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CHALLENGES IN FETAL SEX DETERMINATION BY ULTRASONOGRAPHY IN CATTLE: THE GESTATIONAL PERIOD FACTOR AND DIAGNOSTIC WINDOW

DESAFIOS NA SEXAGEM FETAL POR ULTRASSONOGRAFIA EM BOVINOS: O FATOR PERÍODO GESTACIONAL E JANELA DE DIAGNÓSTICO

DESAFÍOS EN LA DETERMINACIÓN DEL SEXO FETAL POR ULTRASONOGRAFÍA EN BOVINOS: EL FACTOR DEL PERÍODO GESTACIONAL Y LA VENTANA DIAGNÓSTICA

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ABSTRACT

Fetal sexing is the procedure performed to identify the sex of the fetus during gestation. In cattle, fetal sex determination by ultrasonography represents an important tool for reproductive management, as it enables strategic herd planning and supports productive and economic decision-making. However, its accuracy may be influenced by the gestational period in which the examination is performed, as well as by embryonic and fetal development, in addition to factors related to the experience and technical skill of the operator conducting the examination, which may directly impact diagnostic reliability and productivity. In this context, the present study aimed to analyze the factors influencing fetal sex determination by ultrasonography in cattle, particularly gestational age and the diagnostic window. Findings from the literature indicate that, despite existing challenges, ultrasonography remains the most feasible and cost-effective method for fetal sexing in cattle production systems.

Keywords: Fetal Sexing. Ultrasonography. Cattle. Gestational Period.

RESUMO

A sexagem fetal é o procedimento realizado para identificar o sexo do feto durante a gestação. Em bovinos, a sexagem fetal por ultrassonografia representa uma ferramenta importante para o manejo reprodutivo, visto que permite o planejamento estratégico do rebanho, além de contribuir para a tomada de decisões produtivas e econômicas. No entanto, sua precisão pode ser influenciada pelo período gestacional em que o exame é realizado, bem como pelo desenvolvimento embrionário e fetal, além de fatores relacionados à experiência e à habilidade do operador responsável pela realização do

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exame, os quais podem impactar diretamente a confiabilidade do diagnóstico e a produtividade. Diante desse cenário, o presente trabalho teve como objetivo analisar os fatores que influenciam a sexagem fetal por ultrassonografia em bovinos, como a idade gestacional e a janela diagnóstica. Os achados da literatura indicam que, apesar dos desafios, a ultrassonografia permanece como o método mais viável e de baixo custo para a sexagem fetal em sistemas de produção bovina.

Palavras-chave: Sexagem Fetal. Ultrassonografia. Bovinos. Período Gestacional.

RESUMEN

La sexación fetal es el procedimiento realizado para identificar el sexo del feto durante la gestación. En bovinos, la determinación del sexo fetal mediante ultrasonografía representa una herramienta importante para el manejo reproductivo, ya que permite la planificación estratégica del rebaño y contribuye a la toma de decisiones productivas y económicas. Sin embargo, su precisión puede verse influenciada por el período gestacional en el que se realiza el examen, así como por el desarrollo embrionario y fetal, además de factores relacionados con la experiencia y la habilidad del operador responsable del procedimiento, los cuales pueden impactar directamente la confiabilidad del diagnóstico y la productividad. En este contexto, el presente estudio tuvo como objetivo analizar los factores que influyen en la determinación del sexo fetal por ultrasonografía en bovinos, especialmente la edad gestacional y la ventana diagnóstica. Los hallazgos de la literatura indican que, a pesar de los desafíos existentes, la ultrasonografía sigue siendo el método más viable y de bajo costo para la sexación fetal en sistemas de producción bovina.

Palabras clave: Sexación Fetal. Ultrasonografía. Bovinos. Período Gestacional.

1 INTRODUCTION

Early identification of fetal sex in cattle is a strategic reproductive management tool that directly impacts the economic efficiency and market planning of rural properties (Kim et al., 2025). In modern livestock, the use of transrectal ultrasonography has been consolidated as the gold standard method for this purpose, allowing phenotypic differentiation between males and females in the first third of gestation (Kim et al., 2025). This information becomes relevant, since the preference for the sex of the calf varies according to the productive objectives of the property, with males being prioritized in meat production systems, while females are largely destined for reproduction (Kim et al., 2025). From the embryological point of view, sexual differentiation in cattle begins in the first weeks of gestation, and is dependent on progressive morphological events that culminate in the migration of the genital tubercle, a key structure for the sonographic identification of fetal sex (Curran et al., 1989).

However, the accuracy of ultrasound diagnosis is strictly dependent on the chronology of embryofetal development, specifically in relation to genital tubercle migration (Sánchez et al., 2021). Although recent advances in molecular and proteomic biomarkers seek alternative methods of sexing, ultrasonography remains challenging due to the need for an accurate "diagnostic window" where the fetus is of adequate size and positioning for the visualization of anatomical structures (Kim et al., 2025; Blacka et al., 2024). Understanding the time constraints and biological factors that influence this technique is critical to minimizing diagnostic errors and optimizing industry profitability (Kim et al., 2025).

Several techniques have been developed for sex determination. Embryo sexing, performed through biopsy or molecular analysis of preimplantation embryos, has high precision, but it is an invasive and high-cost method, making it unfeasible for application in production systems (Kim et al., 2025). Amniocentesis, in a similar way, is also an invasive procedure, which limits its use. Thus, among the available methods, the use of ultrasound stands out for being easily applicable in field conditions, providing fast results, and presenting excellent cost-benefit (Kim et al., 2025).

2 METHODOLOGY

This study is characterized as a narrative literature review, structured with the purpose of synthesizing and evaluating contemporary scientific evidence about the

challenges in fetal bovine sexing by ultrasound. The theoretical foundation was built by searching the PubMed database, using the descriptors "Fetal Sexing", "Cattle" and "Ultrasound". These terms were articulated through the Boolean operators AND and OR, following the terminology criteria of the Medical Subject Headings (MeSH). The selection covered scientific articles published in the last five years, with full text available and written in Portuguese or English, focused on the central theme. Productions with low adherence to the theme, duplicates and studies with methodological fragility were excluded. The selection process involved the screening of titles and abstracts, followed by the technical analysis of the full texts, with the final organization of the information in a descriptive and critical manner.

3 RESULTS AND DISCUSSION

The efficiency of fetal sexing by ultrasound in cattle is governed by the interaction between the stage of biological development and the technical skill of the operator.

- **The Optimal Diagnostic Window**:D and according to clinical evidence, the period between days 55 and 80 of gestation is considered the ideal window for performing ultrasound sexing (Kim et al., 2025). Before this interval, the genital tubercle — the precursor structure of the penis or clitoris — has not yet migrated to its definitive position (near the umbilical cord in males and near the base of the tail in females), which makes safe differentiation impossible (Sánchez et al., 2021). After 80 days, the increase in the volume of fetal fluids and the descent from the uterus to the abdominal cavity make it difficult to reach the transducer and the manipulation necessary to obtain the appropriate imaging planes (Kim et al., 2025).

Historically, the feasibility of fetal sexing via ultrasonography is based on the chronology of genital tubercle migration, initially described by Curran et al. (1989). Although sexual differentiation begins early, the diagnostic accuracy presents a progressive behavior: around day 49 of gestation, the success rate is still limited, rising progressively until it reaches levels close to 100% from day 56, when the genital tubercle reaches its definitive anatomical position (ALI; FAHMY, 2008). This dynamic shows that the diagnostic window is not a fixed point, but a biological interval in transition, in which premature interpretations can result in misclassification of the external genitalia.

- **Biological Foundations and Markers of Differentiation**: Gonad differentiation and external morphology are complex processes that occur simultaneously with

molecular changes in the amniotic fluid and maternal plasma (Sánchez et al., 2021). Although ultrasonography focuses on external morphology, studies indicate that the presence of specific microRNAs and the profile of amelogenin are already established in the period in which image sexing is performed (Sánchez et al., 2021; Blacka et al., 2024). In addition, cow physiological factors, such as the composition of the reproductive tract mucus, can influence the gestational environment, although their direct relationship with ultrasound imaging accuracy still lacks further investigation (Huang et al., 2024).

- **Maternal and Technical Determinants of Diagnostic Accuracy:** In addition to the rigorous chronological factor, the accuracy of fetal sexing by ultrasound is influenced by a complex interaction between individual matrix characteristics and management variables, which can compromise image quality and diagnostic reliability. The body condition score (BCS) and the anatomical conformation of the cow have a direct impact on uterine accessibility; Animals with excessive perirectal fat deposition or large body size often have greater depth of the reproductive tract, which makes it difficult to bring the transducer closer to the fetus. Added to this, the presence of intestinal gas or a large volume of fecal content acts as an acoustic barrier, generating artifacts that obscure subtle structures such as the genital tubercle. In these circumstances, obtaining adequate image planes requires high technical dexterity and careful manipulation to position the conceptus in contact with the active face of the transducer (Curran; Pierson; Ginther, 1991; Kim et al., 2025). The estimation of gestational age based exclusively on the date of Artificial Insemination (AI) is a critical limitation in the clinical routine, due to the asynchrony between induction and effective ovulation. As the window for identifying genital tubercle migration is restricted, biological variations of only 3 to 5 days may be decisive for diagnostic accuracy (Curran et al., 1989; Sánchez et al., 2021). In this context, the literature recommends the integration of fetal biometry — specifically crown-rump length — as a prerequisite to mitigate risks of premature phenotypic classifications (Ali; Fahmy, 2008). Additionally, evidence suggests that the uterine microenvironment and cervical mucus influence the gestational trajectory, reinforcing that the accuracy of the test is inseparable from individual maternal physiology (Huang et al., 2024).

- **Technical Challenges and Economic Impacts**: Key practical challenges include fetal positioning, uterine depth, and fetal motility during the examination. Failure to correctly diagnose sex within the recommended window can result in lost market opportunity, especially in production systems that use sexing to value replacement females or males for feedlot (Kim et al., 2025). Systematic adoption of ultrasound for sexing has been shown to significantly increase profitability on Hanwoo cattle farms, serving as a model for how diagnostic accuracy converts into economic value (Kim et al., 2025).

Other challenges that imply in the diagnosis of fetal sexing is the mucus composition of the cow's Reproductive Tract, which undergoes changes in viscosity, protein content and profile of microRNAs present, which can impact embryonic viability and interfere with the quality of the ultrasound image, in addition, excessively dense mucus can act as a heterogeneous medium, reducing image sharpness (Huang et al., 2024). Another important factor is that ultrasonography is one of the lowest cost procedures compared to the application of Protogemics in the early determination of fetal sex in cattle, which has a high cost, requires specialized laboratory equipment and professionals trained in proteomic analysis (Sánchez et al., 2021).

- **Imaging and Biomolecular Approaches to Fetal Sexing: Limitations and Applicability:** Although ultrasonography is the predominant method, the emergence of molecular technologies offers new perspectives for early gender identification. Recent studies explore the presence of microRNAs in maternal plasma and amniotic fluid as biomarkers of gonadal differentiation (Sánchez et al., 2021), in addition to the use of proteomics via amelogenin for sex estimation (Blacka et al., 2024). However, when comparing these techniques with imaging, a critical counterpoint is observed: although molecular methods allow diagnosis at very early stages and have high sensitivity, their routine application is limited by the high operational cost and the need for sophisticated laboratory infrastructure. In contrast, ultrasonography, despite being strictly dependent on a diagnostic window and operator skill (Kim et al., 2025), offers an immediate (*point-of-care*) and superior cost-benefit ratio for extensive and intensive production systems. In this context, ultrasonography remains a central tool in the bovine reproductive routine, while molecular methods are configured, in the current scenario, as complementary strategies or applicable to high value-added systems. Future

integration between imaging and biomolecular approaches may represent a promising advance in reducing diagnostic errors and anticipating productive decision-making.

In an integrated way, the findings show that diagnostic accuracy results from the interaction between embryofetal maturation, precision in estimating gestational age, and maternal conditions that impact image quality (Curran et al., 1989; There; Fahmy, 2008; Kim et al., 2025). Small temporal variations, especially associated with ovulatory asynchrony, or maternal anatomical limitations can shift the test to a biologically borderline period, increasing the risk of error even when the technique is properly performed (Sánchez et al., 2021; Huang et al., 2024). Thus, ultrasound interpretation requires a careful approach and integration between biometric parameters and maternal-fetal physiological knowledge.

4 CONCLUSION

Fetal sexing by ultrasound in cattle has established itself as a strategic tool in reproductive management, being widely used due to its practical applicability and cost-benefit in production systems (Kim et al., 2025). However, its diagnostic accuracy is intrinsically conditioned to the chronology of embryofetal development, especially to the migration of the genital tubercle, directly linked to the optimal diagnostic window (Sánchez et al., 2021).

The literature shows that the ideal diagnostic window, predominantly between 55 and 80 days of gestation, does not represent only a technical operational interval, but also reflects the synchronization between the anatomical consolidation of the primary marker and the capacity for sonographic detection (Ali; Fahmy, 2008; Kim et al., 2025). Early examinations can result in misinterpretations due to incomplete migration of the genital tubercle, while late evaluations are influenced by increased uterine volume, uterine depth, and difficulty in fetal manipulation (Kim et al., 2025).

In addition to the temporal factor, diagnostic accuracy is multifactorial. Maternal characteristics, such as body condition score and anatomical conformation, as well as technical variables related to operator experience and image quality, have a direct impact on the reliability of the examination (Kim et al., 2025; Huang et al., 2024). In addition, evidence indicates that molecular alterations, such as microRNA expression and amelogenin profile, are already established during the period corresponding to image

sexing, although such methods still have operational limitations for routine field application (Huang et al., 2024).

In this context, it is concluded that fetal sexing by ultrasound should be understood as a biologically dependent and technically sensitive procedure, whose efficacy is associated with the gestational period factor, diagnosis window, and the integration between embryological knowledge, fetal biometry, and professional training (Kim et al., 2025). Although biomolecular approaches represent promising prospects for early diagnosis, ultrasonography remains, in the current scenario, the most viable and economically sustainable method for cattle production systems (Sánchez et al., 2021).

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