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EXOGENOUS CONTROL OF FOLLICULAR WAVE EMERGENCE IN CATTLE

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ABSTRACT

Variability in ovarian response to superstimulatory treatments and in the interval from $PGF_{2\alpha}$ treatment to estrus in cattle is largely attributable to the status of follicular wave development at the time of treatment. To date, most treatments designed to control follicular wave development have been based on removal of the suppressive effect of the dominant follicle, either physically (by electrocauterization or ultrasound-guided follicle ablation) or hormonally (by GnRH or estradiol and progestogen treatment), and thereby induce the emergence of a new follicular wave at a specific time after treatment. Treatment of progestogen-implanted cattle with estradiol-17β (E-17β) resulted in suppression of the dominant follicle and emergence of a new follicular wave 4.3 ±0.1 d later. Superstimulatory treatments initiated 4 d after E-17β treatment in progestogen-implanted cattle resulted in a superovulatory response comparable to that of cattle in which superstimulatory treatments were initiated on the second follicular wave. In another study, induced follicular wave emergence, regardless of the stage of the estrous cycle, resulted in similar superovulatory response and higher fertilization rates in heifers than when superstimulatory treatments were initiated 8 to 12 d after estrus (traditional approach). Finally, estrus synchronization treatments with E-17β plus progesterone and $PGF_{2\alpha}$ have resulted in synchronous estrus and ovulation. Overall, it appears that treatment with E-17β and progestogen in combination may be used to effectively control and synchronize follicular wave development and may have important implications in artificial control of ovarian cyclicity and superovulation.

INTRODUCTION

Ovarian asynchrony and variability in response to treatments remain the most limiting factors to the widespread implementation of advanced reproductive technologies in cattle, despite considerable progress in recent years (5,7,34). The status of follicular wave development is responsible for a large portion of the variability in ovarian response to superstimulatory

Key words: bovine, follicular wave emergence, estradiol, superovulation, estrus synchronization

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treatments and in the interval from $PGF_{2\alpha}$ treatment to estrus (26,34). The advent of real-time ultrasonography has provided a means to determine follicular status prior to gonadotropin treatments. However, it is difficult to precisely determine the status of follicular development on the basis of a single ultrasonographic examination and it is often not practical to serially examine individual animals in a commercial setting. An alternative approach is to control the development of a follicular wave so that superstimulatory treatments may be initiated at the most favourable time, i.e. when the number of follicles capable of responding to exogenous gonadotropins is maximal. Similarly, estrus synchronization programs may be adapted so that induction of luteolysis is coincident with the presence of a growing dominant follicle. The purpose of this paper is to address theoretical and practical aspects of bovine follicular development with respect to artificial control of follicular wave emergence and its application in superovulation and estrus synchronization.

FOLLICULAR WAVE DYNAMICS IN CATTLE

Ovarian follicular development in cattle is a dynamic sequence of organized events which has been described as wave-like (29,38,43). A wave of follicular development has been defined as a synchronous development of a large number of follicles 4 to 5 mm in diameter, followed by selection and growth of the dominant follicle and suppression of the subordinates (20,21). Most bovine estrous cycles have 2 or 3 follicular waves. Wave emergence was detected, on average, on the day of ovulation (Day 0) and Day 10 for two-wave cycles and on Days 0, 9 and 16 for three-wave cycles (20,21). However, there was a great variation in the proportion of animals exhibiting 2 or 3 waves per cycle and in the day of wave emergence, particularly of Wave 2. The genetic and environmental factors which influence this variability have not been clearly defined.

The dominant follicle of a follicular wave becomes larger than the other follicles and, through the production of steroidal and non-steroidal substances, suppresses the development of subordinate follicles and prevents the emergence of the next follicular wave (4,27,28). It has been recently shown that the apparent time of selection of the dominant follicle was coincident with a significant decrease in FSH concentrations (3), whereas, the emergence of a follicular wave is preceded by an increase in FSH concentrations, 1 or 2 d earlier (2). Determination of ovarian factors which inhibit gonadotropin secretion or interfere with folliculogenesis is important in the development of new approaches to the exogenous control of follicular development.

SYNCHRONIZATION OF FOLLICULAR WAVE DEVELOPMENT

There are several possible ways to synchronize follicular development. Most investigations have focused on removal of the suppressive effect of the dominant follicle (physically or hormonally) to allow the emergence of a new follicular wave at a specific time after treatment. The basis of this theory was derived from studies in which cauterization of the dominant follicle or its suppression with steroid-free bovine follicular fluid during the growing phase was followed by a premature FSH release and emergence of a new follicular wave (2,27,28).

Follicle Ablation

Studies in which the dominant follicle was electrocauterized showed that removal of the dominant follicle during its growing phase hastened the emergence of the next follicular wave (4,28). Based on these observations, a study was designed to test the hypothesis that ultrasound-guided follicle aspiration (used for oocyte retrieval in IVF programs), as a method of follicle ablation, would induce the synchronous emergence of a new follicular wave and synchronous ovulation after treatment with $PGF_{2\alpha}$ in heifers (9). Follicle ablation consisted of aspiration of all follicles $\geq$5 mm in diameter in heifers at unknown stages of the estrous cycle; all heifers (ablation and control group) received $PGF_{2\alpha}$ 4 d later. Daily ultrasonography revealed that follicle ablation resulted in a synchronous emergence of a new follicular wave 1.5 d later, and tightly synchronized ovulation after $PGF_{2\alpha}$ treatment. Ultrasound-guided transvaginal follicle ablation may be a useful method of manipulating follicular wave dynamics for estrus synchronization or oocyte retrieval and has been shown to be useful for superstimulation (17).

Hormonal Treatments

The ablation procedure was directed at removing the suppressive effect of the dominant follicle on the emergence of a new wave. Hormonal approaches have been directed at causing luteinization or atresia of the follicles present at the time of treatment. This has been accomplished by using hCG (40) or GnRH analogs (31,40) to induce follicle luteinization or ovulation, or by progestogens and estradiol to cause atresia of the dominant follicle (12).

Results of recent ultrasonographic and histologic studies have shown that treatment with a GnRH analog causes antral follicles to undergo atresia or induces ovulation and subsequent formation of a new CL (47). A new dominant follicle was identified ultrasonographically (by retrospective analysis) within 3 to 4 d after GnRH treatment and this follicle became the ovulatory follicle after $PGF_{2\alpha}$-induced luteolysis (6 d after GnRH treatment; 47). In another study, GnRH treatment followed by prostaglandin 7 d later increased the number of heifers showing estrus within a 5-d period, and enhanced precision of synchrony 2 to 3 d after $PGF_{2\alpha}$ compared to heifers receiving $PGF_{2\alpha}$ alone (45). A 6- or 7-d interval between GnRH and $PGF_{2\alpha}$ resulted in a high degree of estrus synchronization with pregnancy rates comparable to those after treatment with 2 injections of $PGF_{2\alpha}$ 11 d apart (23,45,47).

Progestogen and Estrogen Treatments

Steroid hormones have also been used to alter follicle growth. Exogenous progesterone has been shown to suppress follicle growth in a dose-dependent manner (1,18,44) and estradiol has been shown to induce follicle atresia (19). The combined effects of estradiol and progestogens on follicular wave dynamics in cattle was inferred from preliminary studies designed to investigate the use of estradiol valerate (EV) and a progestogen ear implant[a] in a superstimulatory regimen in beef cows (10). Ultrasonographic examinations revealed that EV treatment at the time of implant insertion (2 d after estrus) was associated with a reduction in the mean diameter of the largest and second largest follicles over a 5-d period followed by an increase in follicle

[a] Syncro-Mate-B, Sanofi Inc., Overland Park., KS, U.S.A.

diameters, presumably from a new follicular wave. In a subsequent study (11), 5 mg EV given during the early growing phase (Day 1; ovulation=Day 0) suppressed the dominant follicle of the first follicular wave and resulted in early emergence of the next follicular wave in heifers without progestogen implants. However, treatment at the mid- (Day 3) or late-(Day 6) growing phase resulted in a delayed emergence of the next wave. This was attributed to an incomplete suppression of the dominant follicle combined with a prolonged effect of the valerate form of estradiol.

Because of the potential adverse effects of the prolonged action of EV, a series of experiments were designed to evaluate the effects of a shorter acting estrogen, estradiol-17β (E-17β). The first experiment was designed to test the hypothesis that E-17β more effectively suppresses the dominant follicle when administered in combination with a progestogen ear implant than when given alone (12). In heifers treated with E-17β plus progestogen ear implants, the dominant follicle ceased to grow 1 d after E-17β treatment and subsequently regressed, resulting in an early emergence of the next follicular wave (Day 5.2 ±0.2). Conversely, E-17β administration to heifers without progestogen implants did not effectively suppress the dominant follicle and emergence of the next wave was delayed (Day 9.8 ±1.1). In this study, the effect of E-17β and progestogen on gonadotropins also was evaluated and results clearly showed that estradiol-induced LH release was not associated with follicle suppression. A post-treatment LH surge was detected in 5 of 6 heifers treated with E-17β alone, and the dominant follicle was not suppressed, whereas heifers with progestogen implants did not exhibit a LH surge after E-17β treatment and the dominant follicle regressed. Furthermore, in the heifers with progestogen implants plasma FSH concentrations decreased by 6 h after E-17β treatment and increased gradually over a period of 24 to 42 h, whereas in heifers treated with E-17β alone FSH decreased and then increased dramatically 12 h later, in association with the LH surge. It was concluded that E-17β was more effective in inducing follicle suppression when combined with a progestogen ear implant. Also, this study provided the rationale for the hypothesis that the suppressive effect of E-17β and progestogen in combination was due to suppression of both FSH and LH secretion, and that gonadotropin suppression must persist for at least 24 h to elicit complete follicle regression. In a second experiment designed to determine an effective dosage regimen of E-17β for suppression of follicular growth in progestogen-implanted heifers, a single dose of 5 mg E-17β was as effective as higher or repeated doses in inducing follicular suppression and resulted in consistent emergence of a new follicular wave 3 to 5 d later (12).

A third study evaluated the efficacy of E-17β and progestogen treatment in synchronizing wave emergence when treatments were given at different stages of dominant follicle development (13). Beef cows and heifers were allocated into 4 treatment groups: untreated control animals and those that were given progestogen implants on Day 2, 5 or 8 and injected with 5 mg E-17β im on Day 3, 6 or 9, respectively. Treatment days were expected to coincide with mid-growing phase (Day 3), late growing/early static phase (Day 6) and late static phase (Day 9) of the dominant follicle of the first wave (21). Day 9 was also expected to coincide with the emergence of the second follicular wave (12). The hypothesis that E-17β plus progestogen treatment results in synchronous emergence of a new follicular wave was supported. Estradiol-17β treatment resulted in an early emergence of the second follicular wave in heifers treated on Day 3 (Day 7.3 ±0.3) and delayed emergence in those treated on Day 6 (Day 10.7 ±0.2) compared to the control group (Day 8.6 ±0.3; P<0.05). Additionally, treatment on Day 9 hastened emergence of the third wave (Day 13.1 ±0.3 vs Day 15.3 ±0.6 for Day 9 and control groups, respectively). The net result was that the interval from treatment to wave emergence was not different among E-17β groups and occurred, on average, 4.3 days after E-17β treatment. These results were consistent with those of the previous experiments (11) and are summarized in Table 1. Combined among experiments, the mean interval from E-17β treatment to follicular wave emergence in 47 animals was 4.3 ±0.1 d, and occurred 3 to 5 d after E-17β treatment in 44 of 47 (94%) heifers and cows treated. We concluded that treatment with E-17β and progestogen in combination can be used effectively to control and synchronize follicular wave emergence and may have important implications in artificial control of ovarian cyclicity and superovulation.

Table 1. Interval from estradiol-17β treatment to the emergence of the next follicular wave in heifers and cows with a progestogen ear implant (3 experiments combined; 11,12).

	5 mg Estradiol-17β			
	Day 1	Day 3	Day 6	Day 9
n	13	15	9	9
Wave Emergence (day)	4.5 ±0.2	4.0 ±0.3	4.6 ±0.2	4.1 ±0.3
(range)	(4 to 5)	(3 to 5)	(4 to 6)	(3 to 5)

Means among groups did not differ.

SYNCHRONIZED FOLLICULAR WAVE EMERGENCE AND SUPERSTIMULATION

Although there have been many reports on dosage regimens and types of gonadotropin preparations for ovarian superstimulation (34), it has been proposed that most of the variability in ovarian response to superstimulatory treatments in cattle is associated with variations in the status of follicular development at the time of treatment (5,7,34,35). In this regard, fewer ovulations were reported when superstimulatory treatments were initiated in the presence of a dominant follicle (17,22,25) or after selection of the dominant follicle (3,39). A higher superovulatory response has been reported when large numbers of small follicles were present at the time superstimulatory treatments were initiated (41). Two recent studies evaluated the responsiveness to superstimulatory treatments initiated with specific regard to follicular wave emergence (6,36). Results demonstrated that treatments initiated on the day before or the day of wave emergence resulted in a higher ovulatory response than treatments initiated 1 or 2 d later. Collectively, results clearly indicate that superstimulatory treatments must be initiated at the time of the endogenous pre-wave FSH surge or wave emergence (before dominant follicle selection) to obtain maximum superovulatory response in a given animal.

Two experiments were designed to determine the superovulatory response following artificially induced follicular wave emergence in cattle. The first experiment tested the hypothesis that E-17β plus progestogen treatment will induce a synchronized crop of follicles as responsive to exogenous gonadotropins as those of the second (spontaneous) follicular wave of the cycle. Beef cows either received progestogen ear implants on Day 0 (ovulation) plus 5 mg of E-17β im on Day 1 and were superstimulated on Day 5 or were not implanted (control) and superstimulated on Day 8 (expected emergence of the second follicular wave; 11). Superstimulatory treatments consisted of 400 mg NIH-FSH-P1 of Folltropin-V[b] administered by either a single sc injection behind the shoulder or divided into twice daily im injections over 4 d. Forty-eight hours after superstimulatory treatments were initiated, 500 µg cloprostenol[c] was injected im and implants were removed 12 h later. Animals were inseminated 60 and 72 h after cloprostenol and slaughtered 7 d later for ova/embryo collection and CL counts. Results confirmed the hypothesis that E-17β treatment of progestogen-implanted cattle will induce a new wave of follicles as responsive to gonadotropin treatments as those of the second follicular wave (Table 2).

Table 2. Response (mean ±SEM) of control cows superstimulated with Folltropin-V on Day 8 after ovulation (Day 0) or cows that were given a progestogen ear implant on Day 0, 5 mg estradiol-17β (E-17β) on Day 1 and superstimulated on Day 5.

	Control		Progestogen plus E-17β	
	Single sc injection	Twice daily im injections	Single sc injection	Twice daily im injections
n	18	16	19	18
CL	22.0 ±3.5	23.7 ±3.7	27.0 ±3.1	16.6 ±3.4
Total ova/embryos	14.2 ±2.5	14.1 ±2.7	13.2 ±1.4	10.6 ±1.8
Fertilized ova	9.2 ±2.0	8.7 ±1.7	9.1 ±1.2	7.6 ±1.7
% Fertilized ova	65	62	69	72
Transferable embryos	5.4 ±1.5	5.5 ±1.7	5.4 ±1.0	3.9 ±1.5
% Transferable embryos	37	39	41	37

Means and percentages were not different

A second experiment was designed to compare superovulatory response following induced synchronized follicle growth by hormonal or physical treatments with a control group of beef heifers. The control group represented the traditional approach with heifers superstimulated between 8 and 12 d after observed estrus. Synchronized wave emergence was induced at unknown stages of the estrous cycle by a progestogen implant plus 5 mg of E-17β 1 d later (progestogen plus E-17β) or by ablating all follicles ≥5 mm in diameter using ultrasound-guided transvaginal follicle aspiration. An additional group of heifers treated with a progestogen ear implant alone also was included (progestogen alone). Superstimulation of the treated groups was initiated 5 d after progestogen implant insertion (4 d after E-17β) or 1.5 d after follicle ablation. Superstimulatory treatments consisted of 400 mg NIH-FSH-P1 Folltropin-V given by a single sc injection behind the shoulder. Cloprostenol treatments, implant removal, AI and ova/embryo collections were done as in Experiment 1. There were no differences among groups in the number of CL and total number of ova/embryos collected (Table 3). However, treatment with progestogen plus E-17β or follicle ablation resulted in a higher fertilization rate than the other treatment groups. Furthermore, the percentage of transferable embryos was higher in heifers treated with progestogen plus E-17β than those treated with progestogen alone.

[b] Vetrepharm Canada Inc., London, ON, Canada.

[c] Estrumate, Coopers Agropharm Inc., Ajax, ON, Canada.

Table 3. Response (mean ±SEM) of beef heifers superstimulated between 8 and 12 d after estrus (control) or following induced synchronized follicle growth with progestogen plus estradiol-17β (E-17β) or follicle ablation at unknown stages of the estrous cycle.

	Control	Progestogen plus E-17β	Progestogen alone	Ablation
n	18	19	19	20
CL	25.4 ±5.3	28.8 ±3.5	24.3 ±3.5	17.2 ±2.4
Total ova/embryos	8.9 ±1.5	13.2 ±2.1	12.4 ±2.2	7.8 ±1.3
Fertilized ova	6.0 ±1.1^{a}	11.2 ±2.0^{b}	7.5 ±1.9^{ab}	6.6 ±1.2
% Fertilized ova	68$^{a\ x}$	84^{b}	60^{a}	85^{y}
Transferable embryos	3.9 ±0.8	6.6 ±1.8	4.4 ±1.6	3.8 ±0.8
% Transferable embryos	44^{ab}	50^{b}	35^{a}	49

^{ab} Means and percentages (progestogen and control groups) with superscripts not in common are different ($P<0.05$).

^{xy} Percentages of fertilized ova (ablation versus control group) are different ($P<0.05$).

A recent study has shown that superovulatory response and especially the quality of embryos collected in heifers with progesterone releasing intravaginal devices (PRID) was influenced by the stage of the estrous cycle at the time of PRID insertion (24). The poor embryo quality in the group of heifers in which PRID were inserted in the early luteal phase was attributed to follicles that were under the influence of the dominant follicle and oocytes that were undergoing degeneration at the time superstimulatory treatments were initiated. Since heifers were implanted with progestogen at random stages of the estrous cycle in the present experiment, it is conceivable that the poor embryo quality in this group was a consequence of having follicles at various developmental stages at the time of superstimulation. In contrast, E-17β treatment induced a new wave of follicles 4 d later and consequently a more uniform group of viable follicles was present at the time of superstimulation. Results from the present study demonstrated that induced follicular wave emergence, regardless of the stage of the estrous cycle, resulted in a superovulatory response at least comparable to the more traditional method of superstimulation 8 to 12 d post-estrus. These approaches obviate the necessity of detecting estrus prior to superstimulation treatments in donor cattle and may in fact result in more viable gonadotropin-responsive follicles.

ESTRUS SYNCHRONIZATION WITH PROGESTOGEN AND ESTRADIOL TREATMENTS

In estrus synchronization programs, the interval from $PGF_{2\alpha}$ treatment to expression of estrus is determined by the stage of development of the dominant follicle at the time of treatment (26,42), which in turn affects pregnancy rates following fixed-time AI (30). Heifers with a viable dominant follicle returned to estrus in 48 to 60 h after $PGF_{2\alpha}$, whereas, heifers exhibited estrus in 5 to 7 d when the dominant follicle had started to undergo atresia. This prolonged interval is a reflection of the time required for a follicle from the new wave to grow and develop to a preovulatory state (26). Various progestogen/progesterone delivery systems and protocols have been developed for estrus synchronization (32,37); however, fertility was usually lower than in untreated cattle when progestogen treatments lasted more than 14 d (32). Alternate, shorter progestogen treatment protocols (7 to 10 d) with $PGF_{2\alpha}$ given before or at the time of termination of treatments have been devised to improve fertility (32,37). However, this has not resulted in sufficient synchrony of estrus/ovulation for fixed-time AI. In addition, pregnancy rates were low when treatments were initiated during the late luteal phase (after Day 14; 8,16). Poor fertility after long-term progestogen treatments or short-term treatments initiated late in the estrous cycle was attributed to prolonged maintenance of the dominant follicle and ovulation of an aged oocyte (8,44). These results point out the need to synchronize follicular development in order to ensure the presence of a viable growing dominant follicle at the time of progesterone withdrawal and/or $PGF_{2\alpha}$ treatment (39).

A series of experiments were designed to determine whether E-17β treatment of heifers with an intravaginal progesterone releasing device (CIDR-B) will reduce variation in the interval from treatment to ovulation, compared to more traditional methods of estrus synchronization in cattle. In a preliminary experiment involving 34 beef heifers (14), treatment with CIDR-B for 7 d plus 100 mg progesterone and 5 mg E-17β im at the time of CIDR-B insertion and 500 µg cloprostenol at the time of CIDR-B removal, resulted in 75% of the heifers ovulating (detected by ultrasonography) between 72 and 84 h after CIDR-B removal. Only 40% of the heifers treated with 2 injections of $PGF_{2\alpha}$ 11 d apart, and 33% of heifers treated with CIDR-B without E-17β ovulated during the same period of time ($P<0.05$). Similar results were obtained in 2 other experiments in beef heifers treated with CIDR-B for 10 d and E-17β administered 1 d after CIDR insertion or Brahman cows treated with CIDR-B for 10 d and E-17β given 2 d after implant insertion (46). A higher degree of synchrony has also been shown when estradiol benzoate was used in combination with CIDR-B for 9 or 10 d (33). In yet another study[d], treatment with E-17β and progesterone on the second day of an 8-d melengestrol acetate (MGA) treatment protocol (with $PGF_{2\alpha}$ on the last day) has resulted in improved pregnancy rates compared to MGA alone. Overall, results of these studies suggest that estradiol treatment in combination with progestogen/progesterone will cause the synchronous development of an ovulatory follicle and may be more efficacious than traditional approaches in synchronization of estrus and ovulation for timed-breeding.

SUMMARY

Imprecision in the degree of ovarian synchrony and variability in response to treatments have continued to be the most limiting factors in the application of new reproductive technologies in cattle. There is sufficient evidence to indicate that superstimulatory treatments must be initiated at the time of follicular wave emergence (spontaneous or induced) to obtain maximum superovulatory response. Synchrony of wave emergence can be accomplished by different approaches such as ultrasound-guided follicle ablation (wave emergence in 1.5 d), or treatments with estradiol and progestogen (wave emergence in 4 d) or possibly GnRH. Superstimulatory treatments initiated at the expected time of the induced wave emergence results in a superovulatory response at least comparable to treatments initiated on a spontaneous wave or to those initiated between 8 to 12 d after estrus. Synchronizing wave emergence in a group of randomly cycling animals obviates the need of estrus synchronization prior to superstimulation

[d] JP Kastelic, unpublished.

and facilitates the management of embryo donors in large scale embryo transfer programs. Finally, incorporation of treatments that synchronize follicular wave emergence in estrus synchronization programs would insure the presence of a growing dominant follicle at the time of termination of progestogen treatments and/or $PGF_{2\alpha}$ treatment and result in synchronous estrus and ovulation, allowing for the effective use of fixed-time AI with high pregnancy rates.

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