EFFECTS OF GENETICS, UNILATERAL OVARIECTOMY, FOLLICLE CAUTERY AND EXOGENOUS GONADOTROPIN ON FOLLICULOGENESIS IN SWINE

by

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CHAPTER I
INTRODUCTION

Economic pressures in recent years have caused livestock producers to strive more than ever to achieve rapid improvements in their animals. The rate of improvement depends upon several factors, and the efficient propagation of superior individuals is one of the most important of these factors. Efforts to improve the reproductive performance of females have included such processes as superovulation, estrous synchronization, and early induction of puberty. Such manipulations of the reproductive process invoke changes in the growth and maturation of ovarian follicles. Several basic questions concerning the dynamics of follicle growth must be answered before these types of alterations can be achieved feasibly and consistently. Some areas which need to be explored include the sequence of events causing follicles to be selected for growth, the length of time required for the completion of follicle development, the stimulatory events which select growing follicles for ovulation, and factors which affect any aspect of the selection, growth and maturational development of follicles.

Surgical ablation of cyclic ovarian structures provides a unique and interesting scenario for the study of female reproduction. The response of the ovaries to such manipulations has yielded much useful insight into ovarian function. Unilateral ovariectomy, follicle cautery, genetically
increased ovulation rate and exogenous gonadotropic stimulation have been utilized in the studies reported herein to examine the processes of follicular development and selection for ovulation in swine.
CHAPTER II
LITERATURE REVIEW

Endocrine Regulation of the Ovary

The reproductive process of male mammals may be regarded as a more or less static system, modified in some species by season, and constantly involved in producing gametes. The female system, on the other hand, is much more complex. Having been born with its entire lifetime complement of gametes, the female system performs an intricate process of sequentially developing these gametes to participate in a timed pattern of reproductive cycles. Brinkley (1981) described the female reproductive system as "a complex of glands, organs, and tissues dispersed throughout the body, and whose function is to ensure the reproduction of the species. Each component is charged with specific roles at various ages and reproductive stages, and each component must perform its assigned role at specific times in concert with all other components."

The hypothalamo-hypophyseal-gonadal axis functions as the major control system for the female reproductive process. This axis includes the hypothalamus, anterior pituitary gland or hypophysis, and the ovary. To coordinate their various roles, these components must communicate, signalling information concerning their functional status at any given time (Brinkley, 1981). Although this axis produces a variety of endocrine signals, for the purposes of this review, only those having major effects on the development of ovarian follicles will be discussed.
These include gonadotropin releasing hormone (GnRH) from the hypothalamus, luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary, and estrogen, progesterone and inhibin from the ovary.

The hypothalamus exerts a profound, yet indirect effect on the ovary. The releasing hormone, GnRH, stimulates the adenohypophysis resulting in the synthesis and release of its hormones. Webb et al. (1981) have demonstrated that continuous hypothalamic support is needed for normal endocrine function of the pituitary in the ewe. Hypothalamic GnRH also serves as a communicative link between the central nervous system and the lower levels of the reproductive system. In this capacity, the hypothalamus responds to stimuli from the central nervous system (i.e., responsiveness to seasonal factors, suckling and copulatory reflex arcs), and also responds to feedback effects of the endocrine secretions of the ovary and pituitary (McDonald, 1980).

The most direct endocrine control of the ovary originates from the anterior pituitary. This gland produces and secretes the two gonadotropic hormones, FSH and LH. These hormones are secreted in a well defined cyclic pattern which has been studied in a number of species. FSH and LH are secreted throughout the estrous cycle, but the proportions and levels of each change during the different stages of the cycle (McDonald, 1980). The time courses of these gonadotropins have been studied recently in the domestic pig by Brinkley (1981) and van de Wiel et al. (1981).
The most dramatic feature of the gonadotropin profile is the preovulatory surge of LH. Concentrations of LH reached peaks on day 0 (day of estrus) of the cycle (Brinkley, 1981; van de Wiel et al., 1981). The duration of the peak was approximately 30 hours. Both studies also cited luteal phase surges of LH between days 8 and 14. These surges were of lower amplitude and shorter duration than the preovulatory surge.

Similar, though less dramatic, patterns were observed for FSH. The FSH surge began shortly after the initial rise in LH. In contrast to LH, however, FSH levels remained elevated much longer. Brinkley (1981) reported a duration of 162 hours. This type of prolonged secretion has been reported also in the ewe (Goodman et al., 1981) and has been well documented in rats and mice (Hoak and Schwartz, 1980; Sasamoto et al., 1981).

Basal and surge secretions of pituitary gonadotropins promote cyclic changes in the development of ovarian follicles to the point of ovulation and corpus luteum formation. These topics will be discussed in subsequent sections. Gonadotropins are also integral to the steroidogenic processes of the ovary. LH can stimulate the release of progesterone from both granulosa cells of follicles and luteal cells of corpora lutea (Brinkley, 1981). LH also may regulate follicular estrogen production by inhibiting androgen production, thus limiting an essential substrate for estrogen synthesis, or by inhibiting ovarian aromatase enzyme activity (Ainsworth et al., 1980). FSH promotes follicular growth and acts synergistically with LH to promote estrogen secretion by the follicle (McDonald, 1980; Hafez, 1980). In addition to their ef-
ffects on sexual behavior and progestational activities, these steroids, estrogen and progesterone, function as the final step in the control of the reproductive cycle. Luteal phase progesterone and the low levels of estrogen produced by the early growing follicle exert a negative feedback, inhibiting the release of pituitary gonadotropins. A non-steroidal component of bovine (Sasamoto et al., 1981) and porcine (Hoak and Schwartz, 1980; DiZerega et al., 1981b) follicular fluid, known as inhibin has been shown also to inhibit FSH secretion. Rising levels of estrogen, produced as follicles mature, feedback in a positive manner promoting the release of gonadotropins, thus initiating the preovulatory surges. The effects of these cyclic endocrine changes on the follicular population of the ovary will be considered in the next section.

Various exogenous hormones have been used to augment or override the normal endocrine regulation of the ovary. Pregnant mare serum gonadotropin (PMSG) was one of the first commercially available gonadotropic materials, and is still a valuable endocrine tool for the study and manipulation of the reproductive cycle. PMSG possesses FSH- and LH-like activities. Its primary use is in the promotion of follicle development. Due to its high sialic acid content, PMSG has a longer circulating half-life and is thus more effective than FSH (Gosling et al., 1979; Hafez, 1980). PMSG has been shown to produce an increase in the number of small antral follicles, with little or no effect on larger follicles (Driancourt, 1979). PMSG acts on the follicle population by reducing the number of atretic follicles (Peters et al., 1975), increasing follicular growth rate and changing the existing relationship
between the number of antral follicles and the number of growing follicles (Mariana, 1976). PMSG has been used successfully to stimulate follicle growth in swine. The maximum response in these animals occurs in the early follicular phase of the estrous cycle (i.e., days 15 to 16; Phillipo, 1968; Hunter, 1972, 1979).

Follicular Development

The developmental process in which female gametes are prepared to participate in cyclic reproductive events is known as folliculogenesis. This process involves the transformation of oocytes grouped in cell nests to small follicles, which are independent units consisting of an oocyte, granulosa cells, and a basement membrane. The small follicles are metabolically and meiotically quiescent and change little in size or appearance throughout the period of their existence. These follicles form the stock from which all developing follicles emerge. At all ages, they comprise the majority of follicles in the ovary, remaining unaltered until signalled to continue development and differentiation.

In domestic animals, including the pig, the ovaries contain small follicles at the time of birth; however, the folliculogenetic process is not necessarily complete at this time and may continue in the neonatal period. In the rat and mouse, folliculogenesis begins in the immediate postnatal period; whereas, in the dog, cat and rabbit, the process is delayed until the second week postpartum. The process begins in the central portion of the ovarian cortex where cells from the rete ovarii contact the oocytes. Rete cells attach to the oocyte surface and trigger
the beginning of follicle formation. The process progresses outward, and as the oocytes in the surface region become involved, cells of the surface epithelium also begin to differentiate and form granulosa cells. The role of hormones in early folliculogenesis remains as yet unresolved; however, interference with normal gonadotropic stimulation has been shown to interrupt the process in fetal monkeys (Peters, 1978). Studies of infant mice showed that deprivation of endogenous gonadotropins leads to abnormality in the ordered growth of follicles (Peters, 1979).

Follicular growth begins as the result of an unknown, perhaps random, initiating event. In young mammals, growing follicles lie in the inner portion of the ovarian cortex, while nongrowing ones are found in the outer regions (Bjersing, 1978). Follicular growth is divided into developmental stages according to the cell layers surrounding the oocyte. A primordial follicle consists of an oocyte surrounded by simple squamous epithelium. The progression to a primary follicle involves the encircling of the oocyte by a single layer of columnar epithelium, which comprises the developing membrana granulosa. A secondary follicle contains from two to six layers of granulosa cells. The final developmental phase, known as the tertiary (Graafian, vesicular, or antral) follicle is characterized by the progressive formation of fluid-filled intercellular spaces. These spaces coalesce to form a single cavity, the antrum. Paralleling the differentiation of the membrana granulosa, an outer follicular compartment, the thecal layer, forms outside the basement membrane and becomes highly vascularized during the course of its development (Greenwald, 1960).
An integral element of follicular development is the attainment of steroidogenic ability. The major steroids produced during follicular development are androgens and estrogens. Steroid production by the follicle is compartmentalized, with the thecal layer producing androgens (i.e., testosterone, androstenedione), while the granulosa aromatizes the androgens to form estrogens (primarily estradiol; Carson et al., 1981). Steroids alter the follicle's ability to respond to gonadotropins by aiding in the induction of receptors. Androgen production is maximal in smaller follicles, while estrogen secretion increases as follicles grow and mature (Carson et al., 1981).

Follicle growth during the estrous cycle is not completely synchronous with the duration of the cycle. Waves of growth have been reported for various species, including domestic animals. Two such waves have been noted in the ewe (Bherer et al., 1976) and the cow (Staigmiller and England, 1982). In the pig, one wave occurs between days 4 and 10 of the cycle, while a second occurs between day 16 and the return of behavioral estrus. During the last 10 to 12 hours preceding ovulation, nonatretic follicles in most species undergo a final period of growth (Bjersing, 1978).

After initiation of growth, follicles continue to develop until reaching one of two fates -- ovulation or atresia. The majority of all follicles are doomed to the latter of these fates. DiZerega and Hodgen (1981) estimate the portion of follicles lost to attrition to be 99.9% of the original population. Byskov (1978, 1979) defined atresia as the process during which the ovarian follicle loses its integrity and
the egg is disposed of by means other than ovulation. Atresia involves the disruption of the granulosa by a process known as pyknosis. Follicles at all developmental stages may be affected; however, the highest rate of atresia is found among the large follicles (Byskov, 1979). The cause of the onset of atresia is unknown; however, the progress of atretic degeneration has been examined and characterized, and is associated with successive changes in the endocrine microenvironment of the follicle. McNatty et al. (1979) stated that the mitotic activity of the granulosa cells and the viability of the oocyte are dependent upon the granulosa cells' retaining their capacity to enrich the follicular microenvironment with estrogens. The cells are able to maintain this functional status only if FSH is present within the follicle. In the absence of FSH, the granulosa cells lose their capacity to produce estrogens and become functionally competent androgen producing cells. High levels of androgens have been associated with the process of atresia (Carson et al., 1981; Nandedkar and Munshi, 1981). High androgen levels, however, are probably secondary to the onset of atresia. A decrease in aromatase enzyme activity is probably the earliest detectable sign of atresia (Carson et al., 1981). This loss of activity leads to decreased estrogen production and increased androgen accumulation which further depresses aromatase activity. Although atresia may be observed at all stages of the ovarian cycle, and thus is apparently not associated temporally with circulating gonadotropins (Carson et al., 1981), pituitary support is needed to prevent atresia in antral follicles (Greenwald, 1961; Findlay and Cumming, 1977; Dufour et al., 1979; Greenwald, 1979).
This prevention of atresia may be due to the stimulatory effect of FSH on aromatase activity (Hafez, 1980; McDonald, 1980). Prostaglandins also have been implicated as factors contributing to the atretic process (Tam et al., 1982).

Ovulation may be regarded as somewhat miraculous, considering the relative proportions of follicles ovulating and undergoing atresia; nevertheless, it is this aspect of follicular development which is of primary importance to the propagation of animals. The successive reduction of potentially ovulable follicles during each ovarian cycle to a number characteristic of the species implies some process of selection (DiZerega and Hodgen, 1981). "A follicle destined to ovulate is derived from a cohort of growing follicles drawn, in turn, from a pool of non-proliferating primordial follicles formed during fetal development. Depending upon the species, typically only a few, or just one, of this cohort escape atresia at various stages of development to reach maturity" (Goodman et al., 1977). This is perhaps the most fundamental similarity of folliculogenesis among mammals (DiZerega and Hodgen, 1981). Many investigations of follicle growth have been undertaken, yet the fundamental question of how follicles are selected for growth and ovulation remains to be completely clarified. DiZerega and Hodgen (1981) proposed that selection is at least a two-step process. First, a follicle(s) growing in the presence of gonadotropins is brought forth from a pool of cohorts which, when exposed to sufficient gonadotropic stimulation, may progress to ovulatory status. Second, a number of growing follicles is chosen from among the others to continue maturation with the ultimate
destiny being ovulation instead of atresia. In other words, a group of follicles is recruited from the pool of pre-antral follicles to continue growth, then the ovulatory crop is selected from these rapidly growing follicles, with the remainder undergoing atresia. A prior step might also be considered, that being the allotment of follicles from primordial reserves to the pool of active pre-antral follicles. This area has received little research interest, yet may profoundly influence the ovulatory process, since the number of small follicles in the ovary has been shown to influence the number which enter the growth phase (Krarup et al., 1969). Furthermore, the number of active pre-antral follicles present at the time of gonadotropic stimulation is one of the factors determining variability in the number of ovulated eggs (Mariana, 1979). Greenwald (1979) reported the presence of a reserve supply of active follicles which can be recruited in addition to the normal complement. It appears that this reserve may be the pre-antral follicles which act as a kind of buffer system between the quiescent primordial follicles and the rapidly growing antral follicles (Dufour et al., 1979). Pre-antral follicles are under control of pituitary gonadotropins; however, endocrine control of this stage of follicular development appears to be of a somewhat static or long-term nature (Dufour et al., 1979). Pedersen and Peters (1971) suggested that the recruitment of follicles from the resting phase may be more hormonally dependent than any other phase of development except for the immediate preovulatory stages. Gonadotropins are needed to maintain normal rates of follicular entry into the growth phase (Cahill and Dufour, 1979; Dufour et al.,
Much evidence has indicated that the secondary surge of FSH is responsible for initiating growth of groups of follicles. This is especially evident in the rat and mouse, in which this surge has been shown to be essential for subsequent ovulation. Porcine and bovine follicular fluids, which contain inhibin, a specific suppressant of FSH secretion, have both produced a blockage of selection of follicles in these animals (Hoak and Schwartz, 1980; Sasamoto et al., 1981). Follicular fluid also has been shown to suppress FSH secretion in primates (DiZerega et al., 1981b). The secondary FSH surge also has an important effect on follicle growth of domestic animals. The initial stimulus for follicle growth in the pig is the FSH peak at days 3 to 5 of the estrous cycle (van de Wiel et al., 1981). In the ewe, the area under the secondary FSH peak is highly correlated with the number of antral follicles, and apparently this peak serves to replenish the stock of antral follicles following their depletion by ovulation (Cahill et al., 1981). However, gonadotropin surges do not appear to be completely necessary for follicle selection in long-cycled animals. Successive obliterations of FSH surges by follicle cautery failed to inhibit ovulation in monkeys (Goodman et al., 1977; DiZerega et al., 1980). Also attesting to the independence of follicle selection and gonadotropin surges is the rapidity with which sheep, swine and cattle can regrow follicles following cauterization of follicles during the estrous cycle (Land, 1973; Clark et al., 1979; Kelly, 1979; Staigmiller and England, 1982). McNatty et al. (1981) suggested that small antral follicles are capable of being mobilized for
estrogen production within just a few hours. Moreover, they documented the growth of such a small follicle (2 mm diameter) to ovulatory size (10 mm) in 30 hours. It appears that, at least in long-cycled animals, gonadotropin surges initiate and sustain major changes in cellular mass (i.e., initiating follicle growth, ovulation and corpus luteum formation); whereas, tonic pulsatile secretion sustains and regulates physiological changes in cells prepared by the surges (i.e., corpus luteum function, ongoing processes of follicle maturation and atresia; Greenwald, 1979; Brinkley, 1981).

Although FSH historically has been considered the primary endocrine regulator of follicle growth, it appears that the final selection of follicles to ovulate or become atretic is LH dependent (Cahill et al., 1981). The ability of a cell to respond to a gonadotropic hormone depends upon the presence of receptors for the particular hormone in the structure of the cell. The presence of LH receptors appears to mark a stage of development when a follicle has been activated and is capable of responding to elevated gonadotropin levels (Webb and England, 1982). Fluorescence localization studies in the cynomolgus monkey showed that, in the early follicular phase of the menstrual cycle, no LH receptors were present, although there were numerous antral follicles. By the mid-follicular phase, a single follicle exhibited thecal binding sites for LH. By the late follicular phase, the dominant follicle clearly had been established, and by the periovulatory period, binding also could be detected in the granulosa cells (DiZerega and Hodgen, 1980b). In the rat, as follicles matured, LH receptors were observed first in thecal,
then in granulosa cells (Channing and Kammerman, 1974; Zeleznik et al., 1974; Richards et al., 1976; Richards, 1980). Similar patterns have been reported in ewes (England et al., 1981; Webb and England, 1982). Staigmiller and England (1982) noted an increase in bovine LH receptors as the estrous cycle progressed and stated that the ovulatory follicle may be unique in possessing granulosa cell LH receptors. Lindsey and Channing (1979) have observed that, as the porcine antral follicle matures, an increase in responsiveness of the granulosa cells to LH and a decrease in their responsiveness to FSH occur. Similarly, Daguet (1979) found an increase in LH receptors as ovulation approached in the sow. McNatty et al. (1981) suggested that an increase in plasma LH immediately preceding the preovulatory surge may be a critical factor for maturing follicles to the level required for ovulation and successful transformation into a fully functional and competent corpus luteum.

Another important aspect of follicular development in polytocous species is the determination of ovulation rate. Since ovulation rate sets the upper limit of litter size, alterations in ovulation rate are of significant economic importance. Ovulation rate is the most important factor determining litter size in sheep, while in mice and swine, embryonic and fetal losses, as well as ovulation rate are determining factors (Bradford, 1979). In swine, ovulation rate is determined by the action of additive genes (Zimmerman, 1979). Zimmerman and Cunningham (1975) selected gilts for increased ovulation rates over a period of five generations, with a resultant difference of 2.5 ovulations between control and selected lines, and a surprisingly high realized heritability.
estimate of $0.45 \pm 0.07$. Progress due to selection, however, is hampered by the lack of variability in the swine population.

The mechanisms through which ovulation is altered by genetic differences are not precisely known; however, differences in follicle populations and endocrine factors have been associated with this process. Clark et al. (1973) detected differences in follicle populations of two swine breeds, Yorkshire and Poland China, noted for different ovulation rates, as early as day 3 of the estrous cycle. Yorkshire gilts, noted for higher ovulation rates, possessed more small, medium and total follicles at both days 3 and 16 of the cycle than did Poland China gilts. In Romanov and Ile de France ewes, the number of small follicles was inversely related to ovulation rate, while the number of larger follicles was related directly. Earlier antrum formation was also observed in the more prolific Romanov ewes. No detectable differences were found in atresia rates between breeds, indicating that the rate of follicular entry into the growing phase (i.e., transfer of small to large follicles) is an important step in the determination of ovulation rate in these ewes (Cahill et al., 1979).

Variations in the sensitivity of the hypothalamo-hypophyseal axis to the negative feedback effects of gonadal hormones on gonadotropin release has been postulated to underlie genetic variations in ovulation rate among some breeds of sheep (Land, 1979a). If this control axis were to become less sensitive to negative feedback, higher levels or longer durations of gonadotropin surges might be expected. Land (1979a) has shown that LH release in more prolific ewes is less sensitive to
negative feedback of estradiol, and that treatment with estradiol during the estrous cycle has less effect on subsequent ovulation rate in these ewes. Further, altering negative feedback by treatment with the anti-estrogenic compound, clomiphene, generally results in increased ovulation rate (Land, 1979b). FSH release apparently is affected also, since higher-ovulating Romanov ewes exhibit a secondary FSH surge which begins earlier and is larger than that of the Ile de France ewes (Cahill et al., 1981). This secondary FSH peak is highly correlated with the number of follicles present at the ensuing estrus (Cahill and Dufour, 1979). Bindon et al. (1981), however, failed to be able correlate ovulation rate with FSH levels in Romanov and Ile de France ewes.

An alternative hypothesis has been presented by McLaren (1962), who proposed that differences in prolificacy may reflect changes in ovarian sensitivity to endogenous hormones. Finding that the response of strains of mice selected for differing fecundity differed at varying hormone levels, she concluded that selection at any one level of hormone (i.e., physiological) may be a poor guide to the animal's response under other conditions. Since ovulation rate appears to be altered by two independent means, altered ovarian sensitivity and altered hypothalamic and/or pituitary sensitivity, McLaren (1962) concluded that, provided the maximal response of the ovary is not exceeded, combination of these two factors in an individual might lead to a further increase in fecundity.

Unilateral Ovariectomy

Hunter (1787) was the first to report the phenomenon of ovarian
compensation following unilateral ovariectomy (ULO), when he noted that litter size did not change when one ovary was removed from a gilt. Although limited in its scope, this experiment was remarkable in its philosophical insight and has served as a precursor of scientific investigation of ovarian function. After ULO, the remaining ovary responds by increasing its mass and also by increasing its ovulation rate to that characteristic of both ovaries for a given species. For the purposes of this review, only compensatory ovulation will be considered, since it is this aspect of the ovarian response which is pertinent to the dynamics of follicle growth and selection.

The ability of the remaining ovary to compensate in ovulation rate after ULO at different stages of the reproductive cycle has been used as an indicator of the timing of follicle selection in various animals. ULO probably has been used most extensively in studies of laboratory animals, including rats, mice and hamsters. Compensation in ovulation rate ceased in rats when ULO was performed after 2000 h of day 3 in 4-day cycling rats and after 0200 h of day 4 in 5-day cycling rats (Peppler and Greenwald, 1970a). Similarly, single hamster ovaries failed to compensate if ULO was performed after 1600 h of day 3 in the 4-day cycle (Greenwald, 1961, 1962). Guinea pigs, which have 16- to 19-day cycles, failed to compensate when an ovary was removed after day 12 (Hermreck and Greenwald, 1964). These studies isolate critical periods for follicle selection at days 3 or 4 in rats, day 3 in hamsters and day 12 in guinea pigs. Similar critical periods have been examined in sheep and swine. This period occurs after day 14 (Land,
1973), but before day 16 in the estrous cycle of the ewe (Findlay and Cumming, 1977). ULO at different stages of the porcine estrous cycle has yielded somewhat contrasting results; therefore, the critical selective time has not been precisely determined. Wiginton (1980) found that complete compensation ceased when ULO was performed after day 13 of the cycle; whereas, Coleman and Dailey (1979) found a compensatory response in gilts treated as late as day 16. Interestingly, this period of follicle selection appears to occur near the time of luteal regression in the ewe (Land, 1973) and guinea pig (Hermreck and Greenwald, 1964). Hermreck and Greenwald (1964) speculated that regression of the corpus luteum and the resultant drop in progesterone levels release the negative feedback on LH secretion, and that LH may cause a wave of atresia, thus preventing further compensation of selection. This is supported by the finding in the hamster that decreased compensation after day 3 of the cycle is paralleled by a wave of atresia among the smaller follicles (Greenwald, 1962).

The ovarian response to unilateral ovariectomy obviously involves an alteration in the normal process of follicular maturation and selection. This presents two pertinent questions for research investigations: 1) What effects does ULO exert upon the follicle population of the ovary, and 2) how are these effects mediated?

In response to the stimulus initiated by ULO, the remaining ovary prepares twice its usual number of large preovulatory follicles in order to compensate in ovulation rate. This apparently is not accomplished by recruiting more follicles for growth, since several studies have found
no change in the total number of follicles growing in the ovary after
ULO (Greenwald, 1961; Brinkley et al., 1964; Short et al., 1968a,b;
Brinkley and Young, 1969). Dailey et al. (1969) reported that the total
number of follicles in the ovary of prepuberal gilts subjected to ULO
at 177 days of age was greater than that in the corresponding ovary of
control gilts; however, it must be realized that this situation may not
be completely analogous to that of mature cyclic animals, since at this
time the hypothalamo-hypophyseal-gonadal axis has not yet attained its
mature functional status. The specific method whereby the remaining ovary
accomplishes this change in follicular maturation appears to be species-
dependent. Two possibilities arise: 1) a passive method in which the
rate of atresia is lowered and more follicles survive, or 2) an active
role in which small follicles are more rapidly or in larger numbers
transformed to larger follicles. Decreased atresia rate appears to be
responsible for ovarian compensation in the hamster (Greenwald, 1961)
and rabbit (Desaive, 1949). An increased proliferation of small to
large follicles has been reported for the rat (Peppier and Greenwald,
1970b) and guinea pig (Hermreck and Greenwald, 1964). ULO has been
shown to initiate more rapid transfer of small to large follicles in the
pig (Brinkley et al., 1964; Short et al., 1968a,b; Brinkley and Young,
1969). This transfer also can be initiated by unilateral destruction of
corpora lutea in the pig (Brinkley and Young, 1969). The mechanism in
the ewe is seemingly a combination of these two possibilities. Dufour
et al. (1979) reported an increase in the number of pre-antral and antral
follicles, accompanied by an increased growth rate of follicles and decreased atresia in most follicular size classes. However, this decreased attrition was not of sufficient magnitude to explain the degree of compensation observed.

Although much disagreement concerning the mechanisms of ovarian compensation has arisen, there can be little, if any, doubt that compensation is a gonadotropin-related event. The pivotal question is whether the remaining ovary compensates due to an increased secretion of pituitary gonadotropins following ULO, or to the availability to the single ovary of the amount of hormone normally perfusing both ovaries. Gibson et al. (1979) found no detectable changes in FSH in serum samples pooled from groups of five mice. McLaren (1966), utilizing a uterine weight bioassay, also failed to show changes in FSH levels. Although the sensitivity of these types of assays in detecting transient changes in FSH such as those that may occur after ULO must be questioned, McLaren (1966) further found that, in groups of mice induced to ovulate using PMSG, the single ovary of ULO mice ovulated as many ova as both ovaries of control mice when both groups were given the same dose level of hormone. In contrast to this observation, Greenwald (1968) found that, in hypophysectomized rats, increased doses of PMSG were required to achieve compensatory ovulation in hemicastrated rats. Although McLaren's (1966) evidence can be interpreted strongly in favor of increased availability of a fixed amount of gonadotropin to a single ovary, recent studies, using more refined assay procedures, have demonstrated transient rises in serum FSH after ULO. Benson et al. (1969) reported an increase in
serum FSH on days 1, 2 and 3 after ULO in rats. Welschen and Dullaart (1974) and Butcher (1977) found FSH levels increased to periovulatory values at 5 and 12 h, respectively, after ULO in rats. Butcher (1977) further stated that a prolongation of the FSH surge was still evident as late as the third post-surgical estrous cycle. Furthermore, if the rise in FSH is prevented from occurring by administration of follicular fluid, compensatory recruitment does not take place (Welschen et al., 1979). Similarly, luteectomy in the monkey was followed by transient rises in FSH (DiZerega et al., 1981a). Short et al. (1968b) found decreased pituitary FSH content after ULO in gilts and presumed this to indicate that the FSH surge continued longer than in intact gilts. Increases in FSH may be attributed to removal of part of the negative feedback effect of progesterone from the CL of the removed ovary (Brinkley and Young, 1969). The endocrine secretions of the follicle also may have an effect; however, estradiol does not appear to be involved (McLaren, 1962; Short et al., 1968b). The nonsteroidal portion of follicular fluid, inhibin, a specific inhibitor of FSH, appears to play a major role in the extended FSH surge noted in hemicastrated rats (Hirshfield, 1982).

Further lines of evidence provide direct support of a gonadotropic role in ovarian compensation and indirect support to the hypothesis that compensation is due to an increase in gonadotropin secretion rather than increased availability to the single ovary. Administration of progesterone, which blocks increased gonadotropin secretion, prevented compensatory ovulation in mice (Gibson et al., 1979), rats (Peppler and
Greenwald, 1970a), and swine (Short et al., 1968a). ULO of anestrous ewes resulted in no compensation during the anestrous season; however, compensation did occur when the breeding season resumed (Mallampati and Casida, 1970). If increased availability of hormone to a single ovary were to be responsible for ovarian compensation, one would expect some degree of response to basal gonadotropin levels even in anestrous or progesterone-treated animals upon removal of one ovary. Hypophysectomy of rhesus monkeys (Cochrane and Holmes, 1966) and pregnant rats (Chatterjee and Greenwald, 1971), as well as intrahypothalamic implantation of metalthiobutylure (an inhibitor of pituitary gonadotropin secretion) in rats (Malven et al., 1971) resulted in failure of ovarian compensation to occur. Compensatory responses in rats (Peppler, 1971) and hamsters (Greenwald, 1960) declined with increasing age. This decline was attributed to decreased levels of gonadotropins and was overcome in the hamster by supplemental injections of PMSG.

Cautery of Follicles

Another surgical procedure which has been useful for the study of follicular kinetics is the electro-surgical cauterization of follicles. In this procedure, the tip of a needle is inserted into a follicle and activated until the follicle is destroyed. Depending upon which follicles are cauterized and when in the ovarian cycle the cauterity is performed, this procedure can be used to study various aspects of follicular development. Two predominant uses of follicle cauterity have evolved: 1) destruction of the dominant follicle(s) to determine its
effects on folliculogenesis, and 2) destruction of all follicles in order to examine the progress of follicular regrowth.

In primates, which are almost exclusively monotocous, follicle cautery has yielded information concerning the growth and selection of the dominant or ovulatory follicle. Goodman et al. (1977) found that cauterization of the dominant follicle on days 8 to 12 of the menstrual cycle of rhesus monkeys blocked ovulation and abolished mid-cycle gonadotropin surges. The failure of ovulation and gonadotropin surges to occur indicated that selection of the ovulatory follicle had already occurred by the time of cautery and that no surrogate was readily available to ovulate in its stead. The contention that selection had already occurred was further supported by the fact that the next gonadotropin surge was delayed for approximately the length of a normal follicular phase. DiZerega et al. (1980) abolished intercycle gonadotropin surges by cautery in two consecutive cycles. The fact that ovarian function in the third cycle was uninterrupted indicated that gonadotropin surges are not necessary for follicle recruitment in this species. In cynomolgus monkeys, the failure of follicles to respond to injection of human menopausal gonadotropin after follicle cautery on day 8 demonstrated the attenuation of follicular responsiveness to gonadotropins after establishment of the dominant follicle (DiZerega and Hodgen, 1980a). Although the ewe can not be regarded as monotocous, similarities exist in that a dominant follicle (or follicles) is selected for ovulation. Bherer et al. (1976) cauterized the largest follicles of ewes at day 14 of the cycle; however, in contrast to the response noted in primates,
the second largest follicle in this situation proceeded to ovulate.

The major emphasis of follicle cautery studies in domestic animals has involved the course of follicular regrowth following cautery of all visible follicles at various times during the estrous cycle. Brinkley and Young (1969) found that after cautery on day 2 of the cycle of the pig, a new complement of follicles had grown by day 13, and that this complement was not different in numbers of follicles or size distribution from controls. Clark et al. (1975) found that after follicle cautery at various stages of the estrous cycle of the pig, the greatest amount of follicular regrowth activity occurred in the period between days 14 and 20. Dailey et al. (1976) found that gilts subjected to cautery regrew fewer small and total follicles; however, these gilts exhibited a reduced rate of atresia and an increased frequency of cystic follicles. Follicle cautery has been used to examine the time of selection of ovulatory follicles. In gilts, cautery at day 14 (Dailey et al., 1976) or day 16 (Kelly, 1979) resulted in a lengthening of the estrous cycle. This was interpreted as an indication that follicles had already been selected at this point and that others were undergoing atresia and unable to respond, thus extra time was needed to recruit smaller follicles to ovulatory status. However, similar lengthening of the cycle after cautery at days 15, 16 or 17 in cows was shown to result from failure of the corpus luteum to regress at the expected time (Chupin and Saumande, 1981); therefore, this procedure may not be a reliable indicator of follicle selection. Finally, destruction of follicles has been used to estimate the time required for follicular regrowth to occur. In the cow,
Staigmiller and England (1982) found that more than 96 h were required for follicles to grow from 5 mm to ovulatory status, since at 96 h after cautery, neither follicle size nor steroid production of new follicles was that expected in ovulatory follicles. In the mare, about 5 days were required for development of a follicle from 5 mm to ovulatory size (Driancourt, 1979). Komkov and Clark (1981) cauterized follicles on the day of estrus in the gilt. At this stage of the cycle, all follicles are assumed to be either ovulatory or atretic, or in the very early stages of growth. Using this regimen, follicle growth can be observed without the inhibitory influence of the corpus luteum (since cautery of ovulatory follicles prevents CL formation). Results of this study indicated that 12.8 days were required for follicle growth in the pig.

Although many aspects of the selection and development of ovarian follicles have been clarified to some extent, a thorough understanding of the various components of this process has yet to be attained. The experiments included in this thesis work have been designed to examine the processes of follicle selection and subsequent follicular development by challenging the ovary with combinations of treatments which affect the selection process. By observing the response of the ovary to such challenges, information concerning the dynamics of follicular development may be obtained, and a more precise understanding of ovarian function may emerge.
CHAPTER III

EFFECTS OF GENETICS AND UNILATERAL OVARIECTOMY ON FOLLICULOGENESIS IN SWINE

Summary

Forty crossbred gilts from the University of Nebraska genetic research herd were randomly assigned to a 2 x 4 factorially-designed experiment. One-half of these gilts were progeny of nine generations of selection for high ovulation rate, while the other one-half were progeny of randomly selected control gilts of similar ancestry. Unilateral ovariectomy (ULO; right ovary removed) was performed on day 13, 15, 17 or 19 of the estrous cycle.

Pre-treatment ovulation rate of the selected gilts (15.5 ± .5) was greater (P < .05) than that of control gilts (13.7 ± .6). Mean treatment cycle length (19.8 ± .3 days), number of small follicles (24.8 ± 2.5) and follicular fluid weight (2.4 ± .1 g) were unaffected (P > .05) by genetic group, day of ULO, or their interaction. Mean number of medium follicles and total follicle numbers were likewise unaffected by genetic group; however, the numbers of follicles in these size groupings declined (P < .05) between days 17 and 19 of the cycle, documenting the progress of selection and atresia. Post-treatment ovulation rate tended to differ (P < .08) between genetic groups and was affected by day of ULO. Mean ovulation rate after ULO on days 13, 15 and 17 (14.1) was greater (P <
Mean ovulation rate after ULO on days 13 and 15 (15.9) was greater
(P < .002) than that observed after ULO on day 17 (10.6). Mean ovu-
lation rate after ULO on day 13 (16.8) was not different (P > .20) from
that observed for day 15 (14.9). These results indicate that follicle
selection may continue through day 15, but ceases before day 17 of the
porcine estrous cycle.

Introduction

Hunter (1787) first reported the phenomenon of ovarian compensation
when he noted that unilateral ovariectomy (ULO) in the pig did not de-
crease litter size. ULO and ovarian compensation have been valuable
tools for studying the timing of cyclic ovarian events, such as the
periodic selection of ovulatory follicles. ULO after day 3 in the ham-
ster (Greenwald, 1960, 1961) or after day 12 in the guinea pig (Hermreck
and Greenwald, 1964) resulted in failure of ovarian compensation to oc-
cur at the next estrus. These studies indicated that critical periods
for follicle selection occur on day 3 in the hamster and day 12 in the
guinea pig.

Ovarian compensation apparently occurs by altering the normal fol-
licle selection process. Another condition in which this process is
thought to be altered is in animals genetically predisposed to higher
than normal ovulation rate. These two processes possibly would compete
for a finite group of follicles available at the time of selection, if
they occurred simultaneously. The objectives of this study were:
1) to compare the follicle selection process in gilts selected for high ovulation rate with that in unselected control gilts and 2) to determine the number of follicles on the right ovaries of gilts of both lines at various stages of the estrous cycle.

**Materials and Methods**

Forty gilts were obtained from the genetic research herd of the University of Nebraska. One-half of these gilts were progeny of gilts derived from nine generations of selection for high ovulation rates, following one generation of relaxed selection. The other one-half were progeny of a randomly selected control line of similar ancestry (Zimmerman and Cunningham, 1975). Gilts were penned in an outside dirt lot adjacent to intact boars and checked daily for behavioral estrus. A gilt was considered to be in estrus (day 0 of cycle) when she adopted an immobile stance and allowed a boar to mount. Gilts were observed through two estrous cycles of normal length (17 to 23 days) before being assigned to one of four treatments: (a) unilateral ovariectomy (ULO) on day 13, (b) ULO on day 15, (c) ULO on day 17, or (d) ULO on day 19 of the cycle.

On the day of surgery, gilts received 1 g sodium thiopentol (Di­pentol; Diamond Laboratories, Inc., Des Moines, IA) intravenously to induce anesthesia which was maintained during surgery by a mixture of oxygen, nitrous oxide and methoxyflurane (Metofane; Pitman-Moore, Inc., Washington Cross, NJ) administered through a closed-circuit system (Dziuk et al., 1964). A mid-ventral laparotomy was performed to exteriorize the ovaries. The number of corpora lutea (CL) of the left
ovary was determined at this time. Following surgical removal of the right ovary, its follicles were measured, utilizing calibrated wire loops, and classified as small (1 to 2 mm diameter), medium (3 to 6 mm) or large (7 to 10 mm; Clark et al., 1973). The CL were counted (and when combined with the number of CL on the left ovary constituted the pretreatment ovulation rate), and then dissected from the ovary. Finally, the remaining portion of the ovary was minced, blotted and weighed to determine ovarian fluid weight, presumed to be mostly follicular fluid.

Gilts were slaughtered within 16 days of the first post-surgical estrus. At this time, the remaining ovary was recovered and the number of CL determined. Although 40 gilts were originally allotted to this experiment, only 33 provided data for statistical analysis. Three failed to establish estrous cycles of normal length, another died before assignment to a treatment group, two developed ovarian cysts, and one failed to return to estrus after ULO.

Data for pretreatment ovulation rate were analyzed as a completely random design analysis of covariance to adjust for the age of the gilts at the post-surgical estrus. Data for the number of follicles and ovarian fluid weight were analyzed by analysis of variance for a 2 x 4 factorial design. Data for ovulation rate at the post-surgical estrus were analyzed by analysis of covariance (2 x 4 factorial design) with age of the gilts as the covariate. Data for CL weight also were subjected to covariance analysis, adjusting for the number of CL. Differences between means were determined by a set of orthogonal comparisons (Table III-1, footnote b; Steel and Torrie, 1980). Data analyses were accomplished by
Results

The mean (standard deviation, SD) age of the gilts at the post-surgical estrus was 375 (±3) days. The estrous cycle length during the cycle when ULO was performed averaged 19.8 (±1.7) days and was similar (P > .05) in length to the cycle before ULO (19.0; ±1.4 days).

Although selection pressure had been relaxed for one generation, a higher (P < .05) ovulation rate (mean ± standard error, SE) was noted during the cycle before ULO in the selected gilts (15.5 ± .5) than in the control gilts (13.8 ± .6). After ULO, the difference between genetic groups was lessened, but the selected gilts (12.6 ± .8) still tended (P < .07) to have a higher ovulation rate than the control gilts (10.4 ± .9). The mean ovulation rate after ULO on days 13, 15 and 17 (14.1) was greater (P < .001) than that observed after ULO on day 19 (3.7) of the cycle (Table III-1). Additionally, the mean ovulation rate after ULO on days 13 and 15 (15.9) was greater (P < .002) than that observed on day 17 (10.6). However, the mean ovulation rate after ULO on day 13 (16.8) was not different (P > .20) from that observed for day 15 (14.9). No observable (P > .80) interaction was found between the genetic groups and day of ULO for the number of CL.

The right ovary was examined for the number of follicles of the different size classes and ovarian fluid weight (Table III-2). The number of small, medium and large follicles, and the total number of follicles, ovarian fluid weight and CL weight were not affected (P > .05)
TABLE III-1. LEAST SQUARES MEANS ± SE AND ORTHOGONAL COMPARISONS FOR THE NUMBER OF CL AFTER UNILATERAL OVARIECTOMY ON VARIOUS DAYS OF THE ESTROUS CYCLE

<table>
<thead>
<tr>
<th>Day of ULO</th>
<th>Number of gilts</th>
<th>Adjusted no. of CL</th>
<th>Orthogonal comparisons&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Probability of a difference&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>13</td>
<td>9</td>
<td>16.8 ± 1.1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>10</td>
<td>14.9 ± 1.0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>8</td>
<td>10.6 ± 1.2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>6</td>
<td>3.7 ± 1.3</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup>The number of CL was adjusted for the age of the animal.

<sup>b</sup>Comparisons were: I—Mean of day 13, 15 and 17 vs day 19; II—mean of day 13 and 15 vs day 17; III—mean of day 13 vs day 15.
TABLE III-2. LEAST SQUARES MEANS FOR THE NUMBER OF FOLLICLES, FLUID AND CL WEIGHTS IN THE RIGHT OVARY AT VARIOUS DAYS OF THE ESTROUS CYCLE

<table>
<thead>
<tr>
<th>Genetic group</th>
<th>Day of estrous cycle</th>
<th>Number of follicles</th>
<th>Ovarian fluid weight(^a)</th>
<th>CL weight(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Small</td>
<td>Medium</td>
<td>Large</td>
</tr>
<tr>
<td>Control</td>
<td>13</td>
<td>29.6</td>
<td>18.6</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>28.2</td>
<td>19.6</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>24.3</td>
<td>24.3</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>16.7</td>
<td>12.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Select</td>
<td>13</td>
<td>23.2</td>
<td>22.0</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>28.4</td>
<td>23.0</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>33.6</td>
<td>14.6</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>14.0</td>
<td>10.8</td>
<td>6.0</td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td>14.8</td>
<td>6.1</td>
<td>---</td>
</tr>
<tr>
<td>Error df</td>
<td></td>
<td>26</td>
<td>27</td>
<td>---</td>
</tr>
</tbody>
</table>

\(^a\) Weights listed in table are expressed in grams.
\(^b\) Adjusted for number of CL.
by genetic group. However, the day of the estrous cycle affected the number of small follicles on the right ovary. The mean number of small follicles for days 13, 15 and 17 (27.9) tended (P < .07) to be greater than the number present on day 19 (15.3). No genetic group by day of cycle interactions were noted (P > .07). The number of medium follicles was greater (P < .01) on days 13, 15 and 17 (20.4) when compared with day 19 (11.7). An interaction (P < .05) existed between genetic group and day of the cycle for the number of medium follicles. The interaction showed that the number of medium follicles increased from days 13 and 15 (19.1) to day 17 (24.3) in the control gilts, while the number decreased for the same days in the selected gilts (22.5 and 14.6, respectively). The total number of follicles (1 to 10 mm) varied with the day of the estrous cycle, also. The total number on days 13, 15 and 17 (48.4) was greater (P < .01) than the number present on day 19 (27.3). No genetic group by day of cycle interaction was detected (P > .90).

The ovarian fluid weight, presumed to be mostly follicular fluid, was greater (P < .05) on day 17 (2.8 g) than on days 13 and 15 (2.2 g). Neither ovarian fluid weight nor CL weight were observed to be interactive between genetic group and day of the cycle (P > .50).

Discussion

The results of this study indicate that the time period for ovarian follicle selection during the porcine estrous cycle occurs before day 17. This is supported by the failure of compensatory ovulation to occur after ULO on day 17. The difference in the time of compensation between
this and the study reported by Wiginton (1980) may possibly be attributable to genetic differences, since the gilts in the present study were of a more diverse ancestry, or to the age differences between the gilts in the two studies. The results of this study are consistent with the work of Coleman and Dailey (1979), who found compensation in ovulation rate through day 16 of the estrous cycle. Additionally, these results confirm those of Clark et al. (1979) and Kelly (1979) in which follicle cautery on day 16, but not on day 14, of the cycle resulted in delayed onset of the next estrus, presumably to allow enough time for new follicles to grow. After day 17 of the cycle, the follicles remaining on the ovary are hypothesized to be of two major types: 1) follicles that have been selected to ovulate at the ensuing estrus and 2) follicles in various stages of atresia. This categorization of follicle types is supported by the evidence that they are incapable of responding to the selection stimulus invoked by ULO (this study and Coleman and Dailey, 1979; Wiginton, 1980), follicle cautery (Clark et al., 1979; Kelly, 1979), or exogenous gonadotropin (Phillipo, 1968; Hunter 1972; Hunter et al., 1976; Hunter, 1979). The ovulatory response to exogenous gonadotropin diminished progressively when given on days 17 or 18 and was rarely augmented after day 19, as compared to the response noted when administered on days 15 or 16 of the cycle. Therefore, the maximum ovulatory response to exogenous gonadotropins is observed during the time when follicle selection is reportedly occurring. The results of this study also complement those conducted in laboratory animals in which a similar time
period exists during the cycle after which ULO fails to promote a compensatory response (Greenwald, 1960, 1961; Hermreck and Greenwald, 1964). This time period has been correlated with the selection of follicles in these animals as well.

It has been well established that caloric intake influences follicular development and ovulation rate in gilts and that the response to increased caloric intake is dependent on the stage of the estrous cycle. Dailey et al. (1972) showed a critical time (4 to 6 days before estrus) in which increased caloric intake exerts its effect on follicular development. Periods of increased caloric intake shorter than 4 to 6 days resulted in a diminished response. This period of 4 to 6 days before estrus corresponds to the critical time for follicular response to the stimuli of ULO and exogenous gonadotropins.

Increased concentrations of circulating gonadotropins, increased sensitivity or receptivity of gonadal tissue to gonadotropic stimuli, decreased atresia rate and increased recruitment of follicles into the larger size classes have all been implicated as components of the mechanisms which increase ovulation rate and produce compensatory hypertrophy or compensatory ovulation. Gonadotropic stimulation, specifically that of FSH, is probably the key to both processes. Ovarian compensation has been linked to a transient rise of FSH after ULO (Benson et al., 1969; Welschen and Dullaart, 1974; Butcher, 1977). Ovulation rate increased by genetic means may involve a more rapid transfer of follicles from primordial reserves to the growing phase and increased sensitivity to FSH, as evidenced by antrum formation at earlier stages.
of follicle development (Cahill et al., 1979). Regardless of the mechanisms involved, this experiment involved both processes simultaneously—a situation which could possibly have resulted in competition for the follicles available for selection. The lack of a significant interaction between genetic group and day of ULO for compensatory ovulation indicates that control and selected animals responded similarly to the treatment; thus, no competition was observed. The only significant interaction was observed for the number of medium follicles in the right ovary. This interaction suggests that the gilts selected for high ovulation rate were progressing through follicular stages more rapidly than the control gilts. Genetic differences in follicular growth rate have been reported by Dailey et al. (1976) and can be further observed by examining the number of large follicles present in the two genetic groups on days 17 and 19 of the cycle (Table III-2). Large follicles tended to appear earlier and in larger numbers in the selected gilts; however, the number of animals with large follicles was too small to be considered for statistical analysis. Furthermore, the degree of difference in ovulation rate between the two lines lessened after ULO, indicating that a critical level where competition would occur may have been approached.

Studies by Kirkpatrick et al. (1968) and Clark et al. (1973) have shown that genetic differences in the number of follicles may be observed as early as day 3 of the cycle between Chester White or Yorkshire and Poland China gilts. In those studies, the gilts with the higher ovulation rate had the greater number of follicles. Therefore,
the selection for genetic differences in follicle numbers may occur early in the cycle. Although no differences in number of follicles were observed between the control and selected gilts in this study, it must be remembered that only the follicles of the right ovary were considered.

The general trend for the number of follicles of the various size classes involved a decrease in small, medium and total number of follicles, and an increase in the number of large follicles as the cycle progressed. Vesicular follicle number is regulated by phasic endocrine secretions. Fewer follicles are observed during the follicular than the luteal phase of the cycle, their number parallelling the functional life span of the CL (Dailey, 1973). An analysis of the numbers of follicles on the various days of the cycle revealed that significant decreases occurred near the time (around day 17) of selection of the ovulatory follicles (Table III-2).

In conclusion, the examination of the porcine estrous cycle by a variety of experimental techniques indicates that the time around day 17 of the cycle is significant in determining the developmental fate of ovarian follicles.
CHAPTER IV
EFFECTS OF FOLLICLE CAUTERY AND EXOGENOUS GONADOTROPIN
ON FOLLICULOGENESIS IN SWINE

Summary

The objective of this study was to determine the effects of exo-
genous gonadotropin (Pregnant mare serum gonadotropin, PMSG) on the time
required for follicular regrowth after follicle cautery on the day of
estrus in gilts. After establishing normal cycling patterns gilts were
assigned randomly to a 2 x 2 factorially designed experiment. On the
day of treatment, all gilts underwent a mid-ventral laparotomy. One-
half of the gilts had all large follicles destroyed by electro-surgical
cauterization, while the other one-half underwent a sham operation. On
the day after surgery, gilts received a subcutaneous injection of either
saline or PMSG. Gilts were monitored daily for estrus and were slaugh-
tered within 16 days of the post-surgical estrus. Serum samples were
obtained during the experimental cycle for progesterone assay. PMSG
produced no effect on cycle length (i.e., time for follicle regrowth),
while follicle cautery produced a shortening of the cycle to 12.8 days.
Ovulation rate was not affected by treatment. Gilts undergoing the sham
operation displayed peak levels of approximately 15 ng progesterone per
ml of serum; whereas, gilts subjected to follicle cautery displayed
basal levels of progesterone less than 1 ng/ml serum throughout the
treatment period.
Introduction

Since alterations of the female reproductive process involve a change in the normal patterns of growth and maturation of ovarian follicles, the length of time required for a follicle to grow imposes a limit upon the ability to change the normal reproductive pattern for economic or research purposes.

Destruction of follicles at various stages of the estrous cycle has been a productive tool for study of follicular development in swine. Cautery of follicles on the day of estrus is a particularly useful means of examining follicle growth, since the follicles on the ovary at this time are either ovulatory, atretic or in the very early stages of development. Cautery at this stage provides a method for study of synchronized follicle growth and, furthermore, removes from the system the inhibitory effects of the corpus luteum, since none is formed when the follicles are destroyed. Komkov and Clark (1981) determined that approximately 13 days are required for follicles to achieve ovulatory status after cautery on the day of estrus.

The objectives of this study were to determine the effects of exogenous gonadotropic stimulation (PMSG) on the length of time required for follicular regrowth after cautery on the day of estrus and subsequent ovulation rate.

Materials and Methods

A large pool of four-way crossbred gilts (Yorkshire x Hampshire x Duroc x Landrace) was obtained from the Texas Tech University swine
research herd and moved to an outside dirt lot. Gilts were penned adjacent to two intact boars, and were monitored daily for evidence of estrous activity. A gilt was considered to be in estrus (day 0 of cycle) when she adopted an immobile stance and allowed a boar to mount. The boar was then removed to prevent mating. Gilts were monitored in this manner through several complete estrous cycles until a normal cycling pattern was established. At this time (12 to 14 months of age), gilts were randomly assigned to treatment groups. The experiment was a 2 x 2 factorial design (Steel and Torrie, 1980), the factors being: 1) sham surgery vs. follicle cautery on the day of estrus and 2) no PMSG (saline vehicle) vs. 500 I.U. PMSG (Gestyl; Organon, West Orange, NJ) administered on the day after surgery (day 1 of the cycle).

All gilts were injected intravenously with 1 g sodium thiamylal (Surital; Parke-Davis, Detroit, MI) to induce anesthesia, which was maintained throughout the surgery by a mixture of nitrous oxide, oxygen and methoxyflurane (Metofane; Pitman-Moore, Inc., Washington Crossing, NJ) administered through a closed-circuit system (Dziuk et al., 1964). A mid-ventral laparotomy was performed to exteriorize the ovaries. In gilts subjected to the sham surgery, the ovaries were simply brought to the surface and examined for the number of large follicles (> 5 mm diameter), while treated gilts had all such follicles destroyed by electro-surgical cauterization. On the following day, each gilt received a subcutaneous injection of either saline or PMSG. After surgery, gilts were again monitored daily for estrus and were necropsied within 16 days of the post-surgical estrus. The number of corpora lutea (CL) on each
ovary was counted to determine ovulation rate. The data were analyzed using the General Linear Models procedure of SAS (Barr et al., 1979).

Since a major premise of this study involved the growth of follicles in the absence of the inhibitory influence of progesterone from the CL, it was necessary to ascertain that follicle cautery indeed prevented CL formation. This was accomplished by monitoring circulating levels of progesterone during the estrous cycle after surgery.

Blood samples were collected via indwelling catheters established by a method modified from the procedure of Ford and Maurer (1978). Catheters were inserted into the anterior vena cava approximately two or three days before the expected time of the experimental estrus and were removed three to four days following the post-surgical estrus. Due to inability to maintain functional catheters in all animals for the duration of the experimental period, there were some variations in the beginning and ending times of blood sampling between individual animals. However, in all cases, sampling continued well past mid-cycle so that any rise in progesterone due to incomplete follicle destruction could be detected. Destruction of a gilt's follicles was considered to be incomplete, and her data were removed from the statistical analysis if progesterone levels exceeded normal basal levels ($\geq$ 1 ng/ml) at any time during the treatment estrous cycle.

Blood samples were collected once daily at 0800 hours. After clotting at room temperature, samples were refrigerated overnight. The serum was separated by centrifugation, frozen and stored at -20C prior to radioimmunoassay. Serum (50, 100 or 200 microliters) was extracted
with 6 ml petroleum ether. Procedural losses were assessed and corrected for by recovery of tritiated progesterone added to each sample. Serum extracts were incubated with 100 microliters GDN-337 anti-serum for 30 minutes prior to addition of tritiated progesterone, and then incubated at room temperature overnight (16 to 20 h). Assay tubes then were chilled in an ice bath for 30 minutes before the addition of dextran-coated charcoal. Tubes were centrifuged at 2000 rpm for 10 minutes at 4°C. The supernatant was decanted and processed for scintillation counting. The progesterone content of each sample was determined from a standard curve (range 0 to 500 picograms) and expressed as nanograms progesterone per milliliter of serum.

Results

Results are summarized in Table IV-1. The average age of the gilts at the time of the post-surgical estrus was 416.8 days (standard deviation, SD; 63.0). The pretreatment estrous cycle length was 19.4 days, and the number of large follicles present at the time of surgery was 16.9. Neither endpoint differed (P > .23) among the treatment groups.

Post-surgical estrous cycle length was not affected (P > .55) by hormone treatment (Vehicle, 15.9; PMSG, 17.0 days) or by the interaction of hormone and surgical treatments. However, cycle length was shortened (P < .003) by follicle cautery (sham surgery, 20.1 days; follicle cautery, 12.8 days). Post-surgical ovulation rate (12.8) was not affected (P > .69) by hormonal or surgical treatment or their interaction.
<table>
<thead>
<tr>
<th>Hormone treatment</th>
<th>Surgical treatment</th>
<th>Estrous cycle length</th>
<th>Number of follicles at surgery</th>
<th>Number of CL after cautery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pretreatment</td>
<td>Treatment</td>
<td></td>
</tr>
<tr>
<td>Vehicle&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Sham surgery</td>
<td>19.2(5)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.3(4)</td>
<td>16.4(5)</td>
</tr>
<tr>
<td></td>
<td>Follicle cautery</td>
<td>20.3(4)</td>
<td>12.5(2)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>15.3(4)</td>
</tr>
<tr>
<td>500 IU PMSG</td>
<td>Sham surgery</td>
<td>19.4(5)</td>
<td>21.0(5)</td>
<td>16.6(5)</td>
</tr>
<tr>
<td></td>
<td>Follicle cautery</td>
<td>18.5(2)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>13.0(2)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>22.5(2)&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

|                      | Standard deviation | Error df |  |  |  |
|----------------------|--------------------|----------|  |  |  |
|                      | 1.15               | 12       |  |  |  |
|                      | 4.50               | 9        |  |  |  |
|                      | 4.15               | 12       |  |  |  |
|                      | 4.37               | 12       |  |  |  |

<sup>a</sup> Number of follicles greater than 5 mm diameter.
<sup>b</sup> Phosphate buffered saline.
<sup>c</sup> LS mean(number of gilts)
<sup>d</sup> Two gilts failed to show estrus, but did ovulate.
<sup>e</sup> Data for gilts remaining after removal of gilts with incomplete follicle destruction.
The time courses of serum progesterone levels for the various treatment groups are depicted in Figures IV-1 through IV-3. In gilts subjected to sham surgery (Figure IV-1), progesterone levels began to rise above basal levels of less than 1 nanogram per milliliter (ng/ml) on days 2 to 3 of the cycle. Peak levels of approximately 15 ng/ml were reached by day 15 of the cycle, with a rapid decline thereafter. In gilts undergoing follicle cautery (Figure IV-2), serum progesterone levels remained at basal levels for the duration of the experimental estrous cycle and began an apparently normal rise following the next estrus. Two gilts (Figure IV-3) in the group subjected to follicle cautery and PMSG exhibited a small rise in serum progesterone during the treatment cycle. Although this rise was not comparable in magnitude to that of animals undergoing sham surgery, the rise above basal levels indicated that cautery had not fully prevented luteinization. Since these gilts did not meet the experimental criterion (i.e., no CL present), their data were eliminated from the statistical analysis.

Discussion

The results of this study indicate that 12.8 days are required for follicles to grow and ovulate after follicle cautery on the day of estrus. This coincides with and confirms results obtained by Komkov and Clark (1981) in a similar experiment. Furthermore, this time requirement for follicle growth supports the contention that porcine follicles begin growth at about day 6 of the cycle (Dailey et al., 1976; van de Wiel et al., 1981), since a follicle beginning growth at this
Figure IV-1.
Serum progesterone in gilts subjected to sham surgery.
Figure IV-2.
Serum progesterone in gilts subjected to follicle cautery.
Figure IV-3.
Serum progesterone in gilts with incomplete follicle destruction.
time and growing for about 13 days would ovulate at day 19 (a normal
time for ovulation in swine). How, then, can these results be recon-
ciled with those of Clark et al. (1979) which indicated that ovulation
was not delayed if follicles were cauterized on day 11.4 of the cycle
(i.e., time for regrowth of follicles was approximately 6 days)? Two
possible explanations arise. First, since follicle cautery was per-
formed at widely divergent points in the estrous cycle, the follicle
reserves available may have been at very different stages of maturity,
and thus required different time periods for maturation. Second, after
cauterization at estrus, the ovary may remain relatively quiescent for
the first 6 days, at which time follicles begin to grow (as suggested
by Dailey et al., 1976 and van de Wiel et al., 1981), and in which
case the time required for growth is 6 days in either instance.

The normal ovulation rate and rise in progesterone levels following
the post-surgical estrus indicate that the ovaries behaved normally,
even though the estrous cycle was shortened by the cautery treatment.
Since ovarian function apparently was normal, the failure of PMSG to
elicit a response is somewhat surprising and may be interpreted in at
least three ways. First, perhaps the dose of gonadotropin given was
insufficient to stimulate follicle growth. Hafez (1980) listed the dose
needed for superovulation in the pig to be 750 to 1500 I.U.; thus,
the 500 I.U. given may have been subthreshold for any type of ovarian
response. Second, the gonadotropic stimulus may have been given at an
inappropriate time with respect to follicle growth. Finally, gonado-
tropic stimulation may affect aspects of follicular growth other than
the rate of growth, and thus may be ineffective in hastening follicular development (Gosling et al., 1979).

The amount of progress that can be realized in attempts to control animal reproduction is constrained, to a variable extent, by any of a number of limiting factors imposed by the ability of the reproductive system to respond to manipulative stimuli. One such limiting factor may be the time of initiation of various stages of follicle growth. Various lines of evidence have indicated that the early follicular phase (days 15 to 17) of the porcine estrous cycle is an important time for the selection of ovulatory follicles (Dailey et al., 1976; Clark et al., 1979; Coleman and Dailey, 1979; Kelly, 1979; Clark et al., 1980; Wiginton, 1980). Dailey et al. (1972), van de Wiel et al. (1981) and the present study indicate that another important stage, the initiation of the final phases of follicle growth, occurs approximately at day 6 of the cycle. This time period may prove to be another critical point in attempts to change the normal pattern of the reproductive process, just as days 15 to 17 of the cycle are the time of maximal responsiveness to PMSG (Phillippo, 1968; Hunter 1972, 1979).

Another limiting factor may be the length of time required for a follicle to develop to ovulatory status. The close agreement between this study and that of Komkov and Clark (1981) indicates that this time requirement of approximately 13 days is an important consideration in the reproductive cycle of swine. Further investigation of the dose level and timing of gonadotropic stimulation will be necessary to determine if the rate of follicle development can be altered or if it imposes
a definite and restrictive limit upon the ability to alter the normal pattern of the reproductive cycle.
CHAPTER V

GENERAL CONCLUSION

Although scientific interest in the reproductive process has been present for centuries, a large portion of our current knowledge of this subject has developed in the last 50 years. Much of the progress in the field can be attributed directly to the development of very powerful and useful research tools such as sensitive radioimmunoassays, advanced surgical techniques, and biochemical procedures for the synthesis of hormones or hormone analogs. These techniques and many others have allowed scientists to answer many questions concerning reproductive function. Yet, at the same time, these research tools have pointed to areas of which knowledge is still very limited. Only when a thorough knowledge of the reproductive process has been attained can one reasonably hope to manipulate the process successfully and consistently. The two experiments included in this study have provided information concerning timing of ovarian events which may be critical to any attempt to alter the normal pattern of reproduction. The ability to achieve such alterations will become increasingly important as world population expands and demands for increased efficiency are placed on the livestock industry. Basic investigations of various areas of animal physiology will be instrumental in enabling the industry to meet this challenge.


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