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Exogenous Steroid Hormone Effects on Litter Size, Early Embryonic Survival and Placental Development in Swine

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EXOGENOUS STEROID HORMONE EFFECTS ON LITTER SIZE, EARLY EMBRYONIC SURVIVAL AND PLACENTAL DEVELOPMENT IN SWINE

By

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ABSTRACT

EXOGENOUS STEROID HORMONE EFFECTS ON LITTER SIZE, EARLY EMBRYONIC SURVIVAL AND PLACENTAL DEVELOPMENT IN SWINE

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The effect of steroid hormones on litter size at term and on placental development at Day 30 to 35 of gestation was studied in swine.

In the first study, progesterone (25.0 mg) and estradiol (12.5 µg) alone and in combination were given to pregnant sows on Days 16 and 17 of gestation. The control animals were injected with 2 ml of arachis oil during the same time period. When compared, the findings demonstrated that estradiol alone, at a level of 12.5 µg, resulted in a significant increase (1.5 and 1.6 pigs/litter, respectively) in the total number of pigs born and pigs born alive. In addition, the effects of two levels of estradiol (3.125 and 6.25 µg) and three levels of estrone (6.25, 12.5, and 18.75 µg) were investigated when injected in sows at the time of implantation (Days 16 and 17). The results did not show any significant effect between control and treatment groups for either total number of pigs born or pigs born alive. These

findings indicate that at the dose used, estradiol and progesterone in combination do not have the beneficial effect on litter size previously observed with estrone and progesterone.

In the second experiment, progesterone (25.0 mg) in combination with estrone (12.5 μg) was administered to sows for a ten-day period starting on Day 14 of pregnancy. The control group received 2 ml of arachis oil at the same time period. The animals from both groups were slaughtered between Days 30 and 35 of gestation, and the reproductive tracts and the contents were examined. A significant increase was found for both allantoic fluid volume and placental surface area in sows injected with the hormones when compared to the controls. Crown-rump length was significantly less in the treatment than in the control group. Amniotic fluid protein concentration, pH and osmolality were significantly greater in the treated sows than in the controls. Allantoic fluid pH was also significantly higher in the treated group. Protein concentration in the allantoic fluid was significantly less in the experimental group than in the control group. Finally, 2 protein bands in the allantoic fluid (Rf values between 0-0.09 and 0.50-0.59) and in the amniotic fluid (Rf values between 0.10-0.19 and 0.20-0.29) were significantly less often observed in the treated group.

TO MY MOTHER'S MEMORY

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INTRODUCTION

Embryonic mortality is a major problem confronting the swine industry. The incidence is highest during early gestation when 30 to 40 percent of the ovulated eggs in the pig are not represented by live embryos at Day 30 of gestation. It is known that a great number of factors affect embryo survival in this species; however, there is disagreement on the cause and the time course of embryonic mortality. It has been suggested that hormonal balance may be one of the most important factors, and hormone therapy has been utilized as a means of solving the problem. Furthermore, reasonable evidence has shown that hormonal therapy is more effective at the time of implantation (15-18 days of gestation) because it assumed that the uterine secretions are related to the hormone concentration in the blood. On the other hand, a number of published papers have shown no relationship between hormone therapy and embryonic survival.

The present study has the following objectives:

1. To examine the effect of estradiol and progesterone in combination, progesterone alone, and three levels of estradiol on litter size when injected at the time of implantation.

2. To verify the effect of three levels of estrone on litter size (injected on Day 16 and 17).
3. To determine the effects of an estrone-progesterone combination on early embryonic survival and placental development.

LITERATURE REVIEW

IMPLANTATION

Within the last decade, studies of mammalian implantation have increased and become more diversified. The primarily morphologic focus of earlier studies has shifted rapidly to more sophisticated multidisciplinary analyses of regulation mechanisms of implantation and their integration with carefully defined morphologic variables. It is understandable, but in some respects unfortunate, that the bulk of recent studies has focused on a mere handful of semidomesticated and laboratory species. In this study, we will correlate findings between pigs and other species.

Type of Implantation

There are three major types of implantation based on the degree of penetration of the uterine mucosa by the implanting blastocyst. They are designated central, ecentric and interstitial. The implantation of the pig blastocyst is described as central or superficial because the chorion does not invade the endometrium, and throughout the entire gestation the chorion and the uterine mucosa may be pulled apart without damage to either. This is the characteristic of the epitheliochorial placenta.

Time of Implantation

Some time after the recognition of the pregnancy and the completion of the distribution and migration of the embryos in the uterine horns, implantation is begun.

In early research, Green and Winters (1946) observed implantation starting as early as 11 days of pregnancy. However, Crombie (1970) found discrete cell-to-cell contacts, held together by coagulated uterine secretion, and region of the trophoblast in close apposition to the maternal uterine epithelium by Day 14. The definite interdigitation of the microvilli, which is the stage at which implantation is considered to be advanced, was not observed until Day 18 (Perry 1969; Crombie 1970; Swift and King 1978). At this time, the trophoblast and uterine mucosa both have developed short microvilli. The trophoblast cells are columnar and tall in areas where there is free space occupied by hystotroph and which will be the future sites of the areolae. In the areas where there is close apposition to the maternal epithelium, the cells are low and cuboidal. The uterine cells on the 20th day of pregnancy become cuboidal in some areas with irregular surfaces, which may assist the attachment of the chorion. The attachment, according to Crombie, was considered completed by the 24th day.

Mechanism of Implantation

In species such as the pig, the blastocyst, while it remains free in the uterine lumen, may produce some substance that is capable of diffusing into the uterine "milk," exerting a local effect on the uterine tissue.

Shelesnyak and Kraicer (1963), working with rats, postulated the role of histamine released from the mast-cells of the uterine connective tissue in inducing the stimulation of a "sensitized endometrum" to transform stroma cells to decidual cells. The injection of estrogen was observed to dissipate the mast-cells of the uterine connective tissue. Twenty-four hours prior to the expected implantation time, Shelesnyak noted that the mast-cells in the rat uterus fell to almost zero. The reduction of mast-cells is presumably indicative of the release of histamine and the increased permeability of the endometrial cells. The fact that this phenomenon was observed in synchrony with the estrogen surge prior to implantation was the basis for Shelesnyak's theory that ovarian-derived estrogen, during the progestation phase of the cycle, is related to the release of histamine and the induction of implantation. This discussion can be summarized in Figure 1.

More recently, Perry *et al.* (1973) noted that pig blastocysts were capable of synthesizing estrogen and hypothesized that they might be involved in the process of

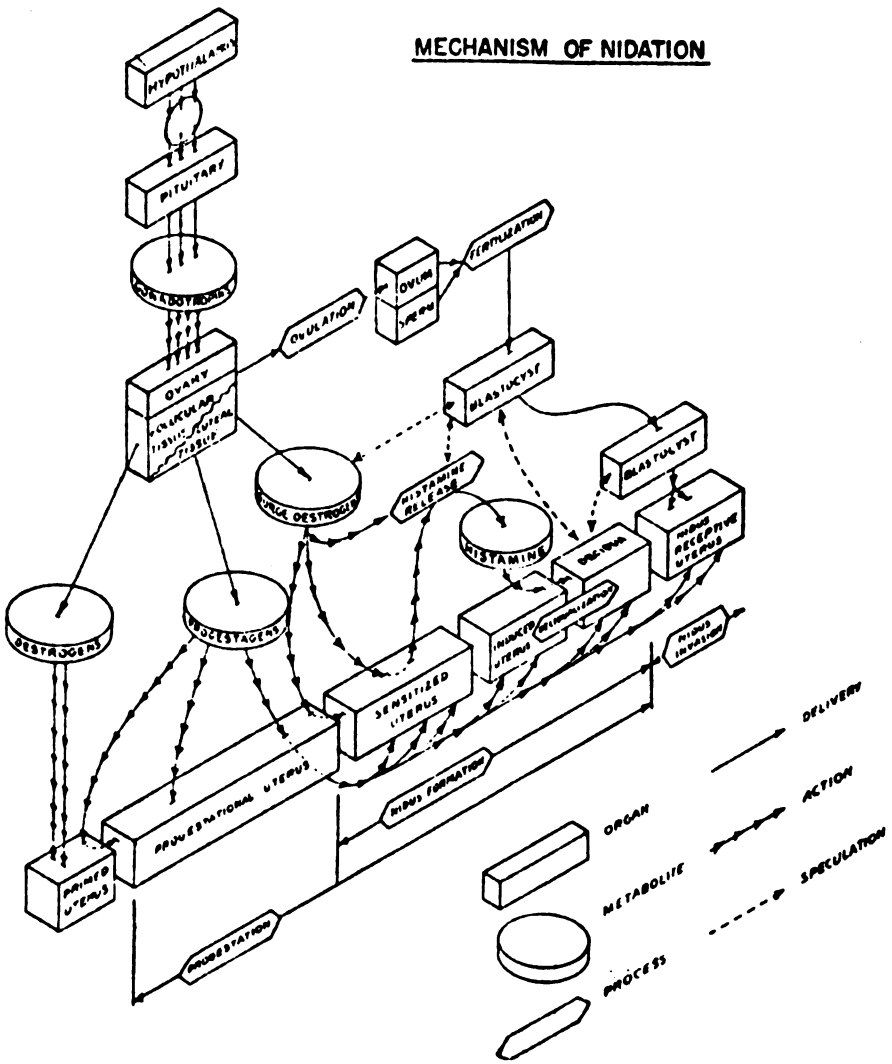


Fig. 1. Mechanism of implantation--from Shelesnyak, M. C. and P. F. Kraicer. 1963.

implantation or the signal whereby the blastocyst announces its presence to the mother. Other experiments with estrogen treatment of adult ovariectomized rats have been shown to cause the release of histamine, prostaglandin and serotonin. This fact was the basis that blastocyst estrogen induces a local increase in capillary permeability by inducing the release of vaso-active substances. This increase in capillary permeability is a necessary prerequisite for the induction of implantation in most species (Dickman *et al.* 1977).

In the central or superficial variety of implantation, the crucial mechanism is the growth and interlocking attachment of the microvilli, which is assisted by the expansion of the allantochorion. The contact becomes even more firm as the gestation progresses, when the loculus or fetal bulk increases and maintains the contact between the villous surfaces of the endometrium and the chorionic sac. In addition to the development of microvilli to assist attachment, another prerequisite to implantation is the formation of sufficient areolae in the chorion to absorb the secretions of the uterine glands (histotroph) and the subsequent development of the placenta and conceptus in the embryonic and fetal periods. Also, the rapid increase in allantoic fluid estrone and estradiol concentration between Day 20 and 30 of gestation is associated with an increase in the allantoic fluid volume, causing an expansion of the chorio-allantoic membranes

and forcing them into close apposition with the endometrium (Knight *et al.* 1977).

Parter (1977) stated that it is still uncertain whether implantation occurs as a result of the uterine epithelium secreting a substance that activates the blastocyst to implant, whether it causes it to synthesize a substance that prevents implantation, or whether the blastocyst, in some way, provokes receptivity in the endometrium. The following is an outline of a contemporary theory about the mechanism of implantation: (1) estrogen priming, (2) progestational changes; estrogenic action: inhibitory factor synthesis → suppressed sensitization → stimulus from blastocyst (positive protamine blue reaction, edema) → degeneration of uterine epithelial cells (not applicable in pigs) → transfer of estromal cells to decidual cells.

EXTRA-EMBRYONIC MEMBRANES

Figure 2 shows the interrelationship between the embryo and extra-embryonic membranes.

Yolk Sac

The yolk sac is first identifiable around the time of gastrulation, when a mass of cells that will become the endoderm segregates from the embryonic mass and reinforces the blastocyst wall. It is soon reinforced by the mesoderm layer and separated from the trophoblast by the

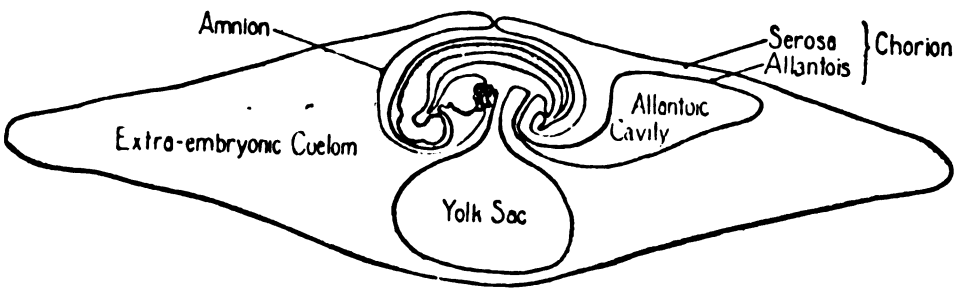


Fig. 2. Interrelationship between the embryo and extra-embryonic membranes--from Patten, B. M. 1958.

extra-embryonic coelom. It extends beyond the head of the embryo and beyond the caudal end. During tabulation, between 12 and 15 days, it becomes enclosed in the embryonic body, after which only the extra-embryonic portion of it is considered the yolk sac. Although mammalian embryos never contain yolk, the yolk sac is not considered a vestigial structure because it is known to have a vital role in hematopoesis when the embryo first begins to produce red blood cells (Marrable 1971). Also, in the early embryonic period the yolk sac serves as an organ purveying nutritive material (histotroph) to the embryo. This function is later taken over by the allantois (Patten 1958).

Amnion

In the early stages, the amnion closely involves the embryo, but is soon filled with a watery fluid which serves to equalize the pressure around the embryos. It first is a formation of the amnio-chorionic fold with the amniotic wall facing the embryo and the chorionic wall against the uterine surface. The limiting sulcus, which is a fold circumscribing the embryonic area, deepens and circles beneath the disc. The dorsal opening diminishes until its closure at about 18 days (Marrable 1971). Ventral to the embryo is a region not limited by the membrane where the arteries, veins, and ducts of the umbilical cord traverse the extra-embryonic membranes and the yolk sac.

Allantois

The allantois is at first free in the chorionic cavity, the extra-embryonic coelom. Like the gut and yolk sac, it consists of inner endoderm and outer mesoderm. From its beginning it is supplied by the umbilical arteries, which are, in turn, prolongations of the dorsal aorta. The allantois expands in such a way that it is crescent shaped and four times the size of the embryo at 19 days. After becoming richly vascular, it comes into contact with the chorion. By 26 days, the chorion becomes increasingly vascular, but between 30 and 40 days the terminal allantoic vessels regress and the blood flow is halted so that the tips are degenerated and referred to as ischaemic extremities (Marrable 1971). As the gestation advances, the allantois assumes a vital anatomical function to the conceptus. Its rich vascular supply is responsible for maintaining exchange with the vessels of the uterus. Its connective tissue is important for supporting this vascular network between the maternal and embryonic circulation.

Allantochorion

The allantochorion is formed when the allantoic splanchopleure fuse with the extra-embryonic somatopleure, or trophoderm. The expanding conceptus brings the chorion into close contact with the uterine mucosa. At first, its

surface is smooth, but later it acquires villi which fit between the ridges of the uterine mucosa. Amoroso (1952) objected to the term villi, maintaining that true villous structures are only occasionally present on the chorion, and even then in a number too small to substantially increase the absorptive surface. The tips of these structures are covered with cuboid epithelial cells, and the uterine fossae into which they fit are lined with columnar cells (Heuser 1927). Soon after the embryo is 12 mm in length, small circular areas appear on the surface of the chorion, which are applied to the mouths of the uterine glands and are obviously involved in the absorption of the uterine milk or histotroph. These structures are called areolae. The chorion is thus divided into areolar areas and intra-areolar areas, which will be discussed in the section on the placenta. The areolar areas of the chorion have a different type of columnar epithelium than the intra-areolar areas. In the areolae, the capillaries do not displace the epithelial cells as they do in the chorionic ridges of the intra-areolar areas (Amoroso 1952). The primary and secondary ridges of the chorion are formed of trophoblastic ectoderm and are supported by allanto-chorionic mesenchyme.

EMBRYONIC FLUIDS

From about 18 days of gestation, fluids accumulate in the amniotic and allantoic cavities. The combined

embryonic fluids expressed as a percentage of conceptus weight is shown in Table 1. We can see that there is a gradual and irregular decline in the proportion of fluids to a value of 25% or less in the last month.

Origin

The origin of amniotic and allantoic fluids is uncertain. Early research (Jacque 1903 and 1904) advanced the hypothesis that the fetal fluids were largely fetal urine. Wislocki (1935) pointed out that the volume of allantoic fluid was 200 ml when the embryo was only 3 cm long, and therefore it was unlikely that such a small embryo could be capable of excreting such a large volume of urine.

McCance and Dickerson (1957) examined the allantoic and amniotic fluids of gilts at different stages of gestation and found that the concentrations of urea and creatinine in the fetal fluids were below those in the maternal or fetal serum, and concluded that it was therefore unlikely that they were of renal origin. Knight *et al.* (1973) reported that the rapid accumulation of water in the allantoic fluid might be associated with an increased permeability of the cells and altered electrolytes movement under the effect of a higher concentration of estrogen. Bazer (1975) stated that the most probable source of protein in the allantoic fluid is the secretion of the uterine glands absorbed and transported by the areolae.

TABLE 1

COMBINED EMBRYONIC FLUIDS EXPRESSED AS PERCENTAGE OF CONCEPTUS WEIGHT

Embryonic Age (days)	Embryonic Fluids (%)
35	69
42	31
49	35
56	40
63	51
70	29
77	47
84	33
84	25
91	21
98	20
98	22
105	23
112	25
112	20
112	22

From Mitchel *et al.* (1931)

Volume

Wide variations have been observed in the volume of the embryonic and fetal fluids, both between different litters and individual embryos of the same litter.

McCance and Dickerson (1957) measured the volumes of allantoic and amniotic fluids at 20, 45, and 65 days. They found that at 20 days the volume of allantoic fluid was small (5.3 ml) and the volume of amniotic fluid was not detectable. At 45 days, the allantoic volume was larger (110 ml) and amniotic fluid was 26 ml. Finally, at 65 days the allantoic fluid volume increased approximately 3-fold (353 ml) of that observed at 45 days. The amniotic fluid was 62 ml. Pomeroy (1960) showed changes in the amounts of embryonic fluids of the pig during the course of gestation (Figure 3). On the average, the amniotic fluid increases to a maximum of about 200 ml. After 75 days it drops to 100 ml or less by the end of pregnancy. The allantoic fluid increases rapidly from 19 days, reaching a maximum between 65 and 70 days, and then decreases sharply by the end of pregnancy.

Knight *et al.* (1977) compared the volumes of amniotic and allantoic fluid of intact gilts (IC) and unilaterally hysterectomized-ovariectomized gilts (UHOX). Their results are shown in Figures 4 and 5, respectively. Measurable amounts of amniotic fluid were not present until Day 30.

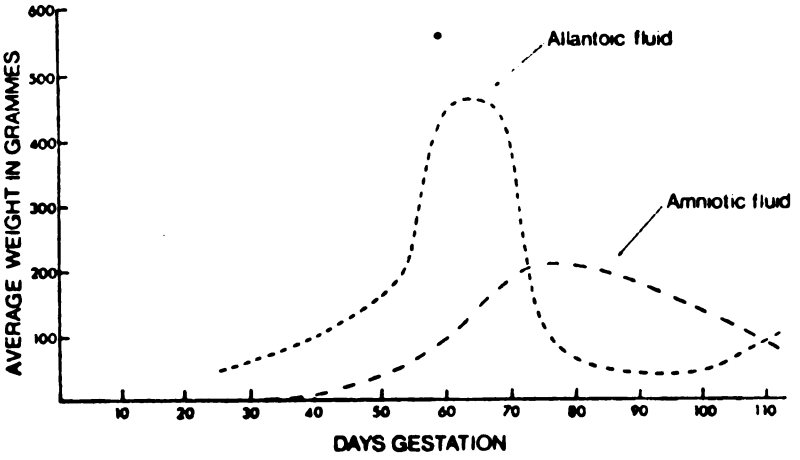
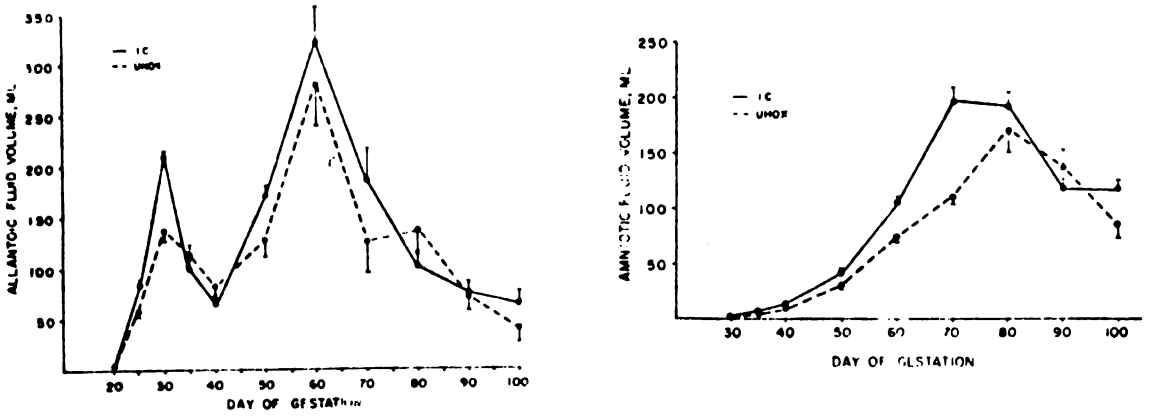


Fig. 3. The amounts of embryonic fluids of the pig during the course of gestation--from Pomeroy, R. W. 1960.



Figs. 4 & 5. The amounts of pig fetal fluids during gestation--from Knight *et al.* 1977.

In IC gilts, the amniotic fluid volume increased from Days 30 to 70, plateaued to Day 80, and then decreased to Day 100. In UHOX gilts, there was a steady increase from Days 30 to 80 and a subsequent decrease to Day 100. The first rapid increase in allantoic fluid volume occurred between Days 20 and 30. Then there was a decrease to Day 40, followed by a second increase to Day 60 and a subsequent decrease to Day 100. This general pattern of change in allantoic fluid volume was similar for IC and UHOX gilts and was correlated with placental length and allantoic fluid total protein.

Composition

The main component of the fetal fluid of the pig is water, ranging from 95% at 35 days to 91% at 112 days (Mitchell *et al.* 1931; Pomeroy 1960).

Table 2 shows the composition of the amniotic and allantoic fluids at several stages of gestation (McCance and Dickerson 1957; McCance and Widdowson 1960). We can see that at 20 days the average osmolar concentration in the allantoic fluid was 256 milliosmols. It declined steadily to an average of 92 at 55-65 days and increased again (237) at 90 days of gestation. The allantoic fluid at 22 days had a pH of 7.0, and this may have been higher before that time. By 46 days it had fallen to 6.0 and remained at about that

TABLE 2

COMPOSITION OF THE EMBRYONIC FLUIDS OF THE PIG

	Amniotic Fluid					Allantoic Fluid						
	Days Gestation					Days Gestation						
	34	41-46	55-65	67	90	20	22	46	41-46	55-65	67	90
Na (mEq/l)	125	126	119	117	111	114	60.5	9.9	13	14	7.9	4.1
K "	21	18.9	9.3	10.0	8.4	14.3	9.5	4.5	8.2	6	12.6	76.7
Ca "	-	-	-	-	-	-	2.9	19.2	-	-	14.5	34.9
Mg "	-	-	-	-	-	-	1.5	0.9	-	-	0.45	5.0
Cl "	103	104	92	94	91	69	36.3	28.4	30	18	19.4	75.8
P (mg/100 ml)	4.0	4.1	6.5	-	-	8.6	-	-	6.6	-	-	-
Urea (m-mol/l)	3.0	4.5	4.8	-	-	3.1	5.6	6.1	8.4	10.3	14.0	17.6
Creatinine (mg/100 ml)	-	0.8	0.3	-	-	1.2	2.2	3.0	3.7	-	2.8	16.5
Total Osmolar Conc. (mosm/l)	296	290	294	273	273	256	149	104	120	100	92	237
pH	-	-	-	7.2	7.2	-	7.0	6.0	-	-	6.1	5.8

SOURCE: McCance, R. A. and Dickerson, J. W. T. (1957); McCance, R. A. and Widdowson, E. M. (1960).

level until the observations ceased at 90 days. Both urea and creatinine compounds had a tendency to increase during gestation. The concentration of sodium ions exceeded that of other elements at Day 20. At 41-46 days the potassium did not fall to the same extent, and neither did the chloride, of the sodium concentrations, and at this age the chloride ions frequently exceeded the sum of the sodium and potassium ions. At 90 days of gestation, except for sodium, all of the other elements had a large increase which was due to the absorption and decrease of the allantoic fluid by that time. The chemical structure of the amniotic fluid changed relatively little throughout these stages of its functional life and was quite different from that of the allantoic fluid. This applied particularly to the pH and the concentration of sodium, chloride and osmolal material.

In a study which examined the composition of calcium, magnesium and phosphorous in the porcine fetal tissues, Ashdown and Marrable (1967) reported that the concentration of calcium was higher in the fetus than in adult pigs. It was 18 mg per 100 ml in the fetus and 11 mg per 100 ml in the maternal serum. This was also true for magnesium: 3.79 mg/ml in the fetus vs 2.60 mg/ml in the sow. Knight *et al.* (1977) observed a rapid accumulation of protein in the allantoic sac from Days 30 to 60 and an equally rapid

decline from Days 60 to 100. The decline in protein concentration may indicate its increased assimilation by the embryo. They also noted a rapid increase in estrogen secretion by the placenta between the 60th and 100th day of gestation.

Wislock and Dempsey (1946) found that the uterine glands of the pig gave strong reactions for calcium. Thus, the histotroph may contribute to the considerable build-up of calcium in the embryonic membranes between 20 and 46 days. It is not known for certain whether the calcification is due to the storage of calcium or whether it is used for the ossification in the developing embryo.

PLACENTA

The placenta of the pig is classified as epitheliochorial. In the early stages, as many as six histologically distinct layers (three maternal and three fetal) separate the two bloodstreams (Figure 6), but with advancing gestation the vascular beds are brought closer together by their increasing command of subepithelial positions and the invasion of the trophoblast by embryonic capillaries (Figure 7). Gellhorn *et al.* (1941) measured the thickness of the uterine epithelium at different stages of gestation and found it to be 18 microns at mid-pregnancy, 13 microns at 100 days, and 10 microns just before term; i.e., almost a 50% decrease. The characteristic of this class of placenta is that anytime

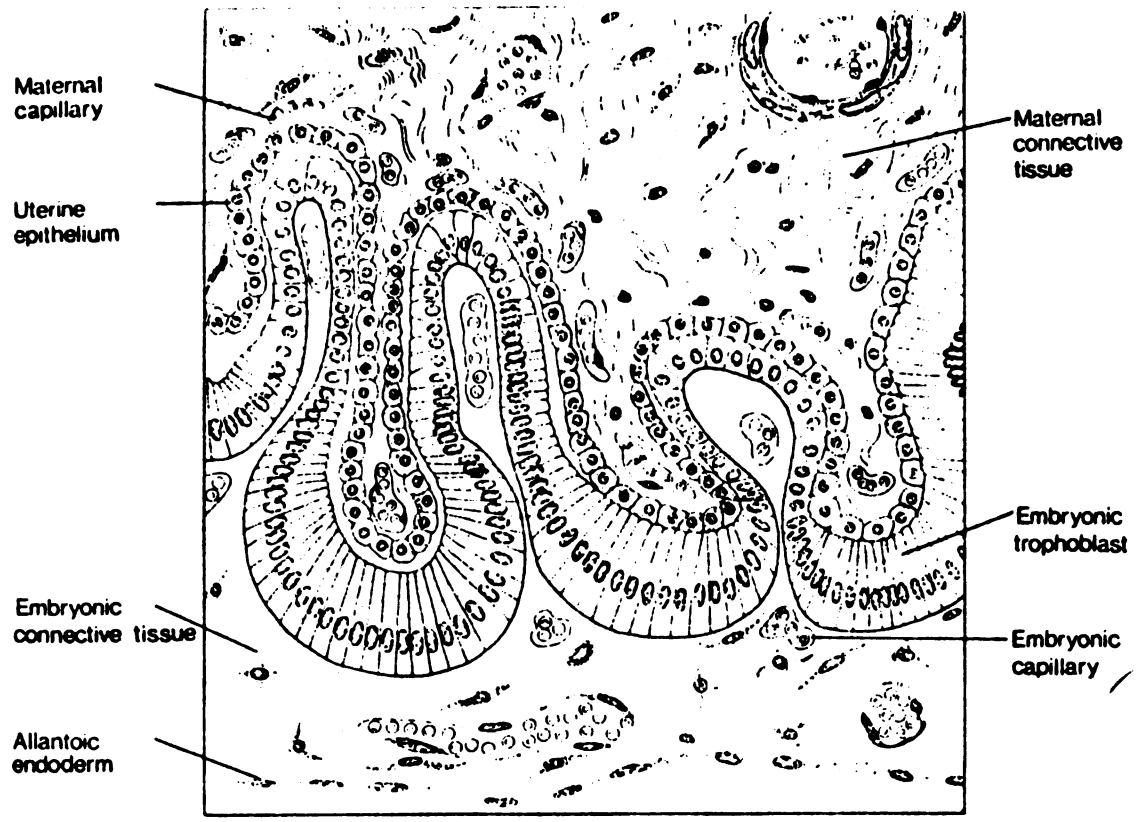


Fig. 6. Vertical section of placental pig--from Marrable, A. W. 1971.

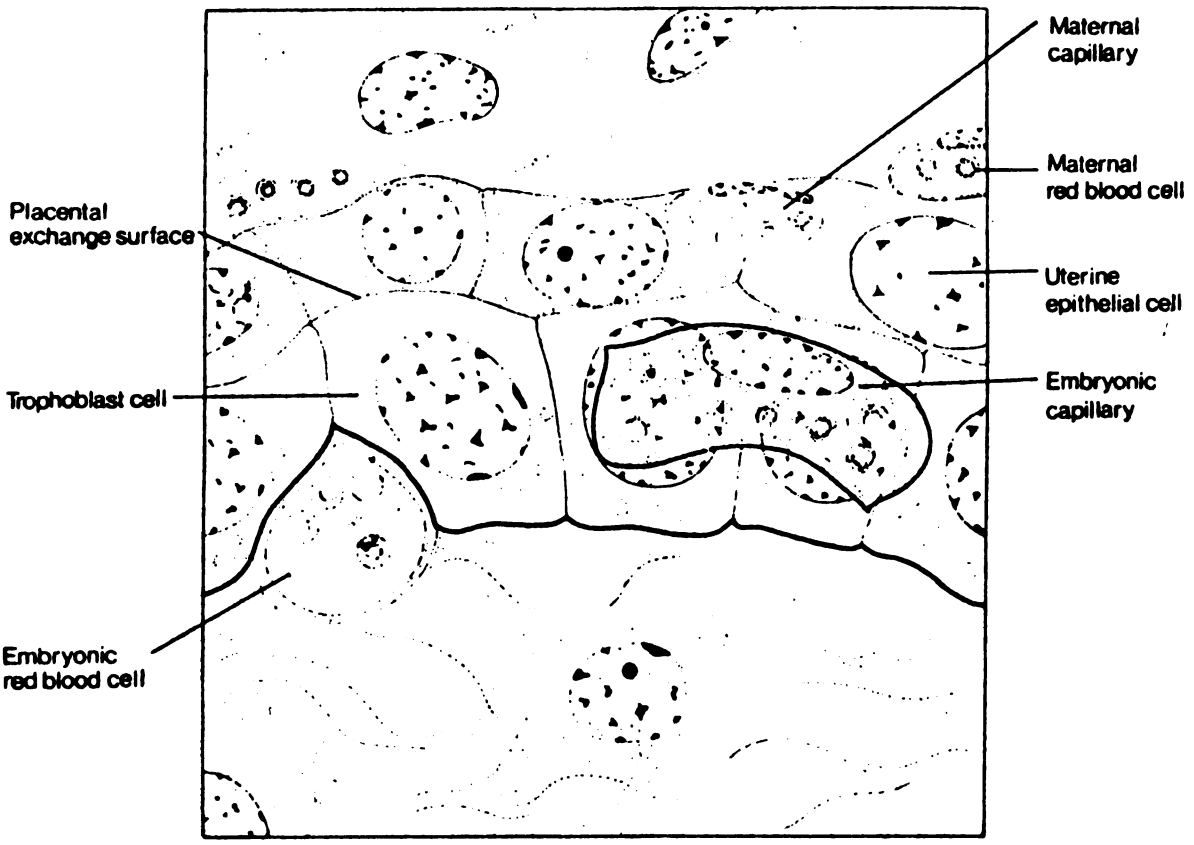


Fig. 7. Placental pig interface showing the proximity of embryonic and maternal blood--from Marrable, A. W. 1971.

throughout the gestation the two portions (fetal and maternal) may be separated from each other without tearing, because the trophoblast does not invade the endometrium. They are anchored together by the interdigiting miniature fingerlike structures called microvilli, although they lack the absorptive function of the true intestinal villi (Amoroso 1952). The chorionic sac lies in such a way as to fit snugly over the alternating fossae and ridges of the endometrium.

Maternal Portion

The maternal portion of the placenta consists of a non-ciliated glandular epithelium. In the glandular areas of the endometrial mucosa (Figure 8), the epithelium is cuboidal or low columnar with large vesicular nuclei and abundant mitochondria. Apically the cell surface exhibits numerous microvilli and occasional cilia resting upon a thick basement membrane (Dempsey *et al.* 1955). The uterine glands become hypertrophied during pregnancy in connection with their histotrophic function. The subepithelial connective tissue becomes increasingly vascular in early pregnancy with the new formation of capillaries (Amoroso 1952). The uterine epithelium remains uniform for a time, but with the differentiation of the endometrial fossae and ridges some of the cells in the depths of the grooves beneath the tips of the trophoblast become cuboidal. Over the summits of the

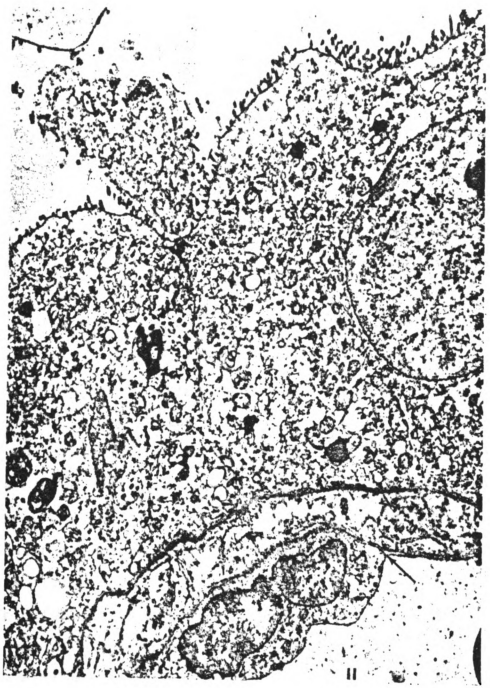


Fig. 8. Section through a uterine gland--from Dempsey *et al.* 1955.

mucosae septa, the epithelial cells are columnar. When these changes have taken place around the 20th day, the irregular epithelial surface is believed to assist attachment (Heuser 1927; Crombie 1970).

Fetal Portion

The embryonic portion of the placenta consists of the trophoblast or chorion which is a single layered epithelium supported by the allantochorion mesenchyme through which the allantoic vessels purvey the histotroph from the circular structures of the chorion. The areola are applied to the mouths of the uterine glands from which the histotroph originates.

By mid-gestation (50 to 60 days), five superficial zones are distinguishable on the allantochorion (Figure 9): a large central placental zone with villi and areolae, two bordering paraplacental zones without villi and areolae, and two ischaemic zones (necrotic tips) which form the extremities of the conceptus (Ashdown and Marrable 1967). The ischaemic zones are seen to lack trophoblast and allantoic endoderm (Marrable 1968). In the interareolar areas, the capillary network is the site of the transmission of the more diffusible substances from the maternal circulation. The relatively less vascular areas of the fossae absorb the less diffusible substances secreted by the uterine glands

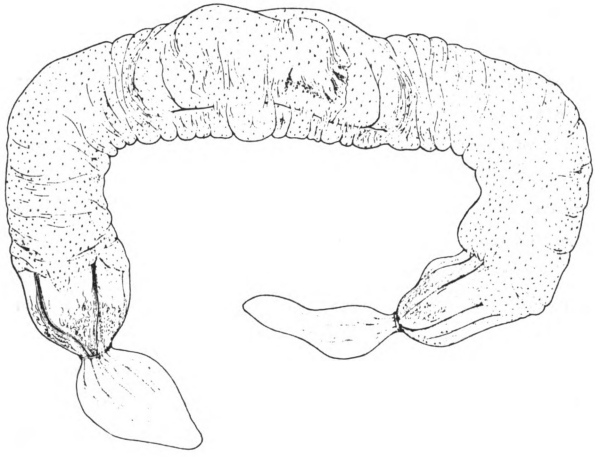


Fig. 9. Chorionic sac of pig showing established surface zonation--from Ashdowns, R. R. and A. W. Marrable. 1967.

and transmitted through the chorionic areolae and the allantoic vessels.

In early gestation, the chorionic vesicles are positioned end-to-end in the uterus, separated by their ischaemic extremities. These necrotic tips may be entwined or one may be invaginated into another, or they may become stuck together by a gummy uterine secretion. By the last third of the pregnancy, the paraplacental zones are adherent to those of neighboring fetuses, but they may be separated without tearing either (Ashdown and Marrable 1967). In a few cases, the embryos may become physically linked by abnormal anastomoses which may link major vessels. This phenomenon is known as parabiosis (Figure 10). Such embryos may be enclosed in a single chorion with each fetus having its own amnion, yolk sac, etc. If a female enters into para-biotic fusion with a male embryo, sometimes it will not develop normally as a female. This is a freemartin, which is rare in pigs.

The microscopic subdivisions of the chorion (Figure 11), which interdigitate with similar structures on the uterine mucosa, have been called microvilli, although they do not serve the absorptive function of the true villi in the intestines (Amoroso 1952; Dempsey *et al.* 1955). The chorionic epithelium rests on a moderately developed basement membrane. Capillaries are sparse beneath the crescentic

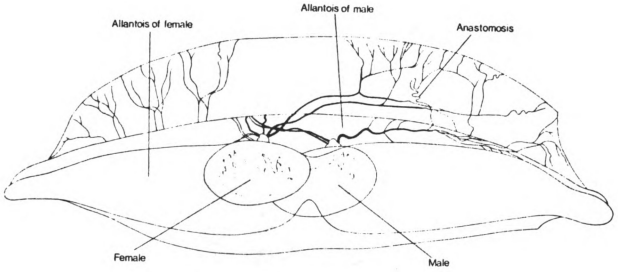


Fig. 10. Parabiotic fusion of sacs: freemartin--from Marrable, A. W. 1971.

Fig. 11. Electron micrograph of placenta, sow. Uterine capillary (1); uterine epithelium (2); interdigitating microvilli (3); trophoblast (4); fetal capillary (5)--from Dellmann, H. D. and E. M. Brown. 1976.

fossae and more abundant under the ridges. At the tips of the chorionic ridges, the fetal epithelial cells are cuboidal. In the fossae, they are columnar. The fetal capillaries enveloped by the basement membrane penetrate between the cells and compress the cytoplasm of the epithelio-chorion in a position which has been called intraepithelial (Bjorkman 1965). After the allantoic vessels have reached the chorion, the hematotrophic nutrition supplements the histotroph by establishing an exchange between the myometrial vascular supply, the allantoic and umbilical vessels. The cells lining the chorionic fossae contain numerous, homogenous, colloid droplets located in the basal part of the cell (Figure 12). The chorionic cells have large ovoid nuclei. In the areolar areas of the chorion, the epithelium is of a simple columnar variety. In the areolar areas, the surface has been described as pseudo-stratified because the epithelial are not all uniform in height (Dempsey *et al.* 1955).

INTRA-UTERINE ENVIRONMENT

Hormonal modification of the intra-uterine environment in swine has been studied (Reddy *et al.* 1958). They found that the mean weight of the reproductive tracts were 22.5% greater in the treated group than the mean weight in the control group. These sows were injected with a combination of estrone (50 µg) and progesterone (50 mg) for ten

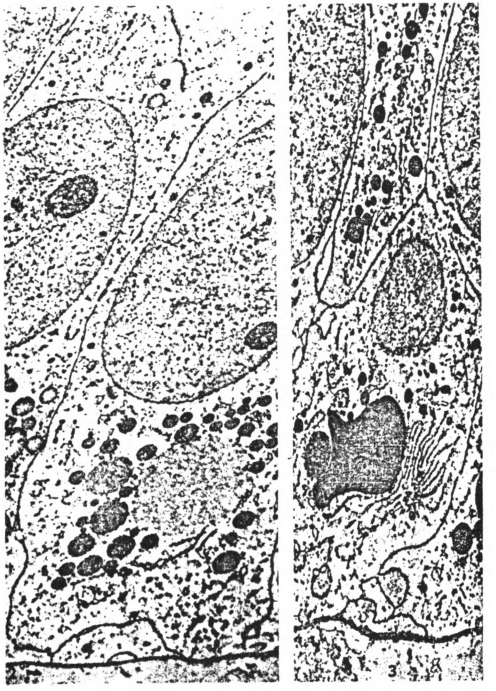


Fig. 12. Colloid droplets located in the basal part of the cell--from Dempsey *et al.* 1955.

consecutive days (from Day 4 through Day 13) and slaughtered on Day 55 of pregnancy. Furthermore, they investigated the effect of several combination levels of progesterone and estrone when injected during early gestation. Their most significative study was when sows were injected with a combination of 25 mg progesterone and 12.5 µg estrone for 10 days, starting on Day 14 of pregnancy. These injected animals were slaughtered on Day 56 of gestation and the following findings were reported: the percentage of mortality was 13.5 in the treated animals compared to 23.3 in the non-treated group of gilts; the mean weights of the reproductive tracts (uterus, vagina and embryos) were 8054 and 5445 grams in the treated and untreated gilts, respectively. Similar results were also observed in the weights of the reproductive tracts without embryos (7156 g in the treated and 4809 g in the untreated gilts). The mean weight of the individual embryos was similar for both groups. It was 79 and 80 g for non-treated and treated animals, respectively. The mean volume of the fetal fluid in the experimental gilts was 539 ml compared with a mean volume of 189 ml in the non-treated gilts. This difference was significant, and the fetal fluids apparently distended the lumen of the uterus, transmitted pressure to the uterine wall, and stimulated growth by hypertrophy. The uteri from the treated gilts were more distended, with a corresponding increase in linear

capacity of 29% over the mean linear capacity of 2610 ml in the non-treated gilts. This difference was not due to random variation within the population, and therefore it is described as a treatment effect.

The development of the pig conceptus has been observed by Marrable and Ashdown (1967). They reported that throughout gestation, there was wide variation in the length and weight of embryos from the same litter. This variation is greater in the second half of gestation than in the first. The embryos at 32, 33 and 36 days of age had crown-rump lengths of 2.8, 3.1 and 3.3 cm, respectively. Weights were notably more variable than crown-rump lengths. The embryos had mean weights of 2.9, 4.0 and 4.1 g at 32, 33 and 36 days of age, respectively.

Knight *et al.* (1974a) studied the effect of two levels of progesterone therapy at a constant level of estradiol on development of the porcine conceptus to Day 40 of gestation. Gilts were either sham-operated or bilaterally ovariectomized on Day 4 of pregnancy. Sham-operated gilts received either corn oil daily (SO-CO) or 3.3 mg progesterone (P) and 0.55 µg estradiol (E_2)/kg/day (SO-HP). Bilaterally ovariectomized gilts received either 1.1 mg P and 0.55 µg E_2/kg/day (OVX-LP) or 3.3 mg P and 0.55 µg E_2/kg/day (OVX-HP). These gilts were treated from Day 4 to Day 40 of gestation when they were hysterectomized so that the uterus and its contents could be examined. From this study, they reported

that treatment had no effect on pregnancy rate, number of live embryos, or percent embryonic survival. However, embryos' weight and crown-rump length were significantly ($P < .05$) greater for the SO-CO group as compared with embryos from gilts in the other treatment groups. Placental length and allantoic fluid volume were significantly ($P < .05$) increased in the SO-HP and OVX-HP treated gilts compared to those of SO-CO and OVX-LP treated groups. The combination of these two factors are critical in determining the placental surface area which achieves contact with the maternal endometrium. Brambell (1933) found that the number of placental areolae is related to placental surface area. Areolae appear by 18 days of gestation and become fixed at about Day 35 of gestation. The number of areolae may be critical since they represent the placental sites of absorption of nutrients from the uterine glands. Placental weights were significantly ($P < .05$) greater in sham-operated compared to bilaterally ovariectomized females. Allantoic fluid total protein per conceptus and per litter was significantly ($P < .05$) less for the OVX-LP groups than the other three treatment groups. Generally, these data indicate that the high progesterone level increased uterine secretory activity and enhanced placental development. This may be accomplished by an increase in allantoic fluid volume which leads to an increase in the placental surface area.

In the more recent studies, Knight *et al.* (1977) investigated the conceptus development in intact control (IC) and unilaterally hysterectomized-ovariectomized (UHOX) gilts. Their findings showed that ovulation rate was equal for the two treatment groups; however, the percent of fetal survival was significantly greater in IC gilts from Day 35 (but not prior to Day 30) to Day 100 of gestation. Placental weight was significantly ($P < .01$) greater in IC gilts. By Day 30, there was an average difference of 10.9 g in placental weight between IC ($\bar{X}$ = 27.6 g) and UHOX ($\bar{X}$ = 16.7 g) gilts, and by Day 100 this difference had increased to 63.5 g (258.0 vs 194.5 g). Placental surface area was also significantly ($P < .01$) greater in IC gilts. By Day 35 of gestation, there was an average difference in placental surface area of 47.4 cm^2 between IC ($\bar{X}$ = 281.5 cm^2) and UHOX (234.1 cm^2) gilts. However, no significant ($P < .10$) treatment effects on either fetal crown-rump length or fetal weight were observed prior to Day 40 of pregnancy. At Days 35 and 40, the crown-rump length in IC gilts was 3.5 and 5.1 cm, respectively, while in the UHOX it was 3.3 cm by 35 days and 4.8 cm by 40 days of gestation. The conceptus weight was equal (3.7 g) for IC or UHOX gilts at 35 days of gestation. By Day 100 of pregnancy, an average difference of 20.5 mm in crown-rump length was found between IC and UHOX gilts. Also, there was an average difference of 169.0g

in fetal weight between IC and UHOX gilts. In conclusion, data from this study suggest that intra-uterine crowding and the associated reduction in endometrium surface area resulted in inhibition of placental development and, in turn, increased fetal mortality and inhibited development of those fetuses which survived.

INTRA-UTERINE PROTEIN

It has been established that embryos of swine must reach the uterine environment for continued development beyond the early blastocyst stage. Published data suggests that the uterine secretions of the pig may contain blastokinin--like factor(s) that are necessary for blastocyst development (Murray *et al.* 1971).

Murray *et al.* (1972), studying the protein content of porcine uterine flushings from Days 2 to 18 and on Day 20 of the estrus cycle, reported that the secretions of the uterus show considerable cyclic variation. The protein content of the uterine secretion remained stable from Days 2 to 9 of the estrus cycle and then began to increase. The increase became marked on Day 12, and the maximum value was reached on Day 15. Then the total protein of the uterine flushings decreased sharply so that by Days 17, 18 and 20 the values were similar to those obtained during Days 6 to 9 of the estrus cycle. The peak protein content coincided

with the time when the reproductive system was under progestational influence. This has been confirmed by Tillson *et al.* (1970) who observed the plasma progesterone maximum from Days 10 to 14 of the cycle. Murray *et al.* (1972) observed five protein fractions in uterine secretion. Three of them did not appear to vary with the days of the estrus and were found by gel filtration in all profiles. These three protein fractions (Fraction I, II and III) had estimated molecular weights greater than 200,000, 200,000 and 90,000 and mean elution volume of 57.0, 72.0 and 85.0 ml, respectively. One additional protein fraction (Fraction V) with a molecular weight of about 20,000 and elution volume of 115 ml appeared in the protein profile, beginning on Day 9 and continuing through Day 16. Fraction V constituted more than 20% of the total protein on Days 12 to 16, and it was quantitatively greater on Day 15. On Days 14 to 16, the protein profile contained another protein fraction (Fraction IV) unique to the luteal phase of the estrus cycle. Fraction IV consisted primarily of a naturally lavender-colored protein with an estimated molecular weight of 45,000. This has been termed the "purple-protein." The pattern of secretion of both Fraction IV and V was such that the presence of functional corpora lutea would be necessary for their secretion. Thus, the rapid decline in Fraction IV and V coincided with the time of corpora lutea regression and reduced plasma progesterone levels.

It is uncertain whether either Fraction IV or V is involved in blastocyst formation. Perry and Rowlands (1962) reported that blastulation of swine embryos occurs between Days 6 and 8 after the onset of estrus, i.e., before Fractions IV and V are produced. However, it has been shown (Green and Winters 1946) that initial blastulation occurs around Day 11 of pregnancy, followed by rapid growth of the blastocyst, particularly elongation and development of the trophoblast. This suggests a possible role of these proteins in embryonic development.

Chen *et al.* (1973) conducted an experiment with ovariectomized gilts which were injected with either progesterone or progesterone and estrogen in order to induce the production of Fraction IV. The results showed that ovariectomized gilts injected with estrogen in combination with progesterone produced significantly more uterine protein than either those injected with estrogen or those in intact animals. They also prepared lamb antiserum against the purified protein for Fraction IV, and they did not get any cross-reaction with extracts of homogenized heart, lung, stomach, intestine, liver, spleen, kidney and oviduct tissue obtained from gilts. In addition, they observed no cross-reaction with pig serum obtained on Day 15 of the cycle. Hence, the results suggested that Fraction IV protein was uniquely located in the uterus and its synthesis or secretion is specifically induced by progesterone.

Knight *et al.* (1973) reported that progesterone was the key hormone controlling the quantitative aspects of uterine protein production in the pig, although maximum total uterine protein values were obtained due to the synergistic action between progesterone and estradiol. In this study, protein profiles of samples from estradiol or corn oil treated gilts revealed only small quantities of uterine protein (Fractions I, II and III). Fractions IV and V were found only in ovariectomized gilts treated with progesterone alone or progesterone-estradiol combination, indicating that progesterone was primarily responsible for the secretion of the specific protein components from the secretory cells of the uterine endometrium.

Knight *et al.* (1974b) observed the effects of varying amounts of progesterone on porcine protein secretion in ovariectomized gilts. They showed that 100 mg of progesterone/45.36 kg/day is required to produce a similar amount of uterine protein secretion as that noted in a sham-operated gilt free of additional progesterone. However, exogenous progesterone dose of 300 mg/45.65 kg body weight/day, showed a marked drop in protein recovered, indicating that the relationship between exogenous progesterone administered and the total uterine protein extracted was curvilinear. Also, the effect of varying progesterone (0 to 150 mg) and holding estradiol constant (0.25 μg) per kilogram body

weight/day on the ovariectomized group was shown. There was a highly significant ($P < .01$) coefficient of correlation between total daily administered progesterone ($r = 0.72$) and estradiol ($r = 0.61$) given and total protein recovered. The correlation coefficient between daily administered progesterone and total protein recovered in sham-operated gilts was 0.78 ($P < .01$). Finally, a negative correlation ($r = -0.44$) between total recovered uterine protein and total daily estradiol was reported in one experiment of sham-operated gilts. This suggested that estradiol either may inhibit or delay uterine secretory activity when the dosages exceed a certain level.

Chen *et al.* (1975), in a study with ovariectomized gilts which were injected with estradiol alone (0.55 µg/kg/day), progesterone (2.2 mg/kg/day) or a combination of progesterone and estradiol for 14 days of gestation, reported that the purple acid phosphatase of the pig uterus is synthesized by the uterine endometrial glands and transported via the placental areolae into the allantoic fluid. Estrogen and low levels of progesterone are associated with synthesis of the acid phosphatase. They also point out that uterine protein is regulated by progesterone. This last statement was later confirmed by Jones *et al.* (1976). They induced protein synthesis <u>in vitro</u> in blastocyst tissue before attachment and found no detectable effect of estrogens on

the quantitative or qualitative pattern of protein synthesis in the pig.

The effects of age upon the quantitative and qualitative uterine specific protein secretory patterns during the induced cycle of non-mated prepuberal gilts were determined by Segerson and Murray (1977). Gilts at 3, 4 and 5 months of age were assigned for this experiment. The control group received saline and the treatment group received PMS followed by the injection of HCG 72 hours. The results indicated that gilts of 4 and 5 months of age secreted significantly ($P < .01$) greater quantities of both total recoverable uterine protein and uterine specific protein than gilts 3 months of age. Only one 3-month-old saline treated gilt produced the uterine specific protein, but five of six gilts 3 months old receiving HCG produced the complete uterine specific pattern. Both saline and HCG treated gilts at 4 or 5 months of age were capable of producing the complete uterine specific protein profile during the induced cycle. Since the present study revealed the presence of the complete pattern of the uterine specific protein in most gilts, perhaps the estrogen secretion resulting from PMS stimulation may have additionally been involved. The quantities of both total recoverable uterine protein and uterine specific protein are less in the immature gilts than quantities observed in the mature gilts within the same breed.

STEROID HORMONE THERAPY

Conflicting results have been reported as to the effects of progesterone and estrogen therapy on embryonic survival in sows. In 1956, Sammelwitz *et al.* treated gilts with progesterone at rates of 50, 100, 200 and 400 mg daily for 26 days following mating. They reported that progesterone at a level of 100 mg daily had little beneficial effect in reducing early embryonic mortality in swine. However, dosages of 200 mg daily or greater caused complete degeneration of corpora lutea.

Later, some workers reported that either raising plasma progesterone levels to nearly twice the normal or reducing them to half did not influence embryonic survival during the first 30 days of gestation in females with normal numbers of ovulations (Haines *et al.* 1957; Spies *et al.* 1959; Webel, Reimers and Dziuk 1975).

The changes in the plasma levels of progesterone of unconjugated estrone and estradiol-17-beta and of estrone sulphate in the sow at the time of implantation, during gestation, and at parturition has been described by Robertson and King (1974). The mean plasma progesterone levels in pregnant sows at Day 14 were 21.9 ng/ml, dropping to 13.0 ng/ml by Day 28. The mean levels of unconjugated estrone and estradiol-17-beta were first present in measurable quantities at Day 80 and rose to peak values just before

parturition. In relation to the concentration of estrone sulphate, it was above the sensitivity of the method at Day 9, but there can be no doubt that the concentration of 60 pg/ml observed at Day 16 represents the beginning of a rise of estrone sulphate in the plasma, which continued to the peak of > 3 ng/ml sometime between Days 23 and 30. The plasma concentration of estrone sulphate then declined to a low value of 35 pg/ml around Day 46. The synthesis of estrogens by the blastocysts begins at about Day 9 and there is a subsequent associated rise in the level of maternal plasma. Estrone sulphate may be a requirement for locally initiating the process of implantation in the uterus and at the same time informing the maternal endocrine system that conception has occurred.

Reddy *et al.* (1958) reported a positive response to progesterone-estrone therapy on embryonic mortality in swine. In this study, two ratios of progesterone to estrone were followed. One group received 2 ml of the hormone solution containing 25 mg of progesterone and 25 µg of estrone (1000:1 ratio). A second group received 2 ml of hormone preparation containing 25 mg of progesterone plus 12.5 µg of estrone (2000:1 ratio). In the latter group, the pregnant gilts were given the oil solution of hormones intramuscularly each day for a period of 10 days, beginning either on Day 4 or

Day 14 of gestation, and this resulted in a highly significant increase in litter size at 55 days of gestation.

Wildt *et al.* (1976) reported that a dose of 25 mg progesterone and 12.5 µg of estrone injected together for 10, 5 or 2 consecutive days during implantation (Days 14 to 23 of gestation) caused a beneficial effect on litter size at term. However, estrone or progesterone alone, as well as higher levels of the progesterone-estrone solution, had no effect on litter size.

Geisert *et al.* (1978) conducted an experiment to determine whether progesterone (25 mg) and estrone (12.5 µg) therapy, injected on Days 15 to 17 of gestation in swine, could reduce embryonic loss at 30 or 70 days gestation. From this study it was reported that progesterone-estrone treatment failed to exert a beneficial effect. By Day 30, the mean value of 12.1 or 13.5 nondegenerating fetuses was recorded from treated and control gilts, respectively. By Day 70, the results were similar in both groups. Furthermore, the percentage survival was lower in the treated group than in the control for either Day 30 or Day 70 of gestation.

Day *et al.* (1963) demonstrated that exogenous progesterone and estradiol, administered subcutaneously from Days 11 to 25 or 17 to 25 of gestation, had a trend toward increased litter size; however, the difference was not significant. In this study, hormone treatment was prepared to

contain three combinations (100:50, 400:200, 500:250) of progesterone (mg) and estradiol (μg) per 100 lb. of body weight.

The levels of exogenous progesterone and estradiol benzoate necessary for optimal embryonic survival rates during the early stages of ovariectomized pregnant swine were determined by Gentry *et al.* (1973). They reported that embryo survival rates were similar in pigs given 0 to 1000 μg estradiol benzoate daily. However, when the level of estrogen was increased to 5000 μg/day, embryo survival rates were reduced. Embryo survival rates were lower in pigs given a level of 40 mg compared with 80 mg of progesterone and it was not increased by doubling (160 mg) the level of progesterone. The group given 80 mg progesterone and 500 μg estradiol benzoate had the highest average embryonic survival rate.

MATERIALS AND METHODS

The effect of steroid hormones on embryonic mortality and on placental development was studied in two experiments using different groups of sows. These experiments were conducted at Michigan State University and were initiated in December 1976 and terminated in April 1978. The first experiment was divided into a series of three trials to study effects on litter size:

(1) administration of estradiol and progesterone, alone and in combination;

(2) administration of two levels of estradiol;

(3) administration of three levels of estrone.

The second experiment dealt with injecting a combination of estrone and progesterone at a 1:2000 ratio and studying its effect on placental development.

STEROID SOLUTION PREPARATION

Hormone solution preparation was based on the technique described by Reddy *et al.* (1958). A stock solution of estradiol or estrone was prepared by dissolving 31.25 μg of either estradiol (1, 3, 5, (10)-Estratrien-3, 17 Beta-diol, Mann Research Laboratories, New York) or estrone (Delta 1,

3, 5 (10)-Estratrien-3-ol-17-one, Sigma Chemical Co., St. Louis) in 200 ml of arachis oil (Planters Company). A quantity of 10 ml of the estradiol or estrone stock solution and 3.125 g of progesterone (Delta^4-pregnen-3.20 dione, Sigma Chemical Co., St. Louis) were added to a 250 ml flask. Arachis oil was then added to give a total of 250 ml of solution. Two ml (the injection quantity) of this solution contained 25.0 mg progesterone and 12.5 µg estradiol or estrone (ratio = 2000:1, progesterone:estrogen). For treatments requiring progesterone or estradiol alone, solutions were prepared by adding either 3.125 g of progesterone or 10 ml of the estradiol stock solution to 250 ml of arachis oil. Two ml of these solutions contained either 25.0 mg of progesterone or 12.5 µg of estradiol, respectively. The three levels of estrone used in this study were prepared as follows: low estrone level--five ml of the estrone stock solution were added to 245 ml of arachis oil; medium estrone level--ten ml of the estrone stock solution were added to 240 ml of arachis oil; high estrone level--twenty ml of the estrone stock solution were added to 230 ml of arachis oil. Two ml of these solutions contained 6.25, 12.5, or 18.75 µg of estrone, respectively.

To prepare the two levels of estradiol treatment, 5.0 or 2.5 ml of the estradiol stock solution were mixed with either 245 or 247.5 ml, respectively, of arachis oil, giving 6.25 and 3.125 µg of estradiol per two ml.

EXPERIMENT 1

Experimental Animals

Experimental animals in this experiment included 127 multiparous sows of four genetic groups (Duroc, Yorkshire, Hampshire, and Yorkshire-Hampshire crossbreed). These animals ranged from 18 to 48 months of age and from 160 to 220 kg in weight. The dams were checked daily with a boar for estrus, and those that showed estrus behavior were mated on the first and second day of estrus. Nine sires of proven fertility and of either the Duroc, Landrace, Hampshire, or Yorkshire breeds were used.

Sows were housed in a gestation barn at a temperature of 15°C. They were individually tied or placed (groups of four) in pens throughout gestation. The sows were fed individually 2.0 kg of a 13% protein, corn-soybean meal diet fortified with minerals and vitamins once daily (in the morning). One week before expected parturition, the sows were taken to the farrowing house and placed in individual stalls. In this house, they were fed twice daily 2.0 kg of a 16% protein, corn-soybean meal ration fortified with minerals and vitamins.

Experimental Design

A summary of experimental groups and the number of sows used in Experiment 1 are shown in Table 3. The

TABLE 3

TREATMENTS, NUMBER OF SOWS, AND DOSAGE THERAPY LEVELS EMPLOYED IN EXPERIMENT 1

Group	Treatment	Number of Sows	Dosage Level of Therapy[a] per Day
1	T_0	20	2 ml of peanut oil
2	T_1	18	Estradiol alone (12.5 µg)
3	T_2	16	Progesterone alone (25.0 mg)
4	T_3	16	Estradiol (12.5 µg) & progesterone (25.0 mg)
5	T_4	10	Estradiol (6.25 µg)
6	T_5	10	Estradiol (3.125 µg)
7	T_6	12	Low estrone (6.25 µg)
8	T_7	13	Medium estrone (12.5 µg)
9	T_8	12	High estrone (18.75 µg)

[a]Injected on Days 16 and 17 of pregnancy.

experimental design in the first experiment consisted of 3 trials. Control animals were injected with 2 ml of arachis oil intramuscularly (anterior to the scapula) on Days 16 and 17 of pregnancy. Data from control pigs were combined from all treatment groups.

Trial 1

Animals assigned to this trial were divided into 3 groups. The first group received estradiol alone at a dose of 12.5 µg. The second group received progesterone alone at a level of 25.0 mg, and the third group received a combination of 12.5 µg estradiol and 25.0 mg progesterone.

Trial 2

Sows treated in Trial 2 received two levels of estradiol. Group 1 received 2 ml of the oil solution containing 6.25 µg of estradiol. In Group 2, the sows received 2 ml of the oil solution containing 3.125 µg of estradiol.

Trial 3

Animals in Trial 3 were allocated randomly to one of three treatment groups, resulting in 12, 13 and 12 sows for Groups 1, 2 or 3, respectively. Sows in Group 1 received 6.25 µg of estrone (low estrone) dissolved in arachis oil as described earlier. The second group was given 12.5 µg of estrone (medium estrone), while the last group received the high estrone level, or 18.75 µg.

Methods of steroid administration in Trials 1, 2 or 3 were similar to the control group. Sows that maintained pregnancy were allowed to deliver and the measures taken included the total number of pigs born, number of pigs born alive, and birth weight per litter or piglet.

EXPERIMENT 2

A total of 22 multiparous sows (18 to 30 months of age and 150 to 210 kg in weight) of Duroc, Yorkshire, Hampshire or Yorkshire-Hampshire crossbred types were assigned for this study. The animals were mated daily on the first two days of estrous to four boars of either the Yorkshire or Duroc breeds. Of the 22 sows, four returned to estrus and the remainder were maintained for the first 30 to 35 days of pregnancy housed individually or in groups of four. A complete 13.0 percent corn-soybean meal ration was fed at a rate of 2.0 kg/animal/day throughout the breeding and gestation periods.

Treatment solution preparations of 25.0 mg of progesterone and 12.5 μg of estrone were made as described earlier. The sows in the treatment group received 2 ml injections (i.m.) daily (0900-1000) for 10 days starting on Day 14 of pregnancy (the first day of estrus = Day 1 of gestation). The sows in the control group were given 2 ml of arachis oil during the same time period.

The sows were slaughtered at the Meat Laboratory of the Michigan State University Department of Animal Husbandry between Days 30 and 35 of pregnancy. The reproductive tracts were recovered and analyzed. The number of corpora lutea, ovarian weight, and position of each conceptus were recorded. Each conceptus was removed from the uterus and placed on paper where a tracing of the chorionic sac was made. The values for placental surface area were later measured with a planimeter. Data on the total amniotic and allantoic fluid volume (ml), weight of fetal membranes (g), weight of fetus (g), and fetal crown-rump length were also recorded.

Allantoic and amniotic fluid samples (10 ml) from the sows used in this experiment were examined for protein concentration, pH, osmolality, and acrylamide gel electrophoresis. The electrophoresis technique was described by Brewer *et al.* (1974). For electrophoretic separation, 7.5% acrylamide lower gel was used in 8 x .4 cm glass tubes with tris-glycine buffer (pH 8.6) and Bromotimos blue as a tracking dye. Proteins were incorporated into the large pore sample gel and then resolved by applying a constant current of 2.0 mA per tube for approximately 2 hours. After each run, gels were stained by 1-hour exposure to Amido black (1% stain in 7% acetic acid) followed by destaining for 48 hours in 7% acetic acid. A complete description of the procedure is given in Appendix A of this thesis.

Rf values for migrating protein bands were calculated and compared for treated and control pigs.

RESULTS

EXPERIMENT 1 (STEROID HORMONE EFFECTS ON LITTER SIZE AT TERM)

In Experiment 1, a mathematical model was used that included breed of sow, breed of boar, parity of the eight treatments. The procedure for least squares was used to obtain estimates of the treatment means, adjusted for the other factors, and to obtain the error mean square. Student's T test was used to compare the mean of each treated group to the mean of the control group.

Conception Rate

The number of sows returning to estrus for each treatment in Experiment 1 is shown in Table 4. Conception rates were high in all treatment groups, except for Groups T_5 (estradiol 3.125 µg) and T_7 (medium estrone 12.5 µg) where 40 and 23 percent of the animals returned to estrus, respectively.

Litter Size

Trial 1 (estradiol 12.5 µg and progesterone 25.0 mg, alone and in combination)

Administration of the progesterone and estradiol combination at a time corresponding to implantation (Days 16

TABLE 4

NUMBER OF SOWS RETURNING TO ESTRUS FROM EACH TREATMENT GROUP IN EXPERIMENT 1 (Injections on Days 16 and 17)

Treatment	Total Sows	# of Sows Returning to Estrus	% Pregnant at First Breeding
T_0 (control)	20	3	85.0
T_1 Estradiol alone	18	3	83.3
T_2 Progesterone alone	16	1	93.8
T_3 Estradiol & progesterone	16	1	93.8
T_4 Estradiol (6.25 µg)	10	1	90.0
T_5 Estradiol (3.125 µg)	10	4	60.0
T_6 Estrone (6.25 µg)	12	2	83.3
T_7 Estrone (12.5 µg)	13	3	76.9
T_8 Estrone (18.75 µg)	12	2	83.3

and 17) resulted in a 2% decrease for total pigs born and 3% decrease for pigs born alive (Table 5). However, this difference from the control was not significant ($P > .05$). Progesterone alone also resulted in no significant increase in total pigs born. Similarly, this treatment had no effect on the number of pigs born alive. A significant ($P < .05$) difference from the control was observed when sows were injected with estradiol alone (12.5 μg) for either total pigs born or pigs born alive. The increase was 12% for total pigs born and 14% for pigs born alive over the control group.

No significant effect ($P > .05$) of estradiol and progesterone, alone or in combination, is shown (Table 6) on birth weight per litter or per pig. The estradiol and progesterone combination group showed a decrease in birth weight per litter by 2% when compared to the control. Similarly, progesterone alone reduced the birth weight per litter by 2 percent. The estradiol alone treatment had no effect on birth weight per litter. In addition, no treatment had any significant ($P > .05$) effect on birth weight per pig. Estradiol alone decreased birth weight per pig by 12%, whereas combination of estradiol and progesterone or progesterone alone increased the birth weight per pig by 13 and 6%, respectively, when compared to the control. Thus, there was no apparent advantage by giving estradiol and

TABLE 5

EFFECT OF ESTRADIOL AND PROGESTERONE, ALONE AND IN COMBINATION (DAYS 16 AND 17), ON LITTER SIZE

	No. of Sows	Total Pigs Born[a]	Live Pigs Born
Control	17	12.7±0.7	11.7±0.7
Estradiol (12.5 µg) & progesterone (25.0 mg)	15	12.5±0.7	11.3±0.7
Estradiol alone (12.5 µg)	13	14.2±0.7*	13.3±0.8*
Progesterone alone (25.0 mg)	15	13.4±0.7	12.0±0.7

[a]Values are least squares regression means ± standard errors.

*Significantly ($P < .05$) different from control group.

TABLE 6

EFFECT OF ESTRADIOL (12.5 μg) AND PROGESTERONE (25.0 mg), ALONE AND IN COMBINATION[a], ON BIRTH WEIGHT PER LITTER AND PER PIG

	No. of Sows	Avg./Sow Birth Wt./ Litter	Avg./Sow Birth Wt./ Pig[b]
Control	17	17.6±1.0[c]	1.6±0.4
Estradiol (12.5 μg) & progesterone (25.0 mg)	15	17.3±1.1	1.8±0.4
Estradiol alone (12.5 μg)	13	17.8±1.1	1.4±0.4
Progesterone alone (25.0 mg)	15	17.2±1.1	1.7±0.4

[a]Injected 16 and 17 days of gestation.

[b]Weight in kilograms

[c]Values are least squares regression means ± standard errors.

progesterone in combination to pregnant sows at the time of implantation (Days 16 and 17).

Trial 2 (two levels of estradiol)

In this study, sows receiving 6.25 μg of estradiol for 2 days showed a decrease of 0.6 pig in total pigs born over the control (Table 7). A similar decrease (0.6) was also shown in pigs born alive for this treatment. However, when sows were treated for the same period (Days 16 and 17 of pregnancy) with 3.125 μg of estradiol, an increase (0.4 and 0.5 pig, respectively) was observed in both parameters, i.e., total pigs born and pigs born alive. The increases or decreases observed in both treatment groups were not significantly ($P > .05$) different from the control group.

Animals in Trial 2 generally failed to respond significantly ($P > .05$) to the 2 levels of estradiol treatment for birth weight per litter (Table 8). Animals treated with 6.25 μg of estradiol showed a 0.3 and 0.6 kg increase for either birth weight per litter or per individual pig, respectively, compared to the control. In addition, a 0.3 kg decrease for birth weight per litter and the same value of that from the control group for birth weight per pig was found in sows which were injected for 2 days with 3.125 μg of estradiol. However, none of these differences were significantly ($P > .05$) different from the control group.

TABLE 7

LEAST SQUARE MEAN VALUES (±SE) FOR LITTER SIZE FROM CONTROL AND TREATED SOWS WITH TWO LEVELS OF ESTRADIOL ON DAYS 16 AND 17

	No. of Sows	Avg/Sow Total Pigs Born	Avg/Sow Pigs Born Alive
Control	17	12.7±0.7	11.7±0.7
Estradiol (6.25 µg)	9	12.1±0.9	11.0±0.9
Estradiol (3.125 µg)	6	13.1±1.1	12.2±1.1

TABLE 8

EFFECT OF TWO LEVELS OF ESTRADIOL ON DAYS 16 AND 17 ON BIRTH WEIGHT PER LITTER AND PER PIG

	No. of Sows	Avg/Sow Birth Wt./ Litter(kg)	Avg/Sow Birth Wt./ Pig(kg)
Control	17	17.6±1.0[a]	1.6±0.4
Estradiol (6.25 µg)	9	17.9±1.4	2.2±0.5
Estradiol (3.125 µg)	6	17.3±1.7	1.6±0.6

[a]Values are least square regression means ± standard error.

These data suggest that there is no benefit to injecting estradiol alone at levels of 6.25 or 3.125 µg into pregnant sows at Days 16 and 17 of pregnancy.

Trial 3 (three levels of estrone)

The least square means (±SE) for litter size at term in Trial 3 is displayed in Figures 13 and 14. They show that levels of 12.5 µg of estrone resulted in the highest total number of pigs born (Figure 13) and pigs born alive per sow (Figure 14). This was 3.1 and 5.1 percent, respectively, over controls. In addition, levels of 6.25 µg of estrone also showed a small beneficial effect on either total number of pigs born (2.4%) or pigs born alive (2.6%). These differences were not significant ($P > .05$) in either of these two treatments. On the other hand, when a level of estrone of 18.75 µg was administered to sows on Days 16 and 17 of pregnancy, a marked decrease in litter size was found. This treatment resulted in a decrease in pigs/litter (1.2) for either total number of pigs born (Figure 13) or pigs born alive (Figure 14), respectively, in comparison with the control group. This decrease observed was not significantly ($P > .05$) greater than the control group.

The results of the effect of three levels of estrone on birth weight per litter and per pig are shown in Table 9. It can be seen that there is no trend for the treated

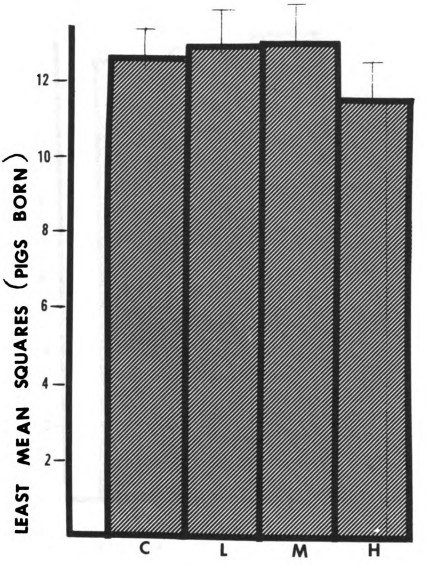


Fig. 13. Least square means (± Standard error) for total number of pigs born from control (C) and treated sows with estrone at low (L), medium (M), and high (H) dose.

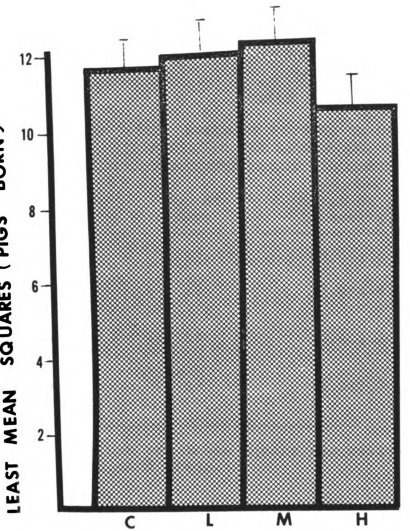


Fig. 14. Least square means for the effect of estrone at low (L), medium (M), and high (H) levels (± Standard error) vs. control (C) on the number of pigs born alive.

TABLE 9

EFFECT OF THREE LEVELS OF ESTRONE ON DAYS 16 AND 17 ON BIRTH WEIGHT PER LITTER AND PER PIG

	No. of Sows	Avg/Sow Birth Wt./ Litter(kg)	Avg/Sow Birth Wt./ Pig(kg)
Control	17	17.6±1.0	1.6±0.4
Estrone (6.25 µg)	10	17.0±1.4	1.4±0.5
Estrone (12.5 µg)	10	17.4±1.3	1.8±0.5
Estrone (18.75 µg)	10	16.2±1.3	1.7±0.5

[a]Values are lease square regression means ± standard error.

animals to have greater birth weights per litter than the control group. The results obtained from these were respectively 16.2, 17.0, or 17.4 kg/litter for high, low and medium estrone against 17.6 kg/litter for the control values. A reduction (0.2 kg) on birth weight per pig was also observed on the low estrone group. However, an increase of 0.1 and 0.2 kg per pig was found in both high and medium estrone treatments, respectively. Neither the reduction nor the increase caused by the levels of estrone in birth weight per pig was statistically significant ($P > .05$).

EXPERIMENT 2 (EFFECT OF A COMBINATION OF ESTRONE AND PROGESTERONE ON PLACENTAL DEVELOPMENT

The findings with regard to fertility of sows between Days 30 and 35 of pregnancy are shown in Table 10. The sows injected with a combination of progesterone (25.0 mg) and estrone (12.5 µg) for 10 days, starting on Day 14 of pregnancy, had either more corpora lutea (1.0) or more live embryos (0.3) per sow than control. On the other hand, the experimental group had a higher incidence of embryonic mortality than the control groups. The mean values for this latter factor were 28 and 25 percent, respectively. None of the differences observed above were statistically significant ($P > .05$).

TABLE 10

FERTILITY OF SOWS RECEIVING 10 DAYS OF ESTRONE (12.5 μg) AND PROGESTERONE (25.0 mg) TREATMENT[a] MEASURED AT DAYS 30 TO 35 OF PREGNANCY

	No. of Sows	C.L./ Sow	Live Embryo/ Sow	Embryonic Mortality(%)
Control	9	15.8±0.7	11.9±1.5	25
Progesterone & estrone treatment	9	16.8±0.8	12.1±1.4	28

[a]Injected from Day 14 to 23 of pregnancy.

The results of the effect of a progesterone and estrone combination on the intra-uterine environment are summarized in Table 11. There was a 34.6% increase of allantoic fluid volume in the treated sows compared to those of the control group. Also, placental area was 16.5% larger in the treated group. Analysis of variance indicated that these differences were highly significant ($P < .001$).

A negative response for the fetal crown-rump length was observed when sows were injected with estrone (12.5 µg) and progesterone (25.0 mg) in combination for ten days. The mean conceptus in the control group was 0.1 cm longer than the experimental group, and this difference was statistically significant ($P < .05$).

There was no significant difference between control (2.5 ml) and treatment group (2.4 ml) for amniotic fluid volume. The weight of fetal membranes was 4.1% higher, whereas the weight of the fetus was 10% lower in the experimental group than in the control group. The differences observed between experimental and control groups are not statistically significant ($P > .05$).

The protein concentration, pH, and osmolality observed in the fetal fluids at Days 30 to 35 of gestation are shown in Table 12. With regard to the allantoic fluid, a significant ($P < .01$) increase was found in the pH of the treated sows compared to the control group. However, the

TABLE 11

EFFECT OF 10 DAYS INJECTION OF PROGESTERONE (25.0 mg) AND ESTRONE (12.5 µg) ON PLACENTAL DEVELOPMENT BETWEEN DAY 30 AND 35 OF PREGNANCY

	Least Square Means±SE per Sow:		Percent Change
	Control (9)	Treatment (9)	
Allantoic fluid (ml)	149.4±7.1	201.1±7.0**	+34.6
Amniotic fluid (ml)	2.5±0.1	2.4±0.0	-4.0
Wt. fetal membranes (g)	29.2±1.3	30.4±1.3	+4.1
Wt. fetus (g)	2.0±0.1	1.8±0.1	-10.0
Crown-rump length (cm)	2.7±0.04	2.6±0.04*	-3.7
Placental area (cm^2)	258.9±8.1	301.5±8.2**	+16.5

*Significantly ($P < .05$) different from control group.

**Significantly ($P < .001$) different from control group.

TABLE 12

LEAST MEAN SQUARES (SE) FOR PROTEIN CONCENTRATION, PH, AND OSMOLALITY OF THE FETAL FLUIDS FROM CONTROL AND TREATED SOWS[a], RECORDED DAYS 30 TO 35 OF GESTATION

Contents	No. Samples		Allantoic Fluid		Amniotic Fluid	
	Cont.	Treat.	Control Sows	Treated Sows	Control Sows	Treated Sows
Protein (mg/ml)	80	38	2.53±0.3	1.36±0.4*	1.41±0.1	3.5 ±0.2***
pH	36	30	6.17±0.04	6.33±0.04**	7.54±0.04	7.66±0.04*
Osmolality (mOsmols/kg)	80	38	131.91±4.0	137.66±5.9	228.97±7.1	260.43±10.4*

[a]Sows treated (estrone 12.5 µg & progesterone 25.0 mg) from Days 14-23 of pregnancy.

*Significantly ($P < .05$) different from the control.

**Significantly ($P < .01$) different from the control.

***Significantly ($P < .001$) different from the control.

opposite was found for protein concentration. The mean protein values were 2.53 and 1.36 mg/ml for the control and treatment group, respectively, and this difference (1.17 mg/ml) in favor of the controls is statistically significant ($P < .05$). In addition, osmolality was not significantly ($P > .05$) different for allantoic fluid from control and treated animals.

In the amniotic fluid, the protein concentration was 3-fold higher in the treated sows than in the controls (3.55 vs 1.41 mg/ml, respectively). Analysis of variance indicated that this difference was highly significant ($P < .001$). Furthermore, a significant ($P < .05$) difference between the groups was found for pH measurement. The pH was 2% higher in the hormone-treated animals (7.66) than in those injected with vehicle alone (7.54). There was a significant ($P < .01$) difference in osmolality between the experimental and control animals. This difference was 12% greater in the treated animals when compared to the control groups.

Table 13 shows the percentage distribution of protein bands in the fetal fluids recovered from pregnant sows between Days 30 to 35. There were eight bands for both allantoic or amniotic fluid for either treated or untreated sows. In the allantoic fluid, there were two protein bands which were more frequently found in the control than in the

TABLE 13

PERCENTAGE DISTRIBUTION SHOWING PROTEIN BANDS OF VARYING Rf VALUES OF 30 TO 35 DAY GESTATION SWINE FETAL FLUIDS

	No. of Samples	Rf Values							
		.00-.09	.10-.19	.20-.29	.30-.39	.40-.49	.50-.59	.60-.69	.70-.79
Allantoic fluid									
Control	73	89(65)[1]	84(61)	58(42)	67(49)	37(27)	96(70)	81(59)	36(26)
Treated	25	68(17)*	88(22)	68(07)	84(21)	28(7)	80(20)*	96(24)	56(14)
Amniotic fluid									
Control	62	84(52)	89(55)	61(38)	76(47)	60(37)	85(53)	82(51)	47(29)
Treated	26	96(25)	69(18)*	23(6)**	92(24)	54(14)	92(24)	77(20)	54(14)

[1]No. of bands for each group

*Significantly ($P < .05$) different from the control group.

**Significantly ($P < .005$) different from the control group.

treated animals. The differences between them (bands with Rf values less than 0.09 and between 0.50 and 0.59) were significant ($P < .05$). With regard to amniotic fluid, the bands number with Rf values between 0.10 and 0.19, and 0.20 and 0.29 were significantly more frequently observed in the control than in the experimental group.

DISCUSSION

The results of Experiment 1 indicate that estradiol (12.5 μg) and progesterone (25.0 mg) combination at this level, imposed during implantation (Days 16 and 17), did not exert a beneficial effect on the litter size in swine as was previously noted with estrone and progesterone. A similar study by Day *et al.* (1963) has demonstrated that a combination of estradiol and progesterone, administered subcutaneously either from Days 11 to 25 or 17 to 25 of gestation, had no significant increase in litter size. In turn, Reddy *et al.* (1958) reported a significant increase in litter size at Day 56 of gestation when exogenous steroids were given to sows for 10 days, starting on Day 14 of pregnancy. Also, Wildt *et al.* (1976) reported that steroid therapy for 10 or 2 consecutive days during implantation (starting Day 14 or 16 of gestation, respectively) caused a beneficial effect on litter size at term. Similarly, field studies by Morcom *et al.* (1976) showed an increased litter size (1.5 pigs) after administration of steroids on Days 16 and 17 of gestation. Reddy *et al.*, Wildt *et al.*, and Morcom *et al.* used estrone instead of estradiol in combination with progesterone.

Estradiol is ten times biologically more potent than estrone. Thus, the present result suggests that this lack of effect with estradiol (in combination with progesterone) to increase litter size might be due to its greater potency than estrone, and a lower level of estradiol might be effective.

No significant effect on either total pigs born or pigs born alive was observed when progesterone alone (25 mg) was injected into the pregnant sows during early gestation. This result is in agreement with those of Wildt *et al.* (1976). Sammelwitz *et al.* (1956) has reported that progesterone alone at a level of 100 mg daily (but not 200 mg) had little beneficial effect in reducing embryonic mortality when given to sows for 26 days following mating.

Additional observations recorded here showed that estradiol (without progesterone) at levels of either 3.125 or 6.25 µg had no significant effect on litter size. However, estradiol at a level of 12.5 µg did. Rigor *et al.* (1963) reported that exogenous estradiol at a dose of either 20 or 100 µg injected daily for 15 days (beginning on Day 9) showed no effect on embryonic survival in the pig.

The role of estradiol in controlling litter size remains unclear. However, a synergistic action of progesterone may cause the effect. There are several lines of evidence which lend credence to this hypothesis. Perry *et al.*

(1973) noted that pig blastocysts were capable of synthesizing estrogen from progesterone and hypothesized that this might be involved in the process of implantation. Furthermore, it is known that exogenous estrogen in rats causes the release of histamine, prostaglandin and serotonin, which induces a local increase in capillary permeability. Dickman *et al.* (1977) reported that the increase in capillary permeability is a necessary prerequisite for the induction of implantation in most species. Considering the results of the present study, it is possible that exogenous estradiol in combination with endogenous progesterone could be reducing embryonic mortality by increasing the number of implantation sites, but this hypothesis requires further study.

In Trial 3 of Experiment 1, it was shown that there was no advantage to giving estrone to swine during the implantation time. This has been found in earlier research by Wildt *et al.* (1976), who reported that injection of a 12.5 µg level of estrone for 10 days during early gestation resulted in no beneficial effect on litter size at term. However, in the present study, estrone at 18.75 µg decreased litter size when it was given to pregnant sows for 2 days (Days 16 and 17). From this, one can conclude that the relationship between exogenous estrone administered and litter size is a sensitive one and that the maximum effective level is 12.5 µg.

In Experiment 2, no significant difference was observed in either ovulation rate, live conceptus or embryonic mortality between control and treated sows at 35 days of gestation. In agreement with the present results, Knight *et al.* (1974b) points out that a combination of progesterone (1.1 or 3.3 mg/kg/day) and estradiol (0.55 µg/kg/day) injected into bilaterally ovariectomized gilts from Day 4 to Day 40 of gestation had no effect on pregnancy rate, number of live embryos or percent embryonic survival. However, preliminary studies (Reddy *et al.* 1958) have shown a treatment effect on the above parameters by the middle of gestation in swine.

The least squares mean for allantoic fluid volume and the mean size of placental area were significantly higher in sows injected with progesterone and estrone than in control sows measured between Days 30 and 35 of pregnancy. These results are in agreement with those of Reddy *et al.* (1958). Also, Knight *et al.* (1974b) found that placental length and allantoic fluid volume were significantly ($P < .05$) increased in the sham-operated, high-progesterone and ovariectomized, high-progesterone gilts compared to those of the control group. McGovern *et al.* (1978) observed no significant difference in either mean volume of allantoic fluid or mean placental surface area per conceptus between treated and control sows by Days 46 to 49 of gestation. Therefore,

we can conclude that the increase in litter size resulting from the injection of estrone (12.5 ng) and progesterone (25.0 mg) in combination may be due to an increase in the number of implantation sites rather than an increase in the uterine placental sufficiency.

The observation that the fetal crown-rump length in the present study is significantly ($P < .05$) greater for the control group as compared with embryos from the sows in the treatment group agrees with previous findings (Knight *et al.* 1974b).

The work described here also indicates that there is no significant hormone therapy effect on either amniotic fluid volume, weight of the fetal membranes or weight of the fetus. Actually, the weight of the fetus and amniotic fluid volume was less in the treated sows than in the control. Reddy *et al.* (1958) found that the mean weight of the individual embryo was similar for both groups. However, Knight *et al.* (1974b) observed significantly heavier embryos for the control than for the treatment group.

Further results indicate that pH in the fetal fluids is significantly higher in the treated sows than in the controls. Also, protein concentration and osmolality of the amniotic fluid were significantly increased by hormone therapy. Osmolality was not significantly affected by the exogenous steroid hormones in the allantoic fluid, whereas

the protein concentration was significantly less in the treated sows than in the control.

These data indicate that the combination of progesterone and estrone at levels of 25.0 mg and 12.5 µg, respectively, increased secretory activity. Furthermore, the reduction of the protein concentration as well as the lack of a significant effect on osmolality by hormone therapy in the allantoic fluid appears to be associated with its increase in volume in the conceptuses from pigs by Day 30 of gestation, as has been shown by Knight *et al.* (1977).

Two protein bands in either allantoic fluid (Rf values between 0-0.09 and 0.50-0.59) or amniotic fluid (Rf values 0.10-0.19 or 0.20-0.29) were significantly ($P < .05$) less often observed in the treated than in the control group in the present study. Murray *et al.* (1972) reported that the presence of functional corpora lutea would be necessary for the secretion of two uterine proteins (Fraction IV and V) during the estrus cycle of swine. This study was confirmed by Chen *et al.* (1973) and Knight *et al.* (1973) who added that progesterone alone or progesterone and estradiol in combination were the key hormones controlling the secretion of the specific protein components (Fraction IV and V) from the secretory cells of the uterine endometrium. Although, in our experiment, we found that hormone therapy resulted in a decreased incidence of two protein bands in fetal fluids

when protein was measured at 30 to 35 days of pregnancy, a possible role of these protein changes in embryonic mortality may exist.

Future work in this field must include studies of the identification of the exact proteins in each band. Furthermore, the effect of the combination of estradiol at a level of 1.25 μg (10% of the dose used in the present studies) and progesterone at a level of 25.0 mg, given to pregnant swine at the time of implantation, should be tested.

SUMMARY AND CONCLUSIONS

Embryonic mortality in swine is of extreme importance during early gestation. The incidence is highest during this time with estimates from 35 to 40%. To test the ability of exogenous steroids to affect litter size and placental development when imposed during the implantation period was the main goal of this study. The following conclusions resulted from the research:

1. Estradiol (12.5 μg) had no effect on litter size compared with estrone injected into intact pregnant sows in combination with progesterone (25.0 mg) on Days 16 and 17 of pregnancy.
2. Administration of estradiol alone at a level of 12.5 μg resulted in a significant ($P < .05$) increase in litter size at term.
3. Injection of high estrone (18.75 μg) to pregnant sows for 2 days (Days 16 and 17) tended to decrease litter size.
4. Progesterone (25.0 mg) given in combination with estrone (12.5 μg) to pregnant sows for a 10-day interval (14 to 23) showed the following placental results when measured at 30 to 35 days of gestation:

a. There was a significant ($P < .01$) increase for both allantoic fluid and placental surface area in treated sows compared to controls.
b. The fetal crown-rump length was significantly ($P < .05$) greater in the control than in the treatment group.
c. Protein concentration, pH and osmolality in the amniotic fluid and pH in the allantoic fluid were significantly ($P < .05$) higher in the experimental animals than in the controls.
d. There was a significantly ($P < .05$) lower protein concentration in the allantoic fluid from treated sows than from the controls.
e. Protein bands with Rf values between 0 and 0.09 or between 0.50 and 0.59 in the allantoic fluid were significantly ($P < .05$) more frequent in the control than in the treatment group.
f. Protein bands in the amniotic fluid with Rf values between 0.10-0.19 and 0.20-0.29 were significantly ($P < .05$) less often observed in the treated group than in the control.

APPENDICES

APPENDIX A

EXPERIMENTAL PROCEDURES FOR ELECTROPHORESIS

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EXPERIMENTAL PROCEDURES FOR ELECTROPHORESIS

For analytical columns, glass tubes 8 cm by 0.4 cm ID, marked 2 cm from one end (the top) are used. Place the tubes in rubber serological stoppers so that they stand upright. Or, if there is some means of keeping the tubes upright, simply wrap the bottoms of the tubes tightly in parafilm, and clamp them in position. (If the Canalco fluorescent light holder is available clamp the tubes to the face of the holder, using the metal spring across the front. Leave the light switched off to prevent bubble formation from lamp heat.) Prepare a Pasteur pipette with a drawn out tip for overlayering and fill it with water. This should deliver about one drop per second.

For convenience and to make storage possible, the ingrediants for gel preparation are made up in several solutions (Table 14), called A (containing concentrated lower buffer), B (stacking buffer), C (concentrated acrylamide and cross-linking reagent for lower gels), D (acrylamide for upper gels), E (riboflavin, for initiation of polymerization), and F (ammonium persulfate, for polymerization).

Prepare 1 ml of lower gel solution per tube by mixing 1 part A to 1 part C to 2 parts fresh F. The solutions should be at room temperature lest bubbles of air form while the gel is polymerizing. Add the F last. Using a disposable pipette, add the gel solution to the 2 cm mark on the tubes, then tap the tubes to insure that no air bubbles have been trapped in them. Carefully overlayer the gels with 4-6 mm of water, using the drawn out Pasteur pipette. The initial sharp boundary line will blur because of diffusion, but a new sharp line will appear ten minutes to one hour later, about 2 mm below the original interface. This indicates that the gel has polymerized. Let the gels stand another 10-20 minutes.

Remove the upper liquid by flicking the tube, and place the tubes near a fluorescent light. An apparatus consisting of three stacked circular 30-watt Cool White fluorescent lamps mounted on an aluminum plate with the ballasts is convenient for polymerization of analytical and preparative gels and can be constructed for about $50.

Upper gel solution consists of 1 part B to 2 parts D to 1 part E to 4 parts water. 0.2 ml of this is pipetted onto the top of each lower gel and is then overlayered with 4-6 mm of water.

Turn on the fluorescent lamp, and let the upper gels photopolymerize for 30 minutes. They will be opalescent. Flick off the liquid. Do not leave the gels exposed to the lamp for excessive periods, as heat from it tends to produce air bubbles in the gels as well as convection in incompletely polymerized regions. The gels must now be used within three hours or problems occur because of diffusion between the solvents in the upper and lower gels.

If stoppers have been used, insert a syringe needle between the stopper and tube to prevent a vacuum from forming when the stopper is pulled off. If there are air bubbles in the bottom of a gel tube, remove them by adding upper buffer with a Pasteur pipette. Dry the outsides of the gel tubes, and push their tops through the rubber grommets (medicine dropper tops) which give a leakproof fit.

Add 200 ml of upper buffer to the lower buffer container. Set the upper buffer container with all its holes plugged with gel tubes or stopper tubes onto the metal rack so that the gel tubes are in contact with the buffer. Add 1 ml of tracking dye to 200 ml of upper buffer, and pour this into the upper buffer container. Remove air bubbles in the tops of the gel tubes by directing a stream of buffer onto the gels with a Pasteur or long-tip pipette.

Connect the electrodes to the power supply: positive polarity to the lower chamber electrode, negative to the upper.

If the Canalco apparatus is used, the gel tubes are inserted into silicone grommets in a plastic dish. Don't force the tubes past the lower edge of the dish, as this tends to tear the grommet.

In preparing samples for disc electrophoresis, it is best to dialyze against the Tris-glycine upper buffer. In any event, the presence of large amounts of salts in the samples should be avoided, as they delay stacking for periods proportional to the amount present.

The dialyzed samples should be made 10% in sucrose or glycerol, then layered with a pipette or syringe onto the upper gels of appropriate tubes. About 50 micrograms of protein is applied per tube. More should be applied if the preparation is very heterogeneous.

Turn on the power supply and run at 2.0 milliamperes per tube for 1.5 to three hours, until the blue tracking dye has migrated to within 2-5 mm of the bottom of the lower gel. Don't run the dye off the gel. Smaller ID tubes should be given less current or they will overheat.

Any tubes may be taken off at any time, but power must be shut off while doing this. The amperage should be cut back after a tube is removed.

Remove the gels from the glass tubes by "rimming." Insert a 2-inch 25 gauge hypodermic needle between the gel and the glass tube. Hold the needle steady and rotate the tube as the needle moves farther into the tube. Rotate the tube when withdrawing the needle also. It will probably be necessary to rim the gel from both ends of the tube. Do not try to use a longer needle as these are hard to control. The needle should be attached to a slow stream of water for lubrication during rimming. Alternatively use an 18 gauge needle insert with a rounded end; gel tubes are rimmed while immersed in water. If the gel is obstinate even after repeated rimming, use a rubber Pasteur pipette bulb filled with water to force the gel out.

Place the gels in the protein stain solution for one hour. Heating the gels in the staining solution at 95°C for 15 minutes provides faster staining. The gels may be destained by allowing them to stand in several changes of 7% acetic acid until clear or a commercial diffusion destainer may be used. Destaining can be done faster by electrophoresis. Place the gels in constricted destaining tubes. These are slightly larger than the tubes the gels were cast in, but pinched off at the lower end to keep the gels in place during destaining. Electrophorese until all the free dye is removed, using 7% acetic acid in both buffer chambers. Alternatively, a transverse destaining apparatus can be used.

TABLE 14

SOLUTIONS FOR ELECTROPHORESIS EXPERIMENTS

Lower Gel			Upper Gel		
A.	1 N HCl	24.0 ml	B.	1 M H_3PO_4	25.6 ml
	Tris	18.2 g		Tris	5.7 g
	TEMED*	0.23 ml		TEMED*	0.46 ml
	Water to 100 ml (pH 8.9)			Water to 100 ml (pH 6.9)	
C.	Acrylamide	30.0 g	D.	Acrylamide	10.0 g
	Bisacrylamide	0.8 g		Bisacrylamide	2.5 g
	Water to 100 ml			Water to 100 ml	
F.	Ammonium persulfate	0.14g†	E.	Riboflavin	4.0 mg
	Water to 100 ml			Water to 100 ml	
H.	50X concentrated Upper Buffer:				
	Tris	30.0 g			
	Glycine	144.0 g			
	Water to 1000 ml				
	Use 20 ml/liter final buffer (pH 8.3)				

1 N HCl = 83 ml conc HCl/liter

1 M H_3PO_4 = 68.3 ml 85% H_3PO_4/liter

Tracking dye: 0.005% Bromophenol blue in water.
Staining solution: 1% Amido black in 7% acetic acid.

*TEMED is N, N, N´, N´ tetramethylethylenediamine. TEMED, acrylamide, methylene bisacrylamide and Tris can be obtained from Eastman Kodak. The other chemicals were purchased from the Fisher Chemical Company.

†Riboflavin and light may be used instead. Use the same concentration of riboflavin as is used in polymerizing upper gels; an excess inhibits polymerization. Note that polymerization is not as complete in riboflavin polymerized gels, and protein Rf's will be greater.

APPENDIX B

PUBLICATIONS BY THE AUTHOR

APPENDIX B

PUBLICATIONS BY THE AUTHOR

Full Paper

Placental and paraplacental surface area in conceptuses from sows treated with progesterone and estrogen during early pregnancy, by P. T. McGovern, C. B. Morcom, W. Ferreira de Sá and W. R. Dukelow. Journal of Animal Science, submitted 1978.

Abstracts

See the following pages for texts of abstracts.

LAPAROSCOPY AND REPRODUCTIVE PERFORMANCE IN *Macaca Fascicularis*

J. P. Mahone, W. Ferreira de Sá,
M. E. Lehnert and W. R. Dukelow

Laparoscopy has been performed on a colony of *Macaca fascicularis* over a period of nearly eight years for repeated ovarian observation before, during and after ovulation. It has been used for pregnancy diagnosis and determination of precise gestation length. Laparoscopy has had no effect on ovulation or cycle length. Of 334 cycles where no laparoscopy was performed, the mean cycle length was 29.4 $\pm$ 11.1 days compared with 30.6 $\pm$ 5.0 days in 252 cycles where laparoscopy was performed. By combining laparoscopic prediction of ovulation time and exposure of the female to the male for a 20-minute period, six pregnancies occurred from 80 matings. Two of these pregnancies were terminated by Cesarean at 92 days. The others resulted in gestation lengths of 164 days, 15 hr; 165 days, 10 hr; 163 days, 3 hr; and 168 days, 12 hr. In later trials with 21 females over a ten-month period, estimates of probable ovulation were made based on menses records and laparoscopy. Females were then exposed to males for periods ranging from several hours to several days. Five pregnancies occurred in animals which had been subjected to extensive laparoscopy. Two abortions occurred (112 and 123 days p.c.), due to unknown causes, to animals which had been laparoscoped 43 and 54 times previously, respectively. Three animals delivered live infants (all males) conceiving after 38, 49 and 47 laparoscopies. The gestation lengths of these three births were, respectively, 147-153 days, 165 days, and 165-171 days. Adhesions can occur after extensive laparoscopy (usually with 30 or more laparoscopies) but by careful technique they can be largely eliminated. If adhesions do occur, they can be corrected by laparoscopic electrocoagulation and cutting.

We conclude that extensive laparoscopy does not interfere with the mechanism of ovulation, normal cyclicity, or normal pregnancy.

Presented at Amer. Soc. Primatologists Meeting, Seattle, Wash., April 1977.

ARTIFICIAL INSEMINATION WITH FROZEN SEMEN USING LAPAROSCOPY IN SWINE

C. B. Morcom, W. Ferreira de Sá[1]
and W. R. Dukelow

The laparoscope has been shown to have a wide variety of uses ranging from ovarian observation to the actual sampling of uterine fluid in swine (J. Reprod. Fert. 44:301, 1975). The present project was designed to determine if pregnancies could be obtained after the laparoscopic deposition of small volumes of frozen-thawed semen directly into the oviduct. Gilts were prepared for laparoscopy by the procedure of Wildt *et al.* (J. Reprod. Fert. 35:541, 1973). The ovary and oviduct were located and the latter was positioned, using laparoscopic forceps and a probe, so that a 22 ga. spinal needle would be directed into the oviductal lumen. This was done as near to the tubo-uterine junction as possible. While the needle was being positioned, liquid nitrogen-stored semen (supplied by Dr. V. G. Pursel, USDA) was thawed in a light aluminum container held at 37°C in a water bath. Approximately 0.30 ml semen was inseminated into each oviduct. Nine gilts were inseminated. Of these, two did not return to estrus. On one (inseminated in only one oviduct) a laparoscopic examination was performed 29 days after insemination. A hysterectomy was performed and five embryos were recovered. A second pregnancy was allowed to go to term and resulted in the birth of four pigs. Present studies are examining various sites of insemination and the optimal time for insemination relative to estrus. This technique is applicable in cases of extremely rare or valuable animals, or where only a small volume of semen is available for shipping or insemination. It also offers valuable opportunities for examining the basic physiological processes of fertilization, capacitation and implantation in swine.

Presented at the 11th Annual Meeting Midwestern Section of the Amer. Soc. of Anim. Sci., Carbondale, Illinois, January 1978.

THE EFFECTS OF EXOGENOUS STEROID HORMONES ON LITTER SIZE, EARLY EMBRYONIC SURVIVAL AND CHORIONIC SURFACE AREA IN PREGNANT SWINE

W. Ferreira de Sá, C. B. Morcom,
P. T. McGovern and W. R. Dukelow

A significant increase in litter size has been found to result from the daily injection of sows and gilts with a mixture of estrone and progesterone (1:2000) both when treatment was given from Day 13 to Day 22 of pregnancy and when treatment was restricted to Days 16 and 17 (Day 1 = day of breeding). The observations recorded here were designed (Trial 1) to determine the effect of the 10-day treatment on embryonic mortality and on the size of the chorionic sac, and (Trial 2) to see if a beneficial effect on litter size could also be obtained by two-day treatment of sows with estradiol and progesterone alone or in combination (1:2000 ratio). In Trial 1, the treated sows were given daily injections of 12.5 µg estrone and 25 mg progesterone in an oil base from Day 14 to Day 23 of pregnancy, and the control sows were given daily injections of the vehicle alone over the same period. The animals were slaughtered between Days 30 and 35, and the reproductive tracts and the contents were examined. No differences in embryonic mortality were evident between the control and treated sows. The mean values per conceptus for the control vs. treated groups were: chorionic surface area, 263 cm vs. 302 cm; weight of fetal membranes, 32 g vs. 30 g; amniotic fluid volume, 2.5 ml vs. 2.5 ml; allantoic fluid volume, 149.4 ml vs. 201.1 ml; fetal weight, 2.0 g vs. 1.8 g; fetal crown-rump length, 2.7 cm vs. 2.6 cm. In Trial 2, four groups of sows were treated on Days 16 and 17 of pregnancy with (a) 12.5 µg estradiol and 25 mg progesterone in an oil base, (b) 12.5 µg estradiol in oil, (c) 25 mg progesterone in oil, or (d) the vehicle alone. The results obtained at farrowing revealed no differences between the four groups with regard to litter size: total number of piglets per sow, (a) 10.8, (b) 10.9, (c) 11.5, (d) 11.1; live piglets per sow, (a) 9.7, (b) 10.7, (c) 10.6, (d) 10.7; total weight of litter, kg, (a) 15.1, (b) 15.8, (c) 15.0, (d) 15.9. The findings indicate that at the dose used, estradiol and progesterone do not have the beneficial effect on litter size previously observed with estrone and progesterone.

Presented at 1978 Joint Meeting of American Dairy Sci. Association and American Soc. of Anim. Sci., East Lansing, Mich., July 1978.

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