Chapter

Physiological and Clinical Aspects of the Endocrinology of the Estrous Cycle and Pregnancy in Mares

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Abstract

The use of advanced reproductive endocrinology can generate important economic benefits for equine breeding farms. Pregnancy in the mare involves considerable endocrine changes, which can be explained in part by the development of different structures such as embryonic vesicles, primary and secondary CL, endometrial cups and development of fetoplacental units. Both the pregnant mare and the fetus adapt to this development with unique mechanisms, such as alterations in the maternal endocrine metabolism and hormonal feedback. Since the ability to produce a viable foal is critical for the broodmare, the maintenance of the gestation implies almost a year of physiological effort. Therefore, the joint knowledge of basic reproductive science and current clinical endocrinology allows veterinarians and breeders to be better positioned to achieve their objectives. This chapter reviews normal and abnormal endocrine patterns during the equine estrual cycle, pregnancy. We also consider hormonal evaluation related to placentitis, abortions, recurrent pregnancy loss, and premature deliveries. Also, several aspects associated with endocrinological control of the reproductive cycle, ovulation, parturition, high-risk mare, and hormone supplementation will be developed.

Keywords: estrous, clinical endocrinology, mare, pregnancy

1. Introduction

The gestation in the mare begins with the fertilization of the ovum, then the implantation of the blastocyst in the uterus followed by the development of the placenta and fetus until delivery. Therefore, gestation is a dynamic and coordinated process involving systemic and local changes in the mare that support the supply of nutrients and oxygen to the fetus for growth and development in the uterus [1]. In part, these changes occur through the secretion of hormones in the placenta, which in turn interact with each other and exert extensive effects on maternal tissues during gestation [2]. These endocrine changes in maternal physiology adaptations to gestational status result from modifications in the maternal environment of steroids such as progesterone (P_4), estrogens, androgens, and other hormones such as relaxin and prostaglandins (PG). However, an inadequate adaptation of maternal physiology can lead to gestational complications, such as restriction or overgrowth of the fetus and premature delivery [3].

Since an understanding of endocrinology in equine species is useful when considering hormone treatment of cyclic and pregnant mares, this chapter considers a basic review and applications of this information in clinical therapeutic situations. For this reason, this chapter aims to provide an overview of the endocrine changes that occur in the mare in response to gestation and to discuss the key role of hormones in mediating pathological processes.

2. Neuroendocrine control of the estrus cycle in cycling mares

The estrous cycle is defined as the interval of time between two consecutive ovulations. The approximate length varies between 18 and 22 days, considering on average a period of 21 days [4, 5]. The current nomenclature stipulates that the estrous cycle consists of two differentiated stages: estrus or follicular phase and diestrus or luteal phase. These phases are characterized by internal modifications of the sexual organs and glandular system as well as behavioral alterations based on the levels of oestradiol (E_2) and P_4 [6, 7].

2.1 Follicular phase

Estrus, heat or follicular phase is characterized by the presence of follicles at different stages of development, and the simultaneous increase in the secretion of E_2. It has a duration of about 5–7 days, with a variability of 3–9 days related to the season. Thus, estrus is extended in autumn (7–10 days) and is shortened considerably, in late spring and early summer (4–5 days). During this period the mare is sexually receptive to the stallion genital tract and is ready to receive and transport of sperm and finally culminates with ovulation [5, 6, 8].

2.1.1 Follicular dynamics

Ovarian follicular development is a complex dynamic process, characterized by marked proliferation and differentiation of follicular cells, providing an optimal environment for oocyte maturation and preparation for fertilization after ovulation [9]. Among the recruited follicles in each follicular wave, dominance takes place and one follicle of the cohort acquires the ability to continue growing while others undergo atresia. The regulation of each wave and follicular selection involves interactions between specific circulating gonadotropins and intrafollicular factors, ensuring that each follicle is properly stimulated to grow or regress at any stage of development [8]. From an experimental point of view, the occurrence of a wave is defined as follicular growth or simultaneous emergence of a variable number of follicles below 6–13 mm in diameter [10, 11]. In the mare, these follicular waves are classified depending on their ability to develop the dominant follicle (primary waves) or, in contrast, generate only small follicles (smaller waves). Thus, the main waves or greater originate several follicles subordinate and a dominant follicle, while smaller waves, the follicles are not larger than 30 mm in diameter and then regress [12, 13].

During each cycle produces 1 or 2 major follicular waves, differentiated according to time of onset at primary and secondary. The primary major wave occurs near the middle of the diestrus, in which the dominant follicle ovulates at the end or near the end of estrus. The largest wave precedes the previous secondary and emerges during late estrus or early diestrus. There are two anovulatory follicular waves followed by an ovulatory surge during the estrous cycle [14, 15].

Steroidogenesis in the ovaries involves both theca and granulosa cells. The antral follicles acquire receptors for follicle-stimulating (FSH) and luteinizing (LH) hormones in the membranes of the granular cells and theca, respectively. Cholesterol passes through theca cell plasma membrane attached to a lipoprotein, is stored in cytoplasmic vacuoles, and is transported to the outer membrane of the mitochondria. The LH is released in a pulsating form from the anterior pituitary gland and binds to its receptor in the theca cell membrane, mobilizing cholesterol. Inside theca cells, the StAR protein helps transfer cholesterol to the internal mitochondrial membrane, where the cytochrome P_{450} (CYP) enzyme system divides cholesterol into pregnenalone (P_5), and subsequently, P_5 becomes to androstenedione (A_4). The A_4 produced in theca cells is transported through the basal membrane to the granulose cells. There FSH supports the steroidogenic pathway and converts A_4 into E_2 [16].

Increased concentrations of estrogen stimulate the secretion of LH, which in turn induces greater estrogen synthesis. This progressive increase in estrogen also promotes the onset of LH receptors in granulosa cells, which facilitates the transition from the antral stage to the preovulatory stage, when the oocyte reaches the final stage of maturation. At 6 days after the emergence of major follicular wave deviation occurs. This event relates to the growth rate difference of the preovulatory follicle size (22.5 mm) compared to the subordinate follicles (19 mm) [12, 13, 17]. Deviation is related to inhibin secretion [12] and i*nsulin-like growth factor-1* (IGF-1) [13, 17]. Specifically, inhibin reduces FSH secretion, making it impossible to continue the development of the subordinate follicle. However, the dominant follicle continues to grow at a constant rate of 2.3 mm per day until reaching a size of 40 mm in response to the increased sensitivity to FSH. As has been mentioned, at this stage of development, granulosa cells also develop receptors for LH required for final oocyte maturation and ovulation after the LH surge [18].

As has been demonstrated in different horse breeds such as Quarter Horse, Arabian, Thoroughbred, and Spanish Purebred, the maximum diameter of the ovulatory follicle usually varies between 40 and 45 mm [19], although the range may be higher (30–70 mm) [7, 20]. Moreover, size differences were established concerning the breeding season or the presence of multiple ovulations. Thus, the follicles reach a size 5–8 mm higher in spring than in summer or autumn and are 4–9 mm lower in multiple ovulations compared to the simple [20, 21].

The highest concentrations of estrogen secreted by the granulosa cells of the preovulatory follicle also induce the appearance of typical behavioral manifestations of estrus. Estrogens are also responsible for reproductive changes that ensure the reception, transport of sperm and oocyte fertilization [4, 6]. After the preovulatory LH surge, ovulation occurs spontaneously 24–48 h before the end of the follicular phase. The ovulatory process brings rapid evacuation of the oocyte and follicular fluid after follicular rupture at ovulation fossa. Once completed, E_2 concentrations return to basal levels and at the same time completing the oestrus behavior in mares [11, 22–24].

2.2 Luteal phase

The diestrus or luteal phase begins at the time of ovulation with the formation of CL, which is responsible for the synthesis of P_4. Unlike the follicular phase, the insensitivity of the corpus luteum (CL) photoperiod makes the length of this period more constant. Most research estimates an average duration of 14–15 days but can be more durable in mid-summer (16 days) than in spring or autumn (13 days) [5, 6].

2.2.1 Formation of corpus luteum

The disorganization of the follicular wall after ovulation allows blood vessels and fibroblasts invade the follicular cavity. Luteinization involves structural and functional changes in granulosa and theca cells. These are the same cells that initially produced E_2 and become into luteal cells that produce P_4. P_4 remains high from day 5 post-ovulation until the end of the diestrus and exerts specific functions related to the preparation of the endometrium to accept and maintain pregnancy, endometrial gland development and inhibition of myometrial contractility [24].

Have been described two types of CL regarding the presence or absence of central blood clot. In a high percentage of cases (50–70%) in place of ovulation, a core clot develops surrounded by luteal tissue. This type of condition is defined as a corpus hemorrhagic. The cavity begins to fill with blood, fibrin, and transudate for the first 24 h, reaching the maximum size at 3 days. Around day 5 post-ovulation CLs that develop a central cavity usually, have a significantly higher size (32.8 mm) to those without it (26.0 mm). The ratio of the maximum diameter of the CL is 65–80% compared to pre-ovulatory follicle size and has an outer wall thickness of 4–7 mm corresponding to the portion of luteinized tissue. As happens with the size, texture also changes depending on the type of CL. The CL that develops the central cavity is denser than those that lack it, in which the structure is more spongy [25]. Usually, the ratio of non-luteal luteal tissue of the corpus hemorrhagic is minimal during the early diestrus and maximum in halfway of diestrus. These events are associated with the gradual decrease of fluid as a result of the production and organization of connective tissue associated with the clotting mechanism [26, 27]. Notably, the formation of one type or another of CL is a random event. The morphology luteal repeatability is not always observed in subsequent ovulation [26–28].

Furthermore, continuous P_4 levels during diestrus reduce the frequency and intensity of gonadotrophin-releasing factor (GnRH) pulses by a feedback mechanism. However, because the pulses of FSH are higher than those of LH, a new follicular wave is developed during this period. In the absence of pregnancy, the end luteal phase culminates with the lysis of CL induced by the $PGF_{2\alpha}$ of endometrial origin and decreased concentrations of P_4 [5, 6]. Luteal regression involves several structural and functional events characterized by decreased vascularization, an increase of connective tissue, hyalinization, atrophy and fibrosis [29].

2.3 Neuroendocrine control of the estrus cycle

Physiological events that occur during the estrous cycle are regulated by the coordinated interaction of various hormones and releasing factors like GnRH, FSH, LH, E_2, P_4, and $PGF_{2\alpha}$, among others [22]. In this section we will describe a synthesis of the most notable changes and the physiological participation that all these factors have during the estral cycle in the mare.

2.3.1 Gonadotrophin releasing factor

The increased photoperiod during spring and summer causes decreased secretion of melatonin. This signal has a positive effect on the pulses of hypothalamic GnRH, which in turn controls the release of gonadotropins [27]. GnRH pulses produced every 45 min originate predominantly LH secretion whereas those occur every 6 h stimulate the secretion of FSH. The high-frequency pulses of GnRH (2 pulses per hour) during estrus favors an increase in LH and FSH decline, while reducing the frequency to 2 pulses per day, leads an increase of FSH and LH inhibition [30]. These endocrine events, allowing the emergence of follicular waves, E_2

synthesis, and ovulation during estrus and appearance of the CL with P_4 release during diestrus [24].

2.3.2 Follicle stimulating hormone

Follicle-stimulating hormone describes two types of secretion patterns during the estrous cycle in the mare: uni or bimodal. The bimodal pattern occurs frequently during the spring transition period and the ovulatory season. The first peak of FHS appears between the 8th and 14th day of the cycle, the moment in which the largest follicle reached a diameter of 13 mm [18]. This initial increase precedes the beginning of the deviation and is associated with increased synthesis of inhibin by the largest follicle [8, 13, 15, 18, 31] and persists until the preovulatory follicle reaches 22 mm of diameter. The second peak of FSH begins on day 15 of the cycle and it is necessary to complete the development of the preovulatory follicle [19, 31]. Unlike the bimodal pattern, the first peak of FSH would be absent in the unimodal pattern [18]. In the latter pattern, FSH levels remain low during estrus, rise in times around ovulation, maintaining increased during diestrus [31].

FSH is also involved in the development of the LH receptors in the preovulatory follicle [32, 33]. At the start of follicular growth, low levels of estradiol exert negative feedback on the hypothalamic-hypophysis axis (HHA) controlling the tonic or basal release of gonadotropin. This mechanism controls the follicular growth and E_2 synthesis continuously preventing ovarian overstimulation. After the period of follicular growth, once the dominant follicle has been selected, the E_2 and inhibin levels are significantly increased. This elevation of E_2 is responsible for the characteristic changes of the genital tract and signs of heat during estrus. Furthermore, this response exerts positive feedback on the HHA, favoring the emergence of preovulatory LH surge, necessary to produce the ovulation. Additionally, the stimulatory effects of E_2 on LH combined to the inhibitory action of inhibin on FSH create the ideal microenvironment for the final maturation of the oocyte, inhibiting the development of immature follicles [4].

2.3.3 Luteinizing hormone

LH levels gradually increase from day 5 to the day of ovulation, when it reaches the maximum concentration [7, 34]. The pre-ovulatory LH surge occurs as a result of the positive feedback mechanism exerted in the adenohypophysis by E_2 concentrations secreted by the granulosa cells of the preovulatory follicle. However, the peak of E_2 is reached 2 days before the LH surge. During diestrus, LH is released in a pulsatile manner, with a frequency of 1.4 pulses per 24 h and for a period of 20–40 min at the central level, or 2–4 h per pulse at the peripheral level [34]. Therefore, P_4 secretion is maintained by basal levels of LH. The decline of LH at the end of diestrus is a result of the combined effect of decreased estrogen positive feedback, and the resurgence of negative feedback induced by P_4 on the HHA. This gonadotropin not only participates in the development and maturation of the primary follicles but also in the development and maintenance of CL during the luteal phase [8, 13, 22].

2.3.4 Estradiol-17β

The ability of estrogen synthesis is dependent on the effect of FSH on granulosa cells. In the absence of P_4, estrogens begin to be actively secreted by the preovulatory follicle 5–7 days before ovulation. This event coincides with the time of departure and reaches the peak 2 days before ovulation [5, 22], and will be responsible for

the preovulatory release of LH. After ovulation, E_2 levels begin to decrease, reaching basal levels at day 5 post-ovulation [13, 19].

Although estrogen levels are directly related to the degree of ovarian activity, sexual receptivity and reproductive tract changes [4, 6, 13, 31, 35] there is no evidence of a direct relationship between the intensity of endometrial edema and E_2 concentration. This situation is much clearer on P_4. Swelling occurs when P_4 levels are <1 ng/ml, so this hormone could be responsible in principle on the intensity of edema, among other behavioral and morphological changes of the cervix and uterus [35]. However, at the time of ovulation inverse correlations are established between E_2 and FSH levels associated with the negative feedback effect of inhibin, as previously referred [31].

2.3.5 Progesterone

The steroidogenic activity of P_4 depends on the action of LH on theca cells. As noted above, levels of P_4 are <1 ng/ml during estrus [19, 36]. After ovulation, it increases progressively and significantly to the 5th or 6th day, with values similar to those of pregnant mares during the first 14 days of gestation. At this time the CL is fully functional and P_4 levels remain high until day 9 [35, 37], consistent with the maximum diameter reached by the CL [7, 20, 35, 37]. However, peripheral concentrations of P_4 are highly variable between mares. This variability is associated with secretory capacity CL and hormonal catabolic rate. Perhaps this fact may explain the differences in P_4 levels between different breeds during the first 5 days of the luteal period, despite the similarity in length of estrous cycles. Among other factors related to variations in levels of P_4 highlights the number of ovulations. In fact, double ovulations induce higher concentrations of P_4 compared to simple ones [35].

P_4 inhibits the secretion and pulsatile release of GnRH and LH but does not modify the pattern of FSH [7, 13, 15]. This event, unlike what happens in other species, enabling a new wave of follicular growth and in some cases the presence of ovulation during diestrus related to high levels of this hormone [18, 22, 38]. After lysis of the CL at the end of diestrus, P_4 is drastically reduced to levels <1 ng/ml, a fact which promotes the mare returns to estrus [19, 36].

2.3.6 Prostaglandin $F_{2\alpha}$

In the absence of pregnancy, the average life span of the CL is controlled by the release of endometrial $PGF_{2\alpha}$ source, establishing a bimodal pattern of discharge around day 13–16 of diestrus. While the first 4-h peak precedes the decline of P_4, the second occurs during and after luteolysis. Luteolysis involves decreased blood supply, leukocyte infiltration, cell disruption and loss of lutein steroidogenic capacity by apoptotic or non-apoptotic mechanisms intended to disintegrate the CL and therefore secretion P_4 [39, 40].

3. Recent advances in hormonal control of estrous cycle

In mares, the natural breeding season extends from spring to early autumn. Until now, various methods have been used to advance the onset of the breeding season or to synchronize the estrus during the reproductive season. Ovulation induction protocols have also been developed for use in artificial insemination or embryo transfer programs [41, 42].

3.1 Gonadotropin releasing hormone

Seasonal reproductive inactivity in mares is due to reduced synthesis and storage in the hypothalamus of GnRH and decreased amounts of FSH and LH in the anterior pituitary gland [27]. Taking this physiological basis into account, it would be expected that the administration of gonadotropins to anestrous mares will restart reproductive capacity.

The administration of a single dose of GnRH to mares causes an increase in the circulating concentrations of FSH and LH [43]. However, constant infusions result in a continuous release of both hormones [44]. An experience conducted in the late 1980s reported that 50% of mares treated during the seasonal anestrous had fertile estrous after infusion of GnRH for 28 days (100 ng/kg; SC). However, this same experiment showed that mares with transitional anestrous were more likely to respond to GnRH than mares with deep anestrous [45].

In another study, daily but not continuous administration of GnRH to induce ovulation in anestrous mares only induced the development of preovulatory follicles [46]. Also, another report [47] showed that the administration of 0.5 mg GnRH three times daily for 7 or 7.5 days induced normal follicular maturation and normal luteinization in anestrous mares. From these studies, it has been demonstrated that the administration of GnRH in diverse protocols is not profitable and requires a lot of manpower. It also results in variable response to treatment among mares, especially deep anestrous mares.

3.2 GnRH agonists

GnRH is known to be responsible for the secretion of FSH and LH, but studies performed to evaluate the efficacy of GnRH-agonists are conflicting. GnRH agonists were used as injections or slow-releasing implants to induce estrus and ovulation in anestrous and transitional mares. The GnRH agonists available for mares include deslorelin, buserelin, and historelin [48].

According to Allen et al. [49] two injections of GnRH agonists each day or continuous administration of GnRH agonists were able to induce follicular development and ovulation in acyclic mares. In the same way, Bergfelt and Ginther [26], demonstrate the same result where mares where about 60% of treated mares with GnRH-agonist ovulated within a 21-day long treatment.

In a study conducted in transition mares for 28 days, Harrison et al. [50] administered buserelin twice daily (40 μg, IM, q 12 h) for 28 days, or as SC implants releasing 100 μg/day. 45% of the mares ovulated between the 10th and 25th day after the start of treatment, in response to the two daily injections. However, 60% of the mares ovulated between 4 and 30 days after implant treatment. The same results were observed when the GnRH agonist was combined with E_2 [51].

Deslorelin has also been used to induce cycle and ovulation in mares. Slow liberation subcutaneous deslorelin implants are effective in increasing LH and accelerating ovulation in mares [52, 53].

It is important to indicate that the response is in correlation with the follicular size at the beginning of the treatment and the depth of anestrus. This means that due to the insensitivity of GnRH, mares that are already in the transition period are more likely to respond to the treatment compared to those who are in deep anestrus [54]. Another negative aspect of GnRH treatment in anestrous mares is the risk of early pregnancy losses due to inadequate luteal function [26].

3.3 Progesterone and progestins

The administration of P_4 suppresses the release of LH from the anterior pituitary gland. Once P_4 supplementation ceases, the so-called "rebound effect" induces follicular maturation and ovulation. Its use in equine reproduction is a common practice and the available protocols include progestogens administered orally or parenterally. However, its use in mares with seasonal anestrous is questionable.

Different studies indicate that mares in deep anestrous or early transition do not anticipate the first ovulation of the year with P_4 treatments [30, 55]. However, it has been shown that, if treatment is carried out at the end of the transition period and the mares have at least one follicle of more than 20 mm in diameter in the ovaries, they show regular post-treatment cycles [56].

Intravaginal devices containing P_4 (CIDR, PRID, and intravaginal sponges) have been used in mares. Indeed, Hanlon and Firth [57] examined the effect of intravaginal devices placed during 10 days in transitional Thoroughbred mares. The results of the experiment showed that the use of P_4 has a positive effect in bringing forward the first estral cycle of the breeding season. Compared to control mares, in the first 21 days of the season, 95.2% treated mares were served and conceived sooner after the start of the breeding season.

Regumate is the most commonly used orally administered progestogen. Its active ingredient is allyl trenbolone, also called Altrenogest. Allen et al. [55] evaluated the effect of oral P_4 treatment in mares with seasonal anestrous. Within 8 days, 88% of the treated mares showed estrous behavior and within 18 days of treatment interruption, 84% had ovulated. Based on these figures, the treatment gave a positive result in the acceleration of cyclicity in mares, but its response depends on the depth of the anestrus.

3.4 Recombinant equine FSH (reFSH) and LH (reLH)

The use of recombinant equine FSH (reFSH) has been reported to induce follicular growth in cyclic mares [58, 59]. A study reviewed in 2013 however determined the efficacy of it in deep anestrous mares to be very successful with ovulation rate of 76.7% in response to FSH treatment followed by human chorionic gonadotropin (hCG) administration [60].

Mares in deep anestrous treated with reFSH alone or reFSH and reLH in combination under natural photoperiod showed a significant increase in follicular development within 6 days on average and all of them ovulated within 10 days. In comparison, the control group needed a significantly longer time for follicular growth and only 30% of the control mares had ovulated at the end of the 14 days used for the experiment [61].

3.5 Dopamine antagonists and prolactin

Studies in sheep found that dopamine antagonists are effective in increasing LH secretion during estrus by inhibiting the release of dopamine in the brain [62]. In mares, the increased release of dopamine during winter anestrous has been confirmed in studies measuring a higher concentration of dopamine in the cerebrospinal fluid during deep anestrous. It has also been shown that inhibition of dopamine D2 receptors may accelerate the onset of the ovulatory season in mares. Sulpiride, domperidone, and perphenazine have been studied [63].

Mari et al. [64] compared the efficacy of sulpiride and domperidone, two long-acting dopamine antagonists, to induce ovarian activity in mares with deep anestrous. The results showed that sulpiride administration was effective in accelerating the transition period and first ovulation in mares with deep anestrous.

On the other hand, as daylight increases, the concentration of prolactin (PRL) also increases. Dopamine is an inhibitor of PRL release, and it has been suggested that the administration of this hormone may help stimulate cycling in mares in anestrus [65]. Various studies have confirmed that the administration of recombinant prolactin from different animal species (equine, porcine and ovine) has a stimulating effect on mares in anestrus. Thompson et al. [66] examined the effect of subcutaneous administration of recombinant porcine prolactin (rpPRL) pony mares for 45 days. About 17 days after the start of treatment, a high percentage of treated mares showed signs of heat and ovulation accelerated by more than 1 month. However, another study examined the effect of a single dose of recombinant ovine prolactin (ovPRL). As a result, significant stimulation of follicular development was observed, but only one mare ovulated [67].

3.6 Induction of ovulation in mares

A reliable ovulation-inducing drug is one that can trigger ovulation within a certain "fixed" period of time. This pharmacological action can provide enormous advantages in anticipating the right time for artificial insemination. Several pharmacological agents such as GnRH and GnRH agonist, hCG, recombinant equine LH, and equine pituitary extracts, prostaglandins and kisspeptin have been used to determine their efficacy in ovulation induction [68].

3.6.1 GnRH

The frequency of GnRH pulses is the main regulator of LH secretion by the adenohypophysis [69]. Because of this stimulation, they can be used as an ovulatory agent and therefore can be used to induce ovulation in mares. On the other hand, due to its natural origin, it does not cause an immune response after being administered in several sessions. There is also little risk of contamination as GnRH is a synthetic product. In the 1990s, several experiments were conducted to evaluate the efficacy of GnRH in ovulation induction in cyclic mares [70, 71]. In one of them, the effect of a single administration of 2 mg of synthetic GnRH was tested but did not affect ovulation induction. However, daily injections of the same compound from day 2 of heat to ovulation resulted in a shortening of the duration of heat and the time for ovulation [72]. Likewise, Duchamp et al. [73] conducted a study to try to identify a more suitable ovulatory agent. To do that, they compared the effect of an intramuscular injection of 2.500 i.u. hCG and 2 mg GnRH (not synthetic). The use of hCG, injected when the follicle reached 35 mm in diameter, induced ovulation in 24 or 48 h. However, GnRH was not effective in shortening ovulation time compared to the control group.

On the other hand, the pulsatile infusion of endogenous GnRH was effective in advancing ovulation time in cyclic mares [70]. Treatments with low doses of endogenous GnRH (2.5 μg) continuous infusion for 14 days demonstrated increased LH and ovulation in all treated mares compared to controls [74].

3.6.2 GnRH-agonist

3.6.2.1 Deslorelin (ovuplant and other products)

Deslorelin is a potent GnRH agonist and is marketed as a controlled-release subcutaneous implant under the trade name Ovuplant™. In the past, several authors have investigated the efficacy of Deslorelin in inducing ovulation in mares [29, 75, 76].

It has been shown that between 84 and 93% of mares ovulate after 2 or 3 days of treatment, respectively [77]. However, adverse effects have been reported for this drug. Mares treated with Ovuplant™ showed a prolonged interovulatory interval and estrual cycles of 3–7 days longer than controls [78]. In this sense, it was suggested that the GnRH agonist may cause a decrease in the regulation of the pituitary gonadotropic cells [79]. Besides, additional studies reported suppression of follicular growth and decreased FSH levels in mares treated with Ovuplant™ [80]. A study conducted by McCue et al. [81] showed that the extraction of Ovuplant™ after 48 h prevented a prolonged interovulatory interval. These authors also observed an alteration in ovulation rates. However, Ovupant™ is currently not commercially available.

A short-term release product of deslorelin was developed in a biocompatible liquid vehicle called BioRelease™ [82]. This product releases deslorelin for approximately 6–36 h. An increase in the number of ovulations within 48 h has been demonstrated (75% vs. 7% for controls). There was also no effect of fertility and the number of coverages per conception decreased in treated mares (1.6 vs. 2.9).

Subsequently, a greater number of injectable deslorelin products have been developed. Many of them are suspensions in saline or sterile water and do not contain any slow-release mechanism. McCue et al. [83] compared several deslorelin formulations and reported that all of the formulations tested in their study resulted in a shortening of the follicular phase, acceleration of ovulation and a similar response to human chorionic gonadotropin (hCG). It is important to note that these studies were conducted in the middle of the breeding season.

3.6.2.2 Buserelin

Different works have also tested Buserelin for its effect of inducing ovulation in mares [84]. Treatment with 40 μg de buserelin (4 doses/12 h) caused ovulation without altering fertility in mares [84, 85]. Also, the effect of treatments with 20 μg or 13.3 μg of buserelin (4 doses/12 h; or 3 doses/6 h respectively) was comparable with treatment with 2.500 IU of hCG (iv).

However, some problems with Buserelin to induce ovulation were also reported [86]. Mares treated with 40 μg iv. of Buserelin (2 times daily), 2.500 IU of hCG (single dose iv) and 2 ml of water distilled as placebo (iv) were compared. The highest ovulation rate was found in hCG treatments where 88% of the mares ovulated between 36 and 48 h. However, Buserelin treatment caused only 22.7% ovulation within 48 h.

Buserelin has also been given during early diestrus to pregnant mares as a means of improving pregnancy rates [87, 88]. These studies used doses of 20–40 mg of Buserelin between days 8 and 12. The results showed that pregnancy rates after ovulation increased by approximately 10%. The exact mechanism of how GnRH increases pregnancy rates is unclear since P_4 does not appear to be increased.

3.6.2.3 Human chorionic gonadotropin

hCG is a glycoprotein hormone and has a biological function like LH. It is composed of two subunits (α-subunit and β-subunit). The biological activity of hCG is determined by β-subunit, which is composed of 145 amino acids [89]. Several experiments have been conducted to test the efficacy of hCG in ovulation induction [73, 90, 91]. The results of these studies showed that administration of 1.500–3.300 IU of hCG to mares with a follicle in the ovary 35 mm in diameter, or after estrus day 2, induced ovulation within 48 h. The administration of hCG to mares

with a follicle in the ovary 35 mm in diameter, or after estrus day 2, induced ovulation within 48 h. The administration of hCG to mares with a follicle in the ovary 35 mm in diameter, or after estrus day 2, induced ovulation within 48 h. However, the adverse effect of consecutive administration of hCG has been reported. The results demonstrate a null effect from the second administration of hCG.

On the other hand, significant levels of antibodies to hCG were also observed after repeated injections [91, 92]. However, there is much conflicting evidence as to whether antibody formation affects the efficacy of hCG [93].

3.6.2.4 Equine recombinant LH

The recombinant equine LH (reLH) was successfully developed and tested for both in vitro and in vivo efficacy [94, 95]. To test the efficacy of reLH in ovulation induction, a study was performed in mares with 35 mm follicles that were treated with 0.3, 0.6, 0.75, 0.9 mg reLH, 2.500 IU hCG and the number of ovulations within 48 h of injection was monitored. With a total of 84 mares of various breeds 28.6, 50, 90, and 80% ovulated within 48 h in response to 0.3, 0.6, 0.75, and 0.9 mg reLH, respectively. Changes in hormonal profiles (LH, FSH, P_4, E_2) in response to 5, 0.65, or 10 mg reLH were similar to those of mares of the control group, except for the early increase in LH after reLH injection. The result of this study indicates that reLH is a drug that induces ovulation in mares with a follicle size of 35 mm in 48 h. It is important to point out that as a synthetic product it offers good potential by having, for example, a low production cost.

3.6.2.5 Equine pituitary extracts

The raw extract of equine gonadotropin (CEG) from the pituitary, contains FSH and LH. These extracts have been tested to determine if they can be used as agents to control the estrual cycles of mares. Also, due to their LH content, the effect of CEG for ovulation induction has been tested. Duchamp et al. [73] showed that 80% of ponies and 57% of mares ovulated 2 days after the administration of 50 mg and 25 mg of CEG, respectively. However, there is one major obstacle to these results; the FSH and LH relationship in cEG is not always consistent. Another important factor to keep in mind is that CEG may be contaminated with other pituitary hormones. Also, the potential transmission of certain associated diseases between animals or between animals and humans [96–98].

3.6.2.6 Prostaglandins

Savage and Liptrap [99], reported on the use of $PGF_{2\alpha}$ was able to induce ovulation in mares. By administering 250 μg $PGF_{2\alpha}$ synthetic (Fenprostalene) 60 h after the onset of estrus, the interval between treatment and ovulation and the duration of estrus were significantly reduced.

Despite these good results, no other $PGF_{2\alpha}$ could be found that could give similar results [100]. It is therefore believed that the prolonged action of Fenprostalene was responsible for these results. Another $PGF_{2\alpha}$ (Luprostiol), has also been shown to induce a release of LH from the anterior pituitary gland [101].

3.6.2.7 Kisspeptin

Kisspeptin is a neuropeptide that induces the secretion of gonadotropins through the stimulation of GnRH secretion and has also been described as having a role in

triggering the onset of puberty [102, 103]. A study in pony mares demonstrated the anticipated ovulation when treated with 10 mg of kisspeptin. Another report identified that the administration of 500 μg and 1.0 mg of kisspeptin induces indistinguishable LH and FSH responses to 25 μg GnRH. However, a single injection of 1.0 mg of kisspeptin (iv) was insufficient to induce ovulation in the mare in heat [104].

4. Hormonal regulation of pregnancy in normal mares

4.1 Progesterone

"Maternal recognition of gestation-MGR" it is essential to establish a complete and uninterrupted interaction between the uterus and the conceptus to prevent the regression of primary CL as a result of the blocking of luteolysis. The mobility of the conceptus within the uterine lumen between days 11 and 15 (or "first luteal response of pregnancy"); [27] seem to compensate for the reduced contact surface due to the relatively small size of the equine trophoblast, demonstrating that restriction of movement only partially leads to early embryo loss [105]. The PGs synthesized and secreted by the concept itself stimulate myometrial contractions that promote their migration through the uterus, avoiding premature regression of CL. Additionally, the longitudinal direction of the uterine folds, as well as the spherical shape of the embryo due to the persistence of the glycoprotein capsule, contribute to facilitating this movement [106, 107]. During the mobility phase and its subsequent fixation uterine high amounts of estrogen, mainly oestrone sulfate (E_1S) by the equine conceptus are synthesized, related to the development of the embryonic and endometrial vasculature and local effects on myometrial activity, uterine mobility and endometrial gland secretion [108, 109].

Embryo implantation begins around day 36 post-ovulation and involves the development of the chorionic band from the trophoblast, whose cells invade the maternal endometrium giving rise to endometrial cups [110]. Ginther [28] reported that the embryonic cup cells produce a hormone called equine chorionic gonadotropin (eCG), formerly known as pregnant mare's serum gonadotropin. This hormone is first detectable systemically between days 35 and 40 of pregnancy. The cups are mature and robustly secreting eCG at approximately days 50–60, but they will subsequently undergo sloughing by days 100–150 in most mares This resurgence phase of P_4 secretion by the primary CL is termed the "secondary luteal phase or output 2," whereas the production by supplementary CL is termed the "third luteal phase" or "output 3". These accessory CLs formed, respectively, causing an increase in P_4 secretion around the 75th day of gestation [27, 28, 111]. Thus, during this period, two secretion peaks of P_4 are described, which gradually decreasing to undetectable levels at the 200 days of gestation [112, 113].

Ovarian P_4 is necessary for the early maintenance of gestation in the mare until 150 days of pregnancy. After the regression of CLs, the placenta is then the organ in charge of maintaining gestation [114]. Several studies describe maximum levels of P_4 during the second and third months of gestation, followed by a significant decrease to minimum values (<1 ng/ml) from mid-gestation to term [115]. Additionally, the presence of eCG causes a change in luteal steroidogenesis. In this case, CL changes from synthesizing only P_4 to secreting also estrogens and androgens, increasing plasma levels rapidly and tripling the basal values [116]. However, it is not until approximately day 35 that systemic estrogen rises. The source of this estrogen is the ovary, more specifically, the CL and possibly follicles. The stimulation of the ovaries by eCG is responsible for the timing of this increase in estrogen. It appears that estrogen is not actually necessary for pregnancy

maintenance, because ovariectomized mares administered only exogenous progestins will maintain pregnancy without the administration of estrogens [28]. The origin of both steroids is found in the primary CL, since their increase takes place before the formation of the secondary CLs and is absent in mares without functional CL. Although the mechanism by which gonadotropin exerts this activity is unknown, an increase in the expression of the enzyme 17α-hydroxylase in charge of the conversion of P_5 into dehydroepiandrosterone (DHEA) and P_4 into A_4 has been described. Both events coincide with the secretion of eCG, they seem to be limited to the first period since they are not detected towards the middle of gestation [116]. The increase in P_4 responds primarily to the growth of primary CL and the development of secondary and accessory CLs [4, 117].

During the period of endometrial cups activity, secretion peaks are described for testosterone (T) and A_4 [118, 119], whose activity may be decisive in uterine processes related to cell transformation associated with decidualization [120]. In addition, estrogen production depends on the increased synthesis and availability of androgens that are subsequently metabolized by the enzyme aromatase, present in luteal tissue even before eCG secretion. Thus, total estrogen levels are like right-handed during the first 35 days of gestation and increase around day 40 due to follicular development before the formation of CL [121]. Additionally, primary gestational CL produces E_1S in response to eCG stimulation [113, 115, 118].

The regression of the endometrial cups to 100–120 days of gestation causes the cessation of eCG secretion and luteal development, observing a progressive decrease in plasma levels of P_4 to reach basal values around 200 days of gestation [115]. Currently, all the luteal structures present in the ovary have completely involuted [27]. From this moment onwards, various metabolites derived from P_4 (progestins) increase in the systemic circulation, that exceed 500 ng/ml during the last weeks of gestation, which subsequently fall in the 24–48 h prior to birth [122].

4.2 Progestagens

Progestins can be subclassified as pregnenes and 5α-pregnenes. The pregnenes includes P_5, P_4 and 5-pregnene-3β,20β-diol (P5ββ), while 5α-pregnenes includes 5α-pregnane-3,20-dione (5αDHP), 3β-hydroxy-5α-pregnan-3-one (3β5P), 20α-hydroxy-5α-pregnan-3-one (20α5P), 5α-pregnane-3β,20β-diol (ββ-diol) and 5α-pregnane-3β,20α-diol (βα-diol). Of them, the most important ones in maternal plasma during this period are the 5αDHP and its derivatives, 20α5P, and βα-diol. The origin of all of them is found in P_5, synthesized mainly in the fetal adrenal gland, with a production rate exceeding 10 μmol/min. In the placenta, P_5 is converted to P_4 and this is transformed into 5αDHP in the endometrium [123]. The pattern of secretion of 5αDHP at beginning of gestation runs parallel to that of P_4, while around 90 days the onset of P_4 decline gives way to fetoplacental synthesis of the different progestogens whose concentrations continue to increase during the second half of gestation. Thus, 20α5P, which is initially at 5 ng/ml, reaches 69 ng/ml at 200 days of gestation and 300 ng/ml at term. In addition, the concentrations of βα-diol increase to 484 ng/ml [112], while 3β5P, P5ββ and ββ-diol reach values of 100, 10 and 100 ng/ml, respectively, towards the end of gestation [124].

The 5αDHP is found primarily at the uterine level during midgestation, but as labor approaches, its distribution changes and is predominantly in fetal circulation. This metabolite is an immediate precursor of allopregnanolone, a potent gamma-aminobutyric acid (GABA) receptor agonist with activity on myometrial relaxation in other species [125–127]. Serum allopregnanolone increases similarly to its precursor, reaching maximum values at the middle of gestation and a term [112]. However, both P_4 and 5αDHP prevent weakly myometrial contractions induced by

oxytocin *in vitro*, suggesting the intervention of the other hormones in the maintenance of uterine quiescence [128]. On the other hand, an umbilical increase of P_4 after 300 days of gestation related to a greater expression in the trophoblast of the enzyme necessary for the conversion of P_5 into P_4 has been described [129].

Simultaneously with the production of progestagens, the feto-placental unit (FPU) synthesizes phenolic estrogens, E_1S and E_2 17β and 17α, through the aromatization of dihydroandrosterone (DHA), DHEA and its precursors (3β-hydroxyl C-19). The estrogens β unsaturated, equilin and echinelin, specific to the equine species, derive from farnesyl pyrophosphate, through a noncholesterol-dependent pathway. In general, the pattern of estrogen secretion during gestation is characterized by the first peak of secretion around day 40 in relation to follicular development before the formation of secondary and accessory CLs and a subsequent increase from day 80, reaching maximum levels around 210 days of gestation [130–132]. Thus, the initial plasma concentrations of E_1S, corresponding to ovarian synthesis and are affected by ovariectomy. On the contrary, the subsequent peak of liberation comes only from fetoplacental synthesis, descending drastically after fetal death [108, 113, 115, 133].

This increase in estrogens temporarily coincides with the hypertrophy of fetal gonads, which together with local expression of the enzyme 17α-hydroxylase, lead to elevated umbilical levels of P5, T and DHEA [134]. At the same time, maternal plasma concentrations of T and DHEA increase after 100 days of gestation, reaching maximum values at 6 months [116, 135] to promote greater perfusion in the fetal compartment and the uterine tonicity [27, 136]. Legacki et al. [112] describe DHEA values that increase since the first 2 months of gestation to at 6–8 months, decreasing afterward.

The mitochondrial cytochrome P450 side-chain cleavage *enzyme* (P450scc), necessary for the conversion of cholesterol into P_5 is present in the glomerulosa and reticularis zone of the fetal adrenals from 150 days of gestation. However, its expression increases noticeably at the end of gestation, is also found in the fasciculata zone, in the placenta, and the utero-placental tissues. At the same time, fetal plasma levels of P_5 and its uteroplacental diffusion are doubled and tripled between 200 and 300 days of gestation and that subsequently descend in the days prior to birth [132, 137]. One of the main metabolites of P_4, the 5α-DHP, returns to umbilical circulation after synthesis in the endometrium, excreting only 30% of its production to the maternal circulation. Thus, it has been suggested that it could play a relevant role within fetoplacental tissues [137].

4.3 Estrogens

Estrogen production can likewise be determined in serum obtained from the mare and used as an indicator of feto-placental health [136]. Although total estrogen levels decrease in term gestation, E_2 increases dramatically hours before parturition with accentuated myoelectric activity at the uterine level, suggesting the involvement of E_2 in myometrial activation [132, 138]. In fact, estrogens promote PGs synthesis and increase endometrial sensitivity to oxytocin, stimulating myometrial contractile activity during delivery [137].

4.4 Cortisol

A few days before parturition, fetal adrenals change from mainly synthesizing P_5 to producing cortisol in response to the stimulation of adrenocorticotropic hormone (ACTH). The increase of fetal cortisol is related to preparing the fetus for extra-uterine life by stimulating different processes necessary for the maturation of

organs such as the liver, thyroid gland, lungs, digestive system, bone marrow and cardiovascular system [137]. In addition, cortisol activates the enzymes responsible for the synthesis of PGs which, without the presence of progestogens, increase continuously stimulating the onset of myometrial contractions. In addition, E_2 favors the uterine response to PGs and may also promote their synthesis [139].

4.5 Prostaglandins

$PGF_{2\alpha}$ play an important role during delivery by promoting myometrial contractibility, along with oxytocin, and cervical ripening and relaxation (PGE_2). Utero-placental tissues are capable of synthesizing PGs and can be found in maternal plasma, fetal plasma and allantoic fluid [140]. However, its bioactivity is controlled by the enzyme 15-hydroxyprostaglandin dehydrogenase (PGDH), which converts the PGs into inactive metabolites, present in the maternal endometrium since approximately 150 days of pregnancy. Since the labile nature of PGs makes it difficult to measure one of these metabolites, 13,14-dihydro-15-keto-prostaglandin F-2α (PGFM) remained at low levels until day 200, then increased to peak pregnancy levels by day 300 and remained at this value until parturition. PGFM uses one of its metabolites as an indicator of its circulating levels, with a term increasingly being described, although it is during the second labor stage when its value increases up to 50 times [141].

4.6 Relaxin

Relaxin is produced by the trophoblastic cells of the placenta and its activity is related to myometrial [137] as well as of the cervix and pelvic ligaments relaxation [142]. Maternal plasma levels increase at the end of gestation and during the second labor stage. After the expulsion of the placenta, it returns to basal values below the detection limit at 36 h, remaining elevated in cases of placental retention [143].

5. High-risk mares and hormone supplementation

5.1 Progesterone

P_4 concentrations above 4.0 ng/ml are considered adequate to support early pregnancy. However, when levels are <2.0 ng/ml, P_4 supplementation is considered [137]. Several types of P_4 products have been used to maintain pregnancies in mares. After oral administration altrenogest is readily absorbed, reaching peak levels after 3–6 h [144]. Altrenogest acts by binding to the P_4 receptors but has little effect on endogenous plasma total progestagen concentrations. Specifically, altrenogest is not metabolized to 5α-pregnanes in the horse [128]. For this reason, the only scientific evidence that altrenogest prevents loss pregnancy in mares is during the first trimester, when it prevented abortion induced by repeated administration of $PGF_{2\alpha}$ (cloprostenol) [145]. P_4 may exert its effects by interfering with PG production stimulated by proinflammatory cytokines. Daels et al. [146] demonstrated that the rise in endogenous $PGF_{2\alpha}$ concentrations was inhibited by altrenogest treatment. Indeed, when early pregnant mares (21–35 days post-ovulation) were exposed to *Salmonella typhimurium* endotoxin all mares supplemented with altrenogest until day 70 remained pregnant, whereas 6 out of 7 mares aborted when altrenogest therapy was discontinued on day 50 [147].

Mares with suspected luteal insufficiency can be supplemented with altrenogest (0.044 mg/kg per os once or twice daily) or P_4 (150 mg/day IM) starting on day

3 after ovulation and continuing until 100–120 days of pregnancy. Long-acting injectable formulations of P_4 and altrenogest are available in some countries [148]. Administration of the GnRH analog, buserelin (40 μg), 10 or 11 days after ovulation has been reported to improve luteal function and reduce early pregnancy loss [149]. Panzani et al. [150] showed that the use of altrenogest improved recipient pregnancy rates compared to untreated controls. A recent clinical study showed a positive effect of altrenogest supplementation on embryonic growth rates between 35 and 45 days after ovulation in Warmblood mares older than 8 years [151]. P_4 may need to be supplemented generally in early pregnant mares showing estrus signs, with a history of repeated pregnancy loss in case of endotoxemia and of stressful events. In mares under P_4 supplementation continuation of pregnancy has to be monitored regularly, since many will lose their pregnancy despite supplementation of P_4 and this will prevent those mares return to estrus [152].

The latter sentence has been checked. It has been reported that the administration of a single dose of 20–40 μg of buserelin between day 9 and day 10 after ovulation increases the number of multiple ovulations and gestation up to 5–10% [153]. Buserelin does not increase circulating P_4 levels or preventing the luteolysis, acting independently of CL in the mare [154]. These effects preventing pregnancy loss that operating between day 9 to day 10 and day 13 to day 14 of pregnancy.

In a recent study Köhne et al. [155] reported that hCG administration for induction of ovulation in mares increased progestin concentration in plasma of early pregnancy as well as the embryo size at the time of the start of placentation. Periovulatory treatment of mares with hCG may thus be a valuable tool to enhance conceptus growth during early pregnancy by stimulation of endogenous P_4 secretion. However, Biermann et al. [156] report that hCG-treatment of mares on day 5 or day 11 post-ovulation influenced peripheral P_4 concentrations due to secondary luteal tissue but did not alter ovarian and uterine blood flow or increase pregnancy rates.

5.2 Progestagens

Several pathological conditions as placentitis, placental separation or fetus as, alteration in umbilical blood flow attributable to a cord pathologic condition stimulates inflammatory and immune responses leading disrupt the endocrine capacity of the FPU and alterations in endocrine profile in plasma maternal attributed to disturbances to the normal synthetic pathway for these pregnanes [126, 157].

Fetal death or imminent fetal expulsión due to uterine torsion, colic, maternal stress, or acute cases of experimentally induced placentitis when the mares abort rapidly (within 7 days of infection) are related with the rapidly declining of P_5 and P_4 (less than the 95%), consistent with failure of the fetus and feto-placental tissues to produce and metabolize progestagens [158, 159].

In mares with chronic placentitis, placental edema, and placentas with poorly developed or sparse microvilli [159, 160] unusually high concentrations of all the progestagens. This pattern indicates that the fetus and the uteroplacental tissues are metabolically active despite the presence of bacteria or their products. In addition, Shikichi et al. [157] demonstrated that mares with a high concentration of progestins and low concentration of estrogens after day 241 of pregnancy were likely to deliver aborted/dead foals with placentitis. These authors demonstrated elevated and low concentrations of progestins and estrogens in the maternal sera of all cases with placentitis in pregnant mares, respectively.

The mare's exposure to ergopeptine alkaloids from the endophyte fungus found on tall fescue grass (fescue toxicosis), ergot alkaloids inhibit fetal

corticotropin-releasing hormone (CRH), inhibiting the normal function of the adrenal gland to produce the cortisol surge and associated changes in pregnane metabolism [137]. In mares with fescue toxicosis, prepartum total plasma progestagen concentrations remain low, their foals have low cortisol concentrations, indicating suppression of fetal adrenocortical activity and P_5 production [161].

Recent studies demonstrated that altrenogest, when given in combination with antimicrobials, pentoxifylline and nonsteroidal anti-inflammatory (NSAIDs) drugs to mares with placentitis, decreased the incidence of abortion [162]. In these cases, altrenogest counteracts uterine contractility induced by inflammation of the fetal membranes. In the same way, in bacterial placentitis, a combination of trimethoprim sulfamethoxazole, pentoxifylline and a double dose of altrenogest (0.088 mg/kg bwt per os s.i.d.) were successful in maintaining pregnancies to term [163], while that untreated control mares aborted. When mares were treated with trimpethoprim sulfamethoxazole and pentoxifylline without altrenogest, only one live foal was born [163, 164]. Despite this, it is not clear what role, if any, altrenogest plays within this multi-treatment approach. However, the mares can still abort while receiving altrenogest treatment in the last trimester of pregnancy.

5.3 Estrogens

In late gestation total estrogen (including E_1S, E_2, and its metabolites, equilin, and equilenin) may be used for fetal and placental health monitoring. However, it is doubtful that total estrogen concentration can predict fetal death as the fetal gonads are unlikely to respond to fetal stress [157, 165].

Since the production of estrogens requires both contributions by the fetus and placental, reduced concentrations in maternal circulation may indicate or predict a stressed or hypoxic fetus that is not producing the estrogen precursors [165]. Indeed, E_2 [166] and E_1S [167] concentrations decreased sharply in mares with placental dysfunction and after the induction of abortion. If the fetus is severely compromised or die in the uterus, maternal plasma E_1S are baseline because of the absence of the C19 precursors secreted by the fetal gonads. However, pregnancies compromised by equine herpesvirus-1 infection or severe colic can present normal or transiently decreased E_1S concentrations [168]. Compared with the adrenal glands, the gonads are unlikely to respond to fetal stress; consequently, so it is doubtful that total estrogen concentrations can predict fetal death. Frequent blood sampling of mares induced to abort with PG between 90 and 150 days of pregnancy indicated that E_1S levels did not decline until within 5 h of abortion [145].

In cases of placentitis at gestational ages between 150 and 280 days, Douglas [169] and Shikichi et al. [157] showed hormonal alterations common as elevated progestogens and low estrogens in mares that aborted. Although the decline in E_2 associated with placental dysfunction is thought to reflect placental disease per se, Esteller-Vico et al. [170] recommended the estrogen supplementation as a means to reduce the risk of abortion associated with placentitis in mares. Recently, Curcio et al. [171] showed that in addition to basic treatment with trimethoprim-sulfamethoxazole and flunixin meglumine, mares with experimentally induced ascending placentitis benefited from E_2 cypionate supplementation. Conversely, altrenogest did not appear to make a difference in outcomes.

After fetal death and stress or fetal weakness, androgens and estrogens levels drop rapidly. For better determination of the health state of the fetus, due to the metabolism of both steroids, it is recommended to monitor androgens and estrogens simultaneously [126].

5.4 Relaxin

Relaxin is a useful biomarker to assess placental health and can be monitored in high-risk mares. Ryan et al. [172] reported a positive relationship between circulating levels of relaxin and poor outcomes in high-risk pregnancies. Relaxin is detectable in the blood after the 80th day of pregnancy without any changes until the second stage of labor. In mares with impaired placental function, in cases of placentitis, placental abruption, hydroallantois, and hydramnios relaxin concentrations decrease below 4 ng/mL [143, 172]. Low circulating levels of relaxin have been reported both in pony mares affected by fescue toxicosis associated with placental disease and agalactia and in Thoroughbred mares, with other forms of placental disease or insufficiency [172].

In the case of placental hydrops, the risk of spontaneous rupture of the fetal membranes increases significantly [173]. Relaxin has been explored as a potential marker of treatment success in placentitis due to its level decrease in cases of spontaneously occurring and experimentally induced pregnancy loss [174].

5.5 Prostaglandins

Placentitis is characterized by the production of proinflammatory cytokines (such as IL-6 and IL-8) and PGs [175, 176]. PG release increases uterine contractility and consequently the risk of premature delivery [138]. Proinflammatory cytokines and the PGs of the FPU increases both in response to inflammation/infection, inducing premature activation of the fetal hypothalamic-pituitary-adrenal (HPA) axis [177], accelerating fetal maturation before parturition [138, 178]. The fetal adrenal produces both progestins and, once sufficiently mature, cortisol. Fetal cortisol, in turn, enhances placental and uterine PGs production, further enhancing uterine contractility and resulting in fetal delivery. Since the maturation of the equine fetus occurs later in gestation [137] this implies that placentitis or maternal disease could be devastating to the newborn foal. However, early fetal maturation likely counterbalances premature delivery and may help improve the chances for foal survival [138, 178]. The supplementation with progestin and PG synthetase inhibitor can maintain equine pregnancy in the presence of PG_{F2} insults [146, 147]. In addition, Esteller-Vico et al. [170] showed that estrogen suppression resulted in a decrease in circulating PGFM, which suggests that estrogens partially regulate PG production during pregnancy since PGFM concentrations were lower but still increased during the last trimester of equine gestation in letrozole-treated mares.

6. Conclusions

Knowledge of the physiological basis of the estrous cycle allows us to understand the interaction of reproductive hormones and the factors or events that interact in the cyclicity of mares. These basic studies have made possible the correct manipulation of the estrous cycle, the advancement of the reproductive season or the synchronization of ovulation. A great contribution in this sense has been possible through the description of the follicular dynamics and the study of the different structures present in the ovaries of the mares throughout the year.

Likewise, the adequate interaction between the ovary, the placenta, and the fetus guarantees the secretion of the correct hormonal patterns necessary for a successful pregnancy. Measurements of progestogens, estrogens, and relaxin, among other hormones, are useful for monitoring the health status of the placenta and fetal viability. This is mainly because placental pathologies or fetal death are mainly due

to alterations of these hormones. On the other hand, the hormonal diagnosis allows temporizing and early detection of pathological conditions to propose an adequate treatment for the maintenance of gestation and with it, the production of a viable foal. Substantial progress has been made in recent years in the identification of risk pregnancies and their treatment.

All this knowledge helps greatly to improve the work of professionals and achievements for the improvement of reproductive outcomes. It is important to bear in mind that the constant production of basic knowledge and applied in equine reproduction will allow in the future to improve and generate new guidelines in reproductive technologies.

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