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# **Relative Roles of FSH and LH in Stimulation of Effective Follicular Responses in Cattle**

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Additional information is available at the end of the chapter

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## **1. Introduction**

The growth development and maturation of ovarian follicles is a fundamental process for effective reproduction in farm animals. Initial stages of follicle growth occur independent of gonadotropic hormones, antral follicles then become responsive to and subsequently dependent on FSH. In heifers, there are usually 2-3 waves of gonadotropin dependent follicle growth during the estrous cycle (Ireland and Roche, 1987; Savio et al., 1988; Sirois and Fortune, 1988; Knopf et al., 1989) each involving emergence of the wave, selection of the dominant follicle (DF) and a period of dominance, followed by either atresia or ovulation of the DF. The objective is to review some of the data on the mechanisms of gonadotropic control of antral follicle growth in cattle.

## **2. Pattern of follicle growth in cattle**

The pattern of follicle growth in cattle has been clearly characterized with the use of ultrasound. Several reports (Savio et al., 1988; Sirois and Fortune, 1988; Knopf et al., 1989; Ginther et al., 1989) have shown that there are either two, three or occasionally four waves of follicle growth during the estrous cycle of cattle. During each wave of follicle growth, a cohort of 2 to 5 follicles emerges to grow beyond 4 mm in diameter to medium (5-9 mm) size classes (emergence). From the pool of medium follicles that emerge a single follicle is selected to become the dominant follicle (selection). Selection is a fundamental process that determines the species-specific ovulation rate in females (Goodman and Hodgen, 1983) thereby playing a major role in determining the number of offspring born per pregnancy. The selected DF continues to grow in size, while other follicles in the cohort undergo atresia. Finally the DF will either undergo atresia (during the luteal phase) or ovulate (during the follicular phase).

### 3. Association between gonadotropin concentrations and follicle growth

The initial stages of folliculogenesis occur independently of gonadotropic hormones. Antral follicles initially become responsive to and then dependent on FSH for their continued growth. In cattle, follicle growth above 4 mm in diameter is considered to be gonadotropin dependent (Campbell et al., 1995).

Associated with each new wave of follicular development, FSH concentrations increase as emergence occurs (Adams et al., 1992; Sunderland et al., 1994; Hamilton et al., 1995). This transient rise in FSH concentrations occurs over a period of 1 to 2 days during emergence of each new wave of follicle growth (Sunderland et al., 1994; Cooke et al., 1997). Thus, in a typical 3-wave estrous cycle, recurrent FSH rises occur on days 0.5 to 1.5, 8 to 10, and 13.5 to 15; each follicular wave lasts for approximately 7 days. Whereas in a 2-wave estrous cycle only the first two recurrent FSH rises occur; with each wave of follicular growth lasting approximately 10 days. The process of selection of the DF occurs during a period when FSH returns to nadir concentrations. It has now been clearly demonstrated that during all physiological states where follicle waves occur, associated transient increases in FSH concentrations coincide with follicle wave emergence (cyclic cattle: Adams et al., 1992; Sunderland et al., 1994; Cooke et al., 1997; pregnancy: Ginther et al., 1996; post-partum cows during anestrus: Crowe et al., 1998; and pre-pubertal heifers: Adams et al., 1994a).

The precise pattern of pulsatile LH during each wave of follicle growth has been less clearly characterized. The earlier studies of Rahe et al. (1980) characterized the pattern of LH secretion on days 3 (early luteal), 10 or 11 (mid luteal) and 18 or 19 of the estrous cycle of cows. During the early luteal phase pulses were low amplitude and high frequency (20-30 pulses / 24 h), in the mid-luteal period pulses were high amplitude and low frequency (6-8 pulses / 24 h) and pre-ovulatory surges occurred on day 18 / 19 with high frequency and high amplitude pulses occurring during the surge. However, this study was performed before characterization of the pattern of follicular growth, so no recognition regarding stage of follicular development was possible. A more recent study demonstrated that LH pulse frequency is at a minimum during the mid-luteal phase (days 7 through 13 of the estrous cycle; 2.7 to 3.4 pulses per 12 h window); LH pulse amplitude increases from early (0.5 ng / ml) to mid luteal phase (1.04 to 1.3 ng / ml on days 8-11); subsequently decreases to 0.7 to 0.8 ng / ml on days 12-14; and recovers to about 1.0 ng/ml from days 15 - 19 (Cupp et al., 1995). However, this study also failed to align animals by stage of the follicle wave before analyzing the LH pulse characteristics. In a study reported by Mihm et al. (1995), the pattern of pulsatile secretion of LH was characterized at three stages of the first DF: first day of dominance (day 5 of estrous cycle), end of growth phase of the first DF (day 8 of estrous cycle) and at emergence of the second follicle wave (loss of dominance of the first wave DF; day 11 of estrous cycle). LH pulse frequency decreased ( $p = 0.08$ ) between day 5 and 8 ( $7.5 \pm 0.4$  vs  $5.7 \pm 0.8$  pulses per 12 hours) and LH pulse amplitude increased ( $p < 0.05$ ) between days 5 and 11 ( $0.45 \pm 0.04$  vs  $1.1 \pm 0.2$  ng / ml). These data are largely confirmed by a similar study (Evans et al., 1997), where LH pulse amplitude and frequency were characterized on days 3 or 4 (early dominance), 7 or 8 (end of growth phase) and 11, 12 or 13 (loss of

dominance) of the estrous cycle. LH pulse frequency was lower at the end of the growth phase than at early dominance, with an intermediate LH pulse frequency at loss of dominance; LH pulse amplitude was greater at the end of the growth phase of the first DF than at either early dominance or at loss of dominance. Thus, while there are good characterizations of the pattern of LH secretion during the estrous cycle, the relationship of LH pulse pattern to the stage of the follicle wave has only been characterized for the first wave, so the precise role of LH in controlling follicular dynamics throughout the entire estrous cycle remains unclear. These data, however, support the hypothesis that LH pulse frequency decreases once a follicle is selected to become dominant, with an associated increase in LH pulse amplitude; an increase in LH pulse frequency and a decrease in amplitude occurs when a non-ovulatory DF undergoes atresia (Roche, 1996) or both frequency and amplitude increase as a DF proceeds to ovulate during the follicular phase (Rahe et al., 1980). Further evidence for the role of increased LH pulse frequency at later stages of follicular development is provided by studies where dominant follicles were maintained for prolonged periods of time during artificial induction of luteal phases using low levels of progesterone, in the absence of endogenous CL, and associated increased LH pulse frequencies (Sirois and Fortune, 1990; Savio et al., 1993; Mihm et al., 1994). Thus the pattern of secretion of LH at the time when a DF is selected is responsible for determination of the fate of that DF. Luteal phase LH pulse frequencies allow dominant follicles to turnover and undergo atresia; whereas, follicular phase LH pulse frequencies are associated with DF that ovulate. Experimental induction of intermediary LH pulse frequencies induces persistent DF that maintains their physiological health for an extended time period, but when ovulated is associated with reduced pregnancy rates (Mihm et al., 1994). This reduction in pregnancy rate, associated with oocytes from persistent DF, appears to be due to loss of developmental competence due to premature resumption of meiosis relative to time of ovulation (Mihm et al., 1999).

#### **4. Models to study the role of gonadotropins in folliculogenesis in cattle**

While the characterization studies mentioned in the previous section relate stage of the estrous cycle (and / or follicle wave) to gonadotropin concentrations, little work has been done to demonstrate cause and effect in terms of how gonadotropins control the process of follicle growth. There are some appropriate *in vivo* models to address these fundamental issues.

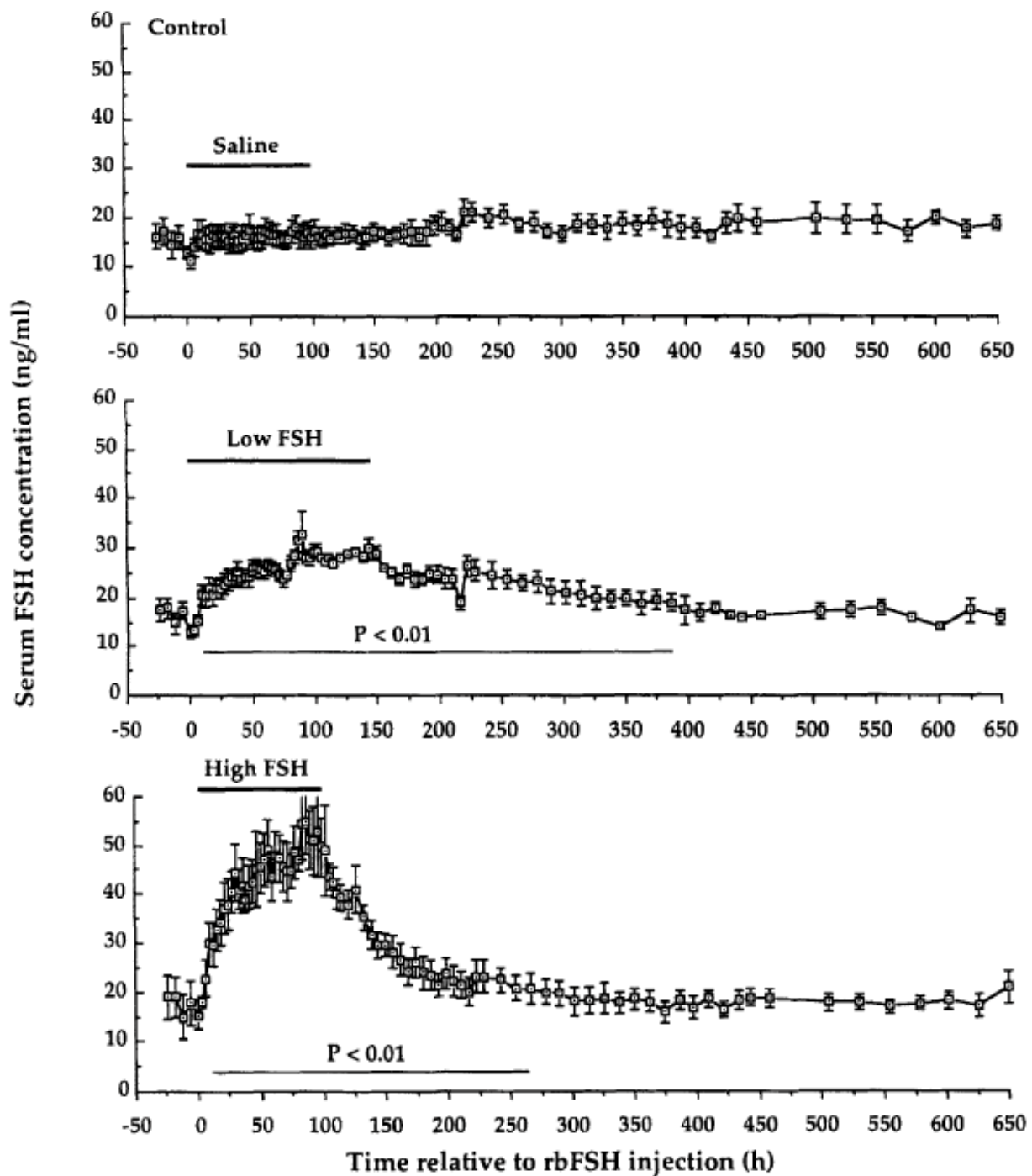
##### **4.1. GnRH immunization**

GnRH immunization involves immunization of animals against GnRH conjugated to a carrier protein (to render it immunogenic) and then administration of this immunogen mixed with an adjuvant to a subcutaneous injection site. For example, Prendiville et al. (1995) used human serum albumin (HSA)-Cys-Gly-GnRH as immunogen in DEAE-dextran adjuvant injected subcutaneously as a primary and booster (28 days post primary immunization) immunization. Vizcarra et al. (2012) has used subcutaneous and intra dermal

immunizations into the mammary gland using various conjugates (GnRH-Ovalbumin, GnRH human serum albumin, or GnRH- keyhole limpet hemocyanin) along with various adjuvants (Freunds complete, Freunds incomplete, DEAE-dextran and mineral oil) in various combinations. Optimal immunization responses (decent antibody titers, estrous cycle suppression and minimal granulomas at the injection sites) were achieved using GnRH-Ovalbumin as adjuvant and Freunds incomplete adjuvant in conjunction with DEAE-dextran. Recombinant DNA techniques to fuse GnRH to carrier proteins has also been used with success as part of the approach to achieving an efficacious vaccination protocol against GnRH (Stevens et al., 2005). Following GnRH immunization pulsatile secretion of LH is reduced (Adams and Adams, 1986; Prendiville et al., 1996), pituitary content of LH and LHRH-receptors are reduced by approximately 50% (Adams and Adams, 1990); resulting in anestrus with follicular growth arrested at  $\leq 4$  mm in diameter for at least 80 days (Prendiville et al., 1995). It has also been demonstrated that GnRH immunization prevents recurrent transient increases in FSH concentrations (Crowe et al., 2001a).

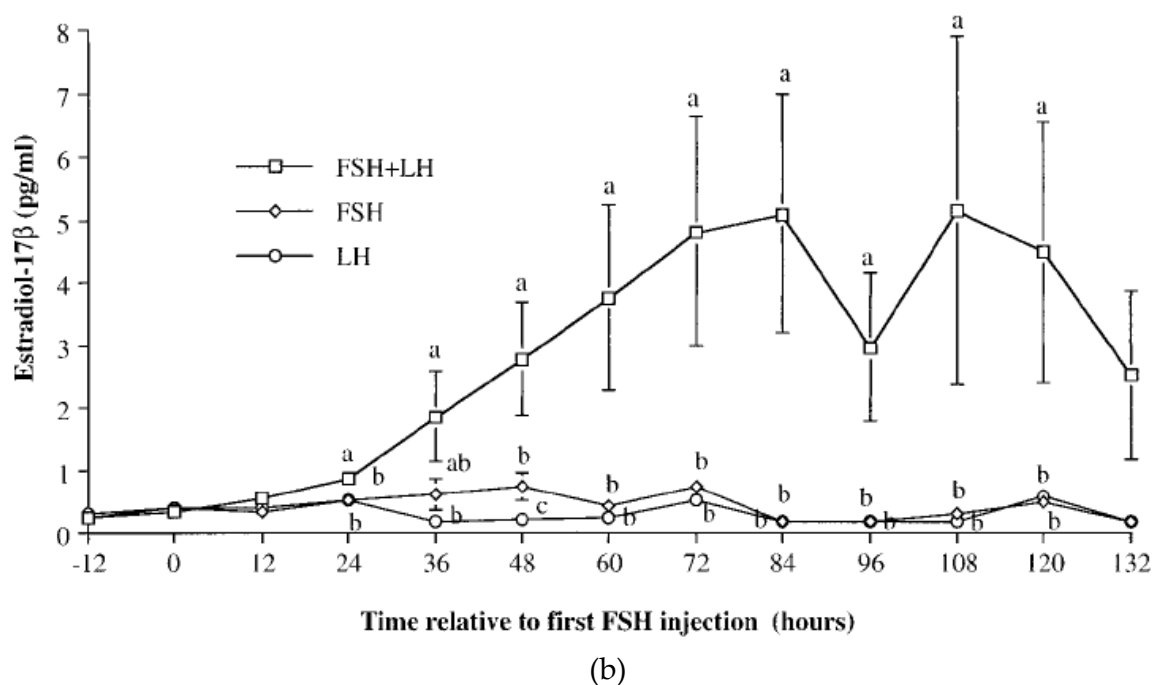
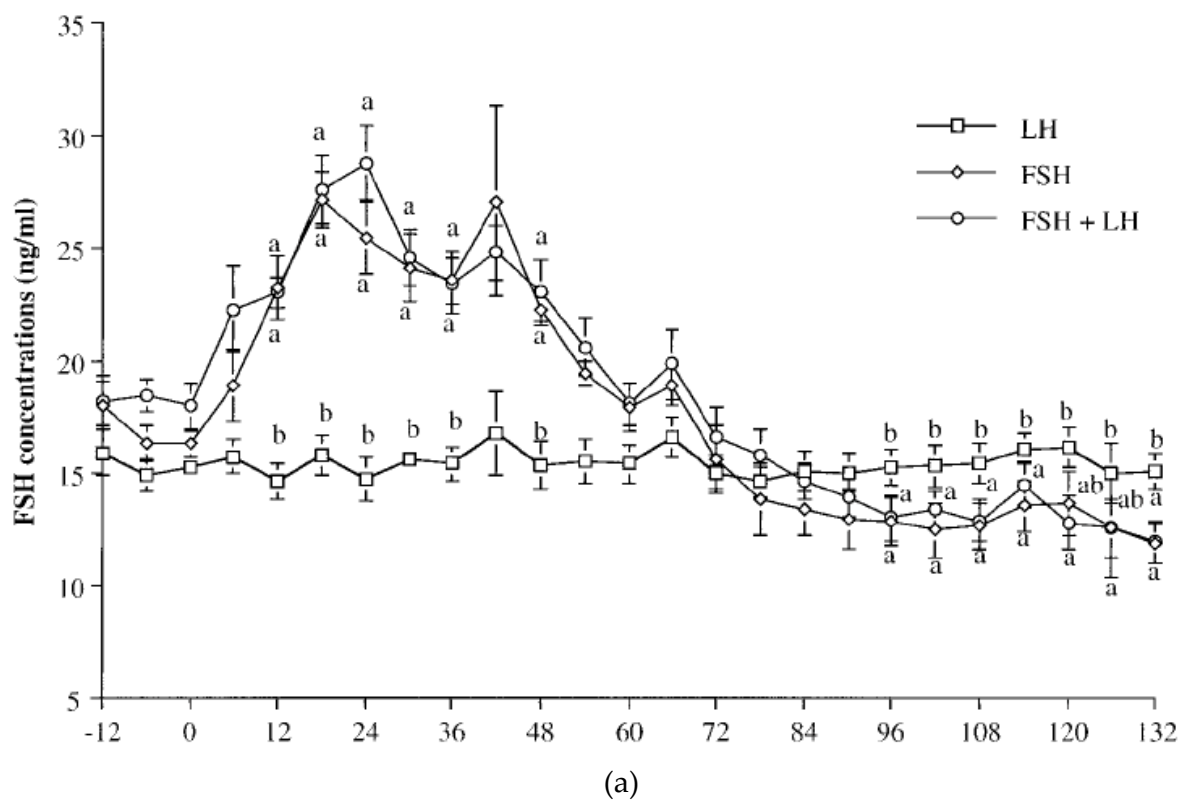
Administration of 12 mg recombinant bovine FSH (rbFSH) as 24 equal doses over 6 days to GnRH-immunized anestrus heifers caused a significant increase in serum FSH concentrations (Figure 1) and emergence of  $4.3 \pm 1.1$  medium (5-9 mm diameter) and  $2.0 \pm 1.1$  large ( $\geq 10$  mm) follicles. Using a higher dose of rbFSH (24 mg administered as 16 equal doses over 4 days) stimulated emergence of  $9.2 \pm 0.9$  medium and  $8.4 \pm 1.2$  large follicles (Crowe et al., 2001a). However, regardless of dose of FSH used, selection of a single DF failed to occur. The failure of selection of a single DF in this study can be explained by either the lack of pulsatile LH secretion or excessive dose or duration of rbFSH treatment. Furthermore, growth of medium and large follicles following FSH treatment was not associated with any change in estradiol concentrations (Crowe et al., 2001a). The lack of estradiol secretion in animals where large follicles are present on the ovary is likely associated with the absence of LH to stimulate the synthesis of androgen precursor required for estradiol secretion from the granulosa cells of large healthy follicles.

A further study was performed to determine the specific roles of FSH and LH in the process of follicle selection (Crowe et al., 2001b). Seventeen GnRH-immunized anestrus heifers, were assigned to receive either i) FSH alone (1.5 mg pFSH i.m.; Folltropin, VetrepHarm Inc., Ontario, Canada; every 6 hours for 48 hours), ii) pulses of LH alone (150  $\mu$ g pLH; Lutropin, VetrepHarm Inc., Ontario, Canada; every 4 hours for 132 hours) or iii) a combination of FSH and LH (at the same doses and schedules as in treatments (i) and (ii), respectively). Ovaries were collected following slaughter 134 - 137 hours after initiation of gonadotropin treatments. Heifers treated with FSH and LH grew substantial numbers of medium and estrogen active large follicles (some of which had associated aromatase activity), those treated with FSH alone grew large numbers of medium sized follicles, but much fewer large follicles (Table 1) and those treated with LH alone grew no follicles greater than 4 mm in diameter. Serum estradiol concentrations were 10- to 14-fold higher in heifers treated with both pFSH and pLH than in heifers treated with either pFSH alone or pLH alone (Figure 2). Follicular fluid taken from heifers treated with a combination of pFSH and pLH had E<sub>2</sub>



**Figure 1.** Mean  $\pm$  SEM FSH concentrations in GnRH-immunized anestrus heifers treated with either saline 4 x/d for 4 d (n = 5), 0.5 mg equivalent (USDA bFSH BP 1) of recombinant bovine FSH (rbFSH) 4 x/d for 6 d (n = 6; 12 mg in total; LOW FSH) or 1.5 mg rbFSH 4 x/d for 4 d (n = 5; 24 mg in total; HIGH FSH). Line with P value indicates period during which a significant elevation above pre-treatment baseline occurred (Modified and reprinted with permission, Animal Science, Crowe et al., 2001a).





**Figure 2.** Changes in mean  $\pm$  SEM serum concentrations of a) FSH and b) estradiol in GnRH-immunized anestrus heifers treated with pFSH alone (1.5 mg porcine FSH; pFSH; injected i.m. 4 x/d for 2d), pLH alone (150  $\mu$ g pLH infused i.v. 6 x/d for 6d) or pFSH and pLH (combination of FSH alone and LH alone treatments; reprinted with permission from Biology of Reproduction, Crowe et al., 2001b).

Day	Medium Follicles		Large Follicles	
	pFSH	pFSH+pLH	pFSH	pFSH+pLH
-1	0	0	0	0
0	0	0	0	0
1	0	1.0 ± 0.6	0	0
2	5.0 ± 1.4a	11.7 ± 2.6b	0	0.2 ± 0.2
3	7.2 ± 2.7	12.2 ± 2.6	0.6 ± 0.4a	7.5 ± 2.4b
4	4.0 ± 2.0	5.4 ± 2.2	2.4 ± 1.0a	13.0 ± 4.4b
5	3.4 ± 1.5	5.8 ± 2.0	3.4 ± 1.1a	12.7 ± 3.2b
6	2.8 ± 1.0	3.5 ± 1.2	4.4 ± 1.6a	14.8 ± 3.0b

**Table 1.** Mean ± SEM number of medium (5-9 mm) and large (≥ 10 mm) sized follicles, detected by daily ultrasonography, in GnRH-immunized anestrus heifers treated with pFSH alone (1.5 mg porcine FSH; pFSH; injected i.m. 4 x/d for 2d) or both pFSH and pLH (FSH as for FSH alone and 150 µg pLH infused i.v. 6 x/d for 6d). Heifers treated with pLH alone (150 µg pLH infused i.v. 6 x/d for 6d) failed to grow follicles > 4 mm in diameter (Crowe et al., 2001b). <sup>a,b</sup>Means, within follicle class and rows, with different superscripts are different (P < 0.05 for medium follicles; P < 0.01 for large follicles).

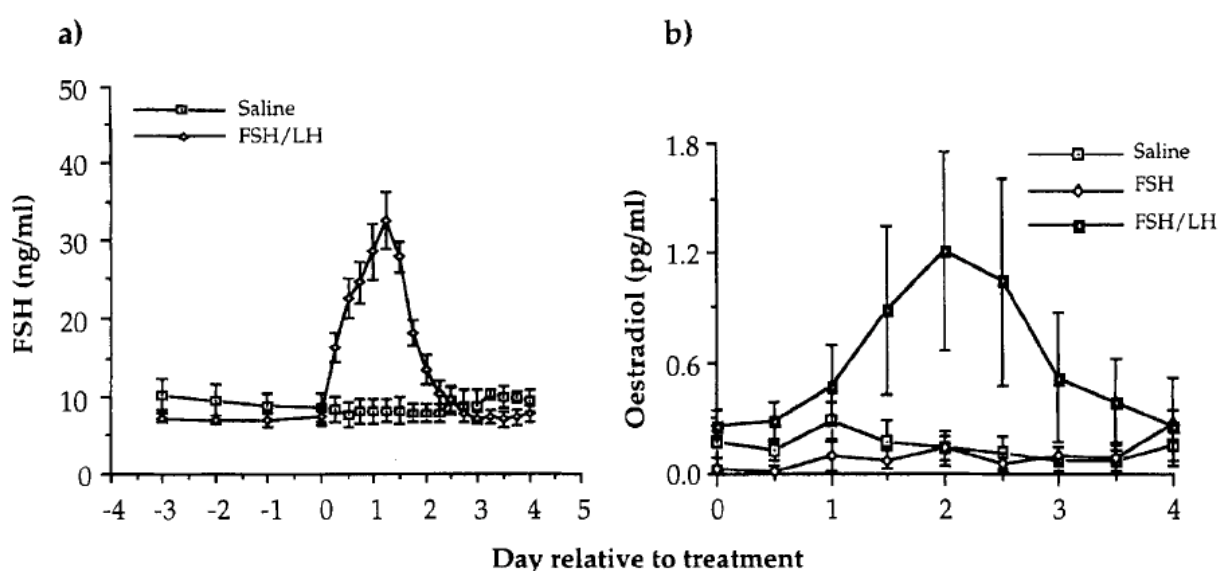
concentrations and E<sub>2</sub>:P<sub>4</sub> ratios that were highly correlated with aromatase activity. This was not the case with follicular fluid taken from heifers treated with pFSH alone. In the study of Crowe et al. (2001b) the dose of FSH used and/or the pattern of administration of LH was still inappropriate to induce physiological selection of a single DF, despite stimulating follicles that were estrogen active using a combination of both FSH and LH. The lack of ability to achieve normal DF selection may reflect an excessive dose of FSH used or an inappropriate pattern of LH infusion. In a further study using GnRH-immunized anestrus heifers administered a lower dose of FSH (1 mg oFSH i.m.; Ovagen, ICP, Auckland, New Zealand; every 6 hours for 30 hours) which gave rise to a transient increase in serum FSH concentration which was similar in both amplitude (although marginally higher peak concentrations were attained) and duration to that seen in cyclic animals (Figure 3a; DJ Cooke and MA Crowe, unpublished observations). A second treatment group received the same dose of FSH coupled with pulses of LH (50 mg pLH; Lutropin; every hour for 48 or 96 hours) generating a high frequency, low amplitude LH pulse pattern similar to that seen during the follicular phase of the normal estrous cycle. A greater (p < 0.05) number of animals treated with FSH and LH produced large follicles (8-12 mm size class) compared with those treated with FSH alone (10/14 vs. 4/14 respectively). As with the previous study (Crowe et al., 2001b) an increase in serum estradiol concentrations was observed in response to FSH and LH treatment compared with controls or with the FSH only group (Figure 3b). Interestingly, the dynamics of this increase in serum estradiol were very similar to that seen during the early luteal phase of the cyclic heifer (Cooke et al., 1997), following emergence of the first follicle wave and selection of the first dominant follicle. Intriguingly, despite an almost "ideal" physiological gonadotropin treatment, and indeed a near perfect follicular response in terms of cohort emergence and presumably activation of steroid biosynthesis, as evidenced by the increase in serum estradiol, a morphologically dominant follicle (as



previously defined, Cooke et al., 1997) was not formed. This may indicate that either i) further manipulation of the replacement pattern of FSH may be required to more precisely mimic a normal recurrent FSH increase in terms of the peak amplitude, the declining phase and / or the nadir pattern of FSH attained, or ii) perhaps a vital "selection factor" has not been provided through mere replacement of gonadotropin support.

#### 4.2. GnRH analogue administration

Similar to GnRH immunization, chronic GnRH agonist administration (Gong et al., 1995) prevents pulsatile release of LH. However, the effect of GnRH agonist on FSH concentrations is variable depending on the treatment regime used. Chronic treatment with 5 or 10 µg buserelin (a GnRH analog) twice a day for 21 days blocks pulsatile LH, but maintains elevated FSH concentrations with follicles progressing to 7-9 mm in diameter (Gong et al., 1995). Furthermore, extended treatment with buserelin infused for a 48-day period using a 28-day minipump followed by replacement with a second minipump for a further 20 days resulted in a reduction of FSH concentrations following insertion of the second minipump and prevention of follicle growth above 4 mm in diameter (Gong et al., 1996). This approach to suppressing gonadotropin secretion from the anterior pituitary gland is more acute than GnRH immunization, but for long-term studies it has the limitation of requiring continuous administration of the GnRH agonist. To date, no studies in the literature are reported where various combinations of gonadotropin hormones have been replaced to cattle treated with this method of achieving a gonadotropin deficient model.



**Figure 3.** Mean  $\pm$  SEM serum FSH (a) and estradiol (b) concentrations in GnRH-immunized anestrus heifers treated with either saline, FSH (1 mg ovine FSH i.m. every 6 hours for 30 hours; panel b only) or FSH and pulsatile LH (50 mg porcine LH every hour for 96 hours; DJ Cooke and MA Crowe, unpublished observations).

### 4.3. Requirement for LH to stimulate androgens and ovulation in post-partum anestrus cows

Post-partum beef cows are an interesting model for study of the mechanisms by which FSH and LH interact to control folliculogenesis. It is now clear that, during post-partum anestrus, recurrent, non-ovulatory follicle waves occur. In cows in good body condition score (BCS) there are typically two non-ovulatory waves of follicle growth before ovulation occurs (Murphy et al., 1990; Crowe et al., 1993), whereas cows in poor BCS have a mean of  $9.6 \pm 1.2$  non-ovulatory follicle waves post-partum before ovulation occurs (Stagg et al., 1995). This lack of ovulation of dominant follicles in post-partum beef cows is associated with a lack of LH pulses, assumed to be required to induce sufficient androgen precursor for FSH stimulated estradiol secretion and subsequently a pre-ovulatory gonadotropin surge and ovulation (Crowe, 2008).

The first post-partum DF is capable of ovulating provided a sufficient gonadotropin signal is available. Crowe et al. (1993) demonstrated that administration of 20 µg Buserelin during the growing/plateau phase of the first postpartum DF in beef cows will induce it to ovulate. Thus, to test the hypothesis that failure of ovulation of dominant follicles during post-partum anestrus in beef cows is due to inadequate LH pulse frequency to stimulate androgen precursor for estradiol synthesis, Duffy et al. (1998) assigned post-partum beef cows to receive either saline, 50 or 100 µg pLH hourly (administration via pulse infusion pumps) for 3 days commencing on the second day of dominance of the first post-partum DF. In 3 of 7 cows receiving 100 µg pLH / hour, ovulation of the first post-partum DF occurred. This result suggests that if sufficient LH is present to stimulate androgen precursor for FSH-induced estradiol production, a positive feedback effect of estradiol on GnRH release can occur, causing an LH surge and hence ovulation of the first DF post-partum. The inconsistent response may be due to a number of possible factors: i) the time of initiation of LH pulses (second day of dominance) may be borderline; ii) the dose of LH may have been inadequate to stimulate sufficient estradiol secretion in some animals or iii) the fact that porcine LH was used in this study rather than bovine LH may have resulted in a lower biopotency of the LH administered than predicted from the dose used. In any event, this study helps confirm the hypothesis that failure of ovulation of early dominant follicles during the post-partum period of beef cows is due to inadequate secretion of LH.

Alternatively studies using nutritional restriction to induce a state of anestrus in cows and then subsequent administration of GnRH has been used to determine the appropriate GnRH pulse frequency to stimulate LH, FSH and ovarian follicular responses. Pulsatile infusion of GnRH once every hour induced ovulation in 6 of 8 nutritionally anestrus cows, whereas continuous infusion of the equivalent amount of GnRH or infusion of a GnRH pulse every 4 hours was much less effective at stimulating resumption of ovulation (Vizcarra et al., 1997). In a further study using nutritionally restricted ovariectomized cows, pulsatile infusion of 2 µg GnRH at a low frequency (once per 4 h) predominantly stimulated LH release only,

where as pulsatile infusion of 2 µg GnRH every hour stimulated LH and FSH. At higher doses of GnRH (4 µg) a downregulation of the LH and FSH responses occurred with LH and FSH release only occurring on the 1<sup>st</sup> and 3<sup>rd</sup> days of treatment (Vizcarra et al., 1999). These studies suggest that concentrations of LH and FSH in blood of cows, and the ratio of LH to FSH, can be altered by the frequency and amount of GnRH stimulation. Changes in the ratio of LH to FSH that occur in cows in different physiological states may be due to the frequency that GnRH pulses are released from the hypothalamus. Furthermore the ability to induce follicle growth and ovulation is dependent on the pattern and frequency of GnRH pulses and the concomitant effect on differential secretion of LH and FSH.

#### **4.4. Delayed follicle selection**

Adams et al. (1993) and Mihm et al. (1997) demonstrated that administration of FSH, before the end of selection, delays the end of selection and attainment of dominance by 1.5 days. Indeed Mihm et al. (1997) concluded that exogenous FSH administered in physiological amounts on days 2 and 3 of the estrous cycle of cattle delayed selection of the first DF and atresia of subordinate follicles and blocked most of the alterations in intrafollicular hormones and growth factors that normally occur during the selection process. These data support the hypothesis that the decline in FSH concentrations to basal levels, following an FSH increase, causes the diverse alterations in FSH-dependent growth factors and hormones within the cohort of pre-selection follicles that lead to the end of the selection process and thus is responsible for differentially inducing both continued growth and enhanced estradiol-producing capacity of the DF and atresia of the subordinate follicles in the cohort.

### **5. Superovulation in cattle**

Superovulation is used as part of commercial multiple ovulation and embryo transfer (MOET) programs as the major method to produce embryos as part of cattle breeding improvement. Superovulatory responses are highly variable ranging from 2 to 80 ovulations and producing 0 to 60 blastocysts following non-surgical flushing. Various factors contribute to the variability in response to superovulatory treatments. These include choice of gonadotropin, animal condition and health, follicle population associated with each wave emergence, presence or absence of a dominant follicle at the time of initiation of gonadotropin treatment, and presence or absence of a CL while treating with FSH.

The options for gonadotropins for use with superovulation in cattle is really between FSH products or equine chorionic gonadotropin (eCG). FSH has a relatively short half-life and must be administered as twice daily injections over 4 days. eCG is predominantly FSH like in action, but has a longer half life and therefore a single injection is sufficient. One problem with eCG is that the prolonged half life can mean that residual FSH activity continues to stimulate follicle growth after ovulation occurs. This has a negative effect on fertilization

rate and/or zygote development (likely due to the high estradiol concentrations from these additional growing follicles). For this reason eCG gives high variability when used for superovulation. Therefore repeated FSH injections are generally the preferred treatment to use in cattle.

Traditionally superovulation treatments commenced between days 8 and 12 post estrus. This required synchronization of estrus and then commencing gonadotropin treatment 8 to 12 days after the onset of estrus. Generally the protocols required observation for the synchronized estrus so that day of commencement of treatment was accurately determined. The initiation of superovulation treatment on days 8 to 12 was considered to be optimal. But the mechanisms as to why were not understood. None of the early studies actually monitored follicular status as this work was generally completed before the advent of ultrasound scanning of ovaries for follicle structures. With the advent of transrectal ultrasonography in the late 1980s studies have characterized the pattern of follicular growth throughout the estrous cycle in cattle (Savio et al., 1988; Knopf et al., 1989; Sirois and Fortune 1988). Days 8 to 12 coincides with emergence of the second follicular wave, and the FSH used for ovarian stimulation augments the spontaneous FSH rise that stimulates emergence of the second follicular wave of the cycle (reviewed by Bo et al., 1995). However the precise day of emergence of the second follicular wave is actually dependent on whether the animal has 2 or 3 waves of follicles per cycle. With animals having a 3 wave cycle the day of emergence of the second wave is typically 1-2 days earlier (ie day 7 / 8 of the cycle) than those having a 2-wave cycle (ie days 9/10 of the cycle). It has been clearly shown that initiation of the gonadotropin treatment for superovulation at follicle wave emergence gives a better response than at a later stage of follicle wave development (Adams et al., 1994; Nasser et al., 1993). Starting the FSH treatment as little as one day after emergence of the wave reduced the superovulatory response compared with commencement on the day of follicle wave emergence (Adams et al., 1994b; Nasser et al., 1993).

One approach to manipulating the time of follicle wave emergence is to ablate all follicles ( $\geq 5$ mm diameter) across both ovaries by transvaginal ultrasound guided follicle aspiration. Then superovulation treatment with gonadotropin can commence 1-2 days later at the time of emergence of the next follicle wave (Baracaldo et al., 2000; Bergfelt et al., 1997). This treatment is quite difficult to implement routinely on farm. Therefore manipulation of follicle waves by hormonal treatments is preferred. Estradiol treatment at the commencement of a progesterone based treatment (CIDR or PRID) will suppress FSH and allow FSH to rebound to stimulate follicle wave emergence 48-72 h after administration of estradiol (Lane et al., 2000). However use of estradiol as part of an estrous synchronization program in cattle is now not permitted in many countries (Lane et al., 2008). While GnRH may be used to control follicle waves at the start of a progesterone based treatment, it will only have an effect if there is a healthy dominant follicle ( $\geq 10$  mm) at the time of GnRH administration, so this is not an ideal option (Lane et al., 2008) to regulate follicle waves before commencement of a superovulatory treatment.

An alternative strategy is to initiate the superovulatory treatment at the start of a synchronized cycle (day 1 after estrus) when the first follicle wave is emerging, but requires use of a CIDR device to be inserted during the gonadotropin treatments, and PGF treatments on day 4 (pm) and day 5 (am). This treatment strategy was reviewed by Bo et al. (2008), and provides an acceptable alternative where estrus observation is not going to be done to aid with superovulation by the conventional approach of initiating FSH treatment on days 10 – 12 of a previously synchronized cycle.

It has been shown that following the administration of an experimental GnRH agonist in cattle, follicles grew to ~8 mm in diameter, when pulsatile LH release was inhibited, and to ~4 mm in diameter, when both FSH release and LH pulses were inhibited (Gong et al., 1996; section 4.2 this chapter). Similarly, when an anti-GnRH vaccine was administered (Prendiville et al., 1995; Crowe et al., 2001a; Crowe et al., 2001b; section 4.1 this chapter), follicles grew to 3 mm, but not larger. The growth of follicles to a larger size resumed upon treatment with exogenous FSH and their growth rate in response to exogenous FSH was similar to controls (Crowe et al., 2001a), but with FSH treatment alone the follicles were not estrogen active (Crowe et al., 2001b). Stimulation with a combination of FSH and pulsatile LH stimulated estrogen active follicles to grow (Crowe et al., 2001b), and this would be an appropriate strategy for superovulation. Both approaches provides for the possibility of preparing donor cows that are in a constant state of readiness with follicles that never achieve dominance unless exogenous gonadotropins are administered.

D'Occhio et al. (1997) developed a model in which two implants impregnated with the GnRH agonist, deslorelin, were inserted to desensitize the pituitary gland to GnRH and block the endogenous LH surge. Each implant released 20 mg of deslorelin per 24 h. Seven days after treatment at random stages of the estrous cycle, superstimulatory FSH treatments were initiated and 2 days later PGF $_{2\alpha}$  was administered; 60 h after the PGF $_{2\alpha}$  treatment, ovulation was induced with an injection of pLH (D'Occhio et al., 1997). This treatment protocol was compared with the EB-CIDR superstimulation protocol in Nelore cows and the number of transferable embryos did not differ (Barros and Nogueira, 2001). Unfortunately, deslorelin implants are not commercially available for use in cattle.

## 6. Conclusions

In conclusion, the mechanisms by which FSH and LH control follicle growth in cattle are complex. Recent models have started to tease apart some of the mechanisms involved. Certainly emergence of follicles > 4 mm in size is FSH dependent and the subsequent fate of selected dominant follicles is dependent on the LH environment present during the dominance phase. Growth of normal estrogen-active follicles beyond 8-9 mm in size is dependent on the presence of adequate LH. Normal luteal phase LH pulse frequencies / amplitudes are required to cause DF turnover (atresia of DF); increased LH pulse frequencies maintained by the presence of continuous progestogens (in the absence of a corpus luteum) will maintain estrogen-active DF for an extended period of time; and follicular phase LH pulse frequencies stimulate final maturation and ovulation of DF.



The precise mechanisms by which follicles achieve the capacity to be selected to become dominant, while subordinates undergo atresia in the face of declining FSH concentrations is still somewhat unclear. However, it can be hypothesized that the follicle destined to become dominant maintains an ability to grow in the presence of declining FSH concentrations due to a number of possible mechanisms: i) increased bioavailability of insulin-like growth factor-I (IGF-I) in that follicle due to reduced total IGF-I binding protein activity (Mihm et al., 1997; Stewart et al., 1996; Canty et al., 2006); ii) expression of LH-receptor messenger RNA (Bao et al., 1997) and LH-receptors (Ireland and Roche, 1983) in granulosa cells of that follicle rendering it more responsive to LH than other follicles in the cohort. Further use of the models discussed in this paper along with identification of expression of key genes involved with these processes should provide further insights in the near future.

Gonadotropin treatments to achieve superovulation are best achieved when administered at the time of emergence of a follicle wave. While strategies have been developed to facilitate this, precise protocols that minimize the need for observation of estrous behavior and achieving optimal superovulatory responses are still being developed.

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