The bovine dominant ovarian follicle

M. C. Lucy

_J Anim Sci_ 2007.85:E89-E99,
doi: 10.2527/jas.2006-663 originally published online Jan 3, 2007;

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://jas.fass.org/cgi/content/full/85/13_suppl/E89
ABSTRACT: Central roles in reproductive biology (e.g., growth and development of the oocyte, steroidogenesis, and ovulation) are played by the ovarian dominant follicle (DF). The DF is different from other follicles because it can escape atresia (the fate of all other follicles), and if exposed to the LH surge, its cells will differentiate into the corpus luteum. The DF was originally studied by looking at the surface of the ovary through a surgical approach. Current studies employ a less invasive ultrasound technique to track the growth and development of the DF. Recruitment and selection, the processes that give rise to the DF, and dominance, the physiological state of the mature DF, are important areas of basic research. Results of these basic studies are easily translated into real-world problems in farm animal reproduction. Superovulation, for example, overrides the selection mechanism and increases the number of ovulations. Understanding the factors that affect the size of the recruited pool of follicles should increase the success rates (i.e., the number of collected embryos) for superovulation. In most animals, the DF is short-lived, existing for long enough to allow for the final maturation of the oocyte. Some DF become atretic because they mature during the luteal phase and are never exposed to the LH surge. For other DF, the LH surge redirects the DF toward its ultimate demise (i.e., luteinization, ovulation, and differentiation into the corpus luteum). The DF is managed pharmacologically within protocols for timed AI. When timed AI fails, there may be abnormal corpus luteum development and early embryonic loss, outcomes that are secondary to inadequate follicular cell maturation and incomplete oocyte capacitation in the DF. Future work on the DF will clarify its underlying biological functions so that a variety of needs in farm animal reproduction can be efficiently managed.

Key words: dominant, follicle, bovine, history

INTRODUCTION

Species differ in the number of eggs (or oocytes) ovulated during reproductive cycles. A single Pacific oyster (Crassostrea gigas), for example, will produce hundreds of thousands of mature oocytes at one time (Tervit et al., 2005). Female farm animals are less fecund, with relatively low rates of ovulation. Ovulation rate is closely tied to the numeric capacity of the mother to gestate and subsequently nurture her young. It would be counter-productive for a cow to ovulate 200,000 oocytes at one time, given her limited capacity to gestate and nurture offspring. Likewise, an oyster (a species without maternal care) could not afford to release only 1 oocyte during the breeding season. In species with a 1-to-1 ratio of mother to offspring, the physiological manifestation of the coordination of ovarian development with maternal care is the dominant follicle (DF).

Essential functions of the DF are to nurture and ultimately release the oocyte and to synthesize hormones that control reproduction. The very fact that the DF does not undergo atresia makes it unique among ovarian follicles. Within the farm species, true DF develop in cattle and horses (Mihm and Bleach, 2003; Fortune et al., 2004; Beg and Ginther, 2006). Follicles in sheep (particularly the prolific breeds) and pigs are typically viewed as nondominant, given multiple ovulation rates for the respective species (Evans, 2003; Hunter et al., 2004). Nonetheless, the preovulatory follicles in sheep and pigs are the product of a selection process, but the final number of selected follicles is greater than 1, and this scenario fails the true definition of dominance. In chickens, follicles are recruited and selected before they enter the follicular hierarchy. Once a follicle enters the hierarchy, the largest follicle (F1) does not suppress the development of other hierarchical follicles (Johnson, 1993). This review will focus predominately on the DF of cattle. Others have recently reviewed the DF of horses (Ginther et al., 2003; Beg and Ginther, 2006) and follic-
ular growth in other farm animals (Evans, 2003; Hunter et al., 2004). There are also several additional reviews on the history (Ireland et al., 2000) and physiology of the bovine DF (Fortune et al., 2004; Berisha and Schams, 2005; Webb and Campbell, In press).

HISTORICAL PERSPECTIVES ON THE DF

Erickson (1966) quantified the number of ovarian follicles in the bovine ovary through the life cycle of the cow. As is typical of mammals, there is a large pool of resting (primordial) follicles in the ovary (>100,000 follicles). Primordial follicles are released from the resting pool throughout the life cycle of the animal. The mechanisms that trigger the initiation of follicular growth are largely unknown (Skinner, 2005). There are a few hundred growing follicles in the bovine ovary at any one time (Erickson, 1966). The most likely outcome for follicles that begin to grow is atresia, which may occur at any stage. The DF must survive and become part of the healthy pool of antral follicles. This is not a trivial task because the rates of atresia are high for follicles >0.5 mm (Lussier et al., 1987). One could argue that selection of the DF is largely complete before the process of follicular selection (defined below) begins.

The fate of the large antral follicles on the bovine ovary has captured the imagination of well-known animal reproductive biologists, including Hammond (1927), who predicted that the DF arises from antral follicles of the preceding estrous cycle; Hansel (Donaldson and Hansel, 1968), who concluded that “continuous growth and regression of follicles occurs during the cycle, the largest size reached by the follicles increasing as the next oestrus approaches”; and Cassidy (Dufour et al., 1972), who marked the largest and second largest follicles with india ink and noted that “...the largest follicle as determined three days before estrus was the one that ovulated whereas before that time another follicle was the one that ovulated.” One of the more insightful papers on the subject was published in 1960 by Erkki Rajakoski (Rajakoski, 1960) of the Royal Veterinary College in Stockholm, Sweden, who examined the ovary of heifers on each day of the estrous cycle. Based on visual examination of the ovary and analyses of follicular health (normal or atretic), Rajakoski made accurate conclusions about follicular waves in cattle:

Follicles ≥ 5 mm are apparently exposed to two growth waves. Nearly a third of all normal follicles ≥ 5 mm were seen during days 3 and 4 of the cycle; clearly a period for the first growth phase. Following upon this growth phase, a large normal follicle persists in the ovaries up to the eleventh day of the cycle, whereas the other growing follicles undergo atresia between the fourth and seventh days. ... The largest normal follicle undergoes cystic atresia after the twelfth day of the cycle. Mean diameter for the largest follicles is much less the days after than before or on the eleventh day and a large cystic atretic follicle is present in the ovaries from the twelfth to the seventeenth day. ... A second wave of follicle growth to exceed 5 mm in diameter commences on the twelfth day of the cycle when the follicles from the first wave have become atretic. ... Once again a large follicle emerges, this time designated to ovulate at the subsequent oestrus. Other follicles which begin to grow during this phase of the cycle appear to undergo atresia (Rajakoski, 1960).

The work of Rajakoski (1960) was supported by that of Ireland et al. (1979), who commented that there was “only one follicle of the medium or large range per pair of ovaries. ... Perhaps, large viable follicles secrete products (i.e., estrogen) that inhibit growth of new follicles until the dominating follicle becomes atretic and disappears.” Matton et al. (1981) added that “growth of medium size follicles occurs only when the largest follicle is either rapidly turning over or had been destroyed.” Ireland and Roche (1983) summarized the underlying physiology of the 2-wave theory by concluding that “at least two periods of growth and atresia of follicles occur between days 3-13 of an estrous cycle in heifers. One is during days 3 to 7 when a single [estrogen-active] follicle 6 mm or more in diameter develops and all other [estrogen inactive] follicles regress. The other is between days 7 and 13 when the [estrogen-active] follicle from day 7 becomes an [estrogen-inactive] follicle and regresses, and another [estrogen-active] follicle develops.”

The terms recruitment, selection, and dominance are typically used to describe the process through which the DF develops (Table 1). These terms were originally coined by Hodgen (1982) in his classic paper. The terms were used to describe folliculogenesis in the primate ovary. Two additional terms, emergence and deviation, were later coined by Ginther et al. (1996) to describe additional features of the process (Table 1). A complete definition of all terms with respect to the bovine was provided by Ireland et al. (2000).

The modern era of study of follicular dynamics in cattle began with the classic publication by Pierson and Ginther (1984). They studied ovarian follicular development in 5 heifers and concluded that there was “1) growth of a large follicle to an ostensibly ovulatory size followed be regression at approximately mid-cycle, 2) selective accelerated growth of the follicle destined to ovulate approximately three days before ovulation, and 3) regression a few days before ovulation of the larger follicles that were not destined to ovulate.” They went on to correctly conclude that ultrasonography “offers much research potential for critical testing of biological hypotheses.”

The original work by Pierson and Ginther (1984) was followed in rapid succession by more detailed ultrasonographic studies of the bovine ovary. These studies were conducted at Cornell University (Sirois and Fortune, 1988, 1990; Turzillo and Fortune, 1990), at University College, Dublin, Ireland (Savio et al., 1988, 1990b), and
Table 1. Terms and definitions used to describe the DF

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recruitment</td>
<td>“... follicle(s) begins to mature in a milieu of sufficient pituitary gonadotropic stimulation to permit progress toward ovulation; a cohort may be of n size”</td>
<td>Hodgen (1982)</td>
</tr>
<tr>
<td>Selection</td>
<td>“... the process by which, typically, a single follicle is chosen; ultimately, it alone may avoid atresia and be competent to achieve timely ovulation”</td>
<td>Hodgen (1982)</td>
</tr>
<tr>
<td>Dominance</td>
<td>“... the means by which the selected (dominant) follicle, or its successor, the corpus luteum, maintains its eminence bilaterally over all other follicles, dictating the course of events in the hypothalamus, pituitary, and ovaries ...”</td>
<td>Hodgen (1982)</td>
</tr>
<tr>
<td>Emergence</td>
<td>“the last day or examination (if more than one examination per day) the future dominant follicle was 4 mm”</td>
<td>Ginther et al.  (1996)</td>
</tr>
<tr>
<td>Deviation</td>
<td>“... the beginning of the greatest difference in growth rates ... between the two largest follicles at or before the examination when the second-largest follicle reached its maximum diameter.”</td>
<td>Ginther et al.  (1996)</td>
</tr>
</tbody>
</table>

at the University of Wisconsin (Pierson and Ginther, 1986; Knopf et al., 1989). The studies included endocrine (progesterone, estradiol, and LH) data that were correlated with ovarian follicular morphology.

According to this early work, cattle have 2 or 3 waves of follicular growth during an estrous cycle. A wave of follicular growth begins with the synchronous growth of a cohort of follicles. The DF is selected from the cohort and establishes dominance. The first-wave DF becomes atretic at midcycle, and a second wave begins afterward. Two-wave cows have a second-wave DF that goes on to ovulate. In 3-wave cows, the second-wave DF becomes atretic and a third follicular wave gives rise to the ovulatory follicle. Three-wave cows have longer estrous cycles than 2-wave cows because their luteal phase is longer. Two reviews of the process were published in the early 1990s (Fortune et al., 1991; Lucy et al., 1992). Follicular waves were also observed to occur in prepubertal heifers (Adams et al., 1994; Evans et al., 1994), in postpartum anestrous (anovulatory) cows (Murphy et al., 1990; Savio et al., 1990a), and during pregnancy (Ginther et al., 1989; Taylor and Rajamahendran, 1991).

Ovarian ultrasonography revolutionized the study of follicular growth in cattle. Before ultrasound, the only method to examine the ovary was visually via a surgical approach. Although multiple survival surgeries were done, surgeries were too infrequent to accurately describe follicular growth. Large follicles on the surface of the ovary were marked with India ink, encircling the base of the follicle, in an attempt to follow the same follicle over time. Using this approach, Dufour et al. (1972) and Matton et al. (1981) could not fully describe follicular waves in cattle. In addition to the capacity for frequent observation, ultrasound enabled the cross-sectional examination of the ovary. The cross-sectional approach was important because small follicles in a cohort were often embedded just below the surface of the ovary. Their true size was impossible to determine from their surface features. Ultrasound technology has improved over time, and newer ultrasound machines are smaller and less expensive (Fricke, 2002). Doppler ultrasound offers the additional ability to measure blood flow to the DF (Acosta et al., 2005; Ginther and Utt, 2006; Miyamoto et al., 2006).

Periods of increased blood FSH concentrations during the estrous cycle were described by several groups in the 1970s (Akbar et al., 1974; Dobson et al., 1978). Schams et al. (1977) used an endoscope to examine the ovary and correlated their visual observations with blood FSH concentrations. Their data were limited, but Schams et al. appropriately concluded that “onset of enhanced follicular growth always seems to coincide with FSH elevations.” Adams et al. (1992) used ultrasonography to fully characterize the relationship between follicular waves and FSH. They demonstrated that a peak in FSH preceded the emergence of a follicular wave. Their work was important because it answered a previously unanswered question about the nature of the signal that triggered follicular recruitment. Blood FSH concentrations increase during and after the LH surge. The postovulatory FSH surge initiates the first follicular wave. A second increase in FSH occurs at midcycle, when the first-wave DF becomes atretic. Three-wave cows have a third FSH rise when the second-wave DF becomes atretic.

**GROWTH AND REGRESSION OF THE DF**

Follicular growth in cattle begins with the transition from primordial to primary follicles. Gonadotropins do not trigger the initiation of follicular growth (primordial to primary follicle transition), but FSH may play a role thereafter because the FSH receptor is expressed in primary and secondary bovine follicles (Bao and Garverick, 1998), and FSH treatment increases preantral follicular growth in vitro (Gutierrez et al., 2000). A variety of locally produced growth factors are also involved. Most notable among these are members of the TGF-beta superfamily (including the bone morphogenetic proteins, their receptors, and growth differentiation factor-9), basic fibroblast growth factor, and epidermal growth factors (McNatty et al., 2005; Knight and Glister, 2006; Webb and Campbell, In press). Insulin-like growth fac-
tors and IGFBP should also be included in this list (Lucy, 2000; Webb et al., 2003; Spicer, 2004). Vascularization of the thecal layer is required for follicular growth and development of the DF (Stouffer et al., 2001; Jiang et al., 2003). The angiogenic factors bFGF, vascular endothelial growth factor, and angiopoietins, therefore, are essential to the DF (Tamanini and DeAmbrogi, 2004; Berisha and Schams, 2005). The mechanisms controlling the initiation of follicular growth and the early stages of follicular development have been reviewed (McNatty et al., 2005; Skinner, 2005; Knight and Glimcher, 2006) and will not be addressed beyond the discussion provided above.

**FSH-Dependent Growth of the DF**

The FSH receptor is expressed soon after formation of the true (cuboidal) granulosa cell layer (Bao and Garverick, 1998). The expression of LH receptors in the theca coincides with the formation of the theca. Garverick et al. (2002) down-regulated FSH secretion in heifers with a GnRH agonist and demonstrated that the bovine follicle can grow to 4 mm without FSH. Follicular growth thereafter was dependent on FSH, and FSH infusion increased mRNA expression for P450 side chain cleavage and P450 aromatase enzymes in granulosa cells and P450 17α-hydroxylase in theca cells. Their results provided convincing molecular evidence that follicles >4 mm in diameter compose the cohort that participates in the follicular wave.

The increase in blood FSH concentrations drives FSH-dependent follicles (4 to 5 mm) into larger diameter size classes and promotes estradiol synthesis by the follicles. The enlarged FSH-dependent follicles are subjected to the selection process. Treating cows with FSH for superovulation subverts the selection process and creates a cohort of DF (Mapleton and Hasler, 2005). The fact that cows can be superovulated clearly demonstrates that follicles in the cohort are fully capable of establishing dominance if FSH support is continued. Likewise, dairy cattle that undergo the abnormal event recruiting multiple ovulations do so because blood FSH concentrations are increased during recruitment (Lopez et al., 2005). The additional FSH allows follicles that would have otherwise been selected against to proceed through developmental changes and become DF.

**Selection of the Bovine DF**

Selection of the DF is a topic that has attracted considerable study. Whereas follicles that participate in recruitment are FSH-dependent, the DF that arises from the recruited pool acquires LH receptors in the granulosa and theca cells and becomes dependent on LH. The transition to LH dependence of the DF was initially shown in the early 1980s in studies of hCG binding to the granulosa cell layer in sheep DF (Webb and England, 1982) and in those of heifers (Ireland and Roche, 1983). Later, in situ hybridization was done in a series of studies that clearly characterized the process throughout the follicular wave (Xu et al., 1995; Bao et al., 1997). These same studies demonstrated that the FSH receptor continued to be expressed in the granulosa cell layer throughout development. The pig is different from the cow in that, in the pig, the FSH receptor is lost in the granulosa layer when follicles shift from FSH- to LH-dependence (Liu et al., 2000).

The shift from FSH- to LH-dependence was viewed as the pivotal event in the development of the DF. In a study from the University of Missouri, Xu et al. (1995) concluded, “the acquisition of LH receptors in granulosa cells may be critical to the establishment and maintenance of follicular dominance, whereas FSH receptors may only play a permissive role.” According to the Missouri model (Figure 1), a follicle that acquired LH receptors before the other follicles could theoretically starve all other follicles of gonadotropins by selectively inhibiting FSH while not inhibiting LH. The selective inhibition of FSH was achieved through the independent regulatory pathways within the hypothalamus and pituitary that control FSH and LH (Pawson and McNeilly, 2005). Whereas FSH secretion is inhibited by estradiol and inhibin, LH secretion is not. The process was viewed as a competition, with a single winner (the DF with LH receptors in granulosa cells). Data from a cDNA microarray study supported the notion that the DF was LH-dependent (Mihm et al., 2006).

The Missouri model made sense, given what was known about blood LH concentrations and survival of the DF (Mihm and Bleach, 2003). A low-progesterone environment (and, consequently, a high LH pulse frequency) caused the DF to persist (Lucy et al., 1990; Siros and Fortune, 1990; Savio et al., 1993). An acute decrease in the LH pulse frequency caused the DF to become atretic (Savio et al., 1993). The LH-dependence of the DF occurred in a low-FSH environment, and hence FSH was viewed as nonessential or permissive for the DF. The exact mechanisms through which LH controlled the fate of the persistent DF were unclear, but the dependence of the DF on LH was well-supported in the existing scientific literature. The underlying mechanisms that trigger LH receptor gene expression in the granulosa cells may involve FSH and estradiol (Segaloff et al., 1990). A caveat of the Missouri model is that LH has not been shown to increase estradiol synthesis in bovine granulosa cells (Fortune et al., 2001). The model, therefore, does not explain the greater estradiol production within the selected DF. The recognized dependence of the DF on LH may arise from LH-stimulated androgen production in the theca cells (Hampton et al., 2004).

Subsequent studies examined earlier time points relative to DF selection and concluded that the DF is selected before the initiation of LH receptor expression in granulosa cells (Evans and Fortune, 1997). An increase in free IGF-I was the critical early event. A link between follicular selection and free IGF-I con-
The bovine dominant ovarian follicle

Figure 1. Models for early events during selection of the dominant follicle (DF). According to the Missouri model, the initiating event is an increase in the LH receptor (LHR) in the granulosa cell layer. According to the Cornell model, the initiating event is the increase in pregnancy-associated plasma protein-A (PAPP-A). In each case, there is an increase in estradiol (E) and inhibin (I) that feeds back negatively on FSH secretion at the hypothalamo-pituitary axis. Beg and Ginther (2006) proposed that both models occur in the DF at about the same time. The mechanisms that control either event (*) are under study. See text for details on each model. A = androgen; FSHR = FSH receptor.

Concentrations had been tied previously to follicular growth in cattle (Echternkamp et al., 2004; Spicer, 2004; Webb et al., 2004). According to the proposed Cornell model (Fortune et al., 2004; Figure 1), an increase in FSH initiates the recruitment of a follicular wave. Within any 1 wave, there was a single follicle with a slight developmental advantage over the other follicles. The FSH causes an increase in an IGFBP-4/5 protease (pregnancy-associated plasma protein-A) that degrades IGFBP-4 and -5 within the follicular fluid. The decrease in IGFBP leads to an increase in free IGF-I within the follicular fluid. Insulin-like growth factor-I is synergistic with FSH for estradiol synthesis within the follicle. The DF grows faster (responding to FSH) and also produces more estradiol. The increase in estradiol decreases FSH and starves the remaining follicles into atresia.

Beg and Ginther (2006) reviewed a series of clever and technically difficult experiments that were designed to elucidate the role that IGF-I played in the selection of the DF. They also reviewed additional studies concerning LH receptor expression during deviation. They concluded their review with a third model that put the Cornell model and the Missouri model together, proposing that the increase in LH receptor expression in granulosa cells and the increase in free IGF-I occurred at approximately the same time. This is a reasonable model because both events (i.e., degradation of IGFBP in follicular fluid and LH receptor expression in granulosa cells) clearly occur in the DF and are probably required for dominance.

Both the Missouri and Cornell models propose that 1 follicle has a developmental advantage over the other follicles at the time of follicular selection. This developmental advantage may be conferred by individual aspects of the follicle at the beginning of the follicular wave, including its vascular bed or the number or health of the granulosa or theca cells. Regardless, there appears to be some randomness to the process and, for the presumptive DF, it helps to be in the right place at the right time.

Pigs and Sheep

As in cattle, pigs undergo a decrease in blood FSH during the preovulatory period (Bracken et al., 2006). Furthermore, the FSH receptor disappears at a relatively immature stage of follicular development (~2 mm...
Figure 2. Development of the dominant follicle (DF) in cattle, sheep, and pigs. An early event in follicular selection is the acquisition of LH receptors in the granulosa cells of the presumptive DF. In this example, the filled circles indicate follicles with LH receptors in the granulosa cells, whereas the open circles indicate follicles without LH receptors in the granulosa cells. In single-ovulating cows and sheep, a single follicle acquires LH receptors. In prolific sheep and pigs, multiple follicles acquire LH receptors in the granulosa cells, and the acquisition of LH receptors in the granulosa cells occurs at a less mature stage of follicular development. More preovulatory follicles are selected in prolific sheep and pigs because more follicles have the capacity to survive during the transition from FSH- to LH-dependence.

The highly competitive event described above for cattle (with a single DF outpacing all others) becomes a team event in the pig ovary. Perhaps a single follicle cannot control FSH secretion. If this is true, then follicles develop collectively under the influence of FSH because their combined effects are needed to suppress FSH secretion. The pig model also includes the development of the LH receptor in granulosa cells at a smaller diameter and before the selection event (Liu et al., 2000; Figure 2). Thus, a cohort of pig follicles may have the capacity to respond to LH when blood FSH concentrations are suppressed. High ovulation rate in sheep arises from physiological mechanisms that may be different for 1 breed or selection line compared with another (Driancourt et al., 1990). In some breeds of sheep, the follicular physiology leading to high ovulation rate is similar to the follicular physiology of pigs, where LH receptor expression in granulosa cells occurs at a smaller diameter (Abdennebi et al., 1999; Mongomery et al., 2001; Juengel et al., 2004). The earlier development of the LH receptor in granulosa cells may enable more follicles to escape atresia during follicular selection. Preovulatory follicles from some breeds of multiple-ovulating ewes also have fewer granulosa cells per follicle, but the collective capacity of the follicles to produce estradiol and inhibin is approximately equivalent to a follicle from a single-ovulating ewe (Juengel et al., 2004). The implication is that a high ovulation rate in sheep can be achieved through an earlier maturation of the preovulatory follicle that enables more follicles to escape from selection. These follicles ovulate at a smaller diameter because of their collective effect on blood estradiol concentrations and the LH surge mechanism.

Final Fate of the DF

Once the DF becomes LH-dependent, its fate is atresia or ovulation. A DF that is not exposed to the LH surge will survive for a short period before becoming atretic (Valdez et al., 2005). During this period, the DF is dependent on growth factors that promote the G1- to S-phase transition of the cell cycle and prevent apoptosis in granulosa cells (Quirk et al., 2004). A DF can be sustained in a persistent state with a low-level progesterone treatment (Sirois and Fortune, 1990; Savio et al., 1993). Although a persistent follicle retains its capacity to trigger an LH surge and ovulate, the oocytes from persistent follicles are less healthy (i.e., exhibit lower fertility), perhaps because the oocyte is prematurely activated (Revah and Butler, 1996; Mihm et al., 1999; Roche et al., 1999). The LH surge converts the proliferating cells of the DF into quiescent cells that are resistant to apoptosis (Quirk et al., 2004).

PRACTICAL OUTCOMES FOR DF RESEARCH

The DF is studied intensely in cattle because there are practical outcomes for the research. These outcomes include methods that are applied to cattle in the breeding herd [e.g., treatment of anestrus (anovular) or cystic ovaries, synchronization of AI, and resynchronization] and also superovulation that is applied to elite females for the purpose of generating multiple oocytes for capturing superior genetics.

Postpartum Cows

Postpartum cows undergo a follicular wave shortly after calving. The DF of the first postpartum follicular wave will ovulate, become atretic, or become cystic
(Savio et al., 1990a; Beam and Butler, 1999). Cows that undergo DF atresia will have additional waves of follicular growth that give rise to DF (Wiltbank et al., 2002). The anovulatory condition can be treated with progesterone, to increase LH pulsatility and follicular maturation, or estradiol, to induce an LH surge and cause ovulation, or both (Rhodes et al., 2003). A cystic follicle arises from a recruitment and selection process and achieves dominance (Hamilton et al., 1995). A protracted dominance phase ensues, however, and the follicle enlarges.

Cystic follicles and anovular follicles are different because there is a disruption of the feedback mechanisms in the hypothalamic pituitary axis within cystic cattle (Vanholder et al., 2006). Cystic ovaries can be treated with progesterone alone to reduce LH pulsatility or with GnRH + hCG to luteinize or ovulate the cyst (Peter, 2004). Kesler et al. (1978) gave GnRH and PGF$_2\alpha$ in a series with a 9-d interval between the GnRH and PGF$_2\alpha$ injection. Their treatment was designed to luteinize the cyst with GnRH and then bring the cows into estrus by regressing the newly formed luteal tissue with PGF$_2\alpha$. The treatment sequence was clearly effective and also foreshadowed the sequential use of GnRH and PGF$_2\alpha$ for timed AI.

**Development of Timed AI**

Perhaps the most important practical outcome for DF research has been the development of follicular synchronization and timed AI programs. Work toward developing a timed AI program in dairy cattle actually began in the 1970s. The state-of-the-art was described in a 1976 review paper that outlined the factors “believed to be important to an ovulatory control system” (Thatcher and Chenault, 1976). Cows or heifers were inseminated at a fixed time after PGF$_2\alpha$, or were treated with GnRH at a fixed time after PGF$_2\alpha$, and inseminated 8 to 12 h later. The results of these trials were viewed as unsatisfactory because “fertility to a timed insemination in the PGF$_2\alpha$ plus GnRH treatment groups tended to be depressed” (Thatcher and Chenault, 1976). We now know that the development of the DF was too inconsistent at the time of the PGF$_2\alpha$ injection.

Thatcher et al. (1989) combined GnRH and PGF$_2\alpha$, in sequence for the specific purpose of synchronizing a follicular wave before a luteolytic dose of PGF$_2\alpha$. They reported their results in a review paper presented to the International Embryo Transfer Society in 1989. Thatcher et al. (1989) concluded that giving GnRH 1 wk before PGF$_2\alpha$, “increased the efficiency of the synchronization program and demonstrated the principle that an estrous synchronization system needs to synchronize ovarian follicular dynamics in addition to CL [corpus luteum] regression.” That work was followed by 2 papers by Twagiramungu et al. (1992a,b) in which GnRH and PGF$_2\alpha$, were given in series to synchronize a follicular wave and estrus.

Two groups, working in Wisconsin and Florida, apparently recognized the utility of combining follicular wave synchronization (GnRH and PGF$_2\alpha$, in sequence) with GnRH and timed AI. The inception of the ideas apparently occurred at about the same time because both groups published abstracts in the *Journal of Animal Science* in 1994. The papers were presented back-to-back in a scientific session on estrous synchronization (Physiology 7; July 13, 1994). The Florida study (Schmitt et al., 1994) was done with heifers and used buserelin, a GnRH agonist. The treatment sequence was buserelin, followed by PGF$_2\alpha$, 7 d later, buserelin after 1 additional day, and timed AI 15 h later. There was a high incidence of short interestrous intervals in timed AI heifers, and the authors correctly concluded that “the ovulatory dose of [buserelin] was given too early (24 h) after PGF$_2\alpha$, for optimal luteinization of the ovulatory follicle and differentiation of the CL.” The Ovsynch protocol (as known today) was presented by the Wisconsin group (Pursley et al., 1994). Heifers and cows were tested, and the results were encouraging in cows. The Wisconsin group then went on to publish their results as a full-length publication (Pursley et al., 1995). They concluded that “ovulation can be synchronized within an 8-h period in lactating dairy cows... . This protocol could have a major impact on managing reproduction of lactating dairy cows, because it allows for AI to be performed at a known time of ovulation and eliminates the need for detection of estrus.”

**Perfection of the Timed AI System**

The Ovsynch protocol can be applied to first-insemination cows and can also be used for resynchronization in cows that are found nonpregnant at pregnancy examination (Sterry et al., 2006). When used as a stand-alone program, the Ovsynch protocol may lead to the ovulation of immature follicles that are a consequence of poor follicular wave synchrony after the first GnRH injection (Lucy et al., 2004). Immature follicles release inadequate estradiol, form subfunctional corpora lutea, and predispose the cow to early embryonic loss (Vasconcelos et al., 2001; Perry et al., 2005). Cows that are between d 5 and 12 of the estrous cycle at the beginning of the Ovsynch protocol have greater pregnancy rates because they are more likely to respond to follicular wave synchronization (Thatcher et al., 2002). Thus, a presynchronization strategy was developed (Presynch-Ovsynch) that employed 2 PGF$_2\alpha$, injections given at appropriate intervals relative to the beginning of Ovsynch (Moreira et al., 2001). The Presynch-Ovsynch gives a consistent improvement in pregnancy rates compared with Ovsynch alone (Thatcher et al., 2006). Ovsynch has been modified in a variety of ways from its original form (Figure 3). These modifications are designed to decrease the number of animal handlings or improve the overall response to the program.

For successful timed AI, somatic and germ cells of the follicle must be managed collectively because they
Lucy

Figure 3. Timed AI programs used for beef and dairy cattle. Numbers below each horizontal line represent days relative to the beginning of treatment (d 0). G = GnRH injection; P = PGF$_{2a}$ injection; CIDR = controlled internal drug (progesterone)-releasing device.

coexist in the DF. A timed AI program for beef that uses a CIDR within the Ovsynch program gave pregnancy rates that were equal to beef cows inseminated at estrus (Schafer et al., 2005). This outcome demonstrates that timed AI can be successful when a relatively simple series of treatments is used. Improving timed AI outcomes in dairy cows may require attention to the underlying fertility problem in lactating dairy cows (Lucy, 2005; Wiltbank et al., 2006).

Superovulation

Superovulatory treatments are most successful when they are applied to cattle that are in the early phases of the follicular wave (Mapletoft and Hasler, 2005). Traditionally, FSH injections are begun between d 9 and 13 of the estrous cycle when the first-wave DF is undergoing atresia and the second follicular wave is beginning. The first-wave DF may also be ablated (DF follicle ablation), or cows can be treated with estradiol or progesterone to synchronize the follicular wave before FSH injections (Mapletoft et al., 2002). Superovulation may be more efficient (in terms of time) today than it used to be, but the success rates (in terms of collected embryos) have not changed appreciably in over 20 yr (Hasler, 2006). Failure to improve superovulation does not reflect a failure to recruit follicles into the preovulatory pool. This can be effectively done with appropriately timed FSH treatments. The limitation of superovulation is the number of follicles in the recruitable pool (i.e., those follicles that have the capacity to respond to FSH). The factors that affect this population are largely unknown. Superovulatory responses may improve when the size of the recruitable pool of follicles can be controlled.

Conclusions

The bovine DF has been a focal point for reproductive biology research for nearly 50 yr. The mechanisms to ensure that a single follicle is recruited, selected, becomes dominant, and ovulates are of primary interest. The selection of the DF involves a change in free IGF-I and an increase in LH receptor expression in the granulosa cell layer. Through these mechanisms, the DF shifts its dependence from FSH to LH and subsequently suppresses FSH to induce atresia in other follicles within the cohort. A DF will assume the fate of the rest of its cohort (i.e., atresia) if it does not initiate the LH surge. The oocyte within the DF has reduced fertility before the DF becomes atretic. Studies of the DF are highly relevant to production agriculture because the DF is manipulated within protocols for superovulation, estrous synchronization, and timed AI. Timed AI procedures are highly effective in beef cows. Timed AI programs continue to evolve for dairy cows, with the hope that the success rates will improve further.
The bovine dominant ovarian follicle

LITERATURE CITED


Hasen, J. P. 2006. The Holstein cow in embryo transfer today as compared to 20 years ago. Theriogenology 65:4–16.


Downloaded from jas.fass.org by on November 6, 2009.


Downloaded from jas.fass.org by on November 6, 2009.
The bovine dominant ovarian follicle


References

This article cites 108 articles, 63 of which you can access for free at:
http://jas.fass.org/cgi/content/full/85/13_suppl/E89#BIBL