Progress in understanding ovarian follicular dynamics in cattle

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Abstract

The study of follicular dynamics began in the mid-20th century, but progress has been particularly rapid in the last two decades through the use of tools that have enabled serial, non-invasive examination. A brief overview of early oogenesis and folliculogenesis is provided as a backdrop to the evolution of our understanding of follicular dynamics during the bovine estrous cycle. Studies to date support the concept that the pair of ovaries acts as a single unit and influences follicular development primarily via systemic endocrine routes involving ovarian and uterine products, the gonadotropins, and their receptors. Dominant and subordinate follicles pass through growing, static and regressing phases that have distinct morphologic and biochemical characteristics; these changes are the basis of efforts focused on diagnosing and manipulating follicular status. An update of research progress highlights recent findings on the repeatability (predictability) within individuals of follicle recruitment and wave pattern (two- versus three-wave cycles), the relationship between oocyte competence and follicular status, and the dynamics of small follicles. Recent studies documented that wave emergence and follicular dominance are apparent earlier than previously reported, and on the basis of periodic endogenous FSH surges and the presence of FSH receptors, the hypothesis that follicles become progressively entrained to waves from the earliest stages of development is introduced. Lastly, recent studies comparing old cows and their young daughters provide a new understanding of the effects of aging on gonadotropins and ovarian steroids, follicular dynamics, ovarian response to synchronization, superstimulation, and oocyte competence.
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1. Introduction—a history of ovarian research

Controversy has been a hallmark of the study of ovarian form and function from the earliest descriptions of the female gonad [1]. In the 5th century B.C., Hippocrates did not ascribe any generative role of the ovary, but rather suggested that generation of a new life was the result of the action of two kinds of semen— one from the male (ejaculate) and one from the female (menstrual blood). A century later, Aristotle characterized the ovary as an imperfect vestige of the male testis with no apparent function. It was not until the mid-1600s that the ovary was recognized for what it was— the producer of eggs. The Dutch physician, Regnier de Graaf, is often cited as the first to recognize the rightful role of the ovary in his ''New treatise concerning the generative organs of women'' published in 1672. The 31-year-old de Graaf, however, was embroiled in bitter arguments with his anatomy professor, Johannes van Horne, and classmate, Jan Swammerdam, who claimed that they had revealed the form (and by extension, the function) of the ovary in a short communication in 1668.

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Their early scientific "abstract", however, was preceded by one of de Graaf's a few weeks earlier, in March 1668. Ironically, the conclusion of a committee tasked to settle the dispute was that neither de Graaf nor Van Horne was the first to have seen eggs in viviparous organisms, but rather another classmate, Niels Steno, who reported in his 1667 treatise that "the testicles of women are analogous to the ovary." All of these early modern scientists, however, held the mistaken belief that the follicle itself was the egg—like a small bird's egg without a shell. It was not until 1827 when an Estonian physician, Karl Ernst von Baer, provided the first description of a mammalian egg from his microscopic study of ovarian vesicles (follicles) in the ovary of a dog. The first studies of the dynamics of follicle development, however, were not for another 100 years.

The purpose of this report is to provide a brief overview and historical background of our understanding of follicle dynamics in cattle, and to highlight some recent advances, primarily from the author's laboratory. It is not intended to be a comprehensive review. For brevity, reference is made to reviews of specific topics rather than original studies. Unless otherwise stated, the information presented is from results of studies on *Bos taurus*.

2. Early oogenesis and folliculogenesis

Oocytes originate as primordial germ cells from the endoderm of the embryonic yolk sac, and migrate by amoeboid movement via the dorsal mesentery of the hindgut to the gonadal ridge [2] by Day 35 of gestation in cattle [3]. Primordial germ cells undergo a limited number of mitotic divisions during migration and upon arrival at the gonadal ridge [2,4]. Primordial germ cells are internalized into the gonadal ridge through its surface epithelium—initially thought to be the source of primordial germ cells and mistakenly named the "germinal" epithelium. During the process of internalization, the primordial germ cells cease mitotic division, become enclosed in germ cell cords (ovigerous cords) composed of epithelial cells which are delineated from the surrounding mesenchymal cells by a basal lamina, and they become referred to as oogonia [4]. Meiosis of oogonia (transition to primary oocytes) begins by Days 75–80 of gestation in cattle and the first meiotic division does not proceed beyond the pachytene stage of prophase-I [3], at which time the chromosomes are decondensed and contained within the nuclear membrane—the germinal vesicle [2].

A single layer of flattened epithelial cells from the germ cell cords condense around the vast majority of surviving oocytes and enclose them to form primordial follicles [3,4]. Oocytes that fail to be surrounded by epithelial cells degenerate [2]. Initiation of follicular growth (activation) begins with the transformation of the flattened pre-granulosa cells of the primordial follicle into a single layer of cuboidal granulosa (follicular) cells—a primary follicle [5]. Proliferation of granulosa cells results in an increase from two to six layers around the oocyte (secondary follicle), to >6 layers of granulosa cells and a fluid-filled antrum (tertiary or antral follicle) [5,6].

3. Follicular dynamics during the bovine estrous cycle

3.1. Follicular waves

The estrous cycle and its phases in cattle were first described by Hammond [7], followed by McNutt [8], and Cole [9]. In 1946, Bullough [10] used a mouse model and described the relationship between ovarian follicular development and hormones. Studies of the dynamics of follicular development were first reported in rats by Mandle and Zukerman [11] and in monkeys in 1951 by Green and Zukerman [12]. Both studies involved a histological approach and both concluded that there was no cyclic variation in follicle numbers. Rajakoski [13] has been credited with the initial proposition of the two-wave theory of follicular growth during the bovine estrous cycle. For three decades after Rajakoski's report, many experiments were done on various aspects of follicular dynamics during the bovine estrous cycle, resulting in contradicting accounts of the nature of follicle development, ranging from the absence of waves to as many as three or four distinct waves per estrous cycle. In later reviews, the two-wave theory of Rajakoski was refuted on the basis that "conclusions were based on qualitative assessment of data without current knowledge of the profile of gonadotropins and of ovarian steroids..." (reviewed in [14]). Evidence was presented to support the concept that follicles are recruited continuously throughout the cycle and the follicle destined to ovulate is selected by coincidence of its stage of maturity (readiness) and the occurrence of the preovulatory gonadotropin surge. However, with the introduction of ultrasonography in the late 1980s, the barrier to our understanding of follicular dynamics was suddenly broken (reviewed in [15]).

Studies using ultrasonic imaging to monitor follicle populations in different size categories or to monitor

individually identified follicles (reviewed in [14]) have convincingly documented that follicular growth in cattle occurs in a wave-like fashion and that the majority of estrous cycles in cattle are comprised of two or three such waves. Follicular wave emergence in cattle is characterized by the sudden (within 2–3 days) growth of 8–41 small follicles that are initially detected by ultrasonography at a diameter of 3–4 mm (Fig. 1) (reviewed in [14,16]). The growth rate is similar among follicles of the wave for approximately 2 days, when one follicle is selected to continue growth (dominant follicle), whereas the remainder become atretic and regress (subordinate follicles). Results of these early studies of follicle dynamics gave rise to the hypothesis that the dominant follicle suppresses the growth of the subordinates in the existing wave, and the emergence of the next follicular wave. Support for this hypothesis was provided in a series of studies involving systemic treatment with the proteinaceous fraction of follicular fluid and by electrocautery of the dominant follicle

Fig. 1. Dynamics of ovarian follicular development and gonadotropin secretion during two- and three-wave estrous cycles in cattle. Dominant and subordinate follicles are indicated as open (viable) or shaded (atretic) circles. A surge in circulating FSH concentrations (thick line) precedes emergence of each wave. A surge in circulating LH concentrations (thin line) precedes ovulation. The LH surge is preceded and succeeded by a period of high-LH pulse frequency as a result of low-circulating progesterone concentrations (i.e., period of luteolysis and luteogenesis, respectively).
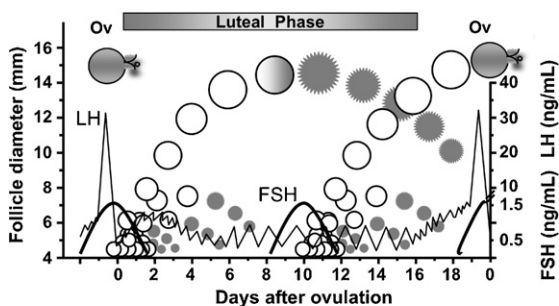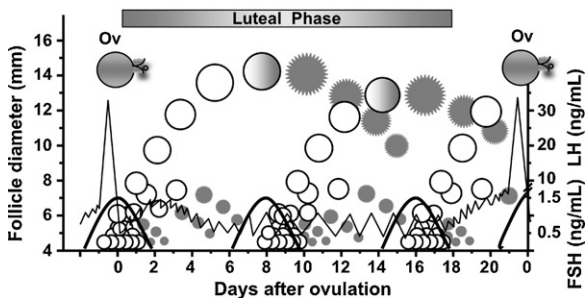
(reviewed in [17]). The applied implications of these findings were immediate and far-reaching, and marked a new era for ovarian synchronization and super-stimulation in cattle [18,19].

3.2. Two- versus three-wave patterns

The majority of bovine estrous cycles (i.e., >95%) are composed of either two or three follicular waves (reviewed in [19]). Some have reported a preponderance (>80%) of either the two- or three-wave pattern, whereas others have reported a more even distribution. In both two- and three-wave estrous cycles, emergence of the first follicular wave occurs consistently on the day of ovulation (Day 0). Emergence of the second wave occurs on Day 9 or 10 in two-wave cycles, and on Day 8 or 9 in three-wave cycles. In three-wave cycles, a third wave emerges on Day 15 or 16. Under the influence of progesterone (e.g., diestrus), dominant follicles of successive waves undergo atresia. The dominant follicle present at the onset of luteolysis becomes the ovulatory follicle, and emergence of the next wave is delayed until the day of the ensuing ovulation. The CL begins to regress earlier in two-wave cycles (Day 16) than in three-wave cycles (Day 19) resulting in a correspondingly shorter estrous cycle (19–20 days versus 22–23 days). Hence, the so-called 21-day-estrous cycle of cattle exists only as an average between two- and three-wave cycles (Fig. 1).

Predictive factors associated with a two- versus three-wave pattern may provide insight into mechanisms controlling the pattern, and have important implications on breeding management and the development of effective protocols for ovarian synchronization. Pregnancy rates in cattle with two- versus three-wave patterns were compared based on the notion that the preovulatory follicle in the two-wave pattern grows for a relatively longer period and may contain a relatively aged oocyte. However, results have been contradictory; pregnancy rates did not differ between two- versus three-wave cycles in some studies [20,21], whereas a lower pregnancy rate was reported for two-wave cycles in another study [22]. There appears to be no breed- or age-specific predilection for a given wave pattern in *B. taurus* cattle. An increase in the proportion of three-wave patterns has been associated with a low plane of nutrition and heat stress (reviewed in [19,23]). In *Bos indicus* cattle, no seasonal effect on wave pattern was detected, but the pattern was influenced by parity (reviewed in [23]). The majority of Nelore heifers (65%) exhibited a three-wave pattern, whereas the majority of Nelore cows (83%) exhibited a two-wave pattern. Others have

reported that up to 27% of estrous cycles in *B. indicus* cows consisted of four waves of follicular development, compared to 7% in *B. indicus* heifers (reviewed in [23]).

In a recent study involving ultrasonographic data from 91 interovulatory intervals [24], two- and three-wave patterns of follicular development were compared to determine the repeatability and predictive characteristics of a given wave pattern. Two-wave cycles were nearly 3 days shorter than three-wave cycles (19.8 ± 0.2 versus 22.5 ± 0.3; $P < 0.01$). The majority of cycles ≤21 days (88%) were of the two-wave pattern ($P < 0.05$), whereas the majority of cycles ≥22 days (78%) were of the three-wave pattern ($P < 0.05$). The proportion of serial cycles in which the pattern remained the same (i.e., repeatability) was more than twofold greater than the proportion cycles that changed patterns (70% versus 30%; $P < 0.01$). The repeatability of wave pattern, and the proportion of two- versus three-wave patterns within the herd were not affected by the season of year. The strongest correlate to the number of waves in an interovulatory interval was the duration of follicular dominance of Wave 1. The duration of dominance (defined as the period of the growing and static phases of the dominant follicle) was 3 days longer and the onset of regression was later in two-wave patterns than in three-wave patterns ($P < 0.01$). Dominance of Wave 1 was associated with a subsequent delay in the attainment of maximum diameter by the dominant follicle of Wave 2, as well as early onset of luteolysis. Therefore, factors that influence the development of the dominant follicle of Wave 1 may be responsible for regulating the wave pattern.

4. Hormonal interplay controlling wave dynamics, ovulation and CL development

4.1. Local versus systemic control

The two ovaries act primarily as a single unit; i.e., each follicular wave includes follicles from both ovaries that respond in unison. In a critical study of intra-ovarian relationships [25], the authors concluded that the dominant follicle suppressed subordinates and new wave emergence via systemic (endocrine) rather than local channels. Only one follicle from the pair of ovaries is selected to become dominant, the side of dominant follicle development was random, and the dominant follicle was equally likely to reside in the same or contralateral ovary to that of the largest subordinate follicle. The side of the CL or dominant follicle of a previous wave had no effect on the side of the ovulatory follicle. Although intrafollicular (autocrine and para-

crine) factors are important for growth, health and demise of an individual follicle, there is no convincing in vivo documentation of one follicle affecting the health/regression status of its neighbors directly by a localized effect.

4.2. Gonadotropins and their receptors

Emergence of a follicular wave and selection of the dominant follicle are temporally associated with a rise and fall in circulating concentrations of FSH (Fig. 1) [17]. Emergence of a follicular wave is preceded by a surge in plasma FSH concentrations in both spontaneous waves and induced waves. Follicular products, especially those from the dominant follicle, are responsible for suppressing FSH release and, therefore, the emergence of the next follicular wave (Fig. 1). At the end of the period of dominance (i.e., at ovulation, or the mid-static phase of an anovulatory dominant follicle), circulating concentrations of FSH begin to rise; they increase 1.5–2-fold over the next 2 days, and peak approximately 12–24 h before emergence of the wave (when the future dominant follicle is 4–5 mm in diameter). If an existing dominant follicle is removed (i.e., follicular ablation), a surge in FSH begins within 12 h, resulting in emergence of a new follicular wave within next 24 h [26]. Selection of the dominant follicle is associated with decreasing blood FSH concentrations during the first 3 days of the wave. The nadir in FSH is reached 4 days after wave emergence, and concentrations remain low for the next 2–3 days. Receptors for FSH are present only on granulosa cells, whereas LH receptors are located on both granulosa and theca cells in the wall of antral follicles. The dominant follicle acquires more LH receptors on its granulosa cells than its subordinates and is therefore able to shift its gonadotropin dependence to LH during the FSH nadir, and continue to grow while the subordinates regress.

4.3. Ovarian and uterine products

The CL is the main source of progesterone; CL morphology and plasma progesterone concentration are good indicators of progesterone synthesis within the CL (reviewed in [15]). Intense angiogenesis, proliferation of granulosa and theca cells from the wall of ovulated follicle, and their differentiation (luteinization) during the first 5–6 days after ovulation (metestrus) results in a progressive increase in plasma progesterone concentration from <1 ng/mL at 3 days after ovulation to approximately 3 ng/mL by 6 days. Plasma progesterone concentration peaks between 10 and 14 days post-

ovulation (>4 ng/mL), followed by decreasing concentrations after 16 days due to luteolysis (hypoxic cell death resulting from hyalinization of blood vessels) induced by $PGF_{2\alpha}$ released from the endometrium of the nonpregnant cow.

Dominant follicles from both anovulatory and ovulatory waves produce estradiol. Theca cells are required for conversion of progesterone to androgens, whereas the aromatase enzyme (to convert androgen to estradiol) is exclusively localized in granulosa cells. After wave emergence, estradiol content in the follicular fluid of the growing dominant follicle increases at least 20-fold by the day of selection (3 days after wave emergence), followed by a threefold decrease by the early static phase of the anovulatory dominant follicle (6 days), before returning to base-line in the early regressing phase (11 days) [15]. Peak estradiol concentration in the follicular fluid of the ovulatory follicle is twice as high as the peak in anovulatory dominant follicles. Exogenous estradiol treatment during the luteal phase induces the demise of the existing dominant follicle, most likely by suppressing LH and FSH, followed by a rebound in FSH and synchronous emergence of a new follicular wave [14,18]. This treatment is the basis of many current protocols for ovulation synchronization for fixed-time AI and superstimulation. In addition to estradiol, which has a major inhibitory action on FSH, growing follicles produce other factors such as IGFs, inhibins and follistatin [27] that also regulate FSH release and availability. Although the dominant follicle plays a major role, all follicles of an emerging wave contribute to suppression of the wave-eliciting FSH surge [28].

The release of both FSH and LH is induced by pulses of GnRH from the hypothalamus, but because FSH release is profoundly influenced by follicular products and because its half-life in cattle is longer than that of LH, episodic release of FSH is less apparent than LH. Pulse frequency and amplitude of LH are influenced by circulating concentrations of both progesterone and estradiol. High levels of progesterone produced by a functional CL during diestrus or pregnancy suppress LH pulse frequency (Fig. 1). When plasma progesterone concentrations were approximately 1.7 ng/mL, six LH pulses per 8 h with an amplitude of 0.2 ng/mL were observed, whereas only 1.8 pulses per 8 h with an average amplitude of 0.34 ng/mL were observed when progesterone concentrations were 5 ng/mL [29]. Therefore, dominant follicles grow larger and remain dominant for a longer interval when LH pulse frequency is elevated (i.e., low progesterone) (reviewed in [14]). Increasing estradiol concentrations with decreasing progesterone after luteolysis increase the LH pulse frequency further, culminating in a large prevulatory LH surge.

5. Oocyte competence and follicular status

Morphological and cellular changes during follicular and luteal development are reflected in biochemical changes at the tissue and plasma levels, as well as in ultrasound image characteristics and the developmental competence of the oocyte (reviewed in [15]). Computer-assisted analyses of ultrasound images of dominant and subordinate follicles at different phases of development demonstrated that pixel heterogeneity of the antrum and wall, and the sharpness of the fluid–wall interface were negatively correlated ($P < 0.01$) with estradiol and the estradiol:progesterone ratio in follicular fluid [15]. Histologically, the wall thickness of Wave 1 dominant follicles decreased dramatically during the late-static (60 μm) and regressing (42 μm) phases, compared to the growing and early static phases (120 μm). Cells of the granulosa layer of the dominant follicle of Wave 1 became loose and degenerate during the late-static phase, and lined by fibroblast-like flattened cells during the regressing phase. The wall of subordinate follicles was thinner and had a lower mitotic index than that of the dominant follicle 3 and 6 days after wave emergence. The dominant follicle of Wave 1 displayed histological evidence of atresia at the time of emergence of Wave 2. Based on these data, follicular dominance is morphologically and biochemically maximal during the mid-growing phase (3 days after wave emergence), and begins to wane early in the static phase (6 days after wave emergence).

A positive relationship exists between early follicular atresia and oocyte competence [30,31]. A significantly greater proportion of cumulus–oocyte complexes collected from subordinate follicles during the early regressing phase were expanded, and a significantly greater proportion of oocytes showed evidence of nuclear maturation than those collected during the growing and early static phases. Furthermore, oocytes obtained from early regressing subordinate follicles (Day 5 of follicular wave) were more likely to develop into embryos in vitro than those obtained from growing (Day 2), early static (Day 3) or late-regressing (Day 7) subordinate follicles. Interestingly, ultrasound image gray-scale pixel values of the perifollicular stroma were lower in ovaries from which oocytes were collected that did not produce embryos compared to those that did produce embryos [31]. A comparison was made recently of the developmental

competence of oocytes that developed under low-versus high-progesterone concentrations during super-stimulation with FSH [32]. Oocytes that developed under a high-progesterone milieu (i.e., low-LH pulse frequency) were less capable of developing into embryos. Interestingly, oocytes that developed under a prolonged-low-progesterone level (i.e., mimicking persistent oversized follicles) failed to ovulate. These findings have provided impetus for further studies to determine if short-term, low-progesterone exposure (i.e., high-LH pulse frequency) during the growing phase of follicle development after FSH superstimulation could be utilized to enhance the proportions of transferable embryos in vivo and in vitro.

6. Dynamics of small follicles

6.1. FSH responsiveness

Whereas the developmental dynamics of follicles ≥4 mm have been well characterized, the dynamics of smaller follicles remain a mystery. In a classic experiment involving assessment of the mitotic index of granulosa cells before and after colchicine treatment [6], it was estimated that small follicles grew more slowly (27 days to grow from 0.13 mm to 0.67 mm) than larger follicles (6.8 days to grow from 0.68 mm to 3.67 mm) and that two estrous cycles were required for preantral follicles to reach the mature antral stage. However, these estimations do not shed light on follicle dynamics in relation to changes in circulating concentrations of gonadotropins and follicular wave emergence.

Similarly, the role of FSH in regulating the wave-like development of follicles ≥4 mm has been clearly defined [17], whereas the role of FSH in smaller follicles, including small antral, and preantral follicles, remains unclear. An early study demonstrated the binding of FSH to the granulosa cells of follicles with only a single layer of granulosa cells [33], but it has been argued that they may not be coupled to the adenylate cyclase-second messenger system during early stages of folliculogenesis, and may be non-functional [34]. However, the growth-promoting effect of FSH on preantral and small antral follicles in cattle produced in vitro [35] and in vivo [36,37] suggests a role for FSH in the development of early stage preantral and antral follicles.

If small follicles are responsive to FSH, it is logical to postulate that their developmental dynamics follow a wave-like pattern in response to periodic endogenous surges of FSH. Until recently, reference to a follicular

wave was limited to follicles ≥4 mm, based simply on the limit of resolution of existing ultrasound equipment. At the microscopic level, there is no morphologic distinction between mid- and late-stage antral follicles <4 mm and those ≥4 mm [5,6]. At the cellular level, both size categories of follicles not only express FSH receptors, but have a similar level of expression on a per granulosa cell basis (reviewed in [38]). The periodic emergence of waves of follicles ≥4 mm in response to periodic surges in blood FSH concentrations [17], and the consistency in the number of follicles ≥2 mm [39], or ≥3 mm [40] recruited into successive waves indicates that follicles may become progressively entrained to waves from the earliest stages of development.

6.2. Wave emergence and follicular dominance

The availability of new ultrasound scanners capable of resolving structures as small as 1 mm permitted a study designed to characterize the developmental pattern of 1–3 mm follicles in cattle, and to determine the stage at which the future dominant follicle first attains a size advantage among its cohorts [41]. Results revealed a change over days ($P < 0.05$) in the number of 1–3 mm follicles, with a maximum ($P < 0.05$) 1 or 2 days before conventionally defined wave emergence
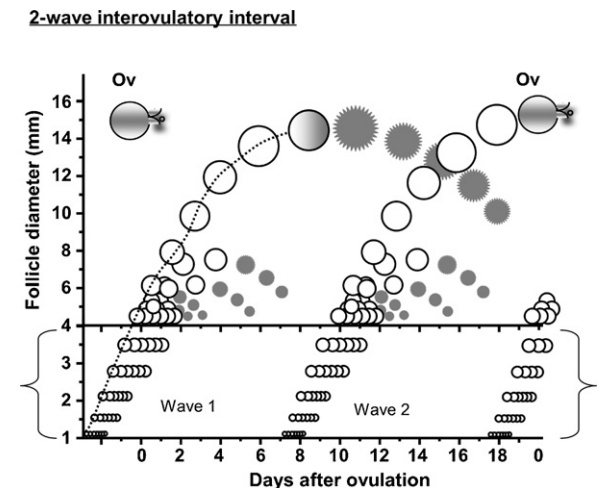

Fig. 2. Ovarian follicular wave pattern detected in follicles as small as 1 mm in diameter (two-wave pattern shown). Small follicles (1–3 mm) in parentheses illustrate wave emergence 2.5 days earlier than previously detected (i.e., at 4–5 mm). Note that the growth rate of the follicle destined to become dominant (dotted line) is similar to others in the wave until about 5 days after wave emergence (beginning at 1 mm), and that the follicle destined to become dominant has a size advantage over those destined to become subordinate at its earliest detection (1 mm).

(dominant follicle first detected at 4 mm), followed 3–4 days later by a maximum ($P < 0.05$) in the number of $\geq$4 mm follicles (Fig. 2). The future dominant follicle was first identified at a diameter of 1 mm and emerged 6–12 h earlier than the first subordinate follicle ($P < 0.01$; Fig. 2). After detection of the dominant follicle at 1 mm (0 h), its diameter was greater than that of the first and second subordinate follicles at 24 h ($P = 0.04$) and 12 h ($P = 0.01$), respectively, when the dominant follicle was 2.4 ± 0.17 mm and 1.7 ± 0.14 mm (Fig. 2). The growth rate of the dominant follicle was greater than that of the first and second subordinate follicles at 120 h ($P = 0.03$) and 108 h ($P = 0.02$), respectively, when the dominant follicle was 9.5 ± 0.30 mm and 8.8 ± 0.49 mm. The authors concluded that: (1) 1–3 mm follicles develop in a wave-like manner in association with surges in plasma concentrations of FSH; (2) 1–3 mm follicles are exquisitely responsive to transient elevations in FSH (i.e., within 6 h); (3) selection of the dominant follicle is manifest earlier than previously documented and is characterized by a hierarchical progression over a period encompassing the entire FSH surge (5 days).

7. Reproductive aging

Endocrine and ovarian characteristics of reproductive aging were characterized in a series of recent studies in which old cows ($\geq$15 year) were compared with their young ($\leq$5 year) daughters [42–44]. Mean circulating FSH concentrations were consistently higher in old cows than in their daughters, and the expected pattern of FSH secretion and wave emergence was maintained in old cows, i.e., each ovarian follicular wave was preceded by a surge in circulating FSH. Despite elevated FSH, fewer 4–5 mm follicles were recruited into each follicular wave in old cows than in their daughters. This interesting inverse relationship between the number of follicles recruited into a wave and the peak concentrations of FSH has also been reported in studies documenting the repeatability of follicles numbers within individuals [39,40].

The two-wave pattern occurred in 60% of the estrous cycles of old cows and the three-wave pattern occurred in the remainder, similar to their daughters [42]. The majority of mother–daughter pairs (six out of nine) had the same wave pattern. The length of interovulatory and interwave intervals did not change with age. The ovulatory follicle of old cows with a two-wave pattern was smaller at the time of ovulation than that of young cows. The diameter of the CL was smaller, and the plasma concentration of progesterone tended to be

lower in old than in young cows. There was no age effect on circulating LH concentrations or LH pulse frequency. The emergence of an additional wave during the IOI resulted in greater follicular attrition in three- than in two-wave patterns [24], providing rationale for the hypothesis that depletion of the follicular reserve and onset of reproductive senescence may occur earlier in individuals exhibiting predominantly three- versus two-wave patterns.

The hypothesis that aging of the hypothalamo–pituitary axis in cattle is associated with a decrease in synchrony of the FSH surge and follicular wave emergence was tested in a recent study involving estradiol/progesterone-based ovarian synchronization [43]. Steroid treatment suppressed circulating FSH in both age groups for 36 h, and the intervals from treatment to subsequent FSH peak (3.7 ± 0.2 days) and wave emergence (4.3 ± 0.3 days) were not different between old and young cows. In a study of the ovarian response to superstimulatory treatment, fewer small (<5 mm) follicles were recruited into the follicular wave, and fewer 6–8 mm, 9–11 mm and $\geq$12 mm follicles developed after ovarian superstimulation in old cows than in their young daughters. On average, young cows had eight more ovulations than old cows.

Results of the latest in the series of studies between young and old cows [44] suggest that fertilization or cleavage rates decline with age. Fewer embryos and a higher proportion of unfertilized oocytes and/or uncleaved zygotes were recovered from old cows compared to their young daughters. This conclusion was supported by the observation that of the total oocytes and embryos recovered per donor, significantly more old cows (10/15; 67%) produced <50% embryos compared to their young daughters (4/16; 25%). The survival of embryos obtained from old cows and their daughters after transfer into young recipients did not differ. As well, pregnancy loss did not differ between embryos transferred from old versus young cows.

8. Summary and conclusions

Wave-like follicle development in cattle is manifest as simultaneous emergence of a group of small (i.e., 1 mm) follicles in both ovaries in response to a surge in circulating FSH concentrations. The largest of the group at its earliest detection (1 mm) usually remains dominant (random distribution in either left or right ovary), but individual follicle growth rates are similar until the dominant follicle reaches 8–9 mm (i.e., 5 days after emergence at 1 mm). Acquisition of LH receptors in granulosa cells of the dominant follicle results in

rapid divergence in growth rates; the dominant follicle continues to grow as the subordinates cease and begin to regress in a milieu of minimal FSH. Growing, static and regressing phases of dominant and subordinate follicles are morphologically distinct (based on histology and ultrasonography) and are reflective of functional status (steroid and protein metabolism and oocyte competence). The vast majority of estrous cycles are composed of two or three follicular waves. Two-wave cycles are consistently shorter (19–20 days) than three-wave cycles (22–23 days). The number of follicles recruited into each wave varies greatly among individuals, but is highly repeatable within individuals. The wave pattern tends to be repeatable within individuals, and duration of dominance of the first wave is predictive of the wave pattern. Reproductive aging in cattle is characterized by an elevation in plasma FSH concentrations, a decrease in the number of follicles recruited into each wave, a lower super-ovulatory response, and a lower oocyte fertilization rate.

Acknowledgements

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