

Ovarian Follicular Dynamics During the Estrous Cycle in the Mare

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ABSTRACT

The mare is polyestrous, seasonal long-day breeder. The endocrinology of the estrous cycle involves a balance between hormones produced by the pineal gland, hypothalamus, pituitary gland, ovaries, and endometrium. Growth of antral follicles in the ovary occurs in wave-like patterns, and is influenced by several factors such as stage of the estrous cycle, season, pregnancy, age, breed and the individual. Variations among mares in regards to the time of emergence and the type of follicular waves (minor or major, ovulatory or anovulatory) can be significant; however, most commonly, a major ovulatory wave begins at mid-cycle and one follicle (occasionally two, rarely three) becomes dominant and ovulates while the other subordinate follicles in the wave regress. During follicular growth, approximately 7–11 follicles per wave emerge at diameters of 5–6 mm and enter a common-growth phase of about 6 days, in which follicles grow at an approximately similar rate and each follicle has the capacity for future dominance. Subsequently, the deviation process occurs, in which several intra-follicular factors increase in the largest follicle of the ovulatory wave but not in the subordinate follicles; and only the dominant follicle continues to grow. To date, the intra-follicular insulin-like growth factor (IGF) system is the main known mechanism that initiates follicular deviation. The intra-follicular changes in the future dominant follicle apparently increase the responsiveness of this follicle to decreasing follicle stimulation hormone (FSH) and increasing luteinizing hormone (LH) concentrations which occur at that time. The other follicles of the wave have the same capacity for dominance, but do not reach a similar preparatory stage before being negatively affected by the changing gonadotropin concentrations. Thus, the largest follicle alone continues to grow, becomes dominant, and eventually ovulates. This paper reviews the current basic understanding of the endocrine regulation of the estrous cycle, growth patterns of ovarian antral follicles, and the hormonal and the intra-follicular aspects of follicular deviation in mares.

Keywords: Mare, Follicular dynamics, Estrous cycle, Endocrinology

INTRODUCTION

Reproductive activity in horses is seasonally dependent, as it is primarily affected by the length of daylight (photoperiod). In mares, an increase in the duration of daylight (long-day photoperiod) activates reproductive activity, while shortening the duration of daylight (short-day photoperiod) triggers its termination. Therefore, the physiologic breeding season, or the ovulatory season, of the mare lasts from early spring, until late summer. Therefore, the duration of the ovulatory season that mares experience depends on the geographical latitude

at which they live; however, genetics, nutrition, climate, and other environmental factors may play a role as well. During the breeding season the non-pregnant mare will have recurring estrous cycles. The estrous cycle is defined as the period from one ovulation to a subsequent ovulation, with each ovulation being accompanied by signs of estrus and plasma progesterone concentrations below 1 ng/ml (1-2).

The equine estrous cycle is commonly described as a combination of a follicular phase, or estrus, and a luteal phase, or diestrus. During estrus, the mare is sexually receptive to

the stallion, and the genital tract is prepared to accept and transport sperm and an oocyte to the site of fertilization in the ampullary region of the uterine tube (3). During the diestrus period the mare is not receptive to the stallion, and the genital tract is prepared to accept and nurture the conceptus. The diestrus period ends with regression of the corpus luteum (CL) which occurs due to prostaglandin F_{2α} (PGF_{2α}) release from the endometrium of the non-pregnant mare (4).

Mean length of the estrous cycle in the mare population during the physiologic breeding season is approximately 21 days, but it can vary greatly (range 18-24 days). The length of diestrus remains relatively constant at 14-15 days and is not affected by the season (5). However, although estrus typically comprises 4-7 days of the cycle, its length is more variable (ranging from 2-12 days or more). In the beginning and at the end of the breeding season the length of estrus may be 7 to 12 days, whereas, around the summer solstice, estrus may last only 3 to 4 days (6). Therefore, the duration of estrus is shortest during the peak of the ovulatory season. The diameter of the largest follicle at the time of luteolysis affects the interval from onset of estrus to ovulation. Larger follicles present at CL regression typically ovulate sooner, thus shortening the associated estrus period (7).

Endocrine regulation of the estrous cycle

The endocrinology of the estrous cycle involves a delicate balance among hormones produced by the pineal gland, hypothalamus, pituitary gland, ovaries, and endometrium. The hypothalamus produces gonadotropin-releasing hormone (GnRH), which is released in brief pulses into the hypothalamic-pituitary portal system, and stimulates the synthesis and release of the gonadotropins, follicle stimulation hormone (FSH) and luteinizing hormone (LH), from the anterior pituitary gland (8). Thus, GnRH secretion elicits the release of both LH and FSH from the pituitary, but apparently the ratio of LH/FSH reaching the circulation is influenced by GnRH pulse frequency and by physiologic feedback from inhibin, estrogens, and progesterone released from the ovaries. Low-frequency pulses of GnRH stimulate synthesis and release of FSH; and high-frequency GnRH pulses stimulate synthesis and release of LH (8). During diestrus, progesterone released from the CL suppresses the high frequency of GnRH release. During estrus, after luteolysis occurs and circulating progesterone concentrations are low, GnRH pulse frequency is markedly increased as the estrogens produced

by maturing follicles increase and positively stimulate the hypothalamus to release GnRH.

The gonadotropins enter the systemic circulation and stimulate the ovaries. FSH stimulates follicular recruitment and growth, and LH stimulates maturation of the follicles, maturation of the oocytes, production of estrogens, ovulation, and luteinization of the CL(2).

Growth pattern of ovarian antral follicles

In the mare, growth of antral follicles occurs in wave-like patterns (9-10). The periodic development of follicular waves continuously occurs and is influenced by several factors such as stage of the estrous cycle, season, pregnancy, age, breed and individual. Hence, different types of follicular waves (minor or major, ovulatory or anovulatory; see Figure 1) with variable levels of activity within waves may develop under specific physiological conditions. In the mare ovary there are approximately 40,000 primordial follicles and 100 growing follicles (2, 11). Antral formation in the growing population of underlying follicles in both horses and ponies occurs when the follicle reaches a diameter of 0.2 to 0.4 mm. Atresia (regression) of follicles is rare until they reach 1 mm (12). Unfortunately, the factors influencing the initial stages of follicular development in domestic animals are less known (13). Furthermore, our understanding of dynamics in the population of small follicles (up to about 5 - 10 mm) in the mare is lacking; however, it is believed that this phase in the horse occurs over a prolonged period of time, as was shown in other species (2, 10, 14-15). It was speculated that small follicles are continuously growing and regressing and thus providing a reservoir for larger follicles in the follicular waves (2).

Most mares were shown to have one or two follicular waves per cycle. The types of follicular waves that develop in mares are major waves (characterized by dominant and subordinate follicles) and minor waves (largest follicle does not attain the diameter of a dominant follicle). Based on transrectal palpation, a single major follicular wave was proposed initially for the equine estrous cycle (16). The wave of follicles dissociates about 6 days before ovulation into a single growing preovulatory follicle and several regressing follicles. Subsequently, the initial transrectal palpation findings were substantiated by ultrasound, based on grouping of follicles into diameter categories (10) and tracking of individual follicles (17-18). It was found that there are profound individual variations as well as breed differences in wave patterns during

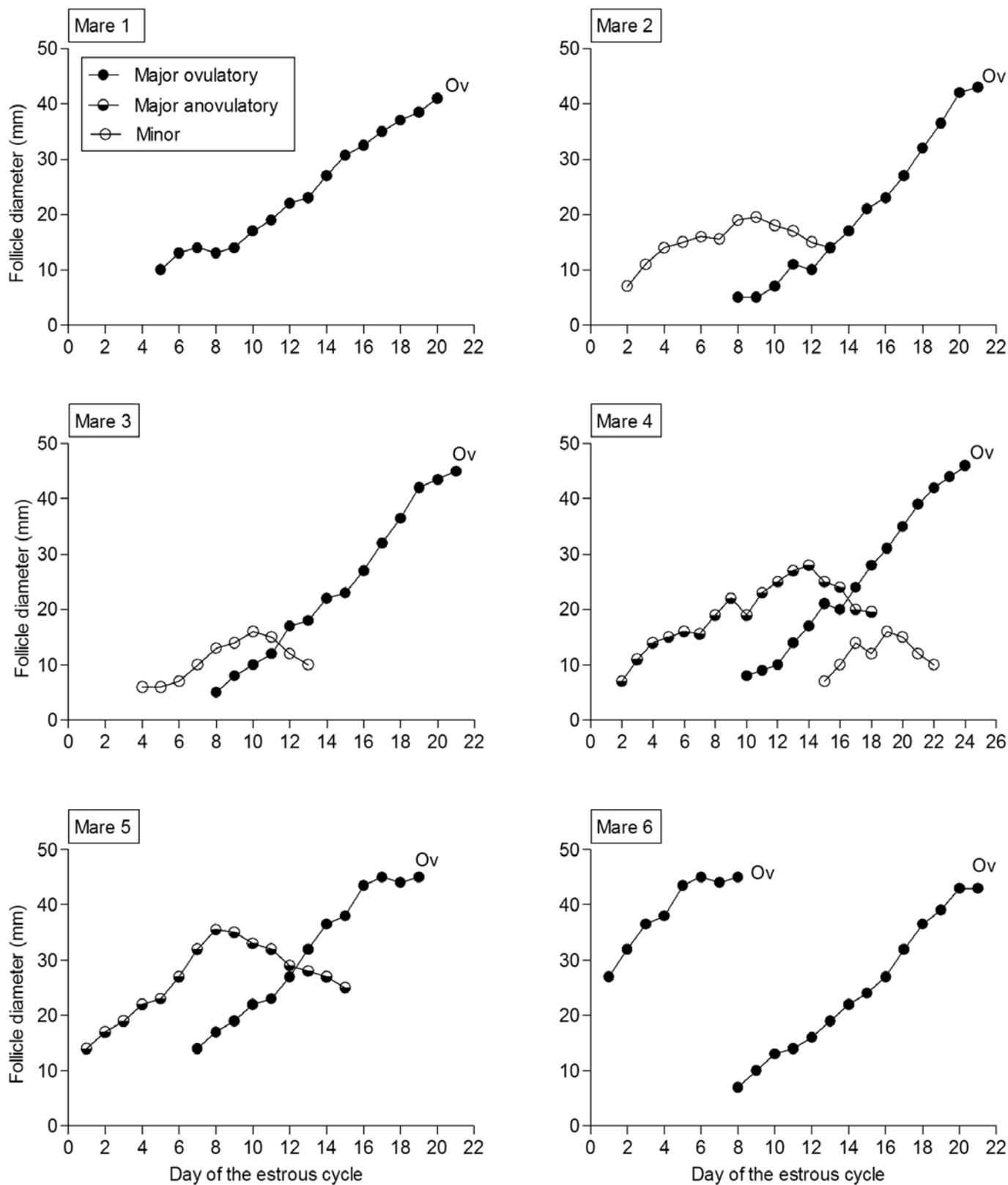


Figure 1: Examples of follicular waves in the estrous cycle of six different mares. The diameter of the largest follicle in the wave is presented. Note the different type of follicular wave: Minor wave; Major anovulatory wave; and Major ovulatory wave.

the estrous cycle (2). In some breeds (e.g. Quarter-Horses, Ponies), usually only one major wave develops in late diestrus and culminates in the estrous ovulation. In other breeds (e.g. Thoroughbreds), a secondary major wave frequently develops in early diestrus, and the dominant follicle may be anovulatory (more common), or occasionally ovulatory (diestrus ovulation; for example, see Figure 1, Mare 6) (19). Minor follicular waves have been demonstrated in mares in variable patterns (20-21). In all horse breeds, however, a major ovulatory wave begins at mid-cycle and one follicle (occasionally two, rarely three) becomes dominant and ovulates.

Development and selection for dominance; follicular deviation

Horses have an effective follicle selection mechanism, so that usually only one antral follicle of an ovulatory follicular wave becomes dominant and ovulates (22-23). Anovulatory follicular waves also occur and are defined as major waves when a dominant follicle develops and minor waves when a dominant follicle does not develop. Among farm species, cattle and horses have the most effective selection mechanism as indicated by a greater frequency of single ovulations than in goats, sheep, and swine (22, 24). A distinctive change in growth rates between the developing dominant follicle and the remaining subordinate follicles is the eminent selection event in a follicular wave, and is defined as deviation.

Major and minor follicular waves develop in mares from the stimulation of an FSH surge (24-26). As aforementioned, the ovulatory wave which emerges in mid-diestrus is most consistent. However, earlier waves in the estrous cycle may or may not develop a dominant follicle (≥ 28 mm) or it may not be detectable (27). The ovulatory waves can be studied ultrasonically for approximately 3 days before the peak of the wave-stimulating FSH surge in mares. Mean numbers of 7-11 follicles per wave emerge at diameters of 5-6 mm over one to several days and enter a common-growth phase of about 6 days (22, 28-29). The common-growth phase extends from the beginning of wave emergence to the beginning of deviation; the end of the common-growth phase and the beginning of deviation are synonymous. During the common-growth phase, the follicles grow at an approximately similar rate and each follicle has the capacity for future dominance (30-31). However, on average, the future dominant follicle emerges one day earlier than the other follicles of the wave; this early emergence results in a size advantage for the future

dominant follicle at the end of the common-growth phase. Follicles that emerge late in a wave reach a smaller maximal diameter, and may reach a plateau or maximum diameter at the end of the common-growth phase (22, 24, 27, 31).

Studies support the conclusion that all, or most, follicles in the same cohort during the common-growth phase have the potential for future dominance (22, 30-31). Ablation of the largest follicle at or shortly after the end of the common-growth phase or beginning of deviation is followed by the establishment of dominance by the second largest follicle in mares (22). Thus, rather than selection of a dominant follicle, the selection or deviation process more literally involves an action against the remaining follicles (24, 27).

In mares, the mean diameters of the two largest follicles at the beginning of deviation are approximately 23.0 and 19.0 mm (22, 28-29, 32). Apparently, when the largest follicle reaches a decisive developmental stage, rapid development of the deviation mechanism blocks the second-largest follicle, and other follicles before they reach a similar diameter. Thus, the mean difference in diameter between the two largest follicles at the beginning of deviation indicates that the destiny of the follicles must be established in <1 day in mares (equivalent to a difference of 3 to 4 mm). Despite the rapid designation of follicle status, a subordinate follicle may maintain adequate viability for one day or more after the beginning of deviation so that it may be rescued to convert to dominant status if the dominant follicle fails or is ablated (24, 27). Therefore, in mares, treatment protocols to increase the number of ovulations (superovulation) commonly employ initiation of stimulatory hormonal treatments, such as equine FSH (eFSH), at the time of deviation; these treatments are given in order to stimulate, or "save", those subordinate follicles to achieve maturation and to ovulate (33-40).

Systemic hormonal aspects of follicular deviation

In mares, the wave-stimulating FSH surge reaches peak concentrations when the largest follicle is about 13 mm (Figure 2) (22, 27, 29). Thereafter, FSH concentrations decrease over several days. During the decline in the wave-stimulating FSH surge and before follicle deviation, growth of follicles is dependent on FSH (41). However, this FSH decline is necessary for the establishment of deviation, as indicated by a delay or prevention of deviation by administering FSH or by increasing endogenous FSH with an anti-inhibin immunization (24, 33, 35, 42). There is a 3-day interval between

peak FSH concentrations and the beginning of deviation. During that 3-day interval, all follicles of the wave continue to require FSH; however, their secretion of estrogens and inhibin contribute to the decline in pituitary FSH release. In association with the beginning of deviation, large follicles are more responsive to declining or low concentrations of FSH than small follicles (24, 43). Therefore, the role of FSH after the peak of the surge involves the continuation of growth and development of all follicles before deviation and the development of the dominant follicle after deviation. Based on several studies, FSH stimulates the production of estradiol, insulin-like growth factor (IGF)-1, inhibin-A, activin-A, and other factors (24, 30, 44-46). These FSH-stimulated factors have intra-follicular roles in deviation.

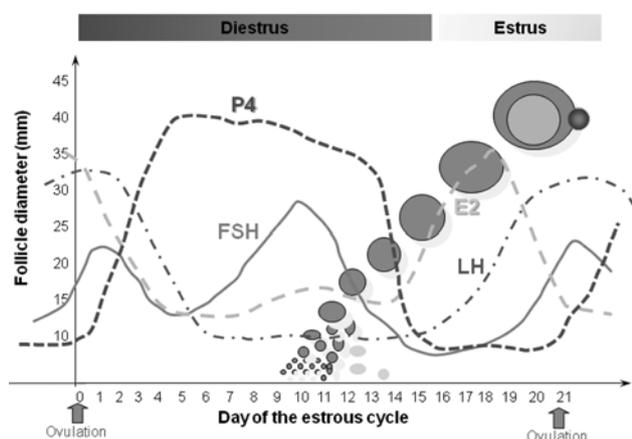


Figure 2: Schematic diagram of the mare estrous cycle endocrinology

Follicular release of estradiol and inhibin, alone and synergistically, suppress the circulating concentrations of FSH. Circulating estradiol in mares begins to increase at about one day before deviation, and remains elevated until 1 or 2 days before ovulation (47). Total inhibin concentrations begin to increase earlier than estradiol, just before the beginning of the declining portion of the wave-stimulating FSH surge (24, 47-49). After the expected day of deviation, total inhibin remains elevated, which is attributable to the dominant follicle. Experimental ablation of the largest follicle at the expected beginning of deviation prevented a further increase in circulating estradiol and a decrease in inhibin; this resulted in a post ablation FSH increase (24, 26-27). Hence, the beginning of the FSH decline at 13 mm diameter follicle in mares coincides with the beginning of an inhibin increase for both minor and major waves (50-51). For major waves, inhibin

(probably inhibin-A) is produced by multiple follicles before deviation and by the developing dominant follicle after the beginning of deviation and is an FSH suppressant during the entire FSH decline. Estradiol begins to play a greater role in FSH suppression near the beginning of deviation.

In mares, concentrations of the periovulatory LH surge begin to increase during the FSH decline, reach maximal concentrations one day after ovulation and then decline for several days (Figure 2) (2, 26-27). Thus, LH and FSH are dissociated during the periovulatory period. It has been shown that the granulosa cells of the future dominant follicle acquire LH receptors before the beginning of diameter deviation (52). Furthermore, experimental reduction of LH encompassing deviation resulted in smaller post-deviation diameter of the largest follicle and lower circulating concentrations of estradiol and total inhibin compared to controls (47, 53). These studies indicated that regulation of the production of inhibin, as well as estradiol, depends at least partly on LH, and that there is an LH-mediated survival advantage for the largest follicle of the wave before cessation of growth by the remaining follicles (24, 27).

Intra-follicular aspects of follicular deviation

Over the last few years our knowledge and understanding of the intra-follicular aspects of follicular deviation in monovular species has increased significantly due to experimental approaches such as follicular fluid sampling (54-56), follicle ablation (29, 31), and injection of potential stimulatory or inhibitory factors into follicles (57-59). Several intra-follicular factors were evaluated and were found to be different between the largest and the second largest follicle before the beginning of deviation. Concentrations of free IGF-I, estradiol, inhibin-A and activin-A are greater in the future dominant follicle than in other follicles before the beginning of deviation (27, 30, 60). However, studies confirmed that free IGF-I is the key factor for the initiation of deviation in horses.

In mares, increased concentrations of free IGF-I in the future dominant follicle, but not in subordinate follicles, is the first noted change before the beginning of diameter deviation. The IGF system includes IGF-I and -II, IGF-binding protein (IGFBPs), and IGFBP proteases (61). Free IGF-I stimulates granulosa cell proliferation and synergizes with gonadotropins to promote differentiation of follicle cells (62). *In vitro* effects of IGF-I in cattle include increased pro-

liferation of granulosa cells and estradiol production (45), enhanced sensitivity of granulosa cells to FSH (62-63), increased secretion of inhibin-A, activin-A and follistatin from granulosa cells (45), and enhanced LH stimulation of androgen synthesis from theca cells (64).

The IGFs exert a pivotal role in the regulation of IGF bioavailability by selectively binding the IGFs and making them unavailable to their receptors (30, 60). The IGFs are inhibitory to gonadotropin-induced follicular growth and differentiation and inhibit the actions of IGFs at the level of target cells (62, 65). Thus, changes in intra-follicular IGFs lead to changes in IGF bioavailability and the up or down regulation of gonadotropin actions on follicular cells (27). Proteolytic activity for BP-2 (66), BP-4 (67), and BP-5 (68) has been reported in dominant follicles during the follicular phase in mares. The IGF proteases degrade the binding proteins and thus increase the bioavailability of IGF-I in follicles (30, 61, 67). A greater proteolytic activity of such proteases was reported in the future dominant follicle at the beginning of deviation and was temporally associated with greater concentrations of free IGF-I (58).

Vascular endothelial growth factor (VEGF) stimulates mitosis of endothelial cells, increases vascular permeability and angiogenesis (69). In horses, there is an early increased vascularity in the future dominant follicle (30). Follicular fluid VEGF concentrations were reported to be higher in the largest follicle than in the second largest follicle on the day after the beginning of diameter deviation (27, 59). However, the earlier temporal relationships before deviation have not been studied in horses. An increase in vascularity would give the follicle an advantage to receive preferential supply of growth factors, gonadotropins, steroid precursors and other nutrients required for its continued development.

There is limited information concerning the expression of key receptors in the ovarian granulosa and theca cells in horses. However, in cattle, estradiol and progesterone receptors are present in both granulosa and theca cells (70), and they are upregulated in the dominant follicle (71). The expression pattern of IGF-I receptors in relation to deviation is not known; however, the expression of IGF-I binding sites in cattle were shown to increase from primary to large antral follicles (72). The induction of LH receptors in granulosa cells is one of the early events in selection of a single dominant follicle in cattle (30, 73). Compared to smaller follicles, the LH-receptor protein content in equine granulosa cells

was greater when the follicles were 15–19 mm in diameter than in smaller follicles, but the results were equivocal with regards to the temporality of differential LH receptor acquisition and the beginning of deviation (52). Changes in FSH receptor activity have not been reported for mares. In cattle, no difference in the expression of FSH receptors was found between small and large follicles (73).

In summary, the mare is polyestrous, long-day breeder. During the estrus cycle, growth of antral follicles occurs in wave-like patterns. Variation among mares in regard to the types and timing of follicular waves (minor or major, ovulatory or anovulatory) can be significant; however, most commonly, a major ovulatory wave begins at mid-cycle and one follicle becomes dominant and ovulates. During follicular growth, at the beginning of dominant status (deviation), several intra-follicular factors increase in the largest follicle of the ovulatory wave (30). However, to date, the intra-follicular IGF system is the main known mechanism initiate follicular deviation. The intra-follicular changes in the future dominant follicle apparently increase the responsiveness of this follicle to decreasing FSH and increasing LH concentrations. The other follicles of the wave have the same capacity for dominance, but do not reach a similar preparatory stage before being negatively affected by the changing gonadotropin concentrations. Thus, the largest follicle alone continues to grow, becomes dominant, and eventually ovulates.

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