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Title: **CHARACTERISTICS OF PREOVULATORY FOLLICLES OF PUBERTAL GILTS**

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Two experiments were conducted utilizing crossbred gilts to evaluate follicle characteristics at puberty and to determine the effect of exogenous estradiol-17ß on embryonic mortality in the mature cycling gilt.

Experiment 1 was conducted to compare preovulatory follicle numbers and sizes and follicular fluid concentrations of progesterone, estradiol-17ß, plasminogen activator and plasmin in the same gilts (n=9) six hours after detected first and third estrus. Relationships between follicular fluid concentrations of steroids and those present in vena cava and ovarian venous blood were also examined at each estrus. Follicular fluid and serum concentrations of steroids were determined by use of radioimmunoassays and a caseinolytic assay was used to assess follicular fluid levels of plasminogen activator and plasmin. Total

estrus) and third estrus. However, gilts at first estrus had more follicles 4-8 mm diameter ($P<0.05$) and 8.1-10 mm diameter ($P<0.01$) than at third estrus. Follicular fluid, ovarian venous sera and systemic sera concentrations of estradiol-17ß were greater in gilts at third estrus than at first estrus ($P<0.05$). Follicular fluid concentrations of estradiol-17ß in gilts at first and third estrus were positively correlated with concentrations of this steroid in ovarian venous sera ($r=.51$, $r=.12$, respectively) and systemic sera ($r=.25$, $r=.80$, respectively).

Follicular levels of progesterone, plasminogen activator and plasmin and systemic serum levels of progesterone did not differ between gilts at first and third estrus. The lower follicular fluid concentration of estradiol-17ß in gilts at puberty compared with that of the third estrus gilt may be attributed to differences in follicular development. The greater asynchrony in follicular development of gilts at puberty may be related to the reported increase in embryonic mortality that occurs in these animals.

Experiment 2 was conducted to determine the effect of exogenous estradiol-17ß on embryonic mortality in the mated third and fourth estrous gilt. Eight gilts were injected intramuscularly with 5 mg of estradiol-17ß on the first day of detected third or fourth estrus. Control gilts ($n=9$) were similarly injected with vehicle only. All gilts were mated to fertile boars and slaughtered on days 30-32 of gestation. Fetuses were examined for viability, weighed and measured (crown-rump length). Treatment had no detrimental effect on fetal survival (control, 85.4% vs treatment, 83.3%) and did not affect weight or length of the fetuses. In treated and control gilts, fetal weight increased with age ($r=.82$, $r=.66$,

respectively) as did fetal length (r=.79, r=.50, respectively). Exposing the oocyte or early developing embryo in the mature gilt to exogenous estradiol did not induce a greater incidence of embryonic mortality.

CHARACTERISTICS OF PREOVULATORY FOLLICLES OF PUBERTAL GILTS

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DEDICATION

This thesis is dedicated to the late Edward Higbee, a quiet giant, who through his example instilled in me an appreciation for all things in life. Most of all I thank him for the fond memories with which he left me that will continually remind me of his good nature. Thank you Grandpa!

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I thank God for all He has bestowed upon me and hope that through my abilities I can bring glory to Him.

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REVIEW OF LITERATURE

It is generally acknowledged that the incidence of embryonic mortality in gilts mated at first estrus exceeds that of mated multiestrous gilts. However, the causes(s) for this increased embryonic mortality in the pubertal gilt remains an enigma. In order to gain an appreciation for the possible underlying causes of embryonic mortality this literature review will focus on physiological and endocrinological changes associated with key events in the reproductive cycle of the gilt. Where appropriate relevant information pertaining to other species of animals will be presented.

Specifically this review will consider the basis for attainment of puberty, folliculogenesis, ovulation and ovarian steroidogenesis. How these reproductive phenomena might impact on early embryo development and survival will be considered and discussed.

Puberty

Puberty is that stage of development when the female and male are first capable of producing gametes that upon fertilization result in viable zygotes. Domestic gilts attain puberty at an average age of seven months although there are breed variations (Christenson and Ford, 1979). Factors such as genetics (Self *et al.*, 1955; Signoret, 1970; Christenson and Ford, 1979) nutrition (Wiggins *et al.*, 1950; Friend, 1973; Wahlstrom and Libal, 1977; Van Lunen and Aherne, 1987), social contact with foreign contemporaries (Mavrogenis and Robison, 1976; Christenson and Ford, 1979) and exposure to boars (Brooks and Cole, 1970; Thompson and Savage, 1978; Kirkwood and Hughes,1979) have been found to affect the age of puberty of gilts. Interactions between these individual factors originally caused some difficulty in deducing which had important influences on puberty (Anderson and Melampy, 1972). For example crossbred gilts usually reach puberty at an earlier age than straightbred gilts (Foote *et al,*. 1956). However, regardless of the genetics of the gilts, social contact and exposure to boars generally enhance the onset of puberty if gilts are fed adequate diets.

In a number of species somatic development has been shown to be negatively correlated with the interval from birth to puberty. Thus it is not surprising that nutrition plays a significant role in dictating age at which gilts reach puberty. Van Lunen and Aherne (1987) have shown that gilts fed an ad libitum diet reached puberty at an earlier age than those with restricted intake. However, average weights of gilts fed ad libitum and those fed limited diets were similar at puberty (135.2 vs. 133.1 kg, respectively). Therefore these investigators theorized that a minimum weight threshold must be met in order for puberty to occur. Similarly, Nelsen *et al.* (1982) found that a minimum age was required for puberty attainment in heifers. Although there is evidence for a minimum weight threshold

for puberty attainment in gilts, this variable alone is not sufficient to guarantee onset of puberty (Kirkwood and Aherne; 1985). Unless gilts of adequate size and age are afforded social contact with other gilts and(or) exposure to a male, as emphasized above, attainment of puberty is usually delayed.

Mechanisms of Puberty Onset: Gonadostat Hypothesis

The current hypothesis governing the physiological onset of puberty as advanced by Ramirez and McCann (1963) is known as the "Gonadostat Hypothesis." This hypothesis maintains that prior to puberty the pituitary secretes gonadotropins in a low sustained manner due to a hypersensitivity of the hypothalamic-hypophyseal axis to inhibitory feedback of ovarian estrogens. When the sensitivity of this axis to estrogen lessens, gonadotropin secretion increases. Of particular importance is luteinizing hormone (LH) which stimulates preovulatory follicular growth and estrogen production; the latter subsequently triggering the ovulatory LH surge.

In the prepubertal lamb, LH pulse frequency is approximately one pulse per 3 hours due to the inhibition by estradiol (Foster and Ryan, 1979). However, during the peripubertal period, as inhibitory effects of estradiol on the hypothalamic-hypophyseal axis decrease, the pulse frequency of LH increases to one pulse per hour. These investigators found a concurrent increase in tonic or basal LH secretion and postulated that it may be these changes in LH secretion that are critical for the final stages of follicular growth just prior to puberty (Ryan and Foster, 1980).

Lutz *et al.* (1984) investigated the gonadostat hypothesis of sexual maturation in the gilt by collecting serum samples at 15 minute intervals over an 8 hour period. These researchers observed an increase in LH secretion asssociated with an increase in estrogen secretion similar to that observed by

Ryan and Foster (1980). These investigators also found that as puberty approached, serum LH concentrations and LH pulse frequency increased while LH peak amplitude decreased (Lutz *et al.*, 1984). These data are also similar to the findings of Ryan and Foster (1980) in the lamb. Collectively, these two studies support the idea that preovulatory follicular development is dependent upon a critical pattern of LH secretion characterized by an increase in tonic LH release.

Diekman *et al.* (1983) characterized endocrinological changes in the gilt during the prepubertal and peripubertal period and found no significant increase in serum LH pulse frequency during the 6 weeks prior to puberty. This is in contrast to the observations of Lutz *et al.* (1984) in the gilt and Ryan and Foster (1980) in the ewe. A possible explanation for this discrepancy in results is that Diekman *et al.* (1983) sampled every 20 minutes for four hours while Lutz *et al.* (1984) sampled blood at 15 minute intervals for 8 hours. Thus frequent sampling for a longer time appears to have provided a more accurate picture of LH secretory patterns in the gilt.

Estradiol Secretion Prior to Puberty

Serum estrogen concentrations in the gilt increase gradually from 8.4 pg/ml at ≥ 57 days prior to puberty to 14 pg/ml at 3-5 days before onset of puberty (Lutz *et al.*, 1984). These results are in agreement with those of Esbenshade *et al.* (1982) who found that average serum estradiol-17ß concentrations 6 days prior to onset of pubertal estrus was 6 pg/ml and increased to a peak value of 20 pg/ml on day 2.5 prior to puberty. After this point serum concentrations of estradiol-17ß decreased gradually until basal levels of 2-4 pg/ml were reached by day 4.5 post-estrus. Increased systemic concentrations of estrogen prior to pubertal estrus can be accounted for by the increase in growth of follicles destined to ovulate and their synthesis of estrogen.

Progesterone Secretion Prior to Puberty

Serum progesterone levels of gilts were observed to be similar at 6 days prior to pubertal estrus and at estrus with values ranging from 0.1 to 0.3 ng/ml (Esbenshade *et al.*, 1982). Upon formation of corpora lutea on day 1 post-estrus, concentrations of progesterone began to increase and peaked at between 7-13 days post-estrus (Henricks *et al.*, 1972). These observations suggest that in the gilt there is no transient increase in serum progesterone occurring prior to puberty, as has been observed in the heifer (Gonzalez-Padilla *et al.*, 1975; Berardinelli *et al.*, 1979) and ewe lamb (Foster and Ryan, 1979; Keisler *et al.*, 1983; Legan *et al.*, 1985). The source of the transient increase in progesterone observed in these latter species is believed to originate from immature corpora lutea which form from the luteinization of immature follicles (Legan *et al.*, 1985). Regression of these immature corpora lutea is via prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Keisler *et al.*, 1983). Legan *et al.* (1985) believes that this transient increase in progesterone is important for synchronizing pubertal follicular development with the pubertal LH surge. If this proposed action of the short luteal phase is correct then the absence of such a phase in the pubertal gilt may be a contributing factor to embryonic mortality. For more detail see section on "The Role of Embryo Asychrony in Embryo Mortality".

Folliculogenesis

Folliculogenesis is the process whereby primordial follicles develop into preantral (primary and secondary) antral (tertiary) and then ovulatory (Graafian) follicles. As stated by Ireland (1987) folliculogenesis is a "spectacular event" when one considers that the primordial follicle may increase in diameter 400 to 600-fold before ovulation and that, in order to supply the ovary with just a few ovulatory follicles at each estrus, between 500 to 1000 primordial follicles begin to develop. Individual primordial follicles that constitute the large population have only one of two destinies, to become atretic (degenerate) or to ovulate, of which the foremost is the predominant pathway.

The first step in folliculogenesis is the initiation of primordial follicle growth and the formation of a growing primary follicle (Foxcroft, 1987). This stage of follicular growth occurs independent of gonadotropin stimulation and is subject only to intraovarian control (Peters *et al.*, 1975). Once the follicle attains a thecal layer and the antrum begins to form, continued development from this point on requires the influence of both follicle stimulating hormone (FSH) and LH (Richards, 1975; Foxcroft, 1987). Such follicles are termed "recruited" by Goodman and Hodgen (1983). These recruited antral follicles make up a proliferating pool in the pig which contains approximately 50 follicles in the 2 - 5 mm diameter range (Anderson, 1980).

In the presence of appropriate endocrine stimulus "selection" occurs, which is the process by which only a few recruited follicles are selected to develop to ovulatory size while the remaining follicles undergo atresia (Goodman and Hodgen, 1983).

As can be easily surmised, the process of folliculogenesis is complex and is characterized by many physical and biochemical changes in the follicle, such as

cellular constitution, gonadotropin responsiveness and steroidogenic capability. Because folliculogenesis is the process directly related to the availability of an ovum, it is deemed appropriate to review some of these changes, especially those of endocrine relevance.

Gonadotropin Receptor Responsiveness during Folliculogenesis

Because gonadotropins play a primary role in follicular maturation, steroidogenesis and ovulation, an examination of the evolutionary changes involved in theca and granulosa cell gonadotropin receptor systems is appropriate. Follicle stimulating hormone and LH are produced by the anterior pituitary and are the primary gonadotropins involved in folliculogenesis (Hisaw, 1947). These gonadotropins bind to their respective plasma membrane receptors in the follicle resulting in an induction of cAMP-mediated enhancement of protein synthesis. Enzymes essential for steroidogenesis and receptors are examples of proteins synthesized in response to gonadotropins.

There is an abundance of information available on the acquisition of gonadotropin receptors and gonadotropin-receptor interactions within the different populations of porcine follicular cells. Quantitation of changes in gonadotropin receptors during follicular development in the gilt has been possible in part because of the large number of follicles present and in part because of the uniformity of the cellular populations. In the majority of experiments, follicles have been analyzed as three distinct groups based on diameter: small (1-2 mm), medium (3-5 mm) and large (6-12 mm).

Thecal cells of porcine follicles contain LH receptors from early stages of follicular development and throughout subsequent folliculogenesis (Hillier, 1987) but these cells never acquire FSH receptors (Carson *et al.*, 1979). These findings are similar to those of Zeleznik *et al.* (1974) who found that immature rat thecal

cells bound only human chorionic gonadotropin (hCG). This hormone and LH bind to the same receptors with similar affinity (Kammerman *et al.*, 1972; Lee and Ryan, 1973; Haour and Saxena, 1974).

In contrast to the single receptor population present in thecal cells throughout folliculogenesis, gonadotropin binding to granulosa cells changes as the follicle matures (Carson *et al.*, 1979). Granulosa cells of preantral follicles possess FSH receptors only (Zeleznik *et al.*, 1974; Rajaniemi *et al.*, 1977) and binding of FSH in granulosa cells of porcine follicles decreases as diameters of the follicles increase. Granulosa cells begin to acquire LH receptors at about the time of antrum formation (Zeleznik *et al.*, 1974; Rajaniemi *et al.*, 1977) and binding of LH to granulosa cells increases as the follicle matures (Channing and Kammerman, 1973; Kammerman and Ross, 1975; Nakano *et al.*, 1977). Ability of granulosa cells of porcine follicles to bind hCG (LH) increases 10 to1000-fold when comparing small and large follicles (Channing and Kammerman, 1973; Kammerman and Ross, 1975). This augmentation of hCG (LH) binding is believed to be due to an increase in receptor sites and not to a change in affinity, which was found to be similar in small and large follicles (Kammerman and Ross, 1975).

Increase in LH binding as follicle diameter increases is in agreement with the data of Channing and Kammerman (1973) who reported that cAMP formation and progestin production were positively correlated during folliculogenesis. Regulation of the induction and aggrandizement of the granulosa cell LH receptors are not clearly understood. It has been speculated that FSH induces the appearance of LH receptors in granulosa cells (Zeleznik *et al.*, 1974; Rajaniemi *et al.*, 1977; Ireland, 1987) and it is possible that estrogens acting as an intrafollicular autocrine regulator act synergistically with FSH (Lindner *et al.,* 1977; Hillier, 1987) in promoting induction of these receptors.

If one were to characterize the importance of folliculogenesis, the

development of the ovum to a stage that upon release could undergo successful fertilization would be of primary importance. The next event of importance in folliculogenesis would have to be the steroidogenic capability of developing follicles.

It has been established that the follicle is an important source of estrogens, progestins and androgens, and as the follicle increases in size, the concentration of these three steroids in the follicular fluid become elevated in a highly significant manner (Eiler and Nalbandov, 1977; Ainsworth *et al.*, 1980). Follicular fluid concentrations of estrogens and androgens peak at about the onset of estrus which coincides with the LH surge and thereafter begin a rapid decline (Eiler and Nalbandov, 1977; Ainsworth *et al.*, 1980). In contrast to the previous two steroids, progesterone continues to increase after the LH surge and causes an inversion of the estrogen:progesterone ratio (Meinecke *et al.*, 1987). The dynamics of intrafollicular steroid concentrations appear to have biological significance because it has been shown that steroids are important for *in vivo* and *in vitro* oocyte maturation and subsequent development of a fertilizable oocyte (Moor and Trounson, 1977; McGaughey, 1977; Moor *et al.*, 1980; Rice and McGaughey, 1981; Racowsky, 1983).

Estrogen Biosynthesis

In the nonpregnant female primary sites of estrogen biosynthesis are the developing follicles of the ovary. In addition, small quantities of estrogen are produced by the adrenal cortex and during pregnancy the fetal-placental unit produces large quantities of these steroids. In considering estrogen biosynthesis by the ovary an elegant study by Falck (1959) was the first to demonstrate that a functional relationship existed between theca interna and the granulosa cells. The results of this study led to the present concept known as the "two cell hypothesis."

Falck transplanted theca interna and granulosa cells plus a piece of vagina, which served as an indicator of estrogen synthesis, into the anterior chamber of a rat's eye. Granulosa cells by themselves always luteinized and were unable to cause cornification of the vaginal transplant. However, if theca interna and granulosa cells were transplanted together they were capable of producing cornification of the vaginal tissue (Falck, 1959). This hypothesis that two cell types are involved in estrogen synthesis, was further investigated by Short (1962) who found that estrogens were present in follicular fluid but were nondetectable in the corpus luteum. Because development of the follicle during estrus is characterized by hypertrophy and hyperplasia of granulosa cells, whereas theca interna cells undergo degeneration, Short (1962) concluded that the theca interna cells were responsible for estrogen production. These investigations stimulated further *in vitro* studies of individual follicular cell types and their involvement in estrogen biosynthesis.

Bjersing and Carstensen (1967) incubated porcine granulosa cells in the presence of both testosterone and androstenedione as substrates and found that granulosa cells were capable of producing estrone and estradiol thus indicating the presence of aromatase within granulosa cells. Additional *in vitro* cell culture studies were performed by Liu and Hsueh (1986) in which immature rat cells were utilized. These investigators found that when granulosa and theca interstitial cells were cultured alone, they produced negligible quantities of estrogen. If progesterone was added to the separate cell cultures, the biosynthesis of estrogen was not enhanced whereas when aromatizable androgens (testosterone and androstenedione) were included in the media, granulosa cells but not theca cells, were able to synthsize estrogens. Further treatment of individual cell types with FSH, hCG or FSH and hCG did not augment estrogen production. When granulosa cells and theca interstitial cells were cultured together there was a

significant increase in estrogen biosynthesis that was enhanced by gonadotropin treatment. In the same combined culture system, exogenous testosterone or progesterone antibody administration decreased estrogen production; their effect was reversed by addition of exogenous testosterone or progesterone but not the progesterone agonist R5O20. These results indicated that exogenous progesterone (and testosterone) were utilized as substrates for estrogen biosynthesis and that the effect of progesterone did not involve the progesterone receptor system.

Further support for the two-cell hypothesis of estrogen biosynthesis was provided by Baird (1977) using an *in vivo* experiment in which the ovaries of ewes in the follicular phase where autotransplanted to a carotid jugular skin loop. Ewes treated with an antisera against testosterone registered a significantly lower secretion of estradiol than controls suggesting that testosterone leaves the ovarian cell (theca or granulosa) before aromatization *in vivo*.

In contrast to the previous findings in support of the two cell hypothesis, Evans *et al.*, (1981) using porcine granulosa and theca cells from pregnant mare serum gonadotropin (PMSG)-treated prepubertal gilts found that on a per follicle basis, theca cells produced estradiol in quantities comparable to the granulosa. The fact that these tissues were collected from gilts induced to ovulate with a gonadotropin not normally present may contribute to the contradictory results.

Mechanism of Action of Estrogens

The suggestion that estrogens were able to regulate gene expression was first proposed by Mueller *et al.* (1957) and since has triggered the interest of many scientists to decifer the mechanisms by which estrogens can exert such an effect. The first model, now recognized as the "Old Model" or "Two Step Mechanism" advanced by Gorski *et al.* (1968) and Jensen *et al.* (1968) stipulated that

estrogen, a lipophilic molecule, was able to diffuse through the cell plasma membrane and would then bind to a cytoplasmic estrogen receptor. Once the extranuclear estrogen-receptor complex was formed it would then undergo "receptor transformation," an alteration of the receptor protein, as it was translocated to the nucleus. Inside the nucleus, the estrogen-receptor complex could initiate or accelerate RNA synthesis and ultimately protein synthesis by induced mRNA's.

Evidence supporting this former theory was the finding that unoccupied estrogen receptors were resident in the cytoplasmic fraction of homogenized estrogen target tissues (Gorski *et al.*, 1968; Jensen *et al.*, 1968). With the use of ultracentrifugation of a sucrose density gradient the estrogen receptor complexes in the cytoplasmic fraction and nuclear fraction were reported to yield a sedimentation coefficient of close to 8S and 9S, respectively. In high ionic buffers these complexes reversibly dissociated into 4S and 5S subunits, respectively, thus indicating that some form of receptor transformation occurred during translocation (Jensen and DeSombre, 1973).

Although this model became the central dogma, there was scepticism with respect to its validity because Zava and McGuire (1977) found that there was a larger fraction of unoccupied estrogen receptors in nuclear preparations. Experiments of King and Greene (1984), in which five monoclonal antibodies for estrogen receptors plus indirect immunoperoxidase techniques were utilized, indicated that both the cytosolic and nuclear forms of the estrogen receptor reside in the nucleus. With advances in technology such as formation of monoclonal antibodies to estrogen receptors and cytochalasin B-induced enucleation to yield cytoplast and nucleoplast fractions, researchers began to reexamine the mechanisms by which estrogens exerted their effects.

The results of experiments by King and Greene (1984) were confirmed by

the enucleation experiments of Welshons *et al.* (1984) using GH_3 cells derived from rat pituitary tumors. These investigators proposed that the previously detected cytoplasmic receptor was an artifact of homogenization. In the absence of steroid the receptor is loosely associated with nuclear components and can be partitioned into the cytosol upon cell disruption. It was therefore suggested that there is no nuclear translocation of receptor as part of the steroid response, but rather and increase in the affinity of the receptor for nuclear components. These findings have led to the "new model" for describing the action of estrogens.

The "New Model" describing the molecular mechanism by which estrogens regulate gene transcription as stated by Greene and Press (1986) begins with diffusion of free estrogens through the plasma membrane. Once inside the cell, estrogen may bind to low affinity binding proteins, not true receptors (Mercer *et al.*, 1981). Estrogen continues its passage either unaided or loosely bound to proteins through the cytoplasm into the nucleus where it interacts with an unoccupied receptor and possibly causes the activation of a dimeric estrogen receptor complex. The activated estrogen receptor complex then becomes tightly associated with nuclear acceptor sites such as specific DNA sequences (Compton *et al.*, 1983; Payvar *et al.*, 1983) ribonucleoproteins (Liang and Liao, 1974) basic nonhistone proteins (Puca *et al.*, 1974) or nuclear matrix (Barrack and Coffey, 1980).

At the present time the biological significance of the above acceptor sites has not been elucidated, and for this reason the field of steroid molecular biology continues to represent an area in which the questions outweigh the answers.

Progesterone Biosynthesis

Biosynthesis of progesterone in the ovary can be considered as somewhat of an intermediate in the "two cell hypothesis." Granulosa cells, which are

responsive to both LH and FSH late in folliculogenesis, are the prominent sites of the conversion of cholesterol to progesterone (Evans *et al.*, 1981; Liu and Hsueh, 1986). Follicle stimulating hormone and LH acting in granulosa cells enhance the uptake of lipoproteins, liberation of cholesterol from lipoproteins, mobilization of cholesterol, and activation of the mitochondrial side-chain cleavage enzymes that convert cholesterol to pregnenolone. These gonadotropins also promote conversion of pregnenolone to progesterone by activation of 3ß-hydroxysteroid dehydrogenase (Ireland, 1987). As stated previously progesterone is not only important as a distinct entity but also as an intermediate in steroidogenesis. Progesterone produced by granulosa cells is utilized by theca cells to form androgens that can further be aromatized into estrogens by granulosa cells in the rat (Liu and Hsueh, 1986).

Mechanism of Action of Progesterone

The mechanism of action of progesterone is in some ways very similar to that of estrogens, especially with respect to its ability to associate directly with components within the nucleus thereby activating genes and stimulating resultant mRNA production (Norman and Litwack, 1987). Progesterone is transported to its target cells bound to a corticosteroid-binding globulin (Mendelson, 1988). Progesterone is released at the target cell, and because of its lipophilic properties, freely diffuses across the plasma membrane into cytoplasm and ultimately into the nucleus (Mendelson, 1988). Once inside the nucleus, progesterone binds to a 6S "A:B" receptor. The occupied activated dimer dissociates and it is postulated that the "B" subunit "searches" the chromatin until it interacts with the appropriate acceptor protein(s). The "A" receptor subunit becomes associated with some effector or initiation site for RNA synthesis which stimulates transcription and the production of proRNA. This proRNA is then modified to liberate mRNA which is

translated into a new polypeptide and ultimately a new protein which can exert its biological effects (Norman and Litwack, 1987).

Selection and Dominance

As indicated earlier, the first step in folliculogenesis is the recruitment of follicles from a pool of gonadotropin-independent follicles. Time of selection of dominant follicles (those follicles destined to ovulate) from this proliferating pool has been well established to be between days 14 -16 of the estrous cycle of the gilt or sow (Foxcroft and Hunter, 1985). This timing of selection is in agreement with the findings that the beneficial effects of "flushing" in increasing number of follicles of ovulatory size in gilts are only consistently observed when this practice commences at least, but no later than, 4 days prior to estrus (Kirkpatrick *et al.*, 1967). Also, after day 16 of the cycle unilateral ovariectomy or electrocautery of follicles on one ovary results in incomplete ovarian compensation (Foxcroft and Hunter, 1985). Likewise endogenous gonadotropins are ineffective in causing superovulation after day 16 of the cycle (Foxcroft and Hunter, 1985).

Once selection occurs, smaller follicles in the proliferating pool undergo rapid atresia and their replacement is blocked (Robinson and Nalbandov, 1951; Clark *et al.*, 1982) The criteria or factors that determine whether or not a follicle continues development toward ovulation are not well understood although there are many possibilities.

Many intragonadal nonsteroidal factors isolated from porcine ovarian tissue have been postulated to play various roles in regulating gonadal function. Examples of these include oocyte maturation inhibitor (OMI), granulosa cell maturation and luteinization stimulator (LS), granulosa cell luteinization inhibitor (LI), LH receptor binding inhibitor (LH-RBI), gonadocrinin and inhibin (Foxcroft, 1987). These factors undoubtedly play some role in follicular maturation in the

porcine ovary. The majority of research to elucidate roles of nonsteroidal components has been performed using tissue collected from gilts whose physiological states were unknown (Foxcroft, 1987). Thus any distinct conclusions as to the direct roles of these compounds as possible mediators of the selection process are premature until studies are performed on individual follicles from animals of known history (Foxcroft, 1987).

The possibility also exists that dominant follicles elaborate some hormonal and nonhormonal factors that may alter gonadotropin release from the pituitary (Ireland, 1987) thereby creating an environment via a feedback system that is suppressive to less developed follicles. Shaw and Foxcroft (1985) observed a significant positive correlation between FSH concentrations post-weaning and ovulation, suggesting that FSH may be involved in determining the final number of dominant follicles. These data are consistent with the proposed ability of the dominant follicle to secrete increased amounts of inhibin (Padmanabhan *et al.*, 1984) and estrogen that act via a long-loop negative feedback to decrease FSH release and thereby suppress growth of other follicles (Ireland, 1987).

Whatever the mechanism involved in selection, it does not result in an absolute preference. As indicated by Foxcroft (1987) there is a range of development which exists within a population of dominant follicles in which some smaller follicles never reach the developmental maturity of other follicles within the same selected pool. There also has been a report of asynchrony of maximal estrogen concentrations in follicles destined to ovulate (Foxcroft, 1987). Because follicular estrogen levels and diameter have been highly correlated (Grant et al., 1989) it is tempting to to conclude that these smaller follicles in the ovulatory pool are producing less estrogen than their counterparts. Whether this discrepancy in maturity has any consequences on subsequent embryonic survival has yet to be investigated.

Ovulation

In response to rising levels of estrogens produced by preovulatory follicles, the pituitary releases an ovulatory surge of luteinizing hormone. This surge of gonadotropin initiates a cascade of events at the follicular level that ultimately result in ovulation. Because there is a possibility that time sequences of ovulatory events or factors liberated during the ovulatory process may influence ultimate viability of the fertilized ova during later development, a review of ovulation is justified.

Hyperemia of the Follicle With Respect to Ovulation

Within a few minutes of the LH surge, there is a significant increase in ovarian circulation (Lee and Novy, 1978). It is well known that the ovulatory surge activates adenylate cyclase which in turn stimulates the synthesis of cAMP in follicular cells (Hunzicker-Dunn *et al.*, 1979). Substances that are believed to directly mediate the rise in ovarian blood flow are histamines, serotonin and possibly bradykinins that cause local vasodilation and increased capillary permeability (Espey, 1980). The relevance of this hyperemia is to increase the migration of polymorphonuclear leukocytes, which serve as a line of defense, and macrophages into follicular tissue (Espey, 1980).

Importance of prostaglandins in the ovulatory process is supported by evidence that administration of indomethacin, an inhibitor of prostaglandin synthase is able to block ovulation (Shimada *et al.*, 1983; Reich *et al.*, 1985). It has been suggested that prostaglandins facilitate ovulation by stimulating contraction of smooth muscle cells surrounding the follicle. However, there is no convincing evidence that contraction of ovarian smooth muscle is essential for ovulation (Espey, 1978). It has also been theorized that prostaglandins may

stimulate the synthesis, release and(or) activation of collagenase-like enzymes involved in ovulation. For a more detailed explanation of the involvement of prostaglandins and collagenase in ovulation, see section on "Plasminogen Activators and Ovulation."

Plasminogen Activator

Plasminogen is an inactive proenzyme that is present in the plasma and extravascular fluids such as follicular fluid (Beers, 1975), saliva (Moody, 1982) and porcine uterine fluid (Fazleabas *et al.*, 1983). Plasminogen, in its native form is a single polypeptide with a molecular weight of approximately 92,000 and contains glutamic acid as the N-terminal amino acid; thus it is termed Glu-plasminogen (Danø *et al.*, 1985). Conversion of plasminogen to the active serine protease plasmin via plasminogen activator is by way of hydrolysis of the arginyl^{560}-valyl^{561} peptide bond. Holvoet *et al.* (1985) have suggested that the activation of Glu-plasminogen *in vivo* yields Glu-plasmin without the involvement of Lys-plasminogen intermediates. Glu-plasmin is characterized by the presence of an activation peptide and two peptide chains known as the "heavy" and "light" chains (Christman *et al.*, 1977). The heavy chain (MW = 60,000), derived from the amino terminus of plasminogen, is linked to the light chain (MW = 20-25,000) which is derived from the carboxy- terminus by a single disulfide bond (Christman *et al.*, 1977). Once Glu-plasmin is formed, it can be converted to Lys-plasmin in a reaction catalyzed by either Glu- or Lys-plasmin in which the activation peptide is removed (Danø *et al.*, 1985).

The majority of plasminogen activators (PA) can be characterized as either urokinase-type PA (u-PA) or tissue-type PA (t-PA), which display differences in function, molecular weight and immunological reactivity (Danø *et al.*, 1985). Christman *et al.* (1977) suggested that there may also be a third category of PA,

the circulating PA, which has a much higher molecular weight than either u-PA or t-PA. These investigators have stated that the circulating PA may not be structurally distinct from u-PA or t-PA, but rather the difference in molecular weight may be explained by binding of inert plasma proteins to other PA's.

Urokinase-type Plasminogen Activator

Sahli in 1885 (Danø *et al.,* 1985) was the first to describe the fibrinolytic activity of urine, which was later suggested to be due to a plasminogen activator (Sobel *et al.*, 1952), and which is now known as u-PA. Urokinase-type PA is a serine protease with a molecular weight of appoximately 50,000 and can be present in both a one-polypeptide chain, which is an inactive proenzyme, or a two-chain active enzyme held together by a single disulfide bond (Danø *et al.*, 1985). The active site of u-PA has been found to be located on the heavy chain (Ong *et al.*, 1976) and is able to cause hydrolysis of the arginine-valine bond of plasminogen. Like most serine proteases, u-PA is released from cells as an inactive proenzyme (single chain u-PA). This proenzyme is unable to initiate proteolysis of plasminogen, but can be converted into the active (two chain u-PA) form by limited proteolytic influence of plasmin (Nielsen *et al.*, 1982).

Tissue-Type Plasminogen Activator

Demuth and Von Riessen in 1928 (Danø *et al.*, 1985) suggested that tissue cells produced an agent that was capable of activating an enzyme in plasma that ultimately caused fibrinolytic activity. This product of tissue cells was later defined as t-PA. Tissue-type plasminogen activator is an acid-stable protease whose fibrinolytic or caseinolytic activity is completely dependent on the presence of plasminogen (Danø *et al.*, 1985). Tissue-type plasminogen activator has a molecular weight of approximately 70,000 and, similarly to u-PA, can be present in

an inactive one polypeptide chain or an active molecule consisting of two polypeptide chains linked by disulfide bonds (Danø *et al.*, 1985).

Plasminogen Activators and Ovulation

Extensive degradation of the follicle wall is requisite in order for ovulation to occur. The ovulatory process does not rely soley on increased hydrostatic pressure within the follicle (Espey and Lipner, 1963) but rather on some form of proteolytic activity (Schochet, 1916).

It has been demonstrated that injection of preovulatory follicles with certain proteolytic enzymes such as collagenase and trypsin can cause follicular rupture similar to the events of ovulation (Espey and Lipner, 1965). Espey (1967) also showed that there was a decrease in tensile strength of follicular strips as ovulation approached and indicated that this may be due to the actions of proteolytic enzymes that caused decomposition of the connective tissue of the follicular wall. This premise was further supported by the evidence that plasmin can also decrease the tensile strength of follicular strips (Beers, 1975).

Plasminogen, the plasmin zymogen is present in follicular fluid in concentrations ranging from 0.2 to 0.7 mg/ml (Beers, 1975). Presence of plasminogen (MW ≈ 85,000) of serum origin in follicular fluid is not surprising because the barrier between serum and follicular fluid admits molecules into the follicular antrum in amounts inversely related to size with an absolute cut-off at 850,000 Da (Shalgi *et al.*, 1973). As indicated earlier, conversion of plasminogen to plasmin is dependent upon plasminogen activator. This component has been identified in follicular fluid in greater amounts than in plasma, indicating that some follicular component is capable of producing plasminogen activator (Beers, 1975). Granulosa cells produce copious quantities of plasminogen activator in the sow (Kokolis *et al.*, 1987); in particular this is true for t-PA in the rat (Beers *et al.*, 1975,

Strickland and Beers, 1976; Canipari and Strickland, 1986; Liu *et al.*, 1987) and u-PA in the mouse (Canipari *et al.*, 1987). This synthesis, as well as the correlated increase in PA activity as ovulation approaches (Beers, 1975; Strickland and Beers, 1976; Kokolis *et al.*, 1987) further supports the involvement of the plasmin-generating system with ovulation.

Although Beers (1975) was unable to purify plasmin from follicular fluid, the following finding suggested that one of the proteases in follicular fluid was plasmin. First, bovine plasmin and the follicular fluid protease are eluted from lysine-sepharose under the same conditions. Second, inhibitors of plasmin that are present in follicular fluid also inhibit the follicular protease. The role of these plasmin inhibitors in follicular fluid as suggested by Beers (1975) may be to prevent unrestrained proteolysis within the follicle. Lastly, the presence of plasminogen and plasminogen activator in the follicle should cause the production of plasmin.

Although the evidence for the role of plasmin in ovulation as described up to this point is all based upon indirect evidence, experiments by Tsafriri *et al.* (1989) have supplied direct evidence supporting the importance of this protease in the ovulatory process. These investigators found that injection of specific antibodies to t-PA and α_2-antiplasmin (the major plasmin inhibitor) into the ovarian bursae of PMSG, hCG-treated rats was able to significantly decrease ovulation rate. Furthermore, nonimmune immunoglobulin G and heat-inactivated α_2-antiplasmin were not effective.

At this point the premise of the role of plasminogen activators in ovulation seems overwhelming. However, data that suggest that ovulation can be inhibited even when PA's are not inhibited (Espey *et al.*, 1985) led some researchers to conclude that plasminogen activators are not a necessary component of ovulation. Because involvement of plasmin in ovulation has been suggested to occur via

activation of collagenases (Werb *et al.*, 1977; Espey, 1980) it is possible that actions of indomethacin and other drugs that block ovulation, but not PA activity, may be due to interruption of collagenase activation (Reich *et al.*, 1985; Tsafriri *et al.*, 1989).

Hormonal Control of Plasminogen Activator in the Follicle

Numerous investigators have conducted experiments to elucidate the hormonal control of follicular PA. Ability of FSH to stimulate granulosa cell PA production and secretion has been well documented (Strickland and Beers, 1976; Liu *et al.*, 1981; Martinat and Combarnous, 1983; Canipari and Strickland, 1986). Nevertheless, the correlation between LH stimulation and PA activity of granulosa cells has become an area of debate.

Martinat and Combarnous (1983) in testing many different hormones (FSH, LH, thyrotropin stimulating hormone, chorionic gonadotropins, prolactin and relaxin) of several species found that granulosa cell secretion of PA was specifically stimulated by FSH, at least in the mammalian species. Similarly, Strickland and Beers (1976) found that inactive granulosa cells could be stimulated to produce and secrete PA by LH, but to a greater extent by FSH. Wang and Leung (1983) stated that in immature granulosa cells, FSH, but not LH stimulated PA production. In contrast to these results, Reich *et al.* (1985), using follicular explants of mature rats in proestrus, which should better represent the intrinsic interaction between granulosa cells, theca cells and oocyte-cumulus complex, found that ovine LH was more potent in stimulating PA activity than ovine FSH. It appears that differences in the ability of follicular components, particularly granulosa cells, to produce PA in response to LH lies in the development of LH receptors in these cells. Induction of LH receptors in granulosa cells only occurs in the later stages of follicular development under the stimulation of estradiol and

FSH (Linder *et al.*, 1977; Hillier, 1987). This may explain the inability or decreased ability of LH to stimulate PA activity in the studies by Martinat and Combarnous (1983; who did not specify follicle stage of granulosa cell harvest), Wang and Leung (1983; who used immature granulosa cells) and Strickland and Beers (1976; who used "inactive" granulosa cells).

Ability of prostaglandins E_1 and E_2 (PGE) to stimulate PA activity in granulosa cells (Strickland and Beers, 1976) suggested the involvement of these arachidonic acid metabolites in ovulation. Indomethacin, an inhibitor of cyclooxygenase, and nordihyroguaiaretic acid, an inhibitor of the lipoxygenase pathway, were able to block ovulation and yet had no effect on granulosa cell PA (Shimada *et al.*, 1983; Reich *et al.*, 1985). It was therefore postulated that arachidonic acid derivatives are not obligatory mediators of LH stimulation of PA activity, as proposed by Canapari and Strickland (1986). Actions of PGE during ovulation may be analogous to the ability of PG's to mimic stimulatory actions of LH on resumption of meiosis and folliculogenesis; both of these processes proceed undisturbed in the presence of indomethacin (Reich *et al.*, 1985). These statements do not discredit the role of PG's in ovulation.

Role of steroids in influencing the production and secretion of PA in the follicle has been investigated by Reich *et al.* (1985). These investigators found that steroids in culture media plus follicle explants did not increase PA activity although estradiol (1μg/ml) was able to increase PA activity in LH-stimulated cells (Reich *et al.*, 1985), possibly by influencing LH receptors in granulosa cells of the follicle explants.

Embryogenesis

Perry and Rowlands (1962) reported that the majority of embryonic mortality in swine occurs before day 19, which is concomitant with the timing of attachment. If this is indeed the case, then a description of preattachment development is this polytoccous species is important. The following section will be devoted to delineation of the time sequence of morphological changes that the ova/early embryo undergo between ovulation and attachment. These events have been reviewed extensively (Hunter 1974; 1977).

Ova Development from Ovulation to Fertilization

Ovulation in response to the ovulatory surge of LH occurs between 38-42 hours after the onset of estrus in the gilt or sow (Anderson, 1980). Within 30-45 minutes post-ovulation (PO), the ova, which are invested in cumulus oophorus cells, are located deep within the Fallopian tube in closest proximity to the fimbria and ovary. If mating has occurred prior to ovulation, denudation or the loss of cumulus oophorus will begin. The process of denudation involves the proteolytic action of hyaluronidase which is liberated by spermatozoa.

Ova reach the ampullary-isthmic junction approximately 1 hour after ovulation and it is at this site that the fertilization process begins. Timing of sperm penetration into the zona pellucida and activation of the second meiotic division of all eggs would probably occur between 6-8 hours after mating in most animals. Restoration of the diploid condition is achieved by 12-14 hours after sperm penetration or 20-22 hours PO. Although it has been reported that the number of spermatozoa attached to the zona pellucida increases during tubal transport, some form of polyspermy blockage must occur. Once a spermatozoan gains contact with the oolemma, a process known as the "zona reaction" commences.

This process is characterized by exocytosis of cortical granules that modify the zona pellucida by making it more resistant to protease digestion and therefore less permeable to sperm (Shapiro *et al.*, 1981).

Embryo Development from Fertilization to the Morula Stage

From the one-cell porcine embryo, the next two divisions proceed quite rapidly resulting in two-celled embryos (22-24 hours PO) and eventually the four cell stage (28-32 hours PO). Although the second mitotic division is usually synchronous yielding the typical four-cell embryo, asynchronous mitosis yielding three-cell embryos is not unusual. Production of two- and four-cell embryos occurs within the isthmus and passage through the uterotubal junction at the four-cell stage is usually complete by 46 hours. Embryos then remain in the upper half of the uterine horn for the next 2-3 days. The eight-cell stage is achieved within a few hours after the embryo transverses the uterotubal junction. From this point on, resulting mitotic divisions show a high degree of asynchrony.

Morulae of 16-32 cells are observed late on the third day of development and are thus located in the proximal half of the uterine horn.

Blastocyst Development and Elongation

Porcine blastocyst formation is reported to begin approximately on the fifth day of embryo development with prominence of the inner cell mass present on the sixth day. Blastocytsts are comprised of a zona pellucida, trophectoderm, inner cell mass and blastocoel or fluid-filled cavity. Blastocoel formation is dependent upon Na^+/K^+ ATPase activity at the basolateral surface of the outer embryonic cells. This enzyme activates the transport of three Na^+ molecules into the cellular matrix and exit of two K^+ molecules, thus creating a solvent-drag gradient that facilitates the movement of water into the embryo forming a blastocoel.

Hatching, shedding of the zona pellucida, occurs late on the sixth day of development. Increased hydrostatic pressure formed by continued Na^+/K^+ ATPase activity and resulting blastocoel expansion and zona lysins of both embryonic and uterine origin all play a role in this event. It has been reported it is only after the embryos have hatched that a marked asynchrony in size is noticeable among embryos. It has not been determined whether or not these differences in trophblast growth are directly related to the ultimate viability of the embryo.

After hatching, the trophoblast proliferates markedly, and at around day 9 the expanding blastocyst undergoes massive elongation resulting in a thread-like conceptus. Intrauterine migration of porcine embryos occurs during days 9 to 12 (Dhindsa *et al.*, 1967) and it is around this time that the embryo begins to produce its own estrogens (Perry *et al.*, 1976). Uterine migration and embryo estrogen synthesis are associated with an increase in myometrial activity (Pope *et al.*, 1982a). Results from Pope *et al.*, (1982c) indicate that both estradiol and histamine are involved with this migration. Estradiol-17ß and histamine may work independently (Pope *et al.*, 1982c) or estradiol may stimlate the release of histamine (Spaziani and Szego, 1958) which then causes increased uterine blood flow (Harvey and Owen, 1979). Increased uterine blood flow could indirectly increase myometrial activity (Wislocki and Guttamacher, 1924).

Commencement of a weak attachment of the embryo to the endometrium is observed in the form of short microvilli extending from the surface of the trophoblast on day 13 of gestation (Crombie, 1970). In concert with the materialization of embryo microvilli is the appearance of microvilli on the endometrial surface (Crombie, 1970). Thus the attachment of the embryo and is well advanced by day 18 (Perry, 1969). Contact between maternal and trophoblastic surfaces remains diffuse and the placenta of the porcine is

epitheliochorial in nature.

Embryo Mortality

As stated previously, Van Lunen and Aherne (1987) theorize that a minimum weight threshold must be met before puberty can be attained. Proximity of actual onset of puberty to this weight threshold depends upon whether gilts have been subjected to the presence of boars or social mixing with other gilts.

In comparison with third-estrous gilts, gilts that have been stimulated to reach puberty early will ovulate fewer ova at their pubertal estrus (Robertson *et al.*, 1951; Andersson and Einarsson, 1980; Archibong *et al.*, 1987a,b) and, if mated, will sustain greater embryonic losses (Warnick *et al.*, 1951; MacPherson *et al.*, 1977; Archibong *et al.*, 1987a). Gilts that exceed the minimum weight threshold before stimulated to attain puberty do not exhibit these characteristics (Dyck, 1971; Knott *et al.*, 1984). In quantifying the percentage of embryonic survival, Archibong *et al.* (1987a) reported that by day 15 post-mating first- and third-estrous gilts had 78.1 and 95.1% embryonic survival, respectively, and by day 30 post-mating embryonic survival decreased to 66.7 and 89.4%, for first and third-estrous gilts, respectively.

Oocyte Contributions to Embryonic Mortality

Archibong *et al.* (1987a) found that fertilization rates of pubertal gilts were similar to those of third-estrous gilts (100 versus 98%, respectively). However, there is evidence that ova ovulated by the pubertal gilt may possess an abnormality that is detrimental to development of the embryo at a latter stage. This possibility is supported by both *in vivo* and *in vitro* studies. Menino *et al.* (1989) surgically collected one- to eight-cell embryos from first- and third-estrous gilts, and upon *in vitro* culture, found that development to the blastocyst and expanded blastocyst stages was significantly lower for embryos of first-estrous gilts (50 and

11%, respectively) than for embryos of third-estrous gilts (75 and 35%, respectively). Archibong (1988) employing embryo transfers reported that percentage embryonic survival was significantly less for embryos from first-estrous donors in comparison with donors that had cycled one or more times (63.9 ± 5.1 versus 80.3 ± 3.9%, respectively).

Uterine Contributions to Embryonic Mortality

Schnurrbusch and Erices (1979) have suggested that attainment of puberty in the gilt stimulates endometrial and myometrial development. This increased uterine development continues with each subsequent estrous cycle and is presumably due to ovarian steroids. Because of the relatively underdeveloped uterus of the pubertal gilt it is possible that embryo wastage may be due to restricted uterine space. Dziuk (1968), however, found that when embryos were restricted to a predetermined uterine space by ligation of the uterine horns, thus creating an abnormally crowded environment, embryos were spaced equidistant from each other and there was no increase in day 30 embryonic mortality over that of controls. It is therefore unlikely that the morphologically underdeveloped uterus of the pubertal gilt is related in a causative fashion to increased embryonic mortality observed in the young gilt.

Nevertheless, the possibility still exists that the uterus of the pubertal gilt provides an environment that is not as supportive to early embryo survival as that of their multiestrous counterparts. In support of this possibility Murray and Grifo (1976) found that prepubertal gilts treated with steroids secreted lower concentrations of uterine specific proteins than did mature gilts. On the other hand, this idea has been refuted by the research of Archibong (1988), in which embryos of first-, second-, third- and fourth-estrous donors were randomly transferred to first- or third-estrous recipients. Percentage viable embryo recovery

did not differ between first- and third-estrous recipients (77.2 ± 3.7 versus 74.1 ± 4.9%, respectively).

The two main reproductive steroids are progesterone and estrogen, both of which are extremely important in maintaining viability of the embryo throughout the early stages of pregnancy. Systemic blood concentrations of progesterone, which is produced by corpora lutea, increases during early gestation until days 12 to 14, after which time it decreases until day 25 when concentrations plateau and remain constant until day 100 of gestation. After day 100, progesterone concentrations decrease slowly until parturition on days 114 to 116 (Guthrie *et al.*, 1974; Knight *et al.*, 1977). Estrogens, predominantly of embryonic origin (Perry *et al.*, 1976; Bazer *et al.*, 1982) are believed to act upon the progesterone-dominated uterus to stimulate the secretion of proteins (Geisert *et al.*, 1982a,b) and carbohydrates (Zavy *et al.*, 1982), which make up the histotroph ("uterine milk") that is important for embryonic nurishment prior to attachment. In considering the possibility that altered steroid production during early gestation might account for the difference in embryonic mortality between first- and multiestrous gilts, Archibong *et al.* (1987a) studied plasma concentrations of progesterone and estrogens in the pubertal and third -estrous gilts at days 3, 15 and 30 post-breeding. These researchers found no significant difference in concentrations of these steroids and therefore higher embryonic mortality in the pubertal gilt does not seem to stem from altered synthesis or secretion progesterone or estrogen.

The Role of Embryo Asychrony in Embryo Mortality

Pope *et al.* (1982b) has reported that transferring day 5 embryos into one uterine horn of day 6 nonpregnant recipients and day 7 embryos into the other horn was not conducive to the survival of the younger embryos. Thus asynchrony among developing embryos had adverse effects on embryo survival. During

trophoblastic elongation embryos synthesize and release estrogens (days 11 and 12) which are important for endometrial protein secretion (Geisert *et al.*, 1982a) and also play a role in maternal recognition of pregnancy (Bazer and Thatcher, 1977). If estrogen is administered at day 9 and 10 of gestation, embryonic mortality is increased (Pope *et al.*, 1986), in comparison to injection of this steroid on days 12 and 13 which has no effect. Therefore it has been proposed that estrogens produced by more developed embryos may adversely affect survival of less developed embryos by altering uterine protein secretion (Gries *et al.*, 1989).

Considering that the principal factor contributing to embryonic asynchrony is variation in the time of fertilization (Hunter, 1972) and the fact that the population of spermatozoa are present at the ampullary-isthmus junction anticipating ova for fertilization, then ultimately asynchrony of embryo development must either reflect asynchronous oocyte transport or asynchrony in follicular development and ovulation. Foxcroft (1987) has reported that in cyclic gilts there is an asynchrony in follicular morphology and biochemical activities within the pool of preovulatory follicles. It is posssible that such asynchrony may account for the incidence of embryonic mortality observed in the cyclic gilt by Archibong *et al.* (1987a).

There is yet no direct evidence indicating greater amounts of developmental asynchrony among preovulatory follicles of the young pubertal gilt in comparison with those of third estrous-gilts. Interestingly, however, is the observation that there is no prepubertal transient rise in serum progesterone in the gilt (Esbenshade *et al.*, 1982) similar to that observed in heifers (Gonzalez-Padilla *et al.*, 1975) and the ewe lamb (Legan *et al.*, 1985). The importance of this transient rise in progesterone has been speculated to be involved in synchronizing pubertal follicular development with the ensuing pubertal surge of LH (Legan *et al.*, 1985). Therefore absence of such a transient progesterone surge in the pubertal gilt may cause greater variation in follicular development and

subsequently greater embryonic mortality.

The Role of Follicular Estrogens with Respect to Embryo Mortality

Andersson and Einarsson (1980) have suggested that the proestrous period in the pubertal gilt may be unusually long in comparison to that of the third-estrous gilt. Another difference between the pubertal and third-estrous gilt at this stage of the cycle involves changes in the synthesis of ovarian steroids prior to estrus. Esbenshade *et al.* (1982) found that serum levels of estrogens peak 2.5 days prior to the onset of estrus in the pubertal gilt whereas Henricks *et al.* (1972) reported that the peak serum level of estrogens in the cyclic gilt occurs 24 hours prior to onset of estrus. Therefore this prolonged exposure of the ova to normal concentrations of follicular steroids or abnormally high concentrations of a particular steroid may have a detrimental effect on the ability of the fertilized ova to develop into normal embryos at a later stage of development.

Butcher *et al.* (1975) showed that it was possible to delay ovulation in the rat with pentobarbital sodium without altering the rise in circulating estradiol-17ß, thereby causing a prolonged period of elevated estradiol-17ß to which the ova were exposed. In a later study, Butcher and Pope (1979) found that this early rise in estrogen relative to the time of ovulation accounted for detrimental alterations of the preovulatory oocyte which was exemplified in greater abnormalities and embryonic death during early gestation. It is possible that the ova of the first-estrous gilt is subjected to prolonged high levels of estrogens, which is yet to be demonstrated directly, and that this may influence embryo viability at a later stage of development.

STATEMENT OF THE PROBLEM

Reproductive performance is an important aspect of any livestock enterprise primarily because of its significant impact on economic returns. At present, most swine producers do not breed gilts until their second or third estrus thus taking advantage of increased ovulation rates and embryo survival. Because replacement gilts are for all practical purposes nonproductive prior to delivery of the first litter of young, any prolongation of the interval between puberty and breeding represents an economic liability. If one considers that a gilt is fed 2.5 kg feed per day for the 42 days between puberty and third estrus, at $0.10 per pound plus a $0.25 maintenance cost per day, this interval represents a $33.60 loss per gilt. If the greater embryo wastage in pubertal gilts can be alleviated, this would improve reproductive efficiency of the pubertal gilt by increasing number of litters farrowed per sow during her productive life span and would eliminate the economic liability mentioned above, thus ultimately increasing the producers' profit margins.

Review of the literature has revealed that embryonic mortality is more prevalent in first-estrous gilts than in gilts mated at any subsequent estrus. At present the cause(s) for this phenomenom have not been unveiled. Results of experiments conducted in our laboratory suggest that ova ovulated by the pubertal gilt may have an inherent inability to develop into normal embryos during early gestation. It is recognized that some death of embryos might be attributed to genetic lethals. However, there is no reason to believe that the incidence of genetic lethals would be greater in pubertal than in the multiestrous gilt. It is presumed that ova of the pubertal gilt, while resident in the follicle, are subjected to a factor that does not impair fertilization but does interfere with subsequent embryonic development leading to mortality.

A study was undertaken to determine whether certain constituents of the follicular fluid of preovulatory follicles of the first-estrous gilt differed from those of third-estrous gilts. In addition a study was conducted to determine whether hormone therapy of third- and fourth-estrous gilts could increase embryonic mortality thus mimicking the phenomenon reported to occur in the mated pubertal gilt.

EXPERIMENT ONE: STEROIDS AND PLASMINOGEN ACTIVATOR CONCENTRATIONS IN FOLLICULAR FLUID OF GILTS AT FIRST AND THIRD ESTRUS.

Introduction

A greater incidence of embryonic mortality in pubertal gilts (first estrus) compared with that of multiestrous gilts has been reported by several investigators (Warnick *et al.*, 1951; MacPherson *et al.*, 1977; Young and King, 1981; Archibong *et al.*, 1987a). The cause(s) for this greater embryonic mortality in the first-estrous gilt has not been elucidated, but factors such as intrauterine environment (Archibong, 1988) and steroid production during early gestation (Archibong *et al.*, 1987a) do not appear to be involved. Consequently, attention is now focused upon the possibility that ova of the pubertal gilt may be endowed with a defect (Archibong, 1988) that allows fertilization, but eventually causes demise of embryos. It is possible that some factor within the intrafollicular environment may induce oocyte abberations that are expressed after fertilization. There is some evidence that estrogens either directly or indirectly may be involved in causing the oocyte defect. Andersson and Einarsson (1980) have suggested that proestrus in the pubertal gilt may be unusually long compared with that of multiestrous gilts. A prolonged proestrus in the pubertal gilt is consistent with the data of Esbenshade *et al.* (1982) who reported that serum concentrations of estradiol-17ß peak approximately 2.5 days before estrus in pubertal gilts whereas Henricks *et al.* (1972) reported that serum estrogens were maximal approximately 24 hours prior to estrus in cycling gilts. In addition, Butcher and Pope (1979) have shown that prolonged exposure of oocytes to estrogen during delayed ovulation in the rat results in a subsequent reduction in embryonic survival.

The present study was conducted to determine whether follicular fluid

concentrations of steroids and plasminogen activator differed at a similar stage of first and third estrus in the same gilts.

Materials and Methods

Animals and Procedures

Nine prepubertal crossbred (Yorkshire x Duroc) gilts (mean weight 76.4 ± 0.7 kg) were checked for estrus three times daily (0830, 1530 and 2230 hours) with an intact boar, but were not mated. Gilts were maintained as a group in total confinement with 14 hours light per day and were fed ad libitum until detection of first estrus. Subsequently gilts were fed 2.6 kg · d^{-1} through third estrus.

Blood samples and follicular fluid were collected from each gilt at both first (95 ± 1.5 kg) and third estrus (121.3 ± 2.7 kg). Six hours after detection of first or third estrus 10 ml of blood was collected from the vena cava immediately prior to induction of anesthesia. Anesthesia was induced by injection of a combination of 3 ml ketamine hydrochloride (Ketaset) and 3 ml xylazine (Rompun) and was maintained by inhalation of a mixture of halothane (Flowthane) and O_2 (20-30% halothane). A midventral incision was made through which the reproductive tract was exteriorized. A sample of venous blood (3 ml) was collected from each ovary and the ovaries were examined to determine the number and size of follicles present. Ovaries of first-estrous gilts were also examined for the presence of corpora albicantia to ensure the pubertal status of the gilt. Diameters of the largest follicles were measured by use of a calipher and only the number and size of follicles greater or equal to 4 mm in diameter were recorded. Follicles ranging in size from 8 to 12 mm in diameter were considered mature and preovulatory (Hafez, 1987). Follicular fluid was aspirated from preovulatory follicles by entering the antrum of each follicle through the adjacent stroma with an 25 guage needle attached to a syringe. This method of collecting follicular fluid was employed to minimize blood seepage into follicular fluid (Eiler and Nalbandov, 1977).

Follicular fluid collected from the preovulatory follicles of each ovary was pooled.

Systemic and ovarian venous blood and follicular fluid samples were stored for 6 hours at room temperature (20 C), followed by 24 hours at 4 C. Samples were then centrifuged at 500 x g for 10 min at 4C and the resulting sera and follicular fluid were stored at -20 C until assayed for steroids (sera and follicular fluid), plasminogen activator and plasmin (follicular fluid).

Radioimmunoassays of Steroids

Systemic serum and follicular fluid samples were analyzed for progesterone using extraction procedures described by Koligian and Stormshak (1977) in which 100 µl of sample were extracted with hexane-benzene and frozen. The resulting acqueous phase was decanted, dried and assayed. In the progesterone radioimmunoassay previously validated by Archibong *et al.* (1987a), 100 µl [^{3}H] progesterone, 100 µl progesterone antibody and 1 ml of dextrane coated charcoal were added to each sample extract to quantify the progesterone concentration. Extraction efficiency was 89% and the intraassay coefficient of variation was 7.7%. Samples of systemic sera, ovarian venous sera and follicular fluid were analyzed for estradiol-17ß in the laboratory of Dr. John Resko at the Oregon Health Sciences University. Estradiol-17ß was separated from estrone and other steroids by use of LH-20 Sephadex columns as described by Resko *et al.* (1975) and was extracted with diethyl ether. The isolated fraction containing estradiol was subjected to radioimmunoassay (Resko *et al.*, 1980). Extraction efficiency was 83% and the intraassay coefficient of variation was 8.8%.

Assay of Plasminogen Activator

Concentrations of plasminogen activator (PA) in follicular fluid were determined by caseinolytic assay (Menino and Williams, 1987) with modifications.

Preliminary results of analysis of samples indicated that a plasmin inhibitor was present in the fluid. Therefore follicular fluid was adjusted to pH 3 by addition of 5N HCl, incubated for 2 hours at 40 C and subsequently returned to normal pH (~7) by addition of 5N NaOH (Loskutoff, 1978). Fifteen µl of follicular fluid or of urokinase standard (0, 0.0009, 0.0045, 0.009, 0.0455, 0.0909 and 0.455 milliunits/ml) were combined with 15 µl of 120 µg/ml human plasminogen and were incubated for 15 min at 37 C. Following incubation 25 µl of incubated mixture were pipetted into 4 mm diameter wells cut in a casein agar gel plate and incubated at room temperature (20 C) for 24 hours. Caseinolytic agar plates were fixed for 15 min with 5 ml 3% acetic acid followed with a tap water rinse. Diameters of caseinolytic zones were measured with an electronic digital caliper. Plasminogen activator concentrations in follicular fluid were determined from an equation of the line calculations for ring area of the lytic zones by log urokinase standard concentrations.

Plasmin concentrations were calculated in a similar manner as plasminogen activator, with the following exception. Fifteen µl of follicular fluid or of plasmin standard (0.0, 2.5, 5.0, 10.0, 50.0 and 100.0 µg/ml) were combined with 15 µl phosphate buffered saline (pH 7) and incubated and assayed as above.

Statistical Analysis

Data on serum concentrations of hormones, number of follicles greater than 8 mm in diameter and PA concentrations in follicular fluid were analyzed by way of a Student's paired t-test. Because of heterogeneity of variance data on follicular fluid and ovarian venous concentrations of estradiol-17ß as well as data on number of follicles less then 8 mm diameter were subjected to a square-root transformation. However, for purposes of clarity the means presented for these characteristics represent untransformed data. Relationships between follicular

fluid concentrations of estradiol-17ß and ovarian venous and vena cava serum levels of estradiol-17ß were examined by computation of simple correlation coefficients.

Results

Gilts sampled at pubertal estrus weighed less than at third-estrus (mean ± SE; 95 ± 1.5 and 121.3 ± 2.7 kg, respectively; $P<0.01$). Number of follicles and follicle diameters present at first and third estrus are presented in figure 1. Mean number of mature preovulatory follicles between 8 - 12 mm in diameter did not differ between gilts at first and third estrus (10.3 ± 0.4 versus 9.0 ± 0.9, respectively). Gilts at first estrus had more follicles between 4 and 8.1 mm diameter ($P<0.05$) and 8 to 10.1 mm ($P<0.01$) than at third estrus. Chi-square analysis (Table 1) indicated that the distribution of follicular diameters was dependent upon estrus at which follicles were measured ($P<0.01$).

Mean concentrations of estradiol-17ß in systemic sera, ovarian venous sera and follicular fluid are presented in figure 2. Systemic sera, ovarian venous sera and follicular fluid concentrations of estradiol at third estrus were greater than at first estrus ($P<0.05$). Concentrations of estradiol-17ß in follicular fluid were positively correlated with levels of this steroid in ovarian venous blood at first and third estrus ($r = .51$ and .12, respectively). Similarly concentrations of estradiol-17ß in follicular fluid were positively correlated with vena cava serum concentrations of this steroid ($r = .25$ and .80, respectively). Concentrations of progesterone in sera and follicular fluid of gilts at first and third estrus are presented in figure 3. Serum and follicular fluid concentrations of progesterone of gilts did not differ between first and third estrus.

Changes in follicular fluid concentrations of plasminogen activator and plasmin are depicted in figure 4. Follicular fluid concentration of plasminogen activator tended to be lower in gilts at pubertal estrus ($4.1 \pm 0.4 \times 10^{-4}$ mU/ml) than at third estrus ($6.1 \pm 1.3 \times 10^{-4}$ mU/ml) although the difference was not significant. Plasmin concentrations (ug/ml) did not differ in gilts between first and third estrus (5.6 ± 1.8 versus 5.1 ± 1.5, respectively).

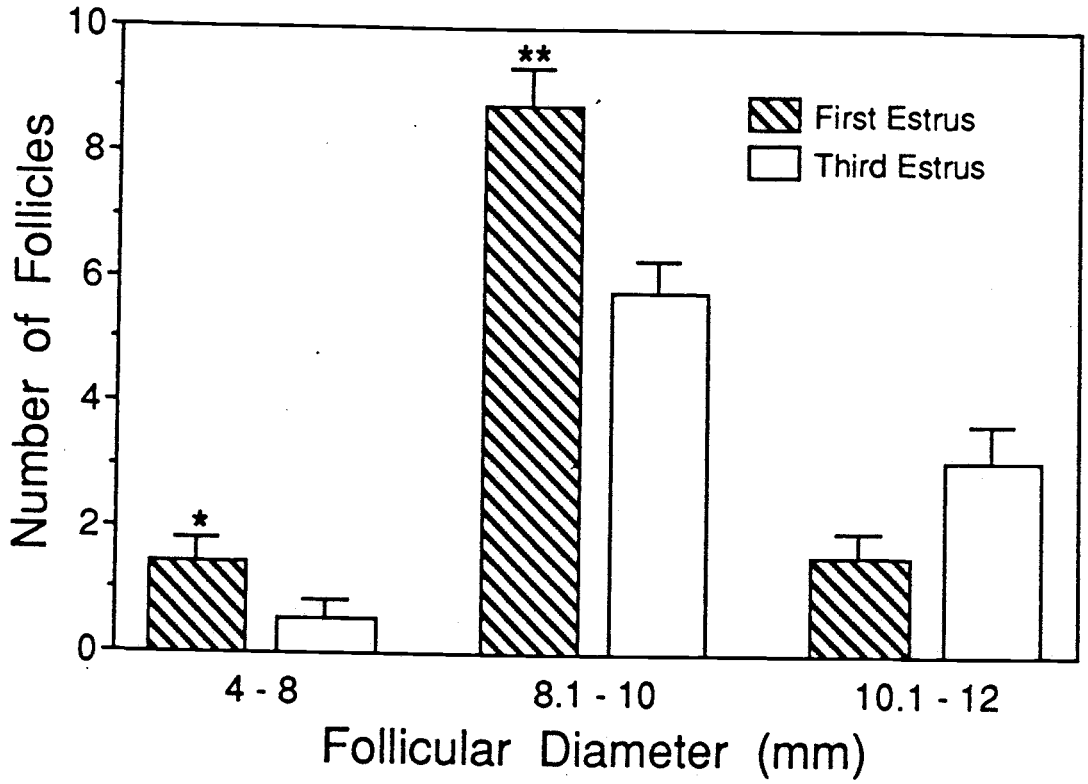

Fig. 1. Mean (± SE) number of follicles 4 - 12 mm in diameter within gilts 6 hours after detected first and third estrus.

*First vs third estrus ($P<0.05$).

**First vs third estrus ($P<0.01$).

Table 1. Distribution of diameter of follicles of first and third estrous gilts 6 hours post-estrus

	Follicle Size (mm)[a]				
Estrus	4.0-6.0	6.1-8.0	8.1-10.0	10.1-12.0	Total
First	5(4.7)[b]	8(7.5)	79(74.5)	14(13.2)	106
Third	1(1.2)	5(5.8)	52(60.5)	28(32.6)	86

[a] Distribution of diameter of follicles differs between gilts at first and third estrus ($P < 0.01$) as determined by Chi-square analysis.

[b] Values are the number (%) within each diameter class. $P < 0.01$.

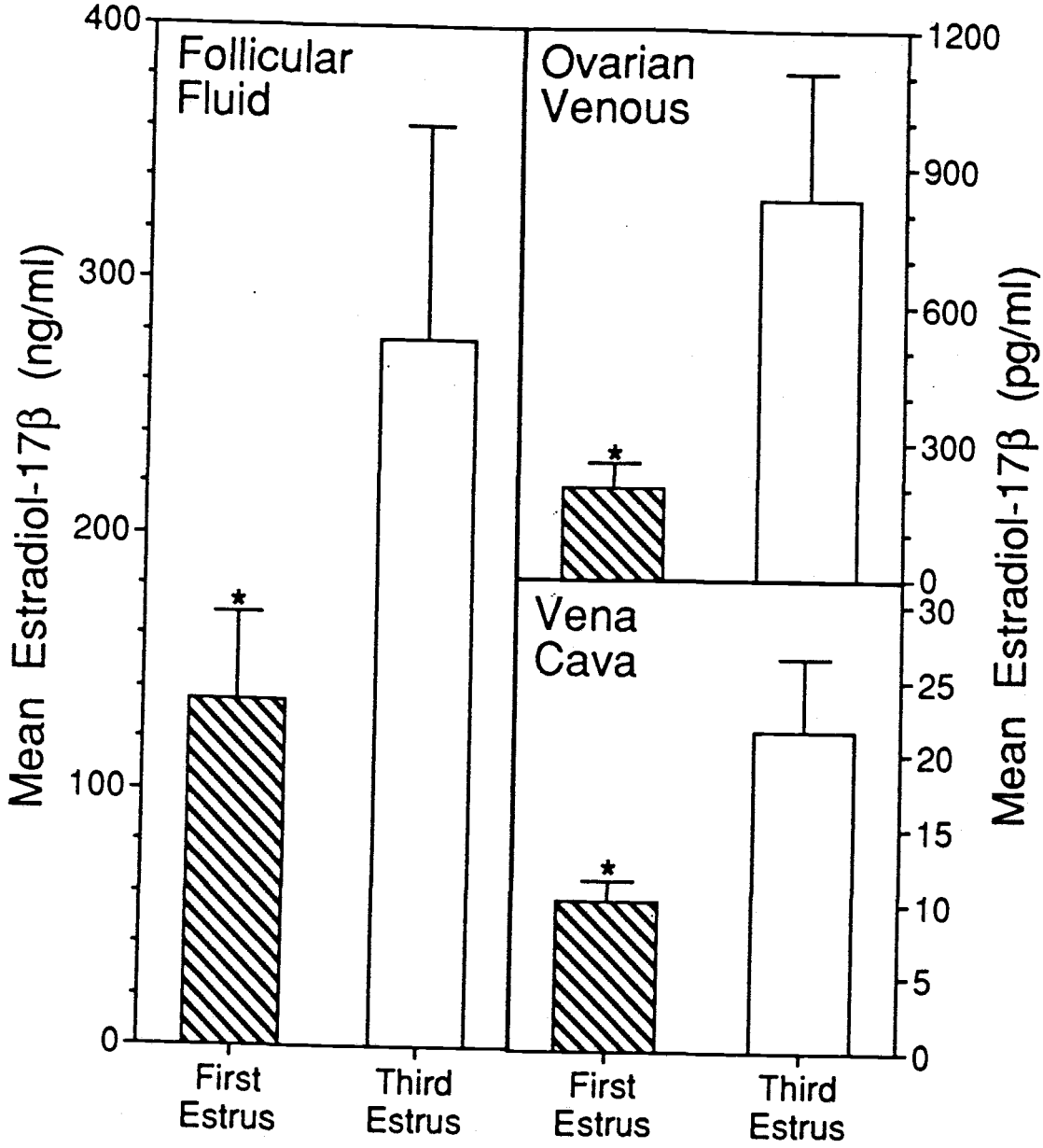

Figure 2. Follicular fluid, ovarian venous and vena cava serum concentrations of estradiol-17β (mean ± SE) in gilts 6 hours after detected first and third estrus.

*First vs third estrus ($P<0.05$).

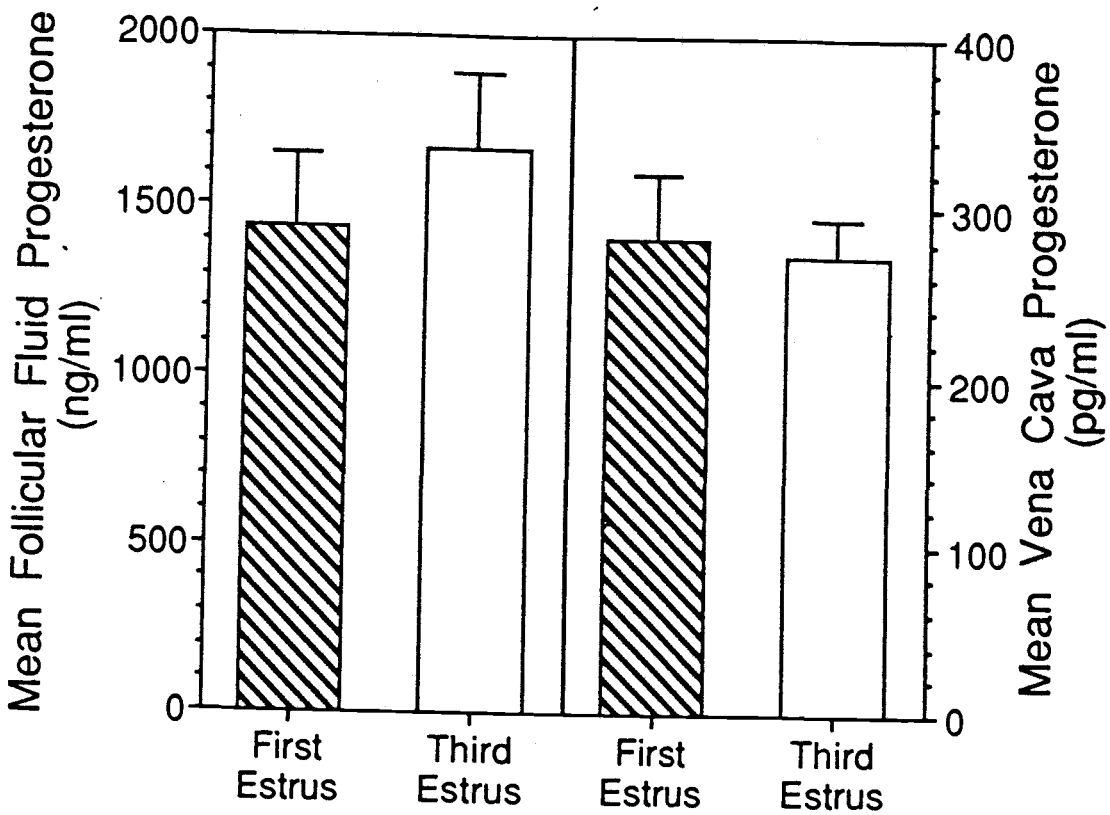

Fig. 3. Follicular fluid and vena cava serum concentrations of progesterone (mean ± SE) in gilts 6 hours after detected first and third estrus.

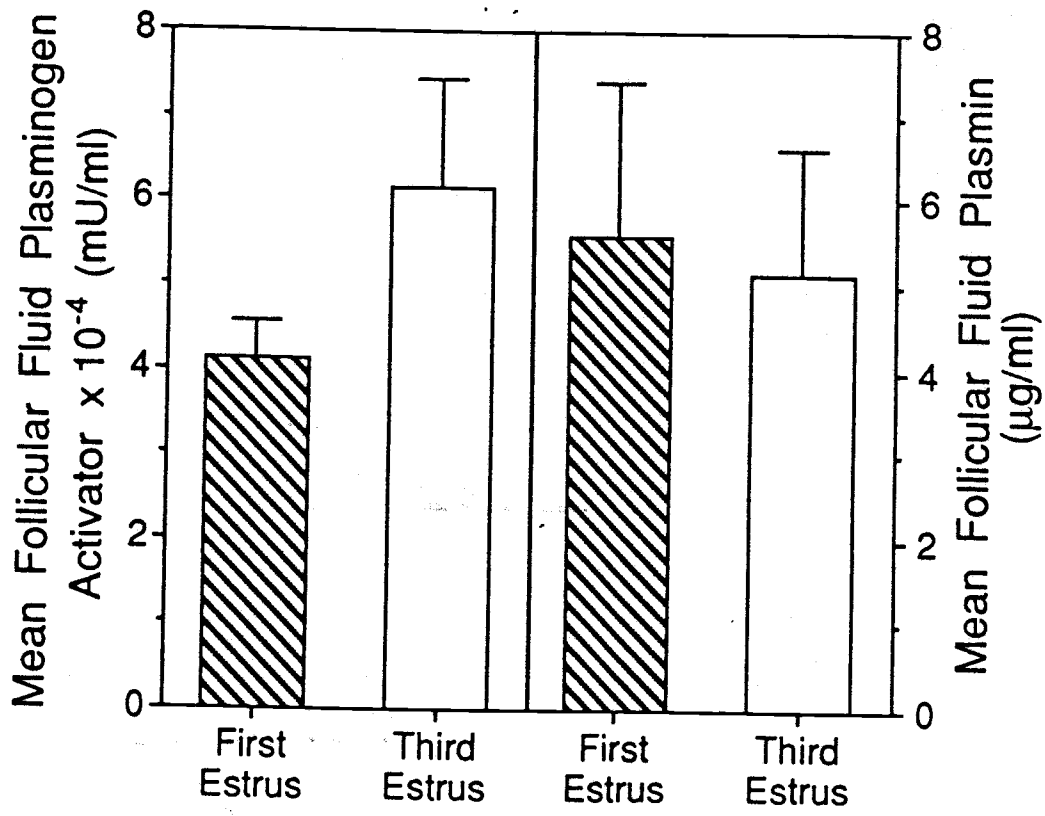

Fig. 4. Follicular fluid concentrations of plasminogen activator and plasmin (mean ± SE) in gilts 6 hours after detected first and third estrus.

Discussion

It has been reported that gilts managed to reach puberty at a young age ovulate fewer ova at pubertal estrus (Robertson *et al.*, 1951; Andersson and Einarsson, 1980; Archibong *et al.*, 1987a) and if mated sustain a higher incidence of embryonic mortality (Warnick *et al.*, 1951; MacPherson *et al.*, 1977; Young and King, 1981; Archibong *et al.*, 1987a) than do multiestrous gilts. Gilts that exceed the minimum age or weight threshold before stimulated to attain puberty do not exhibit these characteristics (Dyck, 1971; Knott *et al.*, 1984). Mean total number of follicles (4-12 mm diameter) per gilt in the present experiment at first estrus was similar to the ovulation rate of pubertal gilts reported by Archibong *et al.* (1987a). This suggests that small follicles would eventually ovulate. There was, however, no increase in the number of observed large follicles at third estrus compared to those present at pubertal estrus. This observation is not consistent with the findings of increased ovulation rates as gilts mature from first to third estrus (Andersson and Einarsson 1980; Archibong *et al.*, 1987a). These data can be explained by the presence of ovarian adhesions in gilts at third estrus which precluded precise calculation of follicle numbers. Although this was the case, one would expect that accessible follicles in these gilts were a reliable representation of the total follicle population.

Considerable variation in porcine blastocyst development has been observed as early as 12 days post-mating (Anderson, 1978). This variation in development may be attributed to the timing of fertilization (Hunter, 1972). Assuming that sperm are present at the ampullar-isthmus junction anticipating oocytes, and duration of ova transport down the ampulla is similar for all ovulated ova, one might conclude that it is an asynchrony in ovulation that ultimately results in variations in embryonic development. This possibility is supported by data of the present experiment. Alhtough there were some follicles in the 10.1 - 12 mm

diameter range in the first estrous gilts the distribution seemed to be shifted toward less maturity in comparison to gilts at third estrus. It is possible that these less mature follicles may not respond to the ovulatory LH surge in a similar manner as more mature follicles therefore resulting in asynchronous ovulation.

Although sampled at the same time after detected estrus, gilts at first estrus had significantly lower follicular fluid, ovarian venous and systemic (vena cava) serum concentrations of estradiol-17ß than at third estrus. In mature gilts (Eiler and Nalbandov, 1977) and those stimulated with PMSG and hCG (Ainsworth *et al.*, 1980) follicular fluid concentrations of estradiol-17ß increased with follicular development until after the ovulatory surge of LH or hCG and onset of estrus and then rapidly decreased. If such a relationship between follicle size and synthesis of estrogen exists in the pubertal gilt then the reduced concentrations of estradiol detected at first estrus, compared with third estrus, may be due to the difference in the population of follicles present at these times. Gilts at first estrus had fewer follicles 10 - 12 mm in diameter than at third estrus. Size of follicles did not appear to be as critical with respect to production of progesterone, plaminogen activator or plasmin because no difference in these follicular constituents were detected between first and third estrus.

The present data with respect to follicular fluid concentrations of estrogen and the levels of this steroid in ovarian venous and systemic serum differ somewhat from those reported by Eiler and Nalbandov (1977). These latter investigators failed to detect a relationship between follicular fluid concentrations of estradiol and serum concentrations of this steroid. However, the data of the present experiment suggest that fluctuations in ovarian venous and systemic levels of estradiol were positively correlated with follicular fluid levels of this steroid at first and third estrus. These data suggest that serum levels of estradiol may indeed represent concentrations of this steroid in follicular fluid, although

caution is forewarned with such an interpretation due to the nonsignificant correlations.

On the basis of results from this study follicular size distribution in gilts at puberty is different than at third estrus. Differences in follicular development are believed to be the cause for lower serum and follicular fluid concentrations of estradiol-17ß found in the pubertal gilt. Wilde*et al.* (1987) stated that a protracted ovulation interval is associated with morphological disparity among littermate embryos. Hence the greater incidence of embryonic mortality found in pubertal as opposed to multiestrous gilts may be due to the larger heterogeneity of follicular development thus resulting in more asynchronous development of early embryos (Hunter, 1974; Anderson, 1978; Pope *et al.*, 1986) which has been found to be detrimental to embryonic survival (Pope *et al.*, 1982b).

EXPERIMENT TWO: ESTRADIOL-17ß TREATMENT OF THIRD AND FOURTH ESTRUS GILTS AT TIME OF DETECTED ESTRUS AND SUBSEQUENT FETAL SURVIVAL.

Introduction

Data presented in the literature by several investigators suggest that follicular estrogen synthesis immediately prior to attainment of puberty in the gilt might be markedly different from that occurring during proestrus in the multiestrous gilt. The report by Esbenshade *et al.* (1982) is of particular interest because these investigators found systemic concentrations of estradiol to be increased aproximately 2.5 days prior to pubertal estrus. Increased secretion of estrogen at this time is consistent with the observation that proestrus in the pubertal gilt is prolonged compared with that of cycling gilts (Andersson and Einarsson, 1980). In contrast to these data, Henricks *et al.* (1972) found that estrogen secretion was maximal on the day prior to estrus in multiestrous gilts. Because the incidence of embryonic mortality is greater in mated pubertal gilts compared with that of mated multiestrous gilts, it is conceivable that this hormone either directly or indirectly might impair the ability of some fertilized ova to develop into embryos capable of surviving beyond early gestation. Therefore, prolonged exposure of the oocytes of multiestrous gilts to estrogen might mimic the endocrine status that prevails in the gilt prior to puberty and thus result in increased embryonic mortality.

An experiment was conducted to determine whether administration of estradiol-17ß to third- and fourth-estrous gilts at detected estrus could promote increased embryonic mortality.

Materials and Methods

Animals and Procedures

Seventeen prepubertal crossbred (Yorkshire x Duroc) gilts (mean weight 76.2 ± 2.4 kg) were checked for estrus using an intact boar once daily until second estrus was attained and subsequently three times daily (0830, 1530 and 2230 hours). At the time of detected third or fourth estrus (day of detected estrus = d 0 of gestation) gilts (mean weight 104.9 ± 8.0kg) were allocated to receive either an intramuscular injection of 3 ml corn oil (vehicle; control; n = 9) or 5 mg estradiol-17ß (n = 8). This dose and route of administration of estradiol has been found to maintain corpora lutea in gilts (Gardner *et al.*, 1963). Gilts were bred at 12 and 24 hours post-treatment and were slaughtered on days 30-32 of gestation. Reproductive tracts were collected 10 min post-mortem and the number of corpora lutea on each ovary was determined. Each uterine horn was opened to expose the fetuses. Total number of fetuses in each horn was determined and a visible heart beat was used as the criterion for assessing viability of fetuses. Percentage fetal survival was calculated by dividing the number of viable fetuses by number of corpora lutea x 100. Fetuses were dissected from the amnion and weighed to the nearest tenth of a gram. Crown rump lengths of fetuses were also determined with a calipher and recorded.

Statistical Analysis

Data on the effects of exogenous estradiol 17-ß on fetal survival, weight and crown-rump length were analyzed by use of Student's unpaired t-test. Relationships between fetal age and weight and length were examined by computation of simple correlation coefficients for both treatment and control

groups. Comparison of homogeneity of correlations were determined by way of a Z test.

Results

Fetal survival data for treated and control gilts are presented in Table 2. There was no difference in ovulation rate nor fetal survival between treated and control gilts. Fetal weight and lengths of control and estradiol-17ß-treated gilts are presented in figures 5 and 6. Age of fetus was positively correlated with weight and length for both control and treated gilts (controls: weight $r = .66$, length $r = .50$; treatment: weight $r = .82$, length $r = .79$). Relationships of fetal age to weight and length were significantly greater in treated gilts than the comparable relationships of these variables in control gilts.

Table 2. Fetal survival of gilts after treatment with estradiol-17β at third or fourth estrus[a]

Item	Control	Treated
Mean Ovulation Rate	12.2±0.5	13.1±0.9
Fetal Survival, %	85.4±3.2	83.3±7.0
Fetuses Missing, %	12.5±2.8	13.5±5.0
Fetuses Nonviable, %[b]	2.1±1.4	3.2±2.5

[a] Values are means ±SE.

[b] Nonviable fetuses represent those that were degenerating or lacking a heart beat at the time of data collection.

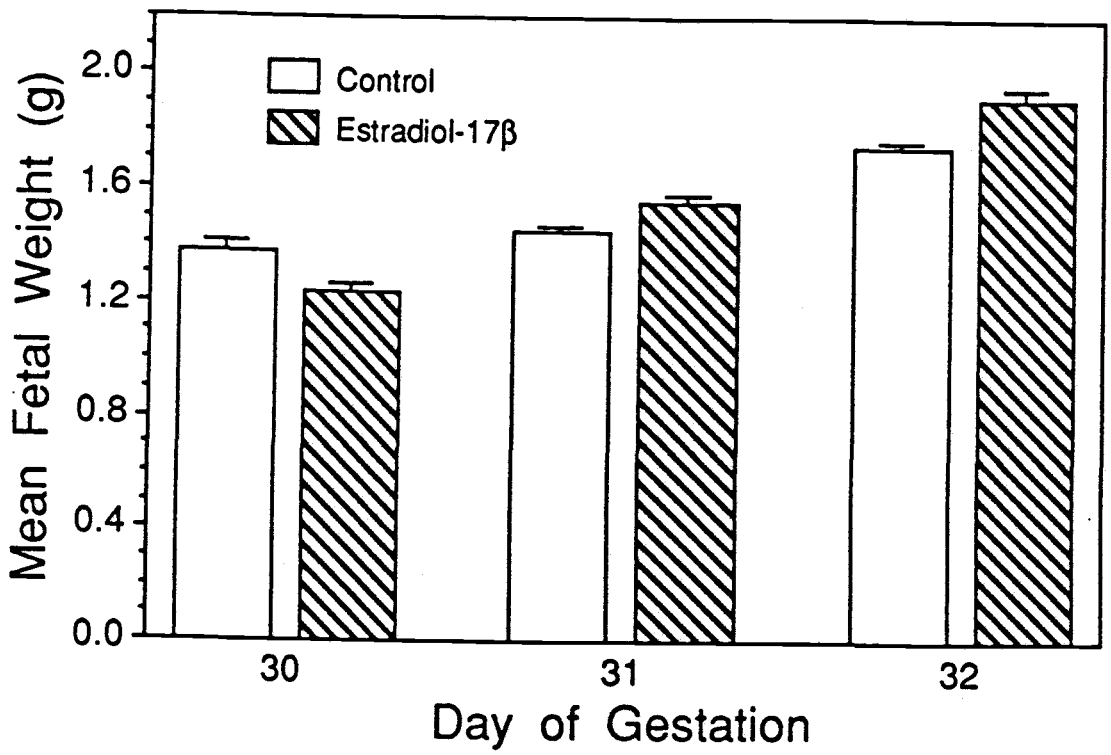

Fig. 5. Weights of fetuses (mean ± SE) collected on days 30-32 of gestation from gilts treated with estradiol-17ß at third or fourth estrus.

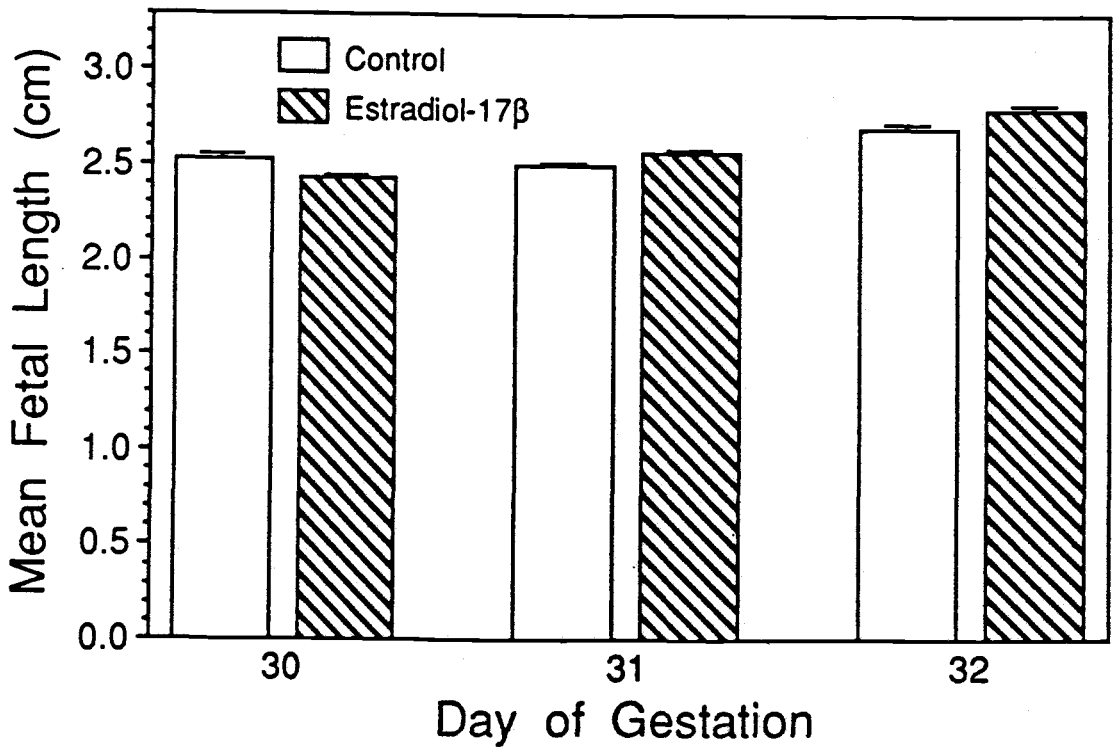

Fig. 6. Length of fetuses (mean ± SE) collected on days 30-32 of gestation from gilts treated with estradiol-17β at third or fourth estrus.

Discussion

Results of the present experiment indicate that injection of estradiol-17ß into multiestrous gilts at detected estrus had no detrimental effect on embryo survival. Percentages of fetuses recovered at slaughter and percentages of viable fetuses were similar among treated and control gilts. Butcher and Pope (1979) experimentally delayed ovulation and mating in rats for 24 hours and found that prolonged exposure of oocytes to endogenous estrogen caused increased embryonic mortality. If intrafollicular levels of this hormone were increased in gilts as a result of treatment, exposure of oocytes to the steroid may not have been of sufficient duration to induce an ovum defect. Alternatively, it is possible that the dose of estradiol-17ß administered was not sufficient to increase intrafollicular concentrations of this hormone. Treatment apparently did not interfere with the process of fertilization or transport of fertilized ova through the oviduct. Others have shown that treatment with estrogens can interfere with normal transport of fertilized ova in the rabbit (Chang and Harper, 1966). Weights and crown-rump lengths of fetuses for stages of gestation at which gilts were slaughtered were similar to those reported by Marrable (1971) and did not differ among control and treated gilts. As expected fetal weights and lengths were highly correlated with fetal age. Homogeneity of correlations between fetal age and weights and lengths was greater for treated than for control gilts, suggesting that treatment may have in some way synchronized embryonic development. Whether such an effect of estradiol is real or simply a reflection of sampling in the present experiment is not known with certainty, but merits further study.

Although results of the present experiment failed to implicate estrogen as a causative factor for increased embryonic mortality the possibility remains that these steroids are detrimental to oocytes in the pubertal gilt. Neither the time of injection of hormone nor the dose may have been optimal for detecting the

inhibitory effect of this steroid on the oocyte in this study.

GENERAL DISCUSSION

Lower follcular fluid concentrations of estradiol-17ß in first-estrous gilts is presumably an indication of a difference in follicular development between gilts at first and third estrus. This assumption is supported by the present findings that there is a greater distribution of follicular development, as indicated by follicular diameters, in gilts at first estrus compared with that at third estrus. Asynchronous follicular development in pubertal gilts may result in dissimilar responsiveness of follicles to the ovulatory LH surge and thus a protracted ovulation interval. Protracted ovulation intervals have been found to be associated with morphological disparity amoung porcine littermate embryos (Wilde *et al.*, 1987). Therefore asynchronous embryo development during early gestation may lead to increased embryo mortality.

Evidence indicates that embryos themselves may play a role in regulating embryonic development in swine. Although embryos begin producing estrogens during the period of rapid trophoblastic elongation between days 11 - 12 of gestation (Geisert *et al.*, 1982b), administration of estradiol-17ß on days 9 and 10 can greatly increase embryo mortality (Pope *et al.*, 1986). These detrimental effects on embryo survival are presumably caused by estradiol-induced alteration of uterine secretions (Gries *et al.*, 1989). In agreement with these findings are the results of asynchronous embryo transfers in which more advanced embryos caused the demise of less developmentally advanced embryos (Pope *et al.*, 1982b). Thus, it seems important that embryonic development occurs in synchrony to support maximal embryo survival.

To support this hypothesis research needs to be conducted to determine the responsiveness of preovulatory follicles to an ovulatory LH surge in both the first- and third-estrous gilt. This would provide knowledge as to whether or not

less developed follicles ovulate simultaneously with more mature follicles. It would also be of significant interest to be able to trace folliculogenesis and ultimately embryogenesis to decipher if there is indeed a relationship between follicle development and early embryogenesis.

The results from experiment two do not support our previous theory that prolonged high levels of estradiol-17ß prior to ovulation are detrimental to embryo survival in the porcine as was reported to be the case in the rat (Butcher and Pope, 1979).

BIBLIOGRAPHY

Ainsworth, L., B.K. Tsang, B.R. Downey, G.J. Marcus and D.T. Armstrong. 1980. Interrelationships between follicular fluid steroid levels, gonadotropic stimuli and oocyte maturation during preovulatory development of porcine follicles. Biol. Reprod. 23:621-627.

Andersson, A.M. and S. Einarsson. 1980. Studies on the oestrus and ovarian activity during five successive oestrous cycles in gilts. Acta Vet. Scand. 21:677-688.

Anderson, L.L. 1978. Growth, protein content and distribution of early pig embryos. Anat. Rec. 190:143-153.

Anderson, L.L. 1980. Pigs. In: E.S.E. Hafez (ed.) Reproduction in Farm Animals. Lea and Febiger, Philadelphia. pp. 358-386.

Anderson, L.L. and R.M. Melampy. 1972. Factors affecting ovulation rate in the pig. In: D.J.A. Cole, (ed.), Pig Production. Butterworths, London. pp.329-366.

Archibong, A.E. 1988. Embryonic Mortality in the Pubertal Gilt. Ph.D. Dissertation, Oregon State University, Corvallis.

Archibong, A.E., D.C. England and F. Stormshak. 1987a. Factors contributing to early embryonic mortality in gilts bred at first estrus. J. Anim. Sci. 64:474-478.

Archibong, A.E., D.C. England and F. Stormshak. 1987b. Ovulation and embryonic survival in pubertal gilts treated with gonadotropin releasing hormone. J. Anim. Sci. 65:752-755.

Baird, D.T. 1977. Evidence *in vivo* for the two-cell hypothesis of oestrogen synthesis by the sheep Graafian follicle. J. Reprod. Fertil. 50:183-185.

Barrack, E.R. and D.S. Coffey. 1980. The specific binding of estrogens and androgens to the nuclear matrix of sex hormone responsive tissue. J. Biol. Chem. 255:7265-7275.

Bazer, F.W., R.D. Geisert, W.W. Thatcher and R.M. Roberts. 1982. The establishment and maintenance of pregnancy. In: D.J.A. Cole and G.R. Foxcroft, (eds.), Control of Pig Production. Butterworths, London. pp.227-252.

Bazer, F.W. and W.W. Thatcher. 1977. Theory of maternal recognition of pregnancy in swine based on estrogen controlled endocrine versus exocrine secretion of prostaglandin $F_{2\alpha}$ by the uterine endometrium. Prostaglandins 14:397-401.

Beers, W.H. 1975. Follicular plasminogen and plasminogen activator and the effect of plasmin on ovarian follicle wall. Cell 6:379-386.

Beers, W.H., S. Strickland and E. Reich. 1975. Ovarian plasminogen activator: relationship to ovulation and hormonal regulation. Cell 6:387-394.

Berardinelli, J.G., R.A. Dailey, R.L. Butcher and E.K. Inskeep. 1979. Source of progesterone prior to puberty in beef heifers. J. Anim. Sci. 49:1276-1279.

Bjersing, L. and H. Carstensen. 1967. Biosynthesis of steroids by granulosa cells of the porcine ovary *in vitro*. J. Reprod. Fertil. 14:101-111.

Brooks, P.H. and D.J.A. Cole. 1970. The effect of the presence of a boar on the attainment of puberty in gilts, J. Reprod. Fertil. 23:435-440.

Butcher, R.L., W.E. Collins and N.W. Fugo. 1975. Altered secretion of gonadotropins and steroids resulting from delayed ovulation in the rat. Endocrinology 96:576-586.

Butcher, R.L. and R.S. Pope. 1979. Role of estrogen during prolonged estrous cycles of the rat on subsequent embryonic death or development. Biol. Reprod. 21:491-495.

Canipari, R., M.L. O'Connell, G. Meyer and S. Strickland. 1987. Mouse ovarian granulosa cells produce urokinase-type plasminogen activator, whereas the corresponding rat cells produce tissue-type plasminogen activator. J. Cell Biol. 105:977-981.

Canipari, R. and S. Strickland. 1986. Studies on the hormonal regulation of plasminogen activator production in the rat ovary. Endocrinology 118:1652-1659.

Carson, R.S., J.K. Findlay. H.G. Burger and A. O. Trounson. 1979. Gonadotropin receptors of the ovine ovarian follicle during follicular growth and atresia. Biol. Reprod. 21:75-87

Chang, M.C. and M.J.K. Harper. 1966. Effects of ethinyl estradiol on egg transport and development in the rabbit. Endocrinology 78:860-872.

Channing, C.P. and S. Kammerman. 1973. Characteristics of gonadotropin receptors of porcine granulosa cells during follicle maturation. Endocrinology 92:531-540.

Christenson, R.K. and J.J. Ford. 1979. Puberty and estrus in confinement-reared gilts. J. Anim Sci. 49:743-751.

Christman, J.K., S.C. Silverstein and G. Acs. 1977. Plasminogen Activators. In: J. Barrett (ed.) Proteases in Mammalian Cells and Tissues. Elsevier Biomedical Press, North Holland.

Clark, J.W., S.G. Brazier, L.M. Wiginton, G.R. Stevenson and L.F. Tribble. 1982. Time of ovarian follicle selection during the porcine estrous cycle. Theriogenology 18:697-709.

Compton, J.G., W.T. Schrader and B.W. O'Malley. 1983. DNA sequence preference of the progesterone receptor. Proc. Natl. Acad. Sci. USA 80:16-20.

Crombie, P.R. 1970. Ultrastructure of the foetal-maternal attachment in the pig. J. Physiol. 210:101-112.

Danø, K., P.A. Andersen, J. Grøndahl-Hansen, P. Kristensen, L.S. Nielsen and L. Skriver. 1985. Plasminogen activators, tissue degradation, and cancer. Adv. Cancer. Res. 44:139-266.

Dhindsa, D.S., P.J. Dziuk and H.W. Norton. 1967. Time of transuterine migration and distribution of embryos in the pig. Anat. Rec. 159:325-330.

Diekman, M.A., W.E. Trout and L.L. Anderson. 1983. Serum profiles of LH, FSH and prolactin from 10 weeks of age until puberty in gilts. J. Anim. Sci. 56:139-145.

Dyck, G.W. 1971. Ovulation rate and weight of the reproductive organs of Yorkshire and Lacombe swine. Can. J. Anim. Sci. 51:141-146.

Dziuk, P.J. 1968. Effect of number of embryos and uterine space on embryo survival in the pig. J. Anim. Sci. 27:673-676.

Eiler, H. and A.V. Nalbandov. 1977. Sex steroids in follicular fluid and blood plasma during the estrous cycle of pigs. Endocrinology 100:331-338.

Esbenshade, K.L., A.M. Paterson, T.C. Cantley and B.N. Day. 1982. Changes in plasma hormone concentrations associated with the onset of puberty in the gilt. J. Anim. Sci. 54:320-324.

Espey, L.L. 1967. Tenacity of porcine Graafian follicle as it approaches ovulation. Amer. J. Physiol. 212:1397-1401.

Espey, L.L. 1978. Ovarian contractility and its relationship to ovulation: a review. Biol. Reprod. 19:540-551.

Espey, L.L. 1980. Ovulation as an inflammatory reaction--a hypothesis. Biol. Reprod. 22:73-106.

Espey, L.L. and H. Lipner. 1963. Measurements of intrafollicular pressures in the rabbit ovary. Amer. J. Physiol. 205:1067-1072.

Espey, L.L. and H. Lipner. 1965. Enzyme-induced rupture of rabbit Graafian follicle. Amer. J. Physiol. 208:208-213.

Espey, L., H. Shimada, H. Okamura and T. Mori. 1985. Effect of various agents on ovarian plasminogen activator activity during ovulation in pregnant mare's serum gonadotropin-primed immature rats. Biol. Reprod. 32:1087-1094.

Evans, G., M. Dobias, G.J. King and D.T. Armstrong. 1981. Estrogen, androgen, and progesterone biosynthesis by theca and granulosa of preovulatory follicles in the pig. Biol. Reprod. 25:673-682.

Falck, B. 1959. Site of production of oestrogen in the rat ovary as studied by microimplants. Acta Physiol. Scand. 47:94-101.

Fazleabas, A.T., R.D. Geisert, F.W. Bazer and R.M. Roberts. 1983. Relationship between release of plasminogen activator and estrogen by blastocysts and secretion of plasmin inhibitor by uterine endometrium in the pregnant pig. Biol. Reprod. 29:225-238.

Foote, W.C., D.P. Waldorf, A.B. Chapman, H.L. Self, R.H. Grummer and L.E. Casida. 1956. Age at puberty of gilts produced by different systems of mating.J. Anim. Sci. 15:959-969.

Foster, D.L. and K.D.Ryan. 1979. Endocrine mechanisms governing transition into adulthood: a marked decrease in inhibitory feedback action of estradiol on tonic secretion of luteinizing hormone in the lamb during puberty. Endocrinology 105:896-904.

Foxcroft, G.R. 1987. Follicular dynamics and its endocrine control in the sow. In: J.R. Roche and D. O. Callaghen, (eds.), Follicular Growth and Ovulation Rate in Farm Animals. Martinus Nijhoff Publishers, Boston. pp.191-206.

Foxcroft, G.R. and M.G. Hunter.1985. Basic physiology of follicular maturation in the pig. J. Reprod. Fertil. Suppl. 33:1-19.

Friend, D.W. 1973. Influence of dietary amino acids on the age at puberty of Yorkshire gilts. J. Anim. Sci. 37:701-707.

Gardner, M.L., N.L. First and L.E. Casida. 1963. Effect of exogenous estrogens on corpus luteum maintenance in gilts. J. Anim. Sci. 22:132-134.

Geisert, R.D., R.H. Renegar, W.W. Thatcher, R.M. Roberts and F.W. Bazer. 1982a. Establishment of pregnancy in the pig: I. Interrelationships between preimplantation development of the pig blastocyst and uterine endometrial secretions. Biol. Reprod. 27:925-939.

Geisert, R.D., R.H. Renegar, W.W. Thatcher, R.M. Roberts and F.W. Bazer. 1982b. Establishment of pregnancy in the pig: II. Cellular remodeling of the porcine blastocyst during elongation on day 12 of pregnancy. Biol. Reprod. 27:941-955.

Gonzalez-Padilla, E., J.N. Wiltbank and G.D. Niswender. 1975. Puberty in beef heifers. 1. The interrelationship between pituitary, hypothalamic and ovarian hormones. J. Anim. Sci. 40:1091-1104.

Goodman, A.L. and G.D. Hodgen. 1983. The ovarian triad of the primate menstrual cycle. Rec. Prog. Horm. Res. 39:1-67.

Gorski, J., D. Toft, G. Shyamala, D. Smith and A. Notides. 1968. Hormone receptors: studies on the interaction of estrogen with the uterus. Rec. Prog. Horm. Res. 24:45-80.

Grant, S.A., M.G. Hunter and G.R. Foxcroft. 1989. Morphological and biochemical characteristics during ovarian follicular development in the pig. J. Reprod. Fertil. 86:171-183.

Greene, G.L. and M.F. Press. 1986. I. Steroid receptor structure (including monoclonal antibodies and new methods of determination). J. Ster. Biochem. 24:1-7.

Gries, L.K., R.D. Geisert, M.T. Zavy, J.E. Garrett and G.L. Morgan. 1989. Uterine secretory alterations coincident with embryonic mortality in the gilt after exogenous estrogen administration. J. Anim. Sci. 67:276-284.

Guthrie, H.D., D.M. Henricks and D.L. Handlin. 1974. Plasma hormone levels and fertility in pigs induced to superovulate with PMSG. J. Reprod. Fertil. 41:361-370.

Hafez, E.S.E., M.-C. Lavasseur and C. Thibault. 1987. Folliculogenesis, egg maturation, and ovulation. In: E.S.E. Hafez (ed.) Reproduction in Farm Animals. Lea and Febiger, Philadelphia. pp. 150-166.

Haour, F. and B.B. Saxena. 1974. Characterization and solubilization of gonadotropin receptor of bovine corpus luteum. J. Biol. Chem. 249:2195-2205.

Harvey, C.A. and D.A.A. Owen. 1979. Effect of histamine on uterine vasculature in rats. Eur. J. Pharmacol. 56:293-296.

Henricks, D.M., H.D. Guthrie and D.L. Handlin. 1972. Plasma estrogen, progesterone and luteinizing hormone levels during the estrous cycle in pigs. Biol. Reprod. 6:210-218.

Hillier, S.G. 1987. Paracrine control in the ovaries. Res. Reprod. 19:1-2.

Hisaw, F.L. 1947. Development of the Graafian follicle and ovulation. Physiol. Rev. 27:95-119.

Holvoet, P., H.R. Lijnen and D. Collen.1985. A monoclonal antibody specific for Lys-plasminogen. Application to the study of the activation pathways of plasminogen in vivo. J. Biol. Chem.260:2106-2111.

Hunter, R.H.F. 1972. Fertilization in the pig: sequence of nuclear and cytoplasmic events. J. Reprod. Fertil. 29:395-406.

Hunter, R.H.F. 1974. Chronological and cytological details of fertilization and early embryonic development in the domestic pig, *Sus scrofa*. Anat. Rec. 178:169-180.

Hunter, R.H.F. 1977. Physiological factors influencing ovulation, fertilization, early embryonic development and establishment of pregnancy in pigs. Br. Vet. J. 133:461-470.

Hunzicker-Dunn, M., S.L. Day, J. Abramowitz, and L. Birnbaumer. 1979. Ovarian responses of pregnant mare's serum gonadotropin-primed rats: desensitizing, luteolytic and ovulatory effects of a single dose of human chorionic gonadotropin. Endocrinology 105:442-451.

Ireland, J.J. 1987. Control of follicular growth and development. J. Reprod. Fertil. Suppl. 34:39-54.

Jensen, E.V. and E.R. DeSombre. 1973. Estrogen-receptor interaction. Estrogenic hormones effect transformation of specific receptor proteins to a biochemically functional form. Science 182:126-134.

Jensen, E.V., T. Suzuki, T. Kawashima, W.E. Stumpf, P.W. Jungblut and E.R. DeSombre. 1968. A two-step mechanism for the interaction of estradiol with rat uterus. Biochemistry 59:632-638.

Kammerman, S., R.E. Canfield, J. Kolena and C.P. Channing. 1972. The binding of iodinated hCG to porcine granulosa cells. Endocrinology 91:65-74.

Kammerman, S. and J. Ross. 1975. Increase in numbers of gonadotropin receptors on granulosa cells during follicle maturation. J. Clin. Endocrinol. Metab. 41:546-550.

Keisler, D.H., E.K. Inskeep and R.A. Dailey. 1983. First luteal tissue in ewe lambs: influence on subsequent ovarian activity and response to hysterectomy. J. Anim. Sci. 57:150-156.

King, W.J. and G.L. Greene. 1984. Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells. Nature 307:745-747.

Kirkpatrick, R.L., B.E. Howland, N.L. First and L.E. Casida. 1967. Ovarian and pituitary gland changes in gilts on two nutrient energy levels. J. Anim. Sci. 26:358-364.

Kirkwood, R.N. and P.E. Hughes. 1979. The influence of age at first boar contact on puberty attainment in the gilt. Anim. Prod. 29:231-238.

Kirkwood, R.N. and F.X. Aherne. 1985. Energy intake, body composition and reproductive performance of the gilt. J. Anim. Sci. 60:1518-1529.

Knight, J.W., F.W. Bazer, W.W. Thatcher, D.E. Franke and H.D. Wallace. 1977. Conceptus development in intact and unilaterally hysterectomized-ovariectomized gilts: interrelations among hormonal status, placental development, fetal fluids and fetal growth. J. Anim. Sci. 44:620-637.

Knott, R.E., D.C. England and W.H. Kennick. 1984. Estrus, ovulation, conception and embryo survival in confinement-managed gilts of three weight groups. J. Anim. Sci. 58:281-284.

Kokolis, N., E. Alexaki-Tzivanidou and A. Smokovitis. 1987. The role of plasminogen activator activity in the ovulation of the sow. In: J.R. Roche and D. O. Callaghen (eds.), Follicular Growth and Ovulation Rate in Farm Animals. Martinus Nijhoff Publishers, Boston. pp. 215-220.

Koligian, K.B. and F. Stormshak. 1977. Nuclear and cytoplasmic estrogen receptors in ovine endometrium during the estrous cycle. Endocrinology 101:524-533.

Lee, W. and M.J. Novy. 1978. Effects of luteinizing hormone and indomethacin on blood flow and steroidogenesis in the rabbit ovary. Biol. Reprod. 17:799-807.

Lee, C.Y. and R.J. Ryan. 1973. Interaction of ovarian receptors with human luteinizing hormone and human chorionic gonadotropin. Biochemistry 12:4609-4615.

Legan, S.J., H. l'Anson, B.P. Fitzgerald and M.S. Akaydin. 1985. Importance of short luteal phases in the endocrine mechanism controlling initiation of estrous cycles in anestrous ewes. Endocrinology 117:1530-1536.

Liang, T. and S. Liao. 1974. Association of the uterine 17ß-estradiol-receptor complex with ribonucleoprotein. J. Biol. Chem. 249:4671-4678.

Lindner, H.R., A. Amsterdam, Y. Salomon, A. Tsafriri, A. Nimrod, S.A. Lamprecht, U. Zor and Y. Koch. 1977. Intraovarian factors in ovulation: determinant of follicular response to gonadotrophins. J. Reprod. Fertil. 51:215-235.

Liu, W.-K., B.D. Burleigh and D.N. Ward. 1981. Steroid and plasminogen activator production by cultured rat granulosa cells in response to hormone treatment. Mol. Cell. Endocrinol. 21:63-73.

Liu, Y.-X., S.B. Cajander, T. Ny, P. Kristensen and A.J.W. Hseuh. 1987. Gonadotropin regulation of tissue-type and urokinase-type plasminogen activators in rat granulosa and theca-interstitial cells during the periovulatory period. Mol. Cel. Endocrinol. 54:221-229.

Liu, Y.-X. and A.J.W. Hsueh. 1986. Synergism between granulosa and theca-interstitial cells in estrogen biosynthesis by gonadotropin-treated rat ovaries: studies on the two-cell, two-gonadotropin hypothesis using steroid antisera. Biol. Reprod. 35:27-36.

Loskutoff, D.J. 1978. Effects of acidified fetal bovine serum on the fibrinolytic activity and growth of cells in culture. J. Cell. Physiol. 96:361-370.

Lutz, J.B., G.B. Rampacek, R.R. Kraeling and C.A. Pinkert. 1984. Serum luteinizing hormone and estrogen profiles before puberty in the gilt. J. Anim. Sci.58:686-691.

MacPherson, R.M., F.D. DeB. Hovell and A.S. Jones. 1977. Performance of sows first mated at puberty or second or third oestrus, and carcass assessment of once-bred gilts. Anim. Prod. 24:333-342.

Marrable, A.W. 1971. The Embryonic Pig: a Chronological Account. Exeter, Great Britain: A. Wheaton & Co. pp. 12-80.

Martinat, N. and Y. Combarnous. 1983. The release of plasminogen activator by rat granulosa cells is highly specific for FSH activity. Endocrinology 113:433-435.

Mavrogenis, A.P. and O.W. Robison. 1976. Factors affecting puberty in swine. J. Anim. Sci. 42:1251-1255.

McGaughey, R.W. 1977. The culture of pig oocytes in minimal medium, and the influence of progesterone and estradiol-17ß on meiotic maturation. Endocrinology 100:39-45.

Meinecke, B., H. Gips and S. Meinecke-Tillmann. 1987. Steroid hormone content of the developing preovulatory porcine follicle. In: J.R. Roche and D. O. Callaghen (eds.), Follicular Growth and Ovulation Rate in Farm Animals. Martinus Nijhoff Publishers, Boston. pp. 207-214.

Mendelson, C.R. 1988. Mechanisms of hormone action. In: J.E. Griffen and S.R. Ojeda (eds.) Textbook of Endocrine Physiology. Oxford Univerisity Press, New York. pp. 28-55.

Menino, A.R., Jr., A.E. Archibong, J.-R. Li, F. Stormshak and D.C. England. 1989. Comparison of in vitro development of embryos collected from the same gilts at first and third estrus. J. Anim. Sci. 67:1387-1393.

Menino, A.R., Jr. and J.S. Williams. 1987. Activation of plasminogen by the early bovine embryo. Biol. Reprod. 36:1289-1295.

Mercer, W.D., D.P. Edwards, G.C. Chamness and W.L. McGuire. 1981. Failure of estradiol immunofluorescence in MCF-7 breast cancer cells to detect estrogen receptors. Cancer Res. 41:4644-4652.

Moody, G.H. 1982. The source of plasminogen activator in human saliva. Arch. Oral Biol. 27:33-37.

Moor, R.M., C. Polge and S.M. Willadsen. 1980. Effect of steroids on the maturation and fertilization of mammalian oocytes. J. Embryol. Exp. Morphol. 56:319-335.

Moor, R.M. and A.O. Trounson. 1977. Hormonal and follicular factors affecting maturation of sheep oocytes *in vitro* and their subsequent developmental capacity. J. Reprod. Fertil. 49:101-109.

Mueller, G.C., A.M. Herranen and K.F. Jervell. 1957. Studies on the mechanism of action of estrogens. Rec. Prog. Horm. Res. 14:95-130.

Murray, F.A. and A.P. Grifo, Jr. 1976. Development of capacity to secrete progesterone-induced protein by the porcine uterus. Biol. Reprod. 15:620-625.

Nakano, R., T. Akahori, K. Katayama and S. Tojo. 1977. Binding of LH and FSH to porcine granulosa cells during follicular maturation. J. Reprod. Fertil. 51:23-27.

Nelsen, T.C., C.R. Long and T.C. Cartwright. 1982. Postinflection growth in straightbred and crossbred cattle. II. Relationships among weight, height and pubertal characters. J. Anim. Sci. 55:293-304.

Nielsen, L.S., J.G. Hansen, L. Skriver, E.L. Wilson, K. Kaltoft, J. Zeuthen and K. Danø. 1982. Purification of zymogen to plasminogen activator from human glioblastoma cells by affinity chromatography with monoclonal antibody. Biochemistry21:6410-6415.

Norman, A.W. and G. Litwack. 1987. Hormones. Academic Press, San Diego. pp. 101-130.

Ong, E.B., A.J. Johnson and G. Schoellmann. 1976. Identification of an active site histidine in urokinase. Biochem. Biophys. Acta 429:252-257.

Padmanabhan, V., E.M. Convey, J.F. Roche and J.J. Ireland. 1984. Changes in inhibin-like bioactivity in ovulatory and atretic follicles and utero-ovarian venous blood after prostaglandin-induced luteolysis in heifers. Endocrinology 115:1332-1340.

Payvar, F., D. DeFranco, G.L. Firestone, B. Edgar, O. Wrange, S. Okret, J.-A. Gustafsson and K.R. Yamamoto. 1983. Sequence-specific binding of glucocorticoid receptor to MTV DNA at sites within and upstream of the transcribed region. Cell. 35:381-392.

Perry, J.S. 1969. Implantation of the blastocyst in the pig. J. Physiol. 200:40P.

Perry, J.S., R.B. Heap, R.D. Burton and J.E. Gadsby. 1976. Endocrinology of the blastocyst and its role in the establishment of pregnancy. J. Reprod. Fertil. Suppl. 25:85-104.

Perry, J.S. and I.W. Rowlands. 1962. Early pregnancy in the pig. J. Reprod. Fertil. 4:175-188.

Peters, H., A.G. Byskov, R. Himelstein-Braw and M. Faber. 1975. Follicular growth: the basic event in the mouse and human ovary. J. Reprod. Fertil. 45:559-566.

Pope, W.F., M.S. Lawyer, B.S. Nara and N.L. First. 1986. Effect of asynchronous superinduction on embryo survival and range of blastocyst development in swine. Biol. Reprod. 35:133-137.

Pope, W.F., R.R. Maurer and F.Stormshak. 1982a. Intrauterine migration of the porcine embryo--interaction of embryo, uterine flushings and indomethacin on myometrial function in vitro. J. Anim. Sci. 55:1169-1178.

Pope, W.F., R.R. Maurer and F.Stormshak. 1982b. Survival of porcine embryos after asynchronous transfer. Proc. Soc. Exp. Biol. Med. 171:179-183.

Pope, W.F., R.R. Maurer and F.Stormshak. 1982c. Intrauterine migration of the porcine embryo: influence of estradiol-17ß and histamine. Biol. Reprod. 27:575-579.

Puca, G.A., V. Sica and E. Nola. 1974. Identification of a high affinity nuclear acceptor site for estrogen receptor of calf uterus. Proc. Natl. Acad. Sci. USA 71:979-983.

Racowsky, C. 1983. Androgenic modulation of cyclic adenosine monophosphate (cAMP)-dependent meiotic arrest. Biol. Repod. 28:774-787.

Rajaniemi, H.J., A.R. Midgley, Jr., J. A. Duncan and L.E. Reichert, Jr. 1977. Gonadotropin receptors in rat ovarian tissue: III. Binding sites for luteinizing hormone and differentiation of granulosa cells to luteal cells. Endocrinology 101:898-910.

Ramirez, D.V. and S.M. McCann. 1963. Comparison of the regulation of luteinizing hormone (LH) secretion in immature and adult rats. Endocrinology 72:452-464.

Reich, R., R. Miskin and A. Tsafriri. 1985. Follicular plasminogen activator: involvement in ovulation. Endocrinology 116:516-521.

Resko, J.A., W.E. Ellinwood, L.M. Pasztor and A.E. Buhl. 1980. Sex steroids in the umbilical circulation of fetal rhesus monkeys from the time of gonadal differentiation. J. Clin. Endocrinol. Metab. 50:900-905.

Resko, J.A., J.G. Ploem and H.L. Stadelman. 1975. Estrogens in fetal and maternal plasma of the rhesus monkey. Endocrinology 97:425-430.

Rice, C. and R.W. McGaughey. 1981. Effect of testosterone and dibutryryl cAMP on the spontaneous maturation of pig oocytes. J. Reprod. Fertil. 62:245-256.

Richards, J.S. 1975. Estradiol receptor content in rat granulosa cells during follicular development: modification by estradiol and gonadotropins. Endocrinology 97:1174-1184.

Robertson, G.L., L.E. Casida, R.H. Grummer and A.B. Chapman. 1951. Age at puberty and related phenomena in outbred chester white and Poland China gilts. J. Anim. Sci. 10:841-866.

Robinson, G.E, Jr. and A.V. Nalbandov. 1951. Changes in the hormone content of swine pituitaries during the estrual cycle. J. Anim. Sci. 10:469-478.

Ryan, K.D. and D.L. Foster. 1980. Neuroendocrine mechanisms involved in onset of puberty in the female: concepts derived from the lamb. Fed. Proc. 39:2372-2377.

Schnurrbusch, U. and J. Erices. 1979. Histomorphologische veranderungen am uterus von jungsauen wahrend der ersten 3 sexualzyklen. Arch. Exp. Vet. Med., Leipzig 33:527-546.

Schochet, S.S. 1916. A suggestion as to the process of ovulation and ovarian cyst formation. Anat. Rec. 10:447-457.

Self, H.L., Grummer, R.H. and L.E. Casida. 1955. The effects of various sequences of full and limited feeding on the reproductive phenomena in chester white and Poland China gilts. J. Anim. Sci. 14:573-592.

Shalgi, R., P. Kraicer, A. Rimon, M. Pinto and N. Soferman. 1973. Proteins of human follicular fluid: the blood-follicle barrier. Fertil. Steril. 24:429-434.

Shapiro, B.M., R.W. Schackmann and C.A. Gabel. 1981. Molecular approaches to the study of fertilization. Ann. Rev. Biochem. 50:815-843.

Shaw, H.J. and G.R. Foxcroft. 1985. Relationships between LH, FSH and prolactin secretion and reproductive activity in the weaned sow. J. Reprod. Fertil. 75:17-28.

Shimada, H., H. Okamura, Y. Noda, A. Suzuki, S. Tojo and A. Takada. 1983. Plasminogen activator in rat ovary during the ovulatory process: independence of prostaglandin mediation. J. Endocrinol. 97:201-205.

Short, R.V. 1962. Steroids in the follicular fluid and the corpus luteum of the mare. A'two-cell type' theory of ovarian steroid synthesis. J. Endocrinol. 24:59-63.

Signoret, J.P. 1970. Reproductive behavior of pigs. J. Reprod. Fertil. Suppl 11:105-117.

Sobel, G.W., S.R. Mohler, N.W. Jones, A.B.C. Dowdy and M.M. Guest. 1952. Urokinase: an activator of plasma profibrinolysin extracted from urine. Amer. J. Physiol. 171:768-776.

Spaziani, E. and C.M. Szego. 1958. The influence of estradiol and cortisol on uterine histamine of the ovariectomized rat. Endocrinology 63:669-678.

Strickland, S. and W.H. Beers. 1976. Studies on the role of plasminogen activator in ovulation. *In vitro* response of granulosa cells to gonadotropins, cyclic nucleotides, and prostaglandins. J. Biol. Chem. 251:5694-5702.

Thompson, L.H. and J.S. Savage. 1978. Age at puberty and ovulation rate in gilts in confinement as influenced by exposure to a boar. J. Anim. Sci. 47:1141-1144.

Tsafriri, A., T.A. Bicsak, S.B. Cajanders, T. Ny and A.J.W. Hsueh. 1989. Suppression of ovulation rate by antibodies to tissue-type plasminogen activator and α_2-antiplasmin. Endocrinology 124:415-421.

Van Lunen, T.A. and F.X. Aherne. 1987. Effect of long-term feed restriction on age at puberty of gilts. Can. J. Anim. Sci. 67:797-801.

Wahlstrom, R.C. and G.W. Libal. 1977. Effect of dietary protein during growth and gestation on development and reproductive performance of gilts. J. Anim. Sci. 45:94-99.

Wang, C. and A. Leung. 1983. Gonadotropins regulate plasminogen activator production by rat granulosa cells. Endocrinology 112:1201-1207.

Warnick, A.C., E.L. Wiggins, L.E. Casida, R.H. Grummer and A.B. Chapman. 1951. Variation in puberty phenomena in inbred gilts. J. Anim. Sci. 10:479-493.

Welshons, W.V., M.E. Lieberman and J. Gorski.1984. Nuclear localization of unoccupied oestrogen receptors. Nature 307:747-749.

Werb, Z., C.L. Mainardi, C.A. Vater and E.D. Harris, Jr. 1977. Endogenous activation of latent collagenase by rheumatoid synovial cells. Evidence for a role of plasminogen activator. New Eng. J. Med. 296:1017-1023.

Wiggins, E.L., L.E. Casida and R.H. Grummer. 1950. The effect of season of birth on sexual development in gilts. J. Anim. Sci. 9:277-280.

Wilde, M.H., S. Xie, K.P. Nephew and W.F. Pope. 1987. The relationship between ovulation and morphological variation of day 11 swine embryos. Biol. Reprod. 33 (Suppl. 1):60.

Wislocki, G.B. and A.F. Guttamacher. 1924. Spontaneous peristalsis of whole uterus and Fallopian tubes of the sow with reference to ovulation. Bull. Johns Hopkins Hosp. 35:246-258.

Young, L.G. and G.J. King. 1981. Reproductive performance of gilts bred on first *versus* third estrus. J. Anim. Sci. 53:19-25.

Zava, D.T. and W.L. McGuire. 1977. Estrogen receptor. unoccupied sites in nuclei of a breast tumor cell line. J. Biol. Chem. 252:3703-3708.

Zavy, M.T. W.R. Clark, D.C. Sharp, R.M. Roberts and F.W. Bazer. 1982. Comparison of glucose, fructose, ascorbic acid and glucosephosphate isomerase enzymic activity in uterine flushings from nonpregnant and pregnant gilts and pony mares. Biol. Reprod. 27:1147-1158.

Zeleznik, A.J., A.R. Midgley, Jr., and L. E. Reichert, Jr. 1974. Granulosa cell maturation in the rat: increased binding of human chorionic gonadotropin following treatment with follicle-stimulating hormone *in vitro*. Endocrinology 95:818-825.