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Higher National Veterinary School. Rabie Bouchama
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TOPIC

Early pregnancy diagnosis methods in cow

Presented by:

Mr. SAMSAR Mamoune Abdelmounaim

Miss TAHAR LAKOUES Hadjer

Miss YEDDOU Meriem Naila

Publicly graduated on 03/07/2024 before the jury:

Mr. SOUAMES Samir	Professor (ENSV)	President
Mrs. AOUANE Nedjma	MCB (ENSV)	Supervisor
Mr. LAMARA Ali	Professor (ENSV)	Examiner

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Dedication

To every single person in my small circle of people...

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To my dear sisters Fatima, Meriem and Houda.

To my dear little brother Mourad.

To my dear auntie Hadjira.

To my cousins, Sam, Lynda, abderrahmmane, Mohamed, Youcef and Nouh.

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To my bestie Naila and her family.

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To group09 members...

To everyone who believed in me..

TAHAR LAKOUES Hadjer

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This thesis is dedicated:

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To my mentors and teachers, who have guided me with wisdom and patience, Shaping not Just my knowledge, but my character.

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May this work contribute, even if modestly, to the vast ocean of knowledge.

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ملخص:
يُعَدُّ التشخيص المبكر للحمل لدى الأبقار عنصرًا أساسيًا في إدارة التكاثر الفعالة للماشية. تحلل هذه الدراسة طرقًا مختلفة تُستخدم للتشخيص المبكر للحمل، بما في ذلك الجس التقليدي، والموجات فوق الصوتية، والاختبارات الهرمونية، وأطقم الاختبار السريعة الجديدة. الهدف الرئيسي هو تقييم التبني، والفعالية، والتحديات المرتبطة بهذه الطرق بين الأطباء البيطريين في الجزائر. تم توزيع استبيان شامل يتضمن 20 سؤالًا على 65 طبيبًا بيطريًا عبر 15 منطقة في الجزائر، مما أسفر عن معدل استجابة بلغ 50 استبيانًا مكتملًا. جمعت الاستطلاع بيانات كمية ونوعية حول خلفيات الممارسين، وأنواع وسلالات الأبقار، وتوقيت التشخيص المفضل، واستخدامهم وإدراكهم لطرق التشخيص المختلفة. أشارت النتائج إلى تفضيل قوي للجس التقليدي، حيث فضَّل 80% من الأطباء البيطريين هذه الطريقة بسبب عمليتها وتكلفتها المنخفضة وسهولة تنفيذها. ومع ذلك، لوحظت بعض القيود مثل نقص الدقة وعدم القدرة على تقييم صلاحية الجنين. كانت الموجات فوق الصوتية، التي استخدمها 46% من المستجيبين، ذات قيمة بسبب دقتها المتزايدة وقدرتها على الاكتشاف المبكر وتقييم صلاحية الجنين، على الرغم من العقبات مثل التكلفة العالية للمعدات والمهارات التقنية المطلوبة. كانت معدلات تبني الاختبارات الهرمونية وأطقم الاختبار السريعة أقل، لكنها كانت معترف بها لفوائدها المحتملة في الكشف المبكر والدقيق عن الحمل. تسلط الدراسة الضوء على الاختلافات الإقليمية في الممارسة وتحدد الاتجاهات التي يمكن أن توجه التحسينات المستقبلية في ممارسات إدارة التكاثر لقطعان الأبقار الجزائرية، بهدف تحسين الإنتاجية والكفاءة في القطاع في نهاية المطاف.

الكلمات المفتاحية:
التشخيص المبكر للحمل، الأبقار، الجس التقليدي، الموجات فوق الصوتية، الاختبارات الهرمونية.

Abstract:
Early pregnancy diagnosis in cows is a critical component of effective cattle reproduction management. This study analyzes various methods employed for early pregnancy diagnosis, including traditional palpation, ultrasound, hormone assays, and newer rapid test kits. The primary objective is to evaluate the adoption, effectiveness, and challenges associated with these methods among veterinarians in Algeria. A comprehensive questionnaire comprising 20 questions was distributed to 65 veterinarians across 15 regions in Algeria, achieving a response rate of 50 completed surveys. The survey collected both quantitative and qualitative data on practitioners' backgrounds, types and breeds of cows, preferred timing for diagnosis, and their use and perceptions of different diagnostic methods. The findings indicate a strong preference for traditional palpation, with 80% of veterinarians favoring this method due to its practicality, low cost, and ease of implementation. However, limitations such as lack of precision and inability to assess fetal viability were noted. Ultrasound, used by 46% of respondents, was valued for its increased accuracy, early detection capability, and ability to assess fetal viability, despite barriers like high equipment cost and required technical skills. Hormonal assays and rapid test kits had lower adoption rates but were recognized for their potential benefits in early and accurate pregnancy detection. The study highlights regional differences in practice and identifies trends that can inform future improvements in reproductive management practices for Algerian cattle herds, ultimately aiming to enhance productivity and efficiency in the sector

Key Words:
Early pregnancy diagnosis, Cows, Traditional palpation, Ultrasound, Hormonal assays.

Résumé :
Le diagnostic précoce de la gestation chez les vaches est un élément crucial de la gestion efficace de la reproduction bovine. Cette étude analyse différentes méthodes utilisées pour le diagnostic précoce de la gestation, notamment la palpation traditionnelle, l'échographie, les dosages hormonaux et les kits de test rapides plus récents. L'objectif principal est d'évaluer l'adoption, l'efficacité et les défis associés à ces méthodes chez les vétérinaires en Algérie. Un questionnaire complet comprenant 20 questions a été distribué à 65 vétérinaires répartis dans 15 régions en Algérie, obtenant un taux de réponse de 50 questionnaires complétés. L'enquête a collecté des données quantitatives et qualitatives sur les antécédents des praticiens, les types et races de vaches, les périodes préférées pour le diagnostic, ainsi que leur utilisation et leurs perceptions des différentes méthodes de diagnostic. Les résultats indiquent une forte préférence pour la palpation traditionnelle, 80 % des vétérinaires favorisant cette méthode en raison de sa praticité, de son faible coût et de sa facilité de mise en œuvre. Cependant, des limitations telles que le manque de précision et l'incapacité à évaluer la viabilité fœtale ont été notées. L'échographie, utilisée par 46 % des répondants, a été valorisée pour son augmentation de précision, sa capacité de détection précoce et son aptitude à évaluer la viabilité fœtale, malgré des obstacles comme le coût élevé de l'équipement et les compétences techniques requises. Les dosages hormonaux et les kits de test rapides avaient des taux d'adoption plus faibles mais étaient reconnus pour leurs avantages potentiels dans la détection précoce et précise de la gestation. L'étude met en lumière les différences régionales dans la pratique et identifie des tendances qui peuvent guider les futures améliorations des pratiques de gestion de la reproduction pour les troupeaux bovins algériens, visant finalement à améliorer la productivité et l'efficacité du secteur.

Mots-clés:
Diagnostic précoce de la gestation, Vaches, Palpation traditionnelle, Échographie, Dosages hormonaux.

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List of abbreviations:

CL= Corpus Luteum

EPF= Early Pregnancy Factor

FSH= Follicle Stimulating Hormone

GnRH= Gonadotrophin Releasing Hormone

LH= Luteinizing Hormone

P4= Progesterone

PAG= Pregnancy-Associated Glycoproteins

Introduction

The early and accurate diagnosis of pregnancy in cows is a critical component of efficient herd management and dairy production. It allows farmers to make informed decisions about breeding programs, nutrition, and overall herd health, ultimately leading to improved reproductive performance and economic gains. Traditional methods of pregnancy detection, such as rectal palpation and observation of estrous behavior, although widely used, have limitations in terms of accuracy and the skill required (Wang et *al.*, 2020).

Advancements in veterinary science and technology have introduced a variety of methods for early pregnancy diagnosis in cows, which cover a range of biochemical tests, such as detecting pregnancy-related glycoproteins (PAGs) in blood and milk, and advanced imaging techniques like ultrasonography (Buragohain, 2017).

This study aims to explore the various early pregnancy diagnosis methods available for cows, comparing their effectiveness, practical application, and potential for integration into modern farming practices.

PART I: BIBLIOGRAPHICAL STUDY

Chapter I: Anatomo-physiological reminders of the sexual cycle in cow

I.1. Genital tract anatomy

I.1.1. Uro-Genital Sinus

I.1.1.1. Vaginal vestibule

The vestibule is a diminutive region within the bovine anatomy that arises from the point at which the urethral opening is situated, and extends towards the rear to merge seamlessly with the labia of the vulva (Hopper, 2021).

I.1.1.2. Vulva

The vulva is composed of the labia, which are situated on either side of the labial fissure. These labia converge dorsally, resulting in the formation of the dorsal commissure. Additionally, the labia also meet ventrally, thereby giving rise to the ventral commissure. Positioned cranially to the ventral commissure, one can locate the clitoris (Budras, 2003).

I.1.2. Tubular Section

I.1.2.1. Oviduct

The oviduct, also known as the uterine tube or fallopian tube, is an essential part of the reproductive system. It serves to transport eggs from the ovary to the uterus and provide the site for fertilization. The oviduct in cows consists of three main parts: the infundibulum, ampulla, and isthmus. The infundibulum is the funnel-shaped opening of the oviduct located near the ovary. It captures the released egg during ovulation. The ampulla is the widest part of the oviduct where fertilization typically occurs. It provides a conducive environment for sperm and egg interaction. The isthmus is the narrowest part of the oviduct, which connects to the uterine horn. It plays a role in transporting the fertilized egg (zygote) to the uterus for implantation. The oviduct in cows is lined with ciliated epithelium and contains smooth muscle that facilitates the movement of eggs through peristalsis and ciliary action (Senger, 2012).

I.1.2.2. Uterus

The bovine uterus possesses a truncated body measuring 3.0 cm in length, accompanied by two elongated uterine horns spanning from 30 to 40 cm, affixed at the point of bifurcation through the intercornual ligament. This particular ligament is segregated into ventral and dorsal components, creating a concavity that serves a valuable purpose in retracting the uterus. The dorsal aspect of the uterine horns exhibits a prominent and unrestrained convex curvature, while the ventral aspect, connected to the broad ligament, displays a smaller concave curvature oriented towards the abdominal cavity. The broad ligaments extend laterally and dorsally to join the dorsolateral abdominal wall, resulting in a distinctive configuration characterized by twisting and curving of the uterine horns. Crucial points of reference for overall orientation during transrectal palpation include the pelvic or pubic brim, the iliac shafts, as well as the obturator and sciatic foramens (DesCôteaux et *al*., 2009).

I.1.2.3. Vagina

The vagina in cows is a part of the female reproductive system, located between the cervix and the vulva **(Figure 1) (Figure 2).** It serves as the site for semen deposition during mating, acts as the birth canal during calving, and allows for the passage of urine. The vaginal walls are composed of multiple layers, including mucosal, muscular, and serosal layers, which provide protection, facilitate copulation, and enable the passage of the calf during birth. Proper management and hygiene are essential to prevent infections and complications like vaginitis or prolapse (Dyce et *al*., 2010).

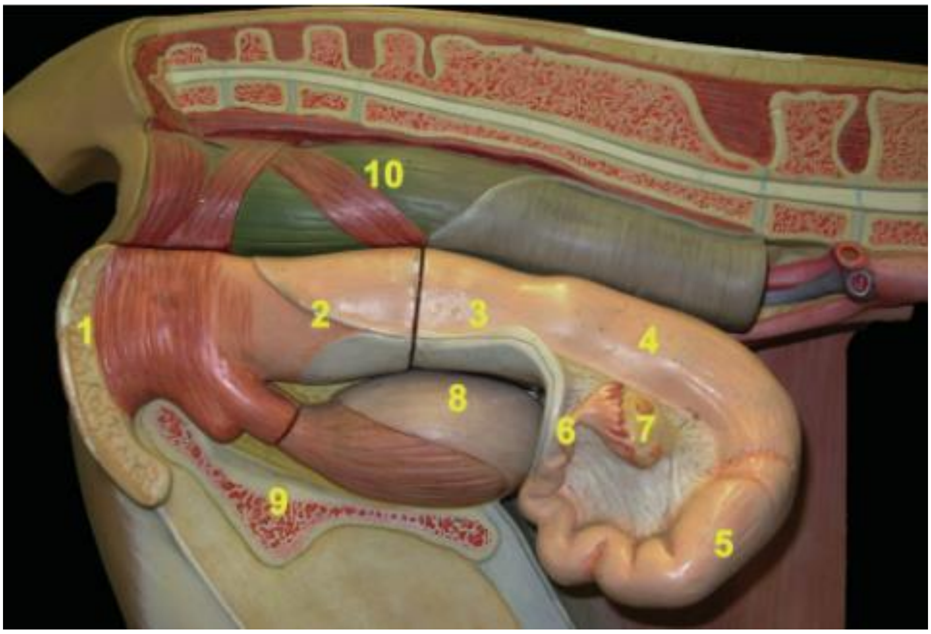

Figure 1: Anatomy of the reproductive tract of the cow (DesCôteaux et *al.,* 2009)**.**

1: Vulva; 2: Vagina; 3: Cervix; 4: Body of the uterus; 5: Right uterine horn; 6: Oviduct; 7: Ovary; 8: Urinary bladder; 9: Ischial bone (cut); 10: Rectum.

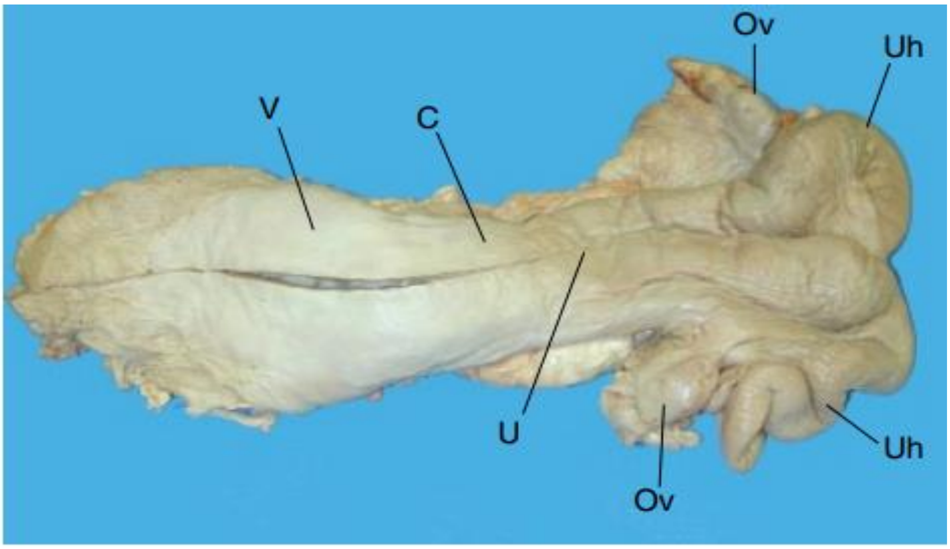

Figure 2: The external anatomy of cow's genital tract (Hopper, 2021)**.**

V: Vagina; C: Cervix; U: Body of uterus; Ov: Ovary; Uh: Uterine horn

I.1.3. Glandular Section

I.1.3.1. Ovaries

The mean dimensions of an ovary lacking functional formations, specifically corpus luteum or a dominant follicle, are approximately 4.0 cm in length, 2.0 cm in width, and 2.5 cm in height. In comparison to heifers, cows possess larger ovaries **(Figure 3)**. The inclusion of corpus luteum, ovarian cyst, or tumor results in an augmented overall size of the ovary. In order to conduct thorough reproductive examinations, it is imperative to consistently assess the ovaries. During pregnancy examinations, the assessment of the ovary permits the practitioner to ascertain the potential for pregnancy based on the presence or absence of CL **(Figure 4)**, the likely position of the pregnancy, the occurrence of double ovulations and the quality of the CL (DesCôteaux et *al.*, 2009).

Figure 3: Active ovary with a corpus luteum and a large dominant follicle (DesCôteaux et *al.*, 2009)**.**

1: Corpus luteum; 2: Dominant follicle; 3: Ovarian stroma; 4: Posterior pole of the ovary.

Figure 4: Inactive ovary (DesCôteaux et *al.*, 2009)**.**

1: Small follicle; 2: Attached border of the ovary; 3: Posterior pole; 4: Anterior pole; 5: Corpus albican.

I.2. Physiology of the estrous cycle

I.2.1. Definition

The estrous cycle can be defined as the regular reappearance at regular intervals of a new opportunity for fertilization and gestation, with the visible reference point being estrus behavior (DesCôteaux & Vaillaincourt, 2012).

The estrous cycle lasts 18-24 days (average of 21 days for multiparous cows) (**Table 1**). During the cycle, morphological, histological, and physiological changes occur periodically within the genital and mammary organs (Noakes et *al.*, 2009).

The cow is a polyestrous species with continuous cycles. Therefore, the cycle is not dependent on seasons (Ball & Peters, 2004).

Table 1: Duration of the different phases of the cow's sexual cycle and timing of ovulation relative to estrus (Perry, 2004).

Species	Proestrus	Estrus	Metestrus	Diestrus	Cycle duration	Ovulation time
Cow	3-4 days	12-18 hours	3-4 days	14-18 days	21 days	10-12 hours post-estrus

I.2.2. Phases of the estrous cycle

The estrous cycle is divided into different phases: Proestrus, estrus, Metestrus, and Diestrus (Gayrard, 2007).

I.2.2.1. Proestrus (Follicular Maturation)

In this phase, the corpus luteum from the previous cycle regresses, and follicular growth is initiated. The uterus slightly enlarges, and the endometrium becomes congested. Only one follicle emerges during this phase, the Graafian follicle containing the ovum (Walker et *al.*, 1996).

I.2.2.2. Estrus (Heat)

Estrus signifies the phase marking the end of follicular maturation and ovulation. This phase is typified by the male's acceptance (Kerbrat & Disenhaus, 2004). Ovulation generally occurs approximately 24 to 32 hours following the onset of estrus (Walker et *al.*, 1996).

Unlike other ruminants where ovulation occurs during estrus, in cows, ovulation occurs approximately 12 hours after the end of estrus (Noakes et *al.*, 2008).

I.2.2.3. Metestrus (luteogenesis)

This phase is characterized by the formation of the corpus luteum from the ovulated follicle. (Noakes et *al.*, 2009).

I.2.2.4. Diestrus

During this phase, the CL is mature and secretes P4. Uterine glands hypertrophy, the cervix closes, and the secretions of the genital tract become weak (Noakes et *al.*, 2009).

I.3. Hormonal control during the first third of gestation

I.3.1. GnRH

GnRH is considered the initiator and fundamental regulator of the reproductive function in mammals, playing a crucial role in regulating biological processes related to reproduction. This peptide is synthesized and released by neurons located in the hypothalamus region, an essential part of the brain involved in regulating many physiological functions. Once produced, GnRH is transported through the portal hypophyseal system, to the anterior lobe of the pituitary gland. When it reaches the pituitary region, GnRH induces the secretion and release of other key hormones, including FSH and LH, which are essential for regulating the reproductive cycle in mammals. These complex hormonal processes are crucial for maintaining hormonal balance and ensuring the fertility of individuals (Kaim et *al.*, 2003).

I.3.2. FSH

Follicle-stimulating hormone is a type of glycoprotein that is produced by the anterior pituitary gland, as indicated by research conducted by INRAP (1988). This hormone plays a crucial role in the regulation of ovarian development and the growth of follicles. Moreover, FSH is responsible for triggering the proliferation of granulosa cells within the ovaries and facilitating the creation of the antrum, a fluid-filled cavity within the ovarian follicle. Additionally, it serves to stimulate the production of estrogen by the follicles, contributing to various physiological processes in the reproductive system. Furthermore, FSH plays a vital preparatory role by initiating the synthesis of receptors for LH.

I.3.3. LH

Luteinizing Hormone is a glycoprotein that is produced by the anterior pituitary gland and plays several important roles: it regulates the last stage of follicle development in conjunction with

FSH, triggers ovulation, and stimulates the creation of the corpus luteum as well as the production of progesterone (INRAP, 1988).

I.3.4. Progesterone

For numerous years, it has been well documented that the level of progesterone in the early stages of pregnancy significantly influences the potential outcome. Several studies have shown that animals experiencing early pregnancy failure exhibit lower levels of plasma progesterone starting around day 12 post-mating (Lukaszewska & Hansel, 1980; Lamming et *al.*, 1989; Mann & Lamming, 1995). Upon reevaluation of Mann et *al.* (1995) data, it was discovered that not only was there an initial variance in milk progesterone levels between day 12 and day 15, but there was also a notably lower concentration of milk progesterone on day 6 in mated cows that did not conceive compared to pregnant cows (**Figure 5**).

A study by Lamming & Darwash (1995) found that a delayed increase in progesterone post-ovulation was linked to a decrease in pregnancy rates in mated animals. These research findings emphasize the negative impact of both a delayed post-ovulatory progesterone rise and low concentrations of progesterone during the luteal phase on the early pregnancy outcome.

Although these studies identify the importance of the concentration of progesterone during early pregnancy, they do not answer the question of how progesterone is exerting its effects. To establish this we must look at the effects of progesterone on the important mechanisms in place during this period. For example, it has now been demonstrated that the concentration of luteal phase progesterone in the cow has a profound influence on the strength of development of the luteolytic signal (Mann & Lamming, 1995).

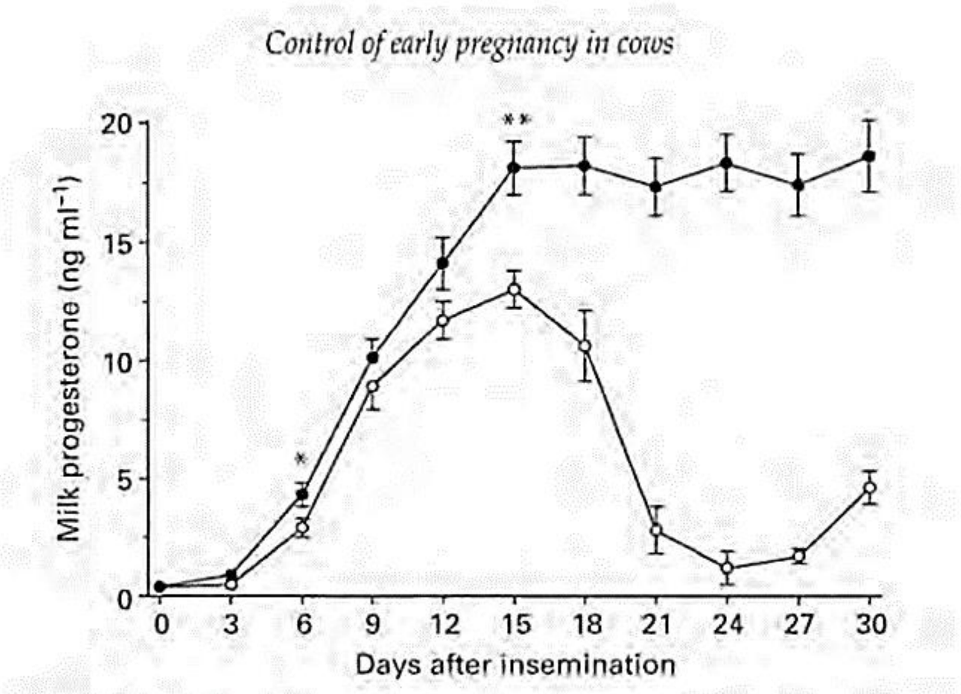

Figure 5: Control of early pregnancy in cows (Mann & Lamming, 1995)**.**

Mean (± sat) milk progesterone concentration after insemination in cows that became pregnant.

(•; n =28) and cows in which pregnancy failed.

(○; n =24). Note the significant differences between pregnant and non-pregnant groups on day 15.

*(P < 0.01) and day 6 (P < 0.05). * P < 0.05; * P < 0.01.*

I.3.5. Estrogen

Most studies suggest that levels of estradiol are similar in mated cows, regardless of whether their pregnancy is successful or unsuccessful (Lukaszewska & Hansel, 1980; Gyawa & Pope, 1992; Mann et *al.*, 1995). A study by Pritchard et *al.* (1994) in beef cows found that cows with higher plasma estradiol levels between day 14 and day 17 had a lower pregnancy rate. However, in some cows, luteolysis had already started during the study, making it unclear whether the elevated estradiol levels caused the pregnancy failure or were a result of unsuccessful embryonic inhibition of luteolysis. Therefore, current evidence indicates that estradiol does not possess the same level of influence as progesterone on early pregnancy outcomes.

Chapter II: Stages of early embryonic development

II.1. Fertilization

After ovulation, the egg moves towards the ovarian bursa due to the suction movements of the auricle and the vibrational movements of the cilia in the tubal cilia. The sperm ascend the genital tract at 15 mm per minute to reach the egg (Derivaux & Ectors, 1980). In the initial segment of the oviduct, the bulb of the tube, multiple sperm cells attach to the egg membrane. However, only one sperm cell can penetrate the egg, which marks the occurrence of fertilization. The pronuclei of the egg and the sperm fuse 45 hours after the beginning of heat (Tainturier, 2003).

II.2. Development in the fallopian tube

Twenty-four hours after fertilization, the egg divides to form 16 cells on 4-5th day while maintaining its size. It is called Morula (**Figure 6**) (Tainturier, 2003).

Figure 6: Photomicrographs of cleavage stages of eggs fertilized in vitro (Anonymous1, 2016).

II.3. Development in the uterus

The embryo moves into the oviduct and eventually reaches the uterine lumen (INRAP, 1988). Small cavities begin to form at the periphery of the morula then they merge to form a cavity known as the blastocyst, the blastocyst consists of three distinct structures:

- A cellular layer around the periphery: trophoblast (future appendages),
- A thickening of this layer: embryonic button and
- A blastocoel cavity

Around days 7 and 8, the blastocoel volume increases, and the cell count goes up from 70 to 180. This causes the trophoblast to apply pressure on the pellucid zone.

On day 9, the embryo experiences a rapid amplification in volume, wherein it expands and subsequently contracts, causing a rupture in the zona pellucida, which releases the blastocysts. After this, the embryonic trophoblast sends out pseudopods that will enter each uterine horn (**Figure 7**) (Bai et *al.*, 2013).

Between days 12 and 14, the embryonic disc develops, and the allantois manifests on the 18th. On the 20th day, Implantation occurs and lasts for several weeks (Tainturier, 2003). Placentation starts on day 21, the uterus upon implantation is in the secretory phase; the blastocyst is implanted in the endometrium along the anterior or posterior wall (Bai et *al.*, 2013).

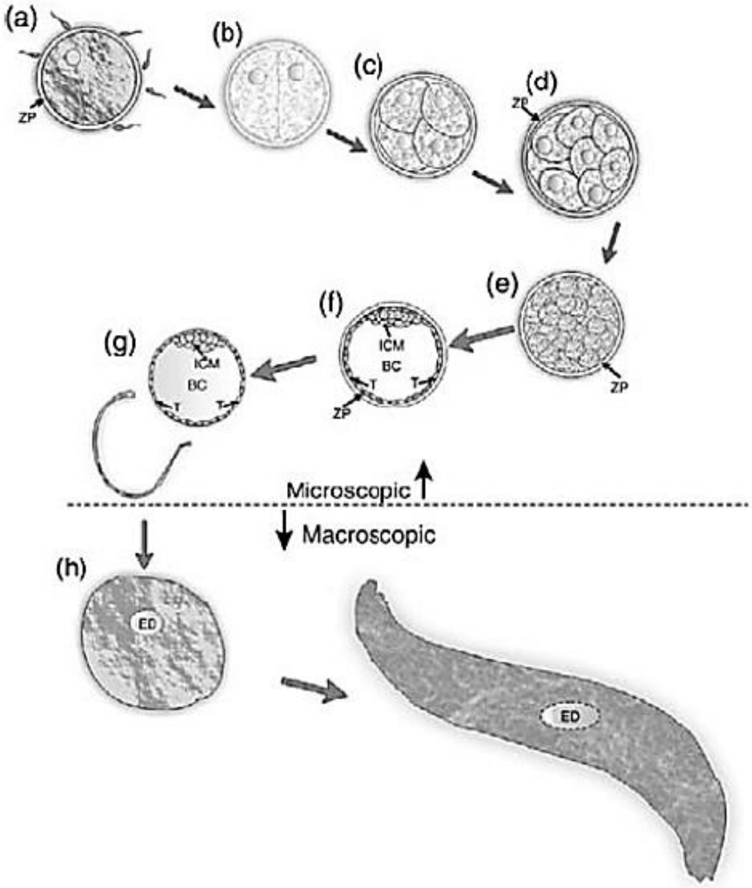

Figure 7: Embryonic development from fertilization to elongation of the chorionic vesicle (Engelhardt & King, 2011).

(a) Fertilization, union of the spermatozoon and the ovum to form the zygote or embryo; ZP = Zona pellucida. (b) First division, begins approximately 24 hours after fertilization, producing an embryo

with two cells (= blastomeres). (c) Second division, occurring less than 12 hours after the first division, producing 4 blastomeres. (d) Third division, occurring a few hours later, producing 8 blastomeres. (e) Subsequent divisions producing a morula with 16 to 64 blastomeres. (f) Divisions leading to the formation of a blastocyst consisting of an embryonic knob (ICM), trophoblast (T), and blastocyst (BC). (g) Pressure inside the blastocyst increases inducing rupture of the zona pellucida, thus allowing the embryo to increase in size. (h) The embryo (= embryonic disc (ED)) grows rapidly within the blastocyst. (i) The spherical blastocyst rapidly elongates into a filamentous shape within the uterine lumen.

II.4. Development of fetal membranes

II.4.1. Yolk sac

The primitive endoderm consists of a new lineage of cells derived from the embryonic knob, it develops rapidly after hatching. Its elongation along the trophectoderm leads to the formation of a central cavity. Then there is an evagination of the endoderm in the ventral part of the embryonic knob: the yolk sac is formed (**Figure 8**). This envelope is transient in mammals; it regresses as the embryo grows (Perry, 1981).

II.4.2. Amnion

The amnion originates from two folds of the chorion (trophectoderm and mesoderm) that merge on the dorsal aspect of the embryo on day 32 of gestation (Noakes et *al.*, 2009). The amnion defines a cavity, known as the amniotic cavity, which holds a whitish or citrine lubricating fluid in which the fetus is immersed (**Figure 8**). It shields the fetus from impacts and desiccation (Perry, 1981).

II.4.3. Chorion

The mesoderm evolves between the yolk sac and the trophectoderm (**Figure 8**). It is the outermost casing of the fetal adjuncts: it encircles the whole embryo and the other adjuncts. It will subsequently contribute to placenta establishment (Perry, 1981).

II.4.4. Allantois

This embryonic adjunct is produced by outgrowth from the cells of the embryo's primitive intestine. It extends between the amnion and the chorion. The allantois shapes a thin-walled, translucent sac, connected to the embryo through the umbilical cord by the allantoic stalk (**Figure 8**). It defines the allantoic cavity, which holds an amber-colored, aqueous fluid. During its expansion, the allantois meets the chorion; its two casings can merge and produce the allanto-chorion. It also contributes to placenta establishment (Senger, 2012).

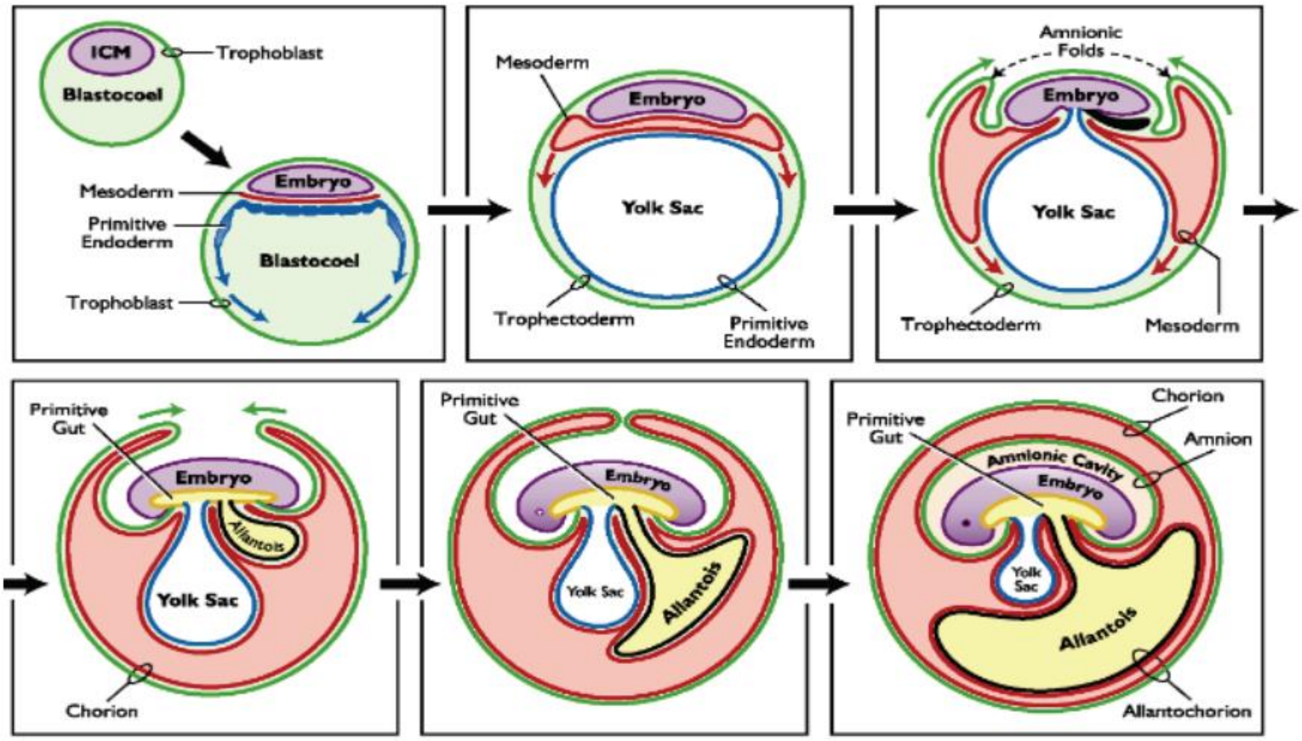

Figure8: Timeline of the formation of embryonic annexes in mammals (Senger, 2012).

II.5. Mechanism of gestational maintenance

Progesterone plays a crucial role in every gestation. It facilitates the complete closure of the cervix throughout gestation through the formation of a mucous plug, the cessation of estrous cycles, and tocolysis (the inhibition of myometrial contractions). P4 fulfills all these roles by inhibiting the synthesis of FSH and LH and repressing estrogen and oxytocin receptors (**Figure 9**) the gestational CL secretes P4, and then the placenta takes over in a process known as placental relay. The transition from the cyclic corpus luteum to the gestational corpus luteum is due to trophoblastin (tau interferon) released by the embryo post-implantation, marking the maternal acknowledgment of gestation (Guérin & Berthelot, 2001).

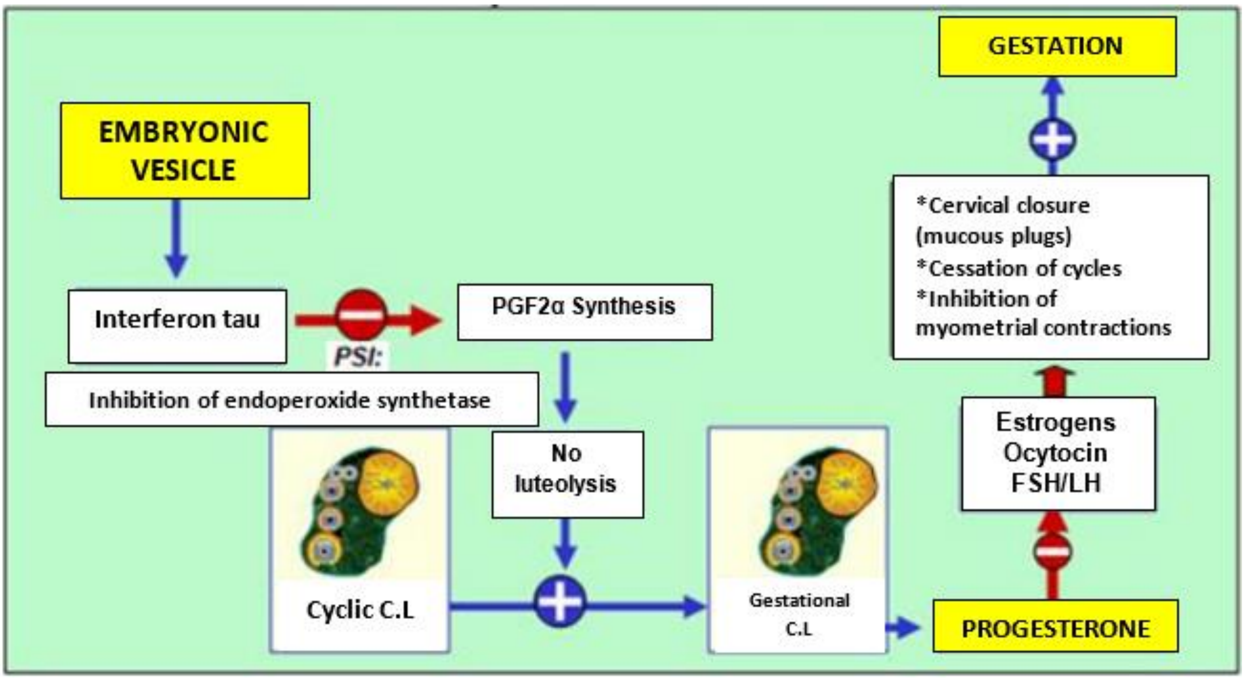

Figure 9: Mechanism of gestation maintenance (Guérin & Berthelot, 2001).

C.L: Corpus luteum

Chapter III: Ultrasonography

III.1. Principle of ultrasound

The principle of ultrasound consists of sending ultrasound waves, which are absorbed and reflected in a specific manner by different tissues. The reflections of these waves, captured by the rectal probe, are converted into visual signals displayed on the screen. Early detection is possible, commencing as soon as 28 days post-insemination (Moumouni, 2018).

III.2. Genesis of echoes

When an audible sound encounters an obstacle, it is reflected, and a returning wave of the same frequency comes back in the form of an echo. The distance between the sound-emitting source and the obstacle can be determined based on the speed of sound propagation.

An acoustic (or tissue) interface is defined as the separating surface between two media of different impedance. Thus, by analogy with an audible sound, when an ultrasound propagating through a tissue encounters a tissue interface, a portion of the ultrasound is reflected and returns to the probe crystals. The probe crystals, deformed by the echo, have the property of generating a current that is recorded and transformed into an image.

The probe is therefore both the transmitter and the receiver. The delay between the emission of the ultrasound and the reception of the echo allows the determination of the distance between the probe and the reflecting tissue interface (**Figure 10**).

In practice, the ultrasound wave that comes into contact with a tissue interface undergoes numerous phenomena which, depending on their importance and combinations, will define the ultimately rendered image. Thus, the analysis of modified ultrasound waves after passing through a medium allows for extrapolating its structure (Hagen et *al.*, 2000; Blond & Buczinski, 2009; Buczinski & DesCôteaux, 2009).

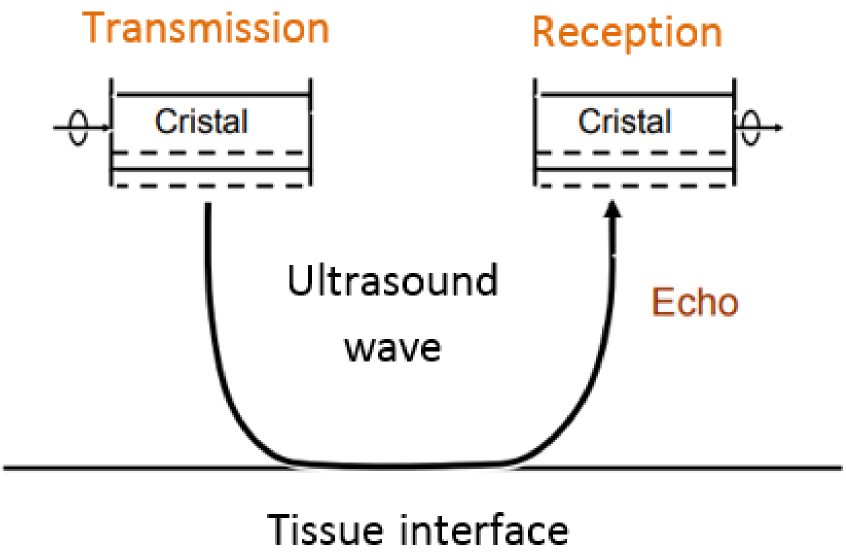

Figure 10: Genesis of echoes (Hagen et *al.*, 2000).

III.3. Ultrasound parameters

III.3.1. Types of probes

III.3.1.1. Linear probes

Provide a rectangular image (**Figure 11**) (**Figure 12**) but can be cumbersome for examining hard-to-reach areas such as intercostal spaces.

III.3.1.2. Sectorial probes

Emit an ultrasound beam that diverges from the convex probe. The resulting image has a shape of «a slice of camembert» (**Figure 11**). This type of probe is more maneuverable but cannot be used transrectally (Lebastard, 2006; Buczinski & DesCôteaux, 2009).

III.3.1.3. Microconvex probes

These are probes whose construction is the same as that of linear probes, but their small size (length) and curved (convex) shape make them resemble sectorial probes (**Figure 12**). Their indications are the same as those of sectorial probes (Boin, 2001).

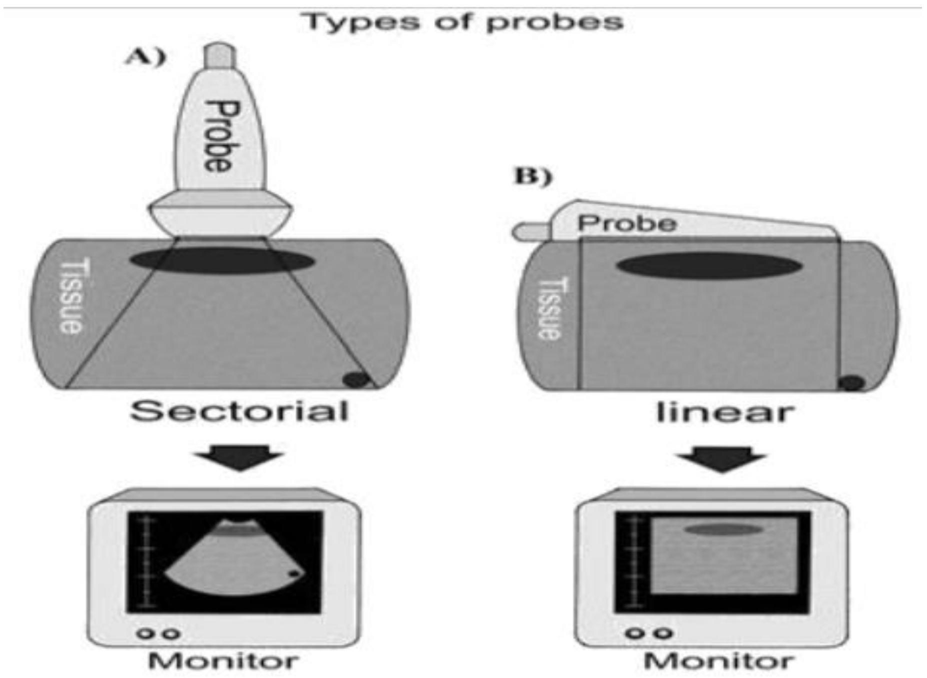

Figure 11: Sectorial and Linear probes (Dibyajyoti et *al.*, 2019)**.**

A= Sectorial probe and B=Linear probe

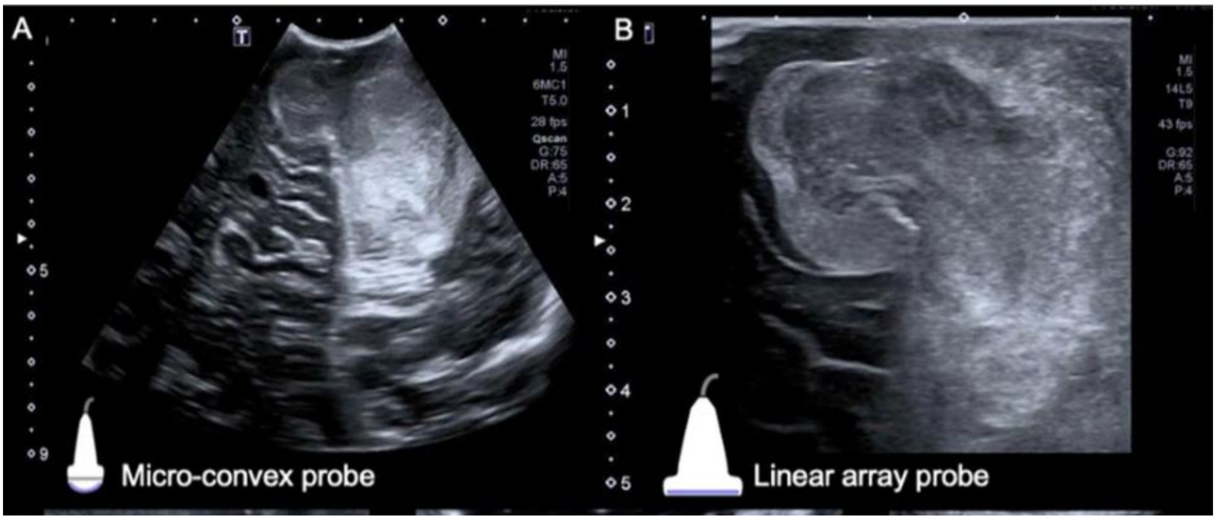

Figure 12: Ultrasound images from different probes (Dixon et *al.*, 2022)**.**

A= Microconvex

B= Linear

III.3.2. Frequency

The frequency is defined as the amount of vibrations of the emitting source (the crystals) per unit of time (second). Its unit of measurement is Hertz (Hz) or cycles per second (the number of times a molecule of tissue vibrates per second) (Hagen et *al.*, 2000).

The frequency varies from 3 to 12 MHz with 5 MHz being the most commonly utilized. A low frequency allows deeper tissue penetration but does not resolve small structures clearly. Conversely, high frequencies enable visualization of small structures with good resolution but limit the depth of examination **(Table 2).**

Table 2: Frequency of probes according to indications in gynecology in cows (Ravary & Radigue, 2003)

Indications	Recommended or possible frequency MHz
Pregnancy diagnosis	5-6 ; 7.5
Gynecology	5-6 ; 7.5
Sex determination	5-6 ; 7.5
Ovarian follicle puncture	7.5
External transabdominal monitoring of gestation	3.5

III.3.3. Echogenicity

The term echogenicity refers to the appearance of the image generated by the ultrasound machine. Indeed, the image depicts different anatomical structures in a grayscale ranging from black to white.

The importance of echoes within the tissue enables classification as anechoic, hypoechoic, echogenic, or hyperechoic based on the number of reflected echoes (from very few to many).

Structures with extremely low echogenicity appear black; these are referred to as anechoic structures, such as luteal cyst with a central fluid (**Figure 13**), early gestational fluids (**Figure 15**), and blood; those with moderate echogenicity appear in shades of gray, referred to as hypoechoic structures such as the kidney, liver (**Figure 14**), and spleen. Dense tissues like bones (**Figure 15**), fibrous tissues, or genital tubercles reflect echoes significantly, thus appearing white; they are then, called hyperechoic structures (Streeter & Step, 2007; Buczinski & DesCôteaux, 2009; Colloton, 2011).

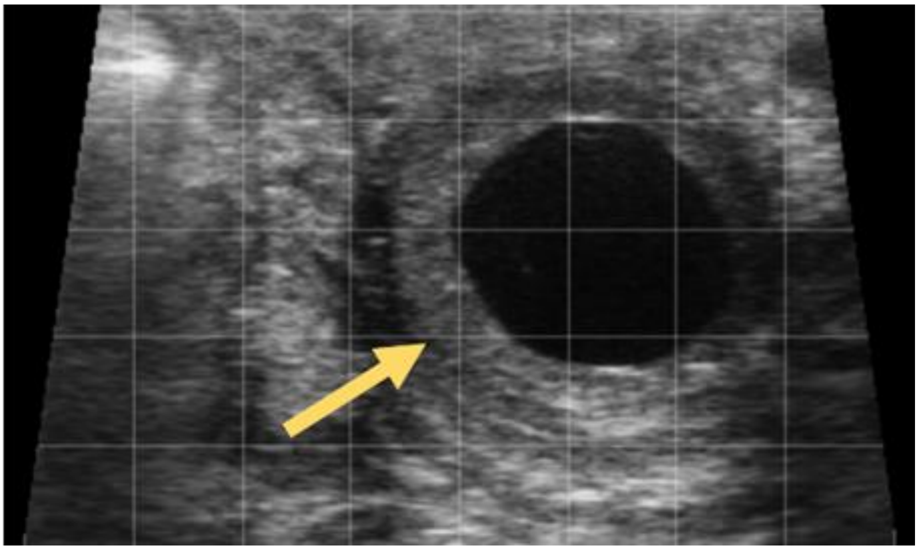

Figure 13: Luteal cyst with a central fluid filled area of 25mm (Anonymous2, 2024)**.**

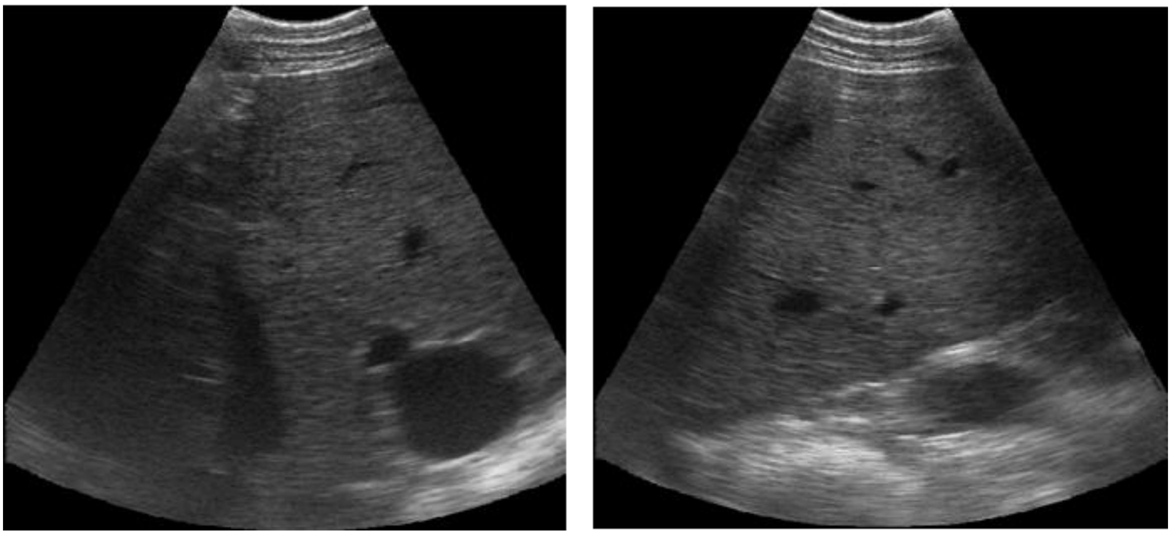

Figure 14: Ultrasonography of the liver (Komeilian et *al.*, 2011).

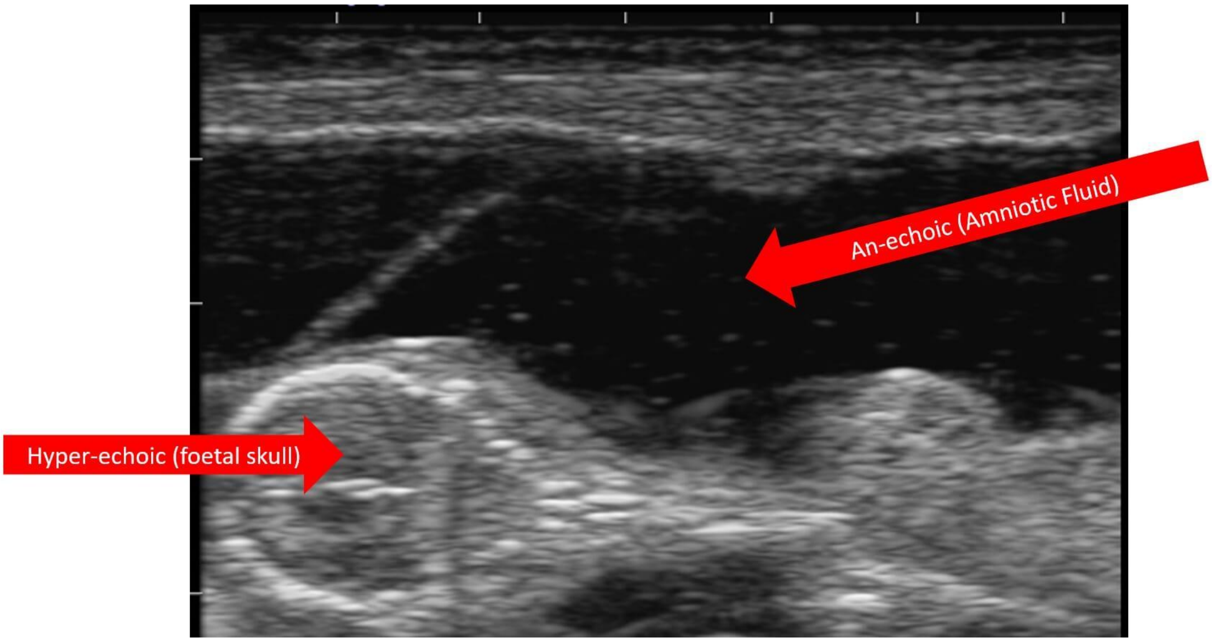

Figure 15: Ultrasonography of fetus (Karvountzis, 2021)**.**

Chapter IV: Early pregnancy diagnosis methods

IV.1. Clinical methods

IV.1.1. Transrectal palpation

The technique of transrectal palpation of the uterus involves manually examining the changes in the uterus, such as swelling due to the presence of a fetus, by inserting an arm through the cow's rectum (**Figure 16**). This procedure can be hazardous since trauma can be inflicted on both cow and fetus and should therefore only be carried out by a trained operator. In non-pregnant cows or during early gestation, uterine horns have similar sizes and diameters. However, from the 40th day of gestation, it becomes possible to distinguish the difference in size between the two horns (Ball & Peters, 2004). This technique is considered an accurate method of pregnancy diagnosis in dairy cattle for a trained veterinarian after day 35 post-breeding (Zemjanis, 1970).

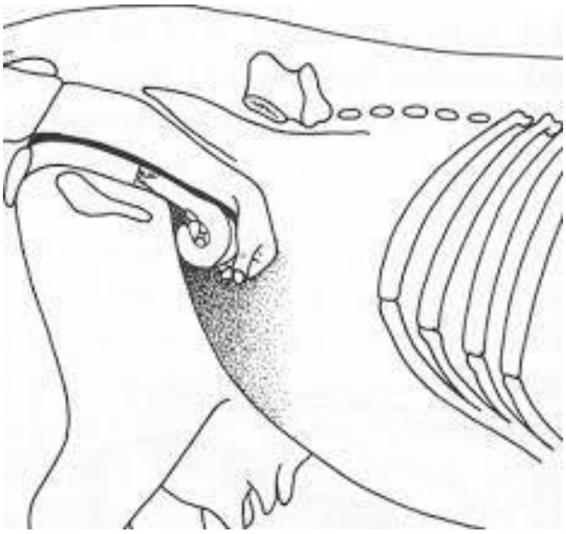

Figure 16: Transrectal evaluation of the bovine genital tract to diagnose pregnancy (Peter et *al.*, 2002)**.**

However, the effectiveness of early diagnosis is contingent upon the breed and reproductive status of the cow. It is noteworthy that an aged cow, regardless of its reproductive state, could exhibit horn asymmetry or a uterus descending into the abdominal cavity because of previous pregnancies.

The veterinarian is required to initially verify the adequate restraint of the cow, employing a glove along with ultrasound gel. While utilizing his non-dominant hand to secure the bovine's tail, he proceeds to gently insert his dominant arm through the cow's rectum (**Figure 17**).

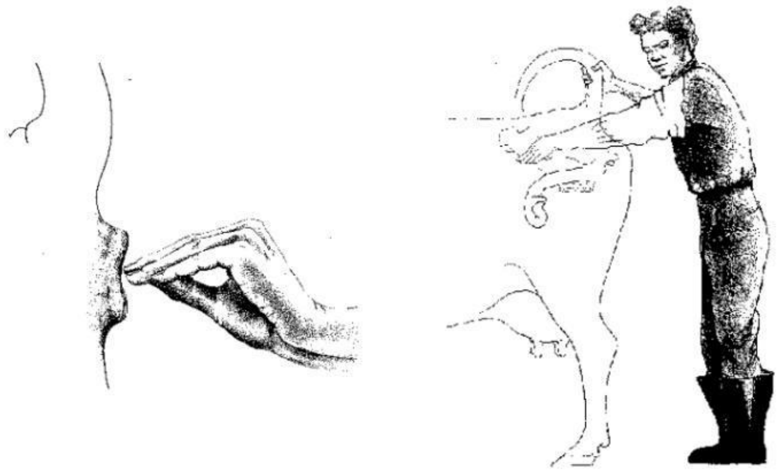

Figure 17: Procedure of the transrectal palpation (Purohit, 2010)**.**

The diagnosis of pregnancy is confirmed by identifying specific structures (**Table 3**); if none is found, the cow is not pregnant or the diagnosis is postponed. Four key structures indicating pregnancy are chorio-allantoic slip, amniotic vesicle, placentomes, and fetus. Complete palpation of the body and both uterine horns must be done before diagnosing as not pregnant (Hopper, 2021).

IV.1.1.1. Membrane slip

By the 30th to 32nd day of pregnancy, membrane slippage can be routinely detected in the gravid horn and in both horns by day 50 to 60 of gestation. Following the identification of the cervix and the retraction of the uterus, each horn can be carefully grasped between the fingers and thumb and slightly elevated (**Figure 18**). During pregnancy, a distinct popping sensation may be felt as the membrane detaches from the uterine walls. At 32 days gestation, it appears like a thread, eventually resembling a small string slipping from the fingers in the gravid horn by day 45 (Hopper, 2021).

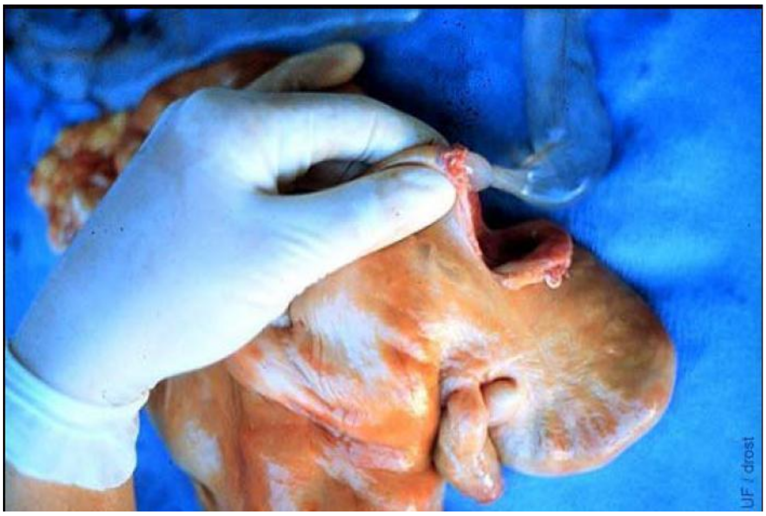

Figure 18: Slipping of the chorioallantoic membrane (Drost, 1982)

IV.1.1.2. Amniotic vesicle

The amniotic vesicle presents as a highly mobile ovoid structure, suspended in the allantoic fluid. The greater density of the amniotic vesicle aids the differentiation between it and the allantoic vesicle. In principle, the palpation of the amniotic vesicle is achievable between the 30th and 65th day of gestation (Arthur et *al.*, 1982).

IV.1.1.3. Fetus

The fetus is palpable around 55-60 days; it can be felt floating in the fluid of the gravid horn.

Table 3: Days of palpation of fetal and maternal structures during pregnancy (Purohit, 2019).

Palpable findings	Gestation days
Palpation of amniotic vesicle	30-65 days
Asymmetry of uterine horns and fluctuation of uterine contents	35-90 days
Palpation of fetal membrane slip	35-90 days
Palpation of fetal bump	65-100 days
Palpation of cotyledons	70 days to term
Fremitus in the middle uterine artery	90 days to term
Fetus and its movements	180 days to term

IV.1.3. Ultrasonography

Ultrasonography is a widely used technique for diagnosing early pregnancy in cattle by using high-frequency sound waves to create live two-dimensional images of the reproductive system and the developing embryo. Ultrasound probes ranging from 3.5 to 7.5 megahertz (MHz) are generally preferred in cows (Çiplak, 2024). An experienced veterinarian can perform this method as early as 28 days after breeding (Ashok et *al.*, 2013).

IV.1.3.1. Ultrasound examination

After the rectum has been emptied and the internal genitalia have been palpated in the usual manner, the handheld ultrasound probe is inserted through the anus (**Figure 19**) and subsequently moved towards the cranial direction along the rectal base (Kahn, 1994).

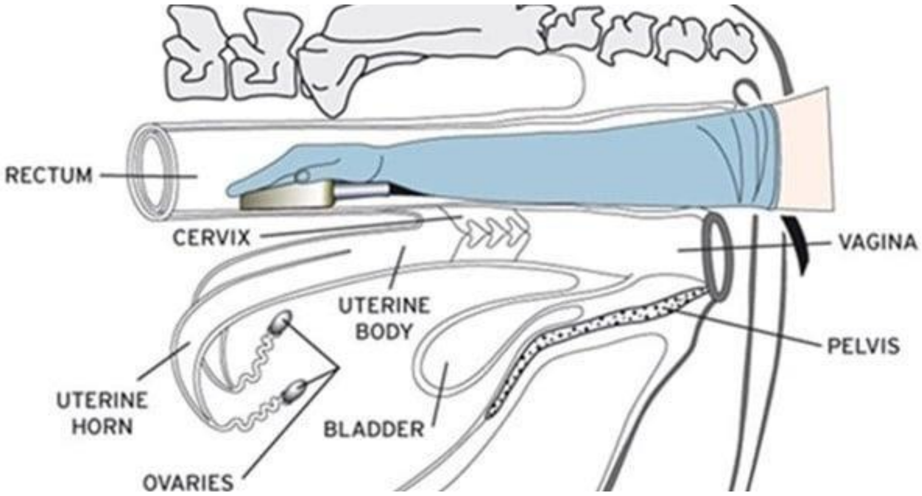

Figure 19: Ultrasound examination of the genital tract using a linear probe (Biagiotti, 2022).

Compared to transrectal palpation, this method provides additional information (**Table 4**) like the fetus's heartbeats, which can be detected at day 21 in cattle and the presence of twins (Kastelic et *al.*, 1988). The gender of the fetus can also be determined at 55-70 days after breeding (Lamb & Fricke, 2004). The result varies depending on the stage of gestation studied, along with the frequency of the probe used, upon which the interpretation of the image relies for example the detection of the bovine embryonic vesicle is feasible using a 7.5 MHz probe as soon as the 9th day post ovulation and with a 5 MHz probe as early as the 12th day (**Figure 20**) and (**Figure 21**) (Boyd et *al*., 1988).

Table 4: identifiable characteristics of the bovine conceptus during ultrasonographic examinations for pregnancy diagnosis, indicating the first day of detection (Szenci, 2021).

Characteristics	First day of Detection in Heifers Curran et *al,1986.* Range	First Day of Detection in Heifers Curran et *al,1986.* Mean ± SEM	First Day of Detection after ET Totey et *al,1991.* Mean ± SD
Embryo proper	19 to 24	20.3 ± 0.3	19.5 ± 0.7
Heartbeat	19 to 24	20.9 ± 0.3	22.6 ± 0.9
Allantois	22 to 25	23.2 ± 0.3	23.1 ± 0.8
Spinal cord	26 to 33	29.1 ± 0.5	33.0 ± 1.5
Forelimb buds	28 to 31	29.1 ± 0.3	32.7 ± 1.3 *
Amnion	28 to 33	29.5 ± 0.5	25.1 ± 1.4
Hindlimb buds	30 to 33	31.2 ±0.3	32.9 ± 1.3 **
Placentomes	33 to 38	35.2 ± 1.0	-
Split hooves	42 to 49	44.6 ± 0.7	-
Fetal movements	42 to 50	44.8 ± 0.8	50.7 ± 1.0
Ribs	51 to 55	52.3 ± 0.5	60.9 ± 1.7

ET: Embryo transfer, * Forelimb, ** Limb buds.

IV.1.3.2. Day 10 to 20 of pregnancy

A small increase in fluid volume may become visible during the period between Days 10 and 17 of the estral cycle after insemination, the fluid will be located in the horn on the same side as the corpus luteum (Curran et *al.*, 1986). It will appear as thin. Anechoic areas that are round in shape in three quarters of all cows and measure 2 to 4 mm in size (Pierson & Ginther, 1984).

Figure 20: Uterus on Day 12 of pregnancy (Kahn, 1994)**.**

Hypoechoic sections (small arrows) through the embryonic vesicle are visible in several places. The larger arrows demarcate the outline of the uterine horn. Ultrasonogram produced with sector scanner at 5 MHz.

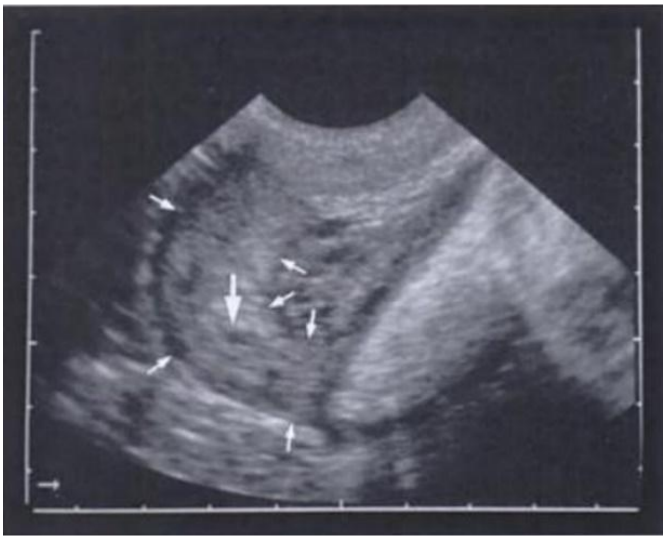

Figure 21 : Bovine uterus on Day 12 of pregnancy (Kahn, 1994)**.**

A Hypoechoic section (large arrow) through the embryonic vesicle is visible in the ventral part of the uterine horn. Small arrows demarcate the greater and lesser curvatures. Ultrasonogram produces with sector scanner at 5 MHz.

IV.1.3.3. Day 21 to 24 of pregnancy

Between Days 21 and 24 of gestation, there is typically a noticeable increase in the volume of fluid contained within the embryonic vesicle, rendering it more readily identifiable through the utilization of ultrasonography (**Figure 22**). The maximum expansion of the embryonic vesicle usually transpires near the amnion, leading to a diameter ranging from 3 to 5 mm and a length of approximately 1 cm by Day 22 of gestation (Kahn, 1994). Before Day 25 of pregnancy, it can often be difficult to find the embryo itself (**Figure 23**). Sometimes its presence can be suspected, but it is difficult to differentiate it from other echoic structures. The length of the embryo between Days 21 and 24 is about 5 mm (Curran et *al.*, 1986).

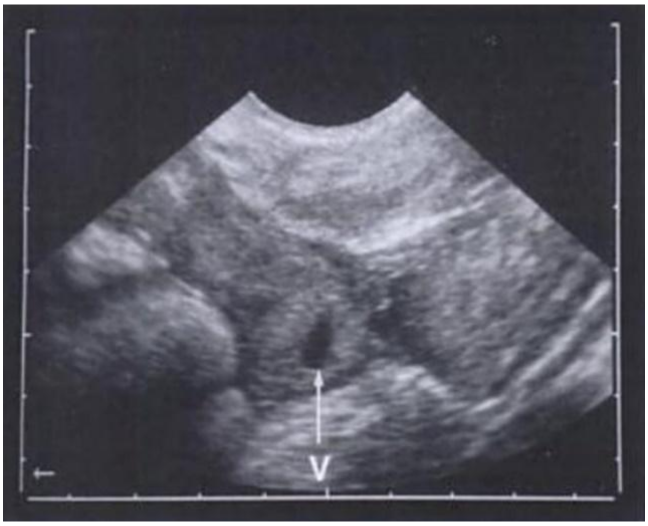

Figure 22: Uterus on Day 21 of pregnancy (Kahn, 1994)**.**

The hypoechoic area of the embryonic vesicle (V) has a size of 9.5 x 4 mm. Ultrasonogram produced with a sector scanner at 5 MHz.

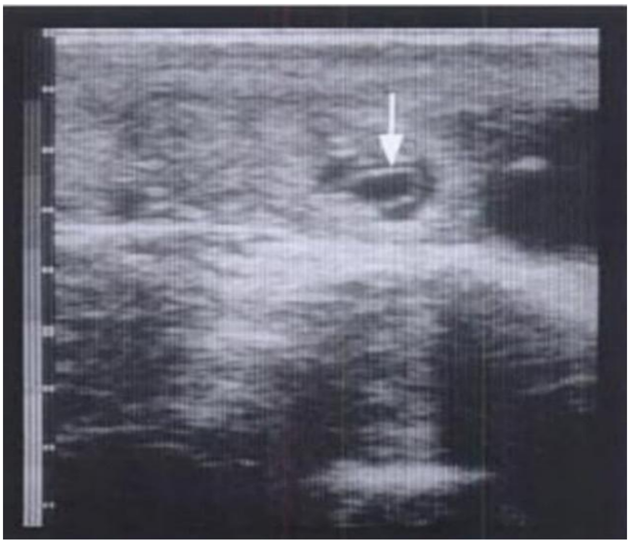

Figure 23: Uterus on Day 24 of pregnancy (Kahn, 1994)**.**

The allantoic membrane (arrow) floats inside the embryonic vesicle

IV.1.3.4. Day 25 to 30 of pregnancy

On day 25 of pregnancy, the embryonic vesicle of the bovine reaches 10 mm at the point of its largest expansion (**Figure 24**) until day 30 the diameter increases to 18 to 20 mm (Chaffaux et *al.*, 1986).

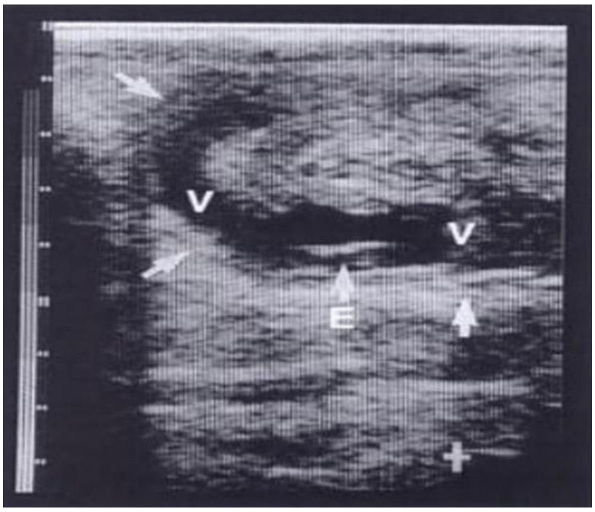

Figure 24: Uterus on Day 26 of pregnancy (Kahn, 1994)**.**

Embryonic vesicle (V) and embryo (E) on Day 25 of pregnancy. The embryonic vesicle extends along the curvature (arrows) into the dorsal portion of the uterine horn.

IV.2. Laboratory methods

IV.2.1. Progesterone assay

Blood P4 tests were established during the 1970s with the objective of early detection of pregnancies (between 20 and 23 days). Nonetheless, it has been noted that elevated progesterone concentrations may be present in instances of a luteal cyst, extended sexual cycles with a prolonged luteal phase, or even pyometra (Robertson & Sadra, 1971).

IV.2.1.1. Assay principle

Progesterone was initially sought in the blood due to its remarkably stable physiologically in short periods, attributed to the presence of storage plasma proteins. However, the complexity of the medium, with the proteins requiring extraction, poses drawbacks. The concentration in whole blood becomes highly unstable post-sampling, with changes occurring over time due to the presence of red blood cells and reversible conversion to 20-a-hydroxyprogesterone (Garnier, 1985). Temperature influences the degradation rate of progesterone in total blood, with higher temperatures accelerating the process (Vahdat et *al.*, 1981). Post-centrifugation, progesterone remains stable in plasma or serum. To ensure optimal sampling and storage conditions for subsequent analysis, two options are viable: prompt centrifugation within an hour of blood collection or delayed centrifugation after refrigeration.

IV.2.1.2.Types of tests

a) Radioimmunology

The method is based on the Antigen-Antibody (Ag-Ac) interaction concept.

1. A rabbit is injected with Bovine progesterone. The rabbit generates antibodies specific to this hormone (Thibier et *al*., 1973).
2. Antibodies are placed in a receptacle with the measured specimen (milk or blood), and excess antibodies are added to ensure some remain unbound.
3. Labeled progesterone (H3 or 1125) is added to saturate remaining unbound antibodies.
4. A surplus radioactive hormone is isolated from the Ag-Ac complexes using techniques (e.g., electrical migration, absorption on a solid substrate (dextran charcoal) or aspiration).
5. The following phase entails the measurement of the radioactivity in the Ag-Ac fraction, the unrestrained labeled progesterone fraction, and the bound fraction by using a liquid scintillation spectrophotometer.
6. The recorded count per minute is correlated with a standard curve. This determines the progesterone level in the sample.

Unfortunately, this testing procedure is limited to specialized laboratories due to the necessity of sophisticated apparatus and the handling of radioactive materials. However, radioimmunology remains the gold standard for progesterone assay owing to its sensitivity, specificity, reproducibility, and accuracy, enabling a quantitative analysis (Thibier et *al.*, 1973; El amiri, 2008).

b) Enzyme-Linked Immuno-Sorbent Assay (ELISA)

The technique known as the immunoenzymatic method is grounded on the concept of attaching an enzyme to an AC or Ag. In instances such as competitive ELISA, cups that have been sensitized by anti-progesterone antibodies are utilized. Upon introduction of the milk/blood sample into the cup, the progesterone present in the milk/blood binds with the antibodies and thus gets deposited at the cup's base. Subsequently, a progesterone-enzyme conjugate is introduced, binding to the unoccupied antibody sites. The higher the amount of enzyme attached to the antibodies, the lower the progesterone content in milk. Through the addition of the substrate, enzyme, and a chromogen, the level of progesterone can be indicated via a colorimetric reaction (Groves et *al.*, 1990).

IV.2.2.Pregnancy-Associated Glycoproteins

Gestation-associated glycoproteins (PAGs) are considered trophoblastic glycoproteins that are classified within the proteolytic enzyme group known as aspartic proteases (Xie, et *al.*, 1991). They are referred to by various designations such as Gestation-specific B protein (PSPB) ((Butler et *al.*, 1982), glycoproteins 1 and 2 linked to gestation (PAG 1 and 2) (Beckers et *al.*, 1988), and pregnancy-specific protein 60kDa (PSP60) (Mialon et *al.*, 1993).

PAGs assays are commonly utilized between days 26 to 30 post-fertilization to identify pregnancy in cattle (Fricke et *al.*, 2016). The assessment of PAGs is feasible through PAG-ELISA techniques using maternal plasma, serum, milk, or whole blood samples (Gábor et *al.*, 2007). If PAGs are present in the sample, they bind to the antibodies. A secondary antibody that is linked to an enzyme is added, which also binds to the PAGs. A substrate is added, which reacts with the enzyme to produce a color change. The intensity of the color change is measured, indicating the presence and amount of PAGs.

- **Positive Result**: A significant color change indicates the presence of PAGs, suggesting the cow is pregnant.
- **Negative Result**: Little to no color change indicates the absence of PAGs, suggesting the cow is not pregnant.

IV.2.3.Early Pregnancy Factor

The penetration of the spermatozoon into the oocyte initiates the formation of a compound, zygotin, which, in the early stages of oogenesis, would trigger the synthesis of a substance recognized as EPF by the ovary containing the corpus luteum (Cavanagh, 1982). The EPF is identified as the most premature factor in pregnancy, which is vital for the embryo's viability; it can be found in the maternal system 24-48 hours after successful fertilization and persists for at least the first half of gestation. It is conceivable that this factor contributes to the reduction of the immunocompetence of lymphocytes at the onset of gestation, thereby facilitating the immunological acceptance of the embryo by the maternal system (Morton et *al.,* 1983). However, this indicator is not exclusive to gestation. EPF is released by tumor cells during their growth and division stages as well as by normal proliferative cells in adult individuals (Quinn et *al.*, 1994) and by activated platelets (Cavanagh, 1991). To date, there is no dependable test founded on EPF detection capable of distinguishing pregnant females.

The serum is prepared and isolated then tested for the presence of EPF using an immunoassay (similar to an ELISA) that detects the specific protein. If EPF is present in the serum, it binds to the antibodies, causing a detectable reaction (such as a color change).

- **Positive**: A color change in the test well indicates the presence of EPF, confirming early pregnancy.
- **Negative**: No color change indicates that EPF is not present, suggesting no pregnancy.

IV.2.4. Bovine interferon tau (boIFNτ)

Bovine interferon tau is a significant protein released by the bovine concept around 16 to 25 days post-fertilization. The presence of IFN-tau in the uterine cavity wash as early as the 12th day of gestation in cows has been documented (Humblot & Dalla Porta, 1984). This particular molecule is produced by trophectoderm mononuclear cells and is selectively discharged into the nearby lymphatic system, where it demonstrates its anti-luteolytic properties (Roberts et *al.*, 1992). An elevation in interferon tau concentration is observed as early as D9-D10, with a notable surge occurring between D17 and D19 when the conceptus experiences rapid shape expansion. Only a minute amount of IFN-t1 enters the bloodstream, and this exceptionally low level impedes the development of a reliable pregnancy diagnostic test (Kubisch, 2001).

After the preparation of blood samples, they are subjected to an immunoassay, where antibodies specific to bIFN-τ are used to detect its presence. This can be done using techniques like ELISA (enzyme-linked immunosorbent assay). The assay results are read to determine the presence and concentration of bIFN-τ in the samples:

- A positive color change confirms pregnancy.
- No color change indicates that the cow is not pregnant.

IV.2.5. Bovine placental lactogenic hormone (BpL)

During a later stage of gestation, binucleated and trinucleated cells located at the microvillus junction of the placenta are responsible for the local production of bovine placental lactogen hormone (BpL), also referred to as somato-mammotropic chorionic hormone (Wooding & Beckers, 1987). The impact of BpL on the development of the mammary gland and lactogenesis is notable. The assessment of BpL can be conducted through radioimmunology labelling (RIA) in maternal serum, with the timing varying greatly among individuals, ranging from the 26th day to the 110th day post-fertilization. Maternal levels of BpL gradually rise, reaching concentrations of 1 to 2 ng/ml around the time of parturition. In contrast to the relatively low levels found in maternal circulation, fetal BpL concentration can peak at 25 to 30 ng/ml by the 90th day of gestation. The delayed appearance of BpL in maternal blood diminishes the utility of this assay for pregnancy diagnosis (Ayad, 2006).

The procedure of the bPLH test consists of various steps . First the blood samples are collected from the bovine jugular vein , the collected blood is placed in a tube, usually containing an anticoagulant to prevent clotting and then centrifuged to separate the plasma (that contains the lactogenic hormone) from the blood cells . An immunoassay, such as an ELISA (Enzyme-Linked Immunosorbent Assay), is commonly used to detect and quantify bPLH levels. Wells of a microtiter plate are coated with antibodies specific to the lactogenic hormone.

Plasma samples are added to the wells and incubated to allow the hormone to bind to the antibodies. After incubation, the unbound substances are washed away and a secondary antibody, conjugated with an enzyme, is added. This antibody binds to the hormone-antibody complex. A substrate for the enzyme is then added, which produces a measurable color change or fluorescence. The intensity of the color or fluorescence is measured using a spectrophotometer or a plate reader. The concentration of bPLH in the sample is determined by comparing the readings to a standard curve generated with known concentrations of the hormone.

IV.3. Rapid kit system

The tests detect the presence of pregnancy-associated proteins in the animal's blood or urine, allowing for quick diagnosis before more advanced methods are required. While these tests are not as accurate as veterinary exams, they can be a useful tool for farmers to identify pregnant animals and monitor their herds. However, it is important to follow the test instructions carefully and confirm any positive results with a veterinarian, as false positives can occur. Additionally, proper training on how to interpret the test results is crucial to ensure accurate diagnosis.

On-farm rapid kits for early pregnancy diagnosis in cows can provide a quick and convenient way to detect pregnancy status. Several types of these kits have been evaluated, with varying levels of accuracy.

IV.3.1. Early Conception Factor rapid test

One type of rapid kit is the Early Conception Factor test, which reportedly detects a pregnancy-specific protein in the serum of cows as early as 3-15 days post-breeding. However, a study found the sensitivity and specificity to be only 51% and 55%, respectively **(Table 5)** (Adams & Jardon, 1999).

Table 5: ECF™ test results versus rectal palpation (Adams & Jardon, 1999).

	Palpation result pregnant	Palpation result open	Total
EFS™ positive	23	40	63
EFS™ negative	22	48	70
Total	45	88	133

IV.3.2. Progesterone ELISA rapid test

These kits (**Figure 25**) have shown varying accuracy, with some studies reporting high reliability, while others have found the predictability to be limited, especially when trying to use a single model across all stages of lactation and pregnancy (**Table 6**) (Nepal et *al.*, 2019).

Nonetheless, the progesterone ELISA test has been recommended for early pregnancy diagnosis and monitoring, as it is a simple and stress-free method for cows (Mehmedi et *al.*, 2021).

Figure 25: Ringbio progesterone ELISA rapid test (Anonymous3, 2015)

Table 6: Results of progesterone kit accuracy in the pregnancy diagnosis in different farm

SN	Farm animals	Number of animals	Types of samples	Days of sampling (Post-insemination)	Accuracy percentage	Remarks	Reference
1	Crossbred Jersey and Holstein dairy cows	20	Blood (serum)	37-40	80 (Positive)	92% sensitivity 57% specificity,	(Sah et *al.*, 2017).
2	Lactating Friesian cow	25	Blood (Peripheral plasma)	21-22	85		(Skemesh et *al.*, 1973).
3	Lactating Holstein cows	91	Milk	20	67.3 (Positive), 87.2 (Negative)		(Cox et *al.*, 2010).
4	Cows	476		21-24	21-24	88.6 (Positive), 93.9 (Negative)	(Reimers et *al.*, 1985).

IV.3.3. PAGs ELISA rapid test

Commercial ELISA blood tests for PAGs are available and commonly used for early pregnancy detection in dairy cattle, starting around 21 days post-breeding (**Figure 26**). These blood-based

PAG tests offer an on-farm rapid kit for diagnosing pregnancy status before the traditional 30-day window for transrectal palpation or ultrasound (Piechotta et *al.*, 2011).

Low PAG concentrations during early pregnancy have been associated with increased risk of embryonic mortality and late fetal losses in cows. Therefore, measuring PAG levels at around day 24 can not only diagnose pregnancy but also predict the likelihood of maintaining that pregnancy until later stages (Piechotta et *al.*, 2011; Reese et *al.*, 2017; Akkose et *al.*, 2019).

Commercial ELISA blood tests for PAG have extremely high sensitivity and specificity for early pregnancy diagnosis in dairy cows compared to ultrasound examination (Piechotta et *al.*, 2011). Compared to traditional methods like rectal palpation or progesterone measurement, PAG-based tests allow for earlier and more convenient pregnancy detection (Szenci, 2021).

***Figure 26: MongGoQ Bovine PAGs pregnancy tes*t** (Anonymous4, 2024)

PART II:
EXPERIMENTAL STUDY

Early pregnancy diagnosis in cows is a crucial aspect of managing cattle reproduction effectively. Various methods are employed for this purpose, ranging from traditional palpation and ultrasound to more advanced techniques such as hormone assays and genomic markers. Each method offers unique advantages in terms of accuracy, cost-effectiveness, and ease of implementation, catering to different needs and preferences in veterinary practice.

I. Objective

The aim of this study is to analyze the collected data to identify prevailing trends, regional differences and compare it with different previous studies. By leveraging both qualitative and quantitative responses, this study seeks to contribute to the enhancement of reproductive management practices in Algerian cattle herds, ultimately aiming to boost productivity and efficiency in the sector.

II. Materials and Methods

This study is based on the analysis of the results we got from a questionnaire, which was distributed to 65 veterinarian in 15 regions in Algeria: Algiers, Blida, Bouira, Sétif, M'Sila, Béjaia, Banta, Boumerdes, Jijel, Taref, Djelfa, Tipaza, Telemcen, Oran and Medea., utilizing both traditional paper formats and digital platforms via Google Forms to ensure broad and convenient participation. It achieved a significant response rate.

II.1. Description of the questionnaire

This questionnaire consists of 20 questions covering various aspects of bovine pregnancy diagnosis:

1. Practitioner background (region, experience, inseminator status)
2. Types and breeds of cows they work with
3. Preferred timing for early pregnancy diagnosis
4. Use and perceptions of different diagnostic methods:
    - Palpation
    - Ultrasound
    - Hormone testing
    - Rapid test kits

5. Advantages and limitations of each method
6. Factors influencing insemination success
7. Common early pregnancy anomalies and pathologies

The questions are a mix of multiple-choice and open-ended, allowing for both quantitative and qualitative data collection. The survey aims to gather insights from veterinarians' field experience, covering technical, practical, and economic aspects of various pregnancy diagnosis methods in cattle. It also explores practitioners' awareness and attitudes towards newer technologies like rapid test kits.

Below is a copy of the questionnaire used in this study:

HIGHER NATIONAL VETERINARY SCHOOL
Questionnaire for Veterinary Practitioners
This questionnaire is part of a final year project entitled:

Early Pregnancy Diagnosis Methods in Cows

Please answer this questionnaire based on your field experience

1) Information about the veterinary practitioner:
   - Region of practice: ……………………
   - Years of experience: …..........................
   - Are you an inseminator? ☐ Yes ☐ No
2) What type of cows do you most often work with?

   ☐ Dairy ☐ Beef ☐ Mixed

3) What breed of cows do you most often work with?

   ☐ Montbéliarde ☐ Prim'Holstein ☐ Brown Swiss

Other:
…………………………………………………………………………………………
………………………………………………………………………………………….

4) At what stage do you perform early pregnancy diagnosis?

   ☐ <1 Month ☐ 1-2 Months ☐ 2-3 Months

Other:
…………………………………………………………………………………………
……………………………………………………………………………………...

5) Do you mainly use palpation for early pregnancy diagnosis?

   ☐ Yes ☐ No

   -**If yes**,
   * At what stage of pregnancy do you prefer to perform palpation?

   ☐ <1 Month ☐ 1-2 Months ☐ >2 Months

   Other: ....

   -**If no**, why not?

   ☐ Lack of technical skills ☐ Lack of early detection ☐ Low accuracy

   Other: ....

6) In your opinion, what are the main advantages of palpation compared to other early pregnancy diagnosis methods?

☐ Most practical Accuracy ☐ Precision ☐ Ease of implementation ☐ Low cost
☐ No equipment needed

Other:
…………………………………………………………………………………………
…………………………………………………………………………………………

7) In your opinion, what are the limitations of palpation as a method for early pregnancy diagnosis?

☐ Precision ☐ Accuracy required ☐ Skills Inability to assess fetus viability
☐ Inability to assess possible structural anomalies of the fetus

Other:
…………………………………………………………………………………………
…………………………………………………………………………………………

8) Do you mainly use ultrasound for early pregnancy diagnosis?

☐ Yes ☐ No

-**If yes**,

*At what stage of pregnancy do you prefer to perform ultrasound?

☐ 25-30 days ☐ 30- 45 days ☐ >45 days

Other: ……………………………………………………………………………………

-**If no**, why not?

☐ Lack of technical skills ☐ No ultrasound machine ☐ Expensive

Other: ……………………………………………………………………………………

9) In your opinion, what are the main advantages of ultrasound compared to other early pregnancy diagnosis methods?

☐ Increased accuracy ☐ Early detection ☐ Easy to use
☐ Evaluation of fetus viability ☐ Determination of the number of fetuses

Other:
…………………………………………………………………………………………
…………………………………………………………………………………………

10) In your opinion, what are the limitations of ultrasound as a method for early pregnancy diagnosis?

☐ Cost of the ultrasound machine ☐ Required skills ☐ Difficulty in interpretation
☐ Maintenance and durability of equipment

Other:
………………………………………………………………………………………………
………………………………………………………………………………………………

11) Do you mainly use hormone testing for early pregnancy diagnosis?

☐ Yes ☐ No

-If yes,

*What do you measure?

☐ Progesterone ☐ PAG ☐ EPF

*What type of sample do you use?

☐ Blood ☐ Milk ☐ Urine ☐ Feces

-if not, why not?

☐ Lack of technical skills ☐ Time-consuming ☐ Expensive for the farmer

Other: ………………………………………………………………………………………

12) In your opinion, what are the main advantages of hormone testing compared to other early pregnancy diagnosis methods?

☐ Increased accuracy ☐ Detection of embryonic mortality ☐ Easy to perform
☐ Early detection ☐ Low risk of false positives ☐ Low risk of false negatives

Other:
………………………………………………………………………………………………
………………………………………………………………………………………………

13) In your opinion, what are the limitations of hormone testing as a method for pregnancy diagnosis?

☐ Unavailability of kits ☐ Stressful during blood sampling ☐ Cost
☐ Time-consuming

Other:
………………………………………………………………………………………………
………………………………………………………………………………………………

14) Do you know about rapid test kits (on the farm)?

☐ Yes ☐ No

-**If yes,** what types do you know?

……………………………………………………………………………………………
……………………………………………………………………………………………
……………………………………………………………………………………………

15) In your opinion, what are the main advantages of rapid test kits compared to other early pregnancy diagnosis methods?

☐ Early detection ☐ Low risk of false negatives ☐ Low risk of false positives
☐ Speed ☐ Ease of use ☐ Mobility ☐ Versatility ☐ Non-stressful

Other:
……………………………………………………………………………………………
……………………………………………………………………………………...….

16) In your opinion, what are the main limitations of rapid test kits as a method for early pregnancy diagnosis?

☐ Unavailability ☐ Cost of the kit ☐ Sensitivity to storage conditions
☐ Detection limit

Other:
……………………………………………………………………………………………
……………………………………………………………………………………………

17) Do you plan to use rapid test kits more frequently if they become available in the future?

☐ Yes ☐ No

18) Based on your experience, what are the most common factors influencing the success of insemination?

☐ Nutritional imbalance ☐ Age of the cow ☐ Lactation stage ☐ BCS
☐ Semen quality ☐ Insemination technique ☐ Timing of insemination
☐ Improper thawing of semen ☐ Poor farming practices ☐ Stress

Other:
……………………………………………………………………………………………
……………………………………………………………………………………………

19) What are the anomalies/pathologies that affect pregnancy in the early stages?

☐ Uterine infections ☐ Hormonal imbalances ☐ Genetic anomalies
☐ Embryonic developmental defects ☐ Negative energy balance
☐ Embryonic developmental abnormalities ☐ Metabolic disorders

Other:
……………………………………………………………………………………………
……………………………………………………………………………………………

20) Do you have any additional comments or suggestions regarding early pregnancy diagnosis methods in cows?
……………………………………………………………………………………………
……………………………………………………………………………………………
……………………………………………………………………………………………
……………………………………………………………………………………………

II.2. Utilization of the questionnaire

After collecting the completed questionnaires, we processed the responses obtained according to each studied parameter. The results and calculations were performed using Excel 2022.

III. Results and discussion

With 50 completed surveys returned, providing valuable insights into the adoption, effectiveness, and challenges associated with different pregnancy diagnosis techniques, we got these results:

III.1. Information about the veterinarian practitioner

III.1.1. Region of practice

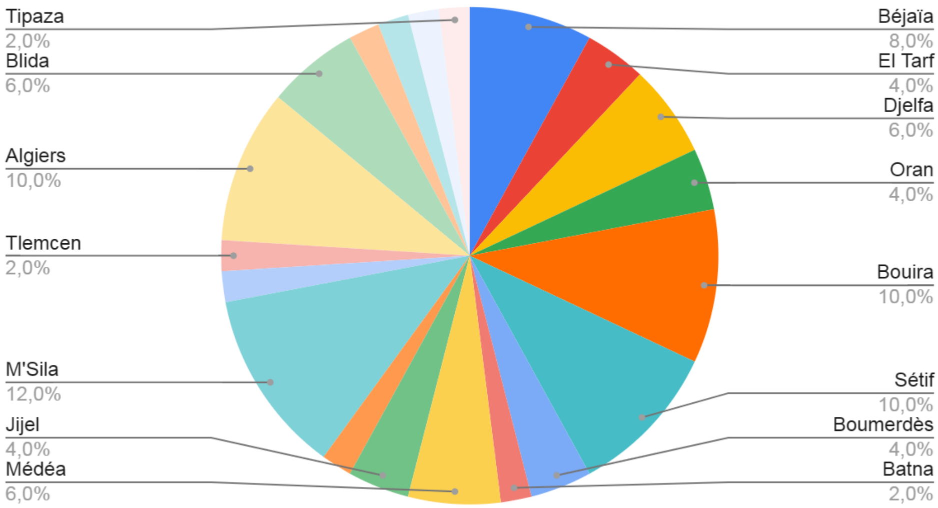

Figure 27: Regions of practice.

- M'Sila has the largest share at 12.0%
- Sétif and Bouira are tied for second at 10.0% each
- Algiers also represents 10.0%
- Béjaia follows at 8.0%
- Several regions have smaller percentages, ranging from 2.0% to 6.0%

This chart represents the distribution of the different answers of the survey across different regions in Algeria. There is a mix of larger urban centers and smaller regions represented.

III.1.2. Years of experience

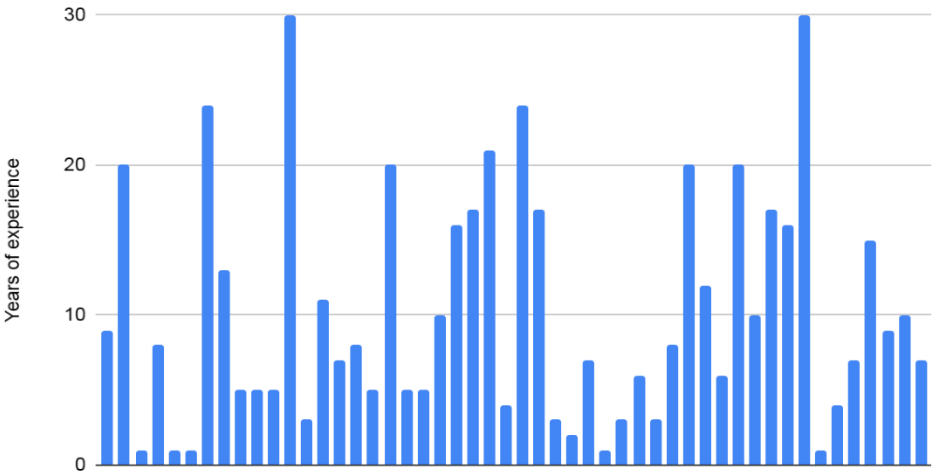

Figure 28: Years of experience.

The data reveals a wide range of experience levels, spanning from 1 to 30 years. The mean experience is approximately 10.7 years, while the median is 8 years, indicating a slight skew towards more experienced professionals. The most common experience level (mode) is 5 years, occurring five times in the dataset. Breaking down the distribution, we see that 33.3% of individuals have 0-5 years of experience, forming the largest group. This is followed by 29.4% with 11-20 years, 23.5% with 6-10 years, and 13.7% with 21-30 years of experience.

This distribution suggests a workforce with a healthy mix of newer professionals and seasoned veterans, providing a balance of fresh perspectives and deep expertise.

III.1.3. Inseminator status

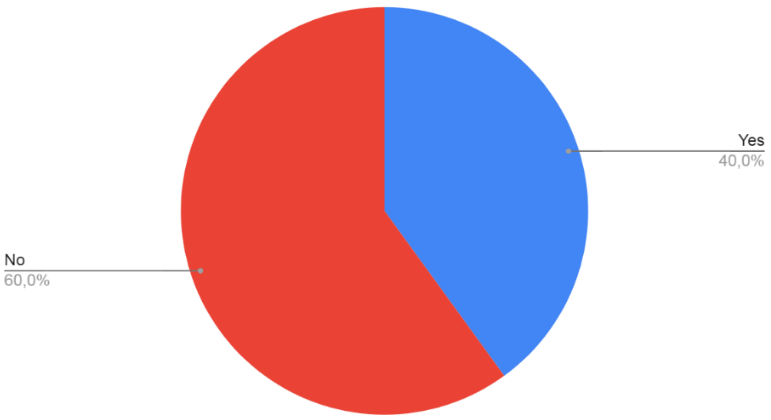

Figure 29: Inseminator status.

A minority of practitioners are qualified as inseminators. This 60%-40% split suggests that artificial insemination is a common but specialized skill within the field.

This proportion is lower than what is seen in some developed countries. For example, a study by de Kruif and Opsomer (2002) in Belgium found that over 80% of bovine practitioners performed artificial insemination. While a study by Rensis et *al.* (2015) in Italy found that over 90% of bovine practitioners were involved in artificial insemination. The lower rate in our study might reflect differences in veterinary education or the structure of agricultural services in Algeria and the specialization of veterinary practitioners in different fields.

III.2. Type of cow

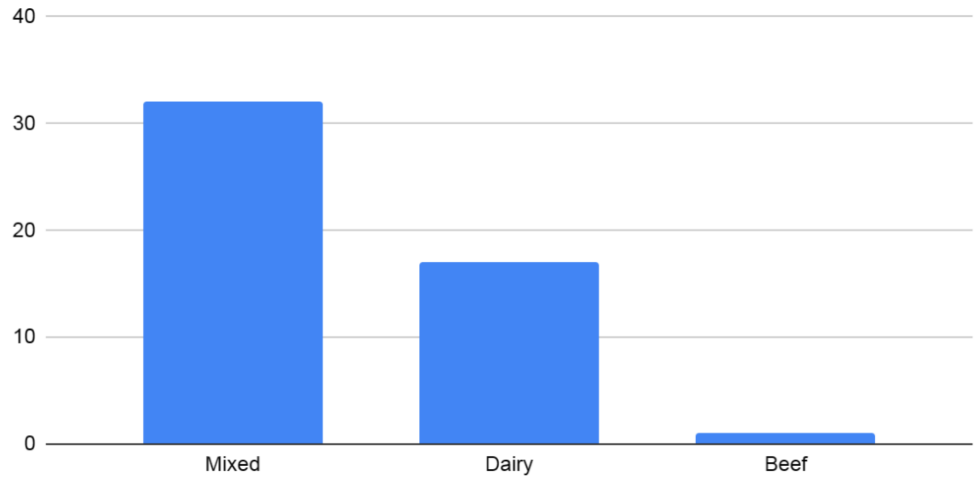

Figure 30: Type of cow.

The figure 30 shows the type of the cow; there is a strong focus on mixed (32) and dairy herds (17), with very little specialized beef cattle practice (1); this aligns with findings from Gherissi et *al.* (2020) in Algeria, who noted the predominance of small-scale mixed farming systems. However, this distribution differs from what is seen in major beef-producing countries. For instance, Jelinski et *al.* (2009) found a more even split between dairy and beef practitioners in Canada. The focus on dairy and mixed herds in Algeria suggests a different agricultural landscape, possibly due to local dietary preferences or economic factors.

III.3. Breed of cow

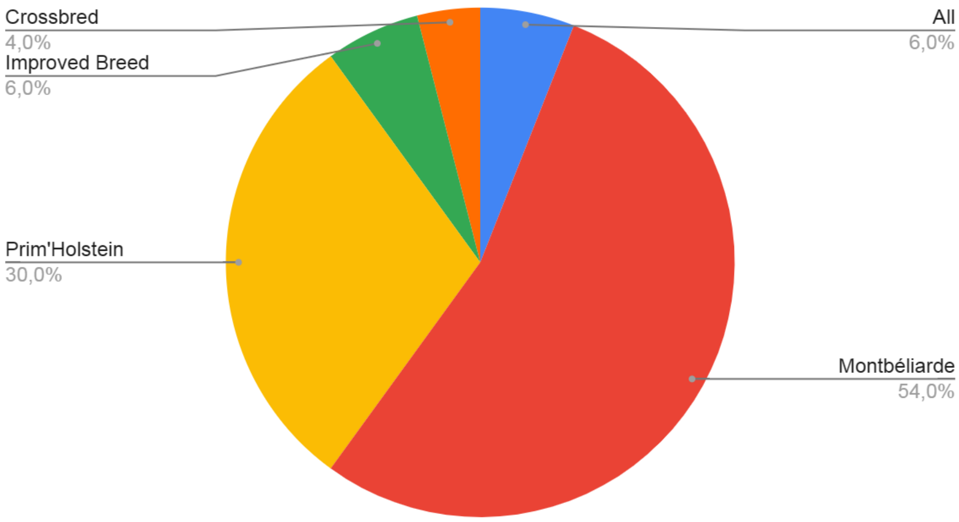

Figure 31: Breed of cow

The figure 31 shows the breed of the cow; Montbéliarde and Prim'Holstein breeds are the dominant breeds, accounting for 80% of the cases veterinarians work with most often. While some (6%) work equally with all breeds, indicating a more generalized practice. Crossbred and improved breeds make up a small but notable portion of the cases 4% and 6% respectively.

These results suggest that these regions likely has a strong dairy industry, as both Montbéliarde and Prim'Holstein are known for milk production. The dominance of these two breeds might indicate a focus on specific traits like milk yield, fat content, or adaptability to local conditions. While the presence of crossbred and improved breeds suggests some farmers are experimenting with genetic improvements or adaptations.

The dominance of Montbéliarde and Prim'Holstein in our survey is consistent with findings from Ouatahar et *al.* (2021) in Algeria. They noted the increasing popularity of these breeds due to their adaptability and high milk yield. However, this differs from global trends where Holstein-Friesian tends to dominate, as reported by Barkema et *al.* (2015) in their review of dairy industry trends.

III.4. Stage of diagnosis performance

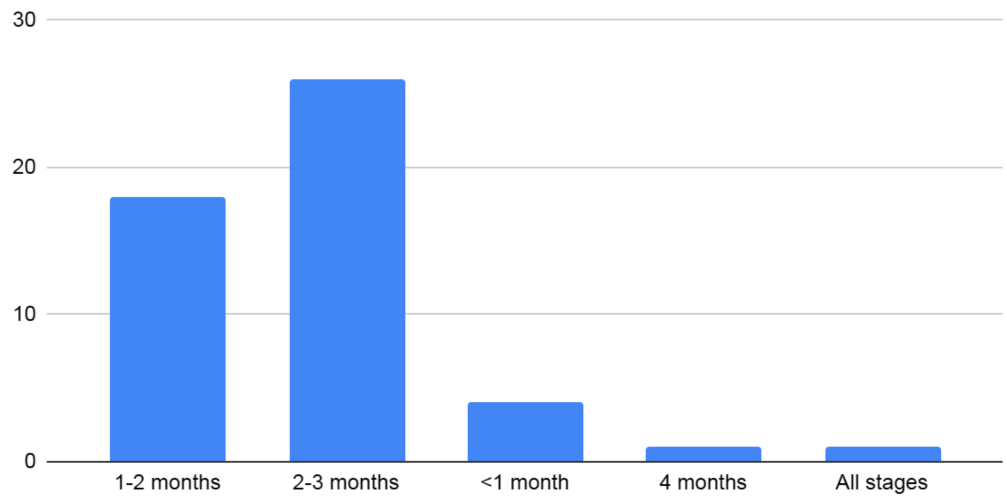

Figure 32: Diagnosis stage.

The vast majority 26 vets (52%) perform diagnosis between 2-3 months of pregnancy, likely due to increased accuracy and reduced risks at this stage. while 18 vets (36%) perform it between 1-2 months. Only 4 vets (8%) diagnose pregnancy before 1 month, suggesting it may require specialized skills or equipment. The single vet performing diagnosis at all stages may indicate a specialized practice or varied clientele. While another vet prefers to perform it at 4 months, it may be due to reduced skills or farmer's preference.

Most veterinarians prefer later pregnancy diagnosis (2-3 months), which is somewhat surprising given modern trends towards earlier diagnosis and differs from recommendations in many developed dairy industries. For instance, Fricke et *al.* (2016) advocate for pregnancy diagnosis as early as 32 days post-insemination using transrectal ultrasonography. The preference for later diagnosis in this survey could be due to available technology, farmer preferences, or local best practices.

III.5. Use of palpation for early pregnancy diagnosis

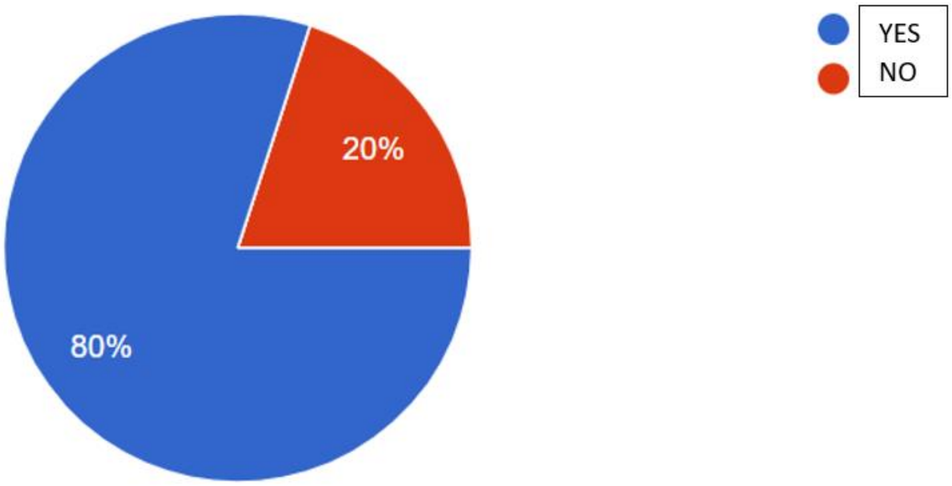

Figure 33: Frequencies of use of palpation for early pregnancy diagnosis.

The study demonstrates that 80% of participants favor palpation as their primary method for early pregnancy diagnosis in dairy cows. This widespread preference highlights palpation's enduring popularity, likely due to its accessibility and established use in the field. However, 20% of respondents opt for alternative methods, indicating a significant minority that relies on different diagnostic approaches. This diversity in practice suggests ongoing exploration and adoption of various diagnostic tools among veterinary professionals.

The high prevalence of palpation use aligns with findings from several studies. Balhara et *al.* (2013) note that rectal palpation remains a widely used method, especially in developing countries, due to its low cost and accessibility. However, this prevalence contrasts with the technological advancements highlighted by Ginther (2014), who emphasizes the revolutionary impact of ultrasound in animal reproduction research.

III.5.1. Preferred gestation stage for palpation

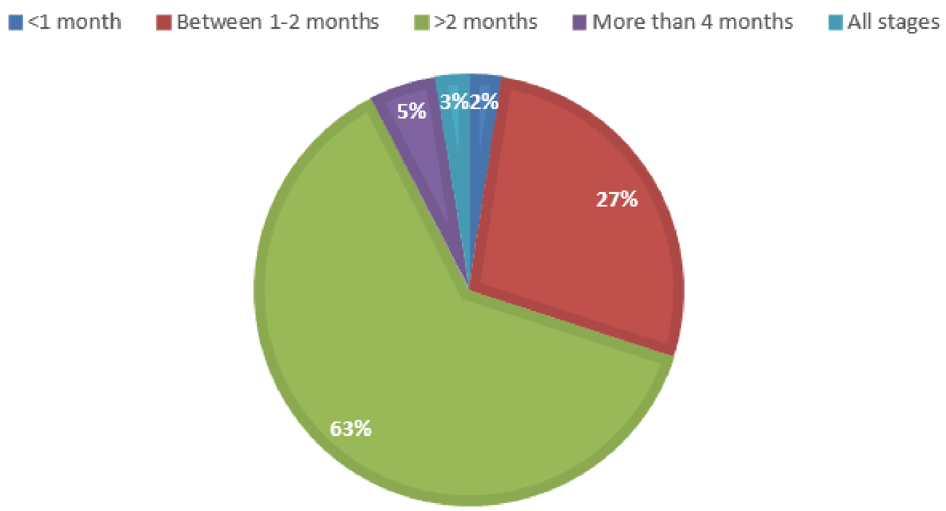

Figure 34: Preferred gestation stage for palpation.

Among those who use palpation, there is a clear preference for later-stage diagnosis. 63% of respondents prefer to perform palpation after 2 months of gestation, while 27% opt for the 1-2 month period. Only a small percentage prefer to palpate before 1 month (2%). This distribution suggests that practitioners generally feel more confident in their diagnosis when the pregnancy is more advanced, possibly due to more easily detectable changes in the uterus at later stages. These findings align with Ball & Peters' (2008) observation that from the 40th day of gestation, it becomes possible to distinguish the difference in size between the two uterine horns.

III.5.2. Reasons for not using palpation

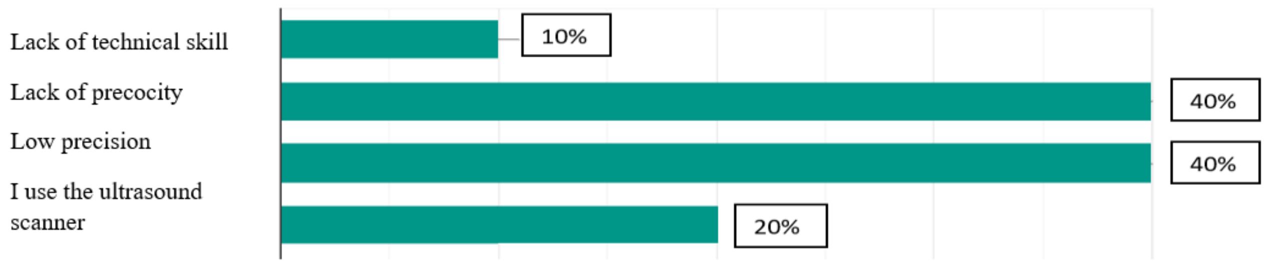

Figure 35: Reasons for not using palpation

The study comes about on reasons for not utilizing palpation adjust with writing discoveries. Concerns almost accuracy and early discovery (each 40% of respondents) are upheld by considers appearing precise palpation is regularly as it were conceivable after day 35 post-breeding (Zemjanis, 1970), with clear uterine horn separation frequently not perceptible until around day 40 (Ball & Peters, 2008). The require for specialized aptitude (10% of respondents) is resounded in writing emphasizing intensive examination procedures (Hopper, 2021). The seen points of interest of other strategies (20% of respondents) are approved by investigate on ultrasonography, which can identify pregnancy prior and give extra data (Kastelic et *al.*, 1989; Lamb & Fricke, 2004).

III.6. Advantages of palpation

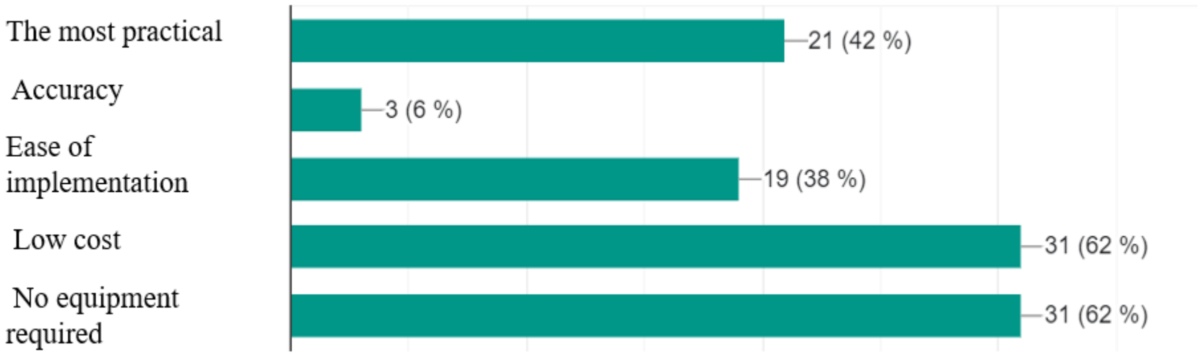

Figure 36: Advantages of palpation.

Respondents cited several advantages of palpation, including practicality (42%), ease of implementation (38%), low cost (62%), and no equipment requirement (62%). These findings are consistent with the literature, which emphasizes the accessibility and established nature of this technique (Ball & Peters, 2008).

III.7. Limitations of palpation

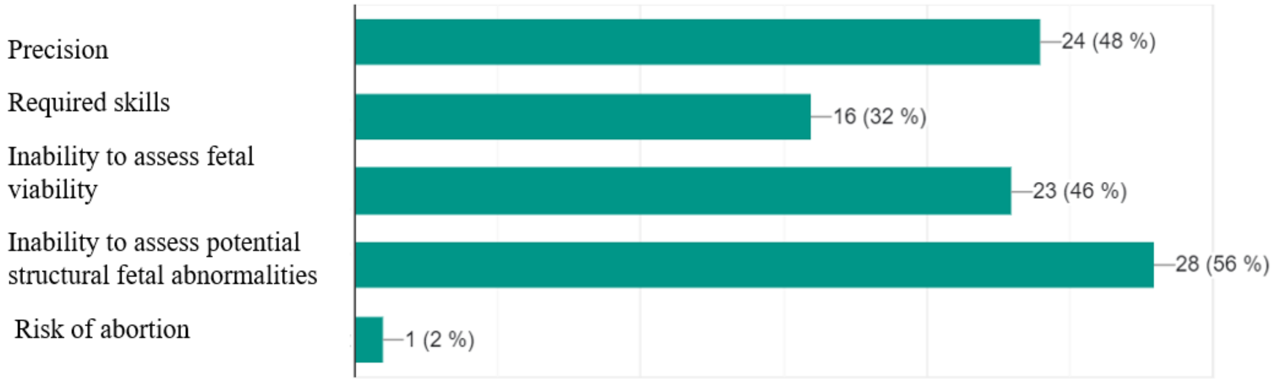

Figure 37: Limitations of palpation

The study highlighted a few confinements of palpation, counting need of accuracy (48%), tall aptitude necessity (32%), failure to evaluate fetal practicality (46%), and failure to distinguish potential basic variations from the norm within the hatchling (56%). These confinements are reliable with the writing. For occurrence, Arthur et *al.* (1982) note that palpation of the amniotic vesicle is as it were attainable between the 30th and 65th day of development, which may contribute to the seen need of accuracy in early determination.

III.8. Use of Ultrasonography for Early Pregnancy Diagnosis

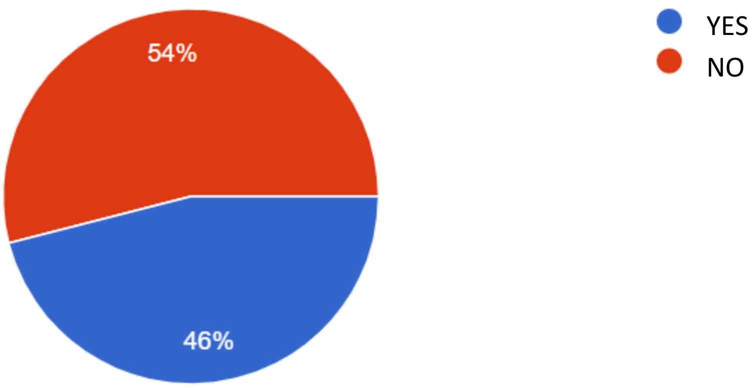

Figure 38: Use of ultrasonography for early pregnancy diagnosis.

The pie demonstrates that 46% of respondents use ultrasonography for early pregnancy conclusion. This tall selection rate recommends an expanding affirmation of the focal points of this innovation in bovine regenerative administration, nearby the progressing utilize of palpation. As famous by Kahn (2004), an experienced veterinarian can perform this strategy as early as 28 days after breeding. (Kahn, W & Volkmann, 2004).

III.8.1. Preferred gestation stage for ultrasonography

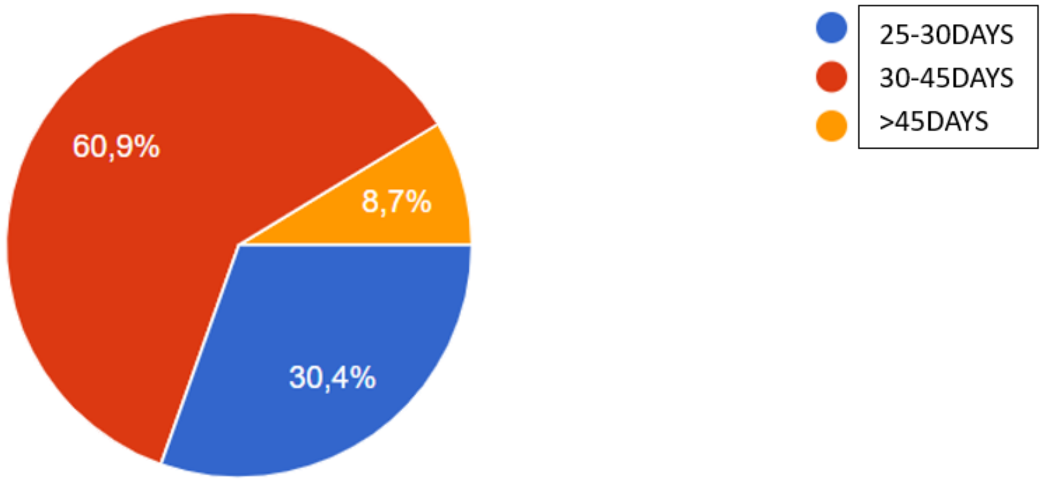

Figure 39: Preferred gestation stage for ultrasonography

Among users of ultrasonography, there is a recognizable inclination for earlier pregnancy diagnosis compared to palpation. Particularly, 60.9% favor to conduct ultrasounds at 25-30 days of incubation, 30.4% select the window of 30-45 days, and as it were, 8.7% hold up until after 45 days. This dispersion underscores ultrasonography's capacity to supply prior and possibly more exact conclusion compared to palpation. Ultrasonography can too give extra information such as fetal gender determination at 55-70 days after breeding (Lamb & Fricke, 2004).

III.9. Advantages of ultrasound

Table 7: Distribution of responses according to advantages of ultrasound.

Advantages of ultrasound	NUMBER	%
Increased precision	37	74
Early detection	43	86
Easy to use	19	38
Evaluation of fetal viability	31	62
Determination of the number of fetuses	36	72
Monitoring ovarian activity in infertility issues for certain cows	1	2
Defining the nature of uterine enlargement (cancer)	1	2

The top advantages cited were:

1. Increased precision (74%)
2. Early detection (86%)
3. Determination of fetal numbers (72%)
4. Fetal viability assessment (62%)
5. Ease of use (38%)

These perceived benefits closely mirror those reported in scientific literature. Ginther (2014) highlighted the superiority of ultrasonography in early and accurate pregnancy diagnosis, while DesCôteaux & Fetrow (1998) emphasized its value in assessing fetal viability and multiple pregnancies. The high percentage of respondents recognizing these advantages suggests a good understanding of ultrasound's capabilities among users.

III.10. Limitations of ultrasound

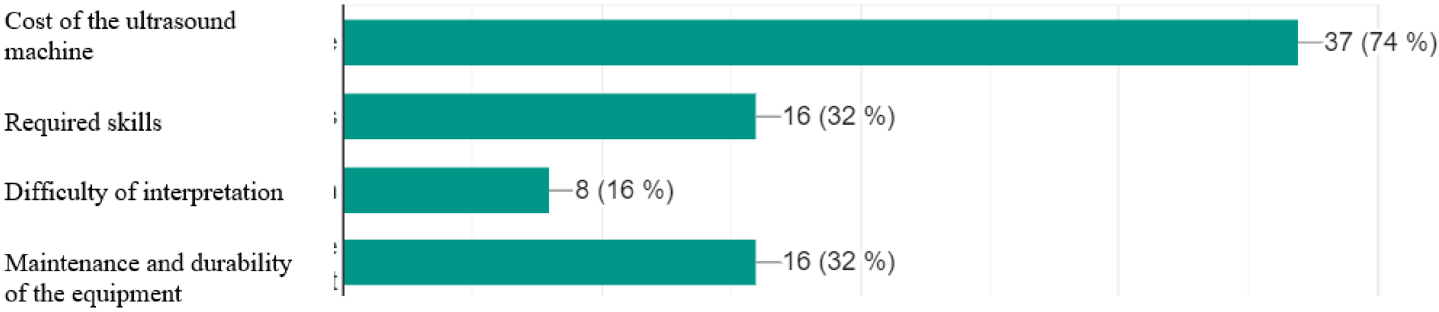

Figure 40: Limitations of ultrasound

The main limitations reported were:

1. Cost of the ultrasound machine (74%)
2. Required skills/competence (32%)
3. Maintenance and durability of equipment (32%)
4. Difficulty in interpretation (16%)

These barriers are consistent with challenges identified in the literature. Mortimer et *al.* (2019) noted that equipment cost and operator skill were the primary factors limiting wider adoption of ultrasonography in bovine practice. The emphasis on cost in our survey (87%) underscores the significant financial barrier that ultrasound technology still presents for many practitioners.

III.11. Use of hormonal assays

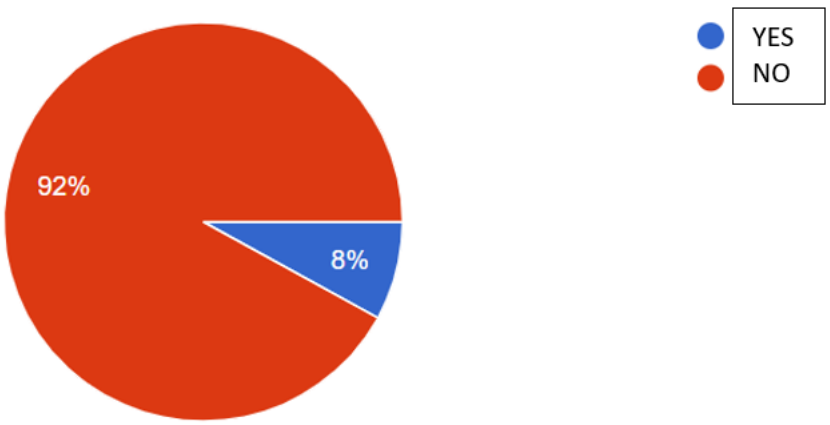

Figure 41: Use of hormonal assays

Only 8% (4 answers) of respondents reported using hormonal assays for early pregnancy diagnosis, while 92% (46 answers) do not. This low adoption rate contrasts with some literature suggesting the potential of hormonal tests. Green (2005) demonstrated the effectiveness of pregnancy-associated glycoprotein (PAG) tests, which can detect pregnancy as early as 28 days post-insemination. The discrepancy between our survey and the literature may reflect slower adoption of newer technologies in the field.

III.11.1. Type of hormone tested

Among the few using hormonal assays, 100% (4 users) test for progesterone. This aligns with the historical use of progesterone as a pregnancy indicator, as described by Roelofs et *al.* (2006). However, it is noteworthy that none of the respondents mentioned using newer tests like PAGs, which have shown promise in research settings (Green, 2005).

III.11.2. Type of sample used

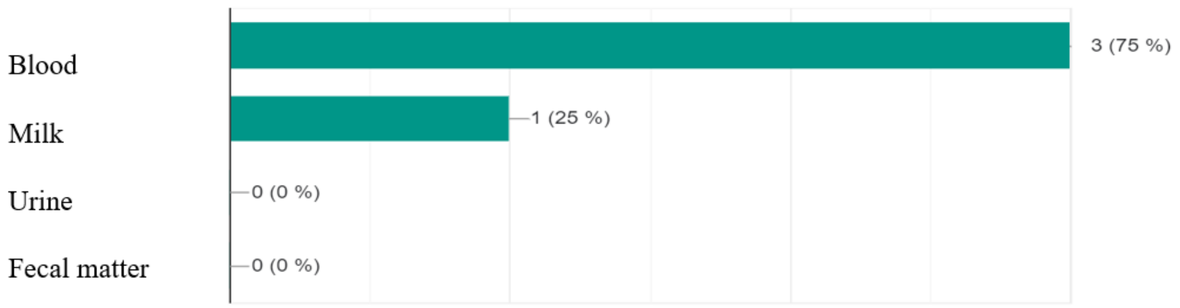

Figure 42: Type of sample used

Of those using hormonal assays, 75% (3 users) use blood samples and 25% (1 user) use milk samples. This distribution reflects the practical considerations of sample collection in field settings. Milk sampling is non-invasive and can be integrated into routine milking procedures, as noted by Roelofs et *al.* (2006), but blood sampling may be preferred for its potentially higher accuracy or when testing non-lactating animals.

III.11.3. Reasons for not using hormonal assays

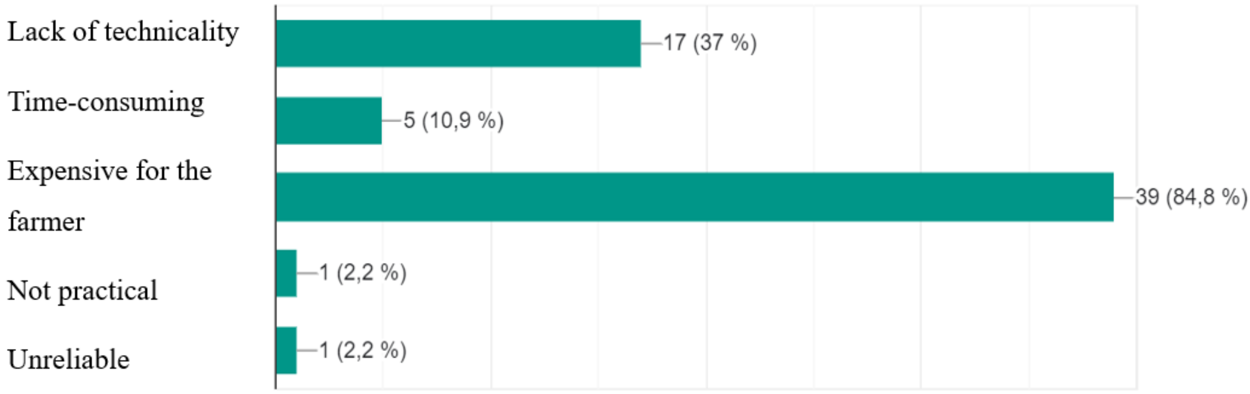

Figure 43: Reasons for not using hormonal assays

The most common reasons given for not using hormonal assays were:

1. Cost to the farmer (84.8%)
2. Lack of technical expertise (37%)
3. Time-consuming nature of the tests (10.9%)

These align with challenges identified by Roelofs et *al.* (2006), who noted that while hormonal assays can be effective, their on-farm implementation faces practical hurdles. The strong emphasis on cost in our survey (76%) suggests that economic factors remain the primary barrier to adoption of hormonal assays in the field.

III.12. Advantages of hormonal assay

Table 8: Advantages of hormonal assay

Advantages	Results
Early detection	23
Increased precision	17
Easy to perform	15
Low risk of false positives	11
Detection of embryonic mortalities	9
Low risk of false negatives	8

Key advantages identified:

1. Early detection: Mentioned 23 times
2. Increased precision: Mentioned 17 times
3. Easy to perform: Mentioned 15 times
4. Low risk of false positives: Mentioned 11 times
5. Detection of embryonic mortalities: Mentioned 9 times
6. Low risk of false negatives: Mentioned 8 times

Early detection is the most cited advantage (46%), highlighting the method's ability to diagnose pregnancy sooner than other techniques. Increased precision is the second most common benefit (34%), suggesting high confidence in the accuracy of hormonal assays.

Ease of use is a significant factor (30%), indicating that the method is practical for field application. Low risks of both false positives and false negatives are noted, further supporting the method's reliability.

The ability to detect embryonic mortalities is seen as an important advantage by some veterinarians. The combination of early detection and increased precision is particularly valued, appearing together in several responses.

This data suggests that hormonal assays are seen as a reliable, accurate, and practical method for early pregnancy diagnosis in cattle. The combination of early detection and precision appears to be the most valued aspect of this method.

III.13. Limitations of hormonal dosage use

Table 9: Limitations of hormonal dosage use

Limitation	Unavailability of kits	Cost	Time-consuming	Stressful during blood sampling
Results	74% (35 times)	60% (31 times)	8% (6 times)	14% (7 times)

Kit unavailability is the most frequently cited limitation (70%), pointing to a systemic issue in accessing necessary while Cost is a close second (62%), indicating that even when available, the method may be prohibitively expensive for routine use. The combination of unavailability and cost is particularly common, suggesting these issues may be interrelated.

The stress on animals during blood sampling is a concern for some vets (12%), but less prominent than logistical issues. Time constraints appear to be a minor but notable concern for a few practitioners.

III.14. Rapid dosing kits

III.14.1. Familiarity with rapid dosing kits

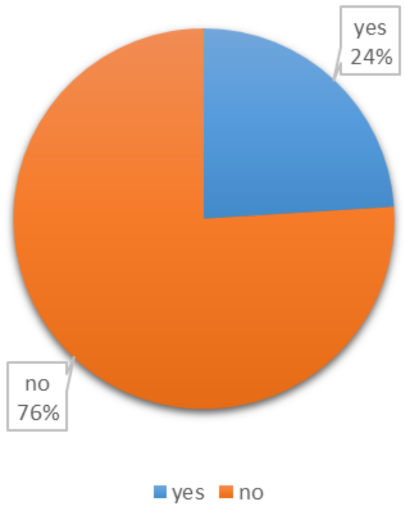

Figure 44: Familiarity with rapid dosing kits

The information presented in the pie chart indicates that 24% of veterinarians acknowledged familiarity with rapid dosing kits by responding to (Yes), whereas 76% of veterinarians indicated lack of awareness by responding to (No). It shows that there is a significant lack of awareness about rapid dosing kits among veterinarians, with only about a quarter familiar with them.

III.14.2. Type of kits

There are 6 responses listed, indicating a range of familiarity with different types of rapid dosing kits among the veterinarians who know about them.

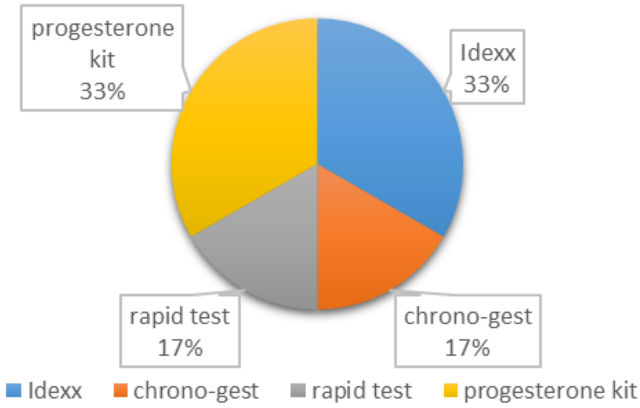

Figure 45: Type of kits

This pie chart illustrates the distribution of different types of pregnancy detection kits used. It shows an equal preference for Idexx and progesterone kits, each accounting for a third of the total usage. Chrono-gest and rapid tests are used equally, each making up 17% of the kit types.

III.15. Advantages of rapid dosing kits

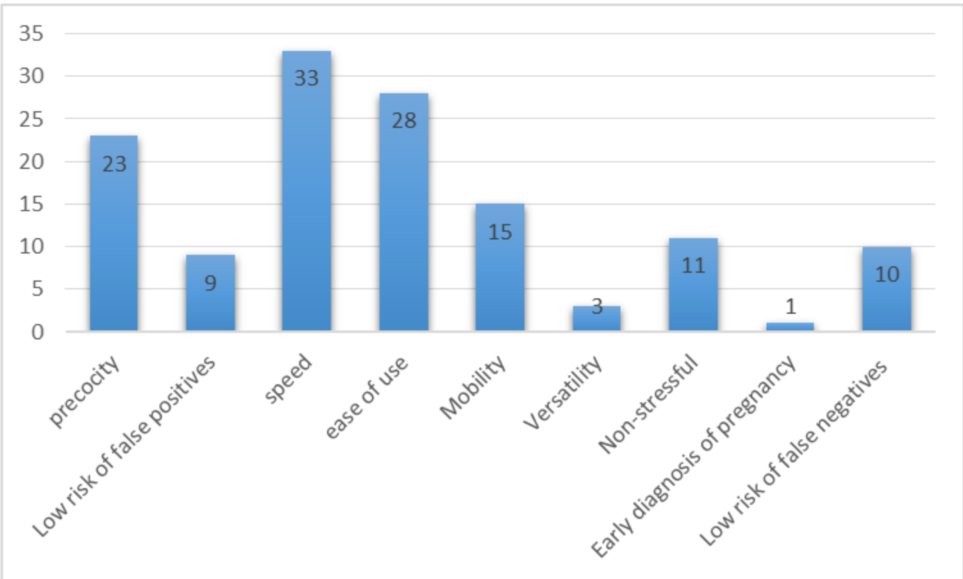

Figure 46: Advantages of rapid dosing kits

This chart is a visual representation of the three key benefits of rapid dosing kits in early pregnancy diagnosis over other methods acknowledged by participants.

The above graph shows that Veterinarians prioritize the speed of use or ease of use, with 33 and 28 responses respectively for Rapid Dosing Kits. These are just emphasizing more on different aspects. The second most cited benefit was precision with 23 responses. Mobiles kits and not stressful are another eight who call these important in the mid-range, while seven find them helpful or a good reality check. Ten respondents recognize the low risk of false negatives but benefits such as low false positives or general applicability were virtually unmentioned (9 and 4 mentions, respectively).

In contrast to all prior evaluations, French studies also emphasize speed and ease of use as a primary benefit. One survey suggested that veterinarians prioritize rapid diagnostics to cut down on time spent diagnosing per case, pivotal in high-volume practices. (Fiocco et al., 2007). Precision is highly valued. In France, 30% of respondents in a similar survey rated precision as a top benefit. The risk of false negatives to the patient was reported as being low and based on French studies, however with less impact than speed / ease. Moreover, a total of 12% answered those (Fiocco et *al.*, 2007).

III.16. Limitations of rapid dosing kit

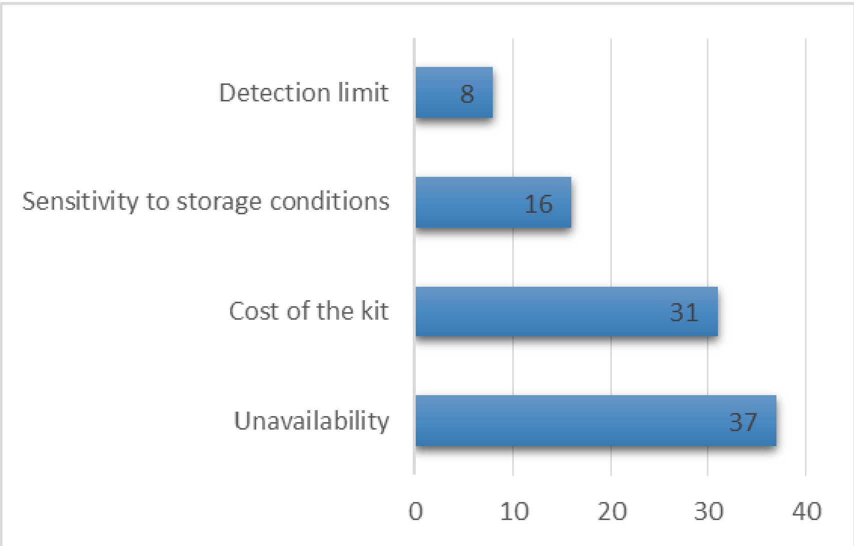

Figure 47: Limitations of rapid dosing kit

The graph below demonstrates respondent perceptions of the restrictions on rapid dosing kits for early pregnancy diagnosis. Lack of availability is the top issue, identified by 37 (3rd) community members. The next limitation noted by 31 respondents is the cost of the kit. Third was issues with sensitivity to storage conditions - 16 responses. In comparison, the detection limit was noted least frequently as a limitation; 8 respondents.

In France, availability overall is much less of a concern than it might be in developing countries. However, it remains a problem in isolated rural areas. A study published by INRAE (French National Institute for Agricultural Research) reported that 25% of rural- area veterinarians identified availability as a limitation .It is undeniable that the cost is still a major obstacle in France. Thirty responses roughly align with the finding of 30% from a survey conducted by the Ministry of Agriculture and Food; which revealed that many veterinarians consider rapid dosing kits to be prohibitively expensive (Nowak, 2021).

III.17. The use of rapid dosing kits in the future

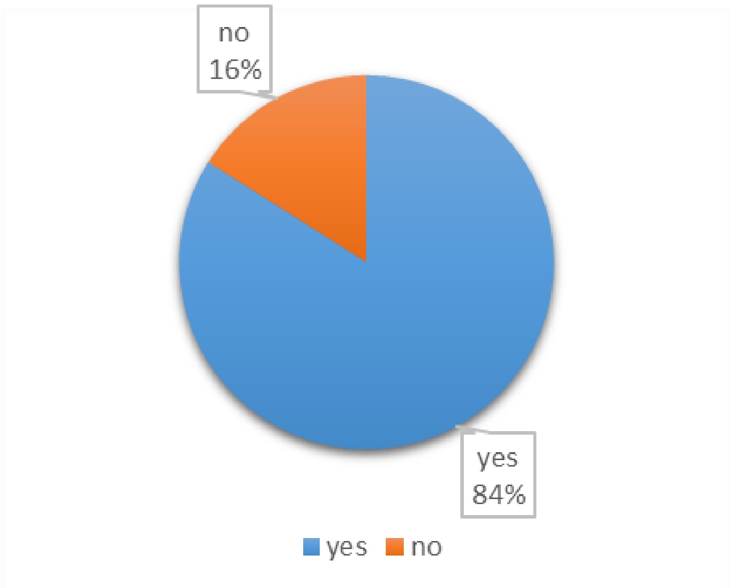

Figure 48: The use of rapid dosing kits in the future

The graph separates itself with 84% of the answers as "yes" representing a massive blue sector, and 'no' homed in on the smallish orange slice for only 16%. According to the data, a strong majority (84%) of survey respondents said they would use rapid dosing kits more often should they be made available down the line. The only discipline where the experts will not increase usage is expenditure (16%).

III.18. The factors most often influencing the success of insemination

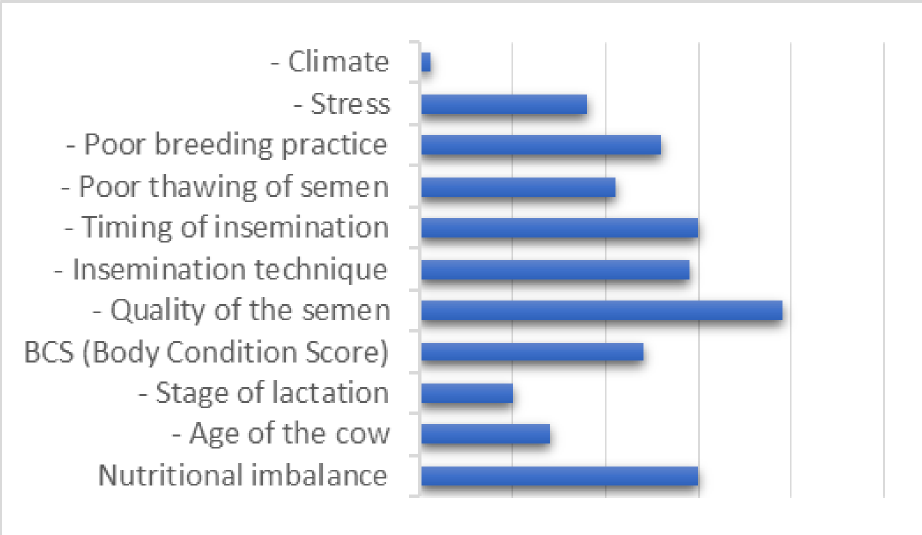

Figure 49: The factors most often influencing the success of insemination.

The graph highlights various factors influencing the success of insemination in cattle breeding. The most significant factors identified are the timing of insemination, the insemination technique, nutritional imbalance, and the quality of the semen. These factors emphasize the importance of accurately identifying the cow's estrus cycle, ensuring proper training and skill of the inseminator, maintaining a balanced diet and nutrition management, and adhering to proper semen collection, storage, and handling procedures. Moderate influencing factors include poor breeding practice, poor thawing of semen, Body Condition Score (BCS), and stress. These suggest that while overall animal welfare and health management are important, they are not as critical as the top factors.

Less significant factors are the age of the cow and the stage of lactation. This indicates that with proper management, successful insemination can occur across various life stages of the cow. Lastly, climate appears to have the least impact among the factors listed, suggesting that it is less crucial compared to other controllable factors in the insemination process.

This information is valuable for farmers and breeders to prioritize their efforts and resources to improve insemination success rates in their herds.

In Algeria, the most significant factors identified were the timing of insemination, the insemination technique, nutritional imbalance, and the quality of the semen. These factors underscore the importance of accurate estrus detection, skilled inseminators, proper nutrition management, and meticulous handling of semen.

In contrast, studies from France, such as those analyzed by (Boichard et *al.*, 1998), highlight similar critical factors but also emphasize genetic influences on conception rates. The timing of insemination, technique, and semen quality are consistently important. Additionally, French research suggests that the economic impact of failed inseminations, including costs related to extra management and losses due to extended open days, is a significant consideration for farmers. This aspect of economic analysis is more detailed in the French studies compared to the findings from Algeria. (Grimard et *al.*, 2006) note that environmental conditions and genetic factors significantly impact first-service conception rates and overall reproductive performance in dairy herds. Both Algerian and French studies recognize the importance of nutrition. However, the French studies place a stronger emphasis on genetic evaluation and environmental variations affecting fertility. For instance, the success rate of artificial insemination (AI) is often influenced by breed, season, and management practices in French studies, which were not as heavily emphasized in the Algerian context.

III.19. The anomalies/pathologies that impact the early stages of pregnancy

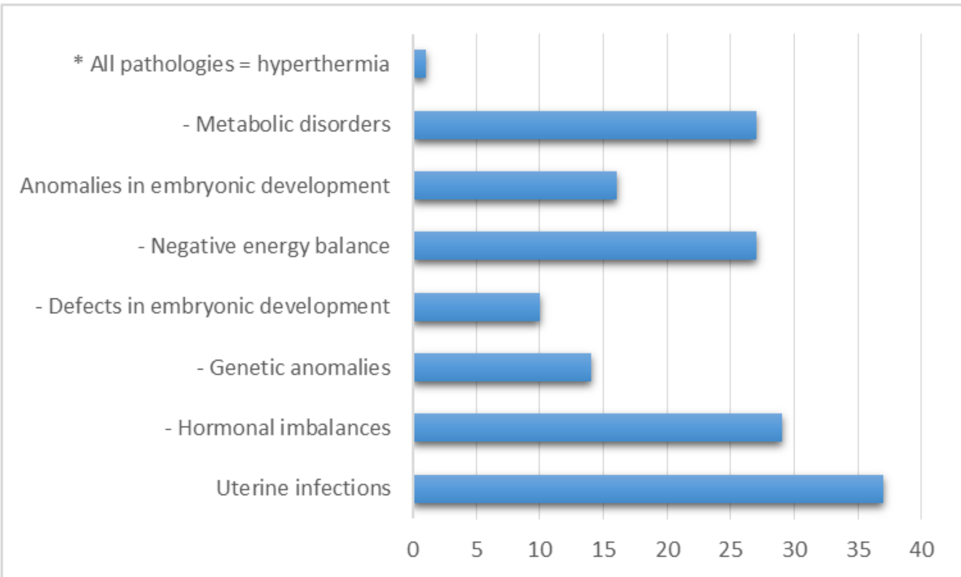

Figure 50: The anomalies/pathologies that affect the early stages of pregnancy.

Various anomalies and pathologies that affect the early stages of pregnancy are shown in this horizontal bar graph. The largest range of the bar in uterine infections, at around 38-40 units.

Hormonal imbalances come in at a close second, with one bar extending about 32-34 units. Negative energy balance is also a huge player, right up there with the other long-term hormonal imbalances. This should also be noted in the context of metabolic disorders, another important factor with an average bar ranging at close to 30 units. Embryonic developmental anomalies have a moderately support bar at about 18-20 units. They are less impactful than developmental anomalies, but these genetic abnormalities can still greatly affect a couple's chances of either having a pregnancy or producing their healthy offspring. Embryonic implantation defects have a decreased influence compared to the above factors but are significant nonetheless. The length of all pathologies such as hyperthermia   is surprisingly low, given that it should in theory be the sum of all other factors. This might be a visual error in the chart or it could represent an alternate metric.

High prevalence of uterine infections is consistent across different countries like the United States with rates ranging from 20-40%, Brazil with a high incidence (35-45%) (Oliveira Padilha et *al.*, 2024) and France with around 30-40%, indicating a common issue in dairy herds globally. Embryonic Developmental Anomalies and Implantation Defects: are less documented in the studies from other countries but are recognized as significant issues affecting pregnancy outcomes.

III.20. Additional comments and suggestions

A total of 12 replies have been recorded. One respondent suggests using ultrasound before 40 days as it is more reliable and less traumatic than palpation. Other comments mention that early diagnosis is less expensive and encourages the use of ultrasound. Due to the elevated occurrence of late embryonic mortality, the final suggestion proposes that confirming pregnancy at 3 months would be more advantageous. The ultrasound appears to be the preferred method for early pregnancy diagnosis in cows, with multiple veterinarians recommending it.

IV. Conclusion

Based on the experimental study presented, we can provide the following conclusion:

The study, which surveyed 50 veterinarians across 15 regions in Algeria, offers valuable insights into the current practices and perceptions of early pregnancy diagnosis methods in cows:

1. Palpation remains the most widely used method (80% of respondents), primarily due to its practicality, low cost, and lack of equipment needed. However, it is generally performed later in pregnancy (2-3 months) due to accuracy concerns.
2. Ultrasound is gaining popularity (46% of respondents), valued for its accuracy, early detection capabilities, and ability to assess fetal viability. Cost and skills remains the primary barrier to wider adoption.
3. Hormonal assays are rarely used (8% of respondents), mainly due to cost and unavailability of kits. However, they are recognized for their potential in early and accurate detection.
4. There's growing interest in rapid dosing kits, with 84% of respondents expressing willingness to use them more if available. Their speed and ease of use are seen as primary advantages.
5. The timing of insemination, insemination technique, nutritional balance, and semen quality were identified as the most crucial factors affecting insemination success.
6. Uterine infections, hormonal imbalances, and negative energy balance were reported as the most common pathologies affecting early pregnancy.

The study reveals a disparity between traditional methods and newer technologies in bovine reproductive management in Algeria. While there is recognition of the benefits of advanced methods like ultrasonography and hormonal assays, practical constraints such as cost and availability limit their widespread adoption. This suggests a need for strategies to make these technologies more accessible and affordable to improve reproductive management in Algerian cattle herds.

The findings also highlight the importance of continued education and training for veterinarians in early pregnancy diagnosis techniques, as well as the need for improved nutritional management and disease control in cattle herds to enhance reproductive outcomes.

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