

APPLICATION OF THE BOVINE MODEL FOR THE STUDY OF OVARIAN FUNCTION IN OTHER SPECIES

Gregg P. Adams*

INTRODUCTION

A research model is a general pattern that scientists use as a tool to investigate a general phenomenon. Research models usually imply the use of specific techniques (e.g., ultrasonography) and experimental units (e.g., animals) with which data were originally gathered and patterns were originally defined. Research models offer a conceptual framework upon which specific hypotheses may be formed and tested, and they permit the extension of a concept into new areas (e.g., different species). Good research models are readily accessible (i.e., abundant and inexpensive), malleable (i.e., easy to work with and adaptable), of broad applicability, and lend themselves to quantitative assessment. Ovarian function is perhaps most studied and best understood in the bovine species. Through the use of ultrasound imaging, studies in the bovine species have served as a template for elucidating physiologic mechanisms related to ovarian function and for characterizing reproductive events in many other species, including humans [5]. The following is intended as an overview of the bovine model for studying ovarian function and its remarkable impact on our understanding of the reproductive biology of many other domestic and non-domestic species. Examples of findings in other species are drawn primarily from studies in the author's laboratory, and hence, is not intended as a comprehensive review.

THE BOVINE MODEL

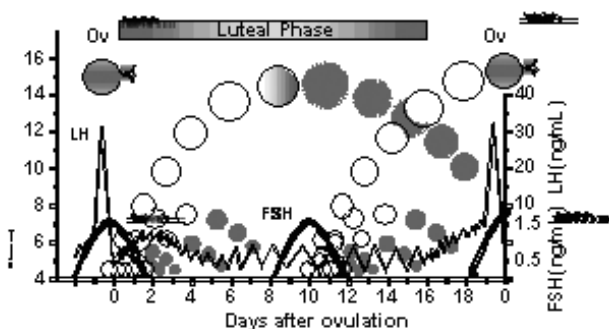
The estrous cycle and its phases in cattle were first described by Hammond in 1927 [27], followed by McNutt in 1928 [44], and Cole in 1930 [20]. In the following decade, Bullough (1946) began the study of the relationship between ovarian follicular development and hormones using a mouse model [18]. Studies of the dynamics of follicular development were first reported in rats by Mandle and Zukerman (1950) [37] and in monkeys by Green and Zukerman (1951) [26]. Both studies involved a histological approach and both concluded that no cyclic variation in follicle numbers existed. Rajakoski [45] has been credited with the initial proposition of the 2-wave theory of follicular growth during the bovine estrous cycle, but for 3 decades after

his report, experiments on follicular dynamics resulted in contradicting accounts of the nature of follicle development during the bovine estrous cycle. In later reviews, the 2-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 5]. 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 1980's, the barrier to our understanding of follicular dynamics was suddenly broken [reviewed in 6, 7 and 54].

Studies using ultrasonic imaging to monitor follicle populations in different size categories or to monitor individually identified follicles [reviewed in 7] 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 to 3 d) growth of eight to 41 small follicles that are initially detected by ultrasonography at a diameter of 3 to 4 mm (Fig. 1) [reviewed in 6,7]. The growth rate is similar among follicles of the wave for about 2 d, when one follicle is selected to continue growth (dominant follicle) while the rest 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 suppresses 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 [2]. The applied implications of these findings were immediate and far-reaching, and marked a new era for ovarian synchronization and superstimulation in cattle [6,14,38].

* DVM, PhD, DACT. Veterinary Biomedical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon Canada S7N 5B4

2-wave interovulatory interval



3-wave interovulatory interval

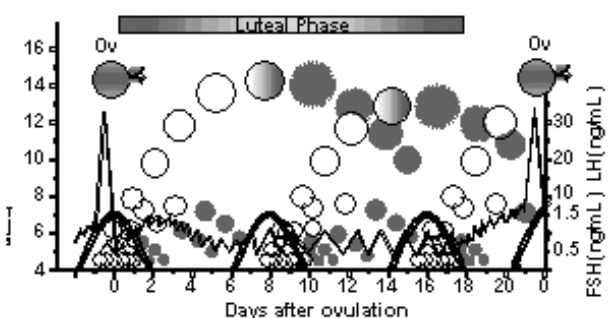


FIG. 1. Dynamics of ovarian follicular development and gonadotropin secretion during 2-wave and 3-wave estrous cycles in cattle. Dominant and subordinate follicles are indicated as open (viable) or shaded (atretic) circles. A surge in circulating concentrations of FSH (thick line) precedes emergence of each wave. A surge in circulating concentrations of LH (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 concentrations of progesterone (i.e., period of luteolysis and luteogenesis, respectively) [8].

2-wave interovulatory interval

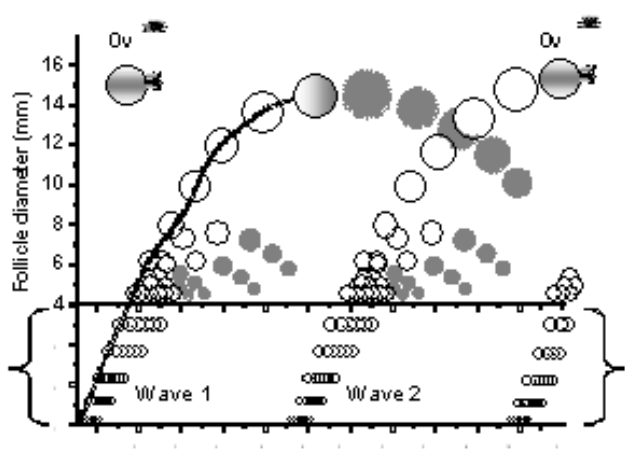


FIG. 2. Ovarian follicular wave pattern detected in follicles as small as 1 mm in diameter (2-wave pattern shown). Small follicles (1 to 3 mm) in parentheses illustrate wave emergence 2.5 d earlier than previously detected (i.e., at 4 to 5 mm). Note that the growth rate of the follicle destined to become 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) [8].

Hormonal interplay: 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 [22], the authors concluded that the dominant follicle suppresses 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 paracrine) 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.

Emergence of a follicular wave and selection of the dominant follicle are associated with a rise and fall in circulating concentrations of FSH (Fig. 1) [2]. 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. Levels rise 1.5- to 2-fold over the next 2 d and peak about 12 to 24 h before emergence of the wave when the future dominant follicle is 4 to 5 mm in diameter. If an existing dominant follicle is removed (i.e., follicular ablation), a surge in FSH begins within next 12 h and results in emergence of a new follicular wave within next 24 h [13]. Selection of the dominant follicle is associated with decreasing levels of FSH in circulation during first 3 d of the wave. The nadir in FSH is reached 4 d after wave emergence and levels remain low for next 2 to 3 d. Receptors for FSH are

present only on granulosa cells while 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. Dominant follicles from both anovulatory and ovulatory waves produce estradiol. Theca cells are required for conversion of progesterone to androgens while the aromatase enzyme for androgen to estradiol conversion 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 d after wave emergence), followed by a 3-fold decrease by the early static phase of the anovulatory dominant follicle (6 d) before returning to base-line in the early regressing phase (11 d) [52]. 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 [6,14,16]. This treatment is the basis of many current protocols for ovulation synchronization for fixed-time artificial insemination 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 [53] 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 [25].

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). Therefore, dominant follicles grow larger and remain dominant for a longer period when LH pulse frequency is elevated (i.e., low progesterone) [3]. Increasing estradiol levels with decreasing progesterone after luteolysis increase the LH pulse frequency further, culminating in a large prevulatory surge.

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- to 3-mm follicles in cattle, and to determine the stage at which the future dominant follicle first attains a size advantage among its cohorts [30]. Results revealed a change over days ($P < 0.05$) in the number of 1 to 3 mm follicles, with a maximum ($P < 0.05$) 1 or 2 d before conventionally defined wave emergence (dominant follicle first detected at 4 mm), followed 3 to 4 d later by a maximum ($P < 0.05$) in the number of ≥ 4 mm follicles (Fig. 2). The future dominant follicle was first identified at a diameter of 1 mm and emerged 6 to 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 to 3 mm follicles develop in a wave-like manner in association with surges in plasma concentrations of FSH, 2) 1 to 3 mm follicles are exquisitely responsive to transient elevations in FSH (i.e., within 6 h), and 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 d).

2-wave versus 3-wave patterns

The majority of bovine estrous cycles (i.e., $> 95\%$) are composed of either two or three follicular waves [reviewed in 7]. Some have reported a preponderance ($> 80\%$) of either the 2-wave pattern or the 3-wave pattern, while others have reported a more even distribution. In both 2- and 3-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 2-wave cycles, and on Day 8 or 9 in 3-wave cycles. In 3-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 2-wave cycles (Day 16) than in 3-wave cycles (Day 19) resulting in a correspondingly shorter estrous cycle (19 to 20 d versus 22 to 23 d, respectively). Hence, the so-called 21-d estrous cycle of cattle exists only as an average between 2- and 3-wave cycles (Fig. 1).

Predictive factors associated with a 2- versus 3-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. In a recent study involving ultrasonographic data from 91 interovulatory intervals [31], 2- and 3-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 d shorter than 3-wave cycles (19.8 ± 0.2 vs 22.5 ± 0.3 ; $P < 0.01$). The majority of cycles ≤ 21 d (88 %) were of the 2-wave pattern ($P < 0.05$), while the majority of cycles ≥ 22 d (78 %) were of the 3-wave pattern ($P < 0.05$). The proportion of serial cycles in which the pattern remained the same (i.e., repeatability) was more than 2-fold greater than the proportion cycles that changed patterns (70 % versus 30 %; $P < 0.01$). The repeatability of wave pattern, and the proportion of 2- versus 3-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 d longer and the onset of regression was later in 2-wave patterns than in 3-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.

Reproductive aging

In a series of recent studies, the bovine model has been established as a valid tool for investigating the process of reproductive aging in humans [34-36]. Endocrine and ovarian characteristics of reproductive aging were characterized by comparing old cows (≥ 15 years) with their young (≤ 5 yr) daughters. Mean circulating FSH concentrations were consistently higher in old cows than in their daughters, as reported in women, 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 to 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 [19,55].

The 2-wave pattern occurred in 60% of the estrous cycles of old cows and the 3-wave pattern occurred in the remainder, similar to their daughters [34]. The majority of mother-daughter pairs (6 out of 9) 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 2-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 versus young cows. There was no age effect on circulating LH concentrations or LH pulse frequency. The emergence of an additional wave during the IOI results in greater follicular attrition in 3- vs. 2-wave patterns [31], and provides rationale for the hypothesis that depletion of the follicular reserve and onset of reproductive senescence may occur earlier in individuals exhibiting predominantly 3- vs. 2-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 study involving estradiol/progesterone-based ovarian synchronization [35]. 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 d) and wave emergence (4.3 ± 0.3 d) 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 to 8 mm, 9 to 11 mm and ≥ 12 mm follicles developed after ovarian superstimulation in old cows than in their young daughters. On average, young cows had 8 more ovulations than old cows.

Results of the latest in the series of studies between young and old cows [36] 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/embryos recovered per donor, significantly more old cows (10/15, 67%) produced $< 50\%$ embryos compared to their young daughters (4/16; 25%). However, the survival rates of embryos obtained from old cows and their daughters after transfer into young recipients did not differ, and pregnancy loss did not differ between embryos transferred from old versus young cows.

FOLLICULAR WAVE STATUS AND SUPERSTIMULATION

The objective of ovarian superstimulatory treatment in cattle is to obtain the maximum number of viable embryos by stimulating growth of antral follicles and ovulation of competent oocytes. However, the variable and unpredictable superovulatory response of the donor animal has remained one of the most limiting factors to successful embryo transfer. Many reports have been published on dosage regimens and types of gonadotropin preparations for ovarian superstimulation, but the results of more recent

ultrasound studies have revealed that the variability in superstimulatory response is primarily associated with i) status of follicular wave development at the time treatment is initiated, and ii) intrinsic number of follicles present at wave emergence within individuals.

Results consistently support the concept that follicular dominance is inversely related to the superstimulatory response. Significantly more large follicles develop and significantly more ovulations are induced when treatment is initiated at the time of wave emergence (before selection of the dominant follicle) than after (reviewed in 6,14,38). In a direct comparison of the superstimulatory response of the first follicular wave of the estrous cycle versus the second, results revealed no differences in the number of ovulations induced or the number of ova/embryos recovered in heifers in which superstimulatory treatments were initiated on the day of emergence of Wave 1 or Wave 2. Hence, an important portion of the variability in superstimulatory response in traditional superstimulatory regimens (initiated 8 to 12 days after estrus) is attributable to variability in wave status at the time treatment is initiated, irrespective of which wave.

The second, and most important contributor to variation in superstimulatory response, is the intrinsic complement of follicles per wave within individual cows. In a recent study [55], cows (n=141) were treated with estradiol and progesterone (1st synchronization) and ranked according to the number of follicles ≥ 2 mm at wave emergence to select the upper and lower 10% of the herd. The high-end and low-end groups were treated with FSH twice daily for 3 days after 2nd synchronization. High-end cows had a significantly greater number of follicles than low-end cows at the time of wave emergence after both the 1st and 2nd synchronizations, and the numbers of follicles at successive wave emergence within individuals were positively correlated. Superstimulatory treatment resulted in more than double the number of large follicles in the high-end group than in the low-end group. Hence, superstimulatory response can be predicted by the number of follicles ≥ 2 mm at wave emergence, and the number of follicles at wave emergence is repeatable within individuals.

OVARIAN SYNCHRONIZATION

The aim of exogenous control regimens is to elicit a desired reproductive status at will, so that diagnostic or interventional procedures can be scheduled to optimize time, labor and results. Past regimens have focused primarily on lengthening or abbreviating the luteal phase through exogenous progestogens or luteolytic agents. However, there is considerable variation in the interval from treatment to estrus and ovulation subsequent to such treatment and much

of the variability has been attributed to the status of the follicular wave at the time of treatment. Studies on the response to a luteolytic dose of prostaglandin given at different times of the estrous cycle indicate that the extant (viable) dominant follicle will ovulate at the time of luteolysis [32]. If luteolysis is induced before the mid-static phase of a dominant follicle (still viable), the follicle will ovulate resulting in a relatively short interval from treatment to ovulation. Conversely, if luteolysis is induced after the mid-static phase of a dominant follicle (defunct), the dominant follicle of the next wave will grow and become the ovulatory follicle, resulting in a longer interval from treatment to ovulation. To reduce the variability of such synchronization schemes, a method of controlling follicular wave emergence is needed.

Follicle ablation

Results of 2 experiments [4,33], in which the dominant follicle was ablated by electrocautery during laparotomy, demonstrated that removal of the dominant follicle hastened the emergence of the next wave. Based on this, transvaginal ultrasound-guided follicle aspiration, as a method of follicle ablation, has been used to induce synchronous wave emergence and ovulation in cattle selected at unknown stages of the estrous cycle [13]. A luteolytic dose of prostaglandin was given 4 days after transvaginal ablation of all follicles ≥ 5 mm in diameter and emergence of a new follicular wave was detected within 2 days of ablation. Although the mean length of the interval from prostaglandin treatment to ovulation was not different between the ablation and control groups (5 days), the variability in the length of the interval was significantly diminished in the former. Addition of LH or GnRH treatment on day 5 after ablation (day 0) plus prostaglandin (day 4) further enhanced ovulation synchrony. Of 23 heifers treated with either LH or GnRH after ablation, all ovulated within the same 24-hour period, and 19 ovulated within the same 12 hour period. Such remarkable synchrony has made transvaginal ultrasound-guided follicle ablation a popular tool for ovarian superstimulation of embryo transfer donors, and synchronization among recipients [reviewed in 38].

Progesterone & Estrogen

It has been known for more than 50 years that administration of progesterone will alter ovarian function in cattle, and in sufficient doses, inhibit ovulation [3]. Exogenous progesterone suppresses the dominant follicle in a dose-dependent manner when given during the growing phase of the follicle, but has no effect on static- or regressing-phase follicles [3]. Exogenous progesterone has no direct effect on plasma FSH concentration, but early demise of growing-phase dominant follicles is followed by an early surge in FSH and early emergence of the next

wave. The suppressive effects of progesterone on the growth of the dominant follicle are mediated by suppression of LH pulse frequency.

In a series of studies, it was shown that estradiol treatment suppressed antral follicle growth [reviewed in 15,38], and suppression was more profound when estradiol was given after insertion of a progestin device. The mechanism of estrogen-induced suppression of follicular growth appears to be a systemic effect, and involves suppression of FSH [16]. Once the estradiol is metabolized, there is an FSH surge, and a new follicular wave emerges. The administration of 5 mg estradiol-17 β (E-17 β) in progestin-implanted cattle was followed consistently by the emergence of a new follicular wave, on average, 4.3 \pm 0.2 days later [15], regardless of the phase of follicular development at the time of treatment.

GnRH OR pLH

Treatment with GnRH or pLH has been used to synchronize follicular wave emergence in cattle, but the synchronizing effect is evident only if ovulation is induced. The ovulation rate in response to GnRH or LH treatment, however, ranged from 22% to 89%, depending on follicular wave status on the day of treatment [39]. In 3 successive experiments [reviewed in 38], GnRH or pLH treatments resulted in fewer ova/embryos than in control animals. Therefore, the use of GnRH or pLH to synchronize follicular wave emergence prior to superstimulation has not been recommended [38].

SHEEP & GOATS

With an approach similar to that used in cattle, and with the understanding derived from the bovine model, later studies on sheep and goats revealed that they too have a wave-like pattern of follicular development during the estrous cycle and during seasonal anestrus. In an initial study involving daily transrectal ultrasonography of Western White Face ewes (Rambouillet x Columbia), the emergence of follicles from a pool of follicles \leq 2 mm was detected on most days of the estrous cycle, but there was a significant increase on Days 2 and 11 (Day 0=ovulation) [47]. Similarly, in another study involving serial ultrasonography of Polypay ewes [24], an organized pattern of development was not detected in follicles that reached only 3 or 4 mm, but follicles that grew to \geq 5 mm emerged at regular intervals during the oestrous cycle, leading authors to conclude that the majority of oestrous cycles consisted of 4 or more follicular waves. The study also revealed a distinct temporal association between the emergence of follicular waves and transient increases in circulating FSH. FSH tended to increase 2 to 3 days before wave emergence and there was close agreement between the number of waves and the number of FSH peaks

during the oestrous cycle (4.1 \pm 0.3 and 4.5 \pm 0.3, respectively) and between the length of the interwave interval and the interval between FSH peaks (4.0 \pm 0.3 days and 3.6 \pm 0.2 days, respectively).

More recent ultrasound data [12,21] confirm a distinct wave-like pattern of follicle development during the estrous cycle in both nonprolific (Western White Face) and prolific (Finn) breeds of sheep. No differences between breeds were found in the pattern of follicular development except that in the Finn, the diameter of dominant follicle was slightly smaller (5.6 \pm 0.2 vs 6.7 \pm 0.2 mm), FSH concentrations were higher around the day of ovulation, the dominant follicle from the penultimate wave of the cycle often ovulated along with that of the ultimate wave, and the ovulation rate was greater (2.7 \pm 0.2 vs 1.8 \pm 0.2). Consistent with the previous study, the number of follicular waves and the number of FSH peaks per cycle did not differ (3.7 \pm 0.2 and 3.8 \pm 0.1), and the interwave interval was highly correlated with the interpeak interval in FSH. In addition, the number of peaks in circulating estradiol concentration did not differ from the number of follicular waves per cycle (3.5 \pm 0.2 and 3.8 \pm 0.1), and the interwave interval was highly correlated with the interval between estradiol peaks. Similar findings have been reported for goats [23] wherein the predominant pattern (75%) consisted of 4 follicular waves emerging at 3- to 4-day intervals during a 23-day estrous cycle. The relationship between FSH and follicular development has apparently not been investigated in goats.

Few changes were noted in the characteristics of follicular waves from the beginning to the end of the anovulatory season in ewes (March to July) [11]. Similar to the ovulatory season, periodic fluctuations in FSH were associated with regular wave emergence throughout the anovulatory season. Circulating LH concentrations were suppressed throughout the anovulatory season, and no differences were detected in maximum follicle diameter, interwave interval or circulating concentrations of FSH during successive follicular waves of the anovulatory period. However, the number of follicles 2 to 4 mm in diameter increased during the early portion of the anovulatory period, only to decrease again to previous levels later in the season [11,48].

A confounding aspect of studying follicular dynamics in sheep and goats is the apparent difference in the nature or magnitude of follicle dominance compared with that of cattle. In sheep, the "wave" pattern has been detected only in follicles destined to grow to \geq 5mm; consequently, very few follicles (i.e., 1 to 3 per wave) are detectable for characterizing the wave pattern, complete with follicle selection and dominance. Indeed, there is some controversy about whether follicle dominance exists in sheep [49], and

if it does, it is certainly less distinct than in cattle. However, the following observations support the notion of the dominance phenomenon in sheep, particularly during the first and last waves of the cycle: 1) emergence of follicular waves associated with a follicle clearly larger than all others were detected during metestrus and pro-estrus in sheep [17,24,47] and goats [23], 2) following prostaglandin-induced luteolysis on various days of the estrous cycle, the proportion of ewes that ovulated the largest follicle at the time of treatment and the interval to estrus varied relative to the day of treatment [29], and 3) follicular and ovulatory responses to superstimulatory gonadotropin treatment were influenced by the status of the follicular wave at the time of treatment, and the presence of a large growing follicle at the time treatment was initiated was associated with lower follicle recruitment, fewer ovulations, and fewer embryos [50]. The latter observations are consistent with those made in cattle where variation in the ovulatory response to prostaglandin treatment and ovarian superstimulation have been attributed directly to the status of follicular dominance at the time treatment was initiated [reviewed in 7,51].

WILD RUMINANTS

Muskoxen

In the first detailed study of ovarian follicular dynamics in a wild species, daily transrectal ultrasonography was conducted on a group of 4 captive muskoxen. Follicular waves were apparent during both the ovulatory [28] and anovulatory seasons (Parker & Adams, unpublished). Only 1 wave was detected during the first (short) cycle of the ovulatory season. During the second (long) cycle of the ovulatory season, 1 musk ox had 3 waves and the remaining 3 animals had 4 waves. Only the dominant follicle of the last wave of the estrous cycle ovulated; the dominant follicle of other waves regressed slowly over a period of a few days. Dominance was clearly manifest in the first and last follicular waves of the oestrous cycle in each of the 4 animals (major waves), whereas the other waves in all but one instance appeared to be minor waves. Wave characteristics and indistinct follicular dominance during diestrus are remarkably similar to that observed in sheep and goats, species to which the musk ox is most closely related. Insight of this kind is important in the design of appropriate artificial breeding systems and in this respect, the muskox may provide a useful model for the endangered takin (*Budorcas taxicolor*). A detailed knowledge of ovarian events may also be critical to the interpretation of the response of wild populations to environmental stress.

Cervids

Using the bovine model as a template, a series of studies was completed recently in the author's

laboratory on the annual pattern of ovarian dynamics in North American elk (wapiti, *Cervus elaphus*), a close relative of the European red deer. In one study [41], the reproductive tract of 13 mature hinds was examined daily by transrectal ultrasonography and blood samples were taken daily between October and January to characterize follicular, luteal, and endocrine dynamics during the breeding season. The pattern was remarkably similar to that of cattle (Fig. 3). Follicle development occurred in waves characterized by regular, synchronous development of a group of follicles in temporal succession to a surge in serum FSH concentration. The mean interovulatory interval was 21.3 ± 0.1 d, but was shorter in hinds exhibiting 2 follicular waves (20 days) than in hinds exhibiting 3 (22 days) and 4 waves (23 days; $P < 0.05$). The interwave interval in 2-wave cycles and between the first and second wave of 3-wave cycles was similar (9 to 10 days). All other interwave intervals in 3- and 4-wave cycles were shorter ($P < 0.05$). The follicle destined to become dominant (selection) was larger ($P < 0.05$) than the largest subordinate follicle one day after emergence, which coincided with the first significant decrease in serum FSH concentration. An inverse relationship was detected between the number of waves and the magnitude of follicular dominance (diameter and duration of the dominant follicle).

In another study [42], transitions from the anovulatory to the ovulatory season ($n = 20$) and from the ovulatory to anovulatory season ($n = 11$), were monitored daily by transrectal ultrasonography in wapiti. The first interovulatory interval was short (9.1 ± 0.3 d) compared with later in the ovulatory season (21.3 ± 0.1) and the last interovulatory interval of the season (21.2 ± 0.6 d). The short cycle was composed of only 1 wave of follicular development whereas subsequent cycles were composed of 2 or 3 waves. Multiple ovulations were detected at the onset of the ovulatory season, but not later. The characteristics of the last IOI of the ovulatory season were similar to those of previous cycles, and transition to anovulation was simply marked by a failure of the dominant follicle to ovulate after a typical luteal phase. The anovulatory season was characterized by continuous, regular emergence of anovulatory follicular waves [40].

Based on protocols developed in the bovine model, and on data gathered previously in wapiti, studies were done to test ovarian synchronization [43] and ovarian superstimulation schemes (McCorkell and Adams, unpublished). Both steroid treatment (i.e., estradiol plus progesterone) and transvaginal ultrasound-guided follicle ablation were effective for synchronizing wave emergence. Successful ovarian superstimulation (average, 12 ovulations per hind) was attributed to effective wave synchronization at the beginning of treatment. Compared to conventional

methods that require 14 days and handling the hinds 6 times, the synchronization protocol reduced the treatment period to 8 days and the number of animal handlings to 4.

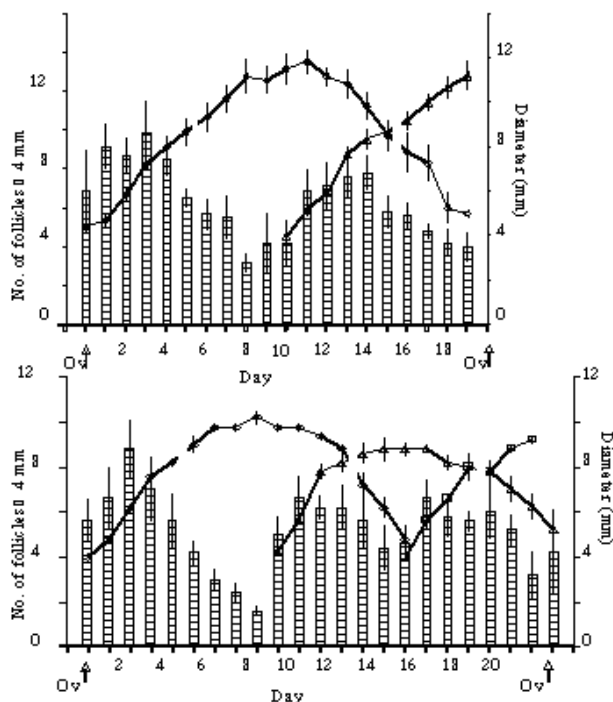


FIG. 3. Follicle and luteal dynamics (mean \pm SEM) in wapiti (North American elk; *Cervus elaphus*) with 2 follicular waves (top, $n = 6$) and 3 follicular waves (bottom, $n = 5$) during the interovulatory interval in the breeding season. The number of follicles ≥ 4 mm in both ovaries are indicated by bars (left axis) and diameter profiles of successive dominant follicles are indicated by lines (right axis). ^{ab}Among days, values with different superscripts are different ($P < 0.05$) [41].

Bison

Studies are currently underway on the annual reproductive pattern of North American bison, using the approach established in the bovine model (McCorkell and Adams, unpublished). The pattern of follicle and luteal development is remarkably similar to that of cattle. The estrous cycle is composed of 2 or 3 follicular waves and is 20 and 22 days long, respectively. Like wapiti, bison are seasonally polyestrous, and the first interovulatory interval of the breeding season is abbreviated (mean, 8 days) and composed of only 1 follicular wave. Critical characterization of ovarian function during successive seasons will enable development of rational synchronization and superstimulation protocols, based on those developed in the bovine model.

CAMELIDS

As induced ovulators, three naturally occurring reproductive statuses exist in llamas and alpacas: 1) nonovulatory, 2) ovulatory but not pregnant, and 3) pregnant. In a study involving ultrasonographic examination of llamas ($n=41$; Fig. 4) daily for a period of ≥ 60 days [1], ovarian follicle development was found to follow a wave-like pattern regardless of reproductive status (nonovulatory, ovulatory nonpregnant, or pregnant) or lactational status (lactating, non-lactating). If ovulation is not induced, the dominant follicle eventually regresses as well, and a new wave emerges so that the ovarian "cycle" repeats itself (Fig. 4). The interval between emergence of successive waves of follicles was longer in nonpregnant animals (20 days) than in pregnant animals (15 days), and lactation was associated with a 2.5 day abbreviation in the interwave interval. Maximum diameter of nonovulatory dominant follicles ranged from 9 to 16 mm and was greater, on average, in nonpregnant animals (12 mm) than in pregnant animals (10 mm). Dominant follicles of successive waves are equally as likely to develop in the ipsilateral as contralateral ovary; i.e., they do not regularly alternate between ovaries [1,57]. Recent ultrasonographic study also documented the wave-pattern of follicle development in alpacas [57]. The mean (\pm sem) interwave interval was 15.4 ± 0.5 days and ranged from 12 to 22 days. The authors concluded that the optimal time of mating might be predicted in alpacas, provided that the emergence of ovarian follicular waves was controlled. The wave-like pattern of follicular development has also been documented in dromedary camels[56], and wave characteristics are remarkably similar to those of llamas. As in llamas, distinct follicular dominance was manifest by a strong inverse relationship between the number of follicles detected and the diameter of the largest follicle. The interwave interval for unmated camels was 18.2 days.

Applied implications of the endogenous ovarian rhythm involve the timing and control of the follicular wave pattern, induction of ovulation, and control of the luteal phase. At any given time, one may expect to find a follicle of ≥ 6 mm in one of the ovaries, but to determine whether the follicle is growing (viable) or regressing (dying) would require more than one examination. Such a determination is of importance for breeding management since an immature follicle (< 6 mm) or over-mature (regressing) follicle are not be capable of ovulation and normal luteal development subsequent to copulation. Based on the bovine model, a recent study was designed to determine the effects of steroids (estradiol plus progesterone), gonadotropin (LH), and ultrasound-guided follicular ablation on follicular wave dynamics in lactating and non-lactating llamas, and to determine the effects of these treatments on pregnancy rates after fixed-time natural mating [46]. The intervals from treatment to

follicular wave emergence and to the day on which the new dominant follicle reached 7 mm (large enough to ovulate), respectively, did not differ between the LH (2.1 ± 0.3 days and 5.2 ± 0.5 days, respectively) and follicle ablation groups (2.3 ± 0.3 days and 5.0 ± 0.5 days), but both were shorter and less variable than in the control group (5.5 ± 1.0 days and 8.4 ± 2.0 days), while the E/P group (4.5 ± 0.8 days and 7.7 ± 0.5 days) was intermediate. A single, fixed-time natural mating was permitted 10 to 12 days after treatment and although ovulation rates did not differ among groups (Control, 93%; E/P, 90%; LH, 90%), the pregnancy rate was higher for synchronized llamas (76%) than for non-synchronized llamas (54%). The results clearly demonstrate that follicular wave emergence can be induced electively, and animals can be synchronized sufficiently to permit fixed-time insemination without the necessity of testing behavioral receptivity.

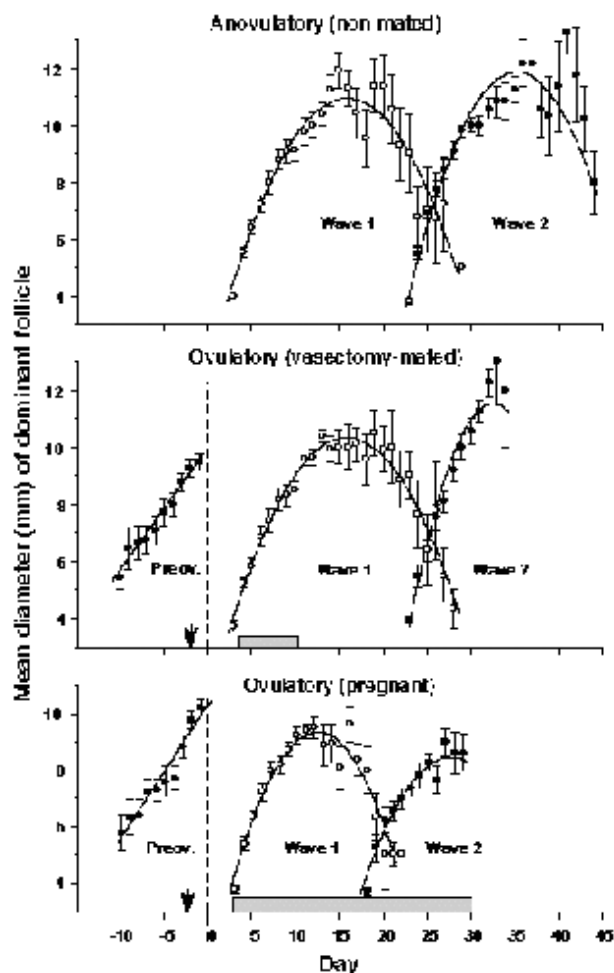


FIG. 4. Mean (\pm s.e.m.) diameter of the dominant follicle for anovulatory, ovulatory non-pregnant, and ovulatory pregnant llamas. The arrow indicates the mean day of mating (ovulation = day 0) and the shaded bars indicate the days of detection of the corpus luteum for the ovulatory groups [1].

WOMEN

A wave phenomenon of ovarian follicular development in women was recently documented in our laboratory [9,10] using an approach based on the bovine model [5]. The ovaries of 50 women with clinically normal menstrual cycles were examined daily using transvaginal ultrasonography for one IOI. Profiles of the diameters of all follicles ≥ 4 mm and the numbers of follicles ≥ 5 mm were graphed during the IOI. Major waves were defined as those in which one follicle grew to ≥ 10 mm and exceeded all other follicles by ≥ 2 mm. Minor waves were defined as those in which follicles developed to a diameter of < 10 mm and follicle dominance was not manifest. Blood samples were drawn to measure serum concentrations of estradiol-17 β , LH, and FSH. Women exhibited major and minor patterns of follicular wave dynamics during the IOI (Fig. 5). Of the 50 women evaluated, 29/34 women with two follicle waves (85.3%) exhibited a minor-major wave pattern of follicle development and 5 women (14.7%) exhibited a major-major wave pattern. Ten of the 16 women with three follicle waves (62.5%) exhibited a minor-minor-major wave pattern, 3 women (18.8%) exhibited a minor-major-major wave pattern, and 3 women (18.8%) exhibited a major-major-major wave pattern (Fig. 5). Documentation of major and minor follicular waves during the menstrual cycle challenges the traditional theory that a single cohort of antral follicles grows only during the follicular phase of the menstrual cycle. Results of these groundbreaking studies are revolutionizing the design of ovulation induction protocols for couples suffering from infertility, and oral contraceptive protocols.

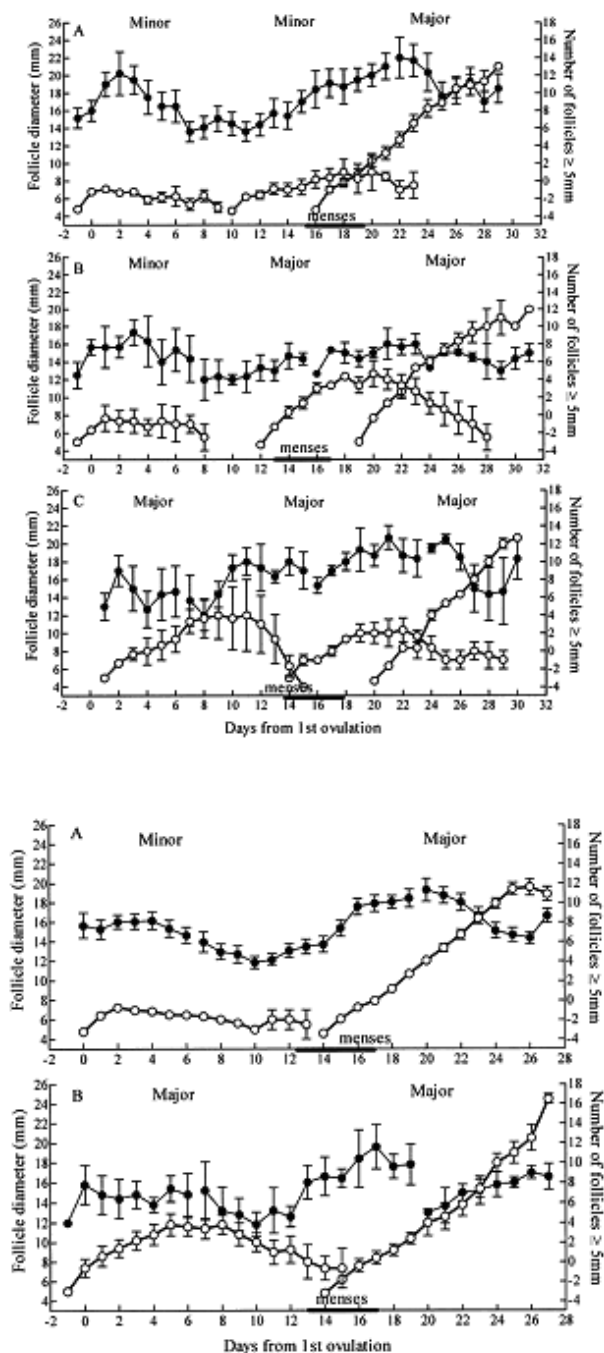


FIG. 5. Ovarian follicular dynamics in women with 2 ($n=34$; left) or 3 ($n=16$; right) waves of follicular development during the menstrual cycle. Solid circles represent follicle numbers, and open circles represent the diameter profile of the largest follicle of each wave. Major waves were defined as those with a distinct dominant follicle, and minor waves were those with no distinct dominant follicle [10].

CONCLUSIONS

The bovine model has had perhaps the greatest influence on our understanding of ovarian function among monovular species. The model has provided a contextual framework from which studies in other species have been designed and conducted. In all monovular species examined to date, follicle dynamics follow a wave-like pattern. Test of hypotheses regarding follicular wave control have provide new insight into the physiologic phenomena of follicle recruitment, selection and follicular dominance, follicular attrition, and reproductive senescence. From comparative study of follicular dynamics among species, some consistent patterns have emerged – such conservation among species provides confidence in our model and increases the power of our observations. Follicular dominance is one example. The suppressive influence of progesterone on dominant follicle growth and wave dynamics was first postulated based on studies in camelids, which have a naturally occurring progesterone-free “cycle”. The magnitude of the dominant follicle profile was greatest, and the interwave interval was longest in the absence of a CL. This, and the observation in cattle that the dominant follicle that develops during metestrus (i.e., low-progesterone) grows to a greater diameter than dominant follicles that develop during diestrus (i.e., second anovulatory wave in 3-wave cycles) or during pregnancy, led to the discovery that progesterone suppresses the dominant follicle during the growing phase in a dose-dependent manner through suppression of LH pulse frequency. Similarly, the prepubertal increase in LH secretion in calves was associated with a progressive increase in the diameter profile of dominant follicles of successive waves and an increase in the interwave interval as the first ovulation approached. In wapiti, the magnitude of follicular dominance differed among seasons and among 2- vs 3-wave patterns, as reflected in the diameter of the dominant follicle and the interwave interval. The maximum diameter of dominant follicle of successive anovulatory waves during the non-ovulatory season was smaller than that of the ovulatory season, (9 mm vs 10 to 12 mm) and the interwave interval was shorter (7 days vs 9 to 10 days). The phenomenon of short, 1-wave cycles at the beginning of the ovulatory season is common to seasonal transition in sheep, musk ox, wapiti, and bison, as well as to pubertal transition in cattle. These are but a few concepts that have arisen from comparative study of ovarian function – concepts that have led to new and effective synchronization schemes for fixed-time insemination and for ovarian superstimulation for in vivo or in vitro embryo production.

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