Chapter 13

# Clinical Use of Progesterone and Its Relation to Oxidative Stress in Ruminants

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Additional information is available at the end of the chapter

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**Abstract**

Studies to determine the physiological effects and functions of progesterone started in the twentieth century. Progesterone is a steroid-structured hormone with 21 carbon atoms originating from cholesterol. The corpus luteum, formed after ovulation in ruminants, secretes progesterone, which plays a role in the continuity of the pregnancy. Progestagens can be used for estrus synchronization in cows and heifers. Similarly, they are used for estrus synchronization during the breeding season or outside the breeding season by taking advantage of the negative feedback effect of progesterone in small ruminants. It is applied for the treatment of embryonic deaths due to luteal insufficiency in cows with high milk yield. In anovulatory anestrus, exogenous progesterone applications can be very useful. Progesterone treatment contributes to the resolution of the anestrus by rearranging hypothalamic functions in cattle with follicular cysts. The oxidative stress index in the luteal phase, when progesterone is high in ruminants, is higher than in the follicular phase. In the critical period of pregnancy, a high index of oxidative stress-induced progesterone causes embryonic death. Factors that cause stress in high milk-yielding cows can affect the amount of progesterone synthesis by inhibiting luteal cell function due to excessive free radical production.

**Keywords:** progesterone, ruminants, oxidative stress, estrus synchronization, embryonic death

## 1. Introduction

Many methods have been developed for controlling reproduction in farm animals. Among these methods, synchronization protocols to increase reproductive efficiency have an important

place. The desired level of pregnancy rates is often not obtained because of the difficulty of following the estrus cycle in each animal on large farms. For this reason, estrus synchronization use in large farms becomes inevitable. Thus, progesterone-assisted estrus synchronizations are implemented intensively in farm animals. In addition, progesterone can also be used for the treatment of reproductive problems such as anestrus, cystic ovarian disease, and luteal insufficiency [1, 2].

This section provides information on the structure of progesterone, its role in physiological events in ruminants, its use in clinical practice, and its relation to oxidative stress.

## 2. The structure and biochemical synthesis of progesterone

Steroid hormones are lipophilic organic compounds with a low-molecular weight derived from cholesterol (**Figure 1**). Steroid hormones are synthesized in the mitochondria and smooth endoplasmic reticulum in gonads, such as the ovary and testis, and then released into the bloodstream. The steroid hormones are broadly classified into three categories based on their physiological functions: glucocorticoids, mineralocorticoids, and sex steroids [3]. Cholesterol is an obligate intermediate used for steroid hormone synthesis by the adrenal gland, ovary, testis, and placenta that can be obtained from three principal sources: de novo synthesis of cholesterol from acetate, cholesterol from circulating high-density lipoproteins, and cholesterol

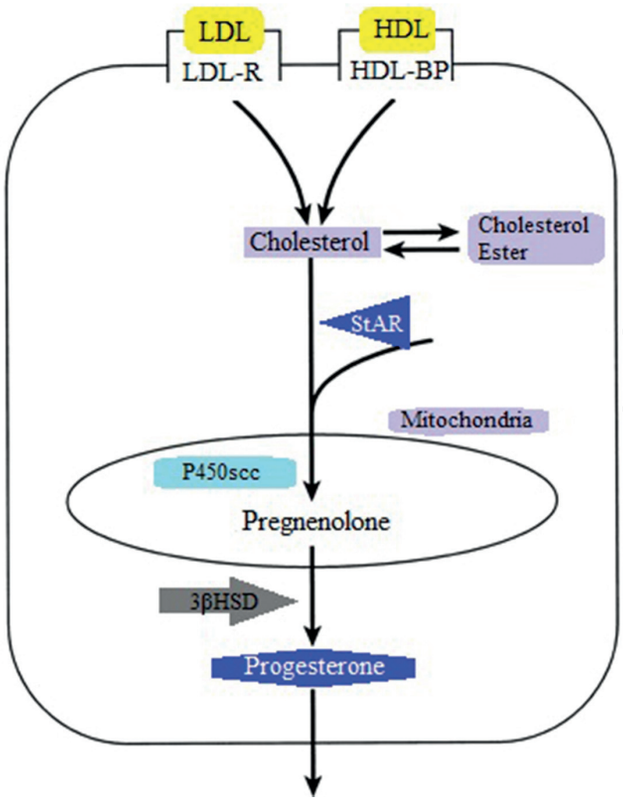


**Figure 1.** Sources of cholesterol for progesterone biosynthesis.

stored in plasma membranes. The major source of cholesterol for luteal cells in ruminants is circulating high-density lipoproteins [3, 4].

Cholesterol is primarily a hydrophobic molecule, and this makes it difficult for cholesterol to diffuse freely through hydrophilic environments such as the cytoplasm. In addition, cholesterol has a hydroxyl group at the 3 position that produces a discrete hydrophilic region making it difficult for the "flip-flop" of cholesterol between membrane surfaces within the lipid bilayer of cellular membranes. Therefore, movement of cholesterol in the circulatory system (lipoproteins) or within the cell is dependent upon transport proteins. Addition of another hydroxyl group at the other end of the cholesterol molecule alleviates the need for transport proteins [5].

Progesterone is a steroid hormone primarily secreted by the corpus luteum and placenta. Production of progesterone in luteal cells is dependent more on transport of cholesterol within the cell than to changes in the activity of steroidogenic enzymes. The P450 cholesterol side chain cleavage enzyme (P450scc) is located on the inner mitochondrial membrane and catalyzes the conversion of cholesterol to pregnenolone [6]. This enzyme catalyzes three oxidation steps: hydroxylations at the 20 and 22 positions and then cleavage between these two carbons. Pregnenolone has two hydrophilic residues that increase mobility through cellular membranes. Pregnenolone diffuses from the mitochondria to the smooth endoplasmic reticulum where it is converted to progesterone by the enzyme 3-β hydroxysteroid dehydrogenase (3β-HSD). This final reaction produces a double bond between the 4 and 5 carbon of the molecule and is the basis for the abbreviation of pregnenolone as progesterone (**Figures 1** and **2**). Progesterone then diffuses from the luteal cells to the bloodstream for transport to target tissues [7, 8].

Progesterone is synthesized from pregnenolone in the corpus luteum, the placenta during pregnancy, and the adrenals as a step in androgen and mineralocorticoid synthesis. Its actions are primarily mediated by an intracellular progesterone receptor, whose numbers increase in the presence of estrogen [9].

The products of hormone synthesis vary with the menstrual cycle; estradiol is the main product during follicular maturation, whereas progesterone is the main product in the luteal phase following ovulation. Progesterone is secreted by ovarian follicular cells prior to ovulation; it is also secreted in larger amounts by the corpus luteum, which forms from follicular granulosa cells following ovulation. The corpus luteum will grow for 10–12 days and then regress if fertilization does not occur; if fertilization does occur, the corpus luteum is maintained for the first 2–3 months of pregnancy. Progesterone plays several important actions in the normal female reproductive cycle. Progesterone prepares the uterus for pregnancy by shifting the endometrium from proliferation to secretion. Withdrawal of progesterone in the absence of pregnancy leads to organized shedding (menstruation) and it helps to mediate sexual response in the brain. After fertilization, progesterone organizes the vasculature of the endometrium to prepare for implantation. It promotes enzymatic digestion of the zona pellucida to allow the oocyte to implant into the uterine wall. In addition, it inhibits contractions of the uterine myometrium (smooth muscle layer) and counteracts the effects of oxytocin on contractility. Progesterone promotes lobuloalveolar growth in the breasts to prepare for lactation, but suppresses premature milk protein synthesis prior to parturition. Some of the effects of progesterone may be related to its ability to antagonize estrogen by decreasing expression of estrogen receptors, e.g. the ability of progesterone to inhibit estrogen-mediated endometrial proliferation. It also has a potent effect as a mineralocorticoid

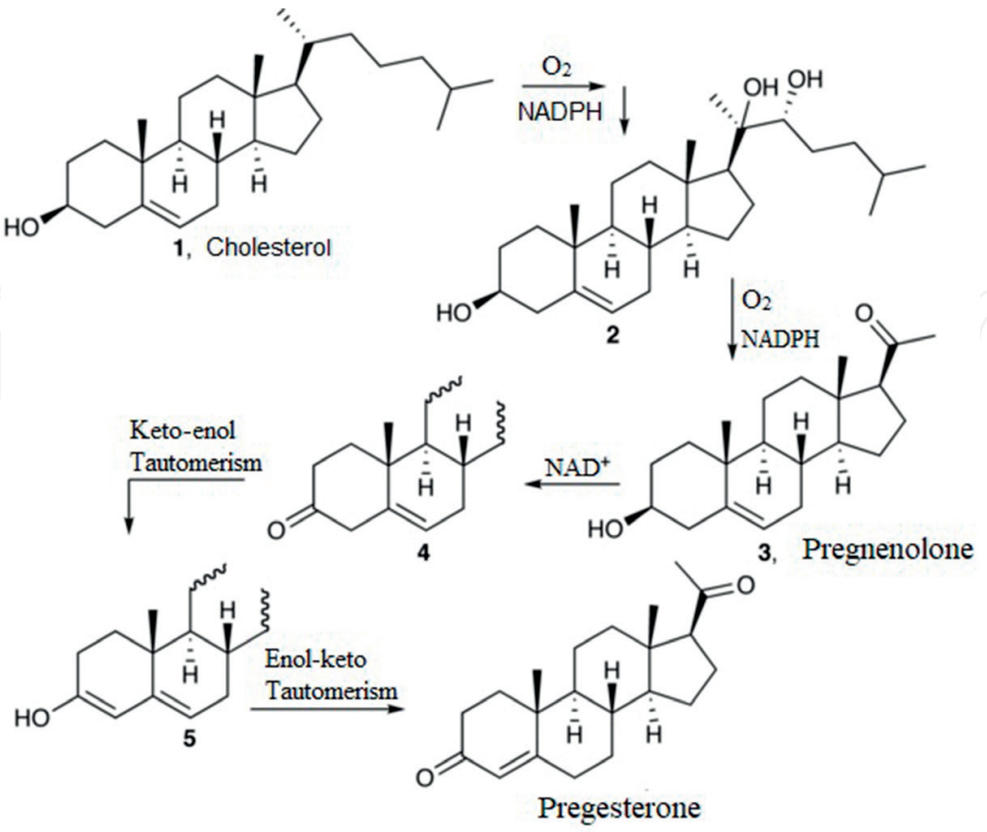


**Figure 2.** Pathway synthesis of progesterone from cholesterol.

receptor antagonist that reduces sodium retention when present and increases sodium retention when progesterone is withdrawn [8, 9].

The role of progesterone in males is less clear, but it is believed to play a role in activating sperm in the female reproductive tract. It has also been implicated as a modulator of male sexual response and behavior [7].

## 3. Progesterone synthesis and secretion during the sexual cycle and pregnancy in ruminants

The corpus luteum formed after ovulation in ruminants is a functional structure formed by membrane granulosa in the wall of Graaf follicles. Hypertrophy and luteinization of the theca interna cells play a role in the continuity of the pregnancy, secrete progesterone for a temporary period, and have endocrine activity. In particular, there is a close correlation between corpus luteum development and blood progesterone levels [10, 11].

Progesterone levels in the proestrus and estrus stages of the estrous cycle in cattle are very low. Newly developing corpus luteum cannot produce sufficient progesterone after ovulation (in the period of metaestrus). Therefore, a significant increase in blood progesterone levels cannot be detected. In the diestrus period, the corpus luteum acquires a functional structure and

synthesizes >2 ng/mL progesterone. The progesterone level is highest during days 8–10 of the estrous cycle in cows. If pregnancy does not occur, prostaglandin F2 alpha (PGF2$\alpha$), which is synthesized in the uterus, passes to the ovarian arteries from the uterine vein inducing corpus luteum degeneration. As a result, the blood progesterone level decreases rapidly to less than 0.5 ng/mL [11]. If pregnancy occurs, blood progesterone levels in the cows can be 6–8 ng/mL on day 21 of pregnancy [12, 13].

In sheep, the corpus luteum following ovulation begins to secrete progesterone after the third day of the estrous cycle. Because the corpus luteum formation is rapid, the blood progesterone level rises rapidly and reaches measurable levels [13]. The progesterone level is the highest (4 ng/mL) between days 9 and 13 of the cycle. If there is no pregnancy, the corpus luteum starts to shrink and the amount of PGF2$\alpha$ in the blood starts to increase on the 12th day of the cycle. On the 14th day of the cycle, the progesterone level is 10 ng/mL but is <0.2 ng/mL on the 16th day of the cycle [14]. Pregnancy in sheep is maintained by the corpus luteum until the 50th day and later by placenta-derived progesterone. Progesterone levels are highest between 60 and 130 days of gestation. As long as pregnancy continues, the concentration of progesterone never falls below 1 ng/mL [12].

The corpus luteum forms after ovulation and progesterone levels begin to increase in goats. Maximum levels of progesterone (6–10 ng/mL) are found in the middle of the cycle. This level starts to decrease on the 15th day of the cycle and descends to basal levels on the 19th day [15, 16].

## 4. Clinical use of progesterone in ruminants

### 4.1. Use of progesterone for estrus synchronization in cattle

Progestagens (ear implants and intravaginal devices [progesterone-releasing intravaginal device (PRID) and controlled internal drug release (CIDR)]) can be administered for 5–20 days for the purpose of estrus synchronization in cows and heifers without uterine infection. Progesterone applications can stimulate puberty in some heifers and initiate normal cyclic activities in anestrus cows. In addition, following the first ovulation after progesterone treatment, normal length sexual activities may continue [2, 17–24].

After the end of progesterone application, synchronized estrus is observed within 3 days [2, 17]. However, when progesterone is used for a prolonged period, the fertility rates decrease due to the persistence of the follicle. In addition, when melengestrol acetate (MGA) is used for estrus synchronization, subfertile estrus occurs initially because of the persistent follicles and artificial insemination is not recommended [24]. Long-term use negatively affects the intrauterine environment and spermatozoon transport. For this reason, estrus synchronization with progesterone for 5–9 days is more suitable for increasing fertility [25, 26].

According to studies conducted in recent years, the pregnancy rate may be 10–15% higher in short-term progesterone use protocols than long-term protocols. However, other researchers argue that there is no difference between pregnancy rates [2, 26–28].

#### *4.1.1. Estrus synchronization with progestagens + prostaglandins*

Progesterone administration allows the synchronization of estrus in ruminants in different stages of the cycle. PGF2$\alpha$ may be injected on the last day of progesterone administration (5–10 days) or 1–2 days before the end of progesterone. After the application of progesterone, estrus occurs and artificial insemination is done [21, 29–31]. Some researchers obtained 54% pregnancy rates in the first estrus in dairy cows (n = 102) given progesterone for 7 days (CIDR) with PGF2$\alpha$ administered on day 6. In addition, 49% pregnancy rates were obtained when a similar protocol was applied to non-cyclic and cyclic heifers [29]. In a similar study, the pregnancy rate was 47% in beef heifers (n = 247) and 59% in dairy heifers (n = 129) [32].

In some studies, artificial insemination was performed according to PGF2$\alpha$ injection and estrus follow-up (4–6 days) 17–19 days after 14 days of MGA application [25, 28]. In this protocol, the purpose of PGF2$\alpha$ injection is to lyse the corpus luteum that can be formed at the end of 14 days of MGA application [33].

#### *4.1.2. Ovulation synchronization protocols + progesterone*

Progesterone is administered as an ear implant or an intravaginal device between days 0 and 7 in order for the Ovsynch (GnRH/PGF2$\alpha$/GnRH) protocol to be successful and the pregnancy rate to increase [27]. In a study performed on heifers (n = 383), a 47% pregnancy rate was obtained [34]. In another study, progesterone administration in Ovsynch did not increase the pregnancy rate in cows with corpus luteum [35]. Others reported that premature estrus was not observed due to progesterone used between 0 and 7 days for ovulation synchronization protocols and full synchronization was achieved [36, 37].

Progesterone administration is also performed in the Cosynch protocol. On day 0, the progesterone device is inserted and gonadotropin-releasing hormone (GnRH) is injected. Progesterone devices are removed during PGF2$\alpha$ injection (day 7). Fixed-time artificial insemination is performed in 48, 56, or 60 hours after GnRH is injected [38–40]. Such protocols may be applied for 12–14 days with progesterone [41, 42].

#### *4.1.3. Five-day Cosynch + progesterone protocols in the heifer*

Ovulation synchronization methods have a high pregnancy rate, especially in cows, although this rate is lower in heifers [2]. Synchronization protocols have been developed to stimulate ovulation, which do not require heat detection. Progesterone administration occurs for 5 days. During progesterone removal, PGF2$\alpha$ is administered. GnRH is administered 72 hours later and fixed-time artificial insemination is performed [19, 21, 22, 26, 27]. A 10.5% higher pregnancy rate can be obtained with this protocol [43]. The pregnancy rate improves between 45.9 and 54.2% in the 5-day Cosynch + PRID protocol and fixed-time artificial insemination with sexed semen in cyclic heifers [26].

It was determined that vaginitis and mucopurulent discharge were observed after use of intravaginal progesterone devices in heifers. The incidence of vaginitis in the heifers may be around 70% or more [22, 23, 26, 44].

### 4.2. Use of progesterone in estrus synchronization in small ruminants

In small ruminants, progesterone suppresses GnRH and luteinizing hormone (LH) release by negative feedback [45]. There may be up to 30-fold increases in LH concentration with a decrease in the plasma level of progesterone. In addition, the dominant follicular LH receptors are sensitive and thus ovulation occurs. As a general principle, these effects of progesterone can be used in estrus synchronization protocols [46–48]. Medroxyprogesterone acetate (MAP), fluorogestone acetate (intravaginal sponge), melengestrol acetate, levonorgestrel, and intravaginal progesterone-releasing devices (CIDR and DICO) are used for estrus synchronization in sheep and goats [48–55].

In sheep, progestagens are used effectively in the control and synchronization of estrus. Equine chorionic gonadotropin (eCG) injection is performed in addition to progestagen administration for 12–14 days in order to obtain high estrus rates and ovulation, especially during anestrus. Nevertheless, these types of manipulations may also vary depending on nutrition, body condition score, lactation, age, temperature, light, and breed [56–60].

In sheep synchronized with MAP during the breeding season, follicle size and LH pulse increase after ram introduction [61, 62]. Intravaginal CIDR or chronolone intravaginal sponge and 500 IU eCG did not affect the LH wave and peak in Tuj sheep outside the breeding season [63].

During the breeding season, estrus start and end times were different in sheep treated with MAP or CIDR for 12 days [64]. High estrus rates and similar fertility rates were determined in sheep that applied short-term (6 days) CIDR-G or fluorogestone acetate (FGA, intravaginal sponge) [52].

Short-term (5 day) FGA and eCG administration produce higher estrus rate than long-term FGA and eCG treatments in sheep during the breeding season [50]. Injections of eCG at different doses (300, 400, and 500 IU) to Awassi sheep in estrus synchronization with progesterone similarly affect fertility parameters [65]. The testosterone antibody, β-carotene, and vitamin E administration did not change the estrus and pregnancy rates 7 days before intravaginal 40 mg FGA administration in Tuj ewes during the non-breeding season [66].

Vaginal sponges with progesterone for 11–14 days were applied to Pirlak ewes during the non-breeding season and 92–100% entered estrus. The pregnancy rate was 37.7–44%. In the study, the fertility parameters did not change for 11 or 14 day vaginal sponge application. Progesterone administration at different day lengths may be effective for the onset of estrus [48].

Short or long-term progesterone treatment for estrus synchronization in goats can be done depending on the breeding season. At the end of progesterone treatment, the protocol is terminated by eCG administration [54, 58, 67–69].

Intravaginal levonorgestrel for 10 days and intramuscular PGF2$\alpha$ administration were used in goats causing high estrus rates (95%) during the breeding season [70]. In a study conducted on Abaza goats during the breeding season, the first estrus pregnancy rate was 73.3% and the pregnancy rate was 93.3% after estrus synchronization using CIDR for 11 days. In addition, the same pregnancy rate was achieved in goats that did not receive any intervention in the study. However, progesterone treatment and estrus synchronization contribute positively to reproductive parameters and increase twinning rates [55].

In sheep and goats, short-term progesterone-impregnated sponge therapy changed the microbial flora of the vagina and formed vaginitis. Staphylococcus is usually detected in these cases of vaginitis [13, 71, 72].

### 4.3. Use of progesterone to prevent embryonic death

Progesterone is the most important hormone for the continuity of pregnancy. Progesterone affects oocyte quality by affecting LH wave frequency and persistent follicle formation. Again, progesterone plays a vital role in influencing the endometrium and creating the appropriate environment for the survival of the embryo [73].

The rate of embryonic deaths in cows may range from 7 to 16% in the first week, 6–44% in the second week, 3–33% in the third week, and 19–42% after the fourth week. Embryonic deaths are caused by genetic and environmental factors. Most of the embryonic deaths, especially due to hormone insufficiency, are the result of luteal insufficiency. For this reason, progesterone or its analogues are applied to reduce embryonic losses before or after artificial insemination [74–76].

In ovulation synchronization protocols, such as Ovsynch and Cosynch, progesterone administration for 7 days between the first GnRH and PGF2$\alpha$ injections reduces the embryonic loss rate due to luteal phase deficiency [13]. Gestational losses were 3.6–6.8% after the 5-day Cosynch + progesterone protocol [26].

Progesterone-assisted estrus synchronization protocols can prevent early embryonic losses and increase the pregnancy rate. In many studies, there are reports that progesterone-assisted applications increase the pregnancy rate and decrease the embryonic loss rate. However, some researchers disagree [2, 35, 77, 78].

One of the most important causes of embryonic deaths in high milk-yielding cows is inadequate embryo development prior to implantation due to insufficient progesterone concentrations. Progesterone application performed between 3, 5, and 10 days after artificial insemination caused a statistically significant increase after CIDR administration compared with the control group. The pregnancy rate was 35% (22/63) in the control group and 48% (32/67) in the progesterone-treated group. The effect of exogenous progesterone is important for the development of pregnancy, especially in cows with first and second lactation [79].

Some researchers report an overall increase of 5% in pregnancy rates following progesterone administration. Progesterone administration time is critical to success. Progesterone treatment for 6 days after artificial insemination can increase pregnancy rates (10% more) [80].

Cows in the CIDR groups, which were administered progesterone for 6 or 12 days after the 5th and 7th days following artificial insemination, had higher pregnancy rates than the control group [81]. Intravaginal progesterone administration for 7 days starting 14 days after artificial insemination can reduce both embryonic deaths and fetal losses [77]. Similarly, post-mating treatment of FGA (intravaginal sponge) in sheep has been reported to reduce embryonic mortality [82].

### 4.4. Use of progesterone in anestrus or anovulatory anestrus

Anestrus is a situation in which a sign of heat cannot be detected in beef or dairy herds. Many forms of functional infertility result in anestrus in cows. This leads to serious economic losses for large farms [73].

Four different types of anovulatory anestrus may be encountered in the postpartum period. The first (type I) is characterized by follicle development remaining "emergent" (~ 4 mm) and not progressing to the "deviation" (~ 9 mm) phase. This type of anestrus is classically referred to as "inactive ovary." In type II, the "deviation" phase is passed, and after the "growth" phase, the follicle undergoes atresia. In type III, the follicle develops to the preovulatory stage but does not ovulate, thus becoming permanent follicles or follicular/luteal cysts. In type IV, the follicles develop and ovulate and the corpus luteum is formed, but the corpora lutea cannot regress and become permanent [13, 83].

In anovulatory anestrus, exogenous progesterone applications can be very useful [84]. The use of intravaginal progesterone devices, such as PRID or CIDR, may induce the restart of cyclic activity in the ovary [13]. Especially at the end of this application, the use of PGF2$\alpha$ and analogues may increase the pregnancy rate [73]. At the end of 10 days of progesterone therapy, injection of eCG, estradiol, or PGF2$\alpha$ is beneficial [83]. In this context, successful results were obtained after 9 days of PRID and intramuscular cloprostenol injection 1 day before PRID removal. Higher pregnancy rates can be achieved by adding progesterone to ovulation synchronization protocols in acyclic cows [13, 85].

### 4.5. Use of progesterone in cystic ovarian disease

Cystic ovarian follicles are non-ovulated preovulatory follicles that maintain long-lasting persistence without any luteal structure on the ovary. With progesterone administration, the number of LH pulses, and thus the LH level, is reduced and maintained at a luteal phase level throughout the cycle [73].

PRID or CIDR can be administered intravaginally for 7–14 days in cows with cystic ovarian disease [86]. In addition, progesterone is frequently used in the treatment of follicular cysts that cannot be mitigated with GnRH or human chorionic gonadotropin (hCG) injections. Progesterone therapy in cows with follicular cysts restarts hypothalamic functions and contributes to the resolution of the problem [83, 87].

Combination of progesterone administration for 9 days with GnRH and PGF2$\alpha$ may improve outcomes in cows with cystic ovarian disease. GnRH is injected and a progesterone-releasing device is applied intravaginally for 9 days. On day 7, PGF2$\alpha$ is administered intramuscularly, and 2 days later, the vaginal progesterone-releasing device is removed. Artificial insemination is done when the cows are in heat [88].

### 4.6. Use of progesterone to induction of lactation

The induction of lactation is a procedure that is applied to infertile heifers and non-lactating cows. The main purpose is to generate profit by initiating milk production. In particular, the combination of progesterone and estradiol in this type of cattle helps to develop the lobule alveolar system. Lactation can be successfully induced in 60% of cows treated with a combination of estrogen and progesterone for 7 or 10 days [89, 90].

When 50 mg progesterone and 20 mg 17$\beta$-estradiol are injected subcutaneously twice a day for 7 days in repeat breeders or aborted heifers, milk synthesis starts 10–21 days after the treatment [91]. In another study, lactation started 11–21 days after the first 17$\beta$-estradiol and

progesterone injection. The highest milk yield was reached 30–35 days after the start of lactation [92].

Lactation can be induced in non-pregnant ewes using progesterone and estrogen treatment. Progesterone and 240 mg estradiol benzoate injection once every 3 days for 60 days increased udder size. At the same time, the development of the udder is stimulated daily by injecting 10 mg dexamethasone trimethylacetate or injecting 5 mg estradiol benzoate and 12.5 mg progesterone for 6 days. After this protocol, milk synthesis starts from the udder with physiological size [93].

## 5. Relationship between progesterone and oxidative stress in ruminants

Oxygen is necessary for metabolism in living organism, but oxygen can be damaging to the living organism when it generates reactive oxygen species [94]. Thus, living organism face an oxygen paradox. During vital biochemical reactions in living organisms, intermediate metabolic products called reactive oxygen species (ROS) are generated that cause oxidative damage in many tissues by reducing oxygen. Oxygen is a potentially toxic molecule that is necessary for aerobic organisms to survive. Oxygen species are called "oxidants" or "free radicals" because of the oxidative destruction they provoke. Free radicals occur in all living organisms that metabolize molecular oxygen [95]. Free radicals carry a single number of unshared electrons in their outer orbitals [96]. They are very short-lived reagents, which disrupt the structure of other electrons in the environment of highly energetic electrons. Therefore, free radicals are dangerous to the organism [97]. Free radicals can occur as a by-product in all parts of aerobic cells, during metabolism, or in pathological conditions and they can cause various changes in the cells. As a result, serious cell, tissue, and/or organ damage can occur [98].

Free radicals are highly reactive molecules. Electrons interact with other molecules in the cell generating oxidative damage. They also damage many biological materials such as proteins, lipids, DNA, and nucleotide coenzymes [99]. There are many defensive mechanisms in place to prevent the formation of ROS and damage to the organism. These mechanisms are generally referred to as "antioxidant defense systems" or "antioxidants" [100]. Antioxidants control the metabolism and free radical levels that occur in normal metabolic or pathological conditions and prevent or repair damage that may be caused by these radicals [101–103].

In the organism, the formation rate of free radicals and the rate of their removal are in balance. This condition, called "oxidative balance," prevents the organism from being affected by free radicals. An imbalance between free radical formation and the antioxidant defense mechanism in favor of free radicals is termed "oxidative stress," which in turn leads to tissue damage [104]. Antioxidants are known to have protective effects on lipids, proteins, nucleic acids, and other macromolecules. Antioxidants affect ROS in four ways: scavenger, quencher, restorative, and chain breaker [103]. All biomolecules are exposed to free radicals. However, lipids are most easily affected [105]. The membranes surrounding the cells and organelles contain a large amount of unsaturated fatty acids. The oxygen molecule has a high affinity for lipids in these unsaturated fatty acids in the cell membrane. The binding of oxygen to the double bonds in the unsaturated fatty acids found in tissues is the result of lipid peroxidation. Lipid peroxidation is

the reaction of unsaturated fatty acids in the structure of phospholipids, glycolipids, glycerides, and steroids in the membrane by free oxygen radicals to various products such as peroxides, alcohols, aldehydes, hydroxy fatty acids, ethane, and pentane [106]. The major intracellular antioxidants found in organisms include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione S transferase, glucose 6 phosphate dehydrogenase, and paraoxonase enzymes. Vitamin E, ferritin, transferrin, haptoglobin, uric acid, ceruloplasmin, glutathione, albumin, bilirubin, and β-carotene are antioxidant defenses in the extracellular environment [107].

Every month, the oocyte grows and begins to develop in the ovary. However, meiosis-I continues only in the dominant oocyte. This process is inhibited by antioxidants causing an increase in ROS. Thus, antioxidants promote meiosis-II [108]. The ROS produced by the preovulatory follicle is considered an important inducer of ovulation [109]. Thus, ROS do not always cause adverse effects [110]. Recently, ROS have been reported to regulate cell function by controlling the production or activation of substances with biological activity [111]. Oxygen deprivation stimulates follicular angiogenesis, which is important for the growth and development of the follicle in the ovary. While follicular ROS increases apoptosis, glutathione (GSH) and follicle stimulating hormone (FSH) act as a balance in the growing follicle. FSH increases in response to estrogen, which triggers catalase production in the dominant follicle, thereby preventing apoptosis [108].

After ovulation, the corpus luteum synthesizes progesterone. Likewise, ROS, which has a key role in reproduction, is also produced in the corpus luteum [108, 112], and antioxidants play an important role in corpus luteum physiology during the cycle [112–114]. When pregnancy does not occur, the corpus luteum shrinks. During pregnancy, progesterone is continuously synthesized [112].

In mammalian species, the main function of the corpus luteum (CL) is the synthesis of progesterone, which is required for the establishment of a uterine environment suitable for the development of the peri-implantation conceptus and the successful progression and maintenance of pregnancy. Progesterone acts on the endometrium to regulate the synthesis of growth factors, cytokines, transport and adhesion proteins, protease inhibitors, hormones, and enzymes, which are primary regulators of conceptus implantation, survival, and development. Thus, compromised CL progesterone production is a potential risk factor for prenatal development and pregnancy outcomes [114].

There should be a rapid decline in the progesterone level for good follicular growth. During the middle luteal phase, superoxide dismutase 1 (SOD1, cofactor Cu or Zn) increases in the CL and decreases during regression [108]. PGF2$\alpha$ is defined as luteolysin because it increases in the CL during regression [115] and inhibits progesterone production by luteal cells. The inhibitory effect of PGF2$\alpha$ on progesterone production by the CL is due in part to increased ROS. In addition, progesterone levels decrease due to the destructive effects of oxidative stressors on luteal cell steroidogenesis [116–118]. ROS can also inhibit progesterone synthesis through inhibition of cytochrome P450, mitochondrial intracellular transport of cholesterol, and degradation of LH receptors [112].

Although the mechanisms of CL rescue from cell death and maintenance of progesterone production are very complex and vary among mammalian species, substantial evidence

suggests ROS are key factors in determining the CL lifespan and antioxidants play significant roles in CL physiology during the estrous/menstrual cycle. Luteal ROS production and propagation depend upon several regulating factors, including luteal antioxidants, steroid hormones and cytokines, and their crosstalk. However, it is unknown which of these factors have the greatest contribution to CL function. In addition, the sequence of events leading to the functional and structural luteal regression at the end of the estrous/menstrual cycle is still not clear. The scarce in-vivo reports studying the CL of rats and sheep have shown the importance of antioxidant enzymes in the control of CL function during the peri-implantation period. As a luteal phase defect can impact fertility by preventing implantation and early conceptus development in livestock and humans, this review attempts to address the importance of ROS-scavenging antioxidant enzymes in the control of mammalian CL function and integrity [119].

The production of ATP is derived from the mitochondrial respiratory chain oxidative phosphorylation, which is the main source of oxygen-free radicals and non-radical ROS. The ROS include superoxide anion (—$O_2$—), hydroxyl radical (—OH), nitric oxide (NO), hydrogen peroxide ($H_2O_2$), and peroxynitrite (ONOO—). ROS are also produced via enzymatic pathways, including the activity of membrane-bound NADH and NADPH oxidases, the activity of xanthine oxidase, the metabolism of arachidonic acid by lipoxygenases and cyclooxygenases (COX), and the mitochondrial cytochrome P450 [119].

The cause of the ROS concentration increase in the regression phase may be a decrease in the SOD1 concentration. A decrease in SOD1 concentration may be due to an increase in PGF2$\alpha$ or macrophages, or a decrease in blood flow to the ovaries [108]. In the CL, luteal cells and phagocytic leukocytes stimulate the production of a superoxide anion. With decreased blood flow to the ovaries, ROS production increases and causes tissue damage. Concentrations of superoxide dismutase 2 (SOD2, cofactor Mn) in the CL increase to clear the ROS produced in the mitochondria during regression. Along with the complete lysis of the CL, the regressor decreases significantly in the SOD2 cells [110]. The SOD1 enzyme is closely related to progesterone production. SOD2 protects luteal cells from oxidative stress induced inflammation [108].

Aerobic cells are equipped with antioxidant enzymes that control ROS production and prevent their propagation to toxic ROS. The conversion of —$O_2$— to $H_2O_2$ by superoxide dismutase (SOD) is the first enzymatic antioxidative pathway. Two different SOD enzymes were identified: copper-zinc-containing SOD (SOD1) is predominantly localized in the cytosol and can be found in mitochondria, and manganese-containing SOD (SOD2) localizes in the mitochondrial matrix. Glutathione peroxidase (GPX) is a group of selenium-containing enzymes that belong to the first antioxidant mechanism preventing the propagation of highly reactive ROS by catalyzing the conversion of $H_2O_2$ to $H_2O$ and $O_2$. NADH and NADPH are key elements in the control of ROS production and maintenance of the cellular redox state. The mitochondrial NADP+-dependent isocitrate dehydrogenase generates NADPH via oxidative decarboxylation of isocitrate [119].

Like any aerobic cells, those of the CL produce ATP through the respiration of $O_2$ with the consequence of luteal ROS production. The rate-limiting step in steroidogenesis in all steroidogenic organs, including the CL, is the transfer of cholesterol from the outer to the inner mitochondrial membrane where it is converted into pregnenolone by the enzyme cytochrome

P450scc. Luteal ROS are generated via enzymatic pathways of the mitochondrial cytochrome P450. In the CL, macrophages and luteal cells produce ROS where they can affect progesterone production. Indeed, there is substantial evidence to indicate that ROS regulate steroid hormone biosynthesis in the CL. The induction of ovarian SOD by LH, which in turn could lead to the production of $H_2O_2$, suggests that this action is involved in the LH stimulation of progesterone secretion in the CL. Thus, ROS can function beneficially to control the production of progesterone by luteal cells over the course of the reproductive cycle and inhibit progesterone synthesis at the end of the cycle. The $O_2$— radical is reported to be involved in the mechanism by which LH stimulates progesterone secretion [94, 119].

Oxidative stress may affect various physiological functions, such as folliculogenesis and steroidogenesis, in the female reproductive system. High ROS levels may also cause adverse pregnancy outcomes or embryonic/fetal losses [120–122] and are implicated in the etiopathogenesis of cystic ovarian disease [123]. ROS and the oxidative stress index in cows may be higher in the luteal phase than follicular phase, especially when progesterone is high. Again, in the luteal phase, the antioxidant status can be high or low. Imbalances, especially in oxidant and antioxidant capacity, can cause cystic ovarian disease by disturbing physiological events necessary for ovulation [124].

High free radical and low progesterone concentrations were detected in cows identified as repeat breeders. Infertility problems such as repeat breeder are encountered due to a low progesterone level in the critical period of pregnancy and the short life of the CL. All kinds of stress factors cause excessive radical production in high milk-yielding cows. This may be a determining factor for the amount of progesterone synthesized by inhibiting luteal cell function [125, 126]. In another study, the complex arrangement of antioxidant enzymes and compounds in the bovine CL was discussed. In particular, the correlation between antioxidant capacity and progesterone concentration was determined in the luteal phase of the estrous cycle. Findings show that antioxidative mechanisms are activated to cope with oxidative stress, which has a negative effect on steroid hormone synthesis [127]. In support of the previous study, it has been suggested that an antioxidant substance (astaxanthin) promotes progesterone synthesis in bovine luteal cell culture. However, attention has been drawn to the fact that the use of antioxidant material at low doses is beneficial [128].

Anestrus is a problem of infertility in which cyclic activity is absent and therefore estrogen-progesterone hormones are not expressed. Non-cyclic Murrah buffaloes were found to have low concentrations of antioxidants such as β-carotene and vitamin E [129]. Oxidative stress biomarkers change in cow milk during the anovulatory and ovulatory estrous cycles. In particular, the SOD levels in cyclic cows are significantly higher than levels in the anovulatory cycle, while the concentrations of lipoperoxides, GSH-Px, and GSH are lower. A low level of lipoperoxides, GSH-Px, and GSH is assumed to be an important event prior to the ovulation response, with high levels of milk SOD concentration in the ovulatory cycle cows [130].

Nitric oxide is synergistic with progesterone and may reduce relaxation by relieving uterine contraction during the paracrine-style secretion phase. In sheep, the regulation of reproductive physiology is related to the effects of oxidative stress [117]. Increased levels of progesterone during pregnancy in sheep and goats as well as increased levels of malondialdehyde (MDA) in

placentomas have been reported [131]. Significant reductions in antioxidant substances may occur in placentomas during early gestation in sheep. These changes in the antioxidant enzymatic defenses of the placenta are thought to be an adaptation to the oxidative stress caused by ROS in early pregnancy [132]. According to the results obtained, pregnancy may be a stressor and it may be beneficial to support progesterone production with antioxidants in order to mitigate oxidative stress effects [131, 132]. The application of antioxidant vitamins in estrus synchronization during the breeding season reduces free radical levels, increases pregnancy performance, and increases the litter size in Tuj sheep [133]. However, β-carotene and vitamin E applications before estrus synchronization did not cause significant changes in plasma MDA levels in sheep during the breeding season [66]. Serum progesterone concentration increases after administration of intravaginal progesterone-releasing devices for estrus synchronization in goats increases oxidants such as eNOS activity, NO, MDA, and total oxidation status total oxidation status decreased [68, 69]. Short-term PRID treatments increase serum progesterone levels but decrease total antioxidant capacity in dairy heifers [23].

## 6. Conclusion

Progesterone is synthesized in the luteal phase and is an important hormone required for the continuity of pregnancy in ruminants. It is widely used in cattle for the purpose of estrus synchronization. In addition, this hormone, which has many uses in clinical practice, continues to be explored in ruminants. As time progresses, more detailed facts about the complex effects of progesterone in the organism, its use in clinical practice in ruminants, and the relationship of progesterone to oxidative stress in ruminants will be revealed.

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