AN ABSTRACT OF THE THESIS OF

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in ANIMAL SCIENCE presented on JULY 24, 1987

Title: EMBRYONIC MORTALITY IN PUBERTAL GILTS

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An experiment was conducted to compare the reproductive performance and serum concentrations of progesterone and estrogen of mated first (n=18) and third (n=18) estrous gilts slaughtered in equal numbers on days 3, 15 and 30 post-mating. Fertilization rate did not differ between mated first and third estrous gilts. Embryonic survival was consistently lower ($P<.05$) among mated first versus third estrous gilts. Ratio of progesterone to estrogen was higher among gilts bred at first versus third estrus on days 15 and 30 but lower on day 3

(stage of gestation x estrous period interaction, $P<.05$). Altered ratio of systemic levels of progesterone and estrogen may be related to early embryonic mortality in mated first estrous gilts.

Day 3 porcine embryos collected from first and multiestrous donors were transferred into synchronous nonmated first (n=40) and third (n=37) estrous recipients to assess influence of uterine environment on embryonic survival in pubertal gilts. Percentage survival of embryos in first and third estrous recipient was not different. However, percentage survival of embryos from multiestrous donors was higher than those from first estrous donors. Embryonic mortality in mated pubertal gilts is likely due to defective ova instead of a hostile uterine environment.

Influence of early ovulation on embryonic survival in first estrous gilts was examined. Gonadotropin releasing hormone (GnRH) was injected intravenously into 10 pubertal gilts after initial mating on first day of detected estrus; control pubertal gilts (n=10) were similarly injected with physiological saline. Blood samples were collected prior to injection, thereafter at 15 minute intervals for 90 minutes and subsequently on day 30 of gestation; sera were analyzed for luteinizing hormone (LH) and progesterone. Treatment with GnRH increased secretion of LH during the 90 minutes after injection ($P<.05$). Ovulation rate was higher in treated than control gilts ($P<.05$) but embryonic survival and serum progesterone concentration were not enhanced on day 30 of gestation. Increased ovulation rate induced by GnRH is not accompanied by an increase in embryonic survival.

Embryonic Mortality in Pubertal Gilts

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of

the requirements for the

degree of

Doctor of Philosophy

Completed July 24, 1987

Commencement June 1988

APPROVED:

Redacted for Privacy

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Date thesis is presented July 24 1987

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ACKNOWLEDGEMENTS

The author wishes to extend appeciation to the Oregon State Agricultural Experiment Station for the provision of funding and research assistantship necessary for the completion of this dessertation. Appreciation is also extended to the United States Department of Agriculture for providing funding for the cooperative agreement between Oregon State University, Corvallis, and Roman L. Hruska U. S. Animal Research Center, Clay Center, Nebraska. A special thanks goes to Drs. Ron Christenson and Bob Oltjen for their support of this agreement.

I wish to express my sincere gratitude to Dr. Fred Stormshak, Professor of Animal Science, O.S.U., for providing me with the opportunity to pursue this degree in his laboratory. He has instilled in me an appeciation of basic research and the importance of creative experimentation in previously unexplored areas of domestic animal reproduction. His confidence in my capability as a scientist has provided me with enthusiasm to strive towards excellence. I am a proud Storm-trooper. My gratitude also goes Dr. David England, Professor of Animal Science O.S.U., for his patience and counseling for the duration of my program, and also for providing me with an assistantship. I would like to extend my gratitude also to Dr. Ralph Maurer, Research Physiologist, U.S. Meat Animal Research Center, Clay Center, Nebraska, for his patience, counseling and supervision of my study in his laboratory.

I wish to thank my colleagues and friends, Patrick Essien, Piper

Klinger, Dr. Jack Rose, John Jaeger, Carrie Cosola, OV Slayden (computer wiz kid), Calvin Walker, Nora Peters, Helen Chesbrough, Jane Williams and Terri Martin for their help during late night blood collection and the preparation of this thesis. A special thank you goes to Dr. Mary Zelinski-Wooten (R.I.A Guru) for her patience and understanding during my radioimmunoassay pupilage under her supervision and to Salah Abdelgadir (CL Abdelgadir) for his invaluable assistance with the preparation of my graphs.

I wish to acknowledge Dr. Kimiyaki Maruyama, Ted Acton, Jenell Dague, Ramona Dana and Ed McReynolds of U.S. Meat Animal Research Center, Clay Center, Nebraska for their tremendous help during surgeries for experiment II. Also acknowledged are: Roy Fancher and Mark Uhden, for their help in animal care at the O.S.U. swine center; Bob Dickson, Clark Meat Science Lab. for performing the slaughter of the experimental animals; Dr. Myron Brown, CEVA Laboratories, Overland Park KS for the generous donation of GnRH; Dr. Gordon Niswender, Colorado State Univ. for the generous donation of LH and estrogen antibodies.

I sincerely thank those closest to me: my family members especially Joe, Maurice senior and junior, Ignatius, Inyang, Agnes, Dan, Margaret, Efana, Yeyen Archibong and Tony Aqua (my favorite cousin) who have given me love and contributed money towards the successful completion of my study. Members of the Lahey family (Allen, Lynn, Mary and Welcome), and Ntekpere family (Uwemedimo, Debra and Princess), who have been very close to me deserve special thanks.

I wish to dedicate this thesis to the following people for providing me the incentive to pursue a doctoral degree in science.

Firstly, my mother, Theresa Archibong, who has always encouraged me to study plants as they relate to the relief of ailments. Secondly, my father, Chief Andrew E. Archibong, though blind, has never ceased to encourage me to aim as high as possible in the world of academics even though he knows it would keep me away from his touch for many years.

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EMBRYONIC MORTALITY IN PUBERTAL GILTS

REVIEW OF LITERATURE

This review will focus on research that has contributed to our understanding of the causes of early embryonic mortality in the gilt. It is established that embryonic mortality is greater in gilts bred at puberty than in those bred at a subsequent estrus. It is presumed that changes in pituitary and gonadal hormone secretion that lead to the onset of puberty may also contribute to a higher incidence of embryonic mortality in gilts mated at this time by adversely affecting the ova and(or) the uterine environment. Therefore an attempt will be made to review the pertinent literature with respect to changes in secretion of hormones and the action of these hormones on target organs during various reproductive states of the gilt.

Hormonal Changes Before and at Puberty

Puberty represents that stage of development at which an animal plays the appropriate role in reproduction and produces gametes that result in viable young. In the gilt, puberty is characterized by the expression of first behavioral estrus and ovulation. Feral gilts are seasonal breeders and attain puberty in late autumn at approximately 8 months of age (Sigoret, 1980). Similarly, domesticated gilts attain puberty at approximately 7 months of age but this age can be affected by genetic (Self et al., 1955; Christenson and Ford, 1979), nutritional (Zimmerman et al., 1960; Friend, 1974, 1976), and climatic factors

(Wiggins et al., 1950; Mavrogenis and Robison, 1976; Love, 1978). Social contact with littermates (Robison, 1974; Mavrogenis and Robison, 1976; Ford and Teague, 1978; Christenson and Ford, 1979) or boars (Brooks and Cole, 1970; Hughes and Cole, 1976; Thompson and Savage, 1978; Kirkwood and Hughes, 1979) has also been shown to affect age of puberty.

Onset of puberty has been ascribed to changes in the sensitivity of the hypothalamic-hypophyseal axis to the negative feedback of ovarian hormones; a dictum referred to as the "gonadostat theory". According to the gonadostat theory, the hypothalamus of the prepubertal animal is highly sensitive to ovarian estrogens that feed back to suppress secretion of pituitary gonadotropins. At puberty, the gonadostat is reset such that the hypothalamus becomes less sensitive to this negative feedback effect of estrogens. As a result, gonadotropin secretion increases to adult levels with consequent follicular development and ovulation. An increase in serum gonadotropin concentrations, in the face of constant or elevated systemic estrogen concentrations, has been observed in lambs just before puberty (Foster and Ryan, 1979; Ryan and Foster, 1980).

Lutz et al. (1984) found a similar increase in secretion of luteinizing hormone (LH), concomitant with an increase in serum estrogen concentrations, just before puberty in gilts. In their study, mean serum LH concentrations, number of LH peaks, LH peak amplitude and mean serum estrogen concentrations, as determined from 15-minute bleeding intervals over an 8-hour sampling period for each gilt, were

grouped into seven common physiological age groups before puberty: group one, 51 to 58 days; group two, 43 to 50 days; group three, 31 to 38 days; group four, 22 to 26 days; group five, 17 to 19 days; group six, 10 to 20 days and group seven, 3 to 5 days. Mean serum concentration of LH decreased slightly from .8 $\pm$ .1 ng/ml at age group one to .6 $\pm$ .1 ng/ml and remained relatively constant through age groups three, four and five. Although not significant, serum LH concentrations increased to .8 $\pm$ .1 ng/ml at age group six and was greater at age group seven (1.0 $\pm$ .1 ng/ml) than during age groups two through five but not age groups one and six. Number of LH peaks per 8-hours (mean $\pm$ SE) was low (2.0 $\pm$ .6) at age one, decreased slowly through age groups two to five, and suddenly increased at age group six (4.0 $\pm$ .6). Mean LH amplitude decreased from 1.9 $\pm$ .1 at age group one to 1.3 $\pm$ .1 ng/ml at age group seven. In general, these data are supportive of the concept that puberty results from an enhanced secretion of gonadotropins.

In contrast, Diekman et al. (1983), utilizing a 20-minute sampling schedule every 4-hours each week for four gilts from 10 to 18 weeks of age and for another four gilts from 19 to 20 weeks of age, did not observe a peripubertal rise in serum LH concentrations. Failure of the latter investigators to observe an increase in LH secretion prior to puberty may be explained by the relative infrequent blood sampling near puberty, compared with frequent sampling undertaken by Lutz et al. (1984). Ovulatory releases of LH were reported to occur on the day of pubertal estrus (day 0) or the day before (day -1) the attainment of

puberty (Esbenshade et al., 1982; Diekman et al., 1983; Lutz et al., 1984). Mean ovulatory surge of LH as measured by the latter researchers varied from 1.0 to 6.2 ng/ml.

Mean serum concentration of estradiol increases gradually during the prepubertal period with a dramatic increase during the peripubertal period. Peak estrogen concentration in the systemic circulation occurs just prior to pubertal estrus. Lutz et al. (1984) observed a gradual rise in serum estrogen concentration from a mean of 8.4 $\pm$ 1.2 pg/ml on day 57 before puberty to a mean peak concentration of 14.0 $\pm$ 1.1 pg/ml just before puberty. Esbenshade et al. (1982) reported a similar estrogen pattern in prepubertal gilts with a mean peak concentration of this steroid (20 pg/ml plasma) occurring at 2.5 days prior to puberty. Over the next 6 days, plasma estrogen concentration declined to a basal level of 4 pg/ml. These data suggest that the increase in systemic estrogen concentration prior to pubertal estrus is due to the initiation of follicular growth.

Initial changes in LH secretion observed by Lutz et al. (1984) occurred while serum total estrogen levels remained relatively constant. These authors argued that perhaps a final maturation in the steroidogenic mechanisms occurred at the level of the ovary that altered the ratio of various estrogens synthesized. This alteration in estrogen synthesis presumably signaled the hypothalamo-hypophyseal system to evoke a change in the mode of LH secretion as was suggested to occur for the onset of puberty in rats (Taylor, 1961; Telegdy and Rubin, 1966). Alternatively, a change in the neuroendocrine mechanisms governing the frequency and amplitude of LH pulsatile release from the

pituitary could have matured independently of estrogen during prepubertal development. Studies with ovariectomized lambs (Ryan and Foster, 1979) and agonadal children (Conte et al., 1975;1981) support this latter possibility because LH release was observed in the absence of the ovaries.

Serum concentrations of follicle stimulating hormone (FSH) are high in young gilts but decline as puberty approaches. This change in FSH secretion is exemplified in the research of Diekman et al. (1983) who showed that mean serum FSH varied from a maximum of 31.3 $\pm$ .6 ng/ml at 10 weeks of age to a minimum of 13.3 $\pm$ 1.5 ng/ml at 14 weeks of age and remained relatively low until puberty. Mean serum concentration of FSH at puberty was 16.8 $\pm$ .8 ng/ml. Frequency of secretory spikes of FSH did not change in gilts 10 to 25 weeks of age (25 weeks = age at puberty). Average amplitude of FSH spikes was greater in gilts at 14 than at 18 weeks of age. No differences in the amplitude of FSH spikes were observed from 19 to 25 weeks of age.

Diekman et al. (1983) also reported a lack of significant changes in mean serum concentrations of prolactin (PRL), frequency of PRL spikes and amplitude of PRL spikes in gilts from 10 weeks of age to puberty, thus revealing a lack of association of systemic PRL to onset of puberty.

Synchrony of episodic secretions of LH, FSH and PRL in gilts from 2 months of age to puberty were reported by Diekman et al. (1983). According to their data, episodic secretion of LH, FSH and PRL were synchronized in gilts 10 to 18 weeks of age. Episodic secretion of FSH and PRL were synchronous in gilts 19 weeks of age to puberty, while

those of LH were asynchronous with those of FSH and PRL from 19 weeks of age to puberty.

Serum concentrations of progesterone were undetectable in the blood obtained from prepubertal gilts 10 to 24 weeks of age (Diekman et al., 1983). No changes were observed in progesterone concentrations immediately before or during the pubertal follicular phase (Esbenshade et al., 1982), thus suggesting that progesterone priming may not be a prerequisite for attainment of puberty in gilts. In heifers (Gonzalez-Padilla et al., 1975) and ewe lambs (Foster and Ryan, 1979; Berardinelli et al., 1979; Keisler et al., 1983; Legan et al., 1985), a transient increase in progesterone secretion has been shown to occur just before puberty. Esbenshade et al. (1982) showed that plasma progesterone levels increased on the first day of postpubertal estrus and reached a peak of 11 ng/ml on day 5 of the first estrous cycle (day 0 = day of onset of estrus) because of corpora lutea formation.

Hormonal Changes During the Estrous Cycle

Estrus is defined as the period when a female animal is receptive to the male and is popularly designated as day 0 of the estrous cycle. Estrus in the pig has been shown to be between 2 to 3 days in duration and is similar among all gilts regardless of the number of estrous cycles experienced (Andersson and Einarsson, 1980; Howard et al., 1983). The combination of physiological events that occur from the beginning of one estrus to the beginning of the next estrus is termed

estrous cycle, which in the pig lasts from 17 to 22 days, irrespective of sexual age (Andersson and Einarsson, 1980).

During the normal estrous cycle, onset of estrus is followed by a small surge of plasma FSH 1 to 9-hours after estrus that has an average duration of 19-hours (Van De Wiel et al., 1981). Maximal plasma levels of FSH ranged between 11.3 to 18.5 ng/ml and were observed 1 to 5-hours after the onset of estrus (Van De Wiel et al., 1981). However, other investigators found that maximum serum concentrations of FSH were attained on day 2 of the cycle (Rayford et al., 1974; Vandalem et al., 1979) and persisted for about 48-hours before declining to basal levels on day 6 (Vandalem et al., 1979). The FSH surge observed by Van De Wiel et al. (1981) at estrus is similar to that reported to occur at estrus in the ewe (L'Hermite et al., 1972). However, Rayford et al. (1974) and Brinkley (1981) found low serum FSH levels at estrus in gilts but these investigators may have missed the FSH surge because of infrequent blood sampling. The surge of FSH at estrus, coincides with decreased systemic estradiol-17β levels, which occur at this time (Brinkley, 1981; Van De Wiel et al., 1981). After the first small surge in FSH, a second rise of this gonadotropin was observed by Van De Wiel et al. (1981) that increased to approximately 20 ng/ml on day 3 after the onset of estrus. They found that this second rise in FSH closely paralleled the rise in plasma progesterone, which began to increase at 27 to 30-hours after the time of maximal plasma LH concentration. Subsequently, surges of FSH appear to occur from day 7 through 16 of the estrous cycle and then decline until the next perceptible rise at estrus (Brinkley, 1981). Redmer et al. (1985) have

shown that it is not the uptake of FSH by the maturing follicles that causes this reduction in systemic FSH, as previously speculated by L'Hermite (1972) in ewes, but rather, inhibin and estrogen produced by the maturing follicle negatively feedback on FSH secretion by the pituitary.

Findley et al. (1974), Aherne et al. (1976) and Ziecik et al. (1982) observed preovulatory LH surges 3 to 8-hours before estrus in weaned sows. Vandalem et al. (1979) observed a preovulatory LH peak 24 to 48-hours after the onset of estrus. The above results indicate that the preovulatory LH surge is not always coincident with the onset of estrus in gilts or sows. This could have serious implications on the timing of mating for maximum fertility and embryonic survival. A single insemination 24 to 30-hours after the onset of estrus, as recommended by Polge (1970) would not be sufficient for maximum fertility, especially when the preovulatory LH surge precedes the onset of estrus. In addition, the conventional 12 and 24-hour mating after the onset of estrus may not guarantee maximum fertility in gilts having a preovulatory LH surge 24 to 48-hours after the onset of estrus.

Mean concentrations of LH declined from 1.4 ng/ml on day 1 (Brinkley, 1981) to less than 1 ng/ml on day 2 in standard size pigs (Ziecek et al., 1982). A similar decline was observed in nulliparous and in multiparous miniature pigs on days 3 and 5, respectively (Howard et al., 1983). Luteinizing hormone levels remained low until day 8 when small surges of LH ($\leq$ 4.2 ng/ml) with a duration of 12 to 24-hours were imminent (Brinkley, 1981). Lower concentrations of plasma LH

persist from day 14 until proestrus (day -2) of the cycle (Brinkley, 1981), with a transient rise from day -1 and subsequent surge at the onset of the next estrus (Niswender et al., 1970; Henricks et al., 1972; Van De Wiel et al., 1981).

Plasma PRL concentrations fluctuate between 5 and 14 ng/ml at proestrus and increase markedly after a sharp rise in plasma estradiol-17β (Van De Wiel et al., 1981). According to these investigators, the initial rise in plasma PRL is followed by large fluctuations, with maximal levels of 19.8 to 77.4 ng/ml observed between day -1 and day 0 of the cycle. Duration of the PRL surge is 50 to 66-hours (Brinkley, 1981; Van De Wiel et al., 1981), and subsequently declines to concentrations of approximately 4.5 ng/ml (Brinkley, 1981). Van De Wiel et al. (1981) postulated that the rise in PRL concentrations at the onset of estrus was caused by increased synthesis and secretion of estrogen. A similar relationship between systemic estrogen and PRL secretion has been observed in rats (Neill et al., 1971) and cows (Karg and Schams, 1974). There appear to be limited changes in plasma PRL concentrations from day 2 through day 13, an interval encompassing most of the luteal phase of the estrous cycle. At the onset of follicular development, about day 14, several large surges of PRL with maximum concentrations between 17.5 and 25 ng/ml with durations of 24- hours were observed. These surges in PRL releases were found to continue through day -3 of the cycle (Brinkley, 1981). Peak prolactin secretion has been reported by Van De Wiel et al. (1981) to be coincident with declining levels of progesterone. It was suggested by

Brinkley (1981) that the PRL secreted during the follicular phase may exert a luteolytic effect on the corpora lutea.

According to Shearer et al. (1972) and Eiler and Nalbandov (1977), estrogen levels remained low following the decline after the onset of estrus. In contrast, a surge of estrogen was observed between days 1 and 5 of the estrous cycle with concentrations of 20 to 30 pg/ml (Henricks et al., 1972). According to Henricks et al. (1972) this surge of estrogen secretion was followed by smaller surges through day 10 of the cycle. Between days 10 and 13, a series of several surges of estrogen secretion of short duration occurred that terminated on day 14 (Brinkley, 1981). Estrogen levels then remained unchanged until days -3 and -2 when a surge occurred that caused the preovulatory release of LH at the onset of estrus (Brinkley, 1981).

A rise in systemic progesterone concentration is generally observed between days 3 and 4 of the estrous cycle (Tillson and Erb, 1967; Stabenfeldt et al., 1969; Tillson et al., 1970; Edqvist and Lamm, 1971; Henricks et al., 1972; Shearer et al., 1972; Parvizi et al., 1976; Brinkley, 1981; Van De Wiel et al., 1981; Magness and Ford, 1983), but the time of maximum systemic progesterone concentrations varies considerably. Maximum levels of this steriod were observed between days 7 and 8 (Stabenfeldt et al., 1969; Henricks et al., 1972; Brinkley, 1981), between days 9 and 12 (Tillson and Erb, 1967; Henricks et al., 1972; Parvizi et al., 1976; Magnes and Ford, 1983) and between days 13 and 14 (Edqvist and Lamm, 1971) of the cycle and ranged from 28 to 59 ng/ml as reported by Tillson and Erb (1967), Edqvist and Lamm (1971), Henricks et al. (1972), Shearer et al. (1972), and Magness and

Ford (1983). Henricks et al. (1972) reported that after the attainment of maximum levels, the concentration of this steroid plateaued for about 2 to 3 days. Systemic progesterone concentrations have been found to slowly decline by day 11 (Magnes and Ford, 1983), followed by a precipituous decline between days 14 and 16 (Henricks et al., 1972; Stabenfeldt et al., 1969; Edqvist and Lamm, 1971; Brinkley, 1981; Magnes and Ford, 1983; Edqvist and Lamm, 1971; Stabenfeldt et al., 1969).

Follicular Development and Oocyte Maturation at Puberty and during subsequent Estrous Cycles

Regulation of Follicular Growth by Pituitary Gonadotropins

The precise time and mechanism by which the pituitary gonadotropins begin to influence ovarian and follicular development are not well understood. Oxender et al. (1979) observed one to a few tertiary follicles in ovaries of gilts 60 to 90 days of age, possibly the stage when follicles become sensitive to gonadotropins. Dependence of follicles at this prepubertal stage on pituitary gonadotropins is supported by the following observations. Casida (1935) and Oxender et al. (1979) found that exogenous gonadotropins were ineffective in stimulating ovarian follicular development in 30-day-old and some 60-day-old prepubertal gilts, respectively. Oxender et al. (1979) however, found that two of four gilts ovulated in response to pregnant mare serum gonadotrpin (PMSG; 30 iu/kg body weight) on day 70 of age.

This latter period of sensitivity of follicles to gonadotropins occurs at approximately the same age as increased secretion of FSH (Diekman et al., 1983). In prepubertal gilts, the number of tertiary follicles peaks at about 130 days of age (about 45 days before onset of puberty) but a large percentage (67%) are atretic (Greenwald, 1978).

During a normal estrous cycle, healthy follicles in response to FSH undergo significant increase in size through mitotic division of granulosa cells and accumulation of exudates that lead to antrum formation and consequently to tertiary follicles (antral follicle). In contrast, atretic follicles, defined as follicles with degenerating oocytes in residence as well as granulosa or theca interna cells with more than 10 pyknotic nuclei per 10 um section, exhibit minimal changes in size and fluid accumulation in response to FSH (Daguet, 1978; Gerard et al., 1979). According to these researchers, by the third day after exposure to FSH during proestrus, two populations of follicles become eminent: those destined to ovulate and those becoming atretic. This may explain why Hunter et al. (1976) obtained superovulation in sows after human chorionic gonadotropin (hCG) administration on day 17 of the cycle, a time when serum FSH is elevated (Brinkley, 1981). A similar injection on days 18, 19 or 20, when serum FSH levels are low (Brinkley, 1981), did not significantly increase ovulation rate. Presumably the hCG injected on day 17 was able to rescue follicles that otherwise would have become atretic.

Clark et al. (1975) showed that in gilts total number of follicles increased from day 2 to 8, remained approximately the same from day 8 to 14 and then decreased progressively until day 20 of the cycle.

These investigators, however, observed that the number of Graafian follicles was low throughout the estrous cycle except at day 20. Follicles that have the potential to ovulate appear to start their growth between day 8 and 14 (Clark et al., 1975). These data suggest that more follicles are lost through atresia earlier and later in the cycle than during midcycle. It is highly unlikely that follicles induced to develop by the FSH surge at estrus are recruited into the ovulatory pool because 2 days have to elapse after exposure to FSH before final maturational changes can be induced by exposure to LH (Daguet, 1978; Gerard et al., 1979). Ovulation occurs 2 days after exposure of follicles to maximal levels of LH at estrus (4 days after initial exposure of follicles to FSH; Daguet, 1978).

Action of Gonadotropins on Follicular Theca and Granulosa Cells

Follicle stimulating hormone has been shown to increase the population of granulosa and theca cells per follicle by increasing their mitotic rates (Daguet, 1978). It also increases the number of gap junctions among granulosa cells (Albertini et al., 1975; Anderson and Albertini, 1976) that permit passage of FSH-induced cyclic adenosine-3'-5'-monophosphate (cAMP) from one cell to another. Follicle stimulating hormone-activated adenylate cyclase in peripheral cells may result in the distribution of cAMP to all granulosa cells including the cumulus oophorus (Thibault, 1977; Racowsky, 1985). Dekel and Kraicer (1978), Eppig, (1979, 1980), Dekel et al. (1979), Bae and Channing (1981), Salustri and Siracusa (1983) and Racowsky (1985) have

demonstrated that cAMP is responsible for the expansion of the oophorus as well as progesterone secretion (Racowsky, 1985).

Daguet (1978) has shown that LH augments an FSH-induced increase in the rate of mitosis in both granulosa and theca cells, and increases cell volume and intercellular space of granulosa cells in tertiary follicles. However, after the preovulatory LH surge the rate of mitosis in granulosa and theca cells declines (Corner, 1919; Daguet, 1978) and the demarcation between the avascular granulosa and theca interna disappears. Concomitantly, the morphology of the granulosa cells adjacent to the basal lamina changes from one of columnar shape to a more rounded shape (Bjersing, 1967; Daguet, 1978). Follicle stimulating hormone also induces the formation of LH receptors in granulosa cells during final growth of the follicle (Channing and Kammerman, 1974; Daguet, 1979). Nakano et al. (1977) reported that FSH binds only to granulosa cells but the pattern of binding is different from that of LH in that the number of FSH receptors per cell does not increase as the follicle enlarges. It has been reported that follicular fluid estradiol concentration increases as pig follicles mature (Hunter et al., 1976; Gerard et al., 1979; Ainsworth et al., 1980). Daguet (1979) also detected high follicular fluid estradiol concentrations just before increased LH binding by granulosa cells and suggested that estradiol may be involved in the induction of LH receptors. Evidence for the involvement of steroids in receptor induction/responsiveness was provided by Veldhius et al. (1982) who reported that estradiol amplified the stimulatory effects of FSH on steroid production by porcine granulosa cells. It has also been

demonstrated that FSH together with insulin, cortisol and thyroxine stimulate de novo synthesis of LH receptors in granulosa cells from small porcine follicles (< 2 mm; Loeken and Channing, 1985).

Specific receptors for prolactin in granulosa tissue from pig follicles have been described and the level of specific binding is greater in small (1-2mm) than in large (7mm) follicles (Rolland et al., 1976; Veldhuis et al., 1980). In vitro cultures of granulosa cells with prolactin produced an inhibitory effect on progesterone accumulation in cells from small follicles and a stimulatory effect on granulosa cells from large follicles (Veldhuis et al., 1980), indicating a possible physiological role for prolactin acting at the ovarian level to control follicular steroidogenesis.

Before tertiary follicle formation, it appears that granulosa cells acquire a small complement of FSH and estradiol receptors. As the number of granulosa cells increase in proliferating follicles, the total number of FSH and estradiol receptors for each follicle increase, followed by a consequent secretion of small amounts of estradiol (Richards et al., 1976). As this proliferative process advances and tertiary follicles containing theca interna develop, the cellular components necessary for the current "two-cell" hypothesis for estradiol production originally proposed for the rat (Falck, 1959) are present (Armstrong et al., 1979; Leung and Armstrong, 1980). The granulosa cells are the predominant site of progesterone synthesis and are responsive to LH, FSH (Evans et al., 1981; Stoklosowa et al., 1982) and prolactin (Veldhuis et al., 1980). Although porcine granulosa cells lack the 17α-hydroxylase enzymes necessary to synthesize

androgens (metabolites for the resident aromatase in granulosa cells) from progesterone or pregnenolone (Bjersing and Carstensen, 1967), substantial LH-induced androgen synthesis by the theca interna of pig follicles has been demonstrated by Stoklosowa et al. (1978), Tsang et al. (1979a), Evans et al. (1981) and Stoklosowa et al. (1982). The major androgen produced by the thecal tissue is androstenedione (Evans et al., 1981) which, after transfer to the granulosa tissue, is converted to testosterone and then aromatized to estradiol under the influence of FSH (Christenson et al., 1985).

In contrast to many other species, the porcine theca interna, in addition to producing androgens, synthesizes estradiol in quantities comparable to levels produced by the granulosa cells (Evans et al., 1981; Haney and Schomberg, 1981; Stoklosowa et al., 1982). Stoklosowa (1982) reported considerable estrogen synthesis by both granulosa and thecal tissue during monolayer culture, but significantly more estrogen was produced when the two cell types were cultured together. In terms of estradiol synthesis by granulosa cells, Stoklosowa et al. (1982) reported significant stimulation by both LH and FSH. In many other species including the rat (Falck, 1959) aromatase activity is confined to the granulosa cells and thus in immature follicles this enzyme is controlled by the binding of FSH to granulosa cells (Richards and Midgley, 1976; Armstrong and Dorrington, 1977; Erickson and Hsueh, 1978). Because thecal cells of the pig also possess an aromatase (Evans et al., 1981; Stoklosowa et al., 1982), the possibility exists that LH may activate the aromatase in this tissue. Thecal tissue is responsive to LH, but not FSH in the production of androgens, which

increase as follicular development advances (Evans et al., 1981). It could be surmised that aromatase activity in the thecal tissue is activated by LH and androgen produced by the thecal tissue is partitioned between thecal and granulosa tissues for aromatization to estradiol.

Due to the close association of the theca interna to ovarian capillaries, this tissue may be a more important source of systemic estrogens (Evans et al., 1981), whereas granulosa cells may contribute more to follicular fluid with consequent effects on intrafollicular function (McNatty et al., 1979; Armstrong et al., 1981). Effects of in vivo removal of granulosa cells from preovulatory follicles on secretion of estrogens are somewhat equivocal. Channing and Coudert (1976) reported little contribution by granulosa cells to ovarian vein estradiol levels after the LH surge in rhesus monkeys, whereas Armstrong et al. (1981) reported a substantial decline in estradiol levels following in vivo removal of granulosa cells from ovine follicles prior to the LH surge. Similar experiments in gilts would be considerably difficult to perform in view of the larger population of follicles in this species. However, data from an in vitro study conducted by Evans et al. (1981) demonstrated that at least in most mature preovulatory follicles, the pattern of estradiol synthesis in response to the presence of androgen is closer to the pattern of systemic estrogen levels in vivo, i.e. rising estrogen levels until the onset of estrus and LH surge or hCG injection, and declining thereafter (Hunter et al., 1976; Eilar and Nalbandov, 1977). In addition, in vivo estrogen production by granulosa cells is dependent on thecal androgen

production, thereby emphasizing the importance of theca interna in the control of estrogen synthesis (Evans et al., 1981). According to these investigators, the probable physiological importance of the granulosa cells as a source of estradiol in follicular fluid, should not be discounted.

Interrelationships Between Follicular Fluid Steroid Levels and Oocyte Maturation

During proestrus in prepubertal gilts (Ainsworth et al., 1980, 1982) and cyclic sows (Eiler and Nalbandov, 1977), follicular fluid concentrations of androgens, estrogens and progestins increase steadily (with progesterone dominating) until the onset of estrus. The increase in these hormones is believed to result from stimulation of steroidogenesis by the ascending limb of the preovulatory LH surge (Niswender et al., 1970; Henricks et al., 1972; Parvizi et al., 1976; Van De Weil et al., 1981), when the follicles are about 7 mm in diameter (Gerard et al., 1979). At this stage, no changes are observed in the dictyate nucleus of the oocyte (Hunter and Polge, 1966; Ainsworth et al., 1980). However, the maturing porcine oocyte is intensively engaged in RNA synthesis and accumulation (Crozet et al., 1981; Motlik et al., 1984). Oocyte cytoplasmic maturation occurs as a result of the temporary increase in intrafollicular estradiol, 17 hydroxyprogesterone or testosterone, induced by the preovulatory LH surge (Thibault, 1977). During fertilization, the matured cytoplasm of oocytes should be able to reduce the disulfide bonds that exist in the

cysteine residues of protamine of the sperm nucleus (Calvin and Bedford, 1971; Mahi and Yanagimachi, 1975; Zirkin et al., 1982; Perreault et al., 1984), which leads to sperm nucleus decondensation. Failure of cytoplasmic maturation to occur results in nondissociation and swelling of the male nucleus after ovum penetration. The sperm head remains unchanged for several hours, and when swelling does occur, grains of condensed chromatin remain in the male pronucleus. Finally, such eggs cleave but do not develop further (Thibault, 1977). Follicular fluid steroid concentrations slowly decline on the first day of estrus and then abruptly decrease as time of ovulation approaches in cyclic sows (Eiler and Nalbandov, 1977). The abrupt decrease in follicular fluid steroid levels occur as a result of the desensitization of the granulosa adenylate cyclase system (Zor et al., 1976), inhibition of 17-20 desmolase and 17β-hydroxysteroid dehydrogenase (Thibault, 1977) induced by maximal LH levels at estrus. In gilts exhibiting estrus for the first time, concentrations of androgens and estrogens follow similar patterns as those observed in cyclic sows. However, follicular fluid concentrations of progesterone after the initial rise during proestrus, stabilize at the onset of pubertal estrus and then markedly increase to about twice their concentration during estrus; about 6-hours before ovulation (Ainsworth et al., 1980). Exposure of oocytes to high levels of progesterone during the protracted proestrus in prepubertal gilts (Andersson and Einarsson, 1980) compared with that of cyclic sows may be related to the higher incidence of embryonic mortality in pubertal gilts (Warnick et al, 1951; MacPherson et al., 1977). This cause of embryonic

mortality is supported by research of Day and Polge (1968) who showed that administration of progesterone to gilts prior to mating caused chromosomal abberations by permitting polyspermic fertilization. Polyspermy in pigs results in embryonic mortality (Bishop, 1964)

Nuclear maturation begins with the resumption of meiosis that occurs concurrently with the loosening of cumulus oophorus from the oocyte, the appearance of an active maturation promoting factor (Jahn et al., 1976) and a substantial decrease in RNA synthesis (Motlik and Fulka, 1986) at about 16 to 18-hours after the onset of estrus. These processes are initiated by the preovulatory surge of LH (Spalding et al., 1955; Eiler and Nalbandov, 1977; Gerard et al., 1979) or can be induced by a single injection of hCG in late proestrus (Hunter and Polge, 1966; Daguet, 1978) in conjunction with the reduced follicular fluid estradiol/progesterone ratio (Gerard et al., 1979). Loosening of the cumulus mass from the oocyte removes the source of oocyte maturation inhibitor. This inhibitor has been identified as a nonspecies specific (Motlik and Fulka, 1986), nonpolar, nonpeptide, hydrophobic, putative maturation inhibitory factor with a molecular weight of less than 1000, synthesized by the granulosa cells, and synergizes with cAMP-dependent processes to inhibit oocyte maturation (Eppig et al., 1983; Down and Eppig, 1984; Racowsky, 1985). Hunter and Polge (1966) have studied nuclear maturation in porcine oocyte extensively. Based on their data, the longest single stage in nuclear maturation after the onset of estrus is that of the germinal vesicle or dictyate nucleus (18-hours). At prometaphase, which occurs between 18.25 to 21-hours after the onset of estrus, the nuclear membrane of

oocytes become irregularly shaped and less well defined than in the germinal vesicle stage, and the chromatin condenses into small lumps or fragments. By 26-hours after hCG injection, the nuclear menbranes disappear and shortly thereafter the first distinct arrangement of chromosomes on the metaphase plate becomes discernable. The assembly of chromosomes on the metaphase plate lasts about 8 to 10-hours after visible resumption of meiotic activity, after which time (36-hours after onset of estrus) the first polar body from a majority of the large preovulatory follicles is extruded.

Mechanism of Ovulation

Hemovascular Events Preceding Ovulation

Preovulatory surge of LH causes a conspicuous change in the ovarian vasculature with a significant increase in ovarian blood flow (Wurtman, 1964; Lee and Novy, 1978). This increase in ovarian circulation is associated with the hyperemic condition that develops in follicles that respond to LH (Espey, 1974). Along with vasodilation, there is an increase in vascular permeability in the follicles (Espey, 1978). These vascular changes cause the follicle to become edematous, a condition that persists through the time of follicular rupture (Szego and Gitin, 1964; Espey, 1967; Bjersing and Cajander, 1974; Parr, 1974 and Cherney et al., 1975). Near the time of ovulation erythrocytes permeate the walls of the blood vessels and form petechiae in the interstitial spaces of the follicles (Parr, 1974; Espey, 1978). These

LH-induced ovarian vascular dynamics are similar to those that occur during an inflammatory reaction and can be mimicked by histamine, but not by FSH or serotonin (Wurtman, 1964).

Several hours after mating, basophils begin to accumulate in the blood vessels around large follicles and a few hours before ovulation these cells accumulate conspicuously in follicles destined to ovulate (Zachariae et al., 1958). The site of accumulation of these cells is characterized by edema, and maximum accumulation is attained a few hours after ovulation at which time the leukocytes pass freely from the vessels to the newly formed corpora lutea (Zachariae et al., 1958). In addition to basophils, other granulocytes and thrombocytes appear in follicles near the time of ovulation (Espey, 1980). Accumulation of these cells well ahead of the moment of follicular rupture signifies that chemotactic substances are being produced even before the tissue has been injured by the actual phenomenon of rupture. Migration of these cells also indicates that reactions similar to those found in inflammation are occurring during the ovulatory process (Espey, 1980).

Thecal tissue that encapsulates mature follicles contains many fibroblasts that become mitotically active as ovulation approaches (Espey, 1980). Specific metabolic factors that stimulate the activation of fibroblasts at this time is not known but could be attributed to the preovulatory LH surge in as much as it occurs at the time of LH dominance. At the time of ovulation, thecal fibroblasts migrate into the stratum granulosum, laying down collagenous support for the mass of developing lutein tissue (Espey, 1978). This movement of fibroblasts suggests that the lutein granulosa produces a

chemotactic agent that attracts the surrounding fibroblasts from the theca externa. The nature of this chemotactic substance is not known. In other tissues, lymphokines and peptides that arise from collagen degradation serve as chemotactic stimuli for fibroblasts (Kang, 1978). However, the granulosa contains neither blood vessels nor collagen; therefore, this layer is an unlikely source of either lymphocytes or collagen fragments.

Biochemical Mechanism of Ovulation

The preovulatory LH surge is followed in about 4-hours by a rapid increase in follicular fluid concentrations of cAMP (Tsang et al., 1979b; Ainsworth et al., 1980). A second less-pronounced rise in follicular fluid concentrations of cAMP (Tsang et al., 1979b; Ainsworth et al., 1980) occurs between 30 and 32-hours after the LH surge (Ainsworth et al., 1980). The initial rise in follicular cAMP mediates the temporary rise in steroidogenic response of gonadotropin-stimulated follicles (Marsh and LeMaire, 1973), with progesterone being the most abundant steroid synthesized (Eiler and Nalbandov, 1977; Ainsworth et al., 1980). Selective progesterone dominance results in the shift of estradiol/progesterone ratio from 2.00 to .115 (Gerard et al., 1979). The role of progesterone in the ovulatory process is to stimulate collagenase activity in the follicle wall (Rondell, 1974), which in the final stages of the ovulatory process causes the follicle wall to become flaccid and distensible (Espey, 1978). Decomposition of follicular connective tissue accelerates rapidly during the final hours

preceding ovulation (Espey, 1980). Lipner and Greep (1971) demonstrated that inhibition of progesterone synthesis prevents ovulation.

The second peak in cAMP occurs at about the same time prostaglandin levels begin to rise in follicles, which in the sow occurs between 30 and 32-hours after the onset of estrus (Tsang et al., 1979b; Ainsworth et al., 1982). Production of prostaglandin $F_{2\alpha}$ (PGF) in the preovulatory follicle may also be mediated by histamine (Espey, 1980). Support for the role of histamine in PGF production comes from the data of Szego and Gitin (1964) who showed ovarian histamine depletion within 2-hours after intravenous administration of LH during the time when follicles first become hyperemic. Further evidence for the role of histamine in the ovulatory process comes from experiments which show that antihistamines, such as chlortrimeton and chlopheniramine, inhibit ovulation in vivo in rabbits (Knox et al., 1979) and in vitro in the perfused rabbit ovary (Wallach et al., 1978). Addition of PGF to the perfusion fluid reverses the antiovulatory effect of chlopheniranamine. Synthesis of PGF in the follicles is apparently not restricted to one specific site. Granulosa cells produce significant amounts of PGF (Plunkett et al., 1975; Erickson et al., 1977; Clark et al., 1978) as do theca cells (Erickson et al., 1977). Prostaglandins, especially of the E-type, stimulate the formation of cAMP in the follicle (Kuehl et al., 1970; Lamprecht et al., 1973; Zor et al., 1973; Nilsson et al., 1974). According to Armstrong et al. (1976), Rigler et al. (1976) and Lee and Novy (1978), prostaglandins stimulate cAMP by a mechanism different from that of LH

because the effects of each are separable and additive. Furthermore, follicles that have become refractory to LH remain fully responsive to prostaglandins (Lamprecht, 1973). These findings suggest that LH and prostaglandins act at different sites in the ovary. Luteinizing hormone could be acting on the granulosa and theca interna, while prostaglandins might stimulate cAMP formation in the fibroblasts in the theca externa and adjacent theca tunic (Espey, 1980). Based on this information, it seems possible that prostaglandins are responsible for the second phase of cAMP formation during the ovulatory process as observed in the rabbit (Espey, 1980). This possibility is supported by the research of Goff and Major (1975), who demonstrated that inhibition of PGF synthesis with indomethacin prevented the second cAMP peak without impairing the initial LH-induced increase in cyclic nucleotide. Maximal follicular fluid concentrations of prostaglandins occur at about 40-hours after the onset of estrus, around the time of ovulation (Ainsworth et al., 1975).

The preovulatory increase in PGF is essential for ovulation. This vital role of PGF is exemplified in the studies of Tsafriri et al. (1973), Yang et al. (1973), Lau et al. (1974), Armstrong and Zamecnik (1975) and Wallach et al. (1975), who found that inhibition of PGF synthesis prevented ovulation, but not luteinization. Exogenous PGF is especially effective in reestablishing the ovulatory process in animals in which PGF synthesis has been inhibited (Armstrong et al., 1973; Diaz-Infante et al., 1974; Wallach et al., 1975; Hamada et al., 1977; Hamada et al., 1978; Wallach et al., 1978). It has been suggested that PGF causes the rupture of the follicle by increasing ovarian

contractility (Espey, 1978). However there is no convincing evidence that ovarian contractions are an essential part of the ovulatory process (Espey, 1978). Prostaglandin $F_{2\alpha}$ has been implicated in the synthesis, release and/or activation of a collagenase-like ovulatory enzyme (Marsh and LeMaire, 1973; LeMaire and Marsh, 1975). Indirect support for this concept comes from evidence that PGF causes the release of lysosomal enzymes in luteal tissue (Kaley and Weiner, 1975). If prostaglandins mediate proteolytic activity in the preovulatory follicle, they probably do so by some mechanism other than by direct activation of latent collagenase (Espey and Coons, 1976). Prostaglandin E_2 stimulates the production of plasminogen activator (Stickland and Beers, 1976), thus increasing plasmin activity. Activation of plasminogen to plasmin has been suggested by Espey (1980) to be a relevant step in the mechanism of ovulation because plasmin is capable of activating procollagenase.

The freeing of the oocyte inside the follicle is the only direct action of both preovulatory LH and FSH surges as evidenced in the study of Thibault et al. (1975), who observed cumulus cell dissociation exclusively in the presence of LH and FSH.

Timing and Duration of Ovulation

Studies by Dziuk and Baker (1962), Dziuk and Polge (1965) and Signoret et al. (1972) have shown that ovulation occurs approximately 40-hours after hCG injection in the gilt. Similarly, spontaneous ovulation in the pig occurs approximately 36-hours after the onset of

estrus (Gerard et al., 1979). The discrepancy of 4-hours could be attributed to failure to detect the onset of estrus precisely in the latter study. Mating has been shown to hasten the onset of spontaneous ovulation in the pig. According to Signoret et al. (1972) the initiation of spontaneous ovulation in gilts mated at the onset of estrus and 6-hours thereafter is 34.1-hours compared with 38-hours in nonmated gilts. The effect of mating on the time of ova release is not well understood. Niswender et al. (1970) suggested that effects of stimuli associated with mating could be mediated by modifications in the patterns of discharge of pituitary gonadotropins that occur within the first 8-hours after the onset of estrus. However, Zimmerman and Nader (1971) reported that two copulations 12-hours apart did not affect the time of onset of spontaneous ovulation. A possibility exists that ovulation occurred earlier in the study of Signoret et al. (1972) because multiple mating with a shorter time interval between matings was used which is coincident with the increasing rate of LH discharge at the beginning of estrus (Niswender et al., 1970).

The data of Signoret et al. (1972) have shown that the phenomenon of multiple matings with a short time interval between matings reduces the duration of ovulation to approximately 1-hour compared with either nonmated gilts (3.8-hours) or gilts mated twice at 12 hourly intervals (Zimmerman and Nader, 1971).

Ovulation Rate

It has been established that ovulation rate is lower at puberty

than any other successive estrous cycle (Warnick et al., 1951; Anderson and Melampy, 1972; Brooks and Cole, 1974; Andersson and Einarsson, 1980), and constitutes one of the major constraints on first litter size (King and Williams, 1984). Delaying mating until second or third estrus allows the use of short-term high-level feeding to increase ovulation rate. The subject of flushing has been extensively reviewed (Anderson and Melampy, 1972; Brooks and Cole, 1974; Den Hartog and van Kempen, 1980). It is firmly established that increasing feed or energy intake before mating will increase ovulation rate, and the optimum period of increased intake appears to be in excess of 11 days. Variable results are obtained when gilts are given increased energy intake on the day before or on the day of mating, while no effect is noted if it is given on the day after mating.

What remains to be determined is whether flushing increases ovulation rate beyond that which would normally be expected, or reverses a nutritional inhibition due to previous low-level feeding. That the latter is a possibility is suggested by the average daily energy intakes of gilts fed high and low energy diets [41.1 and 22.5 MJ metabolizable energy (ME)/day respectively; Den Hartog and van Kempen, 1980]. Energy intake for the restricted gilts was, however, far below what would normally be fed in commercial practice. Anderson and Melampy (1972) suggested that optimal ovulation rate is obtained by about a 25 MJ ME/day increase in energy intake. Thus, for gilts previously fed only 17.6 MJ ME/day, the final daily energy intake would be 42.6 MJ ME which is close to that recommended by the NRC (1979) for a 100 kg gilt.

Source or level of protein appears to have little effect on ovulation rate (Fowler and Robertson, 1954; Zimmerman et al., 1967). Over a short period (1 cycle) protein deprivation has little effect on ovulation rate. However, prolonged feeding (4-6 cycles) of a protein-free diet results in a significant reduction in ovulation rate (McGillivray et al., 1964).

Morphological and Histological Changes at Puberty and Subsequent Estrous Cycles of the Gilt

Puberty in any species is synonymous with the attainment of reproductive competence. Schnurrbusch and Erices (1979) reported that puberty in the gilt was characterized by enhanced uterine growth as reflected by increased thickness of the endometrium, and myometrium, and increased height of glandular epithelium and glandular area. In order to be reproductively competent, an animal must have a fully developed uterus. Growth of the uterus is influenced by its first major exposure to estrogen (Baulieu et al., 1971), which in the gilt occurs approximately 2.5 days prior to the attainment of puberty (Esbenshade et al., 1982). Significant endometrial and myometrial growth were observed to continue during each succeeding estrous cycle (Schnurrbusch and Erices, 1979) probably as a result of exposure of the uterus to elevated estrogen during each cycle. These investigators, however, did not observe any changes in glandular epithelial height, number of glands and glandular area after puberty. Based on the differences observed in the morphology and histology of the uterus

between pubertal and multiestrous gilts, Schnurrbusch and Erices (1979) recommended that gilts should not be mated initially at pubertal estrus but rather at the second estrus. This implies that the uteri of pubertal gilts may not be competent enough to support embryonic development as efficiently as those of multiestrous gilts and thus may contribute to a higher incidence of embryonic mortality.

Establishment of Pregnancy

Mating in the pig occurs during estrus which lasts from 24 to 72 hours (Bazer and First, 1983), and if the gilt or sow becomes pregnant a gestation period of 114 to 115 days follows. Hormonal profiles associated with the first 14 days of the estrous cycle and pregnancy are essentially identical, after which time functional corpora lutea (CL) must be maintained for the duration of pregnancy. Loss of CL function at any stage of gestation leads to abortion within 24 to 36 hours (Belt et al., 1971). Plasma progesterone concentrations of 30 to 40 ng/ml on day 12 to 14 of gestation decrease to 10 to 25 ng/ml by day 25 of gestation (Guthrie et al., 1972; Robertson and King, 1974; Knight et al., 1977), and then remain relatively constant through the remainder of the first trimester of gestation.

First Trimester Embryological Development of the Pig

There are several key events in conceptus development during

pregnancy. The pig embryo is transported from the oviduct into the uterus at about the 4-cell stage (60 to 72 hours after the onset of estrus; Bazer and First, 1983). The embryo reaches the blastocyst stage by day 5 and the zona pellucida is shed (hatching) between days 6 and 7 (Bazer and First, 1983). The exposed blastocyst expands from .5 to 1 mm diameter at hatching to 2 to 6 mm on day 10 and then elongates rapidly to the filamentuous form by day 16 (Geisert et al., 1982b). Between the 9 to 10 mm spherical stage and the 100 to 200 mm long filamentuous stage, pig blastocysts elongate at a rate of 30 to 45 mm/hour, apparently by cellular remodelling and not cellular hypertrophy (Geisert et al., 1982b). However, continued elongation and growth of the conceptus from approximately 200 mm to 1,000 mm on day 14 to 16 does involve cellular hypertrophy (Geisert et al., 1982b). The factor(s) responsible for initiation of blastocyst elongation is not known. Failure of blastocyst elongation may lead to death of the embryo early in gestation or compromise placental development (Perry and Rowlands, 1962; Knight et al., 1977).

Rapid expansion and development of the allantois occurs between day 18 and 30 of gestation (Bazer and First, 1983). According to these investigators, fusion of the chorion and allantois takes place between day 30 and 60 of pregnancy, and by day 60 to 70 placenta development is complete. Placentation in the pig involves interdigitation of microvilli on the surface of the trophoblast and epithelial cells lining the uterine endometrium. Because placentation is superficial in the pig, the direct transfer of nutrients (histotroph) from glandular

epithelium and endometrial surface to the chorioallantois occurs at least through the second trimester of gestation.

Plasminogen activator (PA) is involved in invasive growth of cells (Ossowski et al., 1973), especially those of trophoblast, and is produced by the pig blastocyct (Mullins et al., 1980). Pig blastocysts transplanted to an ectopic site, e.g., uterine stroma (Samuel and Perry, 1972) or kidney capsule (Samuel, 1971) are highly invasive. Within the uterine lumen, however, pig blastocysts do not undergo invasive implantation, rather they attach. Fazleabas et al. (1982) purified and characterized progesterone-induced protease inhibitors secreted by the pig uterine endometrium that are believed to protect the uterus from proteases , such as PA, released by the trophoblast. It is possible that the PA may act within the conceptus to promote cellular remodelling during blastocyst elongation (Bazer and First, 1983), but is prevented from affecting the uterus by uterine production of protease inhibitors that appear to coat and be taken up by trophectoderm (Fazleabas et al., 1982).

Estrogen Synthesis and Intrauterine Migration

Pig blastocysts produce estrogens (Perry et al., 1976), the synthesis of which begins at the 10 mm spherical stage (Geisert et al., 1982a), around day 11 and 12 of gestation (Perry et al., 1976), and which is coincident with the occurrence of intrauterine migration of blastocysts (Dhindsa et al.,1967). Estrone sulfate in maternal plasma

indicates that estrogen secretion by the pig conceptus during the first trimester of pregnancy is biphasic with peaks on days 10 to 12 (Stoner et al., 1986), and 16 to 30 (Robertson and King, 1974; Stoner et al., 1986).

The popular explanation for intrauterine migration (transport) of embryos includes an involvement of the uterine musculature (Pope et al., 1982c). Increased synthesis of estradiol by the porcine embryo was found to occur concomitantly with migration of the embryos and increased myometrial activity in vitro (Pope et al., 1982b).

The association between estradiol synthesis and migration of the embryos may be more than coincidently related. Pope et al. (1982c) observed that neither the embryo nor estradiol directly stimulated the myometrium in vitro as was observed in vivo. However, uterine flushings of days 6,9 and 12 pregnant gilts increased the frequency of myometrial contractions in vitro during a 5-minute exposure period. Furthermore, flushings of day 12 gilts overcame the inhibitory effects of indomethacin, suggesting an indirect action of estradiol on myometrial function through synthesis of prostaglandins (Pope et al., 1982b). Alternatively, the action of estradiol on the uterus may be mediated in part though histamine release (Pope et al., 1982c). Histamine, by increasing uterine blood flow (Harvey and Owen, 1979), could indirectly increase myometrial activity (Pope et al., 1982c) sufficiently to initiate migration of the embryos.

Initially, embryos remain in the vicinity of the tip of the uterine horn until day 6, and then are transported through the horn

during days 7 and 8. By day 9 of gestation, some embryos enter the uterine horn opposite to the horn of origin and by day 12 fully occupy both horns (Dhindsa et al., 1967). Number of embryos migrating does not influence the rate of passage. Furthermore, migration of embryos is a random process with slightly more embryos remaining in the horn of origin than migrate to the contralateral horn (Dziuk et al., 1964).

It has been demonstrated that embryos do not influence the migration of other embryos within the uterine horn. These inferences are based on the data of Dziuk et al. (1964) who showed that when embryos from mated black donors were transferred into the tip of one uterine horn of a recipient gilt and embryos from mated white donors were placed in the tip of the other horn, fetuses of both colors were found mixed in both uterine horns.

Intrauterine Spacing of Embryos

Although migration of an embryo may not be influenced by other embryos, spacing within the uterus seems to be affected by other embryos. By restricting embryos to a predetermined uterine space through ligating the uterus at appropriate locations, Dziuk (1968) showed that at 30 days or later, fetuses were spaced equidistant from each other regardless of availability of uterine space. Crowding did not affect the proportion of embryos that survived to day 30 of gestation (Dziuk, 1968). Equidistant spacing between embryos, regardless of the total space available, indicates that spacing is not

entirely passive but that embryos can exert a repelling effect on each other (Dziuk, 1985). An embryo could create space around itself by promoting localized uterine contractility which would tend to oppose contractility in adjacent portions of the uterus and hence prevent the migration of embryos towards each other (Dziuk, 1985). Dziuk (1985) argued that if several embryos in the same uterus were each generating such repelling contractions, embryos would move to positions at the greatest distance from the origin of such contractions. Furthermore, any capacity to affect local uterine activity must occur after intrauterine migration and mixing but before final attachment. This period of spacing must be no longer than a few hours near day 12 of gestation (Dziuk, 1985). Insufficient estrogen production by the embryos may contribute to embryo wastage due to inadequate intrauterine migration and subsequent improper intrauterine spacing.

Maternal Recognition of Pregnancy

Corpora lutea of the pig must be maintained for continuous progesterone secretion if pregnancy is to be established and maintained to term. In the pig, therefore, maternal recognition of pregnancy is due to signals, presumably from the conceptus, that prevent the uterus from exerting a luteolytic effect on the CL. Possibly, estrogen produced by the embryo prevents PGF secretion from the uterine endometrium into the uterine venous drainage, where it could gain access to the CL and cause luteolysis (Bazer and Thatcher, 1977).

Studies of utero-ovarian vein plasma concentrations of PGF indicate that they are elevated during the period of luteolysis in nonpregnant gilts, whereas no significant changes appear to occur in pregnant gilts between days 12 and 25 of gestation (Bazer et al., 1982). The stable metabolite of PGF, 13,14- dihydro-15-keto PGF (PGFM) has also been studied in systemic plasma of nonpregnant and pregnant gilts by Shille et al. (1979). Their results support those previously observed for utero-ovarian vein plasma PGF in that PGFM concentrations in systemic plasma were elevated during luteolysis in nonpregnant gilts but not in pregnant gilts between days 12 and 20 of gestation.

Kidder et al. (1955) reported that diethylstilbestrol (DES) injected on day 11 of the estrous cycle prolonged the life of the CL. Frank et al. (1977) observed that utero-ovarian vein basal and peak PGF concentrations, as well as number of PGF peaks were lower between days 12 and 20 of the estrous cycle in gilts treated with estradiol valerate on day 11 through 15, compared with that of control gilts. The reduced utero-ovarian vein PGF levels in estradiol valerate-treated gilts were associated with an interestrous interval of 146.5 days compared with 19.0 days for control gilts.

The theory of maternal recognition of pregnancy is based on evidence that estrogen, produced by the blastocysts from days 12 to 30 of gestation or injection of estradiol valerate, changes the uterus from an endocrine to an exocrine organ with respect to the secretion of PGF (Bazer et al., 1982). The precise mechanism whereby estrogen alters the secretion of PGF is not known. In pregnant gilts PGF is

secreted into the uterine lumen and subsequently sequestered to the endometrium to prevent drainage into the uterine venous circulation to affect the CL (Bazer and First, 1983). That the luteotropic effect of estrogen is exerted at the level of the uterus in swine is supported by several lines of evidence: (1) removal of the uterus from intact gilts without hormone therapy, prolonged CL maintenance (Bazer and First, 1983); (2) CL of pregnant gilts (Diehl and Day, 1974) and estradiol valerate-treated gilts (Kraeling et al., 1975) are susceptible to the luteolytic effect of exogenous PGF; (3) production of PGF by the endometrium of pregnant gilts is similar to that of nonpregnant gilts (Zavy et al., 1980) and (4) prostaglandin $F_{2\alpha}$ is secreted into the uterine lumen and transferred from there to uterine venous circulation (Lamotte, 1977). Other evidence suggests a direct effect of steroids and unspecified embryonic factor(s) on luteal function in the pig (Chakraborty et al., 1972; Ball and Day, 1982ab).

The fate and role of PGF sequestered within the uterine lumen of pregnant gilts have not been studied extensively. Walker et al. (1977) reported that conceptus membranes, especially the amnion, have a high capacity for metabolizing PGF to PGFM, which is believed to be biologically inactive. On the other hand, Kennedy (1980) suggested that PGF is required for attachment of the embryos to the uterine wall.

Uterine Secretions During Pregnancy

The term histotroph refers to secretions (histopoietic material)

and detritus (histolytic material) present within the uterine lumen for nourishment of developing conceptuses (Bazer and First, 1983)). In domestic animals, implantation is noninvasive and uterine endometrial gland secretion of histotroph appears to be required for a major portion of pregnancy (Bazer and First, 1983). Murray and Grifo (1976) demonstrated that the concentrations of these uterine secretions were consistently lower in prepubertal gilts induced to ovulate than in cyclic gilts. It is conceivable that if this pattern of uterine secretions were to hold true for gilts that attain puberty naturally, enough histotroph would not be produced, and thus embryonic survival could be compromised on the part of mated pubertal gilts compared with multiestrous gilts.

The most extensively studied protein in porcine uterine secretions is uteroferrin. Uteroferrin is a progesterone-induced glycoprotein secreted by uterine gland epithelial cells (Fazleabas et al., 1985). This protein has a molecular weight of 35,000, a pI of 9.7, acid phosphatase activity, and contains a molecule of iron (Schosnagle et al., 1974; Bazer and First, 1983). The primary role of this protein appears to be the transport of iron from the uterine glands to the conceptus (Buhi et al., 1982).

Uteroferrin is transported from uterine glands across the placental areolae by nonreceptor-mediated pinocytosis and is released into the placental venous system (Renegar et al., 1982). Upon entering the umbilical vein, it is transported to the fetal liver where cells of the reticuloendothelial system bind and endocytose uteroferrin by a

receptor-mediated process involving coated pits, coated vesicles and receptosomes (Renegar et al., 1982). It is speculated that binding of uteroferrin to endothelial and(or) Kupfer cells is dependent on the mannose residues in the carbohydrate portion of the molecule (Bazer and First, 1983). Presumably, uteroferrin is directed to lysosomes where the iron is released to ferritin and transferred to erythroblasts, by a process called ropheocytosis (Bazer and First, 1983) for synthesis of hemoglobin. The fetal liver of piglets is the primary site of erythropoiesis throughout gestation (Bazer and First, 1983).

Uteroferrin not bound by the liver is cleared through the kidney, into the fetal allantoic sac via the urachus (Renegar et al., 1982). Allantoic fluid is known to be a rich source of transferrin (Buhi et al., 1982) because iron bound to uteroferrin is transferred to transferrin in allantoic fluid. Transferrin is then taken up by the allantoic epithelium and enters the fetal circulation (Ducsay et al., 1982) where it provides iron for hematopoiesis and other metabolic needs.

Discovery of uteroferrin suggested that additional carrier proteins might exist in uterine flushings from cyclic pigs. Several reports have shown that retinol and retinol-binding proteins exist in serum (Goodman, 1974) and in various tissues, including the endometrium, in a number of mammalian species (Ong and Chytil, 1975; Chytil et al., 1975). Retinol is also an essential nutrient for the maintenance of reproductive function in cyclic animals (Thompson et al., 1964). Adams et al. (1981) confirmed the existence of a retinol

binding protein(s) in uterine secretions from pigs during the luteal phase of the cycle. According to these investigators, vitamin A content of uterine secretions increases concomitantly with retinol binding protein, which has an affinity for retinoic and oleic acids but not retinyl esters and retinal. Hydrolytic enzymes (lysosomes, cathepsin activities B,D and E, leucine aminopeptidase and proteases) and regulatory proteins (protease inhibitors) have been found in uterine secretions of ovariectomized pigs treated with progesterone (Bazer and First, 1983). Glucose phosphate isomerase and plasminogen activator are the only proteins studied in uterine secretions that appear to be regulated by estrogen (Bazer and First, 1983).

Riboflavin has been identified as the compound causing pig uterine flushings collected between day 6 and 8 of either the cycle or pregnancy to have a yellow coloration (Murray et al., 1980; Moffatt et al., 1980). High riboflavin content of uterine flushings occurs during the period of initial blastocyst expansion. The significance of this association is not understood because riboflavin may affect many aspects of cellular function. Active immunization of rats against a chicken riboflavin carrier protein resulted in reversibly suppressed embryonic survival during the preimplantation period (Murty and Adiga, 1982). Riboflavin transport protein, which may be essential for transplacental riboflavin transport, has also been described for the cow (Merrill et al., 1979).

Glucose is found in the uterine flushings of the pregnant pig, cow, mare and ewe, and fructose is detectable after day 12 of gestation

in gilts and mares (Zavy et al., 1982). These sugars increase in the pregnant uterus with advancing gestation. Glucose phosphate isomerase activity is responsible for the interconversion of glucose-6-phosphate and fructose-6-phosphate and appear to be modulated in endometrial tissue by estrogen (Zavy et al., 1982). According to these researchers, fructose is produced by the trophoblast and chorion from glucose via sorbitol. Fructose may be important because it can be sequestered in the reproductive tract and may be preferentially metabolized through the phosphogluconate pathway to generate $NADPH+H^+$ and ribose sugars essential for biosynthesis in rapidly proliferating tissues (Zavy et al., 1982).

Ascorbic acid is also present in uterine secretions of pregnant gilts and increases quantitatively as pregnancy advances (Zavy et al., 1982). Ascorbic acid is known to affect collagen synthesis, catecholamine and indoleamine biosynthesis, iron absorption and metabolism and to have a sparing effect on B-complex vitamins and vitamins A and E due to its antioxidant role (Bazer and First, 1983).

Maintenance of Pregnancy

Distribution of embryos is critical to maintenance of pregnancy in the pig. When each uterine horn is ligated midway between the tip of the uterine horn and the body of the uterus on day 4 postmating, and the ligatures subsequently removed on days 8, 9, 10 or 11 the embryos

migrate and distribute and a normal pregnancy ensues (Polge and Dziuk, 1970). According to these researchers, when ligatures are removed on day 12, the embryos rarely occupy the uterus beyond the ligature and few pregnancies continue, while removal of ligatures on day 13 yields no pregnancies unless supplemental CL are induced or progesterone is administered. The proportion of uterus unoccupied is inversely related to the probability that pregnancy will continue (Dhindsa and Dziuk, 1968b). These scientists observed that when one-half of the uterus was unoccupied, pregnancy did not continue, whereas, when one-quarter and one-eighth of the uterus was unoccupied, 20 to 30 and 50 to 60% of pregnancies continued, respectively. Thus these data reflect the sequestering of PGF by the endometrium in those portions of the uterus occupied by the embryos. Prostaglandin $F_{2\alpha}$ produced in unoccupied portions of the uterus caused demise of the CL depending on the size of the unoccupied portion of the uterus. Creation of conditions in which all embryos come from one ovary as in unilateral oviduct ligation or unilateral ovariectomy provides evidence that embryos do not reach the contralateral horn until day 9 and therefore one-half of the uterus is unoccupied until day 9 (Dhindsa et al., 1967). Pregnancy under these conditions occurs readily as in females with both horns occupied from day 4 of gestation. Based on occupancy of the uterus, therefore, the determination by the dam that pregnancy should continue is not made until after day 9. However, when embryos are permanently restricted to one horn by ligatures placed near the body of the uterus, pregnancy fails in nearly every case unless supplemental CL are induced

(Christenson and Day, 1971) or embryonic extracts are infused (Ball and Day, 1982a,b).

Evidence thus far indicates that the critical period for signaling of maintenance of pregnancy is around day 12 of gestation, during which time the embryo grows and elongates very rapidly (Geisert et al., 1982b), apparently establishes the space between itself and other embryos and can no longer migrate (Dziuk, 1985). Once an embryo has exchanged a signal for the maintenance of pregnancy with its mother, removal of embryos from one uterine horn after day 14 does not disrupt pregnancy (Dhindsa and Dziuk, 1968a) and demonstrates that the effect of the unoccupied uterine segment is minimal or absent after day 14 of gestation. Moreover, in animals with some embryos removed, levels of progesterone were similar to those of unaltered pregnancy. The presence of inanimate objects in the uterus is not effective, as various items have been inserted to extend the life of the CL to no avail (Anderson and Melampy, 1967). Administration of estrogen to the dam before day 14 of the cycle or pregnancy will cause an extension of the life of the CL and pseudopregnancy may persist for nearly as long as pregnancy (Ford et al., 1982; Geisert et al., 1982c). Synthesis of estrogen by the porcine embryo increases markedly between day 11 and 12 of gestation (Perry et al., 1976; Bazer et al., 1982), and the level of estrone sulfate in maternal plasma from days 20 to 28 (Horne et al., 1983) and 30 (Stoner et al., 1982) of gestation is positively correlated with the number of embryos. Based on these observations, it seems logical, therefore, that feto-placental estrogen or its effects

are a signal to maintain the CL and, consequently, pregnancy (Saunders et al., 1983).

The partitioning of PGF to either the uterine lumen in the case of pregnancy or to the uterine venous drainage in nonpregnant pigs has been postulated as a determining factor or signal for maintenance of pregnancy (Bazer and Thatcher, 1977).

Because a minimum of about four embryos are required at day 12 to maintain pregnancy, it would seem reasonable that each embryo may contribute part of a total signal to maintain the function of the CL and production of progesterone (Polge et al., 1966). This assumption turned out to be incorrect. A greater number of fetuses did not cause an increase in the concentration of plasma progesterone above that in females with fewer fetuses (Webel et al., 1975). Apparently there is a qualitative rather than quantitative signal from the embryos to maintain progesterone production and, once the female is pregnant, the concentration of progesterone is unaffected by the number of fetuses.

STATEMENT OF THE PROBLEM

Reproductive performance is an important aspect of any livestock enterprise because of its impact on economic returns. Present methods of swine production with emphasis on intensive management result in increased costs for labor and other operational factors that contribute to overhead. Under this type of management system, gilts are not bred until second or third estrus. Because gilts are nonproductive prior to initial mating, the interval from puberty to mating constitutes an economic liability. Improved reproductive efficiency by bringing gilts into production at puberty would increase the number of litters farrowed per sow during her productive years and thereby increase the producer's profit margin.

Review of the literature has revealed that embryonic mortality is more prevalent in gilts bred at first estrus than at any subsequent estrus. The physiological basis for the higher incidence of embryonic mortality in first estrous gilts is not known. It is possible that ovarian response to pituitary gonadotropins in the pubertal gilt, in terms of ovulation and steroidogenesis, may not be synchronous with the ability of the uterus to provide a satisfactory environment for embryonic development. Maturation of the uterus is an important developmental phase that ensures embryonic survival. Consequently, investigation of the hormonal and uterine factors in first versus multiestrous gilts may provide insight as to the cause(s) for the higher incidence of embryonic mortality in the pubertal gilt. Research undertaken by this author constituted an attempt to delineate the role of ovarian steroid production, timing of ovulation and uterine

environment in contributing to the greater incidence of embryonic mortality in the pubertal gilt.

EXPERIMENT I: FACTORS CONTRIBUTING TO EARLY EMBRYONIC MORTALITY IN GILTS BRED AT FIRST ESTRUS

Introduction

A major factor limiting production in swine herds is reduced reproductive performance of pubertal gilts. Compared with their multiestrous counterparts, pubertal gilts ovulate fewer ova (Robertson et al., 1951; Andersson and Einersson, 1980; Knott et al., 1984) and if mated, sustain greater embryonic losses (Warnick et al., 1951; MacPherson et al., 1977). The physiological basis for the higher incidence of embryonic mortality in gilts mated at first estrus is not known. Although the uterus of the pubertal gilt may be relatively underdeveloped, it is unlikely that embryo wastage in these animals can be attributed to limited uterine capacity. Results of several studies indicate that up to 30 days (d) of gestation, the uterus of the gilt can accommodate approximately twice as many fetuses as are normally present (Dziuk, 1968; Rampacek et al., 1975).

Most prenatal mortality in swine occurs before attachment of the embryos in the uterus on d 19 of gestation (Perry and Rowlands, 1962). During the preattachment period the embryo relies on histotroph produced by the uterus for survival (Bazer et al., 1982; Bazer and Roberts, 1983). Ovarian steroids play an important role in regulating uterine secretions prior to embryo attachment (Murai et al., 1981; Geisert et al., 1982a,b) and several studies have characterized the

secretory patterns of progesterone and estrogen during early gestation in the gilt (Tillson et al., 1970; Guthrie et al., 1972). However, there are no reports in which secretion of ovarian and placental steroids during early gestation of gilts mated at first estrus has been examined as an underlying cause for the greater incidence of embryonic mortality in these animals.

The objectives of this study were to compare the reproductive performance and serum concentrations of progesterone and estrogen of gilts bred at first estrus with those of gilts bred at third estrus. It was anticipated that the acquired data would provide some insight into the cause(s) for increased embryonic mortality in gilts bred at first estrus.

Materials and Methods

Thirty six prepubertal Yorkshire gilts (initial body wt, 72 to 77 kg) were utilized in an experiment of 2 x 3 factorial design. Gilts were fed ad libitum a commercial diet containing 15% protein until the attainment of puberty. Subsequently they were fed 2.5 $kg.head^{-1}.d^{-1}$ of diet containing 16% protein.

Prepubertal gilts were checked twice daily for behavioral estrus by use of a vasectomized boar. On the day of detected estrus (d 0 of gestation), gilts were assigned in replicate to be mated at first estrus (n=18) or to be retained for mating at the third detected estrus

(n=18) with equal numbers within each category to be slaughtered on d 3, 15 and 30 of gestation. Gilts were slaughtered on d 3 to assess fertilization rate while those on d 15 and 30 were killed to determine the extent of embryonic mortality. Blood samples (approximately 25 ml) were collected from the right external jugular vein of each gilt immediately prior to slaughter according to the method of Schwartz and Smallwood (1977). Ovaries and reproductive tracts were removed from gilts 15 to 20 minutes (min) after slaughter and placed on crushed ice until processed. Blood samples were allowed to remain at room temperature (25 C) for 12 hours (h), then stored at 4 C for 48 h and subsequently centrifuged at 500 x g for 10 min at 4 C. The resulting sera were stored at -20 C until assayed for progesterone and estrogen as described below.

Ovulation rate for each gilt was determined by counting the number of corpora lutea in each ovary. Pubertal status was confirmed by the absence of corpora albicantia in the ovaries of first estrous gilts. Oviducts from gilts slaughtered on d 3 of gestation were flushed with physiological saline and the flushings were examined microscopically for number of fertilized ova (fertilization rate = no. of cleaved ova / no. of corpora lutea x 100). An ovum was considered to be fertilized if blastomeres of equal size were present and if sperm cells were detected in the zona pellucida. Uteri from gilts killed on d 15 were gently flushed with physiological saline to recover embryos. The flushings were examined with a dissecting microscope for number of surviving embryos. Conceptuses in uteri of gilts slaughtered on d 30

of gestation were exposed by an incision made along the length of each horn and examined for viability. Embryos on d 15 and 30 were considered to be viable if they exhibited characteristics described by Marrable (1971). Percentage embryonic survival on d 15 and 30 of gestation was determined as follows: no. of surviving embryos / no. of corpora lutea x 100.

Radioimmunoassays

Sera were analyzed for progesterone and unconjugated estrogen using radioimmunoassays previously validated in our laboratory and described by Koligian and Stormshak (1977) and Zelinski et al. (1982), respectively.

Mean recoveries of various quantities of progesterone (25, 50, 100 and 200 pg) added to 100 ul of pooled serum obtained from barrows were: 27.9 $\pm$ 1.8, 63 $\pm$ 9.4, 107.5 $\pm$ 4.3 and 209.8 $\pm$ 13.4 pg/100 ul. Sensitivity of this assay was 10 pg/tube ($P<.05$, $n = 10$). Intra- and interassay coefficients of variation for the progesterone assay were 6.8 and 15.8%, respectively. Mean recoveries of various amounts of estradiol-17β (4, 6, 10 and 20 pg) added to 1 ml of pooled serum obtained from barrows were: 4.67 $\pm$ .19, 7.25 $\pm$ .31, 11.14 $\pm$ .32 and 22.25 $\pm$ 2.04 pg/ml). The antibody (rabbit antiserum against estradiol-17-succinyl-bovine serum albumen, lot-E-2; donated by Dr. G. D. Niswender, Colorado State University) used for the radioimmunoassay of estradiol was found to have 33% cross-reactivity with estrone;

therefore the values reported will be referred to as estrogen. Sensitivity of the estrogen assay was 2 pg/tube ($P<.05$, $n = 10$). Intra- and interassay coefficients of variation for the estrogen assay were 4.9 and 10.8%, respectively.

Statistical Analyses

Data on serum concentrations of hormones and percentage embryonic survival were analyzed by two-way analysis of variance with differences among means tested for significance by use of orthogonal contrasts. Data on breeding weight and ovulation rate of gilts were analyzed by use of unpaired Student's "t" test.

Results and Discussion

Reproductive characteristics of gilts bred at first and third estrus are presented in table 1. Gilts mated at pubertal estrus weighed less ($P<.01$) than those mated at third estrus. Weight of gilts at puberty in the present study was comparable with that previously reported for gilts at this station (Knott et al., 1984). Gilts gained an average of .56 kg/d between first and third estrus ($\bar{x} = 46$ d), which also compares favorably with the gain of $.60 \pm .10$ kg/d reported for pubertal gilts (Knott et al., 1984).

Number of ovulations in pubertal gilts was less ($P<.05$) than that of gilts mated at third estrus. These data are in agreement with those

TABLE 1. REPRODUCTIVE PERFORMANCE OF GILTS BRED AT FIRST VERSUS THIRD ESTRUS

Item	Estrus at which bred	
	First	Third
Mean breeding wt., kg	84.5 ± 5.7**	114.3 ± 9.0
Mean no. of ovulations	12.17 ± .89*	14.5 ± .38
Fertilization rate, %	100	98
Embryonic survival, %		
15 d post-mating	78.1 ± 2.7*	95.1 ± 2.4
30 d post-mating	66.7 ± 13.9*	89.4 ± 6.6

* $P < .05$.
** $P < .01$.

of Robertson et al. (1951), Andersson and Einersson (1980) and Knott et al. (1984) who found ovulation rate to increase with succeeding estrous cycles.

Fertilization rate did not differ among gilts bred at first and third estrus. Robertson et al. (1951) and Perry and Rowlands (1962) reported fertilization rates between 93 to 96% in gilts and sows, which were similar to those observed in this study. These data suggest that fertilization rate is not a major factor that contributes to the incidence of embryonic mortality in gilts.

Regardless of whether gilts were bred at first or third estrus, embryonic losses were evident by d 15 and increased by d 30 of gestation. However, first estrous gilts sustained greater ($P<.05$) embryonic mortality at each of the stages studied compared with that of third estrous gilts. Increased incidence of embryonic mortality in gilts bred at pubertal estrus with a body weight of between 70.5 and 75 kg has been reported by George and England (1974). Embryonic mortality occurring in gilts bred at third estrus was considerably less than that observed by other investigators (Perry and Rowlands, 1962; George and England, 1974) and may have been due to the limited number of observations in the present study.

Serum concentrations of estrogen (pg/ml; figure 1) were similar in gilts bred at first or third estrus on d 3 (48.8 ± 7.9 vs 50.3 ± 4.4) and d 15 (39.8 ± 3.3 vs $40.1 \pm .9$) but lower ($P>.05$) in first compared with third estrous gilts on d 30 (54.3 ± 6.4 vs 70.0 ± 8.1). Concentrations of estrogen, irrespective of sexual age at mating, were

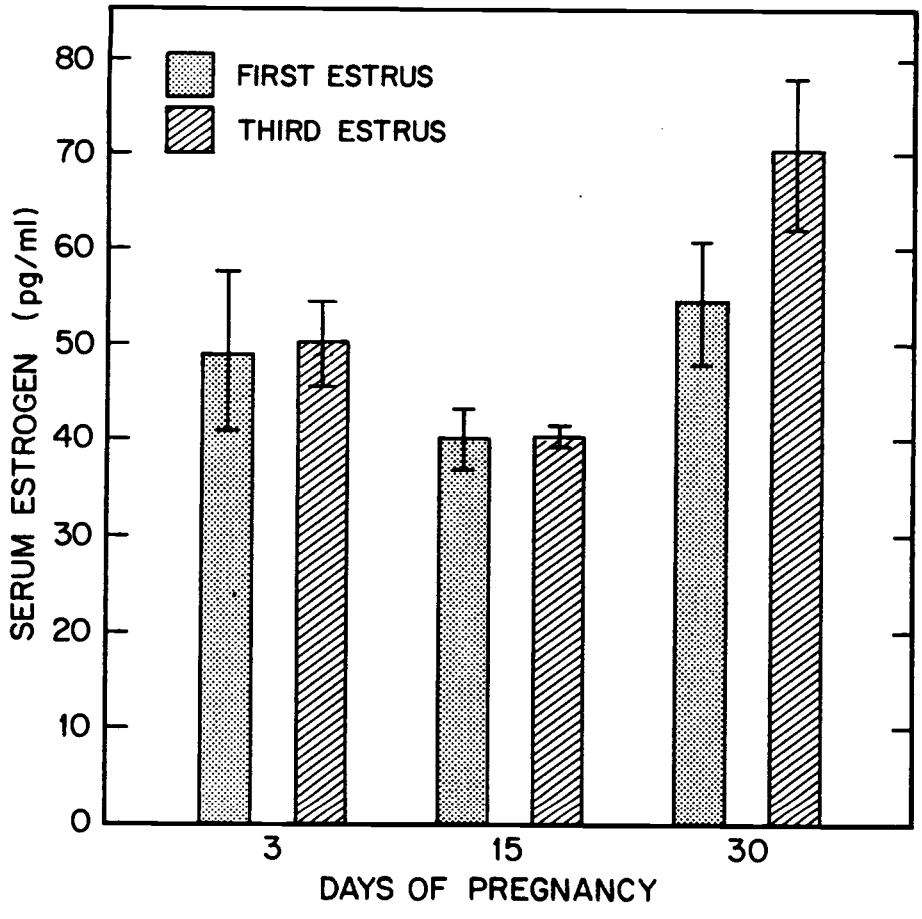

Figure 1. Mean serum concentrations of estrogen in gilts mated at first or third estrus on d 3, 15 and 30 of gestation.

higher on d 30 ($P<.05$) than on d 3 and 15 of gestation with no differences evident between the two earlier stages studied. Changes in serum estrogen levels were comparable with those reported by Guthrie et al. (1972) although they were generally higher than those found by these investigators. The higher values may be attributed to cross-reactivity of our antibody with estrone.

Similarly, serum concentrations of progesterone (ng/ml; figure 2) tended to be lower ($P>.05$) in gilts bred at first vs those bred at third estrus (d 3, $7.8 \pm .5$ vs $8.4 \pm .7$; d 15, 20.2 ± 4.4 vs 30.0 ± 4.2; d 30 12.3 ± 3.3 vs 16.8 ± 2.5). In both categories of gilts, serum concentrations of progesterone were lowest on d 3 and highest on d 15 of gestation ($P<.05$) concomitant with changes in luteal development that occur during this interval. It should be noted that serum levels of progesterone in both categories of gilts were lower on d 30 than on d 15 ($P<.01$); these changes are in agreement with those observed by Tillson et al. (1970), Guthrie et al. (1972) and Magness and Ford (1983). These data indicate that the increased loss of embryos in the gilt mated at pubertal estrus is not caused by failure of the gravid uterus to be exposed to sufficient quantities of progesterone. Perhaps embryonic mortality in the mated pubertal gilt is due to inadequate priming of the uterus by low systemic levels of progesterone prior to pubertal estrus (Esbenshade et al.,1982). Moore (1985) has shown that progesterone priming before induced estrus in ovariectomized recipient ewes enhanced survival of transferred embryos.

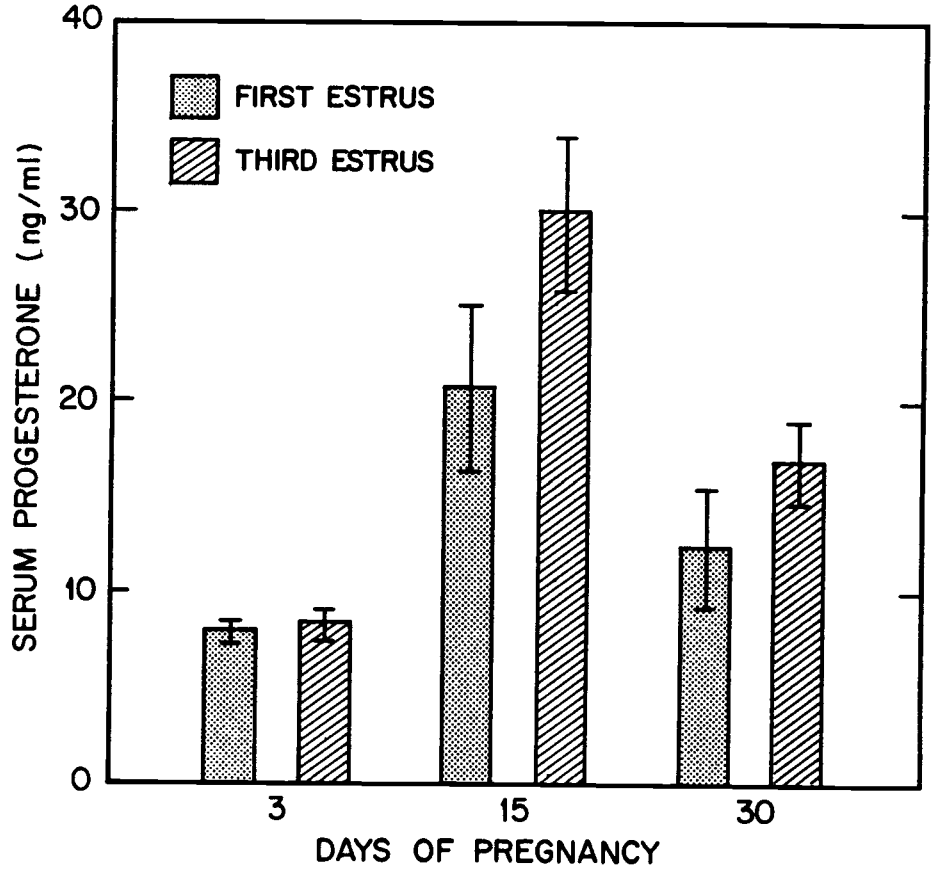

Figure 2. Mean serum concentrations of progesterone in gilts mated at first or third estrus on d 3, 15 and 30 of gestation.

The ratio of progesterone to estrogen was calculated for each gilt and averaged for all gilts within estrous category for each stage studied (figure 3). In comparison with gilts mated at third estrus, gilts bred at pubertal estrus had a higher ratio of progesterone to estrogen on d 15 (439 $\pm$ 71 vs 210 $\pm$ 17) and 30 (597 $\pm$ 106 vs 179 $\pm$ 50) but a lower ratio on d 3 (187 $\pm$ 37 vs 449 $\pm$ 123) of gestation (stage of gestation x estrous period interaction, $P<.05$). These data permit the suggestion that deviation from the secretory pattern of ovarian steroids characteristic of more mature gilts may be related to early embryonic mortality in gilts bred at pubertal estrus; however, it is not known whether these ratios reflect a cause or effect of embryonic mortality.

Interpretation of the significance of these changes in the ratios of progesterone to estrogen is beset by the lack of knowledge of the precise stage at which embryonic mortality occurs in the progesterone dominated uterus. It is conceivable that the low progesterone to estrogen ratio on d 3 of gestation in gilts bred at pubertal estrus may have resulted in an altered uterine secretory activity that was out of synchrony with the stage of embryonic development and thus set a stage for the demise of the embryos. Synthesis of estrogen by the porcine embryo increases markedly between d 11 and 12 of gestation (Perry et al.,1976; Bazer et al., 1982). It has been demonstrated that estrogen synthesized by embryos promote their migration or spacing (Pope et al., 1981c) and also modulates the effect of progesterone on uterine secretion of protein (Geisert et al., 1982a,b). If embryo wastage in the pubertal gilt is substantial before d 11, the estrogen produced by

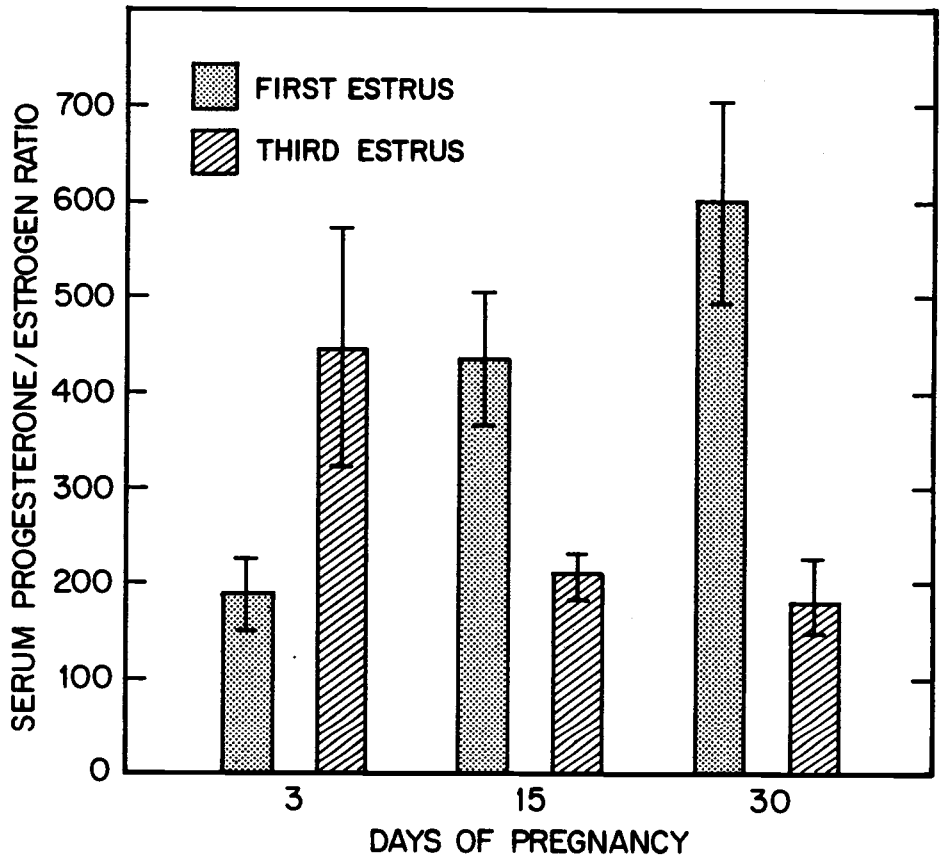

Figure 3. Mean ratios of progesterone to estrogen in gilts mated at first or third estrus on d 3, 15 and 30 of gestation.

surviving embryos may not be sufficient to ensure either proper spacing and(or) secretion of adequate histotroph to prevent further reduction in embryo number. Because the placenta serves as a major source of estrogen after d 12 of gestation, a reduced number of surviving embryos after this date would be reflected by a higher ratio of serum progesterone to estrogen.

These data have demonstrated that gilts mated at pubertal estrus have a higher incidence of embryonic mortality when compared with gilts mated at third estrus. Progesterone and estrogen concentrations during gestation were not different between gilts mated at first vs those mated at third estrus but the ratios of progesterone to estrogen were different at all stages of gestation studied. Whether the differences in progesterone to estrogen ratios are related to greater embryonic losses sustained by the pubertal gilt compared with that of third estrous gilts requires further investigation.

EXPERIMENT II: INFLUENCE OF THE UTERINE ENVIRONMENT ON EMBRYONIC MORTALITY IN PUBERTAL GILTS

Introduction

Gilts mated at pubertal estrus are capable of becoming pregnant and farrowing normal piglets. However, when compared with multiestrous gilts, pubertal gilts ovulate fewer ova (Robertson et al., 1951; Andersson and Einarsson, 1980; Experiment I) and if mated to boars of established fertility, sustain a higher incidence of embryonic mortality (Warnick et al., 1951; George and England, 1974; MacPherson et al., 1977; Experiment I).

The cause(s) for the higher incidence of embryonic mortality in gilts mated at pubertal estrus is not known. Although fertilization rates of gilts mated at pubertal estrus approximates that of gilts mated at third estrus (Experiment I), it is possible that the population of ova released by the pubertal gilt have a higher incidence of abnormalities that are expressed only at some later stage in embryonic development. Koenig et al. (1986) reported that chromosomal abnormalities may account for a substantial portion of embryonic mortality in gilts. Alternatively, the extent of uterine development of the gilt at puberty may not be adequate for embryonic development and survival. Schnurrbusch and Erices (1979) have shown that the uterus of the gilt is not mature morphologically and histologically until attainment of second estrus. These investigators found the

endometrium and myometrium to markedly increase in thickness during the course of the first three estrous cycles. Similarly, Murray and Grifo (1976) demonstrated that concentrations of uterine specific proteins were consistently lower in prepubertal gilts induced to ovulate compared with that of cyclic gilts. It is not known, however, whether the limited degree of morphological development and secretory ability of uteri of pubertal compared with that of multiestrous gilts creates an environment that is less suitable for embryonic development.

The primary objective of this study was to determine, by use of embryo transfer, whether the uterine environment of the pubertal gilt restricts embryonic development. A secondary objective was to ascertain whether embryonic mortality was affected by the maturity of the donor (first- vs multiestrus) of the embryos transferred.

Materials and Methods

One hundred-fifty six prepubertal gilts at the Roman L. Hruska U.S. Meat Animal Research Center, 113 to 154 d of age, were housed in groups of 10-14 per pen and were fed a restricted diet of 2.5 kg. $head^{-1}.d^{-1}$ containing 15% protein throughout the experiment. Gilts were checked twice daily for estrus by the application of hand pressure on the gilts' back in a pen adjacent to a boar. To obtain synchrony of transfer, the first 24 gilts detected in estrus (first day of estrus = d 0 of cycle) were allowed to cycle beyond first estrus in order to establish a population of potential third- estrous recipients and

second and third-estrous donors. Subsequent gilts detected in estrus were assigned to be used as first- estrous recipients or first- to fourth-estrous donors. Assignment of gilts as recipients was dependent upon whether there were synchronous donors available; i.e., gilts that were detected in estrus on the same day. Consequently, 40 first-estrous gilts were used as recipients and 15 of 24 gilts allowed to cycle beyond first estrus were used as third-estrous recipients only. To increase the population of third-estrous recipients, 15 gilts that had previously served as first- or second-estrous donors and seven gilts that had served as first- and second-estrous donors were used as recipients at third estrus. Donor gilts were mated to boars of established fertility 12 and 24 h after the onset of estrus.

Embryos were transferred 48-72 h after the onset of estrus. Before surgery, gilts were isolated without feed or water for 12-16 h. Anaesthesia was induced by injection of 10% sodium thiopental (1 g sodium thiopental in 10 ml of sterile physiological saline) into an ear vein and was maintained with 3% halothane and oxygen delivered at the rate of 1.5 liters/ min. The entire abdominal area of each gilt was scrubbed with 10% betadine solution and disinfected with 70% ethanol after which the reproductive tract was exposed by a midventral laparotomy. Embryos were collected by flushing each oviduct toward a flared Silastic-tube cannula (i.d.= 1.58 mm; o.d.= 3.18 mm) inserted into the infundibular end of each oviduct with the flared end secured in the ampulla with a size-2 nylon suture. The anterior quarter of each uterine horn was also flushed toward a similar catheter located 1-

2 cm posterior to the uterotubal junction when less than 60% of the embryos were recovered from the oviducts. Medium used for flushing and transfer was Dulbecco's phosphate buffered salt solution supplemented with 10% fetal calf serum, antibiotic/antimycotic agent (Gibco Laboratories, Grand Island, NY; 100 units/ml) and glucose. The medium was sterilized by pressure filtration (0.2um pore diameter; Nalge Co., Rochester, NY). Recovered embryos were washed in 8 ml of medium and incubated at 37 C for no more than 30 min before being transferred. Between 6 -10 embryos at the 1-8 cell stage were transferred into both ampullae (3-5 embryos/ampulla; oviduct to oviduct transfer) or both anterior uterine horns (3-5 embryos/uterine horn; uterine horn to uterine horn transfer) of nonmated synchronous first- or third- estrous recipients ($\bar{x} \pm$ SE embryos per recipient: first-estrus, 8.7 ± 0.2; third-estrus, 8.4 ± 0.2). Only embryos normal in appearance as characterized by the presence of blastomeres of equal size encompassed by an intact zona pellucida were transferred. Embryos at the 1-cell stage were transferred only when sperm were observed in their zona pellucida. During embryo transfers, the ovaries of recipients were examined for number of corpora lutea and pubertal status was confirmed in first-estrous donors and recipients by the absence of corpora albicantia in their ovaries.

Approximately 25 ml of blood were collected from the jugular vein of recipients on d 3, 12 and 30 of gestation by the method of Schwartz and Smallwood (1977). Blood samples were stored at 4 C for 6 h, subsequently centrifuged at 500 x g for 10 min at 4 C, and sera

collected and stored at -20 C until analyzed for progesterone and total estrogens.

All recipients (40 first- and 37 third-estrous recipients) were slaughtered between d 30 and 40 of gestation, uteri were collected, and total number of attached conceptuses and viable fetuses were recorded. Fetuses recovered at slaughter were considered to be viable if they exhibited characteristics described by Paton (1948) and Marrable (1971). Percentage attached conceptuses was calculated by dividing the total number of viable and degenerating fetuses by number of embryos transferred x 100. Percentage viable fetuses was calculated by dividing the total number of viable fetuses at slaughter by number of embryos transferred x 100.

Radioimmunoassay of Ovarian Steroids

Concentrations of progesterone were measured in sera collected on d 3, 12 and 30 of gestation, while concentrations of total unconjugated estrogens (estradiol-17β+ estrone; hereafter referred to as total estrogens) were measured in sera collected on d 12 and 30 of gestation only, by use of radioimmunoassays validated in Experiment I. Intra- and interassay coefficients of variation for progesterone were 6.8 and 15.8% and for estrogens 4.9 and 10.8%, respectively.

Statistical Analyses

Percentage of gilts pregnant between first- and third-estrous recipients was compared using Chi-square analysis while data with respect to ovulation rate and percentages of attached conceptuses and viable fetuses were analyzed by least-squares analysis of variance. Percentages of viable fetuses resulting from transfer of embryos from first- vs second-, third- and fourth-estrous donors were compared by the use of SAS (1985) general linear model least-squares analysis of variance. Serum concentrations of progesterone and total estrogens of first- and third-estrous recipients were analyzed by split-plot analysis of variance with differences among means tested for significance by use of orthogonal contrasts.

Results

Reproductive characteristics of first- vs third-estrous recipients are shown in table 2. Number of ovulations in first- estrous recipients was less ($P<.05$) than that of third-estrous recipients. Percentage of gilts that maintained pregnancy until slaughter did not differ between first- and those third-estrous recipients that were never used as donors. However, when gilts were used as donors once or twice before serving as recipients at third- estrus, pregnancy rates declined from 60 to 40 and 14.2%, respectively. Inclusion of the latter gilts in the computation of overall pregnancy rate for third-

TABLE 2. COMPARISON OF REPRODUCTIVE CHARACTERISTICS ($\bar{x} \pm$ SE) OF FIRST VERSUS THIRD ESTROUS RECIPIENTS[a].

Item	First estrus	Third estrus
No. of recipients	40	15[b], 15[c], 7[d]
No. of ovulations	8.9 ± .7	11.4 ± .7*
% Pregnant	67.5*	60[b], 40[c], 14.2[d]*, 43.2[e]
% Attached embryos	77.2 ± 3.8	74.1 ± 4.9
% Viable embryo	70.2 ± 4.6	69.1 ± 6.0

[a] Embryos from donor gilts were transferred to the recipients on d 3 postmating.

[b] Served as third-estrous recipients only.

[c] Served as donors once.

[d] Served as donors twice.

[e] Percentage pregnancy in all third estrous recipients; P<.01.

* P<.05

estrous recipients resulted in a decline in pregnancy rate compared with that of first-estrous gilts (43.2 vs 67.5%; P>.05). Mean percentages of attached conceptuses and viable fetuses of first- compared with those of third- estrous recipients did not differ, indicating that the uterine environment of first- and third-estrous recipients were similar in their ability to support early embryonic development.

Among pregnant recipients, 18 first- and nine third-estrous gilts received embryos from first-estrous donors while nine first- and seven third-estrous recipients received embryos from donors that had exhibited at least one normal estrous cycle ($\bar{x} \pm$ SE, 20.7 $\pm$ .2 d; table 3). Uterine environment of recipients (first- vs third-estrous) did not affect percentages of viable fetuses regardless of their source (first- vs $\geq$ second-estrous donors). Because of the lack of uterine effect on the viability of the embryos, data on embryos were pooled according to donor estrous-groups. Mean percentage of viable fetuses of first-estrous origin was less than those of donors that had experienced two to four estrous cycles (64.2 $\pm$ 4.8 vs 80.0 $\pm$ 5.9%; P<.05).

Serum concentrations of progesterone (ng/ml; figure 4) were lower among first-estrous recipients at all three stages of gestation studied (d 3, 6.92 $\pm$ .73 vs 9.53 $\pm$ 1.39; d 12, 27.32 $\pm$ 1.39 vs 34.44 $\pm$ 2.28; d 30, 26.01 $\pm$ 1.73 vs 31.18 $\pm$ 1.75; P<.05). In each category of recipients, serum concentrations of progesterone increased significantly (P<.05) from d 3 with greatest levels occurring on d 12,

TABLE:3. COMPARISON OF SURVIVAL (x ± SE) OF FIRST VERSUS ≥ SECOND-ESTROUS EMBRYOS BASED ON NUMBER OF PREGNANT RECIPIENTS.

Total no of embryos transferred	Type of transfer	No of recipients	Percentage viability: Uterine environment	Percentage viability: Source of embryos
160	First estrus to first estrus	18	63.4 ± 5.5	
75	First estrus to third estrus	9	65.0 ± 7.8	64.2 ±4.8*
78	≥ Second estrus to first estrus	9	83.1 ± 7.8	
62	≥ Second estrus to third estrus	7	76.8 ± 8.8	80.0 ± 5.9

* P<.05.

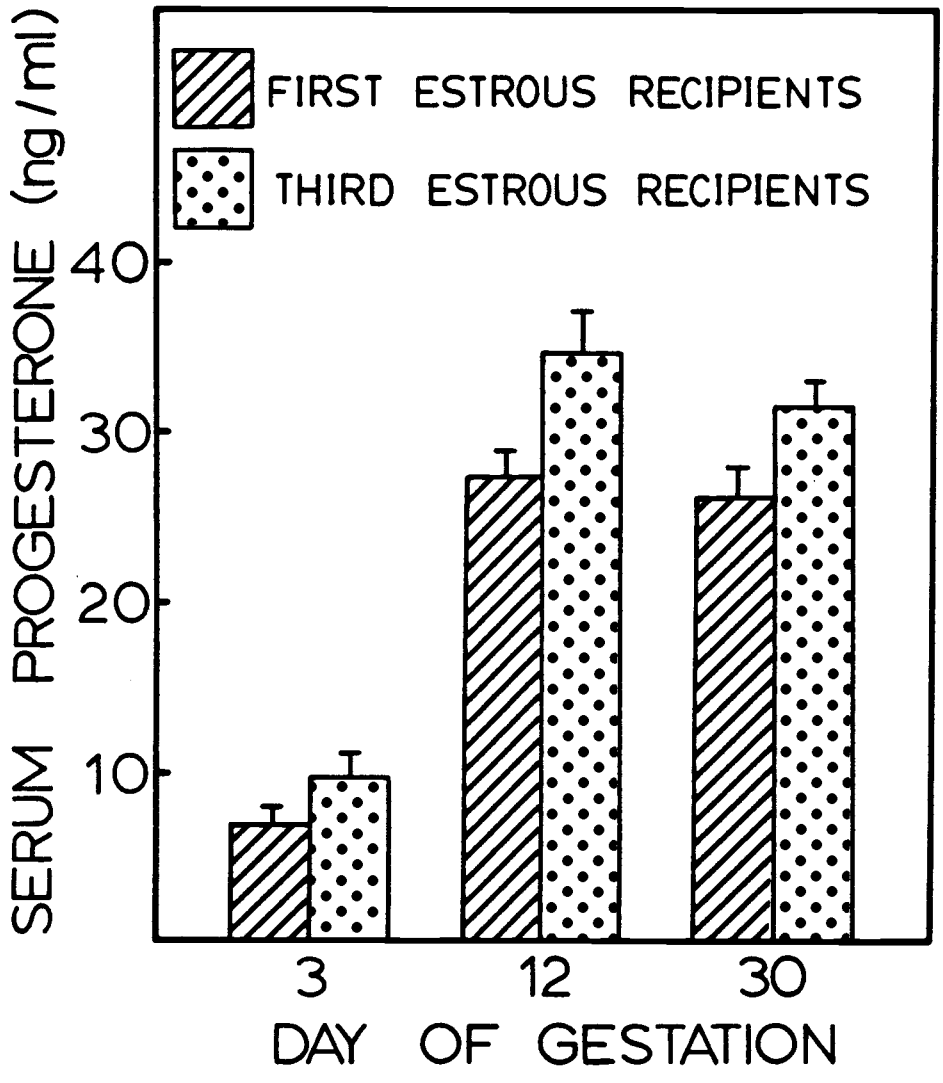

Figure 4. Mean serum concentrations of progesterone in recipient gilts on d 3, 12 and 30 of gestation.

followed by a nonsignificant decline on d 30 of gestation. However, serum concentrations of total estrogens (pg/ml) did not differ among first- and third-estrous recipients (d 12, 3.52 $\pm$ .21 vs 3.27 $\pm$ .15; d 30, 4.89 $\pm$ .29 vs 4.60 $\pm$ .29; figure 5). Serum concentration of total estrogens was higher ($P<.05$) on d 30 than on d 12 of gestation in each group of recipients.

Discussion

In the present study, ovulation rate increased from first to third estrus. Similar data on ovulation rate have been reported by Robertson et al. (1951), Andersson and Einarsson (1980) and in Experiment I.

Percentage pregnancy among first- and third-estrous recipients that were used as recipients only and those that served as donors once ranged from 50 to 67.5 % and is similar to the values reported by Bazer et al. (1968; 1969; Pope et al., 1971; Holtz et al., 1987). The lower overall percentage pregnancy among third-estrous recipients (43.2%) was influenced by the low rate of pregnancy (14.2%) in those recipients that had previously served as first- and second-estrous donors. Anterior uterine horns (about 2.5 cm from the uterotubal junction) of each donor were punctured with a gavage needle in order to flush the oviducts through the uterotubal junction. This process of serial embryo recoveries may have traumatized the uteri of those third-estrous gilts that were previously used as donors twice to the extent that they could not accomodate pregnancy.

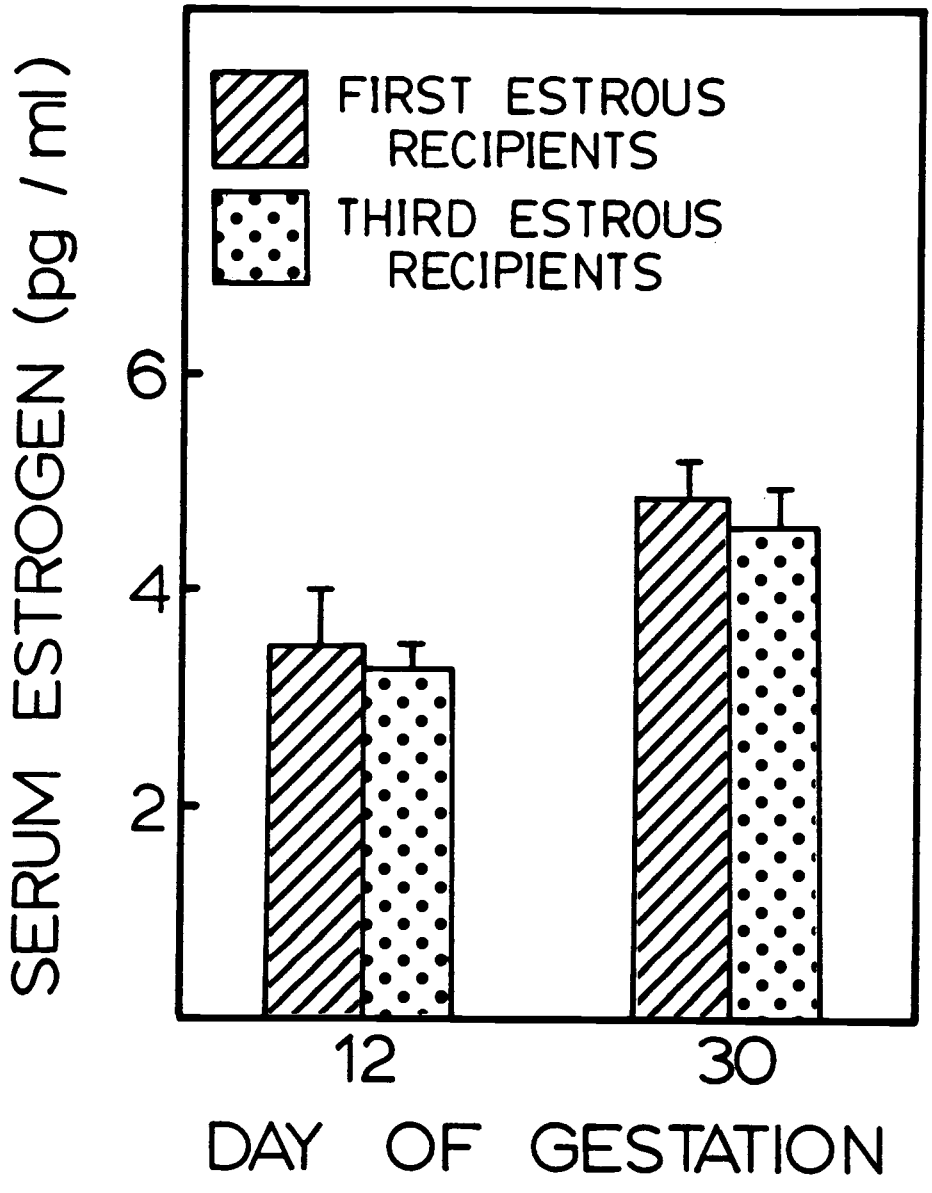

Figure. 5. Mean serum concentrations of total estrogens estradiol-17β + estrone) on d 12 and 30 of gestation.

Uterine environments of first- and third-estrous recipients were not different in their ability to support embryonic survival as determined at the time of slaughter. The porcine uterus under the influence of progesterone produces large quantities of histotroph for the maintainance of pregnancy (Bazer et al., 1982). Estrogen is responsible for the rapid release of histotroph from the uterine endometrial glands and the modulation of the magnitude of progesterone-induced protein synthesis around d 11 of gestation (Geisert et al., 1982), the time the conceptuses first secrete estrogens (Perry et al., 1973; Bazer et al., 1982; Geisert et al., 1982). If losses of embryos were equivalent among first- and third-estrous recipients up to d 11, then it may be assumed that the uteri of these recipients would be subjected to similar quantities of estrogen produced by the embryos. If this premise is true, then similar amounts of histotroph may have been released from the endometrial glands, which could explain in part why embryonic survival rates of first- and third-estrous recipients were not different when identical numbers of embryos were transferred. On the other hand, only normal appearing embryos at identical stages of development were transferred into recipients regardless of sexual age. Thus, the possibility exists that the similar rates of embryonic survival observed in first- and third-estrous recipients resulted from the transfer of preselected embryos.

Although only embryos at identical stages of development that were normal in appearance were transferred, percentage survival decreased among embryos from first-estrous donors compared with those from donors

that had one or more estrous cycles. Because the uterine environment was not a factor in causing increased embryonic mortality in embryos from pubertal gilts, this latter finding suggests that the embryos of pubertal gilts may have been defective. It has been demonstrated that proestrus in peripubertal gilts is usually prolonged (Andersson and Einarsson, 1980) during which time ova in residence could have a protracted exposure to follicular steroids. In cyclic gilts and sows, concentrations of estrogens, progestins (Eiler and Nalbandov, 1977; Gerard et al., 1979; Ainsworth et al., 1980) and androgens (Eiler and Nalbandov, 1977; Ainsworth et al., 1980) increase steadily (with progesterone being predominant) during proestrus and remain elevated until onset of estrus. On the first day of estrus in normal cyclic sows, follicular fluid concentrations of steroids slowly decline and then abruptly decrease as time of ovulation approaches (Eiler and Nalbandov, 1977). In gilts exhibiting estrus for the first time, concentrations of estrogens and androgens followed patterns similar to those observed in cyclic sows (Ainsworth et al., 1980). However, follicular concentrations of progestins, after the initial rise during proestrus, stabilized at the onset of first estrus in stimulated prepubertal gilts and then markedly increased to about 200% of their concentrations at estrus; about 6 h before ovulation (Ainsworth et al., 1980). Lower survival rate of embryos of first-estrous gilts may be due to more ova in these gilts sustaining chromosomal aberrations (Koenig et al., 1986) as a result of prolonged exposure to high intrafollicular progesterone around the time of ovulation. This is

supported by the data of Day and Polge (1968) who showed that treatment of gilts with progesterone before mating induced chromosomal aberrations by allowing polyspermic fertilization. Bishop (1964) has described this type of embryonic death as unavoidable and under normal circumstances should be regarded as nature's way of eliminating unfit genotypes in each generation. On the other hand, it is possible that some of the ova released by first-estrous gilts at ovulation may have been cytologically immature. Such ova can be penetrated by sperm and achieve cleavage but do not develop further (Thibault, 1977). This may account for the lower survival rate among first-estrous embryos compared to those from gilts that had cycled beyond first estrus.

Higher systemic concentrations of progesterone observed among third-estrous recipients at all stages of gestation studied compared with their first-estrous counterparts may be attributed to the greater number of corpora lutea in the former group of gilts, but did not have any effect on embryonic survival. Webel et al. (1975) and de Sa et al. (1981) have shown that either increasing plasma progesterone concentrations by injections of exogenous steroid or by superovulation or reducing systemic concentrations of progesterone by unilateral ovariectomy did not affect embryonic survival. Mean serum concentrations of progesterone for all recipients on d 3 of gestation was similar to that reported by Tillson et al. (1970) and Guthrie et al. (1972) for the same period. This indicates that the luteal phase of the cycle had been established at the time transfers were conducted. Maximum serum concentrations of progesterone were observed on d 12 of

gestation, similar to the observations of Magness and Ford (1983). The slight decline in serum progesterone levels on d 30 of gestation observed in this study is similar to that observed by Guthrie et al. (1972), and is characteristic of continued luteal maintenance in the gilt.

Serum concentrations of total estrogens reported in this study were similar among first- and third-estrous recipients at the two stages of gestation studied. The rise in estrogen levels from d 12 to 30 of gestation in pregnant recipients is an indication of the establishment of pregnancy. Similar concentrations of total estrogens observed in first- and third-estrous recipients on d 30 of gestation was likely due to the equal number of embryos present. Stoner et al. (1986) found a clear relationship between estrone sulfate concentration and litter size around d 30 of gestation. The pattern of estrogen secretion in this study was similar to that observed by Guthrie et al. (1972).

In conclusion, data from this experiment demonstrated that the uterine environment of the pubertal gilt is capable of supporting embryonic development. It appears that defective oocytes are likely causes for the decline in embryonic survival of mated first-estrous gilts.

EXPERIMENT III:OVULATION AND EMBRYONIC SURVIVAL IN GnRH-TREATED PUBERTAL GILTS

Introduction

It has been established that embryonic mortality in gilts bred at pubertal estrus is greater than that of gilts mated at third estrus (Warnick et al., 1951; MacPherson et al., 1977; Experiment I) The reason for this disparity in embryonic survival is not known with certainty, but cannot be attributed to a hostile uterine environment in the pubertal gilt (Experiment II).

Ova of pubertal gilts are fertilized at approximately the same rate as those of older gilts (Experiment I). However, fertilization followed by normal cleavage does not preclude the possibility that ova of pubertal gilts carry some induced defect that is expressed at a later stage of embryonic development. Andersson and Einarsson (1980) reported that peripubertal gilts have a longer proestrus than those that have cycled beyond second estrus. This observation is consistent with the finding that serum concentrations of estradiol in peripubertal gilts peak 2.5 d before puberty (Esbenshade et al., 1982) whereas in cyclic gilts peak levels of this steroid occur 24 h before onset of estrus (Henricks et al., 1972). Consequently, oocytes of pubertal gilts may become defective due to prolonged exposure to steroids and(or) other agents produced by the maturing follicle. Inducing early ovulation in the pubertal gilt by administration of appropriate

exogenous hormone on the first day of detected estrus might therefore reduce the incidence of embryonic mortality in this animal. Thus, the objective of the present study was to evaluate the effect of gonadotropin releasing hormone (GnRH) on ovulation and embryonic survival in pubertal gilts.

Materials and Methods

Twenty prepubertal gilts (73 to 75 kg body weight) were fed ad libitum a balanced commercial diet containing 15% protein until attainment of puberty. Subsequently they were fed a restricted diet of 2.5 kg $head^{-1}$ d^{-1} containing 16% protein.

Prepubertal gilts were housed adjacent to a boar and checked twice daily for estrus by application of hand pressure on each gilt's back in the presence of a boar. Gilts were mated to boars of established fertility initially at the time of detected estrus (d 0 of gestation) and subsequently at 12 and 24 h after detected estrus. After initial mating, gilts were assigned in replicates to a treatment (n=10) and control (n=10) group, weighed, and the treated gilts injected via an ear vein with 200 ug of GnRH while control gilts were similarly injected with physiological saline (vehicle). Blood samples (approximately 25 ml) were collected from the anterior vena cava (Schwartz and Smallwood, 1977) of
five treated and five control gilts (chosen randomly) immediately prior to injection (0 min), thereafter at 15 min intervals for 90 min, and

subsequently from all gilts just before slaughter on d 30 of gestation. Blood samples were
held at room temperature (25 C) for 12 h, then at 4 C for 48 h after which time they were centrifuged at 500 x g for 10 min at 4 C. The resulting sera were stored at -20 C until assayed for luteinizing hormone (LH) and progesterone.

Ovaries and uteri were removed from gilts 15 to 20 min after slaughter and placed on crushed ice until processed. Ovulation rate of each gilt was determined by counting the number of corpora lutea in each ovary. Pubertal status was confirmed by the absence of corpora albicantia in the ovaries of both control and treated gilts at slaughter. Corpora lutea from both ovaries of each gilt were then enucleated and weighed. Conceptuses were exposed by an incision made along the length of each uterine horn and examined for viability. Fetuses were considered to be viable if they exhibited characteristics described by Marrable (1971). Number of viable fetuses was recorded and percentage viable fetuses on d 30 of gestation was determined as follows: no. of viable fetuses / no. of corpora lutea x 100.

Radioimmunoassay

Sera collected from gilts before and after injection of GnRH or vehicle on the day of detected estrus were analyzed for LH only, while sera collected from all treated and control gilts on d 30 of gestation were analyzed for progesterone. Sera were analyzed for LH and

progesterone using radioimmunoassays previously described by McCarthy and Swanson (1976) and Koligian and Stormshak (1977), respectively.

Recovery ($\bar{x} \pm SE$) of various quantities of LH (.1, .2, .3, .4, .6, .8, 1, 2, 4, 8 and 10 ng) added to 200 ul of pooled serum obtained from sows at the midluteal phase of the estrous cycle were: .13 $\pm$ .02, .23 $\pm$ .02, .30 $\pm$ .03, .39 $\pm$ .01, .55 $\pm$ .03, .74 $\pm$ .04, .98 $\pm$ .04, 1.96 $\pm$ .10, 3.93 $\pm$ .05, 7.30 $\pm$ .20 and 11.08 $\pm$ .64 ng, respectively. The antibody (# 566, prepared against a porcine LH fraction; donated by Dr. G.D. Niswender, Colorado State University) utilized was previously shown to be highly specific for porcine LH (Niswender et al., 1970). Sensitivity of this assay was .1 ng/tube ($P < .01$, $n = 10$). The intraassay coefficient of variation for the LH assay was 2.5%. There was no interassay coefficient of variation for LH because all samples were analyzed in the same assay. The assay for progesterone was previously validated for porcine sera in Experiment I. Intra- and interassay coefficients of variation for the progesterone assay were 6.8 and 15.8%, respectively.

Statistical Analyses

Data on serum concentrations of LH were analyzed by split-plot analysis of variance. Data on serum concentrations of LH before GnRH and vehicle injection, ovulation rate (number of corpora lutea), number of attached conceptuses, percentage viable fetuses, weight of corpora

lutea and serum concentrations of progesterone were analyzed by use of unpaired Student's "t" test.

Results and Discussion

Gilts treated with GnRH weighed 98.9 $\pm$ 2.0 kg ($\bar{x} \pm$ SE) while control gilts weighed 91.0 $\pm$ 3.6 kg at puberty. These weights did not differ statistically and were comparable to those of pubertal gilts at this station previously reported by Knott et al. (1984).

Serum concentrations of LH prior to injections in both GnRH-treated and control gilts (7.2 $\pm$ 1.5 vs 4.3 $\pm$ 2.1 ng/ml; figure 6) did not differ ($P>.05$) and were similar to peak concentrations of this hormone reported by Niswender et al. (1970) at the onset of estrus in gilts. These initial serum concentrations of LH suggest that gilts in the present experiment may have already been releasing near maximal quantities of LH at the time of the injections. Treatment with GnRH increased secretion of LH during the 90 min after injection (time x treatment interaction; $P< .025$). The higher serum concentrations of LH after GnRH treatment are in agreement with the data of Chakraborty et al. (1973), Andersson et al. (1983) and Lutz et al. (1985) with the highest serum concentrations detected at 75 min after GnRH injection. In control gilts, serum concentrations of LH after injection of saline were similar to those detected prior to injection. These data are in agreement with those of Niswender et al. (1970) who determined that

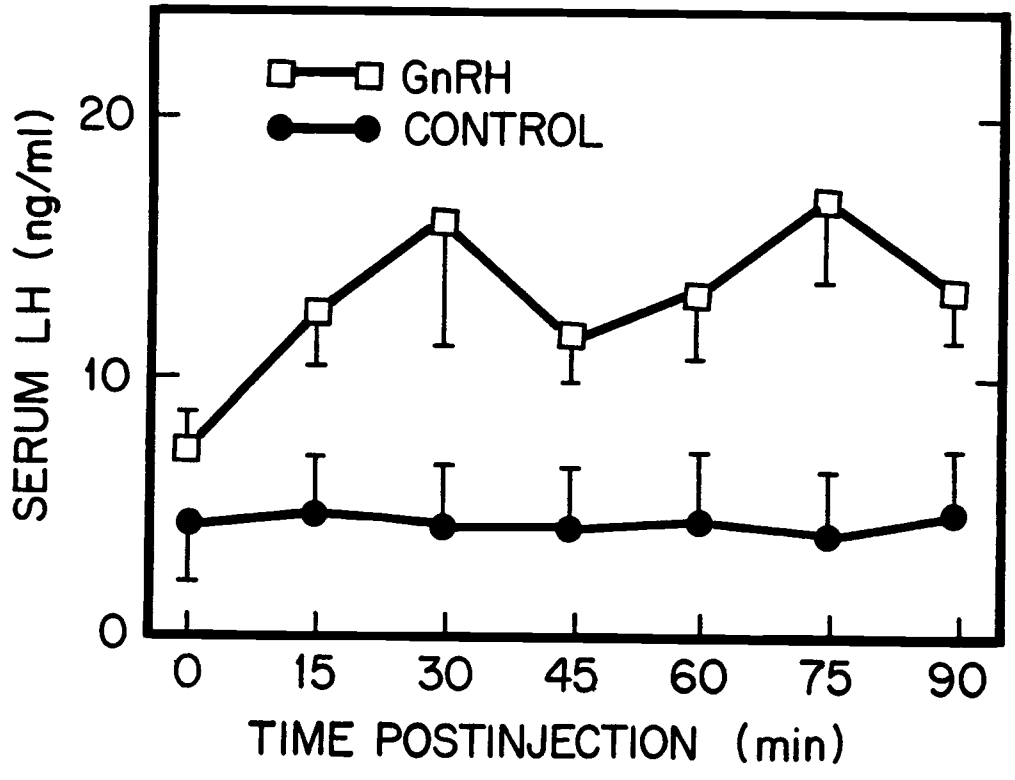

Figure 6. Changes in serum concentrations of LH (mean ± SE) in control and GnRH-treated gilts on the first day of pubertal estrus. Vehicle (saline) or GnRH (200 ug) were injected iv at time 0.

serum concentrations of LH remained elevated for 8 h after peak concentrations at the onset of estrus.

Gonadotropin releasing hormone-induced increase in secretion of LH was accompanied by a concomitant increase ($P<.05$) in ovulation rate (table 4). These data confirm, in part, those of Edqvist et al. (1978) and Lutz et al. (1985) who reported that exogenous GnRH caused ovulation in prepubertal gilts. The increase in ovulation rate in GnRH-treated gilts in the present study resulted from the ovulation of ovarian follicles that otherwise would not have ovulated. This suggests that in the normal prepubertal gilt, the population of follicles capable of being ovulated is greater than the actual number of ovulations that occur in response to the quantity of gonadotropin released.

Mean number of attached conceptuses recovered on d 30 of gestation as well as the percentage of viable fetuses did not differ between GnRH-treated and control gilts (table 4). Number of surviving embryos in pubertal gilts was expected to increase with increased number of ovulations as reported in Experiment I. In this latter study embryonic survival in third estrous gilts, which had similar numbers of ovulations as those gilts treated with GnRH in the present study, was greater than that of gilts mated at puberty. Because embryonic survival did not accompany increased ovulation rate in GnRH-treated gilts it is possible that ova in these gilts may have already been defective before GnRH treatment. Alternatively, ovulation of extra follicles due to GnRH-treatment may have resulted in asynchrony of

TABLE 4. REPRODUCTIVE CHARACTERISTICS OF GnRH-TREATED AND CONTROL GILTS (Mean ± SE)

Item	Control	GnRH
Ovulation rate	12.1 ± .6	14.5 ± .7*
No. of attached conceptuses	10.5 ± .7	10.9 ± .9
% Viable fetuses	83.5 ± 5.0	74.7 ± 6.9
Corpora lutea wt, mg	389.5 ± 11.3	402.8 ± 16.3
Serum progesterone, ng/ml	22.1 ± 2.9	25.0 ± 2.6

* $P < .05$.

development among embryos. The few additional ova ovulated as a result of GnRH-treatment may have been fertilized later than normal. Consequently, there may have existed a mixed population of embryos differing sufficiently in age such that the uterine environment favored survival of embryos resulting from early ovulation and fertilization. Pope et al. (1982a) demonstrated that older embryos create a uterine environment that is not conducive to development of younger embryos. Asynchronous cohabitation of embryos at diverse stages of development, coupled with other agents that normally impact on embryonic survival may have contributed to the demise of more embryos among the GnRH-treated gilts.

Control and treated gilts did not differ in mean weight of corpora lutea (table 4). Similarly, mean serum levels of progesterone (ng/ml; table 4) did not differ between GnRH-treated and control gilts. These data are in agreement with those in Experiment I in which an increase in number of corpora lutea was not accompanied by a significant increase in serum concentrations of progesterone on d 30 of gestation. Data from the present study indicate that uteri of control and treated gilts should have been exposed to similar systemic concentrations of progesterone. Whether progesterone-stimulated production of some uterine factor essential for embryonic survival (Bazer et al., 1982; Bazer and Roberts, 1983) was limiting relative to the greater embryo population resulting from higher ovulation in GnRH-treated gilts is not known.

Results of this study indicate that administration of GnRH to

gilts mated at the onset of pubertal estrus induced increased secretion of LH and number of ovulations. However, the increased ovulation rate was not accompanied by increased embryonic survival or litter size.

GENERAL DISCUSSION

Results of these experiments confirmed reports in the literature that embryonic mortality is higher in gilts bred at first than in those bred at third estrus. Progesterone and total estrogen concentrations were not significantly different between the two classes of gilts. However, the ratios of progesterone to estrogen were different at all stages studied, probably as a result of limited number of embryos present in first estrous gilts. Attempted initiation of early ovulation with exogenous GnRH increased LH secretion, which resulted in higher ovulation rate in treated pubertal gilts. Increased ovulation rate neither increased percentage embryonic survival nor serum concentrations of progesterone as compared with controls. The uterine environment of recipient pubertal gilts was not different from that of their multiestrous counterparts but survival of embryos of first estrous gilts was lower than that of multiestrous gilts. Based on these data, the factors that influence the higher incidence of embryonic mortality in pubertal gilts appear to reside in the ova.

A proportion of embryonic loss is caused by lethal genes in both pubertal and cyclic gilts. Embryo losses resulting from this type of defect are innate and cannot be avoided. Speculatively, the events that lead to the maturation of oocytes in pubertal gilts may not be identical with those of cyclic gilts. Additional losses in embryos prevalent in pubertal gilts may be due to inability of the gilt to release LH shortly after the estrogen peak, which occurs 2.5 days prior

to puberty compared with 24 hours before estrus in cyclic gilts. This results in a protracted proestrus and exposure of oocytes in residence to high levels of progesterone at the time of ovulation. Exposure of oocytes to high levels of progesterone at a comparable time in cyclic gilts or sows has been shown to cause chromosomal aberrations by permitting polyspermic fertilization. Embryonic survival did not improve when ovulation was induced at puberty because the oocytes in these gilts were already exposed to high progesterone levels. Investigation into induction of LH release shortly after peak estrogen secretion as observed in cyclic gilts might contribute to an understanding of the factors influencing embryonic mortality in mated pubertal gilts.

A high percentage of tertiary follicles developed prepubertally are lost to atresia. Therefore, some of the follicles in pubertal gilts may have only recently been recruited and consequently ova shed before their cytoplasmic maturation was complete. In such ova, fertilization may be followed by a prolonged interphase between sperm penetration and sperm nucleus decondensation, and thus, when the embryos finally undergo cleavage, they do not develop further. This could explain the observed poor survival of embryos of first estrous gilts when transferred to uteri of either pubertal or third estrous gilts. Because such embryos undergo the early cleavage division, they may have been transferred along with normal embryos. These embryos subsequently degenerate but the reasons for degeneration are not understood. A possibility exists that they lack the ability to signal their presence by producing estrogen that influences the uterine glands

in their immediate vicinity to release histotroph. There is some experimental evidence suggesting that administration of estrogen to mated pubertal gilts on day 12 of gestation improves embryonic survival.

Future research should be undertaken to confirm the observation that embryos of pubertal gilts have a higher mortality rate than those of cyclic gilts by separately culturing first and third estrous embryos in vitro, observing their daily development and comparing percentage mortality. If confirmed by this technique, an additional study might be conducted to determine whether microinjection of blastomeres from embryos of multiestrous gilts into first estrous embryos can improve their survivability. The rationale for this latter experiment is that such blastomeres, assuming they are genetically normal, would be metabolically competent and thus produce substances that may otherwise be limiting in the embryos of the first estrous gilt.

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