed: [5]). Substantial amounts of ATP are stored in secretory vesicles in the chromaffin cells and can be released.

The secretory vesicles of the chromaffin cells also contain multiple biologically active neuropeptides. These include neuropeptide tyrosine (NPY), Met-enkephalin. Calcitonin gene-related peptide (CGRP), and Substance P [6]. Moreover, NPY is expressed in chromaffin cells [7].

Secretory vesicles also contain bioactive peptides including the following peptides:

- Adrenomedullin (ADM)
- Bombesin
- Calcitonin gene-related peptide (CGRP)
- Dynorphin(s)
- β-endorphin1–31
- Leu-enkephalin
- Met-enkephalin
- Natriuretic peptides - atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP).
- Neuropeptide Y (NPY)
- Neurotensin
- Secretoneurin
- Substance P
- Transforming Growth Factor-β
- Vasostatin I and II

together with an intrinsic renin-angiotensin system (reviewed [5]). The vesicles also contain chromogranins/secretogranin, namely chromogranin A (CGA), chromogranin B (secretogranin I), and secretogranin II (SgII) (reviewed [5]). In addition, chromaffin secretory vesicles contain proteases and peptidases, including the following:

- Cathepsin L processing pro-enkephalin and other neuropeptides
- Aminopeptidase B acting to remove arginine and lysine residues in the processing producing Met-enkephalin and other bioactive peptides
- Prohormone processing proteases PC1/3 and PC2 processing pro-enkephalin and other neuropeptides.
- Metallopeptidase carboxypeptidase E also contributing Met-enkephalin processing
- Tissue-type plasminogen activator.

(reviewed [5]). Parenthetically, it is noted that Met-enkephalin and NPY are found in the same secretory granules in the medullary cells [8]. This suggests that they are released in tandem.

3.2 Catecholamine synthesis and release

Figure 2 summarizes the synthesis and release of EP and NE in medullary cells.

The rate-limiting enzyme in catecholamine synthesis is tyrosine hydroxylase (**Figure 2**). EP-chromaffin cells contain phenylethanolamine N-methyltransferase, while NE-chromaffin cells lack the enzyme [5]. Shifts in synthesis of EP are achieved via increased/decreased activity and/or expression phenylethanolamine N-methyltransferase. For instance, hypoxia inducible factor (HIF)2α decreases the synthesis and, consequently, release of EP [9].

3.3 Nervous control of the medulla

3.3.1 Overview

Synthesis and release of EP and NE in medullary cells is stimulated by acetyl choline and other signaling molecules (see **Table 1**).

The release of catecholamines, neuropeptides, and other signaling molecules from chromaffin cells is controlled by terminals of the splanchnic nerve with acetyl choline acting via cholinergic nicotinic receptors, supposedly the primary neurotransmitter. Nerve terminals contain acetyl choline, NPY, substance P, CGRP, pituitary adenylate cyclase activating polypeptide (PACAP), nitric oxide synthase (NOS) [5, 6, 20], and gamma-aminobutyric acid (GABA) [21]. In addition to its metabolic role, adenosine triphosphate (ATP) is a co-transmitter with acetyl choline in splanchnic nerve terminals in the medulla (reviewed [7]).

3.3.2 Cholinergic control of the medulla

Acetyl choline acts predominantly by stimulating exocytosis and influencing the activity and/or expression of tyrosine hydroxylase, the rate limiting enzyme in

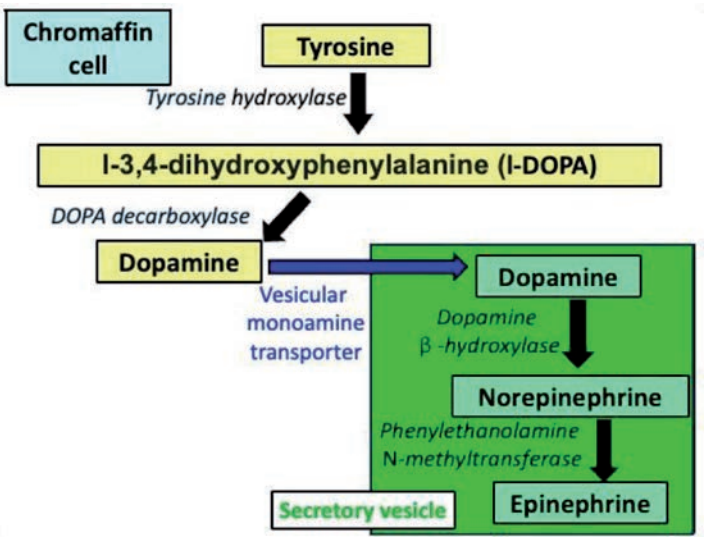


Figure 2.
Synthesis of epinephrine and norepinephrine.

Type of hormone, factor and treatment	Species	Effect	Reference
Neurotransmitters			
Acetyl choline/cholinergic agonists	Bovine	↑	[10, 11]
ATP	Bovine	↑	[12, 13]
Histamine	Bovine	↑	[10]
Gamma-aminobutyric acid (GABA)	Bovine	↑	[14]
Neuropeptides			
Bradykinin	Bovine	↑	[13]
Endothelin-1	Bovine	↑	[15]
Neuropeptide Y (NPY)	Bovine	↑[X]	[16]
Substance P	Bovine	↑	[17, 18]
Other factors			
Nitric oxide (NO)	Canine	↓	[19]

[X] *Augmenting effect of acetyl choline.*

Table 1.
Neurotransmitters and neuropeptides influencing the release of norepinephrine and/or epinephrine from chromaffin cells.

catecholamine synthesis. Stimulation of the splanchnic nerve in dogs is also followed by increased blood flow to the adrenal medulla and secretion of EP and NE, the effect being blocked by nicotinic antagonists [22].

3.3.3 NPY and adrenomedullary functioning

In mice with the NPY Y1 receptor deleted, there is increased basal release of both norepinephrine and epinephrine together with nicotine-induced epinephrine release from chromaffin cells *in vitro* [23]. However, NYP stimulated the release of both NE and EP from chromaffin cells *in vitro* [23].

3.3.4 CRHs and epinephrine/norepinephrine release

CRH stimulates the release of both EP and NE from sheep adrenal medullary tissue *in vitro* [24]. Intriguingly, AVT increases the release of EP but depresses that of NE [24]. Oxytocin decreased the release of EP and NE [24].

3.3.5 Immune cells and adrenal medullary functioning

Immune cells can influence EP release from bovine chromaffin cells [25]. Cytokines influence the release of catecholamines. For instance, based on studies with human chromaffin cells, interleukin-1β (IL-1β) stimulates catecholamine release indirectly, with IL-1β stimulating the release of NPY, which, in turn, induces release of EP and NE [26]. The effect of IL-1β is mediated by MAPK, protein kinase C (PKC), protein kinase A (PKA), and nitric oxide (NO) [26] (1).

3.3.6 Endothelial cells and adrenal functioning

Intra-adrenal endothelial cells influence chromaffin cell functioning. For instance, endothelin-1 stimulates release of EP and NE from chromaffin cells [15]. Moreover,

endogenous nitric oxide (NO) of endothelial cell origin inhibits nicotinic cholinergic stimulation of EP and NE release from canine adrenal medulla [19].

3.4 Actions of epinephrine and norepinephrine

There are major metabolic effects of catecholamines. Plasma concentrations of glucose are increased, reflecting hepatic glycogenolysis and gluconeogenesis in pigs [27] and dogs [28]. Both EP and NE increase lipolysis as reflected by increases in plasma concentrations of free fatty acids in pigs [27] and plasma concentrations of glycerol, with NE being more effective than EP in dogs [28].

There are multiple cardiovascular effects of EP and NE, including the following:

- Increased heart rate
- The vascular pressure in the small vessels in the forelimb of dogs is markedly increased by EP and NE.
- Blood pressure increased with NE being more effective than EP
- Blood flow to adipose tissue decreased with EP being more effective than NE.
- Adipose tissue vascular resistance increased with EP being more effective than NE.
- Skeletal muscle vascular resistance increased with NE being more effective than EP

in dogs [28, 29].

4. The hypothalamo-pituitary-adrenocortical (HPA) axis

4.1 Overview

Synthesis and release of cortisol (livestock) and corticosterone (poultry) is predominantly controlled by ACTH, this hormone being released from corticotropes in the anterior pituitary gland. ACTH results from the proteolytic cleavage of pro-opiomelanocortin (POMC). Other peptides derived from POMC include melanocyte-stimulating hormones (MSH) and β-endorphin.

Pituitary release of ACTH is controlled by releasing hormones, CRH and vasopressin/vasotocin together with neuropeptide W, this being summarized in **Figure 3**.

4.2 Synthesis of glucocorticoids in the zona fasciculata

The adrenocortical cells of the bovine zona fasciculata produce the glucocorticoid cortisol, but little or no aldosterone (see **Figure 4**).

Cholesterol side-chain cleavage enzyme catalyzes the limiting step in glucocorticoidogenesis, that is, conversion of cholesterol to pregnenolone.

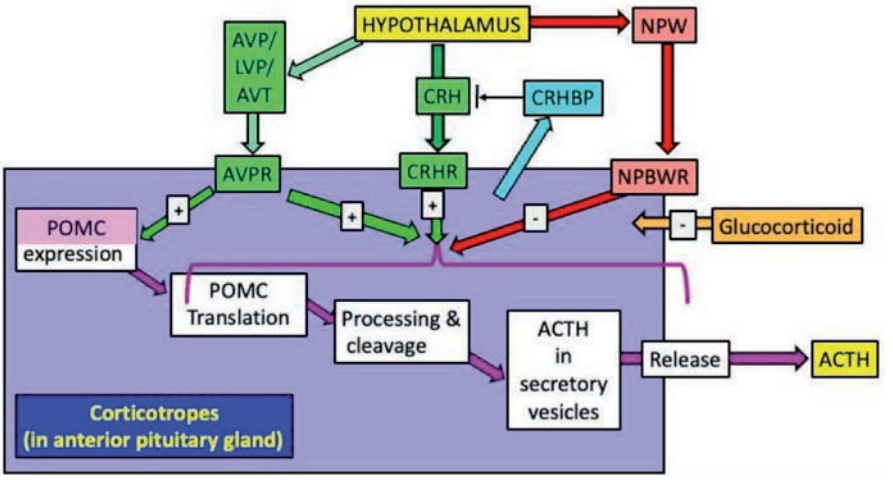


Figure 3.
Control of adrenocorticotropic hormone (ACTH) synthesis and release. [Key: AVP – arginine vasopressin, AVPR - arginine vasopressin receptor, AVT – arginine vasotocin, CRH – corticotropin releasing hormone, CRHBP - corticotropin releasing hormone binding protein, LVP – lysine vasopressin, NPBWR – neuropeptide B/W receptor NPW – neuropeptide W, POMC – proopiomelanocortin].

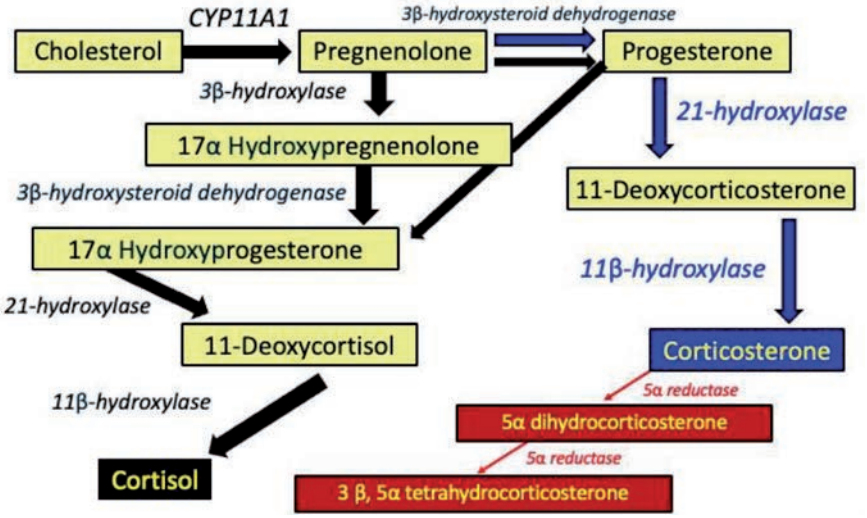


Figure 4.
Synthesis of glucocorticoids in the zona fasciculata (adrenal cortex).

4.3 HPA controlling glucocorticoid production

4.3.1 CRH and AVP controlling ACTH synthesis and release

Plasma concentrations of ACTH are controlled by CRH and AVP. These act via G protein-coupled receptors, namely CRHR1 and arginine vasopressin receptor (AVPRV1b) [a.k.a. vasopressin receptor 1b (V1b on corticotropes)]. In response to the stress of isolation and partial restriction of movement, expression of CRH and AVP in the paraventricular nucleus of the hypothalamus and of POMC and AVPRV1b

in the anterior pituitary gland are increased [30]. These effects are attenuated by intracerebroventricular (i.c.v.) infusion of the neurosteroid, allopregnanolone [30]. In addition, CRH and AVP stimulate the release of immunoreactive ACTH from pars intermedia cells in sheep [31].

AVP but not by CRF alone stimulates both the release of ACTH together with POMC expression in sheep anterior pituitary primary culture [32, 33]. Glucocorticoids decrease POMC expression [32]. Insulin challenges increase plasma concentrations of ACTH and cortisol in conscious adult sheep with increases in the concentrations of both CRH and arginine vasopressin in the hypophyseal portal blood vessels [34]. CRH stimulates the release of ACTH from anterior pituitary cells, with this effect increased in the presence of lysine vasopressin in pigs [35]. Angiotensin II did not affect the release of ACTH from pig anterior pituitary cells (pig: [35]). **Figure 5** summarizes the structure of arginine vasopressin, lysine vasopressin, and arginine vasotocin.

In non-mammalian vertebrates, there is a second CRH, namely CRH2. This is expressed in the hypothalamus and anterior pituitary gland, and like CRH1, CRH2 stimulates POMC expression in chicken [36]. In addition, a third homologous peptide, Urotensin I, binds to the chicken CRHR when they are expressed in COS-7 cells [37].

4.3.2 CRH-binding protein (CRHBP)

There is a CRH-binding protein (CRHBP) that may act as an inhibitor of the HPA axis. It is expressed in the hypothalamus, pituitary gland, gonads, and cerebrum and blocks the effects of CRH [38]. Synthetic glucocorticoid dexamethasone increases the expression of CRHBP in the pituitary gland [38]. Expression of CRHBP in the pituitary gland is also increased by stressors (withholding feed or restraint) [38].

4.3.3 Neuropeptide W and ACTH release

Neuropeptide W (NPW) inhibits ACTH release in chickens. There is expression of NPW in the hypothalamus and its receptor neuropeptide B (NPB)/NPW receptor 2 (NPBWR2) in the anterior pituitary gland [39]. NPW inhibits the stimulatory effects of CRH and/or AVT on POMC expression and plasma concentrations of ACTH [39].

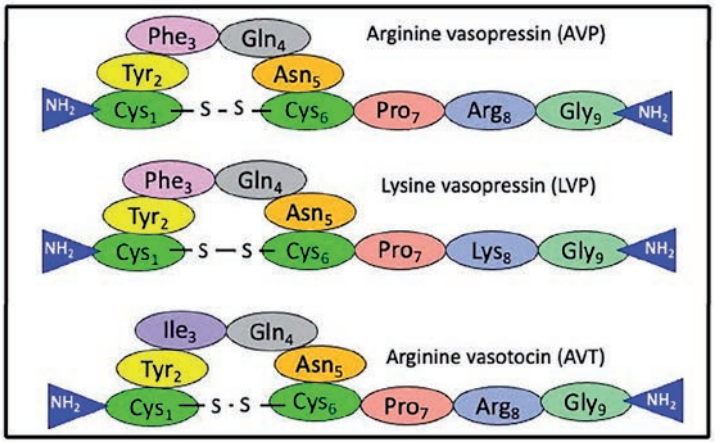


Figure 5.
Comparison of the structures of arginine vasopressin (livestock, dogs, cats and horses), lysine vasopressin (pigs), and arginine vasotocin (poultry).

The synthetic glucocorticoid, dexamethasone, increases the expression of NPBWR2 but reduces POMC expression in pituitary cells [39].

4.3.4 Stress activating the HPA axis

Plasma concentrations of corticosterone are increased by multiple stressors in chickens (**Table 2**).

Examples of HPA activating stresses in livestock include the following:

- Thermal stress [43]
- Social stress [43]
- Immune-related stress (exposure to pathogens) [43]
- Insulin induced hypoglycemia in sheep (34, 44)
- Transportation in sheep [44]
- Hypoxia in sheep [45].

Parameter	Treatment	Effect	Reference
Plasma conc. of Met-enkephalin	Restraint	↑	[40]
Plasma conc. of Met-enkephalin	Water withheld	→	[41]
Plasma conc. of Met-enkephalin	Feed withheld	↓	[41]
Plasma conc. of Met-enkephalin	Darkness	↑	[41]
Plasma conc. of Met-enkephalin	Crowding	↑	[41]
Plasma conc. of Met-enkephalin	Morphine injection	↓	[42]
PENK expression (adrenal glands)	Restraint	↑	[40]
PENK expression (adrenal glands)	Water withheld	↑	[41]
PENK expression (adrenal glands)	Darkness	↓	[41]
PENK expression (adrenal glands)	Crowding	↓	[41]
PENK expression (adrenal glands)	Morphine injection	↓	[42]
PENK expression (ant. Pit. gland)	Restraint	↑	[40]
PENK expression (ant. Pit. gland)	Water withheld	↑	[41]
PENK expression (ant. Pit. gland)	Darkness	↑	[41]
PENK expression (ant. Pit. gland)	Crowding	↑	[41]
PENK expression (ant. Pit. gland)	Morphine injection	↓	[42]
Plasma conc. of corticosterone	Restraint	↑	[40]
Plasma conc. of corticosterone	Water withheld	↑	[41]
Plasma conc. of corticosterone	Feed withheld	↑	[41]
Plasma conc. of corticosterone	Darkness	↑	[41]
Plasma conc. of corticosterone	Crowding	↑	[41]
Plasma conc. of corticosterone	Morphine injection	↑	[42]

Table 2.
Effects of multiple stresses on plasma concentrations of Met-enkephalin and corticosterone together with expression of PENK in the adrenal glands and anterior pituitary glands in chickens.

4.3.5 Other controls of glucocorticoid production

4.3.5.1 Overview

There is cross-talk between the medullary cells and adrenocortical cells. Co-culturing bovine adrenocortical cells with chromaffin cells was accompanied by increases in cortisol production [46]. During sepsis in humans, there is "*local intra-adrenal regulation of adrenal stress response*" [4]. There are multiple peptides/proteins that affect the production of cortisol (**Table 3**).

4.3.5.2 Catecholamines and cortisol production

EP stimulates both cortisol release from bovine adrenal cells and expression of cholesterol side-chain cleavage cytochrome P450, 17 alpha-hydroxylase cytochrome P450, 21-hydroxylase cytochrome P450, and 11 beta-hydroxylase cytochrome P450 [52, 53].

Type of hormone, factor, and treatment	Species	Effect	Reference
Peripheral hormones			
Adrenocorticotropic hormone (ACTH)	Cattle	↑	[47]
Angiotensin-II	Cattle	↑	[47, 48]
β-endorphin	Cattle	→	[49]
Intra-adrenal peptides and hormones			
Adrenomedullin	Human	↑	[50, 51]
Epinephrine	Cattle	↑	[52, 53]
Neuropeptide Y (NPY)	Human	↓	[54]
Cytokines			
Interleukin (IL)-3	Cattle	↑	[55]
IL-4	Cattle	↑	[56, 57]
IL-6	Cattle	↑	[55, 58]
Leukemia inhibitory factor (LIF)	Cattle	↑	[56, 57]
Tumor necrosis factor-alpha (TNF-α)	Cattle	↓	[58]
Growth factors			
Epidermal growth factor (EGF)	Sheep	↑	[59]
Insulin-like growth factor I (IGF-I)	Cattle	↑	[60, 61]
IGF-II	Cattle	↑	[61]
Transforming growth factor beta 1 (TGF-β1)	Cattle	↓	[62]
Releasing hormones			
Corticotropin-releasing hormone (CRH)	Cattle	↑	[63]
Somatostatin	Cattle	→	[49]

Table 3.
Effect of hormones, peptides, cytokines, and growth factors on cortisol production by adrenocortical cells in vitro.

4.3.5.3 Neuropeptide Y and adrenocortical functioning

Neuropeptide Y (NPY) influences cortisol production. Plasma concentrations of cortisol are depressed by NPY *in vivo* and from adrenal slices containing both cortical and medullary tissue cells but not from adrenocortical cells [64]. Based on the ability of a β-adrenergic antagonist to block the effect of NPY, this was interpreted as NPY stimulating chromaffin cells to release EP, which, in turn, depressed cortisol production [64].

4.3.5.4 Other peptides and other intra-adrenal signaling molecules and glucocorticoid synthesis

Chromaffin-produced peptides affect synthesis and release of glucocorticoids. For instance, both ADM and CGRP enhance corticosterone release from rat adrenal glands by increasing blood flow to the adrenal gland, essentially mitigating the mass action inhibitory effect [50].

4.3.5.5 Hormones and other signaling molecules and glucocorticoid synthesis

There are other effects of other hormones, peptides, and prostaglandins on corticosterone production by adrenocortical cells *in vitro* based on studies in chickens and rats (**Table 4**). Thyroid hormones inhibit the synthesis of corticosteroid, at least in chickens [71]. Prolactin suppresses intra-adrenal metabolism of corticosterone in chicken adrenal cells [72]. Moreover, GH *in vivo* increases in young chickens [73]. In chickens, the intestinal mucosa inactivates corticosterone via 11β-hydroxysteroid dehydrogenase and 20β-hydroxysteroid dehydrogenase [74].

4.3.5.5.1 Growth factors and glucosteroidogenesis

Adrenal cortical functioning is influenced by growth factors, with TGF-β1 decreasing 17α-hydroxylase activity and expression in adrenal cells *in vitro* [62].

4.3.5.5.2 Cytokines (immune cells) and adrenal cortical functioning

Cytokines influence adrenal cortical functioning. For instance, cortisol production by bovine adrenal cells is increased by either interleukin (IL)-3 or IL-6, these acting, respectively, via the lipoxygenase and cyclo-oxygenase pathways [55, 58].

Species/hormone and/or peptide	Direction of change	Reference
Rat		
Adrenomedullin	↓	[65]
Adrenomedullin	↑	[50]
Calcitonin gene-related peptide (CGRP)	↑	[50]
Chicken		
Calcitonin	↓	[66]
Parathyroid hormone.	↑	[67, 68]
Parathyroid hormone-related peptide	↓	[69]
Prostaglandins (PG) - PGA1, PGB1, PGB2, PGE1, PGE2 and PGF2α	↑	[70]

Table 4.
Effect of hormones, peptides, and prostaglandins on corticosterone production by adrenocortical cells in vitro.

Another example of cytokines influencing adrenal cortical functioning is tumor necrosis factor-alpha (TNF-α), this inhibiting both basal and ACTH stimulated cortisol production from bovine zone fasciculata cells [58]. Immunoreactive TNF-α is detected in most of the cells of the bovine adrenal cortex and some in the medulla [75]. ACTH decreased the release of TNF-α from cortical cells but did not affect medullary cells [75].

4.4 Other sources of hormones in the HPA axis

Chicken leukocytes have been reported to release ACTH, with stimulation enhanced in the presence of CRH [76]. Glucocorticoids are synthesized by placenta in sheep (cortisol) [77], cumulus-oocyte complex in cattle (cortisol) [78], endometrium of uterus in cattle [79], and thymus and bursa Fabricius in chickens (corticosterone) [80].

4.5 Actions of glucocorticoids

The primary metabolic effects of glucocorticoids are to enhance breakdown of proteins employing amino-acids to synthesize glucose and increase plasma concentrations of glucose by the process of gluconeogenesis. Moreover, glucocorticoids increase lipolysis, releasing fatty acids and glycerol from adipose tissue and consequently increasing plasma concentrations of free fatty acids. Treatment with the synthetic glucocorticoid, dexamethasone, decreased nitrogen retention, essentially protein synthesis minus degradation and subsequently deamidation of amino-acids, and increased excretion of nitrogen (*i.e.* as urea) in pigs, there being concomitant increases in urinary concentrations of urea [81]. Corticosterone is reported to increase gluconeogenesis and proteolysis in young broiler chickens [82]. Moreover, administration of corticosterone to young broiler chickens was followed by increased plasma concentrations of glucose [83]. In addition, corticosterone administration increased circulating concentrations of free fatty acids together with those of triiodothyronine and NE but did not affect those of glucose (laying hen: [84]). There are chronic effects of glucocorticoids on gastrointestinal functioning, with, in pigs, the administration of cortisol increasing both pancreatic and intestinal amylase [85]. Glucocorticoids increase hepatic glutamic oxaloacetic transaminase activity (pigs: [85]). In contrast, corticosterone administration increases blood concentrations of total protein and albumin (broiler chickens) [86].

Glucocorticoid administration can depress growth such as is the case with cortisol in fetal sheep [87] and corticosterone in broiler chickens [86]. Moreover, dexamethasone *in vivo* decreases circulating concentrations of the growth-related hormone, insulin-like growth factor I (IGF-I), in pigs [81].

Glucocorticoids act to reduce immune functioning. For instance, cortisol administration to pigs is accompanied by decreases in the number of cells per unit volume of blood, including the following: lymphocytes, eosinophils, B cells, T-cells, and increases in neutrophil numbers per unit volume [88]. Similarly, *in vivo*, corticosterone decreases the weights of the two primary immune organs, namely the bursa Fabricius and thymus weights in broiler chickens [86]. Moreover, *in vivo* corticosterone decreases duodenal and ileal expression of toll-like receptor 5 (TLR5) [86].

5. Met-enkephalin and other opioid peptides

5.1 Overview of Met-enkephalin

Other neuropeptides are released from adrenal cells. Examples include Met-enkephalin. This is a pentapeptide with a sequence of methionine- tyrosine–glycine-glycine–phenylalanine (MYGGF) resulting from proteolytic cleavage of pro-enkephalin (PENK) (reviewed [89]). Met-enkephalin is produced in and released from multiple tissues, including the hypothalamus, anterior pituitary gland, and adrenal glands (reviewed [89]). In addition to Met-enkephalin, the proteolytic cleavage of PENK generates Leu-Enkephalin and a series of peptides containing Met-enkephalin motifs.

5.2 Control of Met-enkephalin release and synthesis

Release of Met-enkephalin from chromaffin cells is controlled by splanchnic nerve terminals releasing acetyl choline locally. There is evidence that adrenal medullary synthesis of NPY and Met-enkephalin are not tightly linked. In rats, administration of monoclonal antibodies to acetyl cholinesterase was followed by 10-fold increase in adrenal Met-enkephalin immunoreactivity (including peptides containing Met-enkephalin motifs) but only a modest (50%) increase in NPY immunoreactivity [90]. The former was blocked by an opioid antagonist and the latter by the addition of muscarinic and nicotinic receptor antagonists [90].

There is tonic inhibition of PENK expression and probably Met-enkephalin synthesis and release via splanchnic innervation of the adrenal medulla. Following unilateral splanchnicectomy in rats, there are large increases in PENK expression and the Met-enkephalin containing peptides [91].

There is evidence also for opioid inhibition of Met-enkephalin release from and synthesis in both the adrenal and pituitary glands. The effects of isolation stress on Met-enkephalin release for either pituitary or adrenocortical tissues are reversed in lambs injected with the opioid antagonist, naltrexone [92]. Similarly, naltrexone attenuates stress-induced shifts in expression of PENK in stressed young chickens [40, 41].

5.3 Stress and Met-enkephalin

Isolation stress is accompanied by decreases in the pituitary and adrenocortical concentrations of Met-enkephalin young sheep [92]. While plasma concentrations of corticosterone are consistently increased by multiple stressors in chickens (**Table 2**), both plasma concentrations of Met-enkephalin and PENK expression in the anterior pituitary gland and adrenal glands exhibit differences depending on the individual stressor (**Table 2**). In addition, plasma concentrations of corticosterone are also increased by lipopolysaccharide [93]. Hypoxia in fetal sheep was followed by increases in circulating concentrations of large peptides containing Met-enkephalin motifs but did not affect Met-enkephalin itself [94].

6. β-endorphin

Another POMC-derived peptide, β-endorphin, is from the anterior pituitary gland in stressed livestock. For instance, circulating concentrations of β-endorphin are increased in sheep by the following:

- Acute hemorrhagic stress [95]
- Tail docking [95]
- Castration [96]
- Insulin induced hypoglycemia [97].

Parenthetically, β-endorphin and N-acetyl-endorphin have also been demonstrated to be present in the rat thyroid [98].

7. Conclusions

The role and functioning of the adrenal glands in response to stress has been established for over 50 years. In livestock species as in other mammals, the adrenal glands have two principal components: the medulla containing chromaffin cells that release the catecholamine hormones, EP and NE, and the cortex with cortical cells producing glucocorticoids (cortisol in livestock and corticosterone in poultry). In contrast, chromaffin and cortical cells are distributed throughout the adrenal glands in poultry. It is presumed that the chromaffin cells are controlled by splanchnic nerve terminals, and the cortical cells' production of glucocorticoids is controlled by the anterior pituitary hormone ACTH. In turn, the release of ACTH is controlled by hypothalamic neuropeptides, CRH and vasopressin/vasotocin.

This communication contends that the situation is considerably more complex, with cross-talk between cortical and chromaffin cells and multiple locally produced neuropeptides, paracrine factors, cytokines, and hormones that influence the release of cortisol and other glucocorticoids, EP and NE. Adrenal cells are a significant source of the endogenous opioid Met-enkephalin and of neuropeptide Y. There is much that remains to be discovered about the functioning of the adrenal glands.

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Chapter 8

Small Animal Thoracoscopy

Yusuf Şen

Abstract

Thoracoscopy is a minimally invasive surgical technique that allows direct visualization, diagnosis, and treatment of various thoracic disorders in small animal patients. This chapter provides a comprehensive overview of the indications, equipment, patient preparation, and operative techniques associated with thoracoscopy in dogs and cats. Thoracoscopic procedures such as partial pericardiectomy, thoracic duct ligation for chylothorax, and lung lobectomy have become routine alternatives to traditional thoracotomy, providing reduced postoperative pain, shorter recovery times, and improved visualization of intrathoracic structures. The paracostal, transdiaphragmatic, and intercostal approaches are described in detail, with emphasis on patient positioning, anesthesia management, and safe trocar placement. Common thoracoscopic findings include pleural effusion, pleural thickening, neoplasia, pneumothorax, and diaphragmatic hernia. Complications such as hemorrhage, pneumothorax, and organ injury remain rare when proper technique and visualization are maintained. Recent advances in thoracoscopic instrumentation, imaging, and perioperative management have significantly expanded its diagnostic and therapeutic applications in veterinary thoracic surgery, establishing thoracoscopy as an essential component of minimally invasive veterinary practice.

Keywords: thoracoscopy, minimally invasive surgery, small animal, VATS, endoscopic surgery

1. Introduction

Thoracoscopy is defined as a minimally invasive operative endoscopic technique used to visualize the thoracic cavity and thoracic structures [1, 2]. Thoracoscopy is a highly useful, minimally painful diagnostic and therapeutic method in the diagnosis of thoracic diseases. Through thoracoscopy, conditions such as pleural effusion, pericardial effusion, intrathoracic tumors, pneumothorax, primary pulmonary diseases, and thoracic trauma can be diagnosed [3].

Due to its minimally invasive nature, thoracoscopy allows examination of the thoracic cavity and the performance of surgical procedures through 5–10-mm incisions without the need for painful and traumatic thoracotomy [2]. Visualization of intrathoracic structures and pathological changes is significantly superior to open surgery because of the advantages of high-intensity light transmission providing excellent illumination, telescopic magnification, and the ability to advance the telescope into regions otherwise inaccessible during conventional surgery [4].

Even very small lesions that cannot be visualized during thoracotomy can be easily detected through thoracoscopy. Structures that are difficult to see with the naked eye become visible and magnified, allowing precise dissection, hemostasis, and preservation of vital anatomical elements. As a result, the patient's operative stress, tissue trauma, and surgical duration are minimized [5].

Thoracoscopy also enables easy sampling for histopathological, microbiological, and cytological examinations [4]. Initially, thoracoscopy was used solely as a diagnostic technique; however, it has later evolved into an operative method applied in small animal surgery. Recently, thoracoscopic surgical procedures have included pericardial window formation, subtotal pericardiectomy, partial and complete lobectomy of lung lobes, removal of foreign bodies, resection of mediastinal masses, lymph node extirpation, thoracic duct occlusion, and division of persistent right aortic arch [6–8].

Although there are certain differences between laparoscopy and thoracoscopy, they share substantial similarities in technique, equipment requirements, and fundamental applications [2, 9].

2. Indications and applications of thoracoscopy

Thoracoscopy can be employed in situations where further diagnostic information is required for thoracic diseases, in the management of intrathoracic pathological formations, or when the patient's condition renders thoracotomy undesirable or contraindicated. Diagnostic thoracoscopy is indicated for pulmonary, mediastinal, or hilar masses; primary pulmonary diseases; spontaneous pneumothorax; pericardial effusions; traumatic assessment of the thoracic cavity; and pleural effusions [4, 10]. It can also be used as a guide in orthopedic procedures such as pectus excavatum repair and rib fracture stabilization [11, 12].

Numerous thoracoscopic surgical applications—such as lung lobectomies [13], diaphragmatic repairs [14], cardiomyoplasty [15], patent ductus arteriosus occlusion [16], thoracic duct ligations [17], persistent aortic arch ligations [6, 8], and laser-assisted operations—have been developed and are now routinely performed in cats and dogs [5, 18, 19].

The management of pleural effusion can be achieved successfully through thoracoscopy, which represents one of the most common applications of this technique in human medicine [4]. Biopsy specimens obtained thoracoscopically allow accurate diagnosis and prognostic evaluation of diseases. Very small inflammatory nodules, early metastatic lesions, and mesotheliomas can be identified *via* thoracoscopy even before they reach sizes visible during open surgery [2].

Thoracoscopy enables biopsy sampling from tumors, assessment of their resectability, and acquisition of detailed information regarding tumor localization and extension. The technique is also used to confirm the diagnosis of lung lobe torsion [3, 20].

In cases of chylothorax, thoracic duct occlusion can be performed using thoracoscopic techniques [13, 17, 21]. In pericardial effusions, thoracoscopy-guided drainage may be performed, and fluid samples can be collected for cytologic, microbiologic, and histopathologic evaluation. A pericardial window can be created through partial pericardiectomy to enable continuous drainage of the pericardial effusion [2, 22, 23].

For the diagnosis and management of spontaneous pneumothorax, thoracoscopy can be used to precisely localize air leakage sites [24]. It also facilitates the evaluation of emphysematous pulmonary degeneration, determining whether the lesions are focal or diffuse and whether multiple lung lobes are involved [3].

By determining whether a neoplastic process is surgically resectable, thoracoscopy helps avoid unnecessary thoracotomy procedures in patients [10].

3. Thoracoscopic system and equipment

The system and equipment required for thoracoscopy are essentially the same as those used for laparoscopy. The basic components of thoracoscopic instrumentation include a surgical telescope, a trocar-cannula unit, a light source, a standard endoscopic set, biopsy collection tools, and thoracoscopic surgical instruments [7].

Virtually any type of rigid telescope can be used for thoracoscopy. The most frequently utilized telescopes are those with diameters ranging from 5 to 10 mm, and the 2.7-mm multi-purpose rigid telescopes. For cats and small dogs, the 2.7-mm telescopes are the most appropriate, whereas 5 -mm laparoscopes are preferred for medium and large dogs, and 10-mm telescopes are reserved for very large dogs. Smaller telescopes transmit less light and therefore are not practical for use in large animals [25].

The field of view of thoracoscopic telescopes may vary between 0° and more than 90°. Telescopes with a 0° angle minimize image distortion and help the operator maintain orientation more effectively. Angled telescopes, on the other hand, provide the advantage of visualizing structures located outside the direct optical axis. A 30° telescope is most commonly used, as it facilitates manipulation within the thoracic cavity where the ribs may otherwise restrict telescope positioning. Viewing angles greater than 30° are not recommended, as they often result in significant loss of orientation [13].

Cannulas used in thoracoscopy differ from those employed in laparoscopy. Since a gas-tight seal is not required for thoracoscopy, airtight cannulas are unnecessary. Furthermore, the distance between the thoracic wall and intrathoracic organs is usually shorter than that between the abdominal wall and intra-abdominal organs. Cannulas serve to protect the telescope, establish pneumothorax, and maintain the thoracoscopic portals. Blunt obturators are suitable for most cannulas and are recommended for entry, as they minimize the risk of trauma to thoracic organs during insertion. Before introducing the trocar into the thoracic cavity, creating positive pressure by introducing a Veress needle prevents organ injury during trocar insertion [3].

A xenon light source is used in thoracoscopy, as halogen lamps fail to provide adequate illumination. A flexible fiberoptic light cable connects the telescope to the light source [13].

The basic thoracoscopic set includes biopsy forceps designed for thoracoscopy, grasping forceps, scissors, palpation probes, pretied ligature loops, and aspiration-irrigation cannulas, either separate or combined. This equipment allows the collection of multiple samples and can be employed in minor surgical interventions. Solid tissue and lung biopsies can be obtained for histology and impression cytology. Collected fluid samples are used for fluid analysis, cytology, and bacterial or fungal culture. These tools and tru-cut biopsy needles are used for specimen retrieval, while long spinal needles are applied for fine-needle aspiration [2].

For thoracoscopic surgery, pretied ligature loops, clip applicators, tissue retractors, linear endoscopic gastrointestinal anastomosis (GIA) staplers, lasers, monopolar and bipolar electrocautery devices, computerized radiofrequency generators, and harmonic scalpels can be used [4].

Markers like indocyanine green, combined with near-infrared camera systems, allow for a much more detailed assessment of the thorax and make surgical procedures safer [19, 26]. Furthermore, with specialized systems such as the Magnetic Anchoring and Guidance System (MAGS), comfortable surgical procedures can be performed using the Magnetic Anchor Technique (MAT) [27].

4. Patient preparation

Patient preparation for thoracoscopy is essentially similar to that for thoracotomy. The surgical site is clipped, asepsis is established, and the area is isolated using sterile drapes. The thoracic wall area prepared for thoracoscopy should be as wide as, or even wider than, that used for open thoracotomy [13].

The patient's position is determined based on the location of the lesion, the region of the thorax to be examined, and the surgical approach planned. Dorsal or lateral recumbency (either right or left) can be used [9].

5. Anesthesia and pneumothorax technique

Before anesthesia, particularly in geriatric patients, blood values, urinalysis, and electrocardiographic measurements should be obtained. In cases of pleural effusion, thoracentesis should be performed and thoracic radiographs obtained prior to thoracoscopy to reduce the risk of anesthesia-related complications [28, 29].

Mechanical ventilation is required during thoracoscopy. The preanesthetic agents, induction drugs, and maintenance anesthetics are the same as those used for thoracotomy. The anesthetic protocol includes the administration of butorphanol and subcutaneous glycopyrrolate as preanesthetic medication, induction with intravenous propofol or mask induction with isoflurane, and maintenance with isoflurane anesthesia [13].

A pneumothorax must be created to enable visualization and manipulation during thoracoscopy. Positive-pressure insufflation is generally not used. Prior to inserting the trocar into the thoracic cavity, a Veress needle is introduced to generate positive pressure, preventing potential organ injury during trocar placement [29].

6. Establishment of thoracoscopic entry sites

Two main approaches are used to access the thoracic cavity during thoracoscopy: the paraxiphoid-transdiaphragmatic and intercostal techniques (**Figure 1**) [10, 29, 30].

These two techniques have comparable advantages and disadvantages. The choice of approach depends not on the superiority of one technique over the other, but on the specific requirements of each case and the surgeon's preference [4].

6.1 Paraxiphoid-transdiaphragmatic telescope port sites

For paraxiphoid entry, the patient is positioned in dorsal recumbency or, alternatively, the sternum is tilted to achieve a ventrodorsal oblique position. The telescope entry site is located by palpating the region between the xiphoid cartilage and costal arch. A skin incision large enough to allow the passage of a cannula is made either to the right or to the left of the xiphoid process. The cannula is then advanced through this area using a blunt obturator [2, 29].

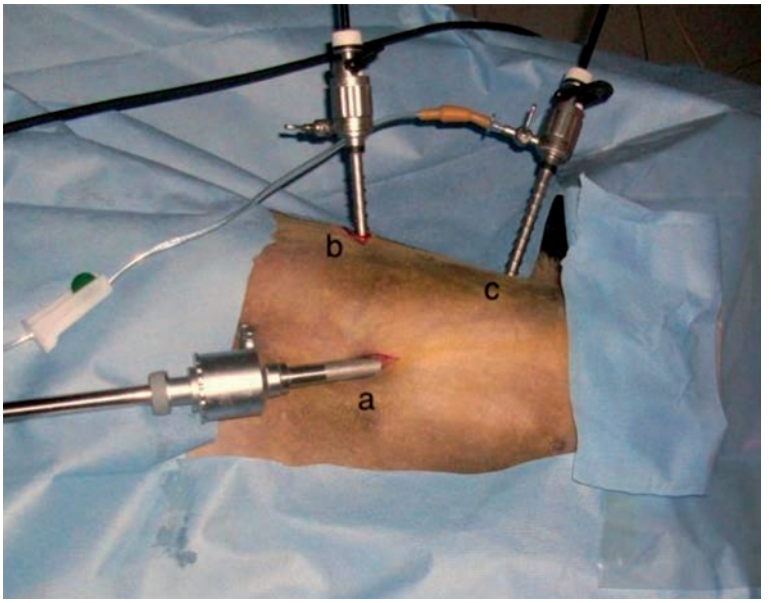


Figure 1.
Thoracoscopic paraxiphoid-transdiaphragmatic (a) and intercostal port sites (b, c).

6.2 Intercostal telescope port sites

The selected entry site should not be directly over the lesion but close enough to permit optimal visualization. Biopsy and other sampling instruments can be placed directly over the lesion following the principle of triangulation [4, 31].

On the lateral thoracic wall, the port is typically established through blunt dissection. A short (2–3 cm) skin incision is made, and blunt dissection is performed with hemostatic forceps. The telescope cannula—either with a blunt obturator or with a guarded trocar—is advanced along the thoracic wall [9]. Once the cannula reaches the pleural space, the obturator is removed, pneumothorax is established, and the telescope is inserted. This is considered a highly safe and reliable technique.

7. Closure of entry sites and pleural cavity management

During closure of the thoracoscopic entry sites, free air within the pleural cavity can be controlled by two primary methods [2].

In cases where only pleural effusion has been aspirated or solid tissue biopsy samples collected, and there is no evidence of air leakage or hemorrhage, the lungs are reinflated under intermittent positive pressure while observing lung expansion through the telescope. Once adequate inflation is achieved, the telescope and cannula are withdrawn, and the incision site is closed [3, 4, 9].

The second technique involves placement of a chest tube through the telescope cannula. The pleural drainage tube is advanced into the thoracic cavity *via* the existing portal, after which the cannula is removed, leaving the tube in place. The thoracic wall layers around the tube are then sutured. Underwater drainage is continued until no air or fluid is observed exiting the tube. In most cases, the chest tube is removed as the patient is extubated or as anesthesia is discontinued. The paraxiphoid portal is

particularly suitable for this method, and chest tubes placed through this site following thoracoscopic pericardial procedures can remain in place for up to 24 hours [10, 29].

In cases of significant thoracic disease with pleural effusion, hemorrhage, or air leakage risk, pleural lavage and extended postoperative drainage may be required. A chest tube should therefore be maintained for several days to allow removal of reaccumulating air or fluid [31].

8. Thoracic pathology

Abnormalities that can be identified by thoracoscopy in cats and dogs include primary and metastatic neoplasms, cranial mediastinal and hilar lymph node enlargement, pericardial effusion and pericarditis, pleuritis (**Figure 2**) ruptured or intact emphysematous bullae, migrating foreign bodies, pulmonary lacerations and contusions, thoracic duct disorders, inflammatory and neoplastic pleural lesions, and diaphragmatic hernias [4].

8.1 Neoplasia

Thoracoscopy plays a major role in determining the stage of disease and evaluating whether surgical excision of an intrathoracic neoplasm is indicated [13, 32–34]. Small, superficial pulmonary and pleuralmasses can be visualized directly (**Figure 3**), whereas deeper parenchymal masses may be identified through associated parenchymal alterations.

Since intrapulmonary nodules are often small and deep, visualization and biopsy sampling may be limited. However, thoracoscopy allows the assessment of lesion location, size, number, and surface morphology. Pleural lesions are better evaluated thoracoscopically than with any other technique. Using optical magnification, small

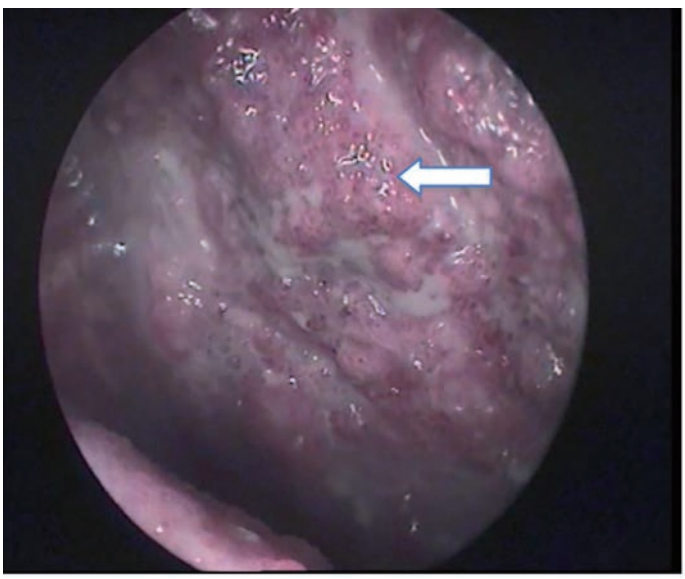


Figure 2.
Pleural thickening/pleuritis in a cat with chylothorax.

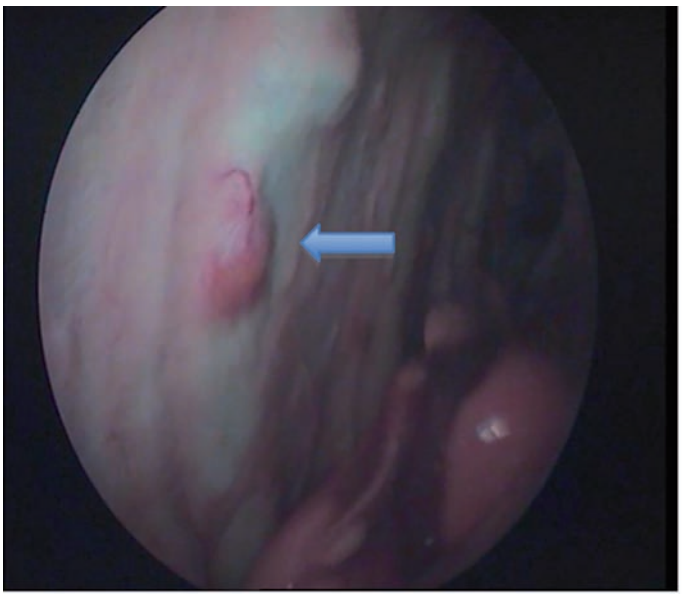


Figure 3.
A mass lesion observed on the parietal pleura in a 10-year-old cat.

pleural or pericardial nodules and plaques can be visualized and accurately biopsied, thereby increasing diagnostic precision [35].

Recent studies confirm the high diagnostic yield of video-assisted thoracoscopic biopsy (VATS) for thoracic neoplasia, with a reported diagnostic accuracy exceeding 95% in small animals [32].

8.2 Pneumothorax

Thoracoscopy allows accurate assessment of spontaneous, traumatic, or foreign body-induced secondary pneumothorax [36–38]. In small animals, trauma is the most common cause of pneumothorax, and in most cases, it may resolve spontaneously without surgical intervention [39].

However, in trauma cases with diffuse or persistent air leakage, pleural drainage or surgical repair may be required. Thoracoscopic techniques are preferred in such patients to minimize additional tissue trauma [37].

Ruptured emphysematous bullae are among the most frequent causes of spontaneous pneumothorax [24, 40]. One of the most challenging aspects of spontaneous pneumothorax management is identifying the air leak before surgical intervention. Thoracoscopy enables direct localization of the air leakage and identification of its cause.

Bullae appear as air-filled, translucent, or collapsed protrusions from the lung surface. Filling the pleural cavity with warm saline through a second portal allows visualization of air bubbles, thereby facilitating lesion localization. Thoracoscopic stapling or sealing of the leaking site provides an effective, minimally invasive alternative to open thoracotomy [4, 9].

Foreign-body-induced adhesions, exudate accumulation, and local inflammation around penetration sites can also be evaluated thoracoscopically, and pathological pulmonary regions can be resected or sealed under direct visualization.

8.3 Hilar lymph node enlargement

Enlarged hilar lymph nodes are visible during thoracoscopy as structures located between partially collapsed lung lobes. Their appearance may range from mildly enlarged to grossly distorted neoplastic masses. Biopsy samples can be obtained for histopathology, or complete excision of the lymph node may be performed thoracoscopically [4]. Now, with markers like indocyanine green and specialized camera systems, lymph nodes can be evaluated in much greater detail [26].

Recent reports emphasize the usefulness of thoracoscopic lymphadenectomy for staging pulmonary neoplasia and systemic diseases in dogs and cats [26].

8.4 Pleural effusion

Thoracoscopy remains one of the most valuable tools for evaluating pleural effusion [2, 15]. Pleural fluid and biopsy samples can be collected for cytology, biochemical analysis, and culture. In neoplastic secondary pleural effusions, thoracoscopy can achieve diagnostic accuracy approaching 100% (**Figure 4**).

The pleural fluid location can be distinguished from solid masses, and adhesions can be identified and removed under direct visualization to improve fluid evacuation [9].

Thoracoscopic visualization also allows targeted biopsy of pleural lesions, providing higher diagnostic yield compared with blind needle sampling [4].

8.5 Pericardial effusion

Thoracoscopy enables the identification of pericardial effusion and the underlying intrapericardial lesions responsible for it. The pericardium may be catheterized, or a drain can be inserted [30]. A rectangular pericardial window approximately

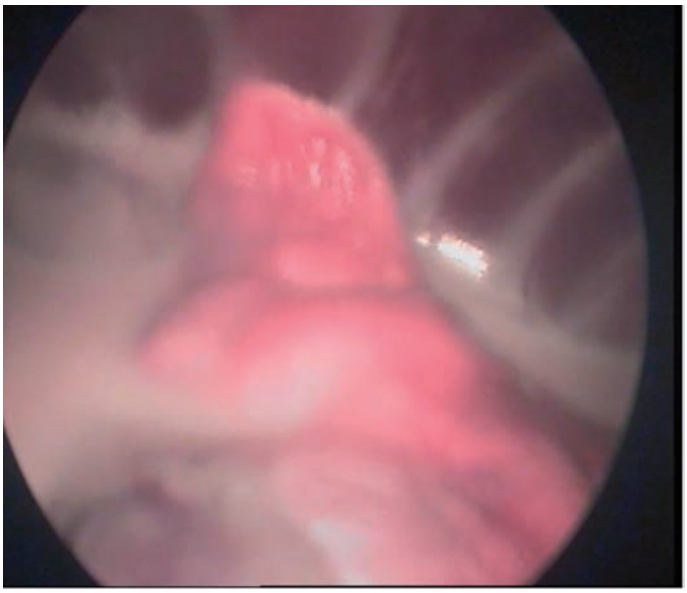


Figure 4.
Thoracoscopic visualization of pleural effusion secondary to chylothorax in a feline patient.

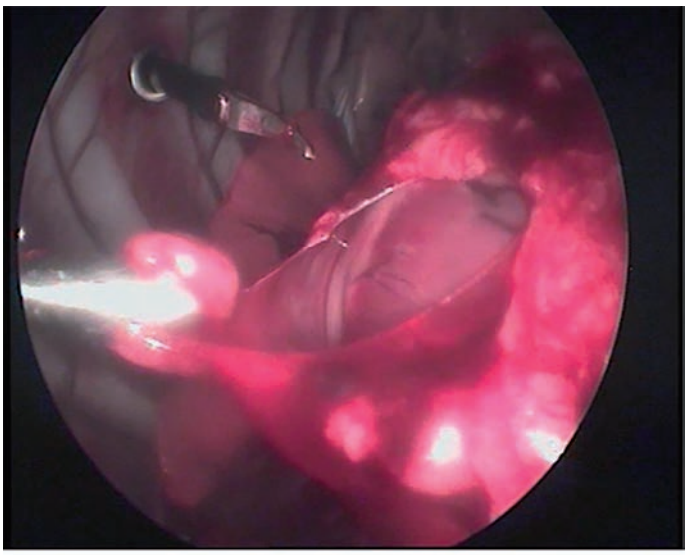


Figure 5.
Thoracoscopic partial pericardiectomy performed in a Pomeranian dog.

2–3 cm wide can be excised from the anterior pericardial surface to facilitate continuous drainage and prevent recurrence of fluid accumulation and cardiac tamponade (**Figure 5**) [9, 41].

Excised pericardial tissue can also be submitted for biopsy or microbial culture. Pericardioscopy allows direct visualization and biopsy of intrapericardial masses. Modern endoscopic energy systems (harmonic and bipolar sealing) further enhance surgical control and safety [9].

8.6 Diaphragmatic hernia

The diaphragm can be thoroughly visualized thoracoscopically, allowing full evaluation of the diaphragmatic surface. Soft-tissue densities in the caudal mediastinum can be examined to identify the herniated structures (**Figure 6**) [4].

Small diaphragmatic hernias can also be repaired thoracoscopically. Thoracoscopic herniorrhaphy reduces postoperative pain and recovery time compared to traditional open repair [14].

8.7 Primary pulmonary diseases

Thoracoscopy allows detailed assessment of pulmonary lobar parenchyma. Biopsies for the diagnosis of diffuse pulmonary disease can be collected using biopsy forceps, Tru-Cut needles, endoscopic staplers, or pretied ligature-assisted apical resections [35].

Thoracoscopy is indicated for performing partial or complete lung lobectomy, diagnosing refractory pneumonia, pulmonary abscesses, and primary lung tumors, and conducting therapeutic resections [42, 43].

The use of thoracoscopy in veterinary pulmonary medicine continues to expand, offering minimally invasive diagnostic and therapeutic advantages similar to those in human thoracic surgery [13].

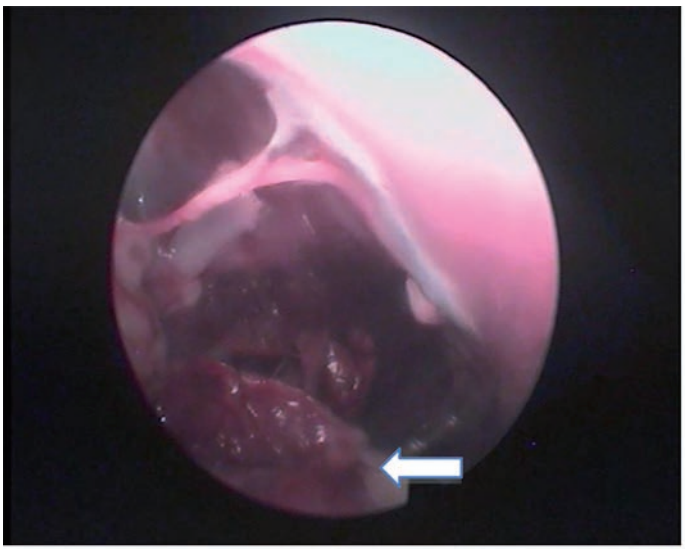


Figure 6.
Thoracoscopic appearance of a hepatic lobe herniating into the thoracic cavity in a cat with diaphragmatic hernia.

9. Contraindications of thoracoscopy

The main limitations in the use of thoracoscopy arise from instrumental constraints, such as suboptimal equipment, lack of adequate instruments for vascular control, and the inherent absence of a true three-dimensional perspective [4]. In cases of clotted hemothorax, adequate visualization cannot be achieved.

Other contraindications include pleural adhesions, hypoxemia, various forms of cardiac instability, and coagulopathies [44]. In such cases, the risks of anesthesia and iatrogenic injury outweigh the diagnostic benefits.

Recent reports emphasize that thoracoscopy should be avoided in patients with severe pulmonary hypertension, uncontrolled coagulopathy, or significant pleural fibrosis, as visualization and safe manipulation are compromised [7].

10. Complications of thoracoscopy

When performed correctly, thoracoscopy is associated with a low complication rate. Potential complications include pneumothorax, lung, cardiac, or great-vessel injury during placement of the Veress needle or trocar, lung laceration, esophageal perforation, or tracheal injury caused by trocar advancement [5, 43, 45].

Additional complications may involve cardiorespiratory disturbances or gas embolism resulting from excessive insufflation pressures. Other non-insufflation-related complications include electrosurgical injuries, hemorrhage, air leakage at entry sites, nerve trauma, and incomplete visualization of anatomical structures [2, 44].

Intraoperative bleeding is uncommon but may result from intercostal vessel trauma or pulmonary parenchymal laceration. Postoperative pain, subcutaneous emphysema, and transient hypoxemia can occur but usually resolve with supportive care [4].

Thoracoscopic complications are significantly less frequent and less severe than those associated with open thoracotomy, particularly with the use of modern blunt-tip trocars, pressure-controlled insufflators, and improved visualization systems [4].

11. Concluding remarks

Thoracoscopy has become an essential minimally invasive surgical technique in small animal practice, offering diagnostic and therapeutic advantages that have significantly transformed thoracic surgery. Compared with traditional thoracotomy, thoracoscopy minimizes tissue trauma, postoperative pain, and recovery time while enhancing visualization of pleural, pulmonary, and mediastinal structures. Its applications in partial pericardiectomy, thoracic duct ligation for chylothorax, lung lobectomy, and pleural biopsy continue to expand with technological improvements and growing clinical experience.

Recent advances in endoscopic imaging, energy-based dissection systems, and perioperative monitoring have further increased the safety and precision of thoracoscopic procedures. As veterinary medicine continues to embrace minimally invasive approaches, thoracoscopy stands out as both a diagnostic and therapeutic cornerstone for managing a wide range of thoracic pathologies in dogs and cats. Continued refinement of techniques, surgeon training, and equipment development will ensure its broader adoption and improved patient outcomes in the future of small animal surgery.

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Chapter 9

Promoting the Well-Being of Laboratory Mice through Environmental Enrichment

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Abstract

The search for the quality of life of animals in captivity has been carried out in laboratories and animal facility in Brazil and around the world. Most of them today use different practices daily, highlighting the environmental enrichment that promotes animal welfare. This practice is of great importance and can happen in a physical, sensory, cognitive, social, and food way, adapted for mice for more reliable results in research, since the animals start to behave in a more similar way to the natural way. The use of animal models such as mice is of great value to scientific knowledge that is continually expanding, proving the importance of using environmental enrichment that tends to promote a more pleasant environment for laboratory animals in various institutions across the country.

Keywords: mouse, environmental enrichment, animal welfare, animal facility, refinement

1. Introduction

The relationship between humans and animals and the maintenance of animals in captivity have existed for a long time, but it was only in the last three decades that the concern for animal welfare and the possibility for animals to express their natural behaviors were recognized as a science [1].

Among the animals used in animal facility, mice are the most common in scientific experiments and research, as approximately 99% of human genes have been

mapped in them, creating a relationship between humans and animals for laboratory experiments. Associated with this, they have several characteristics that make them an important ally in health sciences, including the fact that they are small, docile, and have many physiological similarities when compared to other animal models, such as amphibians, insects, birds, and other mammals [2].

Currently, the most used animal models in scientific research are mice. Mice are induced to show expected and desired behavioral patterns during an experiment, but the literature sometimes describes several undesirable behavioral patterns, such as anxiety, stress, depression, aggression, eating disorders, and compromised immunity [3, 4].

Since mice raised in captivity spend most of their lives in cages, it is extremely important that environmental enrichment is implemented to meet the physiological and ethological needs of these animals. This allows them to be subjected to potentially stressful procedures without any alteration in the experimental results.

The legislation regarding animal welfare in Brazil requires that, when used in experiments, a minimum of discomfort is preserved, thus generating positive benefits for both the animal's welfare and the research results. According to the European legislation, the environmental enrichment of animal housing compartments must be adapted to the needs of the individual and the species to which it belongs. The enrichment strategies of the facilities must be reviewed and updated regularly [5].

This work aims to present the possibilities for improvement in the pursuit of the welfare of mice that are outside their natural habitat and housed in friendly experimental facilities. This is achieved through the implementation of environmental enrichment, aiming for the quality of life and the maintenance of the natural behavior of these animals in captivity.

2. Theoretical framework

2.1 Environmental enrichment

The maintenance of animals in captivity is an activity that has existed for thousands of years, accompanying the history of the relationship between humans and animals. According to Andrade et al. [6], before Christ, philosophers like Aristotle and Hippocrates, at the beginning of animal experimentation, observed the functioning of animal and human organs and noted similarities and differences, contributing to the start of the science of animal experimentation. Since scientists were prohibited from performing autopsies on human cadavers, studies were conducted on animals that provided a closer approximation of human physiological functions [6].

In 1925, Yerkes recognized the importance of animal welfare through environmental enrichment, along with Hedge, in the years 1950–1969, by describing the importance of the animal having the opportunity to express its natural behavior with the implementation of objects added to its environment [7]. It was only from 1970 that the implementation of environmental enrichment began in zoos and animal facilities [8]. And, concomitantly, some ethical debates were about the conduct of experiments on animals. However, this topic had already been questioned between the years 1710 and 1776, when the English physiologist Ferguson warned about the cruel treatment to which animals were subjected in experiments without the major ethical implications. For him, all torture and suffering should be avoided as much as possible [9].

According to Saad et al. [8], science has recognized and mentioned animal welfare and the importance of environmental enrichment because animals are beings capable of having feelings such as joy, pain, and suffering [10]. The perception that experimental animals are sentient beings directly influenced the development of environmental enrichment strategies, aiming not only to minimize pain and suffering but also to promote the physical and emotional well-being of animals used for scientific purposes [2, 11].

Henriques and Sampaio mention the publication of "The Principle of Humane Experimental Technique" by two English researchers, Russell and Burch [12], where the concept of the 3Rs was introduced in 1959, with the goal of changing the view of the time and leading to the reflection that the use of animals in experiments could be minimized. Thus, the 3Rs term referred to considerations about:

- Replacement: The substitution of one species for another lower on the zoological scale or for microorganisms, or, if possible, for non-biological material. This should be understood as a substitute for procedures involving the use of *in vitro* methods, physicochemical techniques, computational or mathematical modeling, or the use of organisms that are known to have limited consciousness. Human studies include epidemiology, post-marketing surveillance, and the ethically approved use of human volunteers, as well as any other method developed based on or using molecular or cellular mechanisms of the phenomenon to be studied.

- Reduction: The reduction in the number of animals used. Studies involving animals must ensure that the results are statistically valid and obtained with the smallest possible number of individuals. Researchers should, whenever possible, seek guidance from the biostatistician at their institution in project preparation, so they know in advance how the data will be analyzed. Another point that should be considered is the sample size. A sample that is too small will not allow the studied effect to be detected with any degree of reliability. Meanwhile, exposure that is too large leads to the unnecessary use of animals. The sample size should be determined using a formal statistical method.

- Refinement: The refinement of techniques to minimize the level of stress and pain caused to the animal during experimentation. The use of analgesia and anesthesia for pain relief and the defined criteria for the application of a humane endpoint should be carefully carried out, evaluated, and described during the investigation. All scientific teaching and research activities must include the environmental enrichment program to be implemented [2, 12–14].

According to Martins et al. [15], in ethical terms, animal welfare and environmental enrichment have become fundamental for laboratory animals, especially related to the issue of refinement.

For Broom and Molento [10], welfare is defined by a set of responses and concepts that encompass the psychological and behavioral state expressed by animals, such as fear, stress, and health, among other reactions. Thus, well-being is related to the animal's various attempts to adapt to the environment it is subjected to.

According to Refs. [6, 16], the evaluation of animal welfare is carried out through two parameters: behavioral and physical, with the behavioral one being the most important in captive animals. Thus, it is observed that animal welfare is closely linked to a correct response to the experimental results.

Environmental enrichment helps with the physical and psychological issues of the animals, allowing them to express their natural behavior. It also contributes to motor stimulation and social interactions, always aiming to improve the biological functions of animals living in captivity [17]. Enrichment can be considered not only as an external means that promotes stimuli but also as a source capable of generating patterns of brain function, since each animal will have different experiences in its enriched environments. There are various techniques used in environmental enrichment to achieve greater stimulation of these animals, such as the insertion of stimuli like those that exist in nature to avoid possible stereotyped and stress behaviors [18].

Elidio et al.'s [19] research demonstrated that with environmental enrichment, mice showed less stress during handling, reduced aggression, better health conditions, less loss of offspring, a higher mating rate, and lower cortisol circulation. According to Lambert et al. [20], with the implementation of environmental enrichment, brain changes correlated with memory and learning were observed, thus showing greater cognitive potential [6]. In older mice, an increase in presynaptic protein levels in the hippocampus was found [20], since brain plasticity is responsive at any age, thus reducing the risks of neurodegenerative changes [18].

Environmental enrichment can enhance certain actions in the immune system, such as increasing the activity of macrophages and the number of innate cells, as described by Souza [21].

However, the authors highlight the importance of considering that biological systems will not always act in the same way. Modifications both in the individual's own organism and in the environment where they live corroborate that certain results are passive to oscillations. Another point raised is that the absence of structures that stimulate the animals' natural behavior decreases well-being and causes stress, reducing the credibility of the study, since stressed animals do not produce reliable results and contradict the principle of the 3Rs [12, 22].

Mieske et al. [11] demonstrated that a stimulating environment can be considered essential for the development of natural behavior and animal welfare of research rodents. Although boredom has never been studied directly and is rarely mentioned, it clearly reflects many studies on the effects of improving living conditions. Chronic boredom, a consequence of living in a barren and confined environment, can pose a health risk to laboratory animals, limiting their validity as model organisms for biomedical research. A stimulating living environment promotes the well-being of laboratory workers, regardless of age and sex.

Among all the advantages generated for studies, some authors question the implementation of environmental enrichment due to its influence on the standardization of research. According to Newberry [17], if there is no correct understanding of species behavior, an inconsistent and inadequate interpretation of the experiment's results will occur. Likewise, the implementation of environmental enrichment will not be applicable when the animal shows behavioral changes due to stress and especially aggression in males, to avoid possible interferences in the result of the experiment.

2.2 Environmental enrichment items

Acrylic igloos (**Figure 1**) are also considered a form of environmental enrichment. In experiments, some of their advantages have been reported, including increased locomotion in non-acrylic-housed animals, a reduction in feeding behavior, and an increase in natural self-grooming behavior. However, aggressive behavior with territorial marking was observed in males [22]. The use of a transparent acrylic material



Figure 1.
Acrylic igloo environmental enrichment item for mice. Animal Facility of the Carlos Chagas Pavilion at the Oswaldo Cruz Institute's Center for Animal Experimentation - Fiocruz.

with multiple, red-colored entries facilitates the visualization of animal corpses, as the animals themselves are enclosed in black [23]. It is important to highlight that mice perceive red as a shade of black, which can contribute to a greater sense of security, reducing stress and improving the comfort and well-being of the animals inside the red igloo [24].

The running wheel was implemented to stimulate physical activity in mice. However, its use is controversial because it can trigger obsessive behavior, leading animals to exercise more than they would in their natural habitat [2]. According to Souza [21], 4 weeks of exercise with a running wheel improved neuroplasticity, cognitive functions, and neurotrophin expression in the organs studied.

Dallagno [25] reported that adult mussels increased their intake of pelleted food without gaining body mass, whereas older mussels showed no such increase or body mass gain. According to National Council for the Control of Animal Experimentation (CONCEA) [2], while a running wheel stimulates physical activity, it can also lead to undesirable obsessive behaviors, causing mice to exercise beyond what is natural for them.

Studies have shown that the sensory, motor, and cognitive stimulation from exercise produces positive results by inducing neuroplasticity mechanisms, as evidenced by increased expression of neurotrophic factors and neurotransmitter receptors in various organs. The neurochemical and neuroanatomical results observed in animals with similar exercise-like enrichments have been associated with changes in anxiety-like and activity-related social behaviors. Recent work demonstrated that physical exercise provided cognitive and emotional regulation benefits and improved contextual fear conditioning in Gfap-hsv-tk transgenic mice [26].

The chew ball is a common item for domestic pets (dogs and cats). The interaction it provides is expected to be stimulated by its shape and the sound it makes when moved. It has shown satisfactory interaction results with younger animal groups. This structure is highly resistant and durable, but it has the disadvantage of a high cost and

difficulty of sterilization [15]. Adult murine mice showed less interest in using the chew ball when first introduced to it and after a five-day interaction period [27].

Polyvinyl chloride (PVC) tubes are durable and help protect insects during the day [2]. According to Santos and Fontes [28], the introduction of PVC tubes in some experiments led to an increase in the number of litters in cages, indicating the improved aspects of animal reproduction. Oliveira [3] reported that PVC tubes were used to reduce episodes of aggression and fighting among groups of animals in a cage, serving a protective role (**Figure 2**).

Absorbent paper (**Figure 3**) immediately piques the interest of mice, stimulating nesting behaviors as well as individual and social interactions. Although it is inexpensive and easy to introduce, its use in cages must be time-limited because it quickly loses its structural integrity as an environmental enrichment item [15].

According to National Council for the Control of Animal Experimentation (CONCEA) [2], adding nesting boxes made from absorbent paper is highly valuable for stimulating the typical nesting behaviors of birds. To maintain its effectiveness, this item should be replaced as soon as it becomes damp with urine or drinking water. However, if the paper towels or lids remain dry and their structure is intact, they can stay in the cages for up to 15 days [27].

Cotton flakes (**Figure 4**) are associated with reproduction, specifically with nest building. They represent a low-cost physical enrichment material, easy to handle,

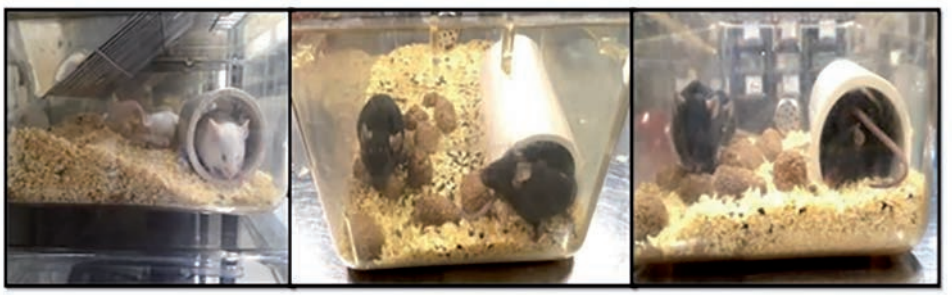


Figure 2.
Enrichment item for Mice: PVC Tube. From the Animal Facility, Carlos Chagas Pavilion, Center for Animal Experimentation, Oswaldo Cruz Institute – Fiocruz.



Figure 3.
Environmental enrichment item: absorbent paper for mice. From the Animal Facility, Carlos Chagas Pavilion, Center for Animal Experimentation, Oswaldo Cruz Institute – Fiocruz.

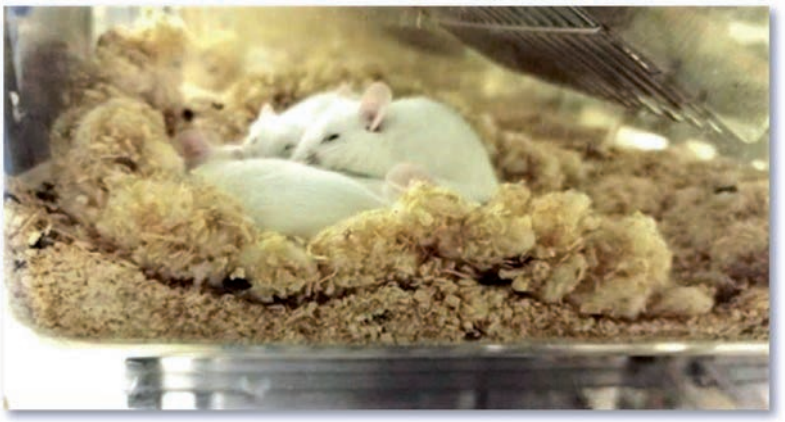


Figure 4.
Environmental enrichment item: cotton flakes. Animal Facility, Carlos Chagas Pavilion, Center for Animal Experimentation, Oswaldo Cruz Institute – Fiocruz.

and can be replaced during cage cleaning or, alternatively, sterilized by autoclaving. Furthermore, they do not cause harm to newborns, have been shown to reduce pre-weaning mortality, and contribute to increased pup body weight [28]. In addition to their benefits for offspring, cotton flakes may also be used as nesting material. According to Rosa et al. [29], cotton was the preferred nesting material among LG/J females, possibly due to its superior nest-building capacity. However, as a shelter/protective object, it was not the preferred option when compared with paper rolls, across females at different life stages (Pubertal, Virgin Adult, and Adult).

The sounds of the tropical forest are characterized as auditory enrichment and may promote increased physical activity, enhanced brain functions, and lifespan extension. These effects were observed both with lower- and higher-range sound exposure, in comparison with the control group. Studies in rodents have indicated that music can increase physiological stress [30, 31]. However, other studies have demonstrated that music induces positive behavior [32] and physiological benefits in rodents [32–34]. It has been suggested that constant background noise throughout the day may provide certain benefits in the housing of these animals, by decreasing excitability and reducing startle responses to sudden noises [35].

To assess the effects of classical music on the general motor activity of unrestrained mice, Cruz et al. [36] employed the open field test. In this assay, no statistical differences were observed in the rhythm of spontaneous movements; however, a reduction in inactivity time was recorded in groups exposed to classical music, indicating an increase in motor activity. Moreover, mice exposed to Mozart's music displayed enhanced exploratory activity, suggesting its potential role in environmental enrichment for captive rodents.

Additional enrichment artifacts, beyond those already mentioned, include paper tubes, shredded paper, wire-free disposable masks, wooden balls, golf balls, bats, and blocks of softwood [2]. Any item used for environmental enrichment should be time-limited to preserve novelty and stimulation, while also preventing the development of stress signals [1].

The type of bedding/flooring used constitutes a fundamental component of the rodent environment, with significant implications for animal health, and should therefore be considered when selecting environmental enrichment strategies.

The quality of bedding/flooring material is closely related to its moisture retention properties, as well as to microbial, traumatic, and aeration effects. Wood shavings have recently been preferred as laboratory animal bedding due to their low cost. However, alternative bedding materials that are dust-free, reduce allergen spread, and minimize ammonia production within cages are available on the market. Despite these advantages, such materials are not always financially viable and/or considered comfortable for mice, and they generally do not impact ammonia generation, differing mainly in urine absorption capacity. In addition to the material itself, the volume of bedding provided is an important factor, since rodents use it in nest building alongside other enrichment items [37, 38].

2.2.1 Sensory indicator

Sensory enrichment encompasses the stimulation of all five senses in animals. These stimuli include visual, auditory, olfactory, tactile, and gustatory inputs (**Figure 5A** and **B**) [28]. In some experiments, music was introduced as an enrichment item aimed at reducing group stress [32–34]. Naturally, animals emit sounds related to courtship, aggression, defense, and maternal care, which can be influenced by ambient noises [28]. Furthermore, environmental sounds may alter physiological and behavioral parameters as well as circadian rhythms in animals [39].

2.2.2 Nutritional/feeding indicator

Food enrichment (**Figure 6**) represents the most immediate way of promoting animal welfare. Providing mice with a varied diet stimulates their natural foraging behavior [17]. However, the standardization of feeding schedules through environmental enrichment may trigger a physiological stress response in animals when alterations occur at any given time [40]. CONCEA [2] emphasized that the diversity of food offerings may interfere with experimental outcomes due to the lack of standardization and the impracticality of feeding management throughout the study. A seed mixture can be scattered on the cage floor; however, mice tend to consume mainly sunflower seeds while disregarding others. Quality control of these seeds is essential, as there is a potential risk of chemical or biological contamination. This practice may be contraindicated for animals used in nutritional or toxicological studies [2].

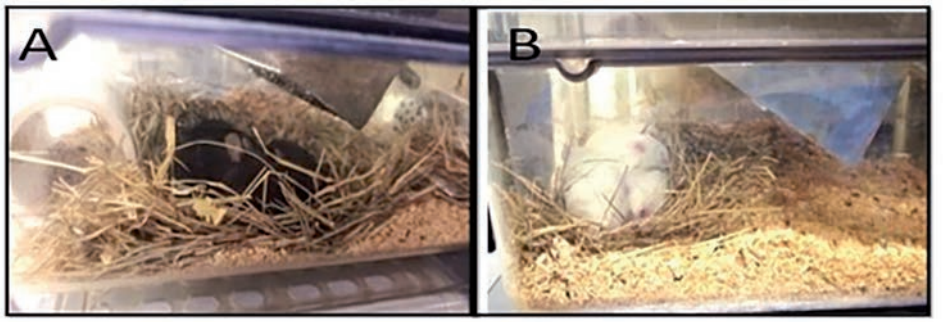


Figure 5.
Sensory environmental enrichment item for mice: hay. Animal Facility, Carlos Chagas Pavilion, Center for Animal Experimentation, Oswaldo Cruz Institute – Fiocruz.



Figure 6.
Environmental enrichment item: seed mixture for feeding. Center for Animal Experimentation, Oswaldo Cruz Institute – Fiocruz.

2.2.3 Social indicator

Mice are highly social species and exhibit improved welfare when housed in pairs or groups. They display more natural behaviors when paired or grouped from the time of weaning, with groups preferably established prior to puberty to prevent stereotypic behaviors and to allow the development of their own social and territorial organization. Positive social stimulation functions as environmental enrichment, enabling mice to express natural behaviors such as grooming and jumping with specifics, thereby expressing emotions and rapidly reducing potential signs of stress [40]. Particular attention must be given to groups composed of male mice. The older the animals, the greater the likelihood of aggressive behaviors. As a rule, same-sex groups should be established before puberty. Territorial and social organization also vary among different mouse strains. Adult Swiss males exhibit greater intolerance in territory establishment and display higher levels of aggression compared to other strains. The addition or removal of an individual may compromise the welfare of the entire group [2].

2.2.4 Human-Animal relationship

According to National Council for the Control of Animal Experimentation (CONCEA) [2], animal handling can be characterized as a form of environmental enrichment (**Figure 7**). This requires clearly described procedures, accessible to all staff involved in animal care, to ensure the positive interactions with animals during cage changes and experimental manipulations, thereby habituating them to routine procedures and consequently maintaining high levels of animal welfare. Refined handling practices that prioritize habituation and socialization of laboratory animals with animal care personnel have a positive impact on welfare and, consequently, on scientific quality [19]. Vilela et al. [41] also reported that animals accustomed to contact with caretakers during physical restraint in the cage and intraperitoneal inoculation exhibited significantly reduced intraspecific aggression (confrontations, attacks, and bites) following these procedures, thereby ensuring animal welfare and ultimately improving the quality of research outcomes.

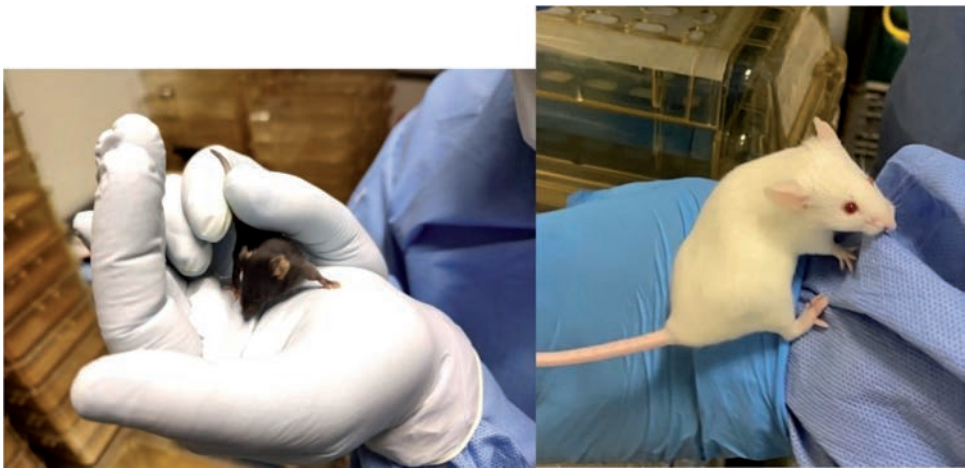


Figure 7.
Environmental enrichment item: human–animal interaction with mice during cage changes. Animal Facility, Carlos Chagas Pavilion, Center for Animal Experimentation, Oswaldo Cruz Institute – Fiocruz.

3. Conclusions

The present study demonstrated that the outcomes of implementing environmental enrichment and promoting animal welfare should be analyzed in a comprehensive and continuous manner, considering both the short- and long-term benefits for animals maintained outside their natural habitat.

The introduction of environmental enrichment should be maintained consistently to provide the ongoing improvements in mouse behavior, thereby reducing negative interventions in response to experimental procedures.

The use of mice according to the 3Rs directly facilitated responses to surveys and indirectly contributed to the welfare of these animals, allowing them to express their natural behaviors without stress and free from abnormal or stereotypic behaviors in cages, enabling interaction with the environmental enrichment items.

Implementing animal welfare through environmental enrichment makes the environment more pleasant and closer to the animals' natural habitat, allowing mice to express their innate behaviors. This, in turn, improves their mental and immune states and consequently enhances research outcomes, thereby preventing the unnecessary use and disposal of mice during research projects.

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We would like to dedicate this work to the memory of Roseli Lopes Simões, a dear colleague and friend, whose dedication, enthusiasm, and companionship left a profound mark on everyone who had the privilege of working alongside her. Her passion for work, generosity, and contagious joy remain an inspiration to us all. She will be deeply missed, but her memory and legacy will continue to live on in every step of our professional and personal journey.

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Chapter 10

The Anatomy and Physiology of the Heart

Tuchakorn Lertwanakarn

Abstract

The heart is a rhythmic and dynamic muscular organ that plays a pivotal role in sustaining life by ensuring continuous blood circulation throughout the body. This chapter presents a comprehensive overview of the heart's anatomical structures and physiological functions in mammals. It begins with the organization of cardiac tissues, including cardiomyocytes and the conduction system, which enables synchronized contraction and efficient hemodynamics. Special emphasis is placed on the electrophysiological properties of cardiac cells, the generation of action potentials, and the roles of ion channels, the cardiac electrical conduction system, generation of electrocardiography and its interpretation, as well as the mechanisms of arrhythmia. The chapter also explores the cardiac cycle, detailing pressure-volume relationships and regulation of cardiac performances. Furthermore, it discusses valve mechanics, the origination of heart sounds, illustrations of phonocardiograms of normal and common abnormal heart sounds. This chapter serves as a fundamental resource for students and professionals in veterinary and biomedical sciences seeking a solid foundation in cardiovascular anatomy and physiology.

Keywords: cardiomyocyte, action potential, electrocardiography, cardiac cycle, cardiac output, phonocardiography, heart sound, hemodynamics

1. Introduction

The heart is a vital organ responsible for the continuous circulation of blood throughout the body, supplying oxygen and nutrients to tissues while removing metabolic waste. Its ability to function as an efficient pump relies on the integration of complex anatomical structures and finely tuned physiological mechanisms. At the core of this function lies the myocardium—a specialized muscular tissue capable of rhythmic contraction, regulated by intrinsic electrical impulses and modulated by the autonomic nervous system. Understanding cardiac performance requires comprehensive knowledge of the heart's structural components, including the chambers, valves, conduction pathways, and coronary vasculature, as well as its physiological response and body's regulation. Hence, this chapter aims to provide an integrative overview of cardiac anatomy and physiology with emphasis on functional relationships between structure and performance. By bridging fundamental concepts with applied clinical understanding, this content serves as a foundation for students, educators, and practitioners in veterinary and comparative cardiovascular medicine.

1.1 The heart as the center of the cardiovascular system

The heart lies in the center of the circulatory system, serving its major function, as muscular pumps—receiving and ejecting blood through an intricate network of blood vessels [1]. Interestingly, the structure and function of the heart among vertebrates vary significantly across taxa. For example, the circulatory system in fish consists of a single-loop circuit, where amphibians and higher taxa possess a double circulatory system, consisting of a systemic and pulmonary circulation [2]. In mammals, the heart is composed of multiple subcomponents, primarily four chambers – the right and left atria and the right and left ventricles. Generally, the left side of the heart is approximately three times larger than the right side, owing to its role in generating higher pressure required for systemic circulation. In contrast, the right side, which pumps blood into the low-pressure pulmonary circulation, has a comparatively smaller chamber size. Additionally, the heart contains four valves, classified into two main groups: atrioventricular (AV) valves and semilunar valves. The AV valves are located between the atria and ventricles, including the mitral valve (or bicuspid valve) on the left side, and the tricuspid valve on the right side. The semilunar valves, found at the exits of right ventricle – pulmonic valve, and left ventricle – aortic valve, are characterized by their crescent-shaped cusps. These valves function to ensure unidirectional blood flow through the heart.

1.2 Structure of the cardiac wall

The cardiac wall is composed of four distinct layers: the pericardium, epicardium, myocardium, and endocardium (**Figure 1**). The *pericardium*, or the heart's protective covering, consists of two sublayers: the fibrous pericardium, which is relatively inelastic and serves to prevent excessive distension of the heart, and a more flexible serous pericardium, which contains parietal and visceral layers. *Epicardium*, a part of the visceral layer, habituates coronary vessels, innervation of autonomic nervous fibers, and lymphatic drainage. Between the layers of pericardium lies a small amount of *pericardial fluid*, which functions to reduce friction between the moving epicardial surface and the pericardial sac [3].

The *myocardium* is the thickest and most functionally important layer, consisting of *cardiac muscle cells* responsible for three essential functions, including contractility, electrical impulse generation, and conduction. The myocardial fibers are arranged in a

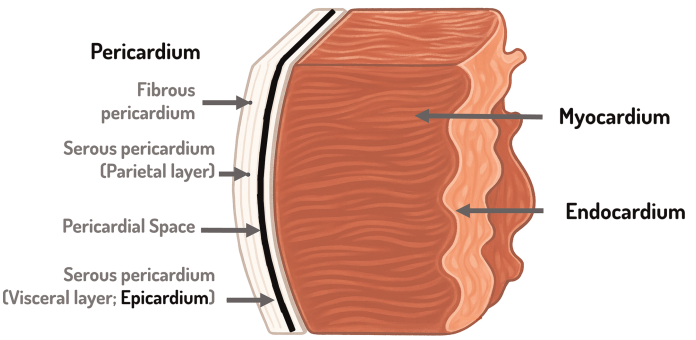


Figure 1.
Components of the cardiac wall layers. The cardiac wall is composed of the pericardium, epicardium, myocardium, and endocardium (Adapted from Ref. [3]).

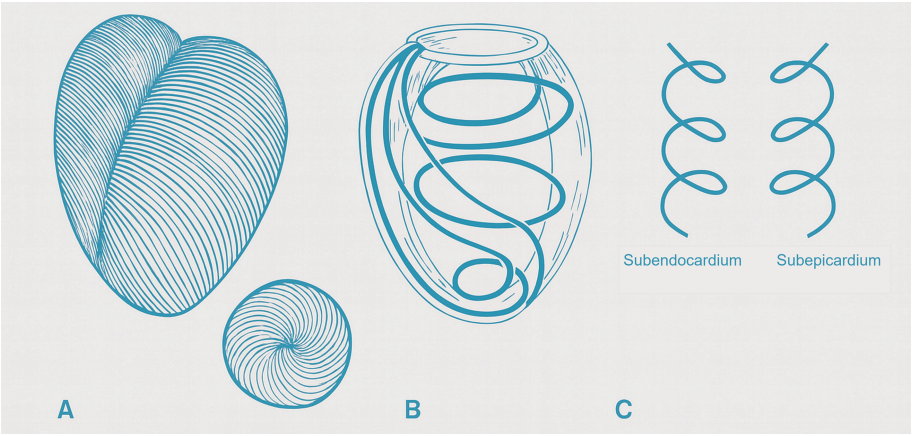


Figure 2.
Orientation of myocardial fiber arrangement in the ventricular wall. (A) Longitudinal and transverse striations of cardiac muscle fibers. (B) Direction of myocardial contraction in both transverse and longitudinal planes, resembling a wringing and twisting motion (C) Contraction patterns of myocardial fibers in the subendocardial and subepicardial layers (Adapted from Ref. [4]).

helical orientation, including longitudinal, radial, and circumferential layers [4]. This spiral arrangement enables a twisting and squeezing motion during contraction, maximizing blood ejection from the ventricles (**Figure 2**). The mechanical action of each contraction also transmits a palpable force to the chest wall, detectable as the *apical beat* during physical examination. The *endocardium*, which is the innermost endothelial layer lining of the chambers, plays an important role in cardiac compliance.

2. Ultrastructure of cardiomyocytes and their role

The heart, a vital organ responsible for the pump of the circulatory system, relies its activity on the function of cardiomyocytes. Most cardiomyocytes are contractile cells, exhibiting striations due to the orderly arrangement of myofilaments. A unique structural feature of cardiac muscle cells is their branched morphology, allowing them to interconnect with neighboring cells via specialized junctions known as *intercalated disks*. Internally, cardiomyocytes are composed of approximately 50% myofibrils, reflecting their primary role in contraction and relaxation. This is followed by mitochondria, which occupied one-third, and the sarcoplasmic reticulum (SR), which makes up about 3.5% of the cell, highlighting the importance of energy and intracellular calcium ions (Ca^{2+}) demand essentially for contraction [5].

2.1 Myofibrils and the mechanism of cardiac muscle contraction

Upon microscopic examination, the cardiac muscle fibers are composed of more than 50% of highly organized cardiac myofibrils (**Figure 3A**). These fibers are responsible for the striated appearance of cardiac muscle, which results from alternating light and dark bands due to the varying densities and alignments of myofilaments. The functional unit of contraction in cardiac muscle is the sarcomere, defined as the segment between two Z-discs (**Figure 3B**). The shortening of these sarcomeres is the fundamental mechanism that drives muscle contraction and, consequently, the pumping action of the heart [2].

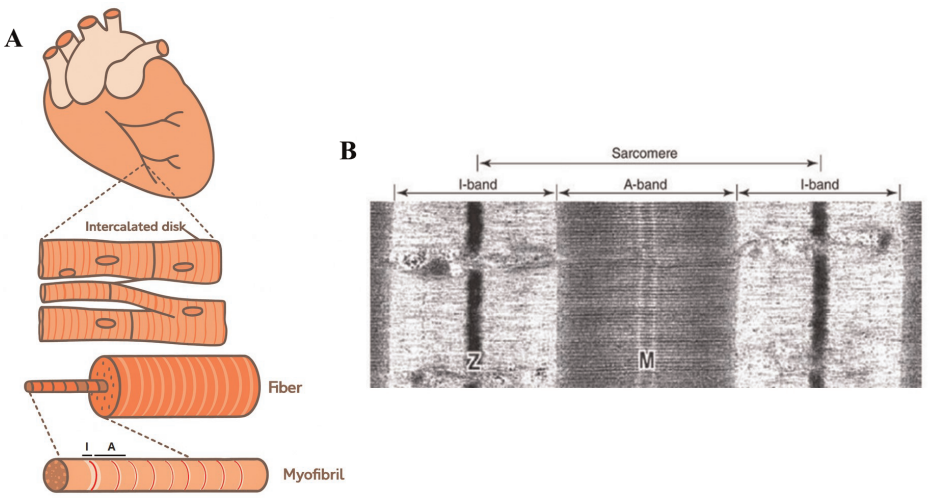


Figure 3.
Organization of the heart and arrangement of cardiomyocyte filaments. (A) Structural organization of the heart, from the myocardium to individual cardiac myocytes (muscle fiber) and their myofibrils. (B) The arrangement of sarcomere, the fundamental contractile unit, extends from one Z-disc to the next and consists of alternating anisotropic (A- band) and isotropic (I-band) regions (Adapted from [2]).

Each sarcomere contains thin filaments (actin) and thick filaments (myosin). The I-band (isotropic) appears lighter under microscopy and contains only actin filaments anchored to the Z-disc. In contrast, the A-band (anisotropic) appears darker and includes overlapping regions of both actin and myosin. The M-line, located at the center of the A-band, serves as the anchoring point for the thick filaments, while the H-zone, a slightly lighter region within the A-band, contains only myosin filaments. Remarkably, the myofilaments are arranged in a hexagonal lattice, with six actin filaments surrounding each myosin filament and vice versa—an architectural feature also seen in skeletal muscle [6].

The thin filaments are composed of double helical strands of actin and regulatory proteins that control muscle contraction, including cardiac tropomyosin (cTm) that block myosin-binding sites in the resting state, and the cardiac troponin complex (cTn), which consists of three subunits: cTnC, which binds calcium ions; cTnT, which anchors the troponin complex to tropomyosin; and cTnI, which inhibits actin-myosin interaction. The thick filaments are composed of myosin molecules, each consisting of two myosin heavy chains (MHC) and associated myosin light chains (MLC). The MHC has a distinct "golf-club" shape with a head region containing an actin-binding site and an ATPase domain, and a tail region that contributes to filament assembly at the M-line. The MLCs include the regulatory light chain (RLC), located near the hinge region and involved in the phosphorylation-dependent regulation of contraction, and the essential light chain (ELC), which stabilizes the MHC structure. In addition, cardiac myosin-binding protein C (cMyBP-C) associates with thick filaments and plays a regulatory role by modulating cross-bridge formation and the force of contraction [7].

2.2 Cross-bridge cycling and contraction mechanism

Cardiac muscle contraction is governed by a process known *as cross-bridge cycling* (**Figure 4**). Initially, the troponin complex is being activated by an increase in

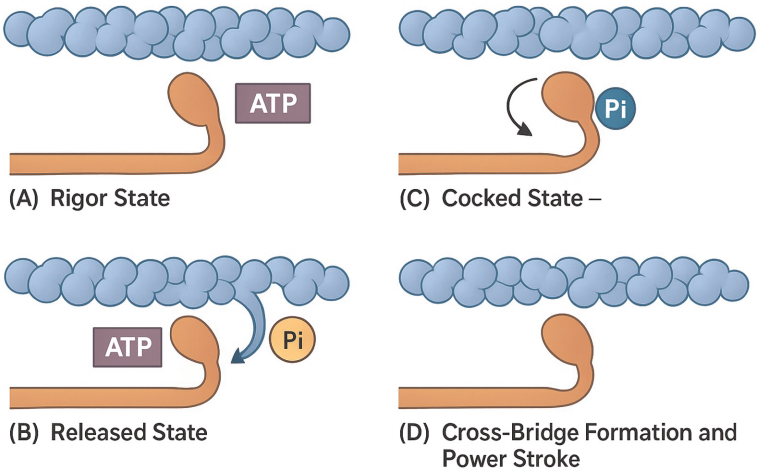


Figure 4.
Cross-bridge cycle of cardiac muscle filaments. (A) Rigor State – myosin head is tightly bound to actin in the absence of ATP. (B) Released State – ATP binds to the myosin head, causing detachment from actin. (C) Cocked State – hydrolysis of ATP repositions the myosin head into a high-energy conformation. (D) Cross-Bridge Formation and Power Stroke – myosin rebinds to actin, releases Pi, and undergoes a conformational change that pulls actin toward the sarcomere center (Adapted from [8]).

intracellular Ca^{2+}, who binds to cTnC and triggers a conformational change in the troponin complex. This change displaces tropomyosin from the actin filament, exposing the myosin-binding sites and allowing myosin heads to engage and initiates the cross-bridge cycle. The cycle consists of five sequential phases. In the *rigor state*, where the ATP is absent, the myosin head is tightly bound to actin. Upon ATP binding, the myosin head detaches from actin and enters the *released state*. ATP is then hydrolyzed by myosin ATPase, producing ADP and inorganic phosphate (P_i), which causes the myosin head to shift into a high-energy conformation known as the *cocked state*. The myosin-ADP-P_i complex then binds to actin, forming a *cross-bridge*. As P_i is released, the *power stroke* occurs in which myosin pulls the actin filament toward the center of the sarcomere. Following this, ADP is released, and the system returns to the *rigor state*, awaiting the next ATP molecule to continue the cycle. The cross-bridge cycle repeats with each ATP influx, enabling sustained contraction. Finially, muscle relaxation occurs when intracellular Ca^{2+} levels fall, allowing cardiac troponin I (cTnI) to rebind actin and enabling tropomyosin to re-cover the myosin-binding sites, thereby inhibiting further cross-bridge formation and ending the contraction [8].

2.3 Sarcoplasmic reticulum, calcium, and the electrical-contraction coupling

As previously described, cardiac muscle contraction is initiated by a rise in intracellular calcium concentration ($[Ca^{2+}]_i$). Conversely, muscle relaxation occurs when $[Ca^{2+}]_i$ decline. Thus, Ca^{2+} is a central regulator of both contraction and relaxation in cardiomyocytes. Within these cells, several specialized proteins and organelles coordinate Ca^{2+} handling (**Figure 5**). One of the most critical components is the *L-type Ca^{2+} channels (LTCC)* located on the cell membrane. These channels are a type of voltage-gated Ca^{2+} channel that open in response to the change in membrane potential, allowing Ca^{2+} influx from the extracellular space into the cytosol.

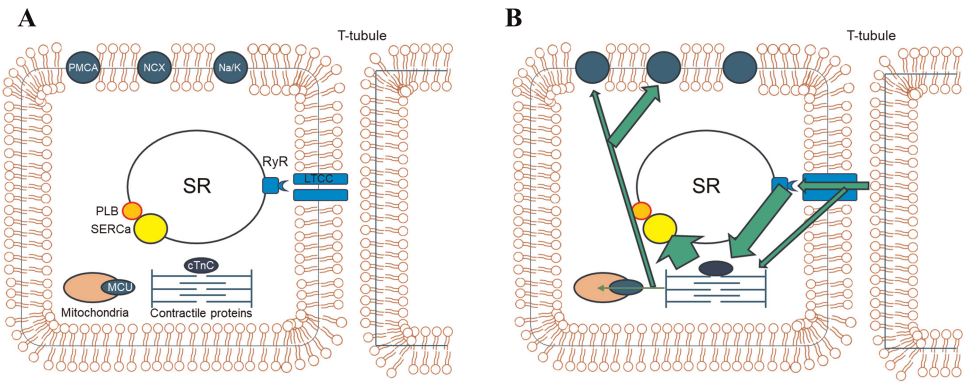


Figure 5.
Regulation of intracellular Ca² dynamics in cardiac muscle cells. (A) Illustration of key Ca^{2+} handling proteins in cardiomyocytes, including the L-type Ca^{2+} channel, ryanodine receptor, cardiac troponin C (cTnC), sarcoplasmic reticulum Ca^{2+}-ATPase (SERCa), phospholamban (PLB), Na^{+}/Ca^{2+} exchanger (NCX), plasma membrane Ca^{2+}-ATPase (PMCA), and mitochondrial Ca2+ uniporter (MCU). (B) Diagram depicting calcium homeostasis in cardiac muscle cells, illustrating the coordinated regulation of calcium influx, release, reuptake, and extrusion (Adapted from [9]).

The *sarcoplasmic reticulum (SR)* plays a pivotal role in *$[Ca^{2+}]_i$ storage and release*. It is structurally divided into two main regions: the subsarcolemmal cisternae, located adjacent to the cell membrane, and the sarcotubular network, which extends throughout the myofibrils. The SR releases Ca^{2+} into the cytosol to initiate muscle contraction. In cardiomyocytes, the subsarcolemmal cisternae form a specialized structure called the *dyad*, where they are coupled with T-tubules. At this interface, *LTCC* on the T-tubule communicate with *ryanodine receptor (RyR)* on the SR via a membrane-anchoring protein called junctophilin-2, which acts like "feet" linking the two membranes. When extracellular Ca^{2+} enters the cells via LTCC, it binds to RyR and trigger a massive release of stored Ca^{2+} from the SR, which is so-called *calcium-induced calcium release (CICR)* process. This amplification mechanism increases cytosolic Ca^{2+} from approximately 10^{-7} M to over 10^{-4} M, making CICR essential for generating sufficient force during each cardiac contraction [9].

In contrast, the removal of Ca^{2+} from the cytosol during relaxation involves four key mechanisms. The primary route is reuptake into the SR through the activity of *sarcoplasmic reticulum Ca^{2+}-ATPase (SERCa)*, which actively transports Ca^{2+} into the SR lumen using ATP. SERCA activity is regulated by a small binding protein namely *phospholamban (PLB)*. Upon phosphorylation, PLB dissociates from SERCA, relieving inhibition and enhancing Ca^{2+} reuptake—highlighting PLB's key role in $[Ca^{2+}]_i$ homeostasis. A second mechanism involves the *Na^{+}/Ca^{2+} exchanger (NCX)* on the sarcolemma. This secondary active transporter utilizes the Na^{+} gradient (maintained by the Na^{+}/K^{+} ATPase) to extrude Ca^{2+} in exchange for Na^{+}. Under physiological conditions, three Na^{+} ions enter the cell for every one Ca^{2+} ion removed. The third mechanism involves the *plasma membrane Ca^{2+}-ATPase (PMCA)*, which directly uses ATP to export Ca^{2+} from the cytosol to the extracellular space. Though it contributes less to total Ca^{2+} removal than SERCA or NCX, it plays a supporting role in maintaining calcium balance. Lastly, excess $[Ca^{2+}]_i$ can be sequestered by the *mitochondrial calcium uniporter*. This channel allows mitochondria to buffer cytosolic Ca^{2+} levels, particularly during conditions of Ca^{2+} overload, helping to preserve cellular homeostasis and prevent cytotoxicity [4].

2.4 Cardiac sarcolemma and ion channels

The *sarcolemma* of cardiac muscle cells functions analogously to an electrical circuit. As a selectively permeable membrane, it restricts the free movement of ions, thereby contributing to the generation of *membrane potential (E_m)*. This electrical potential arises from the ionic concentration gradients between the intracellular *and* extracellular fluids, using specific membrane proteins including the Na^+/K^+ ATPase pump, NCX, and PMCA. The *Na^+/K^+ ATPase pump* actively extrudes three Na^+ ions in exchange for two K^+ ions, thereby establishing a resting electrochemical gradient. The NCX operates as a secondary active transporter, using the Na^+ gradient to remove Ca^{2+} from the cell in a 3:1 exchange ratio. Meanwhile, the PMCA actively expels Ca^{2+} during diastole, contributing to intracellular calcium clearance. Although ion movement continues at rest, the *resting membrane potential* remains stable due to the overall balance of ionic charges across the membrane. The E_m can be calculated using the *Goldman-Hodgkin-Katz equation* as shown below [4]:

$$E_m = \frac{RT}{zF} \ln\left(P_K \frac{[K]_o}{[K]_i} + P_{Na} \frac{[Na]_o}{[Na]_i} + P_{Cl} \frac{[Cl]_o}{[Cl]_i} + \ldots\right)$$

Where R = Universal gas constant = 8.31 J/(K·mol), T = Absolute temperature = 310 K (physiological body temperature), z = Valence (charge) of the ion, F = Faraday's constant = 96,485 coulombs/mol and ln x = 2.303 × $\log_{10}$x.

At the resting stage of cardiomyocytes, K^+ channels remain open, rendering the membrane highly permeable to K^+ (high P_K), while the permeability to other ions is negligible. Consequently, the *resting E_m* closely approximates the *Nernst potential for K^+ (E_K)*, typically around *−90 mV*. Therefore, alterations in extracellular K^+ concentration significantly influence membrane excitability. In *hyperkalemia*, elevated extracellular K^+ ($[K^+]_o$) causes E_m to become less negative, facilitating depolarization and enhancing excitability. Conversely, *hypokalemia* lowers $[K^+]_o$, but reduced K^+ permeability results in less negative E_m, which can paradoxically also increase excitability [4].

2.5 Ion channels and excitability

Ion channels are transmembrane proteins that enable selective ion flow across the sarcolemma, governed by electrochemical gradients. Two major types of ion channels involved in cardiac physiology are (1) *Voltage-gated ion channels* (e.g., Na^+, K^+, and Ca^{2+} channels), which open in response to changes in membrane potential, and (2) *Ligand-gated ion channels*, such as the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, which open upon binding cyclic nucleotides or during hyperpolarization. Voltage-gated ion channels comprise four domains, each containing six transmembrane segments (S1–S6), which collectively form a selectivity filter for specific ions. A voltage sensor located on S4 segments responds to changes in E_m, triggering channel to open or close.

Notably, voltage-gated Na^+ and Ca^{2+} channels feature two gating mechanisms: an activation gate (m) and an inactivation gate (h). Upon depolarization beyond threshold, the activation gate opens, allowing ion influx and thus promoting excitability. As membrane potential rises, the inactivation gate closes, terminating ion flow—a key property in controlling action potential generation. Hence, this dual-gating mechanism yields three functional states: *Resting state* — closure of m gate, which blocks the

Na^+ influx, *activated state* — both gates open, allows the Na^+ influx, and *inactivated state* — closure of h gate, blocking Na^+ entry from any further activation. The period during which Na^+ channels are inactivated defines the *refractory period (RP)*, which includes the effective (absolute) refractory period and relative refractory period. In cardiac cells, the RP is nearly as long as the entire action potential duration, covering the time course of contraction and relaxation. This design prevents summation or tetanus and ensures the complete of muscle twitch [4].

When ion channels open, they increase membrane conductance (G_m) or *permeability*, allowing ions to move upon their *electrochemical gradients*, producing a current (I_{ion}). The driving force for a given ion is defined as the difference between the membrane potential (V_m) and the ion's equilibrium potential (E_{ion}). For example, the Na^+ equilibrium potential (E_{Na}) is approximately +61.5 mV. When a cell at rest (−90 mV) opens its Na^+ channels, a strong inward driving force (~151.5 mV) causes rapid Na^+ influx, generating I_{Na} and leading to *depolarization*. As V_m approaches E_{Na}, the current diminishes, and Na^+ channels close. Conversely, when K^+ channels open, the outward I_K returns the cell to a negative E_m (*repolarization*) or even more negative than normal (*hyperpolarization*), reducing its excitability [5].

2.6 Types of cardiac action potentials

Cardiac muscle cells can be categorized based on their *action potential characteristics* into two types: *Pacemaker cells*, which generate spontaneous action potentials, and *Working (non-pacemaker) myocytes*, responsible for contractile force [10].

1. *Pacemaker cells (e.g., sinoatrial [SA] and atrioventricular [AV] node)*

2. Pacemaker cells have a *resting* E_m of −55 to −60 mV and generate *spontaneous depolarizations* (**Figure 6**). Their action potentials have three phases:

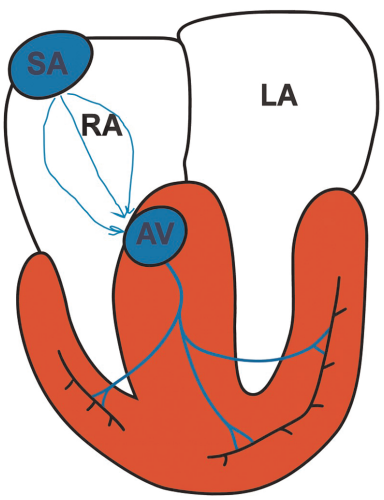


Figure 6.
The cardiac conduction system. The conduction pathway of the heart begins at the sinoatrial (SA) node, which generates electrical impulses that travel through the internodal pathways to reach the atrioventricular (AV) node. From there, the signal is transmitted downward through the left and right bundle branches, into the Purkinje fibers, and finally to the ventricular muscle cells, coordinating a synchronized contraction of the ventricles (Adapted from [11]).

3. *Pre-potential phase (Spontaneous depolarization)*: Initiated by *funny channels (HCN)* and *T-type Ca^{2+} channels*, allowing Na^+ and Ca^{2+} influx, gradually depolarizing the cell.

Rapid depolarization phase: Once the threshold is reached, *L-type Ca^{2+} channels* open, with a rapid influx of Ca^{2+} and an increase in V_m.

Repolarization phase: The opening of *K^+ channels* lead to K^+ efflux, which causes hyperpolarization and reactivates HCN channels to begin a new cycle.

Working myocytes (e.g., atrial and ventricular myocytes).

Working myocytes display a stable resting potential (~−90 mV) and follow a *five-phase action potential* (**Figure 6**):

1. *Phase 4 – Resting potential*: The membrane potential remains unchanged while the ions are re-stabilized by Na^+/K^+ pump, NCX and PMCA.

2. *Phase 0 – Rapid depolarization*: Triggered by Na^+ influx through open Na^+ channels, causes a rapid change in V_m.

3. *Phase 1 – Early repolarization*: The inactivation gate of Na^+ channel closes, while transient K^+ channels are opened. Thereby, K+ effluxes and the V_m reduces in a short period.

4. *Phase 2 – Plateau phase*: A balance of Ca^{2+} influx via L-type channels and K^+ efflux through the opening of K channels, maintaining a steady V_m.

5. *Phase 3 – Rapid repolarization*: Closure of Ca^{2+} channels and sustained K^+ efflux return V_m to the resting level and completing the cycle (**Figure 7**).

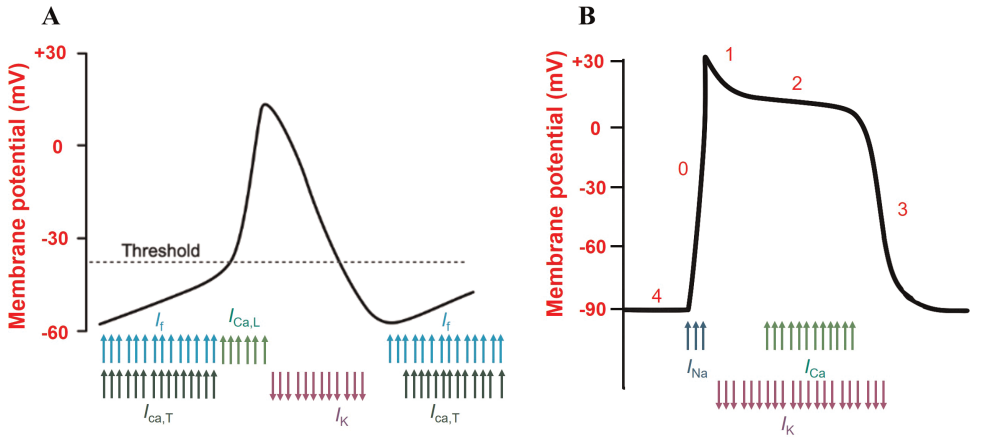


Figure 7.
Action potentials and ion fluxes in two types of cardiomyocytes. (A) Pacemaker cells display a spontaneous, rhythmic action potential with three distinct phases—pre-potential, depolarization, and repolarization—primarily driven by the activity of HCN channels, T-type and L-type Ca^{2+} channels, and K^+ channels. (B) Working myocytes exhibit a five-phase action potential with rapid depolarization, plateau, and repolarization, corresponding to coordinated ion fluxes through Na^+, Ca^{2+}, and K^+ channels (Adapted from Ref. [10]).

3. Regulations of cardiomyocyte performances

Before exploring the regulatory mechanisms of cardiac muscle cell function, it is essential to define four key physiological terms that reflect cardiac performance: *Inotropy* refers to the contractile strength of the heart muscle; *Lusitropy* denotes the ability of the myocardium to relax; *Chronotropy* is related to the control of heart rate, reflecting the SA node activity and *Dromotropy* pertains to the conduction velocity of electrical impulses, particularly from the atria to the ventricles via the AV node. These properties are modulated in response to intrinsic and extrinsic stimuli. *Intrinsic factors* primarily involve myofilament kinetics, including calcium transients, myofilament activation, and the crossbridge cycling process. In contrast, *extrinsic factors* include variations in heart rate (force-frequency relationship), sarcomere length (length-tension relationship), and neurohormonal cytokines [12].

Among the intrinsic factors, *intracellular calcium* ($[Ca^{2+}]_i$) plays a pivotal role in initiating and modulating cardiac contraction. The maintenance of Ca^{2+} homeostasis is governed by a range of calcium-handling proteins, including the L-type calcium channel (LTCC), ryanodine receptor (RyR), cardiac troponin C (cTnC), sarcoplasmic reticulum Ca^{2+}-ATPase (SERCa), sodium-calcium exchanger (NCX), and plasma membrane calcium pump (PMCA). Dysregulation of these proteins alters calcium availability and subsequently affects cardiac function [4].

- *LTCC*: Enhanced LTCC activity increases Ca^{2+} influx, promoting CICR and elevating intracellular calcium transients, resulting in stronger contractions (positive inotropy). Conversely, diminished LTCC function reduces calcium entry and CICR, thereby weakening contractile force (negative inotropy).

- *RyR:* Stimulation or upregulation of RyR facilitates greater Ca^{2+} release from the SR, increasing cytosolic Ca^{2+} concentration and amplifying myocardial contractility (positive inotropy).

- *SERCa*: The activity of SERCa, regulated by PLB, is vital for reuptake of Ca^{2+} into the SR during relaxation. Increased PLB phosphorylation enhances calcium reuptake, improving both relaxation (positive lusitropy) and subsequent contraction (positive inotropy). In contrast, inhibition of SERCa or excessive PLB activity impairs calcium clearance, reducing both relaxation and contractility (negative lusitropy and inotropy).

- *NCX*: Under physiological conditions, NCX extrudes one Ca^{2+} ion in exchange for three Na^+ ions, driven by the Na^+ gradient established by the Na^+/K^+ pump. During diastole, NCX extrudes Ca^{2+} to facilitate relaxation. However, inhibition of the Na^+/K^+ pump leads to the accumulation of intracellular Na^+, causing NCX to reverse its mode of action and import Ca^{2+} in exchange for Na^+ efflux, enhancing intracellular Ca^{2+} and increasing contractility (positive inotropy).

- *cTnC affinity*: Normally, cTnC binds two Ca^{2+} ions at its N-terminal region to trigger contraction. Reduced calcium affinity requires higher Ca^{2+} concentrations for activation and may result in premature calcium dissociation, diminishing contractile strength (negative inotropy). In contrast, increased Ca^{2+} affinity enhances binding stability and promotes stronger contractions (positive inotropy).

Apart from calcium dynamics, the ATPase activity of the myosin head is crucial for regulating the velocity of crossbridge cycling. Higher ATPase activity accelerates both contraction and relaxation. Additionally, phosphorylation of contractile proteins such as cMyBP-C, MHC, MLC, cTm, and cTnI enhances myofilament kinetics and contractile performance. Conversely, dephosphorylation leads to diminished contractility. These mechanisms represent calcium-independent modulation of cardiac muscle performance [12].

For extrinsic factors, one of the key factors is the sarcomere length prior to the contraction, termed *resting sarcomere length*, which reflects the *preload of the heart*. The relationship between sarcomere length and the force of contraction follows the length-tension relationship, also known as the *Frank-Starling law*. This principle states that myocardial contractile force increases with sarcomere length up to an optimal range, where maximal overlap between actin and myosin filaments allows for the highest crossbridge formation. Another important extrinsic factor is the *force-frequency relationship*, which describes how contractile force changes in response to the *heart rate*. As stimulation frequency increases, cardiac muscle may show either enhanced contractility (positive staircase or Bowditch phenomenon) due to the greater CICR. However, at excessively high frequencies, incomplete Ca^{2+} reuptake into the SR leads to reduced SR calcium content and weaker contractions (negative staircase or Woodworth phenomenon). Following a pause, SR calcium stores can be replenished, resulting in a stronger contraction upon resumption (recuperative effect) [4].

Cardiac cells also respond to various chemokines which exert their effects by binding to specific G-protein coupled receptors (GPCRs). Among GPCRs, those coupled to G_s stimulate adenylyl cyclase (AC), increasing cyclic AMP (cAMP) production. This activates protein kinase A (PKA), which phosphorylates multiple intracellular targets, enhancing Ca^{2+} availability and improving contractility and relaxation (positive inotropy and lusitropy). A key example is the β_1-adrenergic receptor (β_1-AR), which responds to norepinephrine from sympathetic nerves and epinephrine from the adrenal medulla. In contrast, receptors coupled to G_i inhibit the AC-cAMP-PKA pathway. Muscarinic M_2 receptors, activated by acetylcholine from the parasympathetic nervous system, reduce both contractility and relaxation (negative inotropy and lusitropy). Intracellular cAMP levels are also regulated by phosphodiesterases (PDEs), which degrade cAMP to inactive AMP. PDE inhibitors enhance cAMP signaling and thus cardiac performances (**Figure 8A**). GPCRs coupled to G_q activate phospholipase C (PLC), which hydrolyzes PIP_2 into diacylglycerol (DAG) and inositol triphosphate (IP_3). DAG activates protein kinase C (PKC), while IP_3 releases Ca^{2+} from the SR. These pathways collectively elevate intracellular Ca^{2+} and promote phosphorylation of contractile proteins, enhancing contractility and relaxation. Examples of G_q-linked receptors include α_1-adrenergic receptors (stimulated by norepinephrine), angiotensin II type 1 receptors (AT_1R), and endothelin-1 type A receptors (ET_AR), all of which contribute to positive inotropy and lusitropy (**Figure 8B**) [13].

4. The heart as a rhythmic organ

The heart is a unique organ capable of rhythmic contraction even when isolated from the body. Due to its intrinsic ability to generate and propagate electrical impulses independently, these impulses stimulate the cardiac muscle cells to contract in a coordinated fashion. This principle is well illustrated in Wigger's diagram, which demonstrates the three key events in cardiac function: electrical changes, mechanical

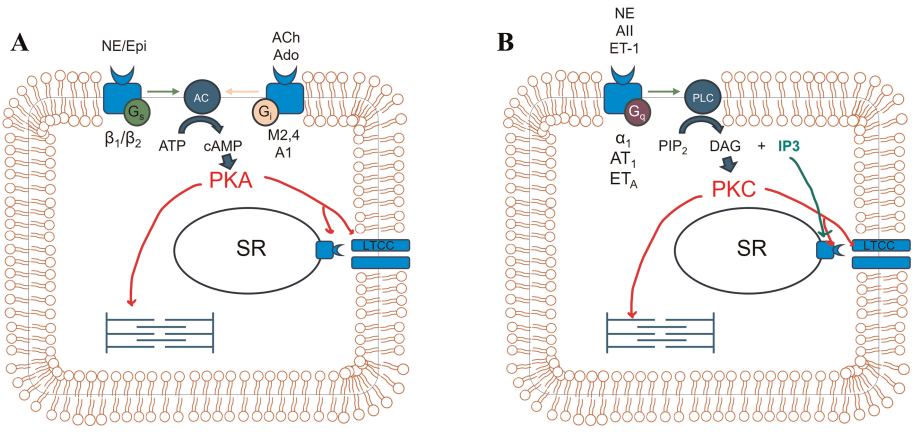


Figure 8.
Important intracellular signaling pathways in cardiac muscle cells. (A) Adenylyl cyclase (AC) converts ATP into cyclic AMP (cAMP), which activates protein kinase A (PKA). This pathway is stimulated via Gs-coupled receptors and inhibited via Gi-coupled receptors. (B) Gq-coupled receptor activation stimulates phospholipase C (PLC), which hydrolyzes phosphoinositol diphosphate (PIP2) into diacylglycerol (DAG) and inositol triphosphate (IP3). DAG activates protein kinase C (PKC), while IP3 binds to ryanodine receptors (RyR) on the sarcoplasmic reticulum (SR), triggering the release of Ca^{2+} and further promotes the cardiac contraction (Adapted from Ref. [8]).

contraction, and hemodynamic flow (**Figure 9**) [6]. Understanding this rhythmic property is fundamental to the study of cardiovascular physiology, with electrophysiology serving as the cornerstone of this field.

4.1 Cardiac conduction system

As previously discussed, the heart contains specialized cells known as pacemaker cells, which can spontaneously generate electrical impulses [11]. In addition to this autorhythmic property, the heart has its own conduction system that enables the rapid and organized transmission of these impulses throughout the myocardium. This coordination is facilitated by gap junctions, which serve as electrical connections between cells, allowing the signal to propagate efficiently from one cell to another. The electrical impulse originates from the sinoatrial (SA) node and travels through the internodal pathways, activating atrial muscles and reaching the atrioventricular (AV) node. It then continues through the bundle of His and down the interventricular septum via the right and left bundle branches, eventually reaching the Purkinje fibers, which distribute the impulse to the ventricular myocardium for synchronized contraction (**Figure 6**).

The SA node, the primary pacemaker of the heart, is located near the foramen ovale at the junction of the cranial and caudal vena cava [4]. Functionally, it acts as the captain, who determines the firing rate and coordinates the rhythm of the entire heart. This electrical activity is generated via HCN (funny) channels, which allow the SA node to spontaneously reach the threshold and generate action potentials. The firing rate varies among species—approximately 70–160 beats per minute in dogs and 120–240 in cats [11]. The SA node is under autonomic control. Sympathetic stimulation releases norepinephrine, which binds β_1-AR, activates G_s proteins, and increases cAMP, leading to greater HCN channel activity, a steeper pacemaker slope, and increased firing rate (positive chronotropy). Conversely, parasympathetic stimulation releases acetylcholine, which binds M_2-ACh receptors, activates G_i proteins, reduces cAMP, and slows the pacemaker rate (negative chronotropy).

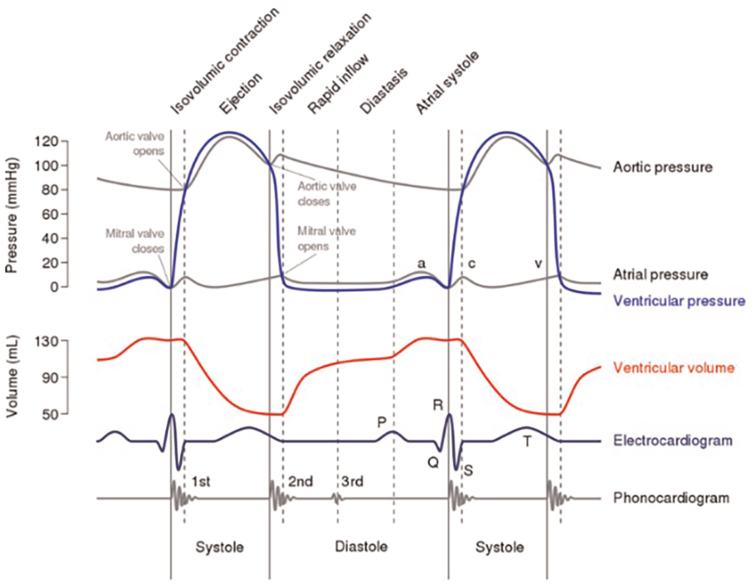


Figure 9.
Wigger's diagram illustrates the sequential changes of multiple physiological events that occur throughout each phase of the cardiac cycle. It begins with electrical events, which can be observed from the electrocardiogram. These electrical signals initiate mechanical events, including changes in pressure and volume within each cardiac chamber, and drive hemodynamic events, or the flow of blood through the heart, resulting in the opening and closing of cardiac valves. These valve movements generate heart sounds, which can be recorded and analyzed through the phonocardiogram.

The internodal pathway, which transmits the signal from the SA to the AV node, consists of three primary tracts: the anterior tract (Bachmann's bundle), the middle tract (Wenckebach's), and the posterior tract (Thorel's) [11]. These pathways ensure depolarization and contraction of the atrial muscle before converging at the AV node. At the AV node, another pacemaker that regulates the conductivity of the heart, It governs ventricular excitation, which is critical for maintaining effective hemodynamics. Located in the central fibrous body between the atria and ventricles, the AV node represents the slowest conduction zone in the heart, allowing a delay that ensures ventricular filling. The AV node also has pacemaker potential, with a firing rate of 40–60 beats per minute. Like the SA node, it is modulated by the autonomic nervous system. Sympathetic stimulation steepens the pacemaker slope, speeding up conduction (positive dromotropy), while parasympathetic input slows conduction (negative dromotropy).

Following AV nodal delay, the impulse travels through the bundle of His and down the right and left bundle branches within the interventricular septum to the apex of the heart. Along this path, the impulse branches into the Purkinje system, ensuring rapid and uniform activation of the ventricular myocardium. This synchronized contraction allows effective ejection of blood from the ventricles into the aorta. When the electrical activity from all these myocardial cells is recorded, it forms a composite vector that can be represented graphically as an electrocardiogram (ECG).

4.2 Generation of the electrocardiogram (ECG)

Electrocardiography is a method for recording the changes in electrical potential across the body, with the heart being the predominant contributor due to its significant electrical activity. The principle of this technique involves detecting the

summation vector of electrical activity in the heart [4]. When an electrical wave propagates in the same direction as the lead vector, it produces a positive deflection on the ECG tracing. Conversely, if the electrical signal moves in the opposite direction, a negative deflection is observed. When the electrical signal travels perpendicular to the lead vector (90° angle), the recorded signal is isoelectric (zero) due to the cosine of 90°. For example, the standard ECG recordings often place the anode near the sino-atrial (SA) node and the cathode near the apex, as the summation of the cardiac electrical vector typically points from base to apex. Therefore, an electrical wave moving toward the apex (cathode) generates a prominent upward deflection, whereas a wave moving in the opposite direction results in an inverted deflection.

When ECG signals are continuously recorded over time, characteristic waveforms corresponding to different phases of cardiac activity can be observed. These include the *P wave*, *QRS complex*, and *T wave*. The *P wave* reflects *atrial depolarization*, initiated by the SA node and propagated through the internodal pathways before reaching the AV node. Because the electrical activity travels toward the apex (and cathode), the P wave is typically upright. The conclusion of the P wave indicates that atrial depolarization is complete, and electrical impulses are momentarily delayed within the AV node. Following this, the impulse proceeds through the AV node and is transmitted via the *bundle of His* into the ventricular conduction system, producing the *QRS complex*. This complex begins with a *Q wave*, which appears negative due to initial depolarization through the right bundle branch away from the cathode. It is followed by a large *positive R wave*, as the signal simultaneously moves down both bundle branches along the interventricular septum (IVS) toward the apex. As the impulse travels from the apex toward the left ventricular free wall, away from the cathode, a negative *S wave* is recorded. The end of the QRS complex signifies that the ventricles are fully depolarized. Lastly, the *T wave* represents *ventricular repolarization*, which may occur from epicardium to endocardium (resulting in an upright wave) or from endocardium to epicardium (producing an inverted wave), depending on the species. *Atrial repolarization* generally occurs during ventricular depolarization and is thus not visible on the ECG unless the heart rate is markedly slow [11].

4.3 Basic interpretation of electrocardiography (ECG)

Before interpreting the recorded electrocardiogram, initial considerations including the animal species and the lead used for interpretation should be concerned. In veterinary medicine, lead II is generally used as its vector direction closely aligns with the heart's mean electrical axis. Additionally, machine settings such as paper speed and sensitivity must be considered, as they affect the assessment of time intervals and waveform amplitudes.

The first step in ECG interpretation involves a general *screening of the ECG* waveform: assessment of *rhythm*, *rate*, and the *origin* of each electrical impulse.

- *Rhythm*: The cardiac rhythm can be categorized as regular or irregular. This is assessed by examining the R-R intervals. Consistent R-R intervals indicate a regular rhythm, while varying R-R intervals indicate arrhythmia.
- *Heart rate* Heart rate is classified into three categories: normal (species-dependent, e.g., 60–160 bpm in dogs, 120–240 bpm in cats), bradycardia (slower than normal), and tachycardia (faster than normal). In most irregular rhythms, HR can be calculated from the number of heartbeats in a 6-second interval and multiply by 10 (e.g., 7 beats in 6 seconds = 70 bpm) (**Figure 10**).



Figure 10.
Estimation of heart rate. Within a 6-second interval (green line), eight heartbeats are shown. Hence, the heart rate of this dog in a minute is approximately 80 bpm.

- *Impulse origin* Normally, impulses originate from the SA node, producing a P-wave followed by a QRS-T complex. Absence of the P-wave with normal QRS may indicate junctional (AV node) origin. A wide and bizarre QRS without a preceding P-wave suggests a ventricular origin, where the signal arises from Purkinje cells or ventricular myocytes (**Figure 11**).

After the screening of ECG interpretation, *detailed ECG* should be analyzed with the normal values (**Table** 1) in the following parameters:

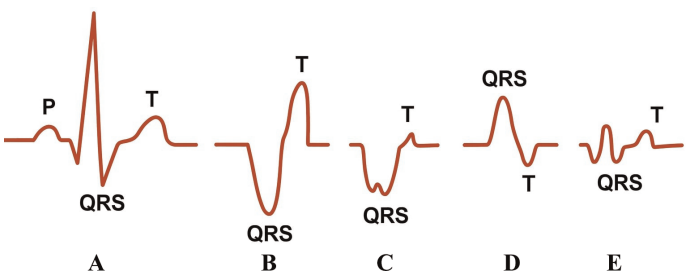


Figure 11.
Display of various QRS complex forms in the ECG. (A) Normal QRS complex; (B–E) Abnormal QRS morphologies indicating ectopic impulse originated from the ventricular muscles (Adapted from Ref. [11]).

	Dog	Cat
Heart rate (bpm)	Adult 70–160 Puppy 70–220	120–240
P-wave duration (sec)	<0.04 (Large breed <0.05)	<0.04
P-wave amplitude (mV)	<0.4	<0.2
PR interval (sec)	0.06–0.13	0.05–0.09
QRS duration (sec)	< 0.05 (Large breed <0.06)	<0.04
R wave amplitude (mV)	< 2 (Large breed <2.5	<0.9
ST segment (mV)	Depression < 0.2 Elevation < 0.15	No depression/elevation
QT interval (sec)	0.15–0.25	0.12–0.18

Table 1.
ECG parameters along with their normal reference ranges in dogs and cats.

4.4 Mechanisms of arrhythmias

Understanding the mechanisms behind arrhythmia is crucial for effective diagnosis and treatment. Generally, arrhythmias are classified into bradyarrhythmias (slow rhythms) and tachyarrhythmias (fast rhythms), based on their underlying electrophysiological abnormalities [14].

4.4.1 Bradyarrhythmias

1. *Reduced automaticity*: This mechanism is often linked to increased parasympathetic activity. Activation of M_2 receptors inhibits the AC-cAMP-PKA signaling pathway, decreasing the activity of I_f and $I_{Ca,T}$ channels, and leads to a shallower pacemaker slope. Therefore, the ECG of the animals may appear as a normal ECG wave with slower heart rate (*sinus bradycardia*), and/or with prolonged PR interval, characterized as a *first-degree AV block* (**Figure 12**).

2. *Dysfunction of the sinoatrial (SA) node*: may manifest as sinus pause, sinus arrest, or SA exit block. In sinus pause or arrest, the P-wave disappears unpredictably, whereas SA exit block features P-wave disappearance in multiples of the cardiac cycle length. When sinus arrest exceeds 5 seconds, systemic perfusion is compromised. In such cases, subsidiary pacemakers in the AV node or Purkinje system may initiate escape beats, identifiable on ECG as "junctional or ventricular escape beat", respectively. These abnormalities are associated with clinical signs such as syncope or lethargy and are collectively referred to as sick sinus syndrome, commonly seen in breeds like West Highland White Terriers, Schnauzers, and Irish Setters.

3. *Conduction blocks*: may occur due to the impairment of electrical signal transmission between the atria and ventricles (AV block) or the transduction within the bundle branch (Bundle branch block).

   - *First-degree AV block* presents as a prolonged PR interval without rhythm disruption.

   - *Second-degree AV blocks* include Mobitz type I, characterized by progressively lengthening PR intervals until a QRS complex is dropped, and Mobitz type II, which shows a fixed P-to-QRS ratio with intermittent QRS absence. The atropine challenge test (0.04 mg/kg IV or SC) distinguishes

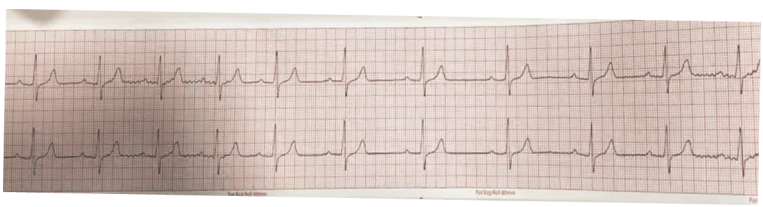


Figure 12.
An electrocardiogram (lead II, 50 mm/sec) was obtained from an English Mastiff presenting with heat stroke. The tracing revealed a prolonged PR interval of 0.14 seconds, consistent with a diagnosis of first-degree atrioventricular (AV) block.

between vagal and structural causes—resolution of the block and increased heart rate indicate vagal origin (**Figure 13**).

- *Third-degree AV block (complete heart block)*, atrial and ventricular rhythms become completely dissociated, producing unrelated P-waves and QRS complexes, which can severely impact cardiac output (**Figure 14**).

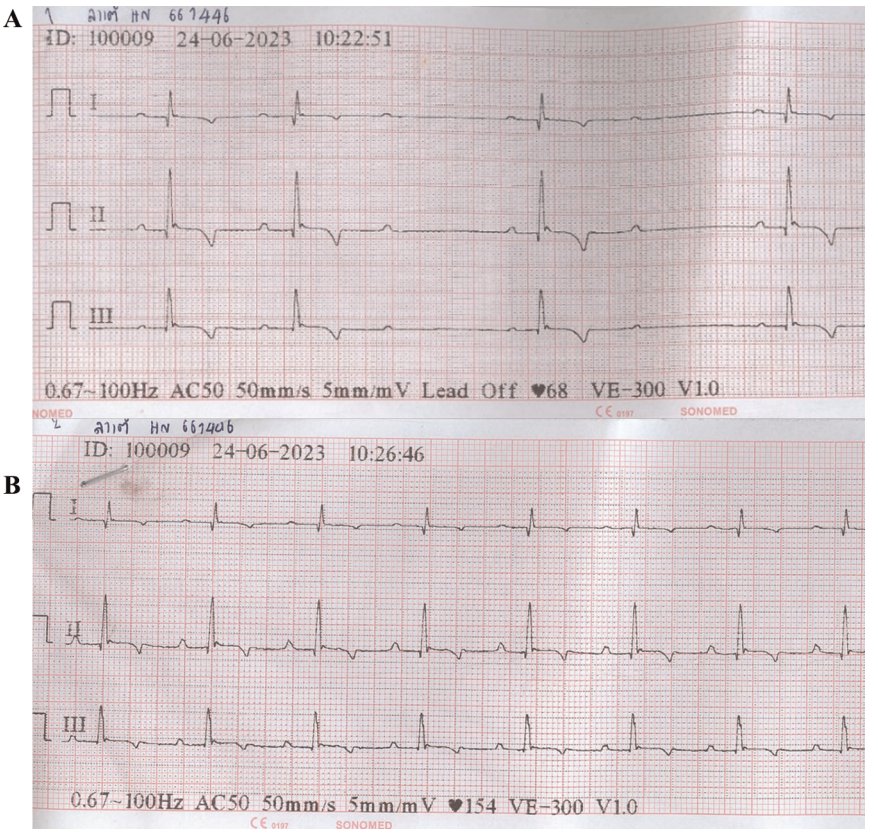


Figure 13.
Electrocardiogram from a Shih Tzu dog undergoing pre-anesthetic evaluation (Lead I–III; 50 mm/s). (A) The ECG shows a slow heart rate with progressively lengthening PR intervals, eventually resulting in a P wave not followed by a QRS complex—findings consistent with second-degree AV block, Mobitz type I. (B) Following intravenous administration of atropine at a dose of 0.04 mg/kg, the heart rate increased immediately, and the previously observed AV block resolved. This response suggests that the AV block was due to increased vagal tone (vagal overtone).

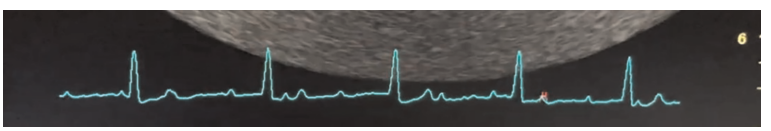


Figure 14.
An ECG (lead II, 50 mm/s) recorded from a cat diagnosed as hypertrophic cardiomyopathy. The completely dissociation between P-wave and QRS complex while demonstrating consistent PP- and RR-intervals indicates complete heat block.

- *Bundle branch block* occurs when the conduction is delayed or blocked within the ventricular pathways. In right bundle branch block (RBBB), electrical impulses travel from left to right, producing an upward initial Q wave followed by a deep inverted R wave, resulting in right axis deviation. In left bundle branch block (LBBB), impulses take an alternative route through right-sided pathways, producing a wider and taller QRS complex with delayed activation.

4.4.2 Tachyarrhythmias

1. *Increased automaticity*: The mechanism is typically triggered by the overstimulation of sympathetic nervous system. Literally, the norepinephrine released from the nerve terminal binds to β_1-adrenergic receptors, activating the G_s-AC-cAMP-PKA pathway. This cascade enhances the activity of both I_f and $I_{Ca,T}$ channels, resulting in acceleration of the heart rate on ECG and represented as *sinus tachycardia*.

2. *Enhanced or abnormal automaticity* may cause non-SA nodal cells to function as pacemakers. Subsidiary pacemaker cells (e.g., AV node or Purkinje fibers) or even ventricular myocytes may develop intrinsic rhythmicity. This results in *ectopic beats or premature complexes*, classified based on their origin: junctional premature complexes lack a P-wave and feature normal QRS complexes; supraventricular premature complexes show normal or altered P-waves with normal QRS complexes; ventricular premature complexes (VPCs) appear as wide, bizarre QRS complexes without a preceding P-wave. The continuation of these abnormalities may lead to more life-threatening conditions like *ventricular tachycardia*, which significantly affects the cardiac hemodynamics (**Figure 15A**).

3. *Triggered activity*. The mechanism occurs because of afterdepolarizations, where the cardiac myocyte is being stimulated during the repolarization phases. For

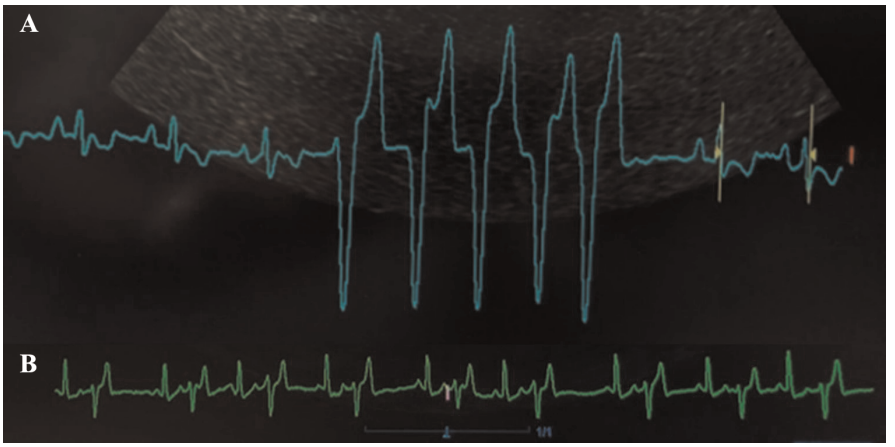


Figure 15.
Examples of tachyarrhythmias in dogs. (A) An ECG (lead II; 50 mm/s) recorded from a dog with mitral valve disease concurrent with heartworm infestations. The bizarre beats revealed five consecutive beats of ventricular premature complexes, indicating paroxysmal ventricular tachycardia. (B) An ECG (lead II, 25 mm/s) obtained from a Yorkshire terrier diagnosed with severe mitral valve disease accompanied with severe left atrial enlargement induced atrial septal aneurysm and atrial septal defect.

early afterdepolarizations (EADs), which occur during phase 2 or 3 of the action potential, it may cause repetitive QRS complexes, such as couplets, triplets, or Torsades de Pointes—a polymorphic ventricular tachycardia that can progress to ventricular fibrillation. *Delayed afterdepolarizations (DADs)*, originating in phase 4, may cause ectopic beats interspersed with normal complexes, such as *atrial or ventricular bigeminy* (**Figure 15B**).

4. *Re-entrant mechanisms* are established when there is unidirectional block, dual pathways, and differing conduction velocities. This causes continuous looping of electrical signals, manifesting as supraventricular tachycardias such as *atrial flutter or fibrillation*. These arrhythmias overwhelm the AV node with rapid impulses, resulting in fast, irregular ventricular responses that compromise cardiac efficiency.

5. Ventricles as muscular pumps

The ventricular myocardium plays a critical role in generating the pressure necessary to propel blood throughout the circulatory system. Functioning like a hydraulic pump, the ventricles must develop sufficient force to overcome vascular resistance and ensure the effective distribution of oxygen and nutrients to peripheral tissues. At the cellular level, the myocardial cells contract and relax—represented by changes in tension (force generation) and cell length. However, when considering the heart as a hollow three-dimensional structure, the tension and length of myocardial function are transformed into pressure and volume of the heart.

As aforementioned, the mechanical activity of cardiac muscle cells can be conceptually divided into relaxation (diastole) and contraction (systole) phases. During the relaxation phase, the heart receives blood and lengthens the resting sarcomere length of the cardiomyocytes. The more blood returns into the heart (venous return), the more muscles stretch. Therefore, the amount of blood in the heart during the end-diastolic phase is considered as the *preload* of the heart. During the contraction phase, the muscle has to generate high pressure to overcome the pressure in the systemic circulation and drive the blood throughout the body. Hence, the arterial pressure is considered as the *afterload* of the heart.

5.1 The cardiac cycle

While the ventricular cardiac cycle can be broadly divided into two main phases, a more detailed breakdown as illustrated by the Wigger's diagram (**Figure 9**). The cardiac cycle would therefore divide into five physiological phases based on electrical, mechanical, and hemodynamic events as follows [6]:

5.1.1 Atrial systole

The contraction of both atria occurs immediately after being depolarized (shown by P-wave on ECG). During this stage, electrical impulses originated from the SA node are transmitted to the AV node via internodal pathways, stimulating atrial muscle contraction. As the atria contracts, atrial pressure rises, and blood is actively propelled into the ventricles. This results in increased ventricular volume and pressure.

5.1.2 Isovolumic contraction

After the impulse is being relayed at the AV node, the signal further transmits into all ventricular muscle via the Bundle branches and Purkinje-fiber system. The ventricles are being depolarized (as shown by the QRS complex on ECG) and contract to generate the pressure. At this phase, the ventricular pressure increases sharply, exceeding atrial pressure and causing closure of both AV valves to prevent backflow. However, because the pressure remains below that in the aorta and pulmonary artery, the semilunar valves remain closed, and no blood is ejected. Consequently, ventricular volume remains constant with the elevation of the ventricular pressure.

5.1.3 Ventricular ejection phase

Once ventricular pressure surpasses arterial pressure, the semilunar valves open, and blood is ejected from the ventricles into both systemic and pulmonary circulations. During the early *rapid ejection*, the ventricular pressure increases rapidly with the reduction in the ventricular volume. Nevertheless, as the ventricles begin repolarizing (as shown by T-wave on ECG), contractile force declines, with the *reduced ejection* rate, even though ventricular pressure remains above arterial pressure.

5.1.4 Isovolumic relaxation

During the relaxation period, when ventricular pressure falls below arterial pressure, the semilunar valves are closed and ended the ejection period. Since the ventricular pressure is still higher than atrial pressure, the AV valves remain closed, and no blood enters or leaves the ventricles.

5.1.5 Early ventricular filling

As ventricular pressure declines further and falls below atrial pressure, the AV valves open, allowing passive blood flow from atria to move into ventricles. This *passive filling* increases both ventricular volume and pressure. Once ventricular pressure approximates or exceeds atrial pressure, the flow slows, leading to a period known as *diastasis*, where there is minimal change in volume or pressure. This phase continues until the SA node initiates the next cycle.

5.2 Factors influencing cardiac output

Before exploring the key factors that influence cardiac function, it is essential to understand several foundational terms. Firstly, *stroke volume (SV)* refers to the volume of blood ejected from the heart with each cardiac cycle. When multiplied by the *heart rate (HR)*, expressed in beats per minute (bpm), the result is the *cardiac output (CO)*—the total volume of blood the heart pumps per minute. Stroke volume is calculated as the difference between *end-diastolic volume (EDV)*, the volume of blood in the ventricle before contraction, and *end-systolic volume (ESV)*, the volume remaining after contraction. Another important concept is *venous return (VR)*, which refers to the volume of blood returning to the heart [2].

Cardiac output is a key measure of the cardiovascular system's ability to meet the body's metabolic demands. It represents the total volume of blood distributed systemically per minute. Given the relationships outlined above, $CO = SV \times HR$ and

SV = EDV − ESV, it follows that cardiac output is directly influenced by EDV, ESV, and HR (**Figure 16**). According to the equations, cardiac output is major regulated by 4 important factors:

- *Preload* refers to the stretch of the ventricular myocardium at the end of diastolic phase, or the *end-diastolic volume (EDV)*, and is influenced by:
    - *Venous return*: An increase in blood volume returning to the heart (i.e. intravenous fluid administration) enhances preload.
    - *Ventricular compliance (lusitropy)*: A compliant ventricle can accommodate greater filling, leading to increased preload. Reduced compliance impairs filling, reduces EDV, and compromises stroke volume.
    - *Diastolic filling time*: Adequate time is needed for ventricular filling during diastole. Tachycardia or arrhythmias may shorten diastolic time and reduce preload.
- *Afterload:* Defined as the pressure the ventricle must overcome to eject blood. High arterial pressure (afterload) increases ESV by impeding ejection, thereby reducing SV.
- *Contractility*: Enhanced myocardial contractility promotes greater ejection of blood, and improves SV. Conversely, weakened contraction leads to elevated ESV and reduced stroke volume.
- *Heart rate*: Heart rate is regulated by the autonomic nervous system and plays a pivotal role in determining cardiac output. Within physiological limits, increases in HR can raise CO. However, excessive heart rates, especially due to arrhythmias, may impair ventricular filling and consequently reduce stroke volume and overall cardiac efficiency.

However, CO is not a static value—it fluctuates based on the metabolic activity of the body. For instance, aged or neutered animals typically have reduced basal

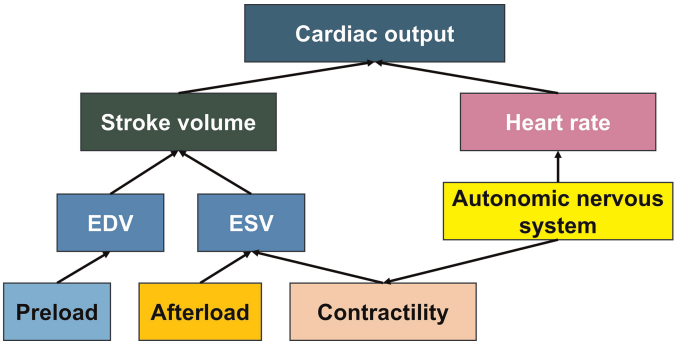


Figure 16.
Factors regulating cardiac performance. Cardiac output is determined by both stroke volume the amount of blood pumped with each heartbeat, and heart rate. Indeed, stroke volume—is influenced by three primary factors: preload (the volume of blood in the ventricles before contraction), afterload (the resistance the heart must overcome to eject blood), and contractility (the intrinsic ability of the myocardium to contract). Meanwhile, heart rate is regulated by the autonomic nervous system, which adjusts cardiac rhythm based on physiological demands.

metabolic rates, whereas those undergoing exercise or experiencing excitement exhibit elevated metabolic demands. Additionally, CO is influenced by body size, with larger animals requiring higher metabolic output than smaller ones. To account for size differences, cardiac output is normalized to body surface area, resulting in the *cardiac index (CI)*, a more accurate measure of cardiac performance. When the heart is underperforming (hypoeffective), such as during excessive parasympathetic stimulation, CO is reduced. Conversely, the heart can be overperforming (hypereffective) by the activation of the sympathetic nervous system.

6. Cardiac valves and heart sounds

6.1 Cardiac valves and their functional mechanisms

As previously discussed, mammals typically possess four heart valves, which are divided into two categories: *atrioventricular (AV) valves*, including mitral and tricuspid valves, separate the atria from the ventricles. The *mitral valve*, which regulates flow between the left atrium and left ventricle, has two leaflets, whereas the *tricuspid valve*, located between the right atrium and right ventricle, has three [5]. These atrioventricular valves are supported by *chordae tendineae* connected to *papillary muscles* within the ventricles to ensure that the valves open toward the ventricles and prevent retrograding blood flow into the atria. During systole, contraction of papillary muscles helps anchor the valve leaflets, preventing them from prolapsing into the atria. In diastole, when the ventricles relax and atrial pressure rises due to venous return, the AV valves open passively, allowing blood to flow from atria to ventricles. For example, the *tricuspid valve* opens to permit flow from the right atrium to the right ventricle, while the *mitral valve* does so from the left atrium to the left ventricle (**Figure 17**).

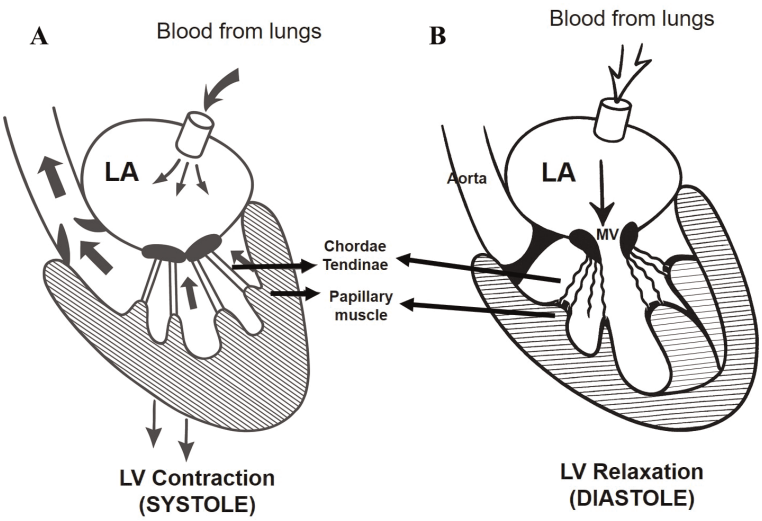


Figure 17.
Illustration of the atrioventricular (AV) valve mechanisms: (A) During systole, even though ventricular pressure rises significantly, contraction of the papillary muscles tethers the valve leaflets, preventing them from prolapsing into the atria. This mechanism helps prevent backflow of blood into the atrial chambers. (B) During diastole, the AV valves open into the ventricles as the pressure in the atria exceeds that in the ventricles (Adapted from [5]).

On the other hand, *semilunar valves*, including *aortic and pulmonic valves*, are consisted of three crescent-shaped cusps. In contrast to AV valves, they lack supporting structures, and the opening and closure are passively responded to pressure differences between the ventricles and the great arteries. During *systole*, while the intraventricular pressure is increasing, it forces these valves to open, and allow the blood to unidirectional pump out of the ventricle into aorta and pulmonary artery, respectively. Upon *diastole*, a reverse pressure gradient causes them to close, preventing backflow into the ventricles.

6.2 Heart sounds

In addition to ensuring unidirectional blood flow through the heart (cardiodynamics), the opening and closure of cardiac valves also generate abrupt changes in blood movement, producing audible *heart sounds*. These sounds result from the sudden deceleration of blood flow as it encounters closed valve leaflets. To detect and analyze these sounds, specialized sound amplification devices such as *cardiomicrophones* can be used. These instruments capture and amplify the sounds and convert them into graphical waveforms known as a *phonocardiogram (PCG)*. The PCG is a valuable tool for identifying abnormal intracardiac flow dynamics and evaluating pathological changes in blood flow patterns. Within each *cardiac cycle*, *four distinct heart sounds* may be detected (See **Figure 9**):

- *S1 (First Heart Sound)*: This sound arises from the abrupt closure of the *atrioventricular (AV) valves*—the mitral and tricuspid valves. As blood impacts the closed leaflets, a short, low-pitched "*Lubb*" sound is generated. It marks the onset of *ventricular systole*.

- *S2 (Second Heart Sound)*: Produced by the closure of the *semilunar valves* (aortic and pulmonic), this sound occurs at the beginning of *ventricular diastole* and is heard as a brief "*Dubb*" sound.

- *S3 (Third Heart Sound)*: Occurring shortly after S2, this soft, low-pitched sound ("*Dhu*") results from the passive, rapid filling of the ventricles during early diastole. While generally inaudible under normal conditions, S3 may become more pronounced in the presence of increased blood volume or *ventricular stiffness*, such as in volume overload states or cardiomyopathies.

- *S4 (Fourth Heart Sound)*: Heard just before S1, S4 arises during *atrial contraction* (atrial systole), when blood is forcefully ejected from the atria into a ventricle that is already partially filled. This sound, described as a soft "*Be*," becomes more prominent when a large volume of blood is pushed against a non-compliant ventricle.

Each of these heart sounds provides important diagnostic insight into cardiac function and pathophysiological conditions. While S1 and S2 are routinely audible during auscultation, S3 and S4 may only be detected under abnormal physiological or pathological circumstances and require attentive auscultation techniques or specialized equipment for proper evaluation.

6.3 Stethoscope and auscultation technique

The unaided human ear has a limited capacity to perceive heart sounds. Typically, to hear human speech, the auditory system requires sound pressure levels above 1/10 Pascal and frequencies exceeding 512 Hz. In contrast, heart sounds often range from 1/1000 to 1/10 Pascal with frequencies between 64 and 512 Hz—rendering them difficult to detect without amplification [4]. This necessitates the use of a device to amplify or transduce sound signals. While cardiomicrophones can serve this purpose, they are less practical for use in animals and may amplify background noise, such as hair or skin friction. These limitations led to the invention of the *stethoscope*, a more versatile and practical tool for clinical auscultation.

A standard stethoscope comprises several components (**Figure 18**):

- *Eartips*: Soft pieces that fit snugly into the ear canal.
- *Eartube*: Metal tubes that connect the eartips to the tubing.
- *Tubing*: Typically rubber or flexible plastic with a lumen that transmits and amplifies the sound wave signals.
- *Chest piece*: The part placed against the body, divided into two sides:
    - *Bell*: Designed to capture low-frequency sounds. (e.g., heart murmurs), often located deeper in the chest.
    - *Diaphragm*: Optimized for high-frequency sounds like those from the *lungs* or *small animal hearts*.

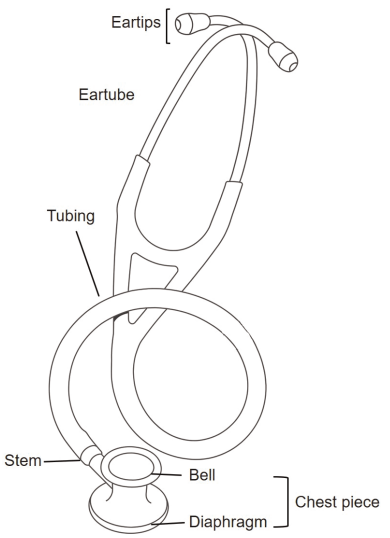


Figure 18.
Diagram illustrating the structure and components of a medical stethoscope.

6.4 Heart sound characterization

Auscultation of the heart begins with precise placement of the chest piece over specific cardiac valve landmarks, as abnormal valve function can significantly alter both blood flow and heart sounds [1]. The *mitral valve* is best heard at the 5th–6th intercostal space on the left side, just caudal to the olecranon. The *aortic valve* is auscultated at the 3rd–4th intercostal space, slightly cranial to the mitral area, while the *pulmonic valve* lies slightly more cranial and ventral. On the right side, the *tricuspid valve* is heard at the 4th–5th intercostal space. In general, atrioventricular (AV) valves —mitral and tricuspid—are best heard during the *first heart sound (S1)*, whereas the semilunar valves—aortic and pulmonic—are more prominent during the *second heart sound (S2)*. This approach is applicable across various mammalian species, including dogs, cats, rodents, cattle, and horses (see **Figure 19**). Besides cardiac auscultation, a stethoscope can also detect tracheal sounds, bronchovesicular sounds, lung sounds, and ruminal contraction sounds. However, the primary focus here is the evaluation of heart sounds, which can be classified by *rhythm* (regular or irregular) and *sound quality* (normal, murmur, or abnormal splitting such as gallop rhythms).

Under normal laminar flow, blood produces soft physiological heart sounds. However, *turbulent or regurgitant flow* results in *heart murmurs*. Murmurs may arise not only from valvular defects but also from altered hemodynamics, as described by the *Reynold's number* (*Re*):

$$\text{Re} = (\text{velocity} \times \text{diameter} \times \text{blood density})/\text{viscosity}$$

Increased *velocity* (e.g., from excitement, valvular stenosis, or outflow tract obstruction), decreased *viscosity* (e.g., anemia), or increased *density* (e.g., hyperproteinemia) can all increase Re and the likelihood of murmurs, even in structurally normal hearts—referred to as *physiological murmurs*. However, if the valvular is abnormally presence, inducing abnormal blood flow, we may hear a *pathological murmur*. These two conditions can be distinguished by structural evaluation such as echocardiography or higher imaging tools [1].

While evaluating heart murmurs, clinicians should follow a structured approach by considering four key aspects. *First, timing*: identify whether the murmur occurs during

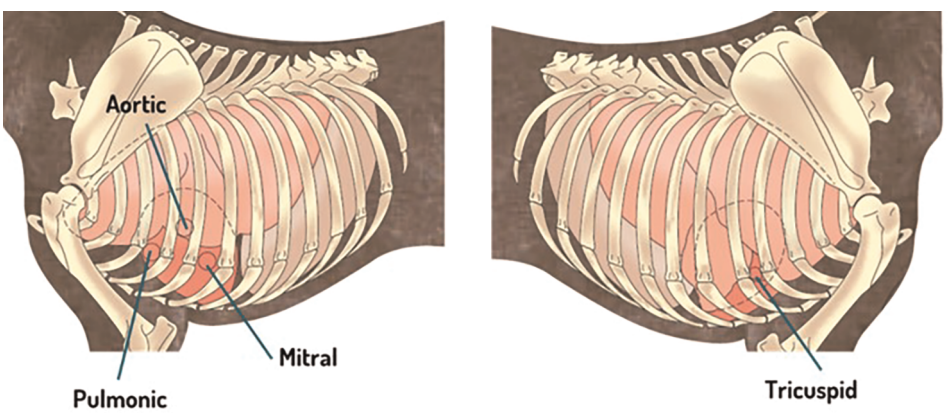


Figure 19.
Auscultation landmarks for cardiac valves in dogs. (A) The left thoracic wall allows auscultation of the pulmonic, aortic, and mitral valves. (B) On the right side of the thorax, heart sounds corresponding to the tricuspid valve can be heard [1].

systole (contraction phase) or diastole (relaxation phase), which helps determine the functional phase of the abnormality. *Second, location*: assess where the murmur is loudest—its point of maximal intensity offers valuable diagnostic insight. For example, a systolic murmur at the heart base suggests aortic or pulmonic stenosis, while a diastolic murmur at the heart base indicates aortic or pulmonic regurgitation. Murmurs over the mitral area during systole point to mitral regurgitation, and during diastole to mitral stenosis. On the right thorax, systolic murmurs suggest tricuspid regurgitation, *while* diastolic murmurs indicate tricuspid stenosis. *Third, the characteristics of the murmur* helps identify the pathology: a holosystolic murmur has constant intensity and is typical of mitral or tricuspid regurgitation; a crescendo–decrescendo murmur increases then decreases in intensity, often seen in aortic or pulmonic stenosis; a diastolic decrescendo murmur softens during diastole, as in aortic or pulmonic regurgitation; and a continuous murmur, such as that in patent ductus arteriosus, spans both systole and diastole and resembles a machine-like sound. *Fourth, murmur intensity is graded* from 1 to 6: grades 1–2 are very soft and require a quiet setting, grade 3 is clearly audible, grade 4 is loud with thoracic radiation, and grades 5–6 are very loud—with grade 6 audible even when the stethoscope is lifted from the chest—and often accompanied by a palpable precordial thrill.

In addition to murmurs, clinicians should recognize *abnormal heart sounds* that reflect underlying pathology [15]. *Split heart sounds*, such as *split S1* (asynchronous closure of mitral and tricuspid valves) and *split S2* (asynchronous closure of aortic and pulmonic valves), indicate discoordination in valve function. The *S3 sound*, occurring during early ventricular filling, suggests increased preload or ventricular stiffness, while the *S4 sound*, heard during atrial contraction, is associated with ventricular hypertrophy or poor compliance. A *systolic click*, a high-pitched sound between S1 and S2, often signals *left ventricular outflow tract obstruction* due to *systolic anterior motion* of the mitral valve—commonly seen in cats with hypertrophic cardiomyopathy. While it can be difficult to precisely distinguish these sounds by ear, *modern electronic stethoscopes* with *phonocardiogram (PCG)* capabilities allow for waveform visualization, improving diagnostic accuracy. Dual-mode stethoscopes that simultaneously record *ECG and PCG* enhance the evaluation of cardiac timing and function (**Figure 20**).

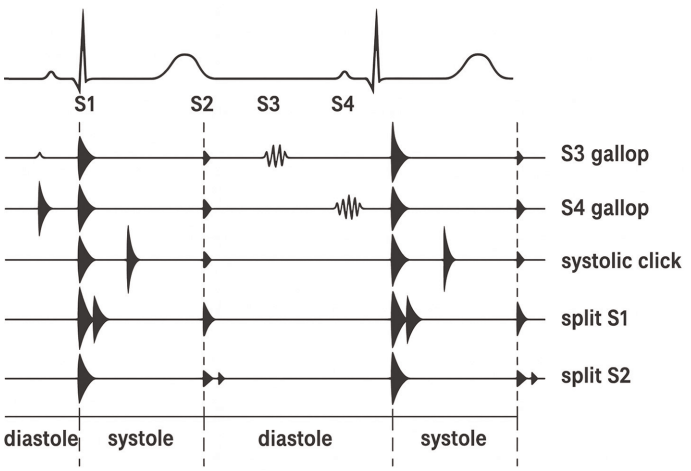


Figure 20.
Illustration of abnormal heart sound waveforms characterized by the presence of more than two audible sounds. These include heart sounds occurring during S3 or S4 (gallop rhythms), splitting of S1 or S2, and systolic clicks occurring between S1 and S2. Such findings indicate structural or hemodynamic abnormalities within the heart [15].

7. Conclusion

The heart is a vital organ whose structure and function are designed to support life by maintaining continuous circulation of blood throughout the body. Each anatomical component—from the myocardial architecture, conduction system, cardiac valves, to the ventricular functions—plays a synergistic role to maintain optimal cardiovascular performance. A comprehensive understanding of the electrical and mechanical events within the cardiac cycle, including electrocardiographic interpretation, hemodynamic assessment, and evaluation of heart sounds through auscultation or phonocardiography, is fundamental to identifying normal physiology and detecting pathological alterations. In veterinary medicine, mastery of cardiac anatomy and physiology provides the cornerstone for diagnosing and managing cardiovascular diseases, while also guiding the advancement of diagnostic tools, therapeutic strategies, and monitoring technologies. With continued progress in the fields of electrophysiology and molecular cardiology, the ability to preserve and restore cardiac function is increasingly refined—underscoring the enduring importance of foundational knowledge in heart structure and function.

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Nomenclature

Afterload	resistance against which the heart must pump
AV node	atrioventricular node; delays impulse before ventricular activation
chronotropy	heart rate modulation
contractility	strength of myocardial contraction
dromotropy	conduction velocity through the AV node
ECG	electrocardiogram; recording of heart's electrical activity
HCN channel	hyperpolarization-activated cyclic nucleotide-gated channel (funny channel)
inotropy	force of myocardial contraction
L-type Ca^{2+} channel	long-lasting calcium channel; critical in plateau and pacemaker depolarization
lusitropy	rate of myocardial relaxation
Na^+/K^+ pump (Na^+/K^+-ATPase)	maintains resting membrane potential by exchanging Na^+ efflux and K^+ influx at a ratio of 3:2

NCX	sodium-calcium exchanger; regulates Ca^{2+} efflux
PCG	phonocardiogram; recording of heart sounds
PMCA	plasma membrane calcium ATPase; actively transports Ca^{2+} out of the cell
preload	volume of blood in ventricles at end-diastole
Reynold's number (Re)	dimensionless number indicating potential for turbulent flow
S1, S2, S3, S4	heart sounds associated with valve closure and blood flow
SA node	sinoatrial node; primary pacemaker of the heart
T-type Ca^{2+} channel	transient calcium channel; involved in early pacemaker depolarization

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Edited by Arnoldo González-Reyna and Kevin Sargent

As animal scientists and producers, we truly believe that animal care is an essential part of our production systems, regardless of the animal species. In addition, animal handling and good production practices provide the cornerstone for the production of high-quality foods for human consumption; animal care and welfare for pet and companion animals are also needed for better human living conditions. Thus, this book is intended to provide current and updated information on several aspects of animal welfare and its impact, and, as such, animal welfare must be given its real importance accordingly. This book is presented in two sections that cover several topics on two main aspects of Animal Welfare and Care: Section I, entitled Animal Production, Health and Welfare, and Section II, entitled Animal Care and Welfare. In Section I, several chapters on animal management, production, and health are presented. In contrast, Section II presents several chapters on basic aspects of animal care, laboratory species, and physiology. As part of the Editorial Team of IntechOpen, we are fully aware that this book is not intended to present a comprehensive review of all aspects of animal welfare; instead, the authors of each chapter have provided a full, in-depth, and updated review of the current situation for each chapter topic. On behalf of the authors and the Editorial Team of IntechOpen, we believe this book will be a useful and practical reference for scientists, academics, students, and producers.

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