To the Graduate Council:

I am submitting herewith a thesis written by James R. Giles entitled "Induction of Puberty and Maintenance of Pregnancy in Prepuberal Gilts." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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INDUCTION OF PUBERTY AND MAINTENANCE OF PREGNANCY IN PREPUBERAL GILTS

A Thesis

Presented for the

Master of Science

Degree

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James R. Giles

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ABSTRACT

The effects of estradiol benzoate (EB) and human chorionic gonadotrophin (HCG) on ovulation, fertilization, and maintenance of pregnancy were tested on 53 prepuberal gilts during the year 1978.

In Experiment I, 8 gilts averaging 170 days of age were injected with 0.4 mg EB twice daily for 3 consecutive days. Human chorionic gonadotrophin was administered (500 i.u.) 36 hours after the last EB injection. Gilts were slaughtered either 31, 55, 77, or 100 hours after HCG injection. Only 39% of the follicles in 2 gilts had ovulated by 77 hours post HCG injection but 83% had ovulated in another gilt by 100 hours.

In Experiment II, 15 gilts averaging 176 days of age were put on trial. Five of these gilts were injected in the same manner as in Experiment I and were maintained in the same pen with 5 gilts and 2 boars which had no hormone treatment. Five more gilts were kept in a pen away from the sight and sound of a boar. There was no difference in age settled between the gilts maintained in the same pen. Two gilts receiving hormone treatment returned to estrus 18 and 21 days after HCG injection and farrowed an average of 8 live pigs while three gilts with only boar exposure farrowed an average of 5 live pigs.

In Experiment III, 30 gilts averaging 150 days of age were alloted equally to 1 of 3 treatments. Twenty gilts were injected with EB and HCG in the same manner as in Experiment I and 10 of

these gilts were artificially inseminated 48 to 72 hours after HCG injection. The other 10 gilts were maintained in an adjacent lot and observed for estrus. Ten gilts were also maintained in a pen away from the sight and sound of a boar. Estradiol benzoate treated gilts experienced first estrus earlier ($P < .01$) than control gilts. Eight of the bred gilts returned to estrus at an average of 37.3 days post HCG injection. Four of these gilts were rebred to a boar and settled an average of 9.8 live feti past 80 days gestation. Progestin levels prior to the start of treatment were all below 1.5 ng/ml of plasma while at day 12 (HCG = day 0) of the induced cycle, progestin levels averaged 18.33 ng/ml (eliminating 3 gilts with levels below 1.5 ng/ml) for gilts receiving EB and HCG treatment. Progestin levels of the 4 gilts bred on the following estrus averaged 23.2 ng/ml on day 10.

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CHAPTER I

INTRODUCTION

In the last few years there has been an increasing demand for pork. Swine are also extensively used in medicine and research. This increasing demand calls for more vigilant management by the producer and an increase in the reproductive efficiency of the breeding herd (Hunter, 1977). Some factors affecting the prolificacy of swine are:

(a) Age at which puberty is attained

(b) Conception rates

(c) Embryonic mortality

(d) Post-natal mortality

(e) Length of lactation periods

(f) Frequency of anestrous sows

Gilts normally attain puberty around 200 days of age depending on several factors such as boar exposure, nutrition, genetics and social effects (Brooks and Cole, 1970; Hughes and Cole, 1975; Tassell, 1967; Mavrogenis and Robison, 1976). Most management practices call for gilts to be bred on their second or third naturally occurring estrus so as to farrow at 1 year of age. If age at first estrus could be reduced then gilts could be bred at an earlier age. The producer could decrease the age at which non-productive gilts were detected and thus the quantity of feed consumed. Certainly the generation interval would be lowered and life-time reproductive efficiency might be increased.

Many researchers have induced prepuberal gilts to ovulate using pregnant mare's serum gonadotrophin (PMSG) and human chorionic gonadotrophin (HCG) (Dziuk and Gehlback, 1966; Baker and Coggins, 1968). However, maintenance of pregnancy in these gilts past 25 days gestation is low.

Hughes and Cole (1976) and Hunter (1977) tried a different approach using estradiol benzoate (EB) and HCG. This study examined time of ovulation, maintenance of pregnancy and progestin levels on day 12 of the induced cycle in prepuberal gilts primed with EB and injected with HCG.

CHAPTER II

LITERATURE REVIEW

I. FACTORS AFFECTING PUBERTY

Many factors affect the age of puberty of gilts. Some of these are age, weight, nutrition, genetics, social and seasonal factors (Dziuk, 1977). As stated before, puberty is often attained by 200 days of age. Age seems to be a more reliable indicator of puberty than does body weight (Robertson et al., 1951; Reddy et al., 1958; Tassell, 1967).

The effects of nutrition are not clear. Robertson et al. (1951) found that full fed gilts tended to reach puberty earlier than limited fed gilts while Self et al. (1955) observed that limited fed gilts attained puberty earlier than full fed gilts. Christian and Nofziger (1952) compared the effects of a high plane of nutrition with a low plane on age at puberty. The low plane females received 70% of the feed intake of the high plane group. The average age at puberty for high plane gilts was 170.2 days and 166.8 days for low plane gilts. However, the high plane gilts averaged 50 pounds heavier than the low plane gilts at puberty. Tassell (1967) concluded that severe underfeeding causes a delay in puberty but moderately underfeeding, while reducing body weight, had little effect on puberty.

Levels of protein seem to be as unclear as plane of nutrition. Robertson et al. (1951) found that gilts receiving a low protein ration

(15%) were 6 days younger at first heat than those which received the high protein ration (20%). Cunningham et al. (1974) noted that gilts fed a 14% protein corn soybean meal diet reached puberty 18.7 days earlier than gilts fed a 10% high-lysine corn diet.

Much of the variability of age at puberty has to do with the genetic background of the gilt. Byrum (1957) demonstrated that Hampshire gilts had a shorter average interval to first estrus than did the Duroc gilts. Hughes and Cole (1975) found that gilts sired by one Landrace boar reached puberty significantly earlier ($P < .05$) than did the offspring of 3 other Landrace boars.

The presence of a boar will often hasten the onset of puberty (Byrum, 1957; Brooks and Cole, 1970; Bourn et al., 1974; Mavrogenis and Robison, 1976; Zimmerman et al., 1976; England, 1977). Brooks and Cole (1970) demonstrated that more gilts reached puberty within a shorter period of time when exposed to a boar at 165 days of age than those not exposed. Bourn et al. (1974) demonstrated that gilts exposed to a boar at 135 days of age attained puberty earlier ($P < .01$) than gilts exposed at 165 days of age (156 vs 173 days, respectively). Gilts exposed to a boar at 140 days of age were younger at puberty (205 days of age) than those where a boar was absent (222 days of age) (Mavrogenis and Robinson, 1976).

Physical contact with a boar may not be the only variable in inducing first estrus in gilts. Sight, sound, and/or smell of the boar may play a big role in bringing pigs into first estrus. Byrum (1957) found that gilts averaging 174 days of age, under dry lot conditions, came into first heat 11.7 days after introduction to a

boar. Gilts under the same conditions but without boar exposure averaged 29.5 days at first estrus. Under pasture conditions, 87.5% of the gilts exposed to a boar came into first heat within 11.4 days after introduction to a boar. None of the pasture gilts without boar exposure came into heat during a 38 day observation period. The difference between the gilts in the dry lot and pasture without a boar, in response to first estrus, may be attributed to the fact that the gilts in the dry lot were within 50 feet of boars and were thus within sound but not sight of the boars while the pasture gilts were neither in sight or sound of the boars.

Transporting gilts from one environment to another when they are about 6 months of age will often trigger the first heat within 5 to 6 days (Signoret, 1970). Bourn et al. (1974) demonstrated that transport in addition to exposure to a boar induced an earlier first estrus than exposure alone. Zimmerman et al. (1976) observed that mixing, transporting, relocating and continuous boar contact for 10 days resulted in 87% of the gilts 165 days of age having ovulated by the end of the treatment. England (1977) noted that combinations of change of location, mixing and boar exposure resulted in estrus in a high percentage of gilts within a short period of time.

There is also a social effect among gilts. Mavrogenis and Robison (1976) observed that age and weight at puberty were reduced in gilts grouped 30 to a pen versus those penned individually.

Wiggins et al. (1950) found that gilts born in the spring were slower to reach sexual maturity than gilts born at other seasons. Mavrogenis and Robison (1976) found that gilts born in the fall reached

puberty at a younger age and a lower weight ($P < .01$) than those born in the spring. They found that gilts born in the fall attained sexual maturity at or close to a minimum age and further reduction was not achieved by boar exposure or other factors. On the other hand, the spring farrowed gilts reached sexual maturity at a much later age which allows a reduction in age at puberty by boar exposure, transporting and other factors.

II. INDUCTION OF PUBERTY

Casida in 1935 did research to determine the age at which the ovaries of pigs might respond to exogenous gonadotrophin stimulation. Primary follicles were found in the ovaries up to 6 weeks of age. Multilayered follicles appeared at 7 weeks and vesicular follicles were beginning to be formed at 10 to 14 weeks of age. Black (1967) confirmed the appearance of vesicular follicles at 10 weeks. Casida (1935) induced ovulation in gilts 107 and 113 days of age using a preparation of pregnant mare serum and also by a follicle stimulating extract of sheep anterior pituitary followed by a luteinizing extract.

Subsequently, many researchers have induced ovulation in prepuberal gilts using pregnant mare's serum (PMS) and a luteinizing agent such as human chorionic gonadotrophin (HCG) (Dziuk and Gehlback, 1966; Baker and Coggins, 1968; Shaw et al., 1971; Schilling and Cerne, 1972; Segal and Baker, 1973; Ellicot et al., 1973; Callaghan and King, 1974; Baker and Downey, 1975; Murray, 1976; Rampacek et al., 1976b).

Dziuk and Gehlback (1966) treated gilts ranging in ages from 95 to 130 days of age with 1 or 2 subcutaneous injections of 500 to

1,250 i.u. PMS followed 96 hours with 500 i.u. HCG intramuscularly and artificially inseminated 24 hours after HCG. Of the 67 gilts treated, 29 (42%) were in heat 24 hours after HCG and 57 of 62 (92%) had ovulated. Eggs were recovered from 18 of 24 artificially inseminated gilts. Of the recovered eggs 68% were fertilized. Three of 18 gilts were pregnant at 23 days but did not carry the feti to term. Baker and Coggins (1968) demonstrated, using gilts 154 to 180 days of age, that the number of ovulations increased linearly with increasing levels of injected mare's serum gonadotrophin (PMSG). The number of immature eggs and the number of polyspermic eggs also increased with increased doses of PMSG. Schlegel et al. (1977) compared doses of PMS and HCG and found that a single injection of 500 i.u. PMS and 250 i.u. HCG to be optimal. They found that although higher dosages gave higher ovulation rates, pregnancy rates dropped severely.

Schilling and Cerne (1972) treated gilts 5 to 6.5 months of age with a single subcutaneous injection of 400 i.u. PMS plus 200 i.u. HCG. Gilts were artificially inseminated on their induced estrus. Of all treated gilts 5.5 months of age, 80% farrowed. Callaghan and King (1974) also claimed that treatment with the PMSG/HCG combination just prior to the onset of puberty may be a successful method for controlling breeding of gilts.

Rampacek et al. (1976b) treated gilts 155, 165, 175, or 185 days of age with 1,000 or 750 i.u. PMSG plus 500 i.u. HCG and then artificially inseminated. The percentage of gilts pregnant at 25 days for 155, 165, 175, and 185 day old gilts were 10, 20, 30, and 50%, respectively, with average litter sizes of 4 to 5 feti.

Baker and Downey (1975) and Guthrie (1977) treated gilts with PMSG and gonadotrophin releasing hormone (GnRH). They found that GnRH had no positive influence on estrus, ovulation, or fertility.

Few workers have studied the effects of estrogen on induction of puberty in gilts. The physiological actions of estrogen work on the hypothalamohypophysical axis to affect the ovary (Schally et al., 1973; Masincupp et al., 1978).

Lantz and Zimmerman (1972) and Pomerantz et al. (1975) demonstrated that estrogen treatment depresses both luteinizing hormone (LH) secretion and pituitary responsiveness to luteinizing hormone-releasing hormone (LH-RH). Lantz and Zimmerman (1972) demonstrated that both a 0.2 mg or 0.4 mg dose of estradiol 17β in mature ovariectomized pigs suppressed LH levels by 24 hours after start of treatment. They observed ovulatory-like release of LH between 72 and 84 hours after first injection on both levels of estradiol, but the peak tended to be higher on the 0.4 mg level. Lantz et al. (1974) noted that estradiol benzoate (EB) injected twice daily, in mature ovariectomized pigs, was more effective than once daily in suppressing LH and that the length of the suppression period increased significantly with the duration of estrogen treatment.

Hughes and Cole (1976) injected gilts 140 days of age, twice daily for 3 consecutive days with 0.4 mg EB. Of the gilts treated, 65% showed estrus within 5 days of the start of treatment. Hunter (1977) injected gilts 165 - 185 days of age with EB in the same manner as Hughes and Cole (1976). He found 6 of the 9 gilts (66.6%) contained stimulated ovaries with some ovulation points 5 days after

the start of treatment. He then further experimented by administering HCG 36 hours after the last EB injection, to enhance ovulation. Gilts were artificially inseminated 24 and 36 hours after the HCG injection. Although none of the gilts settled on the induced cycle, 5 out of 7 had an average of 7.2 live feti at 50 days of gestation.

III. MAINTENANCE OF PREGNANCY

In order to have an understanding of the maintenance of pregnancy in prepuberal gilts, one must understand the relationships between the pituitary, ovary, uterus, and embryo. In the pig, the pituitary is essential for maintenance of pregnancy (Kraeling and Davis, 1974; Dziuk, 1977), indicating that the corpus lutem (CL) of pregnancy requires continuous pituitary luteotrophic support (Kraeling and Davis, 1974). Ovariectomy at any time during gestation causes immediate abortion or resorption of the feti unless progesterone (P) is administered (Dziuk, 1977). This indicates that the placenta of the pig fails to secrete enough P to maintain pregnancy. Removal of the uterus before day 10 of the estrous cycle will prolong regression of the CL (du Mesnil du Buisson, 1961; Anderson et al., 1961; Rampacek et al., 1976a; Puglisi et al., 1978). Anderson et al. (1961) demonstrated, using mature gilts, that CL were maintained in 4 out of 5 animals when only the uterine body and cervix were present. They also showed that more than 25% of 1 uterine horn was necessary to cause luteal regression. This is unlike the prepuberal gilt where as little as 15% of 1 uterine horn or the uterine body left intact will cause regression of the CL (Puglisi et al., 1978). Rampacek et al.

(1976a) and Puglisi et al. (1978) suggest that there could be an excess of uterine luteolysin and/or an increased sensitivity of the induced CL to the uterine luteolysin in the prepuberal gilt. This could explain the difference in the amount of uterine tissue necessary to cause luteal regression between mature and prepuberal gilts. Luteal function in the hysterectomized prepuberal gilt seems to be similar to that of the pregnant sow (Rampacek et al., 1976a). They showed that plasma progestin concentrations on day 29 for hysterectomized gilts were 71% of the concentrations on days 10 through 14. Tillson and Erb (1967) demonstrated that plasma P concentrations in pregnant sows between days 25 to 30 of gestation were approximately 65% to 75% of the concentrations found between days 10 through 14. Segal and Baker (1973) noted that the size and P content of the CL of prepuberal gilts hysterectomized on day 8 of their induced cycle was similar to those of prepuberal gilts pregnant at day 20.

Dhindsa and Dziuk (1968) concluded that embryos must be present in both uterine horns between days 10 and 12 for pregnancy to be maintained. After day 12 only the presence of embryos in 1 horn is needed to maintain pregnancy. They demonstrated that pregnancy could be maintained in unilaterally pregnant gilts in which unilateral pregnancy was established by killing embryos in 1 horn after day 12. None of the gilts in which unilateral pregnancy was established between days 4 and 10 maintained pregnancy. Longenecker and Day (1972) maintained pregnancy in more than half the gilts on trial by infusing extracts of the complete conceptus and embryonic membranes into the sterile horn of unilateral pregnant gilts. Chiboka et al.

(1976) demonstrated that both swine placentas and feti were necessary for maintenance of pregnancy.

Much work has been done on the effects of steroids on embryonic survival and maintenance of pregnancy in mature pigs. This led the way for the use of hormones in maintenance of pregnancy in prepuberal gilts induced to ovulate.

Reddy et al. (1958) limited embryonic mortality to only 13.5% as compared to 23.3% for controls by injecting sows with 25 mg P + 12.5 µg estrone (2,000:1) daily from day 14 to 24 of gestation. Gentry et al. (1973) maintained pregnancy in ovariectomized mature pigs by giving 80 mg P + 500 µg EB (160:1). The embryo survival rate in these pigs was 82%. Gentry et al. (1973) also demonstrated that EB levels of 0, 125, 250, 500, and 1,000 µg per day did not significantly affect embryo survival rates with an adequate level of progesterone. Ellicott and Dziuk (1973) maintained pregnancy in mature ovariectomized pregnant gilts with a dose of P as low as 28.6 ng/day. No ovarian estrogen was necessary after day 10 for maintenance of pregnancy. Plasma P levels on days 45 and 60 of gestation in 1 intact gilt were 15.3 and 16.7 ng/ml. The mean P concentration in 6 ovariectomized gilts implanted with P was 6.0 ng/ml on either day. They stated that a concentrationof 4 ng/ml of plasma P appeared to be minimal for the maintenance of pregnancy.

Neville et al. (1971) induced ovulation in 140 day old gilts using PMSG + HCG and then artificially inseminated them. Fifteen gilts received 150 mg P on day 19 after artificial insemination and every 10 days subsequently over the next 70 days. Four of these pigs

farrowed an average of 6.5 pigs. Rampacek et al. (1976b) maintained pregnancy in 5 of 9 gilts (177 - 198 days of age) using daily injections of 200 mg P and 100 µg EB beginning 12 days after induction of ovulation.

Gardner et al. (1963), Chakraborty et al. (1972), and Kraeling et al. (1975) found that the daily injection of estrogen beginning by at least day 11 in the mature gilt would maintain functional CL until at least day 30 in the presence of a non-gravid uterus. Daily injections of estrogens alone did not maintain the CL or pregnancy in the prepuberal gilt like it does in the mature pig (Segal and Baker, 1973; Rampacek and Kraeling, 1978). Segal and Baker (1973) observed that the daily injection of 5 mg EB from day 11 through day 17 in the intact or bilaterally hysterectomized prepuberal gilts did not maintain the CL or pregnancy in most cases. Rampacek and Kraeling (1978) tried 5 or 10 mg EB from day 10 to day 15 but observed the same results.

Christenson and Day (1971) found that induction of a second set of CL prior to day 11 in unilateral pregnant gilts significantly increased ($P < .05$) the percentage of gilts with a non-pregnant uterine horn that maintained pregnancy to days 25 and 37 of gestation. Shaw et al. (1971) and Ellicott et al. (1973) attempted to maintain pregnancy in prepuberal gilts by inducing a secondary set of CL before day 12 of the induced cycle. Shaw et al. (1971) found no gilts pregnant at day 50. However, Ellicott et al. (1973) found 1 of 11 gilts pregnant at day 60.

Segal and Baker (1973) induced prepuberal gilts to ovulate

and then injected them daily from day 7 through day 25 of gestation with either 100, 200, or 500 i.u. HCG daily. Increasing the amount of HCG injected increased the number of gilts that contained live embryos at day 25 and the number of CL per gilt.

Salisbury et al. (1975) attempted to maintain plasma progesterone levels in hysterectomized prepuberal gilts by injecting luteinizing releasing factor (25 μg) from day 12 through day 19. Levels were maintained on day 20 in 2 of 5 treated gilts at 81% and 71% of the levels on day 12. However, Puglisi et al. (1978) stated that Salisbury and his team did not remove the uterine body and thus left part of the luteolytic tissue intact. Rampacek et al. (1976b) compared LH-RH to HCG in an attempt to form CL which would continue to support pregnancy past the first month of gestation. They found, as well as Baker and Downey (1975), no positive influence over HCG using LH-RH.

IV. PROSTAGLANDIN $F_{2\alpha}$

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) seems to be the natural luteolysin in the ewe (Goding, 1974; Scaramuzz and Baird, 1976). It acts by causing the cessation of progesterone secretion and structural regression of the corpus lutem on day 15 or 16 if an embryo is not present (Goding, 1974).

There is evidence to believe that exogenously administered $PGF_{2\alpha}$ is luteolytic in other species including the pig (Gleeson, 1974; Hallford et al., 1975). Duncan et al. (1961) observed that endometrial flushings from days 16 and 18 of the estrous cycle of the mature pig had an inhibitory effect on <u>in vitro</u> P synthesis by

luteal tissue. This is the time when rapid CL regression takes place if the pig is not pregnant. Christenson and Day (1972) found that infusions of endometrial extracts from days 13 to 17 of the estrous cycle reduced pregnancy rates in unilateral pregnant gilts with a second set of induced CL. Gleeson and Thorburn in 1973 found peak levels of utero-ovarian prostaglandin F at the time of luteal regression in the cyclic sow. Gleeson (1974) measured P concentrations after infusing $PGF_{2\alpha}$ into the uterine vein at 125 µg/hour for 24 hours on days 11 to 12 in unilaterally ovariectomized sows. Progesterone concentrations increased from 10 ng/ml to 26 ng/ml within 5 hours after commencing infusion but, by the conclusion of the 24 hour infusion period, P concentrations were less than 2 ng/ml and the concentration remained low for at least 3 days. Kraeling et al. (1975) showed that, in the mature pig, $PGF_{2\alpha}$ caused regression of CL that was maintained beyond the normal luteal phase of the estrous cycle by EB treatment.

The luteolytic mechanisms of $PGF_{2\alpha}$ is still obscure. Pharris (1970) postulated a vascular effect where luteolysis was due to the constriction on the utero-ovarian vein which results in a reduced ovarian blood flow. Janson et al. (1974) suggested that $PGF_{2\alpha}$ may cause a vascular redistribtuion within the ovary, with a decreased blood flow to the CL and a subsequent increase in blood flow to the follicles. Henderson and McNatty (1975) suggested that $PGF_{2\alpha}$ inhibits further synthesis of cyclic AMP and thus initiates functional luteolysis.

Although $PGF_{2\alpha}$ will induce luteolysis in the pig, the CL is resistant to this compound for the first 11 days of its life

(Gleeson and Thornburn, 1973; Diehl and Day, 1974; Hallford et al., 1975; Moeljono et al., 1976a).

Since it is known that the PGF concentration is much higher in the non-pregnant pig as compared to the pregnant pig (Moeljono et al., 1976b, 1977), they suggested that the presence of the pig blastocysts alters the PGF secretion pattern between 13 to 17 days of pregnancy. Perry et al. (1976) stated that the pig blastocycts begin to produce estrogen by day 12, and hypothesized that this estrogen may be the substance acting as the embryonic signal to the CL coinciding with the time of maternal recognition of pregnancy. Moeljono et al. (1977) and Frank et al. (1977) suggested, however, that estrogen may reduce the secretion of the potential luteolysin into the uterine vascular bed.

V. UTERINE PROTEINS

Brambell (1933) suggested that uterine secretions may be of importance throughout gestation. Krishnan and Daniel (1967) postulated that some uterine mechanism limits the number of embryos that may undergo implantation or placentation through a process of selection which favors development of the embryos which compete more successfully for some biochemical factor necessary in the key steps of embryonic development. Bazer et al. (1969) also suggested that 25 day litter size was limited by a biochemical factor required for continued development. Since it is known that most embryonic mortality in the pig occurs prior to day 25 of gestation (Pomeroy, 1960; Biggers, 1969), the limited biochemical factor might be present during this period.

Murray et al. (1972) and Squire et al. (1972) demonstrated the quantitative and qualitative changes of porcine uterine secretions during the estrous cycle. Murray et al. (1972) found the average total protein secretion was relatively constant during the first 9 days of the estrous cycle. Total protein began to increase on day 10 and maximum values were found on day 15 (Murray et al., 1972; Squire et al., 1972; Knight et al., 1973), or at about the time of maximum progesterone secretion (Tillson et al., 1970). Masuda et al. (1967) noted there was a sharp decrease in protein secretion with the time of CL regression. Squire et al. (1972), Knight et al. (1973), and Chen et al. (1975) demonstrated that the quantitative and qualitative secretion of the uterine proteins is under the influence of progesterone. However, Murray and Grifo (1976b) suggest there may be other factors which regulate uterine protein secretion since they did not find a constant relationship between uterine protein secretions and progesterone levels.

Five protein fractions are secreted by the uterus of the mature gilt with 2 fractions appearing between days 9 and 16 of the estrous cycle (Murray et al., 1972; Squire et al., 1972). These proteins, appearing during the luteal phase of the estrous cycle, contain a purple colored, basic, glycoprotein which comprises about 15% of the total protein (Murray et al., 1972; Squire et al., 1972).

Murray et al. (1972) noted that this purple, basic, glycoprotein was associated with the time of rapid blastocyst development. Chen and Bazer (1973), using sheep antiserum to the purple protein, suggested that it was involved in some aspect of placental development.

Later, Bazer et al. (1975) and Chen et al. (1975) suggested that the uterine epithelial cells and that placental areolae serve as sites of absorption and transport of this protein across the chorio-allantoic membranes and into the allantoic fluid.

Murray and Grifo (1976a) found that gilts do not possess the ability to respond to exogenous gonadal steroids by secreting a complete adult pattern of uterine specific proteins until about 140 days of age. They also observed that gilts 112 days of age secreted a partial pattern of uterine proteins which indicates that the ability to secrete all uterine proteins is not developed at the same time, but occurs during the period from 4 to 5 months of age. Later, Segerson and Murray (1977) demonstrated that non-mated prepuberal gilts 90 to 150 days of age have the ability to produce the uterine specific proteins during an induced cycle while treating with HCG daily from day 12 through 16. They suggested that the uteri of these gilts were not completely immature. Since both groups found that the quantity of induced protein was much less than that in mature gilts, they suggested that the quantitative differences may be related to the pregnancy failure found in prepuberal gilts.

CHAPTER III

METHODS AND PROCEDURES

I. OVULATION TIME AFTER HCG

During the winter of 1978, 8 crossbred gilts averaging 170 days of age were injected intramuscularly (i.m.) with 0.5 mg estradiol benzoate (EB) twice daily for 3 consecutive days. Estradiol benzoate (0.4 mg) was dissolved in 1 ml sesame oil containing 1% benzyl alcohol. An injection (i.m.) of 500 i.u. human chorionic gonadotrophin (HCG) was administered 36 hours after the last EB injection. Gilts were slaughtered 31, 55, 77, and 100 hours following HCG in an attempt to determine time of ovulation. Reproductive tracts were collected and examined. A macroscopic inspection of the uterus and uterine horns was performed. Vascularity, size, weight, and thickness (uterine walls) was approximated for determination of estrogen effect. Ovaries were examined and follicles greater than 5 mm, ovulation points and corpora lutea (CL) were count ed.

II. USE OF EB AND HCG PLUS BOAR EFFECTS TO INDUCE CYCLING

During the spring and summer of 1978, 15 crossbred gilts averaging 176 days of age were randomly allotted to 1 of 3 treatment groups (5 gilts per group).

Group A gilts were injected (i.m.) with 0.4 mg EB in 1 ml

sesame oil/benzyl alcohol solution twice daily for 3 consecutive days. Human chorionic gonadotrophin was injected (i.m.) 36 hours after the last EB injection at a dose of 500 i.u. per pig.

Group B gilts were maintained in the same pasture lot with Group A gilts but received no treatment. Two boars were also maintained in this lot and allowed to breed any gilts in heat. Gilts which conceived and maintained pregnancy farrowed in crates and were maintained according to normal farm management practices.

Group C gilts were penned in a pasture lot away from the sight or sound of boars. They were observed daily for signs of estrus.

Each gilt was fed approximately 5 pounds of a 13% gestation ration per day (Table I) after they were put on trial. Data were analyzed by analysis of variance and comparisons made by orthogonal contrasts.

III. INDUCTION OF FIRST ESTRUS WITH EB AND HCG FOLLOWED BY ARTIFICIAL INSEMINATION

During the spring and summer of 1978, 30 crossbred gilts averaging 150 days of age were randomly allotted to 1 of 3 groups (10 gilts per group). Twenty of these gilts were injected (i.m.) with 0.4 mg EB in sesame oil/benzyl alcohol solution twice daily for 3 consecutive days. Gilts were injected i.m. with 500 i.u. of HCG 36 hours after the last EB injection.

Ten of these gilts were artificially inseminated once or twice from 48 to 72 hours post HCG injection (Group A). Four gilts which returned to estrus were hand-mated to a boar during their

TABLE I. Gestation Ration (13%)

Ingredient	Pounds
No. 2 Yellow Corn	753
Soybean Oil Meal (44%)	100
Dehydrated Alfalfa Meal (17%)	100
Dicalcium Phosphate	20
Limestone	10
Vitamin Trace Mineral Premix[a]	10
Salt	5
Antibiotic	2
	1,000

[a]Provides 400,000 USP units Vitamin A; 100,000 IC units Vitamin D-3; 500 i.u. Vitamin E; 400 mg Riboflavin; 1,000 d-Pantothenic acid; 2,000 mg Niacin; 1,500 µg Vitamin B-12; 100 mg Vitamin K (MSBC); 20,000 mg Choline Chloride; 1.2% Zn; 12.% Fe; 0.8% Mn; 0.12% Cu; 0.02% Co; 0.008% I; and 0.001% Se for each pound fed.

subsequent estrus periods. Semen was diluted with an equal volume of a commercial extender (International Boar Semen: Insemia-Aid). Penicillin G Potassium and streptomyocin sulfate were added at a dose of 1,000,000 i.u. and 1 gm, respectively, to 1,000 ml.

The remaining 10 of the 20 treated gilts composed Group B. These gilts were not bred but were maintained according to normal management practices and observed for estrus daily.

Group C gilts (10) were given no injections and were maintained in a pasture lot away from the sight and sound of boars. These gilts were also observed daily for estrus. All gilts were fed approximately 5 pounds of the 13% gestation ration.

Blood samples were collected in heparinized tubes via anterior vena cava puncture by the method of Schwartz and Smallwood (1977). Blood was collected at the start of treatment, 12 days after HCG injection, and in 4 gilts, 10 days after breeding of a boar. Samples were chilled in an ice water bath and centrifuged at 2,000 X g for 10 minutes. Plasma was removed and frozen at -20°C until analyzed for progestins by radioimmunoassay (RIA) procedures.

Data were analyzed by analysis of variance and comparisons made by orthogonal contrasts.

IV. PLASMA ANALYSES

Plasma from gilts in Experiment III was analyzed for progestins by radioimmuniassay in 3 assays: RIA I, RIA II, and RIA III. Plasma

taken from gilts in all 3 groups prior to the start of treatment was assayed in RIA I. Plasma from gilts in Groups A and B, taken 12 days after HCG injection or 10 days after breeding by a boar was analyzed for progestins by the same procedure in RIA II. Since several of the progestin concentrations in RIA II were above the sensitivity of the standard curve, another assay was performed. In RIA III plasma from Group A gilts was diluted 1:10 with plasma containing less than 60 pg progestins per ml and aliquots were assayed for progestins. The radioimmunoassay procedure for progestins is as follows.

Reagents

Progesterone 1,2,6,7-^{3}H(N) (New England Nuclear)

Progesterone (4-Pregnen-3,20-Dione) (Steraloids Inc).

Anti-Progesterone-11-BSA Serum from sheep #337 (Dr. G. D. Niswender, Colorado State University)

Assay Buffer (PBS-Gel)

32.7 g $Na_2HPO_4.7H_2O$

10.5 g $NaH_2PO_4.H_2O$

18.0 g NaCl

2.0 g Na Azide

Add distilled water to a total volume of 2 liters

Add 0.1% gelatin. Store at 4°C.

Dextran-Coated Charcoal Solution

0.625 g Norit A (Matheson-Coleman-Bell)

0.0625 g Dextran T-70 (Mann Research Labs)

100 ml assay buffer

Store at 4°C.

Aquasol-2 (New England Nuclear)

Petroleum Ether (Fisher Scientific Co.)

Preparation of Assay Label

Dilute 250 μCi of tritiated progesterone in assay buffer until 100 μl contains 20,000 counts per minute (cpm). Store at 4°C.

Preparation of Standards

Stock = 1.0 mg/ml in Methanol (MeOH)

Intermediate = 1:10 stock:methanol = 0.1 mg/ml

Solution A = 1 ml of Intermediate + 99 ml of MeOH = (1 μg/ml)

Solution I = 2 ml of solution A + 198 ml of assay buffer = (1,000 pg/0.1 ml)

Solution II = 100 ml of solution I + 100 ml of assay buffer = (500 pg/0.1 ml)

Solution III = 100 ml of solution II + 100 ml of assay buffer = (250 pg/0.1 ml)

Solution IV = 100 ml of solution III + 100 ml of assay buffer = (125 pg/0.1 ml)

Solution B = 1 ml of solution A + 9 ml of MeOH = (100 ng/ml)

Solution V = 2 ml of solution B + 248 ml of assay buffer = (80 pg/0.1 ml)

Solution VI = 100 ml of solution V + 100 ml of assay buffer = (40 pg/0.1 ml)

Solution VII = 100 ml of solution VI + 100 ml of assay buffer = (20 pg/0.1 ml)

Solution VIII = 100 ml of solution VII + 100 ml of assay buffer = (10 pg/0.1 ml)

Solution IX = 100 ml of solution VIII + 100 ml of assay buffer = (5 pg/0.1 ml)

Dilution of Antibody

Lyophilized antibody is diluted with assay buffer several hundred fold to obtain the dilution that will bind 50% of the total assay label added. A dilution of 1:1,000 was found to bind 50% of the 11,500 cpm. Antibody was diluted 1:100 with assay buffer and stored at -20°C until needed.

Radioimmunoassay (RIA) Procedure

1. Prepare a protocol sheet. Each assay should also contain standards, unknowns, and quality controls

		Reagents						
Tube #	I.D.	Sample	Vol.	PBS-Gel	Ab	Assay Label	Charcoal	Total
1-2-3	Total	-----		+	--	+	+	
4-5-6	Bkg.	-----		+	--	+	+	
7-8-9	B_0	-----		+	+	+	+	

2. Extraction of Plasma Samples

   a. Aliquots of plasma are added to 16 X 150 mm disposable culture tubes in dupblicate (Becton-Dickson)

   100 µl for RIA #I

50 µl for RIA #II

100, 200, and 300 µl of diluted plasma (1:10) for RIA #III

b. Add 250 pg of progesterone to 2 plasma samples relatively free of progestins for a cold recovery determination.

c. Add approximately 1,300 cpm assay label to 2 plasma samples, containing less than 60 pg of progestins per ml of plasma, for a hot recovery. Also add directly to 2 small scintillation vials (Beckman BioVial) the same amount of assay label for an estimate of the total cpm added to the 2 hot recovery tubes. Add 3 ml of Aquasol-2 to these scintillation vials.

d. Add 3 or 4 ml petroleum ether (RIA #I and #II - 3 ml; RIA #III - 4 ml) to all plasma samples and vortex for 1 minute. Samples containing hot and cold recoveries are allowed to stand for 45 minutes prior to adding petroleum ether.

e. Freeze the aqueous layer in each sample using liquid nitrogen and pour off the organic phase into a 12 X 75 mm RTU tube (Becton-Dickson).

f. Place the tubes in a water bath (40° - 50°C) under a fume hood and dry down under a stream of nitrogen.

g. Rinse the walls of each tube twice with 200 µl of petroleum ether and dry down again.

3. Add 200 µl assay buffer, vortex for 1 minute and allow to stand at room temperature for 2 hours.

4. Prepare Total, Bkg., B_0 and standard tubes while you are waiting. Add assay buffer to bring expected total volume in all tubes up to 700 µl.
5. Pour contents from hot recovery tubes into scintillation vials and add 3 ml Aquasol-2.
6. Add 100 µl of antibody to each tube except Total and Bkg. Vortex each tube briefly and allow to stand for 1 hour.
7. Add 100 µl of assay label to all tubes, vortex gently, and incubate approximately 16 hours at 4°C.
8. Add 200 µl of the dextran-coated charcoal suspension (stir constantly with magnetic stirrer) to each tube except Total tubes, vortex briefly and allow to stand for 15 minutes at 4°C. (This entire step is carried out in the cold room at 4°C.)
9. Centrifuge all tubes (Beckman TJ-6 centrifuge) for 10 minutes at 2,000 rpm and pour off the aqueous phase into scintillation vials. Add 3 ml Aquasol-2 and shake by hand.
10. Place all tubes in the scintillation counter (Searle Isocap/ 300 Liquid Scintillation System 6868) and count for 1 minute.

Quality Control

The addition of hot and cold recovery tubes to the protocol list have already been described in RIA procedure 2b and c. However, PBS-Gel and plasma blanks were also added to each assay in order to

determine if any interfering compounds cross-react with the antibody. In order to demonstrate parallelism, 100, 200, and 300 µl aliquots of plasma were assayed in RIA III).

Statistical Analyses

The RIA results were analyzed according to the "logit log" method of Robard et al. (1969) on a Hewlett-Packard calculator (model HP-98135A) and printer (model 9871A) using a taped program (09815-14254) from Hewlett-Packard Calculator Products Division.

From previous assays it was determined that values located on the ends of the linear curve of the logit transformation disagree with the same areas on a hyperbolic type curve (%B_0 vs dose). The coefficient of variation between the linear and hyperbolic curves in the area greater than 80% B_0 but above the sensitivity of the curve was 17.3 $\pm$ 13.6% (see Figure I). The coefficient of variation for the same curves in the area less than 20% B_0 was 6.0 $\pm$ 4.5%, while between the 2 ends (less than 80% B_0 and greater than 20% B_0) the coefficient of variation was 4.6 $\pm$ 4.3%. Because of this variation toward the ends of the linear curve, only the calculated values between 20% and 80% B_0 were used while all other values were said to be greater or less than certain values.

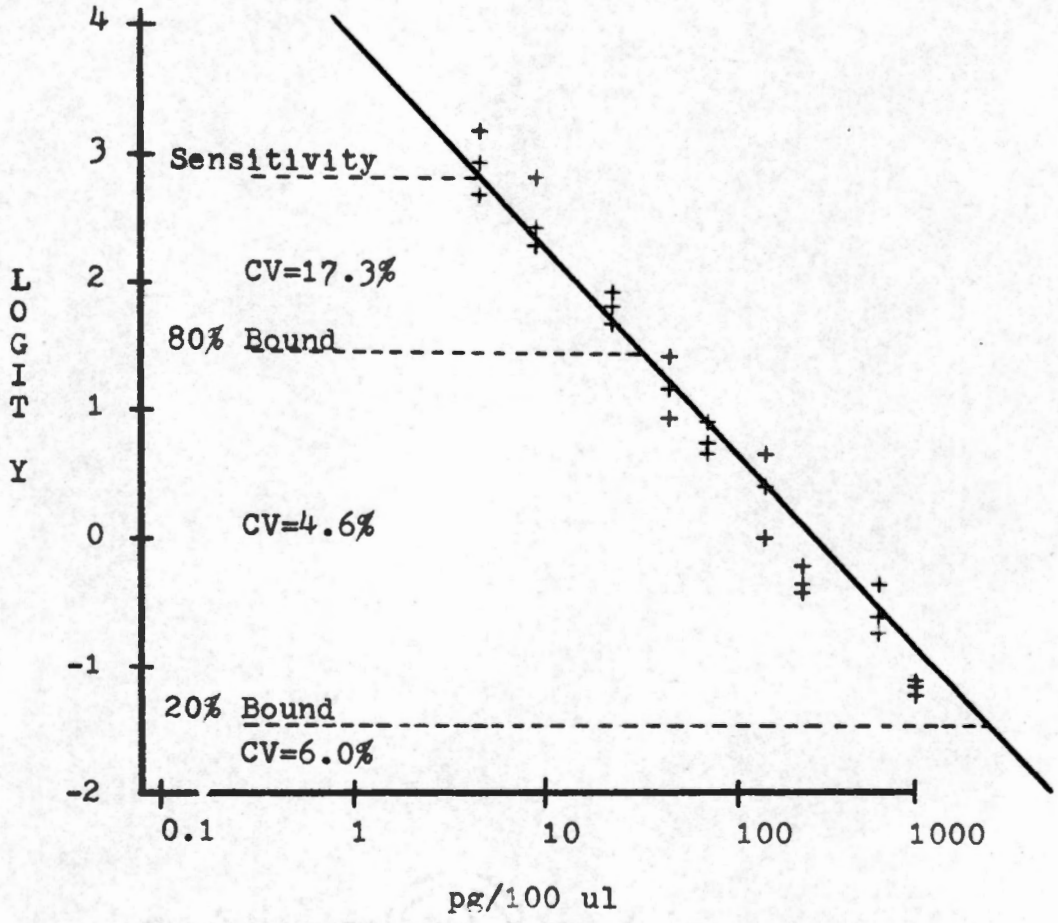

FIGURE I. Differences in progestin concentration, expressed as coefficient of variation (CV), between linear (logit log) and hyperbolic (%Bo vs pg) curves.

CHAPTER IV

RESULTS AND DISCUSSION

I. EXPERIMENT I: OVULATION TIME AFTER HCG

In this experiment an attempt was made to define time of ovulation in prepuberal gilts primed with EB and given an HCG injection 36 hours later. The results of this experiment are summarized in Figure II. All gilts were approaching estrus on the afternoon of the second day of EB injections. By the morning of the third day all gilts were in standing heat. However, they were out or very close to going out of heat by the time HCG was administered. An inspection of the reproductive tracts showed all gilts, except one, with highly vascularized reproductive tracts and thick uterine walls. This gilt had an infantile reproductive tract with no follicles on the ovaries greater than 3 mm. This gilt was removed from the experiment. Examination of the ovaries in the remaining 7 gilts indicated ovulation to be highly variable with no ovulations occurring at 33 hours post HCG injection and only 27% of the follicles greater than 5 mm having ovulated by 55 hours post injection. Of the follicles in 2 gilts slaughtered at 77 hours post HCG injection, 39% had ovulated. Only 1 gilt was slaughtered 100 hours post HCG injection. Data from this gilt indicated that 89% of the follicles had ovulated by this time.

Hunter (1977) used EB and HCG in the same manner as was used to induce first estrus in prepuberal gilts. Slaughter data from 2

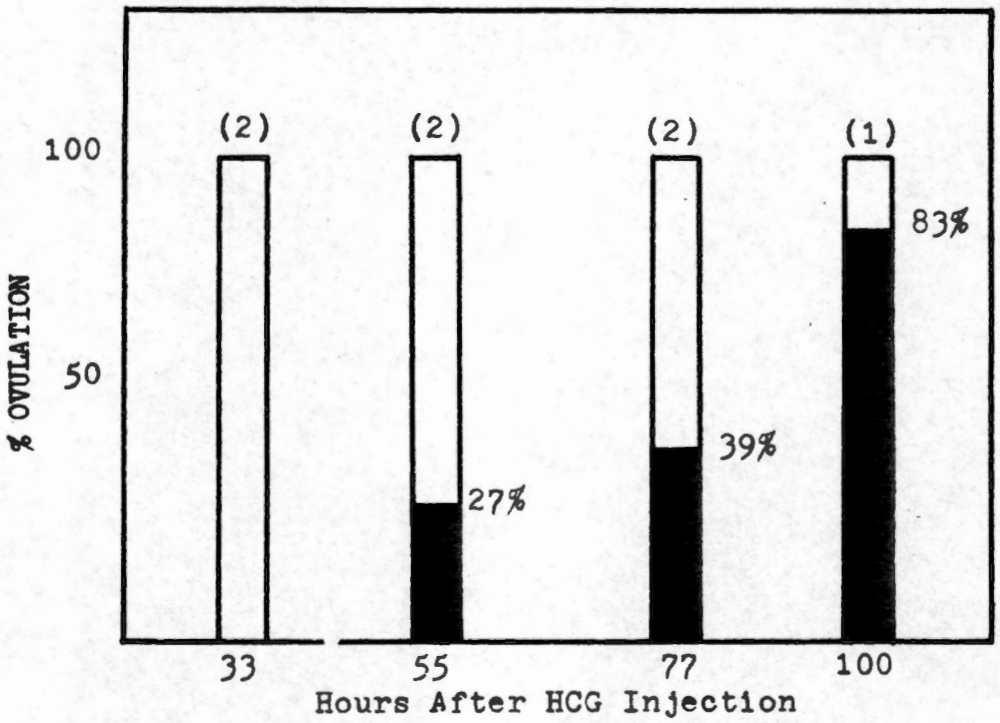

FIGURE II. Ovulation points expressed as a percentage of follicles (greater than 5 mm), (n) = number of gilts.

gilts indicated that ovulation had occurred between 96 and 120 hours after HCG injection.

Hunter (1972) demonstrated that ovulation occurred between 44 and 46 hours after an injection of 500 i.u. HCG given during proestrus. From this experiment there seems to be much difference in time of ovulation between prepuberal gilts primed with EB followed by HCG and mature pigs given HCG during proestrus. However, most researchers consider ovulation to occur approximately 40 hours after an HCG injection in prepuberal gilts primed with PMSG (Dziuk and Gehlback, 1966; Baker and Coggins, 1968). Since the time of ovulation in prepuberal gilts primed with PMSG and injected with HCG is in close agreement with that of mature gilts given HCG, perhaps the lack of agreement may be due to the mode of action of EB and/or dose.

Estradiol benzoate and PMSG affect the ovary but through different pathways. PMSG acts directly on the ovary to cause follicular stimulation while EB acts indirectly on the ovary by way of the hypothalamo-pituitary axis (Schally et al., 1973; Masincupp et al., 1978). Estradiol benzoate, at the dose given, might have an inhibitory action upon the hypothalamus and/or pituitary causing a decrease in the release of gonadotrophin releasing hormones and/or the release of the gonadotrophins from the pituitary. This is the case with high doses of estrogen in mature animals (Nalbandov, 1976; Chakraborty et al., 1972). This would affect growth of the follicle which is under the influence of FSH and LH (Nalbandov, 1976). If an LH surge occurs in these prepuberal gilts like Lantz et al. (1974) demonstrated in ovariectomized gilts (6 months of age)

with similar EB treatment, it may occur too soon to cause ovulation in these follicles. However, this does not explain the failure of HCG to cause ovulation (Murphree, 1979).

II. EXPERIMENT II: USE OF EB AND HCG AND/OR BOAR EXPOSURE TO INDUCE CYCLING

This experiment was designed to distinguish any effects of EB treatment and/or exposure to a boar on age at first estrus, fertilization and maintenance of pregnancy. Results are demonstrated in Table II.

Age at first estrus was reduced ($P < .01$) in gilts in Group A as compared to gilts in Groups B and C. There was also a difference ($P < .05$) in age at first estrus between Groups A and B. Hormone treated gilts demonstrated first estrus 19.6 days earlier than Group B gilts. However, 3 gilts in Group B were bred and settled by the boars on their first estrus which was an average of 5.5 days earlier than gilts in Group A. This indicates that 3 of the gilts in Group B actually attained puberty at an earlier age than gilts which received hormone treatment. However, this effect was not significant.

Gilts in Group A were in standing estrus on the third day of EB injections but were going out of heat by the time of HCG injection. Gilts were bred at the induced estrus by the 2 boars. However, 2 of these gilts returned to estrus at 18 and 21 days after HCG injection. These gilts were bred at this estrus and farrowed 8 live pigs at 114 and 115 days after breeding. The 3 remaining gilts in this group did not return to estrus until after the boars had been removed

TABLE II. Effects of EB and HCG on Early Breeding of Gilts

	EB + HCG + Boar	Boar Only	Control
Age at start of treatment[b]	176.2 ± 9.5	179.0 ± 8.2	173.6 ± 4.3
Age at first estrus[b]	179.2 ± 8.6[a]	185.6 ± 8.0	205.4 ± 19.7
No. of gilts settled[b]	2	3	
Age settled[b]	195.5 ± 4.9	190.0 ± 15.1	
Gestation length[b]	114.5 ± 0.7	114.3 ± 0.6	
Live feti[b]	8 ± 0	5 ± 3.5	
Dead feti[b]	1.5 ± 2.1	3.3 ± 2.3	

[a]Values significantly different from other values in the same line not bearing the same superscript ($P < .05$).

[b]Means ± S.D.

from the pen which was greater than 33 days post HCG injection. These 3 gilts demonstrated a second estrous cycle of normal duration.

Three gilts in Group B which received no hormone treatment were bred by the boars on their first estrus at an average of 190 days of age. These 3 gilts farrowed an average of 5.0 live pigs between 114 and 116 days of age. They also had an average of 3.3 mummified or stillborn feti. Two remaining gilts in this group demonstrated first estrus after the boars were removed or at 222 and 224 days of age. The 5 control gilts demonstrated first estrus at an average of 205.4 days.

One gilt in Group A had 2 mummified feti while 2 of the 3 gilts in Group B had mummified feti. An outbreak of SMEDI (still-births, mummified feti, embryonic deaths and infertility) virus had occurred at this farm approximately 6 months prior to the birth of these gilts. Failure to pass these gilts through the gestation lots could have affected their resistance to the virus. No tests were made to diagnose SMEDI virus in these gilts. If these gilts were infected with the virus, fertility would have been affected and this could be the reason why only 5 of 10 gilts settled.

Gilts in Group A had no problem farrowing. However, 2 of 3 Group B gilts farrowed over a 5 to 7 hour period with oxytocin being administered. Parturition could have been affected by the age of the gilts or by the presence of mummified feti since both gilts were the youngest gilts in the experimental groups to farrow and both contained mummified feti.

No gilt showed the characteristic post-weaning estrus soon

after weaning. However, they did return to estrus within 30 days of weaning. Failure to show estrus soon after weaning could have been related to their poor body condition.

III. EXPERIMENT III: INDUCTION OF FIRST ESTRUS WITH EB AND HCG FOLLOWED BY ARTIFICIAL INSEMINATION

Injecting gilts with EB was successful in inducing estrus. Gilts in Groups A and B experienced first estrus at an earlier age ($P < .01$) than gilts in Group C (Controls) (Table III). Gilts in Groups A and B were in standing heat on the third day of EB injection and were out or very close to going out of heat by the time of HCG administration. These data agree with Hunter (1977).

Data from Hunter (1977) and from the results in Experiment I indicate that ovulation in prepuberal gilts, primed with EB and injected with HCG, occurred past 77 hours post HCG injection. Hunter (1977) artificially inseminated gilts (primed with EB) 24 and 36 hours after HCG injection. However, no gilts settled at this estrus. Gilts in Experiment II were bred by a boar prior to or on the day of HCG injection but no gilts settled at this estrus. In order to have viable sperm in the reproductive tracts at time of ovulation, gilts in Group A were artificially inseminated 48 to 72 hours after HCG injection. However, no gilts settled at this estrus. Excluding 2 gilts which did not return to estrus during the experiment, the average for the induced cycle (Group A) was 37.3 ± 19.7 days, which could be indicative of a pregnancy. However, 4 gilts in Group B also had an abnormally long induced cycle and these gilts were not bred.

TABLE III. Induction of First Estrus with EB and HCG Followed by Artificial Insemination

	Group A	Group B	Group C
Age at start[a]	146.4 ± 3.2	146.1 ± 3.0	146.3 ± 2.8
Weight at start[a,b]	161.3 ± 16.0	165.2 ± 12.7	148.9 ± 32.4
Age at first estrus[a]	153.4 ± 3.7[c]	151.1 ± 3.0[c]	226.9 ± 48.3

[a]Values are means ± S.D.

[b]No significant difference in treatment means.

[c]Values significantly different from other values in the same line not bearing the same superscript ($P < .01$).

Progestin values (see Table IV) indicate that corpora lutea in at least 6 gilts in Group A were still functional by day 10 (see "Results and Discussion: Plasma Analyses"). Semen was often checked for motility and dilution of sperm. It was noted in several cases that sperm was not motile within 15 minutes after collection. It was possible that the extender used (Insemia-Aid) for diluting the semen failed to keep sperm alive.

Four gilts in Group A were bred to a boar during the following estrous cycles and settled an average of 9.8 live feti with 0.5 dead feti (see Table V). These gilts were slaughtered at an average of 86.5 days of gestation. This is similar to the data in Experiment II except no mummified feti were found.

Gilts in Group B were not bred during the induced estrous cycle. One gilt (16-3) remained in constant estrus for most of the experiment. Four other gilts had abnormally long induced cycles (greater than 50 days). However, their second estrous cycle was of normal duration.

Gilts in Group C received no treatment. Three of these gilts were slaughtered at the end of the experimental period because they had not demonstrated estrus. Two of these gilts had infantile reproductive tracts with ovaries containing only small follicles (less than 5 mm) and no corpora albicantia (CA). The other gilt had a reproductive tract which looked to be infantile but the ovary contained 1 CL and no CA.

TABLE IV. Progestin Concentrations

Gilt No.	Pre-TMT[a]	Induced Estrus[b]	Other Estrus[c]
Group A			
21-1	< 0.5	2.74	
24-2	< 0.5	< 1.5	
16-5	< 0.5	> 40.0	15.95
15-6	< 0.5	> 40.0	24.90
17-2	< 0.5	> 30.0	
2-1	< 0.5	> 30.0	
18-4	< 0.5	32.1	29.3
20-2	< 0.5	4.51	
21-3	< 0.5	15.04	
22-1	< 0.5	4.95	22.7
Group B			
5-3	< 0.5	> 20.7	
15-5	< 0.5	> 20.7	
16-1	< 0.5	> 20.7	
16-3	< 0.5	< 1.5	
18-7	0.53	> 20.7	
19-3	< 0.5	6.15	
21-5	< 0.5	9.76	
21-9	< 0.5	1.5	
23-2	< 0.5	4.50	
24-5	< 0.5	9.02	
Group C			
9-2	0.93		
10-7	< 0.5		
15-3	< 0.5		
16-4	< 0.5		
18-2	< 0.5		
18-3	< 0.5		
19-4	< 0.5		
21-2	1.02		
22-5	< 0.5		
24-6	< 0.6		

[a]Values taken 3 days prior to the start of the test.

[b]Values taken 12 days after HCG injection.

[c]Values taken 10 days after natural service by a boar in the following estrous cycles.

TABLE V. Maintenance of Pregnancy in Gilts Treated with EB and HCG

	Group A Gilts
Age settled[a]	213.0 ± 13.3
Age slaughtered[a]	295.8 ± 1.5
Weight slaughtered[a]	265.5 ± 4.8
Days gestation slaughtered[a]	86.5 ± 11.1
Live feti[a]	9.8 ± 2.8
Dead feti[a]	0.5 ± 1.0

[a]Values are means ± S.D.

IV. PLASMA ANALYSES

All but 3 plasma samples taken prior to the start of Experiment III contained less than 0.5 ng of progestins per ml of plasma. One gilt in Group B had 0.53 ng/ml while 2 in Group C had 0.93 and 1.02 ng/ml (see Table IV).

Progestin levels in 8 gilts of Group A taken 12 days after HCG injection averaged 21.2 ng/ml. However, they ranged from 2.74 to greater than 40 ng/ml. These gilts returned to estrus after the induced estrus at an average of 37 days post HCG injection. One gilt (24-2) in Group A had a progestin level lower than 1.5 ng per ml. For the duration of the experimental period she never demonstrated estrus except for the induced cycle. It is possible she never ovulated during this cycle. One other gilt in this group had progestin levels greater than 30 ng/ml. However, she did not return to estrus during the experimental period.

Four gilts in Group A were bred to a boar during the subsequent estrous periods. Blood was collected on day 10 after breeding. All gilts settled at this estrus. Progestin concentrations ranged from 15.95 to 29.3 ng/ml and averaged 23.2 ng/ml.

Five gilts in Group B had induced cycle lengths averaging 33 days post HCG administration and progestin levels averaging 15.5 ng/ml, ranging from 6.15 to greater than 20.7 ng/ml. Two gilts had cycles 56 and 57 days in length. These gilts had progestin levels of 4.5 (23-2) and less than 1.5 (21-9) ng/ml. Two other gilts in this group did not exhibit a second estrus. They had progestin levels of 9.76

ng/ml (21-5) and greater than 20.7 ng/ml (18-7). Another gilt (16-3) exhibited constant estrus and had a progestin level less than 1.5 ng/ml. This was possibly due to cystic follicles. Progestin values of Groups A and B taken during the induced cycle could not be compared due to the fact that some of the samples in Group A were re-evaluated in RIA III and this increased the 20% B_0 limit, thus increasing the maximum dose.

Plasma progesterone levels in mature gilts reached their peak by day 10. They remained at a fairly high concentration to at least day 14. During the next 2 days a precipitous decline occurred and by day 16 progesterone concentrations were comparatively low (Tillson and Erb, 1967; Stabenfeldt et al., 1969; Tillson et al., 1970). These researchers considered day 1 = ovulation; however, in this study where HCG = day 0, ovulation could have occurred on day 3 or 4 (see "Results and Discussion: Experiment I," pp. 29-32). This means that day 10 in this study would correspond to day 13 or 14 in other research. This could explain why some of the progestin values are low and yet others are well within the range for day 14. However, progestin levels and number of CL are highly correlated during early pregnancy (Dziuk et al., 1972; Wettemann et al., 1974; Webel et al., 1975) and the difference in these data might be due to a difference in number of CL. Rampacek et al. (1976b) found several prepuberal gilts where progestin levels had fallen below 5 ng/ml by day 14. They classified these gilts as the most immature. Ovulation could be induced but their CL regress early and the ovaries revert to the prepuberal stage. Four gilts in Group A and 2 gilts in Group B (excluding 16-3) had progestin

levels less than 5 ng/ml. Four of the 6 gilts had an abnormally long interval between the induced estrous period (greater than 50 days) and the first spontaneous period, and one of these did not return to estrus for the duration of the experiment.

V. EVALUTION OF RIA

Accuracy

Accuracy of the 3 assays was tested by recovery determinations. Extraction efficiencies for labeled and unlabeled progesterone were performed in each assay with plasma containing undetectable amounts of progestins. The recovery efficiency using 250 pg of cold hormone in 50 to 300 µl of plasma averaged 76%; however, 63% was obtained in RIA I and III while 102% was obtained in RIA II. The extraction efficiency with tritiated progesterone in 50 to 300 µl of plasma averaged 68%. All samples were corrected for recoveries with either labeled or unlabeled extraction efficiencies depending on which was lower.

Sensitivity

Sensitivity of the standard curve, defined as "the smallest amount of steroid standard that is significantly different from zero at the 95% confidence limit," (Abraham et al., 1977) was 5 pg in RIA I and II and 20 pg in RIA III. However, because the values extrapolated from the ends of the linear curve on the logit log transformation deviated from the results of a hyperbolic curve, only the values between 20% to 80% of the bound were taken as definite doses (see "Materials and Procedures: Statistical Analyses," p. 27).

Determinations were made on plasma, considered free of progestins, and PBS-Gel to obtain blank values. Since all blank values were below the sensitivity of the curve no corrections were made.

Precision

The within-assay variance was evaluated by the coefficient of variation ($CV = \frac{S.D.}{X} \times 100\%$) on a set of 10 duplicate measurements in the same assay. The average for all assays was 4.8% (see Table VI). The between-assay variance was evaluated by duplicate measurements on plasma from a gilt in the luteal phase of the estrous cycle. The between-assay coefficient of variation was 13.7%.

Parallelism

Four plasma samples were assayed in RIA III to check the parallelism of different volumes. In each of 4 samples, duplicate aliquots of 100, 200, and 300 µl of diluted plasma [sample (1): progestin free plasma (10)] were assayed for progestins. Results were analyzed on a per ml basis and all dilutions of the same sample should have the same concentration per ml (see Table VII).

Differences between dilutions were determined by two-way analysis of variance. Doses at the 100 µl dilution were different ($P < .01$) from the 200 of 300 µl dilutions in 3 of 4 samples while no significant differences were found between the 200 and 300 µl dilutions in the same samples. Analyzing this data prior to correction by recovery and dilution factors, but adjusting all data to equal 100 µl (1:10 dilution) gave very similar results. These data demonstrate that the 200 and 300 µl dilutions were very similar in

TABLE VI. Evaluation of Radioimmunoassays

Assay	Total CPM	BKG	B_0	$\%B_0$	Dilution Factor	Hot Recovery	Cold Recovery	Within-Assay CV in %
RIA I	15,018 ± 255	730 ± 45	6,998 ± 117	43.9	10	.70	0.63	2.6 ± 2.3
RIA II	12,773 ± 262	544 ± 45	5,665 ± 22	41.9	20	.72	1.02	7.5 ± 7.4
RIA III	13,142 ± 231	569 ± 27	5,660 ± 181	40.5	100 50 33.3	.61	0.63	4.4 ± 3.4

TABLE VII. Effect of Different Volumes on Progestin Concentrations

Sample No.[a]	100 µl ng/ml	200 µl ng/ml	300 µl ng/ml
37	33.6	30.6	22.8
52	33.4	25.8[b]	24.1[b]
53	33.9	26.5[b]	27.3[b]
54	28.3	21.7[b]	23.8[b]

[a]Differences among sample means not statistically significant.

[b]Values significantly different from other values in the same line without a superscript ($P < .01$).

3 of 4 samples while the 100 µl dilution averaged 7.0 ± 1.5 ng/ml greater than 3 of the 4 samples with 200 and 300 µl dilutions. In all cases pertaining, averages of the dilutions with the least coefficient of variation were taken as the mean dose for that sample.

VI. SUMMARY

Treatment with estradiol benzoate (EB) and human chorionic gonadotrophin (HCG) was effective in inducing first estrus in young gilts at an earlier age than gilts receiving no hormone injections ($P < .01$). However, time of ovulation was highly variable. Slaughter data from 8 gilts indicated that only 39% of the follicles greater than 5 mm had ovulated by 77 hours post HCG injection but 83% of the follicles in a different gilt had ovulated by 100 hours.

Treated gilts did not settle on their induced estrus after being bred by a boar. However, 2 of 5 gilts did settle in their following estrus at 195.5 days of age and farrowed 8.0 healthy looking pigs each.

Treated gilts, artificially inseminated 48 to 72 hours after HCG, did not settle at this induced cycle. However, 4 of 10 gilts did settle during the following cycles at an average age of 213 days. These gilts were slaughtered past 80 days gestation and contained an average of 9.8 live feti. Progestin levels prior to the start of the experiment were all below 1.5 ng per ml. Progestin levels on day 12 of the induced cycle averaged 18.33 ng/ml (eliminating 3 samples which were below 1.5 ng/ml), while those taken 10 after natural service by a boar averaged 23.2 ng/ml.

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