1965

Luteal function and embryonic survival in swine

John Richard Schultz

Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Part of the Animal Sciences Commons, Physiology Commons, and the Veterinary Physiology Commons

Recommended Citation

Schultz, John Richard, "Luteal function and embryonic survival in swine " (1965). Retrospective Theses and Dissertations. 3316.
https://lib.dr.iastate.edu/rtd/3316

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
This dissertation has been microfilmed exactly as received 66-3007

SCHULTZ, John Richard, 1935–
LUTEAL FUNCTION AND EMBRYONIC SURVIVAL IN SWINE.

Iowa State University of Science and Technology
Ph.D., 1965
Physiology

University Microfilms, Inc., Ann Arbor, Michigan
LUTEAL FUNCTION AND EMBRYONIC SURVIVAL IN SWINE

by

John Richard Schultz

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of The Requirements for the Degree of DOCTOR OF PHILOSOPHY

Major Subject: Animal Reproduction

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University Of Science and Technology Ames, Iowa 1965
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>2</td>
</tr>
<tr>
<td>EXPERIMENTAL PROCEDURE</td>
<td>12</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td>18</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>31</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>33</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>38</td>
</tr>
</tbody>
</table>
INTRODUCTION

Litter size in swine is determined by the ovulation rate, the quality of the ovulated eggs, the ability of the eggs to become fertilized and develop into viable zygotes and the ability of the uterus to receive and implant the embryos and carry them through pregnancy. Although a certain amount of embryonic death occurs throughout pregnancy, a particularly critical stage in the development of the embryo occurs at the time of implantation. It is also during this time that the fate of the corpora lutea is determined. These glands remain functional for a relatively short interval in the non-fertile cycle but continue to persist in the event of pregnancy. Progesterone, the hormone of the corpus luteum, is necessary for the establishment of pregnancy and the presence of the embryos is associated with the increased functional life of the gland. Thus, aberrations in this relationship may be associated with the higher incidence of embryonic death at this time. The objective of this study was to investigate the effects of feeding level and an orally effective progestin on reproductive performance in swine. In vitro studies of the changes occurring in the uterine endometrium with respect to progesterone metabolism were also conducted to investigate differences between the pregnant and non-pregnant state at the time of implantation.
LITERATURE REVIEW

Embryonic Survival

Attention was first drawn to the high incidence of embryonic mortality in pigs by Hammond (1921) who described the death of embryos in the latter stages of gestation. Corner (1923) concluded that embryos may suffer degeneration and abnormality in uteri which are free of disease. These studies indicated that prenatal loss in the pig is approximately 30 percent. In sows and gilts, it is estimated that the fertilization rate approaches 95 to 100 percent (Perry and Rowlands, 1962; Nalbandov, 1964); therefore, embryonic death is an important factor in reproductive performance. Warnick et al. (1951) present evidence that a complete lack of fertilization is only a minor cause of repeat breeding in gilts. A comparison of the ovulation and fertilization rates in slaughtered gilts with the conception and farrowing rates of their sisters indicated that embryonic death is an important cause of the discrepancy between ovulation rate and litter size. Casida (1953) has estimated that 37 percent of all embryos actually formed died before birth.

Perry (1954), after an extensive study of slaughterhouse material, concluded at least 40 percent of all ova ovulated are lost before parturition. Examination of the material revealed that about 85 percent of the loss was incurred during the first
half of gestation and approximately 75 percent was incurred before the 25th day. It was suggested that most of the embryonic loss probably occurs at about the time of implantation between the 10th and 20th day. Lerner et al. (1957) observed 25 percent embryonic mortality in gilts slaughtered on the 17th day compared with 33 percent mortality in gilts slaughtered on the 25th day. This suggested that the greater part of the mortality had occurred even before the 17th day. After examining sows at different stages of gestation, similar results were reported by Pomeroy (1960). An over-all loss of 38 percent of the ova was noted and the bulk of this loss had occurred by the end of the third week of pregnancy.

It is clear from these investigations that most of the loss occurs by the 25th day of pregnancy. Implantation has been completed and the embryos have developed sufficiently to be evaluated for gross abnormalities, thus this time may be conveniently chosen for necropsy.

The influence of level of nutrition on embryonic death in the pig has been investigated extensively. Continuous unlimited feeding or a high plane of nutrition has been found to increase the ovulation rate but also to increase the embryonic death up to 25 days (Robertson et al. 1951; Christian and Nofziger, 1952). Self et al. (1955) observed an increase in ovulation rate following a short period of full feeding. In two trials, embryonic death of 68 percent and 47 percent was observed in
full-fed gilts compared with 43 percent and 20 percent for corresponding limited-fed animals. On a full-feeding program, Haines et al. (1959) reported 22.1 percent embryonic death at 25 days compared with 11.6 percent under limited feeding. Similar effects on embryonic death were reported by Gossett and Sorensen (1959); however, ovulation rates on high and low levels of energy intake were found to be comparable. Sorensen et al. (1961) reported that gilts on a high energy ration ovulated 1.3 more ova than did animals on a low energy ration. Embryonic survival was 56.9 percent in the high energy group and 71.8 percent in the low energy group. McGillivray et al. (1962) found an average of 9.5 viable embryos in animals which were starved for a duration of 12 days before mating until slaughter at days 25 to 30. Corresponding animals receiving a normal feed intake during this period exhibited an average of 10.1 viable embryos and animals on a high energy intake had an average of 11.9 living embryos. In another study McGillivray et al. (1963) found no appreciable difference in embryonic survival in animals fed a high energy intake post-breeding compared with animals receiving a normal energy intake.

The importance of progesterone in the establishment and maintenance of early pregnancy is well established (Allen and Corner, 1929); however, it is possible that progesterone may not always be at the appropriate level or in the proper ratio with other hormone levels. This has prompted several investi-
gations of the effects of exogenous progesterone on embryonic survival in swine. In a study on intact gilts, Reddy et al. (1958) demonstrated a decrease in embryonic mortality following the administration of progesterone and estrone. It was reported that a 1:1000 ratio of estrone and progesterone was decidedly inferior to a 1:2000 ratio in reducing early embryonic mortality. Using ovariectomized gilts as experimental animals, Day et al. (1959) also demonstrated the significance of a proper estrogen-progesterone ratio in the maintenance of pregnancy. Thirteen out of 17 bilaterally ovariectomized animals injected with 100 mg. of progesterone and 50 mcg. of estradiol benzoate per 100 lbs. body weight were pregnant at slaughter. Embryonic mortality in these animals was 11 percent less than that observed in intact gilts.

In a study of the effects of energy intake and exogenous progesterone on prenatal survival, Haines et al. (1958) found that neither the ration nor the hormone treatment had a significant influence on the number of embryos, live pigs or embryonic survival. The effects of an implant containing estradiol benzoate and progesterone caprate in a 2000:1 ratio was investigated by Day et al. (1963). No statistically significant improvement in embryonic survival was observed although there was a trend toward increased litter size.

The administration of an orally active progestin was found to cause a significant reduction in embryonic mortality only
when mortality of the control animals approached the 30 to 35 percent level (Morrissette et al. 1963). Animals given 1 mg. of 17-α-acetoxyprogesterone and 0.5 mcg. diethylstilbestrol per pound of body weight exhibited an average of 3.39 dead embryos compared with an average of 4.89 dead embryos in the control group.

Exogenous progesterone has also been found to increase embryonic death (Spies et al. 1959). Progesterone treatment also decreased the average corpus luteum weight significantly in every case. Sammelwitz et al. (1961) found a progressive destruction of corpora lutea with increasing doses of progesterone. No corpora lutea were visible at autopsy on the 23rd to 28th day after mating in animals receiving 400 mg. of progesterone daily.

Results of these investigations indicate that exogenous progesterone does not have a pronounced beneficial effect on embryonic survival in intact swine. However, in most instances, embryonic survival in the untreated animals has been comparatively high and a potential beneficial response could not be demonstrated. Kendall and Hays (1960) found that progesterone reduced embryonic death in intact rats on multiple nutrient deficient diets. McClure (1961) demonstrated that daily treatment with progesterone and chorionic gonadotrophin protected pregnancy in fasted mice. Pregnancy was maintained six days longer in the treated mice compared with control mice.
fasted from the end of the third to fifth days after mating. It was suggested that the pathogenesis of embryonic mortality caused by fasting involved a failure of hypophyseal gonadotrophic function.

Luteal Function

Following ovulation the ruptured follicle undergoes luteinization and is transformed into a corpus luteum. The mammalian corpus luteum represents a transitory gland of internal secretion, the main function of which is the production of progesterone. These glands remain functional for a relatively short interval in the non-fertile cycle but continue to persist in the event of pregnancy. Continued function of these glands is necessary for the maintenance of pregnancy in the pig. du Mesnil du Buisson and Dauzier (1957) found that ovariectomy at any stage of gestation leads to abortion in the sow. Duncan et al. (1961) found that progesterone concentration of luteal tissue was highest 48 days after conception. It has also been demonstrated that significantly more progesterone is present in the peripheral blood of pregnant pigs during the first two-thirds of pregnancy than in non-pregnant females in the mid-luteal phase of the cycle (Short, 1960). Although prolonged function of the corpora lutea is associated with pregnancy, little is known of the factor or factors involved in determining the life span of these glands.
Although prolactin has been identified as the luteotrophic hormone in the rat, it is not luteotrophic in the sheep (Moore and Nalbandov, 1955), cow (Smith et al., 1957) nor in the pig (Sammelwitz et al., 1958; Duncan et al., 1961; du Mesnil du Buisson et al., 1964). Brinkley et al. (1964) found that hypophyseal blockade achieved by high daily doses of progesterone which began on the day of ovulation was unable to prevent the formation of morphologically normal corpora lutea. It was suggested that the hypophyseal hormone necessary for corpus luteum maintenance is released either before or simultaneously with the release of LH. du Mesnil du Buisson and Léglise, (1963) found that hypophysectomy of pigs on the first day of estrus prevented neither ovulation nor the formation of corpora lutea. Hypophysectomy during the luteal phase of the estrous cycle did not induce the early regression of the corpora lutea.

It has been known for several years that luteal regression is delayed in many species following removal of the uterus. An extensive review of this subject has been presented by Reynolds (1949). Loeb (1923) observed an increase in the life span of corpora lutea in the guinea pig following hysterectomy during the luteal phase of the estrous cycle. An extension of this work was done by Rowlands and Short (1959) who observed appreciable amounts of progesterone in the persisting corpora lutea. Hysterectomy of the gilt during estrus or days 5, 10 and 14 resulted in maintenance of the corpora lutea (Anderson
et al. 1963). These glands also persisted in a majority of animals hysterectomized at day 16; however, removal of the uterus at day 18 was followed by estrus and ovulation in a few days. Autotransplants of the uterus in pigs (Anderson et al. 1963) or in guinea pigs (Butcher et al. 1962) resulted in a normal or only slightly prolonged estrous cycle in these species. Melampy et al. (1964) observed an inverse relationship between the quantity of endometrial glands and the duration of pseudopregnancy in rats with autotransplanted uteri. A functional endometrium appears to be necessary for the initiation of luteal regression in rats, guinea pigs and pigs with autotransplanted uteri. The influence of the pituitary on luteal maintenance in hysterectomized animals has been demonstrated by du Mesnil du Buisson and Léglise (1963). It was found that hypophysectomy of pigs hysterectomized early in the estrous cycle resulted in regression of the corpora lutea.

On the basis of the available experimental evidence, it appears that the physiologic events leading to ovulation in the pig are sufficient to cause the formation and maintenance of the corpora lutea during the non-fertile cycle. Continued persistence of these glands is associated with the pituitary and the physiologic state of the uterus.

Bradbury et al. (1950) have stated that in some species apparently there are luteolytic substances, especially in the endometrium, which hasten involution of corpora lutea in non-
fertile cycles. Duncan et al. (1961) found that aqueous preparations of endometrium from uteri obtained on days 12 and 13 of the estrous cycle were capable of increasing progesterone production by the gilt corpus luteum in vitro, whereas preparations made from uteri obtained on days 16 and 18 had an inhibitory effect on steroid synthesis.

A local action of the uterus on the corpora lutea is suggested by results of du Mesnil du Buisson (1961). In gilts mated following severing one horn from the uterine body, early failure of pregnancy occurred in the intact horn. Pregnancy was maintained if the nongravid horn was removed by day 14; however, the pregnancy was terminated if the sterile horn remained after day 16. Anderson et al. (1965) observed unilateral regression of corpora lutea on the side of the nongravid horn in gilts that became pregnant. This effect was also observed when the anterior one-half or quarter of a nongravid horn remained. These results suggest that the nongravid horn is responsible for the termination of pregnancy by the initiation of a luteolytic action.

An investigation of the role of estrogen and progesterone utilization by the uterus with regard to the mechanism of cessation of corpus luteum function was conducted by deJongh and Wolthuis (1964). There was no difference between ovariectomized and ovariectomized-hysterectomized rats in regard to their vaginal smears after injection of estradiol alone. When combina-
tions of estradiol and progesterone were injected, the estrogenic effects were inhibited in the ovariectomized-hysterectomized animals as compared with similarly treated ovariectomized animals. A still more marked inhibition was obtained after traumatizing the uterine endometrium. It was suggested that while the uterus does not utilize appreciable amounts of estrogen, it does utilize considerable quantities of progesterone. It was further suggested that the sparing effect of progesterone in the hysterectomized animals maintained the release of either a pituitary luteotrophic factor or inhibited the release of FSH and LH, thereby delaying the onset of the next estrous cycle.
EXPERIMENTAL PROCEDURE

Embryonic Survival Studies

Cross-bred gilts 6 to 8 months of age were used as experimental animals. They were checked daily with vasectomized boars to detect occurrence of estrus and duration of their estrous cycles. The first day of estrus was designated as day 1. All animals were hand fed 1.82 kg. per day of a corn-soybean meal ration (percent composition: ground yellow corn, 81.45; 50% soybean meal, 13.40; dicalcium phosphate, 1.75; calcium carbonate, 0.80; iodized salt, 0.50; trace mineral premix, 0.10; and a vitamin mixture, 2.00) for a duration of at least three estrous cycles prior to treatment. Gilts were maintained in dry lot, individually fed in feeding stalls during the treatment period and hand-mated to at least two different boars at the time of fertile mating. The treatments were imposed at the end of the estrus prior to fertile mating and were terminated by slaughter 26 days after mating (± 1 day). Corpora lutea and live embryos were counted at the time of slaughter; only embryos exhibiting a visible heart beat were considered viable. Macerating or under-developed embryos or avascular membranes were recorded as embryonic deaths.

In Experiment 1, 32 gilts of Yorkshire x Landrace x Poland China breeding were used in an investigation of the effects of two levels of feed intake during two reproductive stages on
ovulation rate and embryonic survival. The high level (H) of feeding in this study was 3.63 kg. per day and the low level (L) was 1.81 kg. per day. The first reproductive stage began at the estrus prior to mating (pre-breeding), while the second extended from the time of mating until slaughter (post-breeding). The four treatments were thus designated HH, HL, LH, and LL where the feed level of the HL and LH animals was changed at the time of mating. The animals were divided into two groups of 16, with four animals per group on each of the treatments. Gilts were individually fed twice daily during the experimental period.

Experiment 2, consisting of four groups of 16 gilts each, was conducted in the same manner. Feeding levels in this investigation were 2.72 kg. per day (H) and 1.81 kg. per day (L). These levels were increased by 0.45 kg. per day in the two groups of animals on experiment during the winter months. In both experiments the number of embryos present in each gilt was expressed as a percent of the number of corpora lutea and this value was then designated as percent embryonic survival. Percent survivals were transformed into angles and analyzed by the method of unweighted means. Animals not pregnant at slaughter were not included in the analysis.

Experiment 3 was designed to study the effect of an exogenous progestin on early embryonic survival and on corpus luteum maintenance and function in gilts on a reduced feed
intake. This experiment involved a total of 60 gilts consisting of two groups of 30 animals. Each group represented different breeding and was located on a different farm. Animals in group 1 were a Yorkshire x Landrace x Poland China cross, while animals in group 2 were from the Farmers Hybrid cross. Ten animals within each group were randomly allotted to one of three treatments. Gilts assigned to treatment 1 (H) received 2.72 kg. feed per day, while the animals allotted to treatment 2 (L) received 0.91 kg. feed per day. Animals assigned to treatment 3 (L+MAP) were also fed 0.91 kg. feed per day; however, 75 mg. 6-alpha-methyl-17-alpha-acetoxyprogesterone (MAP; Repro-mix, The Upjohn Co.) was added to the daily ration beginning on the fourth day after mating. The experimental period extended from the estrus prior to fertile mating until slaughter as in the previous experiments, but animals in this study were maintained on the same feed level throughout the treatment period and were fed once daily. Corpora lutea from the gilts pregnant at slaughter were dissected free of the ovarian stroma and weighed. Representative slices from each gland were blotted dry, pooled and stored in 95% ethanol for later analysis of progesterone content. Collection of luteal tissue was accomplished at 4°C. The data from this experiment were analyzed by the method of fitting constants. Tests of significance between treatment means were made on the basis of planned comparisons.
Progesterone Determination

Progesterone analyses were based on a procedure utilizing 95% ethanol extraction, aluminum oxide column adsorption chromatography, thin layer chromatography and ultra-violet adsorption spectrophotometry. Tissues were homogenized and extracted for three one-hour periods with 75 ml. of 95% ethanol at 75°C. The ethanol extracts were then prepared for aluminum oxide column adsorption chromatography described by Duncan (1961). Following evaporation to dryness under reduced pressure, the 25% chloroform-n-hexane fraction from the column was transferred to a conical centrifuge tube with five 2 ml. volumes of n-hexane and then dried under a stream of nitrogen.

Thin-layer chromatography isolation of progesterone was based on the procedure described by Armstrong et al. (1964). A slurry of silica gel was prepared by mixing 35 g. of Merck Silica Gel G with 70 ml. H₂O. Fifty mcg. of fluorescein was added to the slurry to impart slight fluorescence to the absorbant. The slurry was applied to five 200 mm. square glass plates to a thickness of 250-300 μ by means of a Brinkmann spreader (Brinkmann Instruments, Inc., Westbury, New York). Plates were activated by heating in an oven at 100°C. for 1 hour and were then stored in a desiccator.

The residue in the centrifuge tube was redissolved in a small volume of benzene:methylene chloride (1:1 V/V) and applied
to one corner of the chromatographic plate. The chromatogram was developed in one dimension in hexane:ethyl acetate (5:2 V/V) then air-dried for a few minutes and chromatographed in the second dimension in methylene chloride:diethyl ether (5:2 V/V). The chromatogram was viewed under ultra-violet light to locate the progesterone spot. This area was scraped onto a tared weighing paper with a sharp spatula. The silica gel obtained from the spot to be analyzed was weighed to the nearest \(\frac{1}{2}\) mg. and an equal weight of silica gel was scraped from a blank area of the plate. The silica gel obtained from the progesterone and blank spots was placed in a centrifuge tube and suspended in 95% ethanol. The suspension was shaken for a minute on a test tube mixer and centrifuged. An aliquot of the supernatant was placed in a 3 ml. cell and measured for absorbance with a Beckmann D. U. Spectrophotometer. Each sample was read against an eluate of a blank area of the same plate at the same dilution. Progesterone content was calculated by applying the equation of Allen (1950) to the observed absorbancies at 230, 240, and 250 m\(\mu\). Two mcg. progesterone could be detected by this procedure. Samples of standard progesterone added to the system resulted in recoveries of 76% to 85% with an average of 82%. Reported progesterone concentrations have been adjusted on this basis unless noted otherwise.
In Vitro Studies

Endometrial preparations were prepared from the uteri of gilts at known stages of the reproductive cycle and pregnancy. The reproductive tracts were obtained immediately after slaughter and all processing prior to incubation was carried out at 4°C. The endometrium was scraped from the uterine cornu, placed in a beaker and thoroughly chopped with fine scissors. Five-hundred mg. samples of the preparation were distributed among the incubation vessels, each containing 5 ml. of chilled Krebs-Ringer bicarbonate buffer and 10 mg. of glucose. One-tenth ml. of 95% ethanol containing 50 mcg. of standard progesterone was added to the vessels. Control samples for determination of steroid recovery were prepared along with those to be incubated but were placed in 75 ml. of 95% ethanol before addition of the progesterone standard. Incubations were carried out with shaking for 2 or 4 hours at 37°C, in a Dubnoff metabolic incubator in an atmosphere of 95% oxygen and 5% carbon dioxide. Immediately after incubation the contents of the vessel were transferred to a flask containing 75 ml. of 95% ethanol, extracted and subsequently analyzed for progesterone as previously described. The time interval between slaughter and beginning of incubation was less than one hour.
RESULTS AND DISCUSSION

Embryonic Survival

The results of the first two experiments are summarized in Table 1. Animals in Experiment 1 receiving the high feed level during the pre-breeding stage exhibited significantly (P<.05) more corpora lutea than did those receiving the low level. The 1.82 kg. increase in feed level prior to breeding resulted in an average of three more ovulations per gilt. There were no significant treatment effects on embryonic survival. Two animals receiving the LL treatment were not pregnant, although their reproductive tracts appeared to be grossly normal at slaughter. The other two gilts not conceiving exhibited cystic follicles. The mean litter size in this experiment was 11.3 embryos.

No appreciable differences in ovulation rate were noted in Experiment 2. The average number of corpora lutea was 12.6 and 11.8 for the high and low pre-breeding groups, respectively. Gilts receiving the high level post-breeding exhibited a significantly higher (P<.05) percent embryonic survival than did gilts on the low level. There was no appreciable interaction between reproductive stages with regard to feeding level, thus, embryonic survival was primarily affected by the level of feeding after mating. No significant effect due to groups was observed. A total of 16 dead embryos was found in the 29 litters.
Table 1. Effect of daily feed consumption on ovulation rate and embryonic survival

<table>
<thead>
<tr>
<th>Stage</th>
<th>Feed level&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
</tr>
<tr>
<td>Pre-breeding</td>
<td>H</td>
</tr>
<tr>
<td>Post-breeding</td>
<td>H</td>
</tr>
</tbody>
</table>

|                | Experiment 1 | |
|----------------|---------------|
| No. animals    | 8             |
| No. pregnant   | 7             |
| No. corpora lutea<sup>b</sup> | 15.4±.44 | 13.3±.50 | 11.1±.51 | 11.5±.61 |
| No. embryos<sup>b</sup> | 13.5±.50 | 11.2±.51 | 10.0±.50 | 10.0±.76 |
| % survival<sup>b</sup> | 88±4.82 | 84±5.21 | 90±4.60 | 87±6.33 |

|                | Experiment 2 | |
|----------------|---------------|
| No. animals    | 16            |
| No. pregnant   | 14            |
| No. corpora lutea<sup>b</sup> | 12.8±.60 | 12.5±.47 | 11.4±.55 | 12.2±.60 |
| No. embryos<sup>b</sup> | 10.9±.53 | 9.0±.56 | 9.2±.40 | 8.4±.70 |
| % survival<sup>b</sup> | 85±5.64 | 72±4.44 | 81±3.48 | 69±6.75 |

<sup>a</sup>Difference between high and low level in Experiment 1, 1.82 kg./day; in Experiment 2, 0.91 kg./day.

<sup>b</sup>Mean ± S.E. Coefficients of variation for Experiment 1 and Experiment 2, respectively: number of corpora lutea, 14.1 and 16.4; number of embryos, 14.4 and 16.9.
of animals receiving the high level during the post-breeding stage and 37 in the 27 litters of animals on the low level. The four gilts on the LL treatment which did not conceive exhibited grossly normal reproductive tracts at necropsy. All other gilts not conceiving exhibited cystic follicles or hydrosalpinx.

The data from Experiment 1 indicate that ovulation rate can be increased by a higher feed level following a period of restricted feeding. Since little difference in ovulation rate was observed in Experiment 2, it appears that a near full-feeding regime is necessary to elicit a significant response. Although embryonic survival in Experiment 1 was not appreciably affected by feeding level, a definite treatment effect was observed in Experiment 2. These results may be partially explained on the basis of the different high level treatments in the two experiments. Animals receiving the different treatments were individually fed but were maintained in the same pen. Thus, when some of the animals were receiving a near full-feeding level as in Experiment 1, the occurrence of coprophagy may have had a greater influence on the response of animals on the low level.

Several groups of workers have demonstrated that continuous unlimited feeding or a high plane of nutrition increased the ovulation rate but also increased the embryonic death up to 25 days (Self et al., 1955; Haines et al., 1959). The high feed levels investigated in this study had no adverse effect on
embryonic survival. However, the treatments were imposed for a relatively short time following a limited feeding period. Furthermore, the high feed level in Experiment 2 was comparable to the limited feeding levels (50 to 70% of the full-fed rate) in the above studies.

Reduced embryonic survival in animals on the low feed level suggested the presence of an intrauterine environment amenable to hormone administration. This prompted an investigation of the effect of an orally effective progestin on embryonic survival and luteal function in limited-fed animals. The data presented in Table 2 show that one H gilt, four L gilts and six L+MAP gilts were not pregnant at slaughter. Animals on the H treatment exhibited significantly (P<.05) more corpora lutea at the time of slaughter than did gilts on the L and L+MAP treatments. The effectiveness of the exogenous progestin in increasing embryonic survival under these conditions is demonstrated by an average of 1.2 more embryos (P<.05) in the L+MAP gilts compared with the L animals. Embryonic survival was 89%, 79% and 92% in animals receiving the H, L, and L+MAP treatments, respectively. Seven of the animals receiving the L+MAP treatment exhibited 100% embryonic survival, while the lowest value observed in these animals was 77%. A total of four dead embryos was found in the litters of animals on the H treatment, nine in litters of animals on the L treatment and two in the L+MAP litters.
Table 2. Effect of daily feed consumption and 6-α-methyl-17-α-acetoxyprogesterone (MAP) on reproductive performance of gilts

<table>
<thead>
<tr>
<th>Item</th>
<th>2.72 kg./day</th>
<th>0.91 kg./day</th>
<th>0.91 kg./day plus 75 mg. MAP&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of gilts</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>No. conceiving</td>
<td>19</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>No. of corpora lutea&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.1±.37</td>
<td>11.2±.34</td>
<td>10.9±.49</td>
</tr>
<tr>
<td>No. of embryos&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.7±.30</td>
<td>8.8±.39</td>
<td>10.0±.34</td>
</tr>
<tr>
<td>Total luteal weight (gm.)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.7±.20</td>
<td>4.6±.21</td>
<td>3.9±.26</td>
</tr>
<tr>
<td>Progesterone concentration&lt;sup&gt;b&lt;/sup&gt;</td>
<td>102±3.1</td>
<td>104±3.3</td>
<td>94±3.1</td>
</tr>
<tr>
<td>Total luteal progesterone&lt;sup&gt;b&lt;/sup&gt;</td>
<td>582±26.6</td>
<td>480±28.6</td>
<td>369±23.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>MAP administered from the 4th day after mating until slaughter.

<sup>b</sup>Mean ± S.E. Coefficients of variation are as follows: number of corpora lutea, 13.7; number of embryos, 13.1; total luteal weight, 5.0; progesterone concentration, 13.1 and total luteal progesterone, 24.4.
Morrissette et al. (1963) found that a significant reduction in embryonic mortality following the administration of orally active progestins occurred only when the embryonic mortality in the control animals approached the 30 to 35% level. These results and those in the present investigation indicate that increased litter size in early gestation may be obtained with the administration of exogenous progestin provided embryonic death is a major factor limiting reproductive performance. Reddy et al. (1958) also observed reduced embryonic mortality in animals injected with an estrogen-progesterone combination and Day et al. (1963) reported a trend in increased litter size following a subcutaneous implant of a combination of an estrogen and progesterone.

All animals not pregnant at slaughter which were treated with MAP did not exhibit estrus during the treatment period. Thus, a level of 75 mg. was sufficient to modify pituitary function under these experimental conditions. Cystic follicles were also observed in each of these animals at slaughter. The cysts were 1 to 3 cm. in diameter and some were partially luteinized.

No statistically significant difference due to treatments was found in luteal progesterone concentration. However, the mean progesterone concentration of 94 mcg./gm. of fresh tissue in the L+MAP gilts was lower than the values of 102 mcg./gm. in the H gilts and 104 mcg./gm. in the L gilts. The similarity
of the values for the H and L animals implies progesterone concentration of the luteal tissue was not associated with embryonic survival. The L+MAP animals had significantly less (P<.05) luteal tissue than did the L gilts. These results indicate an inhibition of luteotrophic support in the progestin treated animals. This inhibition was expressed by less luteal tissue and perhaps to a lesser extent by a reduction in progesterone synthesizing ability. Total luteal progesterone content was 582 mcg., 480 mcg. and 369 mcg. for the H, L, and L+MAP animals, respectively. The lower value for the L+MAP gilts suggests that much of the progestin support in these animals was of exogenous origin. No significant effect due to the breed groups was found in any of the parameters studied.

These results indicate that in animals on a reduced feed intake, the administration of an orally effective progestin results in an intrauterine environment more compatible with survival of the embryos. Although the progesterone concentration in the luteal tissue was found to be unassociated with embryonic survival, the secretion rate of these glands may have been different. This is also suggested by the beneficial effect of the administration of exogenous progestin; however, this effect may be mediated in part by an alteration in pituitary function.
In Vitro Studies

Endometrial preparations from uteri obtained on days 8 to 18 of the estrous cycle and days 17 to 19 of pregnancy were studied. Progesterone recoveries following incubation of these preparations are presented in Table 3. Progesterone utilization represents the difference in recoveries between the incubated and unincubated samples. An average of $40.0 \pm 3$ mcg. progesterone was recovered from the unincubated samples following the addition of 50 mcg. standard. Preparations obtained on days 15 to 18 of the cycle utilized an average of 5.1 mcg. progesterone during the two-hour incubation period. The response was markedly uniform in all preparations within this stage. No appreciable quantity of progesterone was utilized by endometrium obtained on days 8 to 14 of the estrous cycle. Thus, progesterone utilization by the endometrium appears to coincide with the period of luteal regression. Progesterone recovery remained unchanged following incubation of similarly prepared myometrial samples taken from each stage. Table 4 presents data showing the effect of incubation time on progesterone utilization by endometrium obtained on days 15 and 17 of the cycle. Most of the utilization occurred during the first two hours, although the reaction would proceed further with additional incubation. Greater utilization could also be obtained by increasing the amount of tissue. One-gram samples of endometrium resulted in a mean utilization of 8.4 mcg. during
Table 3. *In vitro* utilization of progesterone by endometrium obtained at different reproductive stages

<table>
<thead>
<tr>
<th>Stage</th>
<th>No. Animals</th>
<th>Added</th>
<th>Unincubated Endometrium</th>
<th>Incubated(^a) Endometrium</th>
<th>Utilization(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-14 cycle</td>
<td>5</td>
<td>50</td>
<td>39.9</td>
<td>39.7</td>
<td>0.2(^{+}) .24(^b)</td>
</tr>
<tr>
<td>15-18 cycle</td>
<td>5</td>
<td>50</td>
<td>40.1</td>
<td>35.0</td>
<td>5.1(^+) .23</td>
</tr>
<tr>
<td>17-19 pregnancy</td>
<td>4</td>
<td>50</td>
<td>40.1</td>
<td>39.4</td>
<td>0.7(^+) .30</td>
</tr>
</tbody>
</table>

\(^a\)Two-hour incubation period  
\(^b\)Mean difference \(^+\) S.E.
Table 4. Effect of incubation time on in vitro endometrial utilization of progesterone

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Unincubated recovery (mcg.)</th>
<th>Two-hour incubation recovery (mcg.)</th>
<th>Four-hour incubation recovery (mcg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40.4</td>
<td>35.8</td>
<td>33.0</td>
</tr>
<tr>
<td>2</td>
<td>38.2</td>
<td>32.8</td>
<td>30.6</td>
</tr>
<tr>
<td>3</td>
<td>39.8</td>
<td>35.2</td>
<td>34.4</td>
</tr>
<tr>
<td>Av. utilization</td>
<td>----</td>
<td>4.9</td>
<td>6.7</td>
</tr>
</tbody>
</table>

a Obtained from days 15 and 17 of cycle.  
b 50 mcg. progesterone added.

These results provide further evidence of the biochemical changes occurring in the endometrium during the late diestral phase of the cycle. Duncan et al. (1961) found that aqueous preparations of endometrium from days 12 and 13 of the cycle stimulated in vitro synthesis of progesterone by luteal tissue, whereas preparations obtained on days 16 and 18 had an inhibitory effect on steroid synthesis. Cooper and Hess (1965) also demonstrated that uterine extracts from the guinea pig inhibited progesterone synthesis by ovarian tissue. The effect of the uterus on luteal activity in vivo has been well established. Corpora lutea in gilts following hysterectomy are not only functional in terms of their capacity to synthesize progesterone in vitro but also increase in activity beyond their
pre-hysterectomy status (Duncan et al., 1961). Bradbury et al. (1950) suggested that in certain species there are luteolytic substances associated with the endometrium which hasten involution of corpora lutea in non-fertile cycles. Removal of this luteolysin by hysterectomy would then be associated with continued persistence of the corpora lutea.

Factors associated with luteal regression during the non-fertile cycle must necessarily be modified to permit the persistence of the corpora lutea in the event of pregnancy. Samples of endometrium obtained on days 17 to 19 of pregnancy did not utilize appreciable amounts of progesterone, thus, the products of conception apparently modified certain biochemical characteristics of the endometrium. Day 17 embryos and their associated membranes, taken immediately after slaughter, were placed in incubation vessels containing endometrial preparations obtained on day 17 of the cycle. Two embryos were placed in each vessel. The effects of embryos and gonadotrophins on endometrial progesterone utilization are summarized in Table 5. The addition of 20 mcg. luteinizing hormone (LH) or 200 mcg. follicle stimulating hormone (FSH) had little effect on progesterone utilization by the endometrium. However, addition of 1000 I. U. chorionic gonadotropin (HCG) to the media resulted in a marked inhibition of progesterone utilization. Since this concentration is beyond physiologic levels, the biological significance of this effect is not clear. The
Table 5. Effect of gonadotropins and living embryos on *in vitro* utilization of progesterone by endometrium

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Progesterone Utilization (mcg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>7.6</td>
</tr>
<tr>
<td>20 mcg. LH</td>
<td>7.0</td>
</tr>
<tr>
<td>200 mcg. FSH</td>
<td>6.2</td>
</tr>
<tr>
<td>1000 I. U. HCG</td>
<td>2.2</td>
</tr>
<tr>
<td>Embryos</td>
<td>3.6</td>
</tr>
</tbody>
</table>

*a* All preparations incubated four hours.

Inhibitory activity may be due to non-specific substances present in the gonadotrophin preparation. Preliminary investigations showed that 100 I. U. HCG had no inhibitory effect.

An inhibition of progesterone utilization was also observed following incubation of embryos with the endometrial preparations. Since no significant change in progesterone recovery was noted following incubation of embryos in the absence of endometrium, it may be concluded that the embryos themselves neither synthesized nor utilized appreciable quantities of progesterone under these *in vitro* conditions. The effect of the embryos on the endometrium may be mediated through metabolic degregation products or non-specific toxicity. The ratio of embryonic and endometrial tissues used in this study is not
proportional to that occurring in utero. However, any change elicited by the embryo on the endometrium in vivo has an opportunity to occur over a period of time which cannot be duplicated in vitro.

In a study of the factors determining the cessation of corpus luteum function in rats, deJongh and Wolthuis (1964) found that there was no difference between ovariectomized and ovariectomized-hysterectomized rats in regard to their vaginal smears after injection of estradiol alone. When both estradiol and progesterone were injected, the estrogenic effects of the latter group were inhibited. The authors suggested that the uterus does not utilize measurable amounts of estrogen but that it does utilize considerable quantities of progesterone. Results of this investigation would tend to substantiate this hypothesis and further suggest that this utilization is greatest during the period of luteal regression. The significance of this utilization on luteal regression in vivo remains to be determined. It is possible that these in vitro results are an expression of a multiplicity of undefined cyclic changes occurring in the endometrium.
SUMMARY

A series of experiments was conducted to obtain information on the factors associated with embryonic death in swine. Two experiments were designed to investigate the effect of two feeding levels on ovulation rate and embryonic survival. Increasing the feeding level from 1.82 kg. per day to 3.63 kg. per day for a duration of one estrous cycle before breeding resulted in an average of three more ovulation points per gilt. A significant decrease in embryonic survival was observed in animals receiving 1.82 kg. per day from the time of mating until slaughter at day 25 compared with animals receiving 2.72 kg. per day.

An experiment was conducted to investigate the effect of an orally active progestin (MAP) on embryonic survival and luteal function in limited-fed animals. Gilts receiving 0.91 kg. feed per day plus 75 mg. MAP had an average of 1.2 (P<0.05) more embryos than did animals receiving 0.91 kg. feed only. Embryonic survival was 89%, 79% and 92% in animals receiving 2.72 kg., 0.91 kg. and 0.91 kg. plus MAP, respectively. No significant differences were detected in luteal progesterone concentration. Animals treated with MAP had significantly less luteal tissue when compared with untreated gilts receiving 0.91 kg. feed per day. Embryonic survival in the MAP treated animals was strikingly uniform.
In vitro utilization of progesterone by the endometrium was found to occur during the period of luteal regression in the non-fertile cycle. Five-hundred mg. samples of endometrium obtained on days 15 to 13 of the estrous cycle utilized an average of 5.1 mcg. progesterone during the two-hour incubation period. This effect was not observed in preparations obtained on days 8 to 14 of the cycle and 17 to 19 of pregnancy. Progesterone utilization by endometrium obtained on day 17 of the cycle was inhibited when incubated with day 17 embryos. No appreciable change in progesterone recovery was noted following incubation of embryos in the absence of endometrium. Inhibition was also achieved with 1000 I. U. HCG; however, neither 20 mcg. LH nor 200 mcg. FSH affected utilization. These results provide further evidence of the biochemical changes occurring in the endometrium during the period of luteal regression.
LITERATURE CITED

Allen, W. M.
1950 A simple method for analyzing complicated absorp-
tion curves, of use in the colorimetric determina-
tion of urinary steroids. J. Clin. Endocrinol. and
Metabl. 10: 71-83.

Allen, W. and Corner, G.
1929 Normal growth and implantation of embryos after
very early ablation of the ovaries, under the
influence of corpora lutea extracts. Am. J.
Physiol. 88: 340-346.

Anderson, L. L., Butcher, R. L. and Melampy, R. M.
1961 Subtotal hysterectomy and ovarian function in

Anderson, L. L., Butcher, R. L. and Melampy, R. M.
1963 Uterus and occurrence of oestrus in pigs.
Nature 189: 311-312.

Anderson, L. L., Rathmacher, R. P. and Melampy, R. M.
1965 The uterus and unilateral regression of corpora
lutea in the pig. Am. J. Physiol. To be published
ca. 1965.

Armstrong, D. T., O'Brien, J. and Greep, R. O.
1964 Effects of LH on progestin biosynthesis in the

Bradbury, J. T., Brown, W. E. and Gray, L. A.
1950 Maintenance of the corpus luteum and physiologic
actions of progesterone. Rec. Prog. Horm. Research
5: 151-194.

Brinkley, H. J., Norton, H. W. and Nalbandov, A. V.
1964 Role of a hypophyseal luteotropic substance in the
function of porcine corpora lutea. Endocrinology

Butcher, R. L., Chu, K. Y. and Melampy, R. M.
1962 Effect of uterine autotransplants on the estrous
cycle in the guinea pig. Endocrinology 70: 442-443.

Casida, L. E.
1953 Fertilization failure and embryonic death in domes-
Christian, R. E. and Nofziger, J. C.

Cooper, E. and Hess, M.

Corner, G. W.
1923 The problem of embryonic pathology in mammals, with observations upon intrauterine mortality in the pig. Am. J. Anat. 31: 523-545.

Day, B. N., Anderson, L. L., Emmerson, M. A., Hazel, L. N. and Melampy, R. M.

Day, B. N., Romack, F. E. and Lasley, J. F.

Duncan, G. W.


Haines, C. E., Warnick, A. C. and Wallace, H. D.
Haines, C. E., Warnick, A. C., and Wallace, H. D.

Hammond, J.

deJongh, S. E. and Wolthuis, O. L.

Kendall, K. A. and Hays, R. L.

Lerner, E. H., Mayer, D. T. and Lasley, J. F.

Loeb, L.

McClure, T. J.


Melampy, R. M., Anderson, L. L. and Kragt, C. L.
du Mesnil du Buisson, F.

du Mesnil du Buisson, F. and Dauzier, L.

du Mesnil du Buisson, F. and Léglise, P.

du Mesnil du Buisson, F., Léglise, P. C. and Anderson, L. L.

Moore, W. W. and Nalbandov, A. V.

Morrissette, M. C., McDonald, L. E., Whatley, J. A. and Morrison, R. D.

Nalbandov, W. V.

Perry, J. S.

Perry, J. S. and Rowlands, I. W.

Pomeroy, R. W.

Reddy, V. B., Mayer, D. T. and Lasley, J. F.
Reynolds, S. R. M.

Robertson, G. L., Casida, L. E., Grummer, R. H. and Chapman, A. B.

Rowlands, I. W. and Short, R. V.
1959 The progesterone content of the guinea pig corpus luteum during the reproductive cycle and after hysterectomy. J. Endocrinol. 19: 81-86.

Sammelwitz, P. H., Aldred, J. P. and Nalbandov, A. V.

Sammelwitz, P. H. and Nalbandov, A. V.

Self, H. L., Grummer, R. H. and Casida, L. E.

Short, R. V.

Smith, V. R., McShan, W. H. and Casida, L. E.

Sorensen, A. M., Jr., Thomas, W. B. and Gossett, J. W.


ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. R. M. Melampy for his guidance in the preparation of this thesis, to Dr. L. L. Anderson for his timely counsel and to Joan Schultz for her continued encouragement and untiring effort in typing of the manuscript.