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**Factors affecting pregnancy maintenance in dairy
cattle: A corpus luteum perspective**

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Abstract

This literature review investigates the relationship between ovarian abnormalities and the successful maintenance of pregnancy in dairy cows. Notable findings during US diagnostics in dairy cows include the presence of additional CL, cavitary CL and CL of various sizes. Each of these ovarian abnormalities may exert varying influences on the outcome of pregnancy, either favorably or less favorably. Furthermore, this review explores different diagnostic methods and their associations with fetal survival rates.

Drawing from articles, reviews, and literature from studies conducted in various countries across the Euroatlantic region, this review underscores the critical importance of precise pregnancy diagnostics in dairy cattle.

Our analysis reveals a strong correlation between the presence of additional CL, elevated P4 concentrations, and the successful maintenance of pregnancy. Similarly, physiological cavitary CL is linked to an increase in luteal tissue and P4 concentrations, thus positively impacting pregnancy outcomes. While the impact of CL size variation warrants further investigation, our findings suggest a positive correlation with pregnancy outcomes. Notably, no significant differences in outcomes were observed between FMS and ASP during RP.

Our review concluded that none of the identified ovarian abnormalities had a detrimental effect on pregnancy in dairy cows. Furthermore, we explore treatment options aimed at achieving specific abnormalities.

Keywords: dairy cows, embryonic/fetal mortality, pregnancy, corpus luteum, progesterone, ultrasound, transrectal palpation

Összefoglalás

Ez az irodalom áttekintés a petefészek rendellenességek és a tejlő tehenek vemhességének sikeres fenntartása közötti kapcsolatot vizsgálja. A tejlő tehenek ultrahangvizsgálata során jelentős megállapítások közé tartozik további CL jelenléte, üreges CL, ill. különböző méretű CL-ok. Ezek a petefészek rendellenességek mindegyike változó hatást gyakorolhat a vemhesség kimenetelére, akár kedvezően, akár kevésbé kedvezően. Emellett az áttekintés különböző diagnosztikai módszereket vizsgál, és azokat kapcsolatba hozza a magzatok túlélési esélyével.

Az Euroatlanti régió különböző országaiban végzett vizsgálati eredmények felhívják a figyelmet a pontos vemhességi diagnózisok fontosságára a tejlő tehenek esetében.

Felmérésünk szerint szoros összefüggés mutatható ki a további CL jelenléte, a megnövekedett progeszteron koncentráció és a sikeres vemhesség fennmaradása között. Hasonlóképpen, a fiziológiai üreges CL összefügg a luteális szövet és a progeszteron koncentráció növekedésével, ami pozitívan befolyásolja a vemhesség kimenetelét. A CL méretváltozásának hatása további vizsgálatokat igényel, de eredményeink pozitív korrelációt mutatnak a vemhességi kimenetelével kapcsolatosan. Fontos megjegyezni, hogy a rektális vizsgálat során alkalmazott módszerek (a magzatburkoknak az ujjaink között átcsúsztatása, ill. az amnionhólyag tapintása) között nem észleltek jelentős különbségeket.

Az áttekintésünk arra a következtetésre jutott, hogy azonosított petefészek rendellenességek egyike sem gyakorolt negatív hatást a tejlő tehenek vemhességére. Emellett megvizsgáltuk a specifikus rendellenességek elérésére irányuló kezelési lehetőségeket is.

Kulcsszavak: tejlő tehenek, embrionális/magzati mortalitás, vemhesség, corpus luteum, progeszteron, ultrahang, rektális vizsgálat

Contents

1. Introduction	6
2. Literature review	8
2.1. Dynamics of the estrous cycle	8
2.2. Physiology of the corpus luteum	9
2.3. Ovarian abnormalities	10
2.3.1. Additional corpus luteum	10
2.3.2. Cystic ovaries	10
2.3.3. Size of the corpus luteum	12
2.4. Pregnancy diagnosis	13
2.4.1. Rectal palpation	13
2.4.2. Ultrasonography	15
2.4.3. Biochemical pregnancy diagnosis	16
3. Objectives	19
4. Methods	19
5. Results	20
5.1. Ovarian abnormalities	20
5.1.1. Additional corpus luteum	20
5.1.2. Cystic ovaries	23
5.1.3. Size of the corpus luteum	24
5.2. Pregnancy diagnosis	26
6. Discussion	29
6.1. Ovarian abnormalities	29
6.1.1. Additional corpus luteum	29
6.1.2. Cystic ovaries	31
6.1.3. Size of the corpus luteum	32
6.2. Pregnancy diagnosis	33
7. Summary	35
8. References	36
9. Acknowledgements	42

Abbreviations

AI = Artificial Insemination

ASP = Amniotic Sac Palpation

CL = Corpus Luteum

E2 = Estrogen

EPFs = Early Pregnancy Factors

FMS = Fetal Membrane Slip

GnRH = Gonadotropin-Releasing Hormone

ISGs = Interferon-Stimulated Genes

LH = Luteinizing Hormone

P4 = Progesterone

PAGs = Pregnancy-Associated Glycoproteins

PGF2 α = Prostaglandin F2 α

PRID = Progesterone-Releasing Intravaginal Device

PSPB = Pregnancy Specific Protein B

RP = Rectal Palpation

TRUS = Transrectal Ultrasonography

US = Ultrasonography

1. Introduction

Reproductive performance and milk production are the primary factors contributing to the profitability of dairy cows. While genetics and nutritional management have led to increased milk production per cow, this development has also resulted in reduced fertility [1]. The economic losses associated with pregnancy failure in high-producing dairy farms are a major concern, particularly when comparing poor fertility rates with good reproductive performance. Research has shown that 90% of pregnancies are lost following pregnancy diagnosis before gestation day 90 [2, 3]. Increased open days and thus prolonged calving intervals further decrease profitability [4]. Detecting lost pregnancies as early as possible is crucial for mitigating intervals between calving. While a fetal loss rate of 10% is generally accepted, an increase in this incidence warrants an intensive investigation of the underlying causes [5, 6]. Therefore, it is necessary to identify potential risk factors for pregnancy loss to reduce associated costs.

Pregnancy loss can occur due to various reasons, including both infectious and non-infectious factors. Among viral diseases, bluetongue, bovine viral diarrhoea virus (BVDV) and bovine herpes virus (BHV-1) are the most common. Bacterial and protozoal diseases, such as trichomoniasis and campylobacteriosis, also contribute to pregnancy loss. Infection of the embryo can occur directly or indirectly through intrauterine or systemic infection following viraemia or bacteraemia [6]. Additionally, clinical diseases like mastitis and lameness can lead to pregnancy loss [2]. Non-infectious causes have diverse origins. Genetic factors such as the quality of the bull's semen or the cow's physiology and anatomy, can contribute to hereditary fertility issues [6, 7]. Environmental factors, including weather changes and exposure to mycotoxin-containing feed, can also play a role. Nutritional factors significantly impact reproductive success by affecting negative energy balance and the body condition score (BCS) of high-producing dairy cows [2, 6]. Maternal factors, such as hormonal imbalances, embryo-maternal interactions, the age of the dam, and uterine space, especially in twin pregnancies, are crucial. Studies have shown that cows with twins have a 3.1 times higher risk of pregnancy loss compared to cows with singleton calf [2, 7].

Veterinarians themselves play an important role in minimizing embryonic loss. By implementing necessary hygienic measures, they can decrease the infection rate during

examinations. Moreover, their training in AI technics, estrus detection and pregnancy diagnosis can improve efficiency [8]. Minimizing trauma caused by pregnancy diagnosis, whether performed by RP or TRUS examination, is of great importance.

Pregnancy diagnosis is a critical tool for confirming pregnancy, identifying non-pregnant animals, and detecting lost pregnancies. Detecting non-pregnant cows is crucial for reducing the number of open days on dairy farms and facilitating re-insemination [9]. Gestation is divided into two periods: the embryonic period, which lasts for the first 42 days, and the fetal period, which extends from the day 42 until parturition [10]. Initial pregnancy diagnosis is typically performed in the late embryonic period, with a follow-up confirmation 4-6 weeks later due to the increased risk of pregnancy loss before the early fetal period [9]. Diagnosing late embryonic and early fetal losses is often challenging due to their multifactorial nature [8].

Understanding the ovarian cycle, follicular dynamics and examining ovarian structures allow for estimating the stage of the estrous cycle and diagnosing pregnancy in cows. Abnormalities observed during these examinations can provide insights into the possibility of pregnancy loss. Such information is vital for making optimal treatment decisions on a case-by-case basis [11].

The aim of the present literature review is to explore abnormalities regarding the CL that can be diagnosed during early pregnancy diagnosis and may contribute to pregnancy loss. Specifically, the focus will be on additional CL, ovarian cysts, and differences in CL size. Various methods for diagnosing these conditions will be discussed, emphasizing the importance of timing and accuracy in pregnancy diagnosis to improve reproductive efficiency [5]. Additionally, the potential risks associated with different diagnostic methods will be analyzed. Finally, treatment options will be discussed, providing farmers with the opportunity to either address or mitigate the identified pregnancy risk.

2. Literature review

2.1. Dynamics of the estrous cycle

The estrous cycle in cattle, driven by the P4 secretion of the CL, is divided into two main phases: the follicular phase and the luteal phase. Each phase is further divided into two parts. The follicular phase consists of proestrus and estrus, while the luteal phase includes metestrus and diestrus [12, 13].

During the proestrus phase, the CL is regressing, while a primordial follicle is activated and grows into a tertiary follicle [12, 14]. There is a shift from P4 dominance, produced by the CL, to E2 dominance, secreted by the growing follicle [12]. Follicular growth occurs in waves, typically two or three waves [15], and at the end of the proestrus, in most cases only one dominant follicle continues to grow while the others regress [14].

The estrus phase is characterized by the absence of the CL and the ovulation of the dominant follicle [12]. Luteolysis, the functional and structural destruction of CL, occurs during this phase [13, 16]. The decrease in P4 concentration removes the negative feedback between gonads and the pituitary-hypothalamus complex, leading to an increase in LH. The absence of P4 and the peak of E2 concentration trigger the LH surge, which results in ovulation. After ovulation, an early CL is formed from the wall of the ovulatory follicle [12, 14].

The luteal phase comprises approximately 80% of the estrous cycle [12]. P4 becomes the main steroid hormone produced and secreted by the growing CL, leading to a shift from E2 dominance to P4 dominance. The secretion of LH is restructured after ovulation [13]. Metestrus begins at ovulation and diestrus ends around luteolysis in the absence of pregnancy [12].

2.2. Physiology of the corpus luteum

The CL is an endocrine gland that forms after ovulation and is responsible for the production of P4. As the CL grows, its ability to secrete P4 increases until it reaches its mature size. If fertilization occurs, the CL does not regress because P4 is necessary for preparing and maintaining the reproduction tract for pregnancy. P4 plays a vital role in the maternal recognition of the conceptus and is essential for maintaining pregnancy [13]. In cattle, the embryo/fetus is dependent on luteal P4 for approximately 200 days of gestation [17].

PGF2 α can induce significant morphological changes of the CL. Physiologically, the uterus releases PGF2 α at the end of diestrus, leading to CL regression [12]. If PGF2 α is administered to an animal, apoptosis of the CL and a decrease in P4 synthesis can be observed 24-26 hours later [13]. This method is commonly used for pregnancy termination [18].

Monitoring ovarian structures, particularly the CL, is important in breeding management practices in cattle. During RP, a CL can be felt approximately five days post-ovulation. In case of pregnancy it becomes smooth, rounded and covered with epithelial cells [19]. However, US is the most used tool for imaging the CL. It provides reliable and accurate assessment of the normality and stage of the estrous cycle. In US images, a physiological CL appears either as a homogeneous, uniform echodense structure or heterogeneous with a central fluid-filled cavity. The serous transudate fluid within the cavity appears as anechoic [12, 14]. In some cases, no fluid-filled cavity is observed [12, 20]. As the CL matures, the central cavity tends to become smaller and undetectable [14].

Ovarian structures, especially the CL, are helpful and important reference structures during the pregnancy diagnosis. The presence of an additional CL, a cavitory CL and the differentiation in size of the CL could raise the concern for an increased risk of pregnancy loss [20]. These changes in relation to the CL may lead to impaired releases in necessary steroid hormones and therefore may affect the success of the reproductive events [12].

2.3. Ovarian abnormalities

2.3.1. Additional corpus luteum

An additional CL in a pregnant cow refers to having a number of CL higher than the number of embryos present [7]. The CL is the main source of P4 in the pregnant cows, which is crucial for the survival and development of the conceptus [13, 16]. The presence of an additional CL raises questions about its impact on pregnancy efficiency.

Studies have shown that pregnant cows with an additional CL are three times more likely to have high plasma P4 concentrations compared to cows with a single CL [21]. Besides, cows with an additional CL are 8.3 times less likely to experience pregnancy loss compared to cows with no extra CL [2]. The increased P4 levels associated with an increased number of CL suggest that an additional CL served as a preventive factor against early fetal loss. The positive association between multiple CL and pregnancy maintenance will be discussed further throughout the paper.

During early pregnancy diagnosis, it is important to compare the number of CL with the number of conceptuses to differentiate between twin pregnancy and additional CL. Detecting two or more CL can result in two types of errors: either diagnosing a singleton pregnancy in a cow carrying twins or diagnosing a twin pregnancy in a cow carrying only one embryo but with two CL. Special attention should be given to cows displaying two CL or twins to accurately differentiate between singleton and twin pregnancies [22].

2.3.2. Cystic ovaries

In high-producing dairy cows, cystic ovarian follicles are a common and significant ovarian disorder that can impact reproductive efficiency to some extent [23]. It is important to clarify the terminology used in relation to cysts. When discussing cystic ovaries, the term primarily refers to follicular cysts, although luteal cysts and cystic CL can also fall under the same classification. The confusion in terminology arises from the difficulty in distinguishing between cysts when detected through RP or US examination [12, 24]. Luteal cysts are also known as luteinized-follicular cysts, as they develop from follicles that fail to ovulate and subsequently become luteinized [12, 25]. The term 'cystic' can describe both pathological and physiological structures, so it is suggested to replace the term 'cystic CL' with 'CL with a cavity' to differentiate between physiological and pathological conditions. The former are cavitory CL, the latter are follicular and luteal

cysts [12]. Cavitory CL originate from follicles that successfully ovulate and form a cavity during CL development [25].

Differentiating between cystic and physiological fluid-filled structures through RP is challenging, and abnormalities resembling cysts are mostly associated with pathological conditions [26]. Cysts identified through RP are characterized by the presence of multiple follicles with a diameter of at least 20 mm persisting in the ovaries for at least 10 days, absence of CL, and lack of uterine fluid. Due to the limitations in sensitivity and specificity of RP, US examination is recommended for better accuracy [25]. A better understanding of cysts and cavitory CL has been achieved by US and rapid P4 assays, as their timing of appearance and P4 production differ significantly [24].

Cavitory CL appear shortly after CL formation and may go undetected due to their small size. They can be distinguished from luteal cysts by their thick walls and the presence of an ovulation crown [24]. The cavity of a cavitory CL is fluid-filled, appearing as a centrally located anechoic area within the luteal tissue. Its size can range from 3 mm to 20 mm, with the maximal dimension depending on the duration of existence [27].

B-mode US can differentiate between a cavitory CL and a cyst, as well as distinguishing between follicular and luteinized cysts. The type of cysts can be determined by wall thickness and the location of blood vessels. Follicular cysts show blood flow within the wall, while luteinized cysts have blood vessels surrounding the wall [28]. Luteinized cysts have thicker walls (>3 mm) and can produce higher levels of P4 than follicular cysts, which have thinner walls (<3 mm). Both structures appear as uniformly anechoic ovarian structures, although luteal cysts may exhibit an echogenic rim [23, 28].

P4 secretion differ significantly between cysts and cavitory CL. Follicular lutein cysts secrete P4 concentrations greater than 0.5 ng/ml, while follicular theca cysts are associated with lower P4 concentrations [24].

The combination of palpation or visualization and P4 measurement is crucial for differentiating between the different types of cysts and guiding the appropriate treatment options.

2.3.3. Size of the corpus luteum

The size of the CL has been a topic of interest in relation to its correlation with P4 concentration and its potential influence on pregnancy maintenance [27, 29, 30]. The size of the CL can provide information about its functionality [30]. It is logical to assume that cows with smaller CL diameters would have lower P4 concentration compared to those with optimally sized CL. A small or poor-quality CL may indicate low P4 levels, which could lead to pregnancy loss [20, 31].

TRUS has an advantage over RP in that it allows for the measurement of ovarian structures diameters. While RP provides only a subjective estimation, US visualization enables more accurate measurements [11].

Typically, the earliest detection of the CL using US can be achieved on day 2, when the CL has a diameter of 1.06-1.63 cm [30]. The CL reaches its maximum around day 9, with a diameter of 2-3 cm [19, 32]. In a study by Kayacik [30], the maximum diameter was found to be between 2.00 and 2.18 cm, occurring between days 6 and 10, depending on the length of the estrous cycle.

In the study by Dovenski et al. [27], PGF2 α was injected prior to size determination, including the regression of the first CL and initiating a new estrous cycle. In comparison to other studies, the growth of the CL in this study appeared to take a longer time. On day 2 after ovulation, the CL had a diameter of 1.2-1.5 cm. The size increases until the maximum CL size was reached on day 12, with a diameter of 2.4-2.8 cm [27].

It is important to note that the CL and P4 concentration increase after ovulation in both pregnant and non-pregnant animals. Between days 6 and 9, the CL grows more rapidly in pregnant animals compared to non-pregnant cows, with a respective change of 117% vs. 27% [33]. Significant differences between the two groups are observable around day 14. While the CL of non-pregnant animals regresses, it remains persistent in pregnant animals.

By day 21-22, the CL of non-pregnant animals may be difficult to identify and could have a diameter of 1.1 cm [27, 33].

2.4. Pregnancy diagnosis

Pregnancy diagnosis serves three main purposes: early identification and intervention in fertility issues, enhancing economic efficiency through the identification of non-pregnant and pregnant cows, and certifying pregnancy status [19]. The timing of pregnancy diagnosis varies, but the primary diagnosis is typically performed between days 28 and 35 of gestation, marking the completion of embryo differentiation [6, 9]. An earlier pregnancy diagnosis can be conducted between days 18 and 20, primarily aimed at identifying non-pregnant cows and reducing the number of open days per cow [8]. However, reconfirmation of pregnancy is crucial when the initial diagnosis is made before day 60 of gestation, as there is a higher risk of pregnancy loss until placentation is complete [7, 20]. The second pregnancy diagnosis, recommended between days 60 and 90 of gestation, confirms normal fetal development [7]. Rechecking pregnancy status is especially important for identifying cows that have experienced embryonic loss, enabling the implementation of management strategies to minimize calving intervals and facilitate prompt re-insemination [26].

2.4.1. Rectal palpation

RP is the oldest and most used method for pregnancy diagnosis, meeting the criteria under most circumstances [9]. Experienced palpators can achieve consistent results with nearly 100% accuracy as early as days 30 and 35 of pregnancy [34]. Traditionally, pregnancy diagnoses are performed using RP from days 40 to 60 after AI [35].

In non-pregnant animals, the first identifier is the cervix, a firm, fibrocartilaginous, cylindrical structure approximately eight centimeters long, located at the edge of the pelvic bone. Palpation proceeds to the uterus and the bifurcation into the horns, with the ovaries located at the edge of each horn. In the pregnant animal, the examiner follows the same palpation route. However, the cervix may be positioned more cranially and/or ventrally, and its fluid-filled nature varies with gestation age [19, 34].

While size and position of the uterus may suggest pregnancy, the definitive diagnosis is based on the identification of four structures considered essential signs of pregnancy: chorioallantoic or membrane slip, the amniotic vesicle, placentomes and the fetus itself [19, 34].

The FMS method can be utilized as early as 28-32 days into pregnancy in the gravid horn, extending to days 50-60 of gestation in both horns. It is commonly employed until day 90 of gestation. The procedure involves separately gasping each horn between fingers and thumb and gently lifting. A popping sensation occurs as the membrane slips from the gasp in the gravid horn. This can be attributed to the absence of attachments between the uterus and fetus in this area. After 10 weeks, the gravid horn becomes more tense, making it easier to slip the membranes of the non-gravid horn as well [19, 34].

The amniotic vesicle, a turgid, fluid-filled sac surrounding the embryo, can be palpated between days 30 and 70 of gestation. During this period, the sac measures between 3.5 and 7 cm and is present in one or both uterine horns, depending on the presence and location of one or two embryos. Subsequently, the sac loses its turgidity, becomes soft, and becomes unsuitable for palpation and pregnancy diagnosis [19, 34].

As of day 55, the fetus itself can be felt floating in the fluid by cupping the horn. Between 5 and 7 months of pregnancy, the fetus descends deeper into the abdomen, making palpation more challenging. However, in the last two months of gestation, the viable fetus can be readily felt again [19, 34].

Placentomes, similar in touch to pea-sized swellings, can be palpated as early as 75-80 days of gestation. At this stage, a minimum of three placentomes should be identified to avoid confusion with the ovaries. As the pregnancy progresses, placentomes become more distinct and serve as significant diagnostic criterion [19, 34].

Around days 85 to 90 after conception, palpation of the hypertrophy of the middle uterine artery on the gravid side becomes possible. This freely movable vessel lies in the region of the shaft of the ilium. In advanced pregnancy, the size and fremitus of the uterine arteries may also aid in a positive pregnancy diagnosis [19].

In the ovary, which is almond-shaped and about 3x2x1 cm in size, palpation can identify the follicle, corpus haemorrhagicum, and the CL. The texture of each structure varies depending on its development stage in the estrous cycle. Early follicles are relatively firm, while more mature follicles become fluctuating and blister-like. The corpus haemorrhagicum, the precursor to the CL, is soft and challenging to palpate. The CL becomes palpable five days after ovulation, measuring 0.5 cm in diameter. It possesses a

protected crown and a liver-like consistency. In non-pregnant animals, the CL is firm and small, while in pregnant animals, it becomes smooth and rounded [19].

2.4.2. Ultrasonography

US has become a valuable tool for pregnancy diagnosis in dairy cows since its introduction in veterinary practice. The ability to visualize changes in ovarian structures in real-time has revolutionized reproductive management [20, 27]. B-mode US, utilizing a linear-array transducer designed for transrectal use, is now considered the ‘gold standard’ for evaluating pregnancy diagnosis [26, 36]. While theoretically, pregnancy can be detected as early as 21 days after breeding through heartbeat detection [20, 37], acceptable results are typically obtained between days 25 and 30 of gestation under field conditions [35]. It is important to note that at the early stages of gestation, the embryo develops rapidly, so waiting a few more days for the first pregnancy diagnosis can provide more accurate results. Compared to RP, US allows for easier confirmation of pregnancy by individuals with less experience [9, 20].

One significant advantage of US over RP is the ability to examine ovarian structures accurately. The presence of a CL can confirm pregnancy and location of the embryo. Twin pregnancies can be identified by the presence of two CL, along with a twin line representing the confluence of the chorioallantoic membranes of each fetus [20]. Additionally, US can diagnose other abnormalities such as additional CL without twin pregnancy, cystic CL and different CL sizes [20]. During the US-guided pregnancy diagnosis, CL and embryo numbers are recorded whenever possible [2].

The US relies on the reflection of sound waves, by different tissues to create an image. Fluid-filled structures, such as follicles, cysts, and fetal sacs, appear black or anechoic as they reflect sound poorly. Solid tissues, including CL, fetal bone, and muscles, reflect sound readily and appear grey or white, referred to as hyperechoic structures [28].

Typically, pregnancy is confirmed in cows when an embryo with a beating heart is visualized [35]. Some studies have also considered the presence of allantoic fluid-filled vesicle in the lumen of the uterine horn [38, 39] and a CL with a minimum diameter of 2 cm as additional indicators of pregnancy [40].

In case of embryonic or fetal death before day 70 of gestation, TRUS would reveal cloudy amniotic and chorioallantoic fluids, along with the absence of a heartbeat and/or separated chorioallantoic membranes. However, it should be noted that embryonic heartbeat may persist for a few days after signs of disturbed gestation are detected [41]. After day 70 of gestation, the appearance of cloudy fluids may be considered normal [36, 37].

Another advantage of US over RP is the ability to stage the gestational age of the embryo or fetus. While RP is subjective and dependent on the experience and sensitivity of the examiner, US allows for direct measurement and accurate assessment of recorded structures, leading to more precise diagnoses in reproductive management [20]. Early pregnancy diagnosis is particularly crucial from an economic standpoint [28]. Furthermore, the examination of ovarian structures and the detection of non-pregnant animals using US provide opportunities for assigning cows to different treatment alternatives [9].

2.4.3. Biochemical pregnancy diagnosis

In addition to RP and TRUS, biochemical tests have been developed for pregnancy diagnosis in cattle. These tests detect P4, pregnancy-associated hormones or pregnancy-specific molecules in maternal circulation. Although their accuracy is not yet sufficient for definitive pregnancy diagnosis, they can still be considered as supplementary diagnostic tools. Pregnancy-associated hormones include PSPB, PAGs, EPFs and ISGs [36].

Progesterone

P4 is a hormone that plays a crucial role in pregnancy. Serum and milk P4 levels increase after ovulation when a CL forms and develops [36]. If no pregnancy occurs, the CL regresses and P4 concentrations decrease. In the presence of a conceptus, the CL is maintained, leading to sustained elevated P4 levels [36, 37]. P4 is primarily produced by the CL during the estrous cycle and secreted by the placenta during pregnancy [9]. P4 concentration may be measured in the laboratory using techniques such as radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA) or, less frequently, latex agglutination (LA) between 18 to 24 days of gestation [35, 36]. Recognizable P4 profiles exist for pregnant and non-pregnant cows, so low P4 level

would indicate non-pregnancy [9, 19]. On-farm qualitative tests for P4 levels in milk are also available but require further validation for accuracy.

Pregnancy proteins

Pregnancy associated glycoproteins

PAGs are proteins released by trophoblasts during implantation, playing a crucial role in maternal-conceptus recognition. Detectable levels of PAGs for RIA and ELISA can be found in maternal circulation around days 24 to 28 of gestation in cattle [36, 37, 42]. Unlike P4, PAGs can be detected throughout the entire gestation. However, their relatively long-half-life (approximately 3-4 days) means that PAGs can remain above the threshold for pregnancy several days following fetal death [43, 44]. This persistence can lead to misdiagnosis and difficulty in estimating the stage of gestation [36]. Moreover, persistent high concentrations of PAGs after pregnancy loss can result in false positive results [9, 45, 46]. Nonetheless, changes in PAG concentrations can still serve as valuable evidence for pregnancy diagnosis [10]. While the practical application of PAG tests is not yet widely available, a study of Szelényi et al. [47] has demonstrated their potential as valuable research tools, with the goal of reducing the need for pregnancy checks compared to the TRUS method.

Pregnancy specific protein B

Like PAGs, PSPB enters maternal circulation. PSPB is a glycoproteins synthesized by the binucleate trophoblastic cells of the bovine placenta [36, 48]. PSPB may be detected in the plasma of pregnant cows between days 15 and 22 after AI [48]. Some cows exhibit a delayed appearance of these proteins, making their use for pregnancy diagnosis more accurate from days 28 to 30 onwards [43]. Like PAGs, PSPB can be detected via RIA and ELISA in the plasma of pregnant cows. Maximum sensitivity with RIA is reported on days 29 to 34 [49], while other reports suggest the best results on days 37 and 38 [43]. An accurate ELISA test can be performed between days 27 and 35 after breeding [42, 45]. Due to the persistence of high concentrations of PSPB with pregnancy loss, false positive results are not uncommon [45]. With a relatively long half-life (approximately 7-8 days) in maternal circulation, PSPB can persist in the blood for up to 80-100 days after calving [36, 44]. The most prominent achievement associated with PAGs pertains

to their pivotal role in advancing and implementing PSPB as a significant biochemical indicator of pregnancy [48].

Early pregnancy factors

EPFs are produced by a viable conceptus and can be detected in the maternal system as early as 24-48 hours after successful fertilization. EPFs can persist for at least half of gestation and its absence is immediate in case of conceptus death. However, the only current test available for EPFs detection is the rosette inhibition test, which is time-consuming and challenging to maintain. Further research is needed to develop accurate on-farm diagnostic test [35, 36].

Interferon-stimulated genes

ISGs are proteins produced in large quantities by blood neutrophils and play a role in maternal recognition of pregnancy in ruminants. ISGs production occurs between days 14 and 21 of pregnancy [37, 50]. However, ISGs are present in low concentrations and cannot be detected in serum or milk. Specific molecular techniques are required to detect ISGs in peripheral blood leukocytes of ruminants [50]. Further research is necessary to develop accurate on-farm diagnostic tests for both EPFs and ISGs [35, 36].

3. Objectives

The objective of the study was to analyze abnormalities related to the CL and their impact on pregnancy regression. Additionally, the study aimed to examine the association between diagnostic methods and pregnancy loss.

4. Methods

This survey included data from a series of previously published studies and articles focused on Holstein-Frisian dairy cows. The majority of these studies were conducted in the Euroatlantic region and date from the late 1990s to the present day.

The selected studies primarily utilized TRUS for pregnancy diagnosis, while reconfirmation often relying on RP. Fetal loss and scanned findings related to the CL were documented. In each study, pregnancy diagnoses were consistently conducted by the same operator. During US examination, the CL was assessed from multiple angles to record its size and the presence of luteal tissues, including cavities. Furthermore, the number of CL was determined when more CL were observed than embryos.

Blood samples were primarily collected from the coccygeal vein, occasionally from the jugular vein, and stored in heparinized tubes. After centrifugation, plasma samples were stored at -20°C until assayed. P4 concentration in the sampled plasma was determined using the radioimmunoassay (RIA) method. Blood sampling was typically performed concurrently with pregnancy diagnosis.

5. Results

5.1. Ovarian abnormalities

5.1.1. Additional corpus luteum

When combining the results of seven studies, six out of seven concluded that there is a positive correlation between an additional CL and the maintenance of pregnancy. One study even found that pregnancy loss was higher in cows without an additional CL compared to cows with an additional CL.

Across all studies, the average percentage of pregnant cows diagnosed with an additional CL ranged from 8.2% to 16.6%, as shown in **Table 1**. For example, Bech-Sàbat et al. [21] reported that out of 199 pregnancies, 24 (12,1%) were diagnosed with additional CL. López-Gatius et al. [2] found 49 pregnant cows with an additional CL out of a population of 601 cows. Similar numbers were supported by the review of López-Gatius et al. [7], where out of five studies, 363 out of 4006 pregnant cows had an additional CL. Furthermore, 10.4% (22 out of 211) of pregnant cows were detected with two CL by Starbuck et al. [51]. In the study of Szelényi et al. [40] even 16.6% (191 out of 1148) of singleton pregnancies were found to have two or more CL.

Table 1 shows that pregnancy losses varied from none to 27.3% in cows with an additional CL. Some studies reported no pregnancy losses in cows with an extra CL, while others showed a decreased rate of pregnancy loss compared to cows with a single CL. For example, López-Gatius et al. [7], reported pregnancy losses of 1.7% in cows with an additional CL compared to 9.9%, in those with only one CL. Similarly, Szelényi et al. [40] found an 11% pregnancy loss in cows with two CL compared to 16.3% in the other group. However the study of Starbuck et al. [51] indicated that more pregnancies were lost in cows with the presence of an additional CL compared to the cows with a single CL (27.3% vs. 9.5%).

In a literature review by López-Gatius et al. [7], results from fourteen selected studies were recorded. The odds ratio of pregnancy loss ranged from 0.12 to 0.32, indicating that cows with additional CL had lower pregnancy losses, ranging from 1.7% out of 363 pregnancies diagnosed with an additional CL compared to 9.9% losses in cows with only one CL. López-Gatius et al. [2] recorded odds ratios of 0.12, suggesting that cows with

additional CL were 8.3 times less likely to lose their pregnancy compared to cows without additional CL.

Higher conception rates in cows with two CL were also found in another study where results of the ultrasound examination showed 86.7% with two CL vs. 38.9% with one CL on days 11-13 [52].

The explanation for increased pregnancy rates in cows with an additional CL may be the higher circulating P4 concentrations found in various studies. Luteal tissue is the primary site of P4 production, which is essential for the maintenance of pregnancy. With an additional CL, there is a higher amount of luteal tissue, resulting in higher P4 concentrations in the plasma. For example, Bech-Sàbat et al. [21] found that cows with multiple CL were three times more likely to have high P4 plasma concentrations (>9 ng/ml). Out of the 24 cows with an additional CL, 9 (37.5%) had high P4 concentration. In comparison, only 52 out of 175 (29.7%) without an additional CL had P4 concentration above 9 ng/ml. A strong correlation between increased P4 concentration and the presence of two CL was also shown by Pirokad et al. [53] at various points in time throughout pregnancy. On days 12, 14 and 17 respectively the P4 concentrations in cows with two CL compared to cows with one CL were recorded as follows: 9.36 ng/ml vs. 5.49 ng/ml; 10.72 ng/ml vs. 6.11 ng/ml and 9.72 ng/ml vs. 5.28 ng/ml. However, plasma concentration did not differ significantly between the cows on days 5 and 7 (2.39 ng/ml vs. 3.18 ng/ml and 3.81 ng/ml vs. 4.99 ng/ml respectively).

However, Starbuck et al. [51] reported that the number of CL did not have a relevant effect on the concentration of P4. They found that P4 concentrations did not differ significantly between cows with one or two CL at different times. For weeks 5.7 and 9 respectively, in cows with one or two CL, the P4 concentrations were as follows: 5.1 ng/ml vs. 5.3 ng/ml; 5.2 ng/ml vs. 5.7 ng/ml and 5.1 ng/ml vs. 6.1 ng/ml. They even observed more pregnancy losses in cows with two CL compared to those with a single CL. Additionally, losses were more frequent in cows with P4 concentrations below 3.8 ng/ml. Only 50% of cows with an average P4 concentration of 2.8 ng/ml maintained their pregnancy. These results suggest that increased P4 levels have a positive effect on pregnancy retention.

Table 1. Correlation between the presence of additional corpus luteum and pregnancy outcomes in cattle

References	Additional CL			Pregnancy loss		Pregnancies
	Class	n	%	n	%	N
Bech-Sàbat et al. [21]	Presence	24	12.1	0	0	199
	Absence	175	87.9	25	12.6	
López-Gatius et al. [2]	Presence	49	8.2	0	0	601
	Absence	552	91.8	64	10.6	
López-Gatius et al. [7]	Presence	363	9.1	6	1.7	4006
	Absence	3643	90.9	360	9.9	
Starbuck et al. [51]	Presence	22	10.4	6	27.3	211
	Absence	189	89.6	18	9.5	
Szelényi et al. [40]	Presence	191	16.6	21	11	1148
	Absence	957	83.4	156	16.3	

5.1.2. Cystic ovaries

Seven studies contributed to the effect of cavitory CL on pregnancy maintenance. Cysts can be differentiated into follicular theca cysts, characterized by increased E2 level or with low E2 and P4 concentration, and follicular lutein cysts with increased P4 levels. The study by Braw-Tal et al. [54] showed that preovulatory follicles are characterized by high E2 (>100 ng P4/ml) and low P4, while subordinate follicles have low E2 and high P4 concentrations (>100 ng P4/ml). Other cysts with both low E2 and P4 were characterized by the loss of granulosa and theca cells. This study concluded that results were based on hormonal and morphological features, but morphological variations are often not recognized.

Dovenski et al. [27] confirmed that a cavitory CL has no association with infertility or a reduction in functionality. After treating cows with PGF2 α for CL regression, the onset of a new cycle was consequently initiated. The newly formed CL was visualized by US every 2 to 3 days. The structure was observed, and the diameter was measured. In addition, P4 measurements took place. Central luteal cavities were generally more common in pregnant cows (75%) than in open cows (64.3%). It was identified earliest as the 4th day after ovulation, with a maximum diameter of more than 11 mm on day 15. Its P4 measurements showed that CL with a central cavity had higher P4 levels in peripheral blood. It was concluded that the presence of a cavity is positively related to the functional activity of CL, thus it may decrease the incidence of pregnancy loss.

A case report of a single cow supports the previously mentioned positive effect of a cavitory CL on pregnancy maintenance. In the early stage of pregnancy, a CL with a large cavity was detected in that cow. On day 92, the cavity was measured at 23.9 mm³ and the P4 was around 11 ng/ml. No harmful effects were observed in this case, and the pregnancy was maintained until the end [55].

Jaśkowski et al. [56] studied the quantitative parameters of cavitory CL through US examination and analyzed P4 concentrations. It showed that cavitory CL were, in general, larger than compact CL, however, in 48% of cavitory CL, the luteal tissue was reduced compared to the same size of the compact CL. Comparing both cavitory and compact CL, the diameter between days 6 and 8 after estrus were 0.65 mm compared to 1.3 mm, respectively. P4 concentrations were 1.5 times higher in cavitory CL compared to non-

cavitary CL, resulting in a positive pregnancy rate of 52% for those with cavitary CL compared to 33% for the others.

Moreover, another article studied CL with cavity by dimension and P4 measurements as well. Results showed that homogeneous CL persisted at a P4 concentration of 6 ng/ml when maximal dimension was achieved, while in cavitary CL, P4 concentrations were still rising. In general, cows with a cavitary CL had higher P4 concentrations than cows without CL with a cavity. It was concluded that cavitary CL has no influence on the estrous cycle or fertility. Additionally, it was observed that small cavities disappeared earlier than large ones in pregnant animals [57].

The study of Perez-Marin [58] confirmed 49.2% luteal cavities in pregnant animals and 57.1% in non-pregnant ones. It showed that with the growth of the CL at the beginning of the pregnancy, the cavity grew as well. However, P4 concentrations in cows without cavitary CL and cows with a CL with a cavity differed only slightly, meaning cows with cavitary CL had only slightly higher levels of P4. The size of both types of CL had no different maximal dimensions. Conclusions were drawn that morphologically and hormonally, the cavity had no effect on the functionality of CL.

Contrarily, Szelényi et al. [40] reported that pregnant cows with a cavitary CL lost more calves than those with non-cavitary CL. In that study, a CL with a cavity was considered if the cavity was at least five mm in diameter. The odds of pregnancy loss were 2.73 times higher for cows with cavitary CL than for those without a CL with a cavity between days 29 and 70. If after the 70th day the pregnancy was maintained in cows with cavitary CL, an increased prevalence of pregnancy loss was observed until drying-off.

5.1.3. Size of the corpus luteum

Several studies concluded that the size of the CL during pregnancy may vary immensely. Theoretically, an increase in size would result in an increase in P4, leading to a positive effect on pregnancy maintenance. Five studies demonstrate a direct correlation between the diameter of the CL and the concentration of P4 in the blood. In each study, the CL's diameter is measured by US, while the P4 concentration is measured via radioimmunoassay (RIA). These studies collectively show a high correlation between the US measurement of the CL and the level of P4 in the blood [27, 30, 32, 33, 56].

Dovenski et al. [27] show that the maximum diameter of the CL is reached on day 12 after ovulation, with a diameter of 24-28 mm. With the increase in size, P4 concentration also increases, starting from day 5 with a diameter of 18-22 mm and P4 levels >0.5 ng/ml, reaching 25 mm in diameter and P4 concentration of 5.2 ng/ml on day 15 in pregnant animals. This study is supported by Kayacik et al. [30], where a maximum diameter of around 21 mm on day 9 was detected, and the increase in size contributed to an increase in P4 from <1 ng/ml to >7 ng/ml.

After the maximum size of the CL is reached, the CL of non-pregnant animals starts to regress, while in pregnant animals, it may continue to grow. This results in significant differences of P4 concentration between pregnant and non-pregnant animals: 5.2 ng/ml vs. 0.9 ng/ml [27].

When comparing the growth of CL in pregnant animals vs. non-pregnant animals after ovulation, results show detectable differences. The growth in size of CL in pregnant animals is faster than that in non-pregnant animals, with a growth rate of 117% vs. 37%. Correspondingly, changes in P4 concentration are observed. The maximum P4 concentration in pregnant animals is 9.8 ng/ml vs. 8 ng/ml in non-pregnant animals. On day 13, the CL of non-pregnant cows starts to regress, resulting in observable differences in P4 concentration by day 22, with 8.2 ng/ml in pregnant animals vs. 3.2 ng/ml in non-pregnant animals [33].

The study by Mann [32] shows that in the early CL development phase, the size is effectively related to the circulating P4 concentration. Between days 5 and 8 after ovulation, both size and P4 concentration increase. However, it seems that after day 8, the size of the CL continues to increase while P4 concentration remains constant. This suggests that once the mature size is achieved, there is no correlation between growth and concentration.

Starbuck et al. [51] found that concentrations of P4 were not correlated with the size of the CL, indicating that the size of the CL has no effect on pregnancy maintenance.

5.2. Pregnancy diagnosis

It is well known that iatrogenic fetal death can be induced through RP. In earlier days, even embryo crushing to induce abortion was performed [19]. Differences in pregnancy confirmation via RP may be done using FMS or ASP. In each of the following studies, pregnancy loss for different periods, including embryonic and fetal periods, was evaluated. However, this information was less important for our review, thus we evaluated pregnancy loss by diagnostic methods in general (**Table 2**). In all the studies used, pregnancy in the control group was examined by TRUS.

Romano et al. [59] studied the difference of RP using the FMS technique and the TRUS examination of pregnancy loss. Out of 520 dairy cows, 258 were detected via FMS between 34 and 41 days of pregnancy, while in 262 cows, a viable embryo was detected via TRUS between days 29 and 32. In both groups, a re-evaluation was performed using TRUS at days 45 and 60 of pregnancy. The results show no significant difference, with 14.7% pregnancy loss in the RP group vs. 13.4% of pregnancy loss in the control group.

Further research was done by Romano et al. [60] to study the effect of performing more FMS on pregnancy loss. Pregnant animals were divided into three groups: one in which 1 FMS was used to detect pregnancy, another in which 2 FMS were performed, and a control group. In the first two groups, RP was performed between days 34 and 43, while TRUS was used at day 31 after estrus in the control group. Each pregnancy was re-checked with TRUS between days 45 and 60. Overall, 14.1% (131/928) of pregnancies were lost between days 31 and 60. Specifically, pregnancy loss rates for the control, FMS1 and FMS2 groups were 14.5%, 12.6% and 14.9% respectively. This concluded that pregnancy loss had no correlation with the number of FMS performed and with the technique used.

Another study of Romano and Fahning [61] determined the effect of rectal ASP on pregnancy loss. In total, 167 cows were included in the control group and 180 cows in the ASP group for pregnancy diagnosis. RP was performed in the ASP group between days 34 and 43. Examination via TRUS was done in all animals on days 45, 60 and 90. In general, pregnancy loss did not differ significantly between the control and the ASP group at each control, with a total of 13.2% pregnancies lost in the control group vs. 12.2% in the ASP group.

Additionally, another study including cows from two different farms compared the ASP method with the TRUS pregnancy diagnosis. All ASPs were performed between days 34 and 45 after AI with a reevaluation 2 to 4 weeks later by TRUS. No significant difference from the previously mentioned study was observed. Pregnancy loss at reexamination showed the following results for the ASP group and the control group: 13.2% vs. 11.5% respectively [62].

Furthermore, a study compared two techniques for pregnancy diagnosis: FSM and ASP with a control group using TRUS. Pregnancy loss was investigated and showed again no significant differences, with rates of 9.5% (25/264), 10.2% (27/266), and 12.6% (34/270), for the FMS, ASP and control group, respectively [63].

Interestingly, the study of Bond et al. [64] investigated the difference in pregnancy loss between cows that were evaluated using TRUS by an experienced clinician and those that were evaluated using RP (ASP and/or FMS) by an inexperienced veterinary student. Re-evaluation of 1,216 dairy cows that were initially diagnosed as pregnant was performed using TRUS around day 70 after AI. In the study group, 26 out of 598 cows (4.25%) lost their pregnancy, while 27 out of 618 cows (4.37%) of the control group did not maintain gestation. These results demonstrate that there is no difference in the risk of pregnancy loss between students and skilled veterinarians. Additionally, this study shows no significant difference between TRUS and RP palpation.

Table 2: Pregnancy loss rates by diagnostic method in different studies

References	Group	Lost pregnancies	%
Romano et al. [59]	FMS	38/258	14.7%
	Control	35/262	13.4%
Romano et al. [60]	FMS 1	33/222	14.9%
	FMS 2	29/230	12.6%
	Control	69/476	14.5%
Romano and Fahning [61]	ASP	22/180	12.2%
	Control	22/167	13.2%
Romano et al. [62]	ASP	24/182	13.2%
	Control	19/165	11.5%
Romano et al. [63]	FMS	25/264	9.5%
	ASP	27/266	10.2%
	Control	34/270	12.6%
Bond et al. [64]	RP	26/598	4.35%
	Control	27/618	4.37%

6. Discussion

The primary objective of this study was to investigate how various CL anomalies in dairy cattle might influence gestation outcomes. The findings revealed that different CL anomalies can have both positive and negative effects on pregnancy maintenance. Across the studies examined, we observed relatively consistent correlation.

6.1. Ovarian abnormalities

6.1.1. Additional corpus luteum

The analysis of data from seven studies consistently demonstrated a positive association between the presence of an additional CL and the maintenance of pregnancy in dairy cows. Most of these studies reported lower pregnancy loss rates in cows with an additional CL compared to those with a single CL. Notably, the incidence of pregnant cows diagnosed with an additional CL ranged from 8.2% to 16.6%, indicating that a substantial portion of pregnant cows may have additional CL. Pregnancy was more likely to be maintained in cows with an additional CL, with pregnancy loss rates ranging from none to 27.3% [2, 7, 21, 40, 52, 53]. It is important to acknowledge that while most studies reported decreased pregnancy loss in cows with additional CL, one study showed a higher loss rate in such cows, possibly due to undetected twin pregnancies [51]. Similarly, Szelényi et al. [40] did not record whether cows with two CL carried twins or singleton pregnancies, thus their study suggested that the number of CL had no effect on pregnancy loss. These varying results highlight the importance of accurately assessing embryo viability and the number of embryos compared to the number of CL.

The explanation for the increased pregnancy rates in cows with additional CL may be attributed to the higher circulating P4 concentrations observed in these cows. Luteal tissue is the primary site of P4 production, and the presence of additional CL can lead to higher P4 levels in the plasma. Studies have consistently demonstrated a positive correlation between increased P4 concentration and the presence of two CL [21, 53]. This suggests that higher P4 levels have a favorable effect on pregnancy maintenance.

However, it is worth noting that Starbuck et al. [51] reported that cows with two CL did not have higher P4 concentrations compared to those with a single CL. Possible reasons for decreased P4 concentrations could be reduced luteal output or increased metabolism.

High-producing dairy cows often exhibit an increased metabolic rate, which can result from their greater intake of dry matter. This heightened metabolic rate also impacts the metabolism of steroid hormones like P4 and E2, leading to reduced plasma concentrations of these hormones [21, 65]. Decreased P4 levels due to an increased metabolism or reduced luteal function can significantly affect fertility and gestation, increasing the risk of pregnancy loss [7]. Thus, the pursuit of higher dairy cow productivity may inadvertently negatively impact reproductive efficiency due to increased metabolic rates, decreased P4 concentrations, and subsequent impaired conceptus maintenance.

Strategies aimed at inducing the formation of an additional CL or supplementing P4 may prove effective in reducing fetal loss in high-producing dairy cows. Our results suggest that pregnant cows with an additional CL had higher P4 concentrations and were less likely to experience pregnancy loss. Consequently, it might be of interest to explore methods for inducing an accessory CL to enhance reproduction success. Stojanov et al. [66] proposed GnRH treatment 5 days after AI, which resulted in a significant increase in both number of cows with two CL and P4 concentrations. Additionally, pregnancy success was higher in the group with an accessory CL compared to the control group. In the treated group, 82% of the cows had two CL compared to none in the control group. P4 concentration measures for treated cows were 5.87 ng/ml compared to 4.21 ng/ml in the control group. Furthermore, pregnancy success was 65% for the cows with an accessory CL compared to 48.3% for the control group.

Another study administered GnRH or P4 during pregnancy diagnosis between days 28 and 34 post-AI. While dairy cows treated with P4 exhibited a significant increase in plasma P4 concentration and a reduction in pregnancy loss, GnRH treatment did not produce the same effect. In fact, P4 treatment was three times more effective than GnRH treatment in reducing pregnancy loss in cows with one CL. This suggests that P4 supplementation can effectively increase P4 concentrations in cows with a single CL, but it may not provide substantial benefits to cows with two CL or more [67]. Additional research supports that intravaginal P4 supplementation potentially reduces pregnancy loss in cows with one CL, while supplementation with GnRH is considered more beneficial for cows with two or more CL [7].

For ovulation synchronization, Pirokad et al. [53] put dairy cows on a 5-day P4-GnRH-PGF2 α protocol. Following this, he administered an additional dose of GnRH on day 5 after the end of the hormonal protocol to induce a secondary CL. Cows that received the additional GnRH treatment exhibited a greater CL number on day 14 post-AI, leading to higher pregnancy rates and reduced embryonic loss.

Furthermore, Musilová et al. [52] supported these findings by inducing GnRH on days 5-7 or 11-13. The treated group has a higher proportion of cows with two CL, significantly higher P4 concentration, and a higher conception rate than the group with one CL.

6.1.2. Cystic ovaries

The results of six studies consistently showed that the presence of a cavitory CL led to increased P4 plasma concentrations [27, 40, 55–58]. While four studies concluded that cavitory CL had no association with the fertility, all of them found increased P4 concentrations in cows with cavitory CL compared to those with compact CL [27, 40, 57, 58].

Dovenski et al. [27] and Grygar et al. [57] found that cavitory CL did not impact fertility rates, but both studies noted an increase in P4 levels in cows with cavitory CL, suggesting that the positive functionality of the CL improved with the presence of the cavity. Jaśkowski et al. [56] found increased pregnancy rates associated with cavitory CL and observed that cavitory CL had increased luteal tissue. This suggests that larger cavities result in more extensive luteal tissue, leading to increased P4 concentrations and improved pregnancy rates.

However, Szelényi et al. [40] reported that cows with cavitory CL experienced more pregnancy losses, highlighting the need for further investigation, especially considering limited availability of cases. Cysts in the ovaries can be categorized into follicular theca cysts (elevated E2 levels), follicular lutein cysts (elevated P4 levels), and other cysts with low levels of both hormones. Accurate classification of cysts based on hormonal and morphological features is crucial but may sometimes be overlooked [54].

6.1.3. Size of the corpus luteum

Numerous studies have consistently demonstrated a strong correlation between the size of the CL and the concentration of P4 in the blood. Typically, larger CL tend to produce higher P4 levels, which can have a favorable impact on pregnancy maintenance. Although Jaśkowski et al. [56] observed an increase in luteal tissue due to the enlarged size of the CL. Starbuck et al. [51] reported findings suggesting that the size of the CL did not necessarily correlate with P4 concentration, implying that CL size might not always be a decisive factor affecting pregnancy maintenance.

A noteworthy prior study of Vasconcelos et al. [68] hypothesized that a reduction in CL size would lead to decreased P4 concentrations, potentially altering conception rates. Their results indicated that smaller ovulatory follicles, measuring 13 mm compared to 15.80 mm in the control group, resulted in smaller CL, measuring 5.89 mm vs. 7.10 mm in the control group on day 14. This size difference led to lower circulatory P4 concentrations (2.48 ng/ml vs. 3.06 ng/ml in the control group on day 14). Furthermore, there was an observed tendency towards luteal volume reduction, which was of considerable importance for P4 production.

It is essential to acknowledge that the interpretation of CL size or its effects can sometimes be operator dependent.

In summary, concerning the three aforementioned issues related to the CL, it becomes evident that suboptimal P4 concentrations can have adverse effects on gestational predictions. The findings from additional studies further support the hypothesis that suboptimal P4 concentrations in high-producing dairy cows compromise pregnancy development [35].

López-Gatius et al.'s [69] study confirmed this hypothesis by inducing the administration of a PRID containing 1.55 g of P4 into 549 animals over a span of 28 days. The results revealed pregnancy loss in 95 (8.7%) of the cows: 66 (12%) in the control group and 29 (5.3%) in the treated group. This translated to a significant reduction in miscarriage by a factor of 2.4 in 549 animals subjected to the treatment.

6.2. Pregnancy diagnosis

Pregnancy diagnosis constitutes a pivotal aspect of reproductive management in dairy cattle. Accurate and timely diagnosis empowers farmers and veterinarians to make well-informed decisions regarding pregnant cows and non-pregnant ones that may be culled. Two commonly employed methods for pregnancy diagnosis are TRUS and RP techniques, which encompass FMS and ASP. A comprehensive understanding of the accuracy and implications of these methods is essential for optimizing reproductive outcomes in dairy herds.

Numerous studies have undertaken a comparative analysis of RP techniques (FMS and ASP) with TRUS for pregnancy diagnosis.

Collectively, the results derived from these studies suggest that there is no substantial difference in pregnancy loss rates between the two methods. This finding carries significant practical implications, indicating that both RP and TRUS are effective tools for diagnosing pregnancy in dairy cattle.

Notably, the results reveal that there was no discernible correlation between the number of FMS procedures conducted and pregnancy loss. This discovery underscores the notion that conducting multiple FMS procedures does not heighten the risk of pregnancy loss, thereby supporting the continued use of this RP technique for pregnancy diagnosis.

Similarly, studies comparing ASP with TRUS have unveiled no significant disparities in pregnancy loss rates. These findings imply that ASP can serve as an effective alternative to TRUS for confirming pregnancy status in dairy cows.

A unique study compared the accuracy of pregnancy diagnosis between veterinary students performing RP and experienced clinicians using TRUS. The results showed no significant difference in the risk of pregnancy wastage between the two groups. This finding implies that trained veterinary students can perform RP effectively for pregnancy diagnosis, and their results are comparable to those of skilled veterinarians using TRUS.

The findings discussed here have practical implications for dairy cattle reproductive management. Farmers and veterinarians can choose between RP techniques (FMS and ASP) and TRUS for pregnancy diagnosis based on factors such as equipment availability and expertise. The results suggest that RP techniques can provide accurate pregnancy

diagnoses without significantly increasing the risk of pregnancy loss. Additionally, involving trained veterinary students in the reproductive management of dairy herds, can contribute to cost-effective and efficient pregnancy diagnosis.

7. Summary

In conclusion, findings regarding ovarian abnormalities, including additional CL, cavitory ovaries, and CL size, have significant clinical implications for dairy cattle reproduction management. The presence of additional CL is associated with improved pregnancy maintenance in dairy cows, likely due to increased P4 production. Farmers and veterinarians may consider methods to induce or support the formation of additional CL in cows to increase reproductive success.

While the effect of cavitory CL on pregnancy maintenance is not entirely clear-cut, its presence may serve as a potential indicator of fertility status. While this study resulted in a positive effect of cavitory CL on pregnancy retention, further research is needed to determine the specific conditions under which cavitory CL can have a certain impact on pregnancy outcomes. Furthermore, accurate classification of cystic ovaries into different types, based on hormonal and morphological characteristics, is crucial for understanding their impact on fertility. Identifying the specific type of cyst can help tailor treatment and management strategies.

The size of the CL seems to be correlated with P4 concentration and may influence pregnancy outcomes, but there are some inconsistencies in the findings. Further research is needed to fully understand the complex relationships between ovarian abnormalities, hormone levels, and pregnancy maintenance in dairy cattle.

In addition, accurate pregnancy diagnosis is essential for effective reproductive management in dairy cattle. The choice between RP techniques and TRUS can depend on various factors, including expertise and available resources. The studies discussed here suggest that RP techniques, such as FMS and ASP, can be reliable methods for diagnosing pregnancy without significantly increasing the risk of pregnancy loss. Further research and continued training can enhance the capabilities of veterinary students and farm personnel in performing RP techniques effectively.

8. References

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