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Hormonal Modification of the Intra-uterine Environment in Swine and its Effect on Embryonic Viability

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ABSTRACT

Embryonic mortality was found to be the major factor limiting the reproductive performance in swine. A therapeutic approach has been developed which alters favorably the uterine environment during certain initial stages of pregnancy.

A preliminary investigation suggested the desirability of employing exogenous progesterone and estrone to promote conditions conducive to lower prenatal mortality and higher embryonic survival. In an endeavor to find the most efficient therapy, various dosages and ratios of the hormones were administered at several different critical stages of pregnancy during a two-year experimental period. Further research revealed that a progesterone-estrogen therapy in the form of daily injections of 25 mg. of progesterone plus 12.5 μg. of estrone (2000:1) per gilt for ten consecutive days beginning on the 14th day of gestation would provide a more favorable uterine environment and thus limit the embryonic mortality to only 13.51 percent. The condition of the uterus that would promote maximum embryonic viability was ascertained by physiological, radiological, chemical and histological criteria. The percentages of embryonic mortality were 21.74, 18.75 and 18.18 in three other treatment groups, compared to 23.3 percent mortality in the non-treated group of gilts. Corresponding increases in the linear capacities of the uteri, volume of the uterine fluids and weights of the reproductive tracts due to treatments were observed. The reproductive performance of gilts that received the ovarian hormones in 2000:1 ratio was strikingly uniform.

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INTRODUCTION

Much of the sterility in domestic animals and in women commonly attributed to failure of the union of gametes may be due to the death and resorption or abortion of the embryos. This pathological morbidity, viz., embryonic or prenatal mortality, can be recognized readily in the living animal only when abortion occurs at advanced stages. However, the fact remains that, in case the dead embryos are reabsorbed in situ or are aborted at an earlier stage, the mortality cannot be diagnosed except on autopsy. Therefore, a thorough investigation of the physiopathology of the embryonic mortality in farm animals might enable a broader approach to the general problem of fertility and sterility.

The prenatal losses in swine vary generally between 30 and 40 percent (Squiers, Dickerson and Mayer, 1952; Rathnasabapathy, Lasley and Mayer, 1956; Lerner, Mayer and Lasley, 1957). These losses appear even more alarming in repeat breeder cattle (Tanabe and Almquist, 1953, 54.1 percent; and Casida, 1953, 59.4 percent). Similar losses have been reported in many other species of farm and laboratory animals. Prenatal mortality is the end result of maladjustment somewhere within the intricate mechanism of fertilization and segmentation, transportation of the embryo through oviducts and uterus, the establishment of placental connections with the maternal organism, and the nutrition of the growing embryo. Embryologists suggest that defective internal environment, usually in the nature of faulty implantation, is the primary cause of morbidity in mammalian embryos; whereas, geneticists believe that the embryo itself may possess the internal defects which are hereditary and may manifest themselves during the earliest stages of embryonic existence. However, it has generally been recognized that in mammals, many factors, both genetic and environmental, may prevent the development of the zygote and that there are definite critical periods and stages in the embryonic development when probability of death is higher and more frequent than in others.

The specific cause or causes of embryonic deaths are not known. A positive

assumption could be that a deficiency of progesterone due to decreased luteal function might be involved. The work of Rowson *et al.* (1953) in cattle and that of Black *et al.*(1953, 1954) in cattle and rabbits indicated the possibility of a deficiency of progesterone in the repeat breeder cows as a cause of embryonic mortality. The chemical investigation of the urinary metabolites of progesterone in swine by Glasgow and Mayer (1957) lends further support to the assumption above. An important question is whether the embryos die first and then the excretion of progesterone becomes less or whether an initial sub-minimal level of progesterone results in embryonic death. If the latter proposition is true, theoretically then, the embryonic death could be prevented by means of progesterone therapy at critical stages during pregnancy.

From the time the ovum is fertilized until the termination of parturition, both the complementary and the antagonistic actions of the estrogen and progesterone are constantly at work. This consideration substantiated the rationality of employing a combination of both hormones as a therapeutic measure. Therefore, this investigation is an attempt to explore the possibilities of insuring a better intra-uterine environment and thereby preventing prenatal losses through the administration of exogenous progesterone and estrogen at appropriate stages of gestation.

REVIEW OF LITERATURE

Synergism Between Estrogen and Progesterone:

The work of Smith (1931) indicates that progesterone maintains a physiological balance of estrogens during pregnancy by promoting their excretion through the kidneys when present in excessive amounts. As gestation advances, the amount of estrogenic substances increases, reaching a maximum at term. In this way, the balance between the estrogens and the progesterone is disturbed. Estrogen becomes dominant and sensitizes the uterus to the oxytocic principle of the posterior lobe and of the pituitary gland and labor ensues. Allan and Dodds (1930) and others expanded this theory. It is entirely possible that at other periods in gestation a deficiency of progesterone or an excess of estrogens might result in spontaneous abortion or premature labor (Falls and Lackner, 1936).

The idea that progesterone cannot by itself produce progestational proliferation was suggested by the observations of Loeb and Kountz (1928) for the guinea pig and Corner and Allen for the rabbit (1929). The animals have to be put in the proper physiological state by the injection of follicular hormone before progesterone can produce its effects. Hisaw and Leonard (1930), by their extensive work with rabbits, showed that the follicular hormone was not only necessary for the beginning of the reaction by the corpus luteum extract but that the follicular effect had to be present if the corpus luteum extract was to prolong the progestational reaction. They stated that estrogens appeared to govern the growth and enlargement of the uterus, while progesterone modified the struc-

tures already formed. The fact that estrogen was a growth promoting substance was indicated by the numerous mitotic figures during its maximum influence; whereas, progesterone, when given alone, did not seem to possess this activity.

Korenchevsky and Hall (1937) obtained a marked synergistic activity with progesterone and estrogen in the ratio of 1500:1 in producing a state of progestation in the rat uterus. B. Hisaw, *et al.* (1937a) and Engle and Smith (1938), working with the Rhesus monkey, demonstrated a synergistic effect of the two hormones on the uterus and cited that the overgrowth was more marked and the progestational state more typical when both hormones were administered. Synergism was shown during gestation by Courrier and Kehl (1938a, b) and by Courrier and Jost (1939a). In the castrated pregnant rabbit, gestation could be maintained successfully by the synergistic action of progesterone and estrogen in doses of 0.5 mg. and 0.66 μg. per day (750:1), respectively. The ratio of progesterone to estrogen was not the same in the rabbit, the hamster, and the rat (Rotchild *et al.,* 1940). Hence it seems logical to assume that the ratio for maximum synergism between progesterone and estrogen varies with different species.

Certain hypotheses have been formulated to explain the mechanism of this synergism between the two gonadal hormones. Pincus (1937) proposed that estrogen and progesterone were destroyed in the organism by the same enzymatic system. If this system was saturated with estrogen, then progesterone would be destroyed at a slower rate and thus its action would be prolonged. Astwood (1938) and Reynolds (1939) suggested that estradiol could assist the action of progesterone by increasing the circulation of blood in the uterus. However, such a hypothesis fails to explain how synergism can sometimes be transformed into antagonism with an increase of estrogen. In a summary of Allen's work (1937), Selye (1947) noted, "The important fact to retain is that progesterone requires the synergistic effect of the folliculoids in order to be fully effective in the maintenance of pregnancy, but large doses of the folliculoids counteract this gestation-maintaining effect of the corpus luteum hormone."

Some Empirical and Rational Therapies of Progesterone and Estrogen:

Lyons (1943) successfully maintained pregnancy in hypophysectomized-oophorectomized rats by means of daily injections of 1 μg. of estrone together with 3 to 4 mg. of progesterone. With the same dosage of estrone and progesterone, pregnancy was also maintained in vitamin B_6 deficient rats (Nelson, Lyons, and Evans, 1951). Pregnancy was maintained in the absence of dietary protein by means of a combination of estrone and progesterone in 100 percent of the treated animals as compared to 80 to 100 percent absorptions in the control animals (Nelson and Evans, 1954). Warwick *et al.* (1943) observed some evidence that the number of embryos living until implantation was increased in rabbits treated with progesterone only, and that the early mortality was decreased by treatment with progesterone and estrogen in combination. They stated, "The most logical assumption is that they (progesterone and estrogen) improved the

uterine environment and thereby prolonged the life of the embryos."

Laing's (1949) results on early foetal death in cattle indicated the possibility of progesterone deficiency about the time of implantation. Field observations of Olds and Seath (1951) and Stewart (1952) confirmed Laing's experimental observations. Herrick (1953) reported that 35 percent of the repeat breeder cows settled on the first service following progesterone therapy, compared to only 5 percent of the controls. Dawson's work (1954) showed that 47 percent of the treated animals settled, against 17 percent of the controls. In both instances above, progesterone therapy seems to be obviously beneficial. Yet in another investigation 44 percent of the repeat breeder cows that were injected with 50 mg. of progesterone per day beginning three days after heat, had normal embryos at 34 days as compared with 33 percent in control animals. When 200 mg. of progesterone were used daily, 38.7 percent of the repeat breeders had normal embryos at 34 days, against 25.8 percent in controls. (Wiltbank *et al.*, 1956).

MATERIALS AND METHODS

Experimental animals:

Swine were employed as experimental animals primarily because they are one of the best suited species of farm animals for this type of investigation. The 94 gilts and 10 boars used in this study were obtained from the herd of the Missouri Agricultural Experimental Station maintained in cooperation with the Regional Swine Breeding Laboratory of the United States Department of Agriculture. The 1955 gilts were from crosses among Landrace (L), Poland China (P) and Duroc (D) breeds, whereas, the 1956 gilts were from reciprocal crosses of the Landrace and Poland China breeds.

Procedure:

The first phase of this investigation was a preliminary trial made during 1955 and designed (1) to assess the beneficial or harmful consequence of the hormonal therapy during gestation (2) to determine the therapeutic dosage and the ratio of progesterone to estrone necessary to produce a beneficial response in the intra-uterine environment and (3) to estimate the most suitable stage of gestation and duration of the hormonal therapy that would aid the developing embryos during the critical period. Mature gilts of 200 pounds body weight were employed in this investigation and they were bred to non-related boars in order to avoid inbreeding of the embryos.

Treatment:

Estrone was prepared as a stock solution. To reduce error and to facilitate the administration of the two hormones as a single injection, the following procedure was adopted. Exactly 62.5 mg. of estrone were dissolved in 200 ml. of sesame oil. Then 10 ml. of the estrone solution were transferred to a 250 ml.

volumetric flask, 3.125 gm. of progesterone were added and the contents were thoroughly mixed. The volume of the hormone solution was then adjusted to the 250 ml. mark on the volumetric flask with sesame oil. Two ml. of this solution contained 25 mg. of progesterone plus 25 μg. of estrone while 4 ml. contained 50 mg. of progesterone plus 50 μg. of estrone. These were the two dosage levels used for the preliminary studies during 1955.

During 1956 two ratios of progesterone to estrogen were employed; in one series of experiments a 1000:1 ratio was used while in a second series the hormone ratio was 2000:1. One group received 2 ml. of the hormone solution containing 25 mg. of progesterone and 25 μg. of estrone (1000:1 ratio) whereas a second group received 2 ml. of hormone preparation containing 25 mg. of progesterone plus 12.5 μg. of estrone (2000:1). The quantity of progesterone administered was exactly the same per milliliter of oil solution in both series; the quantity of estrone was reduced to give the solution containing the 2000:1 ratio of hormones.

During 1955 and 1956 the pregnant gilts were administered the oil solution of hormones intramuscularly each day for a period of 10 days. The experimental animals were subjected to as little disturbance as possible during treatment. During 1955 the 10-day hormone treatments were begun at four different stages of gestation in four different groups. The four stages were the 4th, 14th, 24th, and 34th day of gestation. During 1956 the treatments were begun on the 4th and 14th day of gestation. The treatments during 1955 and 1956 are summarized in Tables 1 and 2.

All the gilts were slaughtered, on or as near to the 55th day post-breeding as possible. Reproductive tracts were immediately removed for a detailed laboratory examination. The following observations were recorded.

1. Linear measurements of the uteri.
2. Weights of the intact gravid uteri.
3. Viable embryos in each horn.
4. Intra-uterine spacing of the embryos.
5. X-ray study of the gravid uteri to assess the growth and the spacing of the embryos.
6. Volume of the uterine fluids.
7. Body length and weights of the individual embryos.
8. Sex and nipple number of each embryo.
9. Ovarian weights; number and weights of corpora lutea.

Microscopic Examination:

Samples of uterine tissue were dehydrated, embedded and sectioned at 6 microns. Sections were stained by Delafield's haemotoxylin with alcoholic-eosin solution as a counter stain. These sections were examined for:

1. The height and the edema of the endometrial epithelium.
2. The size and the appearance of stromal nuclei.
3. Endometrial glands and the presence of glycogen particles.

TABLE 1--TREATMENTS EMPLOYED DURING 1955 CLASSIFIED BY DOSAGE LEVELS AND STAGES OF PREGNANCY

Group	Treatment	Dosage Level of Hormonal Therapy per Day	Ratio of Hormones	Stage of Gestation for the Hormonal Therapy
1	A^{4}	25 mg. progesterone + 25 mcg. estrone	1000:1	From 4th through 13th day of gestation (10 consecutive days)
2	A^{14}	25 mg. progesterone + 25 mcg. estrone	1000:1	From 14th through 23rd day of gestation (10 consecutive days)
3	A^{24}	25 mg. progesterone + 25 mcg. estrone	1000:1	From 24th through 33rd day of gestation (10 consecutive days)
4	A^{34}	25 mg. progesterone + 25 mcg. estrone	1000:1	From 34th through 43rd day of gestation (10 consecutive days)
5	B^{4}	50 mg. progesterone + 50 mcg. estrone	1000:1	From 4th through 13th day of gestation
6	B^{14}	50 mg. progesterone + 50 mcg. estrone	1000:1	From 14th through 23rd day of gestation
7	B^{24}	50 mg. progesterone + 50 mcg. estrone	1000:1	From 24th through 33rd day of gestation
8	B^{34}	50 mg. progesterone + 50 mcg. estrone	1000:1	From 34th through 43rd day of gestation

TABLE 2--TREATMENTS EMPLOYED DURING 1956 CLASSIFIED BY DOSAGE LEVELS AND STAGES OF PREGNANCY

Group	Treatment	Dosage Level of Hormonal Therapy per Day	Ratio of the Hormones	Stage of Gestation for the Hormonal Therapy
1	C^{4}	25 mg. progesterone + 25 mcg. estrone	1000:1	From 4th through 13th day of gestation (10 consecutive days)
2	C^{14}	25 mg. progesterone + 25 mcg. estrone	1000:1	From 14th through 23rd day of gestation (10 consecutive days)
3	D^{4}	25 mg. progesterone + 12.5 mcg. estrone	2000:1	From 4th through 13th day of gestation (10 consecutive days)
4	D^{14}	25 mg. progesterone + 12.5 mcg. estrone	2000:1	From 14th through 23rd day of gestation (10 consecutive days)

Chemical Composition of the Embryos:

Chemical composition of the embryos of each horn was determined separately during 1956. A representative sample of the homogenate of all embryos of a single horn of the uterus, prepared by using a Waring blender, was measured into pre-weighed moisture cups. The samples were immediately weighed and dried at a constant temperature of 55° C in an oven. After the samples were fairly solidified they were transferred to a vacuum oven at 75° C and dried for 12 hours. They were then cooled in an efficient desiccator under vacuum and weighed. The samples were redried and reweighed until constant weights were obtained. Loss in weight was considered as moisture content.

The dry matter was then ground into a fine powder with a glass mortar and pestle. Approximately 2 gm. of the dry matter was subjected to ether extraction using a Soxhlet extraction apparatus. Extraction was made with an anhydrous ether-ethanol mixture (1:3) for 16 hours; the process was repeated until constant weights after cooling were obtained. Loss in weight of the extracted dry matter represented the ether soluble or lipid fraction. Moisture and fat free samples of the embryo homogenate were analyzed by the Kjeldahl method for total nitrogen in order to calculate the protein content of the embryos.

RESULTS AND DISCUSSION

A preliminary investigation was conducted during 1955 to evaluate the desirability of the hormonal approach to the problem of embryonic mortality. The preliminary trials were especially important to establish the absolute amounts of hormones necessary, the ratio of progesterone to estrone yielding maximum response, the critical stage of the gestation period for the initiation of treatments, and the length of time the hormonal therapy should continue in order to obtain a beneficial response.

A summary of the treatments incorporating these considerations and the results are presented in Tables 3 and 4. The hormone treatments were initiated on the 4th, 14th, 24th and 34th days post breeding in the four experimental groups and were continued for a 10-day period. Among the eight groups treated at four different stages of gestation with daily injections of 25 mg. of progesterone + 25 μg. of estrone, the embryonic death losses of 13.16, 20.69, 29.41, 30.00 and 31.43 percent in the six treated groups were lower than the 35.82 percent prenatal mortality found in the non-treated animals. The coefficient of variation of 126 percent among control gilts indicates a remarkable individual variation in the incidence of prenatal death losses. This variation was decreased in all the treated gilts.

The mean weights of the reproductive tracts in the 25 mg. and 50 mg. progesterone-level groups were 5783.56 gm. and 6865.00 gm. respectively, both being greater than the mean weight of 5603.04 gm. in the control group (Table 5). The means of the linear capacity (sum of the lengths of the two horns) of the

TABLE 3--MEANS AND COEFFICIENTS OF VARIATION OF THE OVULATION RATE, LITTER SIZE, AND PRENATAL MORTALITY IN LxPxD GILTS DURING 1955 FALL

Treatment Group	Number of Gilts	Ovulation Rate		Litter Size		Prenatal Mortality		Percent Mortality
		Mean	C.V.	Mean	C.V.	Mean	C.V.	
Controls	28	12.46	.51	8.00	.29	4.46	1.26	35.82
Treated with Progesterone and Estrone 1000:1								
*Treatment A_4	3	9.67	.12	7.67	.39	2.00	1.00	20.69
*Treatment A_{14}	3	13.00	.08	8.33	.35	4.67	.81	35.89
*Treatment A_{24}	4	11.25	.29	7.00	.31	4.25	1.11	37.78
*Treatment A_{34}	3	11.33	.13	8.00	.57	3.33	.97	29.41

*Treatment A_4 - Treated with 25 mg. progesterone + 25 mcg. estrone daily from 4th day post breeding for 10 consecutive days.

*Treatment A_{14} - Similar treatment from 14th day post breeding for 10 days.

*Treatment A_{24} - Similar treatment from 24th day post breeding for 10 days.

*Treatment A_{34} - Similar treatment from 34th day post breeding for 10 days.

TABLE 4--MEANS AND COEFFICIENTS OF VARIATION OF THE OVULATION RATE, LITTER SIZE, AND PRENATAL MORTALITY IN LxPxD GILTS DURING 1955 FALL

Treatment Group	Number of Gilts	Ovulation Rate		Litter Size		Prenatal Mortality		Percent Mortality
		Mean	C.V.	Mean	C.V.	Mean	C.V.	
Controls	28	12.46	.51	8.00	.29	4.46	1.26	35.82
Treated with Progesterone and Estrone 1000:1								
*Treatment B_4	1	10.00	--	7.00	--	3.00	---	30.00
*Treatment B_{14}	4	14.00	.21	9.25	.14	4.75	.77	33.93
*Treatment B_{24}	3	12.67	.19	11.00	.27	1.67	.34	13.16
*Treatment B_{34}	3	11.67	.22	8.00	.33	3.67	.57	31.43

*Treatment B_4 - Treated with 50 mg. progesterone + 50 mcg. estrone daily from 4th day post breeding for 10 consecutive days.

*Treatment B_{14} - Similar treatment from 14th day post breeding for 10 days.

*Treatment B_{24} - Similar treatment from 24th day post breeding for 10 days.

*Treatment B_{34} - Similar treatment from 34th day post breeding for 10 days.

TABLE 5--MEANS AND COEFFICIENTS OF VARIATION OF THE WEIGHTS OF THE REPRODUCTIVE TRACTS; TREATED AND UNTREATED ANIMALS

Treatment Group	No. of Tracts	Mean Weight of the Genital Tract in Grams	Coefficient of Variation	Percent Increase
Controls	23	5603.04	.0863	
Treated with 25 mg. Progesterone plus 25 mcg. Estrone	9	5783.56	.1740	2.11
Treated with 50 mg. Progesterone plus 50 mcg. Estrone	8	6865.00	.2967	22.52

uteri along with coefficients of variation are given in Table 6. The 13 pregnant uteri from the gilts treated with daily injections of 25 mg. progesterone plus 25 μg. estrone were very uniform in size as evidenced by their linear capacities of 2834.33, 2858.00, 2869.00 and 2885.20 ml. representing a percentage increase of 9.65, 10.57, 10.99 and 11.62, respectively, over the mean uterine capacity of the non-treated gilts. The uteri from the treated groups receiving a 50 mg. level of

TABLE 6--MEANS AND COEFFICIENTS OF VARIATION OF THE LINEAR CAPACITY OF THE UTERI; TREATED AND UNTREATED ANIMALS

Treatment Group	No. of Gilts	Mean Linear Capacity in Millimeters*	Coefficient of Variation	Percent Increase
Controls	28	2584.86	.1820	
Treatment A_4	3	2834.33	.3076	9.65
Treatment A_{14}	3	2858.00	.1763	10.57
Treatment A_{24}	4	2885.20	.1409	11.62
Treatment A_{34}	3	2869.00	.3120	10.99
Treatment B_4	1	2659.00	--	2.87
Treatment B_{14}	4	2757.50	.1979	6.68
Treatment B_{24}	3	3488.33	.3307	34.95
Treatment B_{34}	3	2967.00	.1129	14.78
All 25 mg. :25 mcg.	13	2863.38	.1930	10.78
All 50 mg. :50 mcg.	11	3005.00	.2320	16.25

*Linear capacity = combined length of both the uterine horns.

progesterone exhibited considerable variation with mean linear capacities of 2659.00, 2757.50, 2967.00 and 3488.33 ml. The corresponding percentage increases were 2.87, 6.68, 14.78 and 34.95 in these four subgroups. Analysis of variation, as presented in Table 7, shows that these increases due to treatments approached significance at the 5 percent level.

TABLE 7--ANALYSIS OF VARIANCE FOR LINEAR CAPACITY OF THE UTERI AT 56 DAYS OF PREGNANCY BETWEEN TREATED AND NONTREATED LxPxD GILTS

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Ratio
Control along with Treatments A and B	(2)	(1,485,987)	742,994	2.54^N
Control vs. Treatments A and B	1	1,173,715	1,173,715	4.00^A
Between Treatments	1	312,272	312,272	1.07^N
Individuals	49	14,352,104	292,900	
Total	51	15,838,091		

N = Not significant.
A = Approaches significance at the 5 percent level.

In order to evaluate critically the effects of the hormonal therapy on the embryos, the weights of the individual embryos were corrected to 55 days of gestation during 1955. Seventy-three percent of the gilts were slaughtered within a range of one day on either side of the 55th day. As the growth of the embryos deviated significantly from linearity (Table 8), regression values of 4.96 gm. per day from 52 to 55 days gestation and 10.31 gm. from 55 to 58 days gestation were employed for the necessary corrections to equate to the 55th day of embryonic weight. The embryonic weights, classified by the treatment groups, are pre-

TABLE 8--TEST OF SIGNIFICANCE OF DEPARTURE FROM LINEAR REGRESSION OF WEIGHTS IN GRAMS ON AGE IN DAYS OF THE EMBRYOS

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F
Total	393	120,250.68		
Days of Gestation	5	53,193.20	10,638.64	61.56***
Individuals	388	67,057.48	172.83	
Days of Gestation	5	53,193.20		
Linear Regression	1	49,600.57		
Deviations from Linear Regression	4	3,592.63	898.16	5.197***

***Very highly significant with $P < .001$.

sented in Table 9. The average weight of the embryos from the non-treated L x P x D gilts at 55 days of gestation was 72.39 gms. with a standard deviation of 13.13 gm. There are no appreciable differences in the mean embryonic weights between groups, indicating only random variations. However, in seven out of the eight treated groups the weights of the individual embryos varied less than did those in the control group, which suggests that perhaps the weaker embryos in the treated groups were aided to a certain extent by the hormonal therapy.

TABLE 9--MEANS, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION OF THE EMBRYONIC WEIGHTS AT 55 DAYS OF GESTATION

Treatment Group	No. of Embryos	Embryonic Weights at 55 Days of Gestation		
		Mean (gm.)	Standard Deviation	Coefficient of Variation
Controls	224	72.39	13.13	.1814
Treatment A_4	23	71.40	18.91	.2648
Treatment A_{14}	25	75.83	11.04	.1455
Treatment A_{24}	28	73.90	6.90	.0934
Treatment A_{34}	24	71.11	10.78	.1515
Treatment B_4	7	78.74	8.10	.1029
Treatment B_{14}	37	68.42	9.70	.1417
Treatment B_{24}	33	67.88	11.30	.1665
Treatment B_{34}	24	76.16	11.81	.1551

Along with the more pronounced increase in the weights and linear capacities of the uteri in the gilts treated with daily injections of 50 mg. of progesterone plus 50 μg. of estrone, there appears to be a tendency for marked variation

(Tables 5 and 6) in the individual response to this higher dosage. Moreover, two of the uteri from the gilts treated with this higher dosage of hormones bore evidence that this dosage level (50 mg. progesterone plus 50 μg. estrone per day for 10 days) had reached the upper limit of dosage level which would result in a beneficial response. Both of the uteri exhibited predominately estrogenic effects; the cervices were greatly dialated with possibilities of ensuing abortion and the endometrium and the fetal membranes were abnormally vascular and hyperaemic. Petichial hemorrhages and scattered areas of congestion were also observed on the embryos. The degree of uterine distention in the higher dosage group was abnormally great, partly as a result of augmented circulation and partly due to the products of conception. This degree of uterine distention is perhaps undesirable. Macroscopic examination presented a convincing impression that the uteri and the embryos from the 25 mg. groups were healthier and anatomically and functionally superior to those of the control and 50 mg. groups.

Although significant differences were not discernable between control and the various treated groups of L x P x D gilts during 1955, the results nevertheless revealed a tendency for a more favorable response in the treated groups, particularly those receiving the lower dosages of hormones. The study showed marked uniformity in the linear measurements of the uteri with about 10 percent increase in size due to treatment, less variation in the intra-uterine mortality in relation to the non-treated animals, and a relatively lower incidence of weaker embryos resulting in apparent uniformity in the weights of the embryos. These results prompted further investigation of the effects of the progesterone-estrone treatment, with a few modifications, upon the uteri and embryos of pregnant swine.

During 1956 the amount of progesterone was kept constant; only the quantities of estrone were changed to produce the progesterone-estrone mixtures in the ratios of 1000 parts of progesterone to 1 part of estrone and 2000 parts of progesterone to 1 part of estrone. Also, the stages of gestation during which the hormones were administered were limited to the 4th through the 13th days and 14th through 23rd days post breeding.

The 4th day of gestation was chosen because the possibility existed that progesterone might prevent the descent of the fertilized ova in the Fallopian tubes if it was administered earlier. In swine, ova pass through the Fallopian tubes in about 3 days and enter the uterus on the 4th day. During the first 10 to 13 days the blastocysts are shifted in the uterine cavity by the intrauterine forces and, after the 13th day, no further displacement is possible since the implantation of the embryos occurs at this time. Therefore, the 14th day of gestation was chosen as the time to begin the hormonal therapy in a second experimental group to avoid subjecting the fertilized ova to any possible alteration in uterine environment prior to implantation.

The biochemical investigation of Glasgow and Mayer (1957) showed a marked decrease in the quantity of the urinary metabolites of progesterone about

the 15th to 25th day of gestation in swine. These results presented additional reasons for the selection of a 10-day period, starting at the 14th day for hormone therapy. Mode of administration and the duration of the treatments were the same as in 1955.

Table 10 summarizes the treatments employed during 1956, along with the means, relative variations of ovulation rate, litter size and embryonic mortality in control and treatment groups. The average litter size in the non-treated groups

TABLE 10--MEANS AND COEFFICIENTS OF VARIATION OF SOME COMPONENTS OF FERTILITY IN CONTROL AND VARIOUSLY TREATED L x P GILTS DURING 1956

Treatment Group	No. of Gilts	Ovulation Rate		Litter Size		Percent Mortality
		Mean	C.V.	Mean	C.V.	
Non-Treated	10	10.30	.130	7.90	.210	23.30
Treatment I[a]	6	11.50	.153	9.00	.337	21.74
Treatment II[a]	3	10.67	.332	8.67	.580	18.75
Treatment III[a]	8	11.00	.128	9.00	.146	18.18
Treatment IV[a]	6	12.33	.151	10.67	.096	13.51

I. Treated with 25 mg. prog. plus 25 mcg. estrone per day from 4th day of gestation for 10 days.
II. Treated with 25 mg. prog. plus 25 mcg. of estrone per day from 14th day of gestation for 10 days.
III. Treated with 25 mg. prog. plus 12.5 mcg. estrone per day from 4th day of gestation for 10 days.
IV. Treated with 25 mg. prog. plus 12.5 mcg. estrone per day from 14th day of gestation for 10 days.

a = Henceforth, the corresponding treatments will be denoted as Tr. I, Tr. II, Tr. III, and Tr. IV

of gilts was only 7.90, compared to 9.00, 8.67, 9.00 and 10.67 for treatments I, II, III and IV. Variance analysis of the litter size, shown in Table 11, proves these differences due to treatments are well beyond the realm of random variation.

Although the sum of the beneficial response due to all four treatments failed to be significant at the 5 percent level, the increase in litter size in the gilts that received daily injections of 25 mg. progesterone plus 12.5 μg. estrone (2000:1) proved to be highly significant ($P<.01$). The superiority of treatment IV over treatment III was also significant ($P<.05$). It is of further interest that the performance of the 14 gilts that received the ovarian hormones in the 2000:1 ratio was markedly uniform as seen by the coefficients of variation. It may be noted that the ovulation rates in the treatment groups were higher than those of the control group. The percentages of mortality were 21.74, 18.75, 18.18 and 13.51 in the treatments groups I, II, III and IV, compared to 23.3 percent mortality in the non-treated group of gilts. The unequal number of gilts in the four groups receiving the hormone treatments was unavoidable due to the non-occurrence of estrus in some of the gilts assigned to these groups for the hormonal therapy. This discrepancy was further aggravated by the return to estrus of 5 of the treated gilts. These were then classified as a separate group.

TABLE 11--ANALYSIS OF VARIANCE OF LITTER SIZE AT 56 DAYS GESTATION SHOWING THE EFFECT OF ALL TREATMENTS TOGETHER AND 2000:1 THERAPIES COMBINED

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F
Control along with All Treatments	(4)	(28.98)		
Control vs. Treatments	1	15.50	15.50	3.13[1]
Between Treatments	3	13.48	4.49	.91
Individuals	28	138.90	4.96	
Controls along with Treatments III and IV (2000:1)	(2)	(28.80)		
Control vs. Treatments III and IV	1	19.2	19.2	9.55**
Between Treatments III and IV	1	9.6	9.6	4.78*
Individuals	21	42.2	2.01	

**P < .01

*P < .05

[1]P > .05 and < .10

The mean weights of the reproductive tracts (uterus + vagina + embryos) were 6098.6, 5934.0, 6527.1 and 8054.1 gm. in groups I, II, III and IV, respectively (Table 12), corresponding to percentage increases of 12.00, 8.98, 19.87 and 47.92 over the mean weight of 5445.0 grams in the non-treated L x P gilts during 1956. Similar increases were also observed in the weights of the reproductive

TABLE 12--MEAN WEIGHTS OF THE INTACT GENITAL TRACTS AT 56 DAYS OF GESTATION AND TEST OF SIGNIFICANCE BETWEEN CONTROL AND TREATED GROUPS

Treatment Group	No. of Gilts	Mean Wt. of the Uterus, with Embryos, plus Vagina, at 56 Days of Pregnancy (gm.)	Coefficient of Variation	T*
Control	10	5445.0	.214	
1000:1				
Treatment I	6	6098.6	.311	.863
Treatment II	3	5934.0	.577	.414
2000:1				
Treatment III	8	6527.1	.250	1.602
Treatment IV	6	8054.1	.146	4.33**

*Control vs. each treatment

**P < .001.

tracts without embryos (Table 14); the percentage increases were 10.15, 10.15, 20.28 and 48.93 in the treatments I, II, III and IV, respectively, over the mean weight of 4804.8 grams in the control group of gilts. Variance analysis for each of these reproductive components, Tables 13 and 15, shows these differences were significant, with treatment IV proving to be distinctly superior to the rest of the treatments.

TABLE 13--ANALYSIS OF VARIANCE OF THE INTACT GENITAL TRACTS AT 56 DAYS OF GESTATION SHOWING THE EFFECT OF TREATMENTS

Component	Degrees of Freedom	Sum of Squares	Mean Squares	F
Control along with All Treatments	(4)	(26,722,986)		
Control vs. All Treatments	1	11,635,006	11,635,006	4.113*
Between 4 Treatments	3	15,087,980	5,029,327	1.178
Individuals	27	76,380,080	2,828,892	
Total	31	103,103,006		
Control along with Treatments III & IV 2000:1	(2)	25,584,635		
Control vs. Treatments III & IV	1	18,049,477	18,049,477	10.267***
Between Treatment III & IV	1	7,535,158	7,535,158	4.286*
Individuals	20	35,158,544	1,757,927	

*F value .05 = 4.21
***P < .005

TABLE 14--MEANS AND TESTS OF SIGNIFICANCE OF THE UTERINE WEIGHTS WITHOUT EMBRYOS AT 56 DAYS OF PREGNANCY

Treatment Group	No. of Gilts	Mean Weights of the Uteri without Embryos (gm.)	Coefficient of Variation	T*
Control	10	4804.8	.221	
1000:1				
Treatment I	6	5292.5	.292	.751
Treatment II	3	5292.3	.567	.463
2000:1				
Treatment III	8	5779.0	.252	1.60
Treatment IV	6	7155.8	.142	4.35***

*Control vs. each treatment.
***Very highly significant with P < .001.

The volumes of the fetal fluids from gilts in experimental groups I, II, III and IV were 2073.3, 2045.0, 2280.6 and 3232.5 ml. as against a volume of 1888.5 ml. in the non-treated L x P gilts (Table 16). The fetal fluids apparently distend the lumen of the uterus, transmit pressure to the uterine wall, and simulate growth by hypertrophy. These fluids also protect the embryo from injury due to external pressures. The uteri from the treated gilts were more distended (Figs. 1 and 2) with corresponding increases in linear capacities of 7.51, 12.82, 17.65 and 28.83 percent over the mean linear capacity of 2610.1 ml. in the non-treated gilts (Figs. 3 and 4). Statistical analyses (Tables 17, 18 and 19) show that these differences were not due to random variation within the population and therefore they are ascribed as treatment effects. A summary of the treatment responses ex-

TABLE 15--ANALYSIS OF VARIANCE OF THE UTERINE WEIGHTS WITHOUT EMBRYOS AT 56 DAYS OF PREGNANCY

Component	Degrees of Freedom	Sum of Squares	Mean Squares	F
Control along with All Treatments	(4)	(21,922,191)		
Control vs. All Treatments	1	9,102,540	9,102,540	4.238*
Between Four Treatments	3	12,819,651	4,273,217	1.989
Individuals	27	57,993,825	2,147,919	
Total	31	79,916,016		
Control along with Treatments III and IV (2000:1)	(2)	(20,769,313)		
Control vs. Treatments III & IV	1	14,644,841	14,644,841	10.436***
Between Treatments III & IV	1	6,124,472	6,124,472	4.364*
Individuals	20	28,066,467	1,403,323	
Total	22	48,835,780		

*Significant with $P < .05$
***Very highly significant with $P < .005$

TABLE 16--MEANS AND TESTS OF SIGNIFICANCE OF THE FOETAL FLUIDS AT 56 DAYS OF GESTATION BETWEEN CONTROL AND TREATED GROUPS

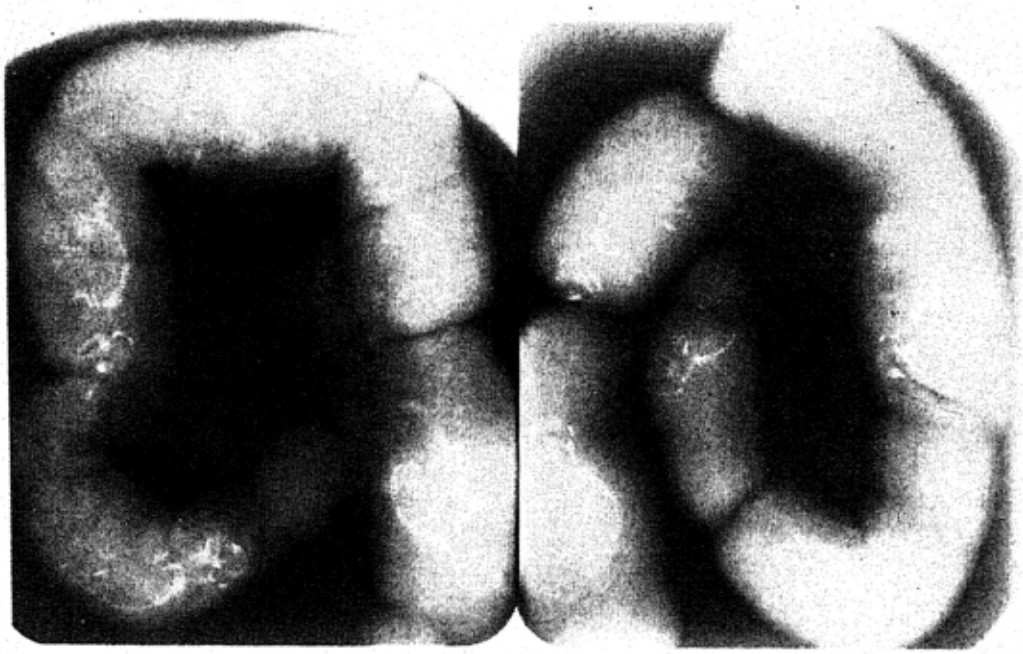
Treatment Group	No. of Gilts	Mean Volume of the Uterine Fluids (ml.)	Coefficient of Variation	T*
Control	10	1888.5	.355	
1000:1				
Treatment I	6	2073.3	.354	.521
Treatment II	3	2045.0	.699	.276
2000:1				
Treatment III	8	2280.6	.312	1.200
Treatment IV	6	3232.5	.219	3.802***

*Control vs. each treatment.
***Significant with $P < .005$.

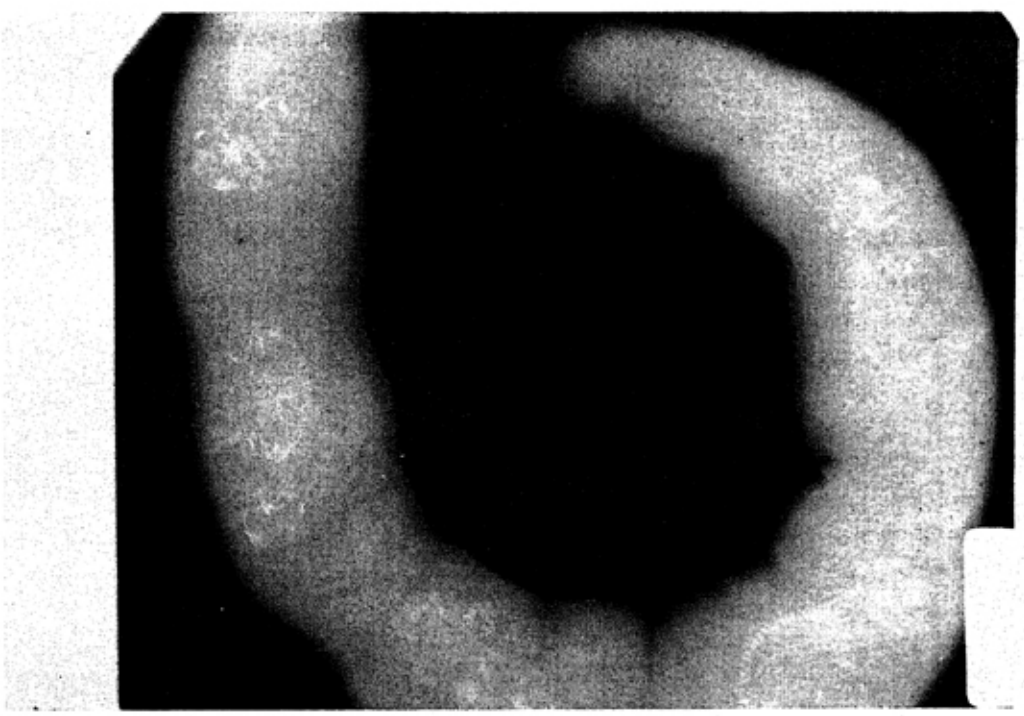
pressed as percent increases over the normal ureri from the control gilts is presented in Table 20. Attention is drawn to the proportional increases in all the observed responses to hormone treatment.

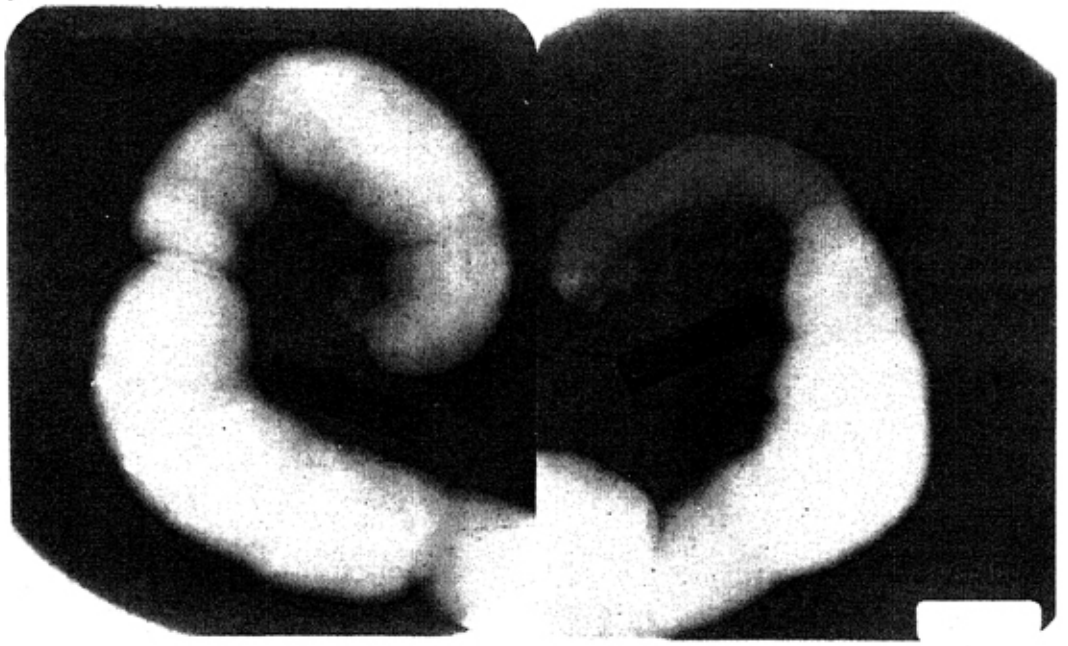
Of the 38 L x P gilts employed during 1956, 5 gilts, treated with daily injections of 25 mg. progesterone plus 25 μg. estrone (1000:1), returned to estrus after the full course of the treatment was completed. These were rebred and were subjected to a similar course of treatment after a second mating. However, they could not be classified under any of the four experimental groups under discussion since the first course of treatment must have influenced their estrous cycles and their subsequent reproductive performances. The prenatal mortality was found to be a high 49.3 percent in this group of repeat breeders.

The reasons for the low percentage of embryonic mortality of 23.3 percent in the control L x P gilts during 1956 are not apparent. Embryonic death losses of 30 to 40 percent were obtained by previous workers as reported in the Review



Figs. 1 and 2—X-ray photographs of uteri from control gilts.





Figs. 3 and 4—X-ray photographs of uteri from treated gilts. Observe the marked distention of the uterus and the spacing of embryos.

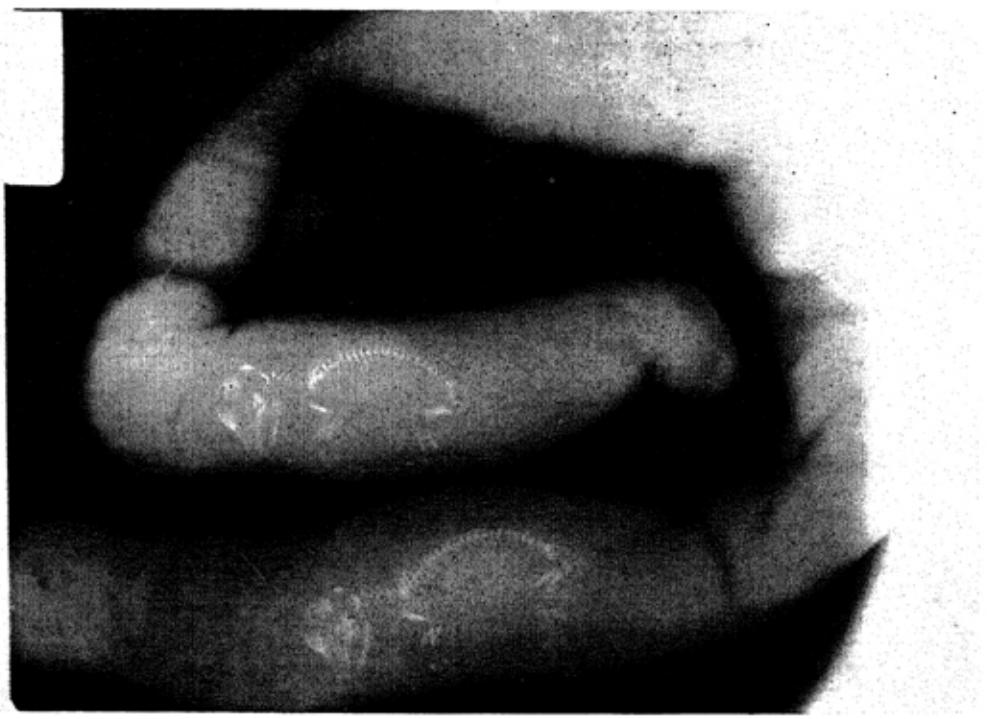


TABLE 17--ANALYSIS OF VARIANCE OF FOETAL FLUIDS AND TEST OF SIGNIFICANT DIFFERENCES BETWEEN DIFFERENT GROUPS

Component	Degrees of Freedom	Sum of Squares	Mean Squares	F
Control along with All Treatments	(4)	(7,852,460)		
Control vs. Treatments	1	2,118,178	2,118,178	3.54[1]
Between Treatments	3	5,734,282	1,911,427	3.19[1]
Individuals	28	16,774,695	599,096	
Total	32	24,627,155		
Control along with Treatments III & IV (2000:1)	(2)	(6,840,513)		
Control vs. Treat. III & IV	1	3,734,001	3,734,001	7.753**
Between Treatments III & IV	1	3,106,512	3,106,512	6.450**
Individuals	21	10,113,962	481,617	
Total	23	16,954,475		

[1]$P < .10$

**$P < .025$

TABLE 18--MEANS AND TESTS OF SIGNIFICANCE OF LINEAR CAPACITY OF THE UTERI AT 56 DAYS GESTATION BETWEEN CONTROL AND THE DIFFERENT TREATED GROUPS

Treatment Group	No. of Gilts	Mean Linear Capacity of the Uteri in mm.	T[1]
Control	10	2610.1	
1000:1			
Treatment I	6	2806.7	.715[n]
Treatment II	3	2944.7	.713[n]
2000:1			
Treatment III	8	3070.7	2.120*
Treatment IV	6	3362.7	3.186**

[1]Control vs. each treatment.

**Highly significant with $P < .01$.

*Significant with $P < .05$.

[n]Statistically not significant.

of Literature. Our 1955 study recorded prenatal death losses of 35.82 percent in L x P x D gilts. It may perhaps be assumed that the hormone treatments would have resulted in more pronounced changes in the reproductive tracts of the experimental animals, on a comparative basis, if a higher incidence of embryonic mortality had occurred in the untreated controls in 1956.

The results obtained in this study demonstrate the uterus is capable of considerable enlargement due to distention by the products of conception and the great quantity of accumulated fluids in the lumen of the uterus. The number of

TABLE 19--ANALYSIS OF VARIANCE OF THE LINEAR CAPACITY OF THE UTERI AT 56 DAYS OF GESTATION

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F
Control along with All Treatments	(4)	(2,394,305)		
Control vs. Treatments	1	1,418,929	1,418,929	3.65[1]
Between Treatments	3	975,376	325,125	
Individuals	28	10,890,910	388,961	
Total	32	13,285,215		
Controls along with Treatments III & IV (2000:1)	(2)	(2,292,226)		
Control vs. Treatments III & IV	1	1,998,556	1,998,556	8.68**
Between Treatments III & IV	1	293,670	293,670	1.28
Individuals	21	4,836,276	230,299	
Total	23	7,128,502		

**Highly significant with $P < .01$.

[1] $P > .05$ and $< .10$.

TABLE 20--PERCENT INCREASES OF SOME REPRODUCTIVE COMPONENTS IN THE TREATMENT GROUPS COMPARED WITH NON-TREATED ANIMALS

		Percent Increase Over the Non-Treated Group			
Component	Control	Treatment I	Treatment II	Treatment III	Treatment IV
Litter Size	7.9	13.92	9.75	13.92	35.06
Weight of the Reproductive Tract (gm.)	5445.0	12.00	8.98	19.87	47.92
Weight of the Tract without Embryos (gm.)	4804.8	10.15	10.15	20.28	48.93
Volume of the Foetal Fluids (ml.)	1888.5	9.78	8.29	20.76	71.17
Linear Capacity of the Uterus (mm.)	2601.1	7.51	12.82	17.65	28.83

embryos present in each gravid uterus to a great extent determines the degree of uterine distention and enlargement, as illustrated by the results already discussed, since the quantity of uterine fluid also increases with an increase in the number of embryos. Among all the treated groups, the one with more embryos (treatment IV) also possessed greater linear capacity and a greater volume of fetal fluids in the uterus. Vascular effects were more pronounced in the uteri from gilts receiving treatments I and II (1000:1) but the uterine distention was more extensive as a result of treatments III and IV (2000:1). Progesterone does not appear to raise the threshold of uterine response to the stimulus induced by stretching due to the products of conception. On the other hand, progesterone seems to aid in such a response. The uterine enlargement associated with the progesterone-estrone therapy cannot be directly and specifically attributed to the therapy alone because the enlargement may be, partly or wholly, the result of distention of the uterus. It may be stated that the extent of the increased capacity and weight of the uterus depends upon the degree of progesterone and estrone

effect on the distention response of the uterus, on the one hand, and the rate of growth of the products of conception, on the other. Increased local circulation is perhaps a supplementary factor which merits consideration.

The growth promoting effects of estrogen upon the uterus are associated with an increased vascularity of the tissues according to Macleod and Reynolds (1938). Allen (1928) states that estrone produces a marked uterine growth. This study indicates that estrone probably retards the distention-induced response of the uterus by restricting the enlargement-promoting influence of the products of conception. This observation is in agreement with that of Reynolds (1942). Although the litter size was precisely the same in the gilts receiving treatments I and III, the higher amount of estrone in treatment I (25 mcgm. per day) lowered the uterine response as shown by a lower uterine weight (Tables 12 and 14), less volume of fetal fluids (Table 16), and a smaller linear capacity (Table 18). The uterine response in treatment III (12.5 μg. estrone per day), as measured by the components given above, was more pronounced than in treatment I.

The weights of the individual embryos were corrected to 56 days of gestation during 1956. Approximately 71 percent of the gilts were slaughtered within a range of one day on either side of the 56th day of gestation. The remaining 29 percent deviated but two days from the 56th day. Since the growth of the embryos during 1956 did not deviate significantly from the linearity (Table 21), a regression value of 8.33 gm. per day was employed as the appropriate correction factor for the necessary adjustment to the 56th day.

TABLE 21--TEST OF SIGNIFICANCE OF DEPARTURE FROM LINEAR REGRESSION OF WEIGHTS IN GRAMS ON AGE IN DAYS OF THE EMBRYOS

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F
Total	314	89,088.7		
Days of Gestation	4	32,177.3	8044.33	43.82***
Individuals	310	56,911.4	183.59	
Days of Gestation	4	32,177.3		
Linear Regression	1	31,433.3		
Deviations from Linear Regression	3	744.0	248.0	1.35[N]

***Very highly significant with $P < .001$.

[N]Not significant.

The mean weights of the embryos from the L x P gilts during 1956 along with the relative variation are shown in Table 22. In spite of the significant increase in the litter size, individual embryonic weights were very similar to the embryos from the non-treated gilts. The variation in the weights of the embryos within each group of the treated gilts is somewhat lower though not to the degree of uniformity obtained in L x P x D gilts during 1955.

It is generally agreed that the weight of the individual embryos in a litter is inversely proportional to the number of viable embryos in the uterus. This was

TABLE 22--MEANS, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION OF THE EMBRYONIC WEIGHTS AT 56 DAYS OF GESTATION IN LANDRACE x POLAND GILTS

Treatment Group	Litter Size	No. of Embryos	Embryonic Weights at 56 Days of Gestation		
			Mean (gm.)	Standard Deviation	Coefficient of Variation
Control	7.90	79	79.44	13.45	.1693
Treatment I	9.00	54	79.99	12.12	.1515
Treatment II	8.67	26	74.00	15.15	.2047
Treatment III	9.00	72	84.26	13.73	.1629
Treatment IV	10.67	64	79.68	12.61	.1582

shown to be true in rabbits and swine. This theory can also be extended to twin sheep, foals and calves in comparison with single ones. Although the underlying mechanism cannot be explained adequately, it may be postulated that the greater uterine distention as a result of larger litter size curtails the nutrition supply to each fetus due to an effect upon the quantity of maternal blood available to each implantation site.

The conditions in the uterus must be optimum for the careful preservation and maintenance of the products of conception. The uterus must be sufficiently quiescent so that it will neither dislodge nor crush its delicate contents. It must nourish the zygote until more efficient vascular connections are established. These considerations stress the necessity for adequate food stores in the uterine tissue and a generous blood supply to replenish depleted nutrients. In other words, optimal chemical mediation is the most important intra-uterine function, presumably performed by the two ovarian hormones, progesterone and estrogen. Under normal conditions the blastocyst is bathed in endometrial secretions which are rich in substrate materials, assuring adequate nutrition. Elaborate provisions must be made in the uterine environment for the fulfillment of all the requirements of the rapidly growing embryo. It is evident that any failure or delay in uterine adaptation will be detrimental to the embryo and result in absorption or abortion.

The proof of macroscopic normality and healthier appearance of the embryos does not rigidly exclude the possibility of undesirable alterations in the physico-chemical status of the products of conception which might prove detrimental to the future growth and development of the embryos. The chemical composition of the embryos and condition of the endometrium as determined by histological examination were considered to be the criteria by which a normal or an abnormal maternal environment could, perhaps, be ascertained in this investigation.

A total of 295 embryos from L x P gilts and 46 embryos from Duroc gilts were employed for the determination of relative quantities of dry matter, ether extract, and crude protein in the embryos. Analyses were made on the contents of each uterine horn. Results are summarized in Table 23. The dry matter, ether extract, and crude protein were 10.64, 2.2 and 6.59 percent in the embryos from the non-treated gilts. These results show that these chemical constituents were very similar in quantity in all the treated groups and did not differ from those

TABLE 23--COMPARATIVE STUDY OF THE DRY MATTER, ETHER EXTRACT AND CRUDE PROTEIN OF THE EMBRYOS BETWEEN TREATED AND NON-TREATED LxP GILTS DURING 1956

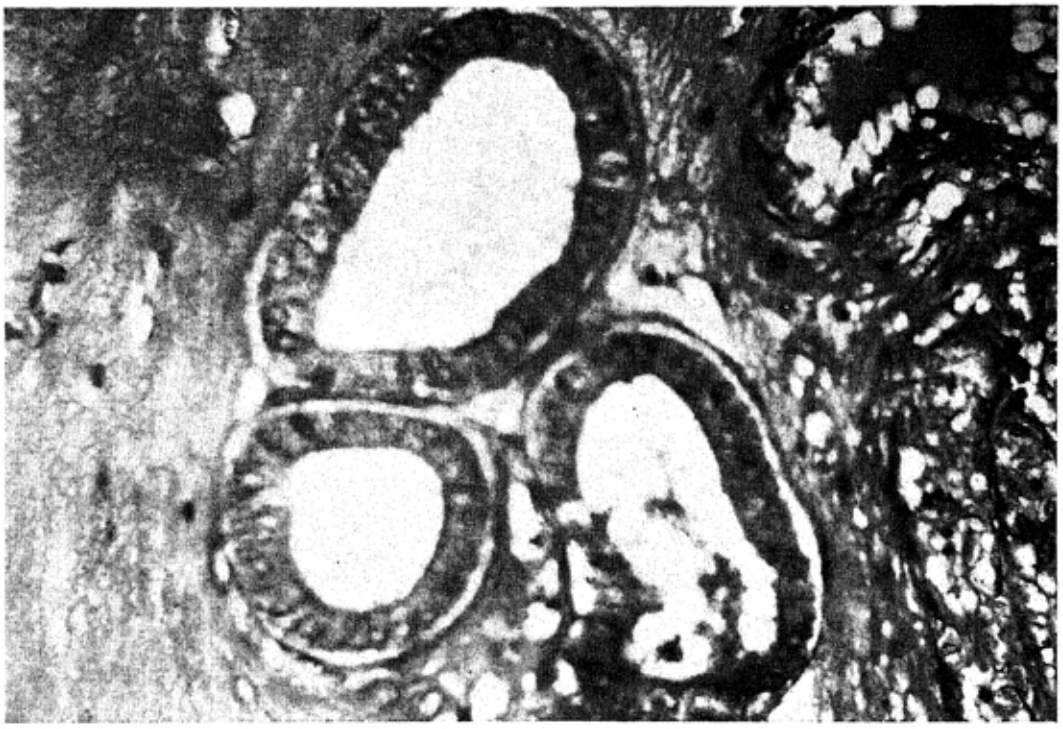
	Embryos of the Right Horn			Embryos of the Left Horn		
Class	Percent Dry Matter	Percent Ether Extract	Percent Crude Protein	Percent Dry Matter	Percent Ether Extract	Percent Crude Protein
Non-treated	10.64 ± .35	2.2 ± .58	6.50 ± .21	10.61 ± .33	2.07 ± .51	6.59 ± .29
Treatment I	10.41 ± .07	2.0 ± .42	7.22 ± .22	10.53 ± .12	2.07 ± .30	6.68 ± .03
Treatment II	10.05 ± .29	1.57 ± .63	6.38 ± .09	10.43 ± .21	2.05 ± .29	6.51 ± .32
Treatment III	10.52 ± .59	2.20 ± .22	6.72 ± .26	10.50 ± .43	2.14 ± .20	6.54 ± .45
Treatment IV	10.30 ± .57	2.37 ± .35	6.46 ± .28	10.15 ± .97	2.29 ± .13	6.51 ± .24
Durocs	10.63 ± .28	2.57 ± .16	6.60 ± .44	10.75 ± .39	2.26 ± .29	6.66 ± .44

of the non-treated gilts. Some of the insignificant differences were considered nothing but random variations. Therefore, it is logically assumed that none of the treatments employed in this investigation during 1956 exerted any deleterious effect on the chemical composition of the products of conception.

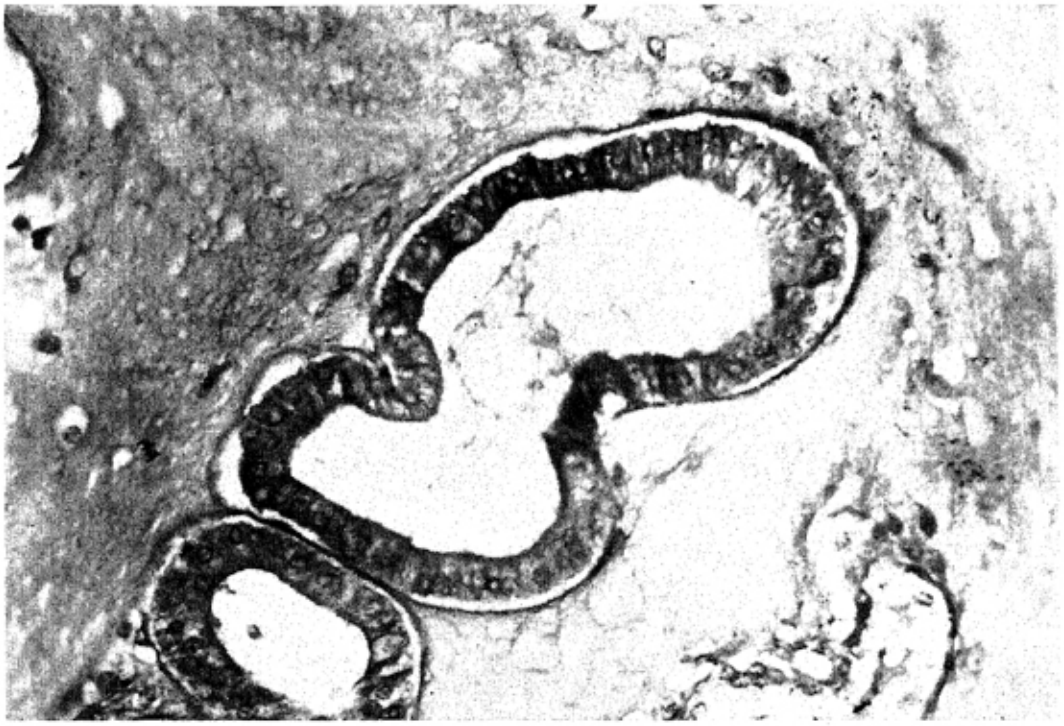
A comparative histological study of the endometrium showed a prevalence of relatively large and conspicuous endometrial glands in all the gilts receiving the daily injections of 25 mg. progesterone plus 12.5 μg. estrone (2000:1); but this was more marked in the group in which the treatments began on the 14th day of gestation (Figure 5). Except in the more superficial part of the functional layer, the glands were wide, tortuous and sacculated, showing considerable amounts of glycogen deposition between the nuclei and the basement membrane (Figure 6); glands were characteristically ladder-like and ragged in appearance due to heavy deposition of glycogen between the cells. The mucous secretion in the glands was thick and abundant. Glycogen deposits were also noticed in the stromal cells and the cytoplasm of the epithelial cells. More nuclei were present in the stroma, indicating stromal edema (Figure 7). Stromal cytoplasm contained lipoid droplets. There were indications of increased cellular constituents in the uterus with marked proliferation of the endometrium. (Figure 8). In the majority of non-treated animals the endometrium was thin, the epithelium was low, and glycogen was less abundant (Figure 9 and 10). The stromal nuclei were fusiform; stromal edema was more marked in the 1000:1 group due to the higher level of estrone injected. It may be presumed that the endometrium was altered favorably, to a certain extent, and that the exogenous progesterone and estrone, particularly in the 2000:1 ratio, provided a more beneficial intra-uterine environment for the growing products of conception.

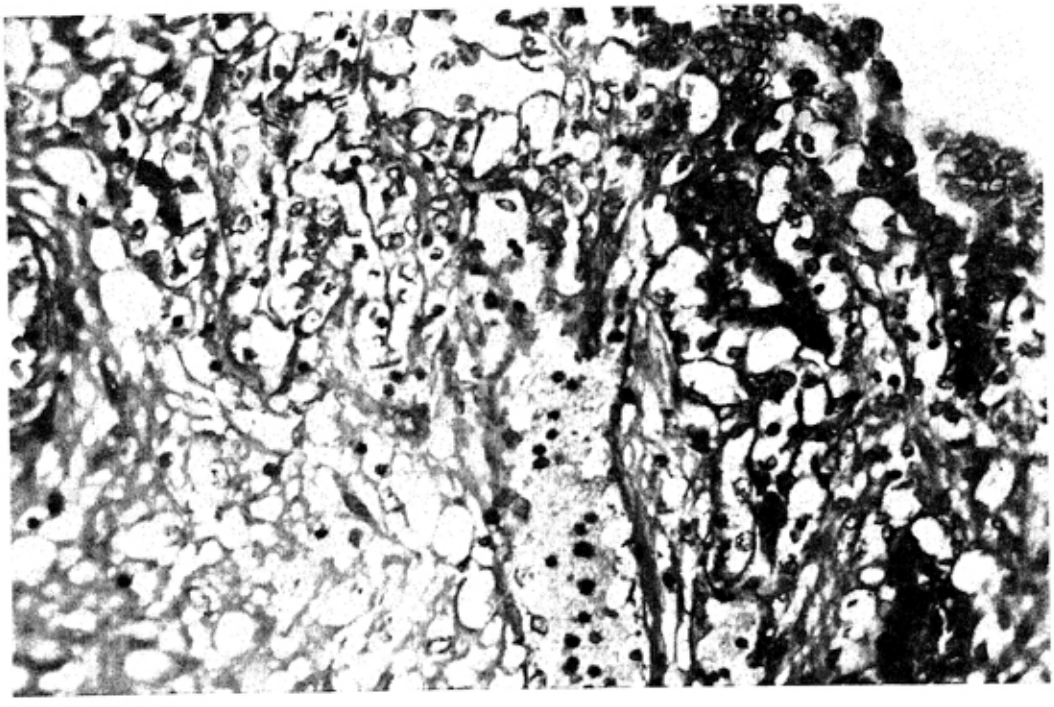
As seems very likely from the results of this investigation, the major factor influencing embryonic growth and development is an optimal uterine environment. But further research will be necessary to comprehend in detail the underlying mechanisms involved in this slightly favorable response. This investigation revealed that progesterone therapy, in the form of daily injections of 25 mg. progesterone plus 12.5 μg. of estrone (2000:1) administered for 10 consecutive days at certain critical stages of pregnancy establishes a more favorable intra-uterine environment for embryonic well being as judged by physiological, chemical and histological criteria already discussed.

Progesterone and estrone in the ratio of 1000:1 were found to be decidedly inferior to the 2000:1 ratio. The uterine response to this more estrogenically potent series indicates that within this range or at some level intermediate between the two series above, there is an inversion of the physiological balance between these two hormones. Estrogen shifts from synergism to antagonism as the dosage of the hormone is increased (Courrier 1950). Therefore, the endocrine requisite of the uterus is ample progesterone plus that amount of estrogen which will synergise most efficiently with the luteal hormone.

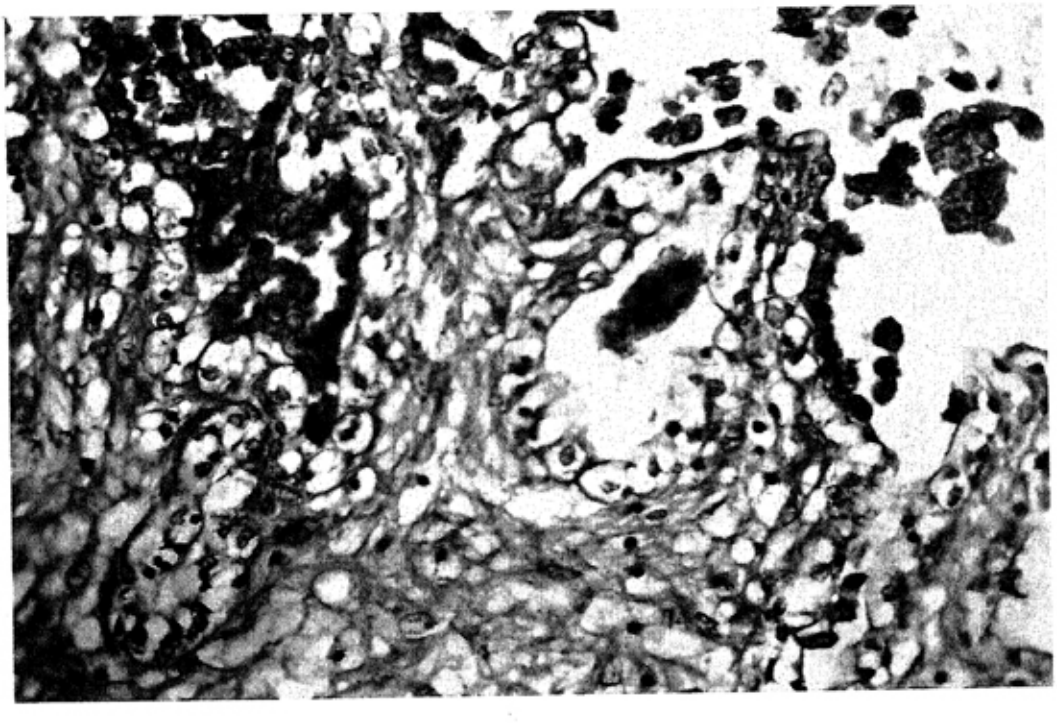


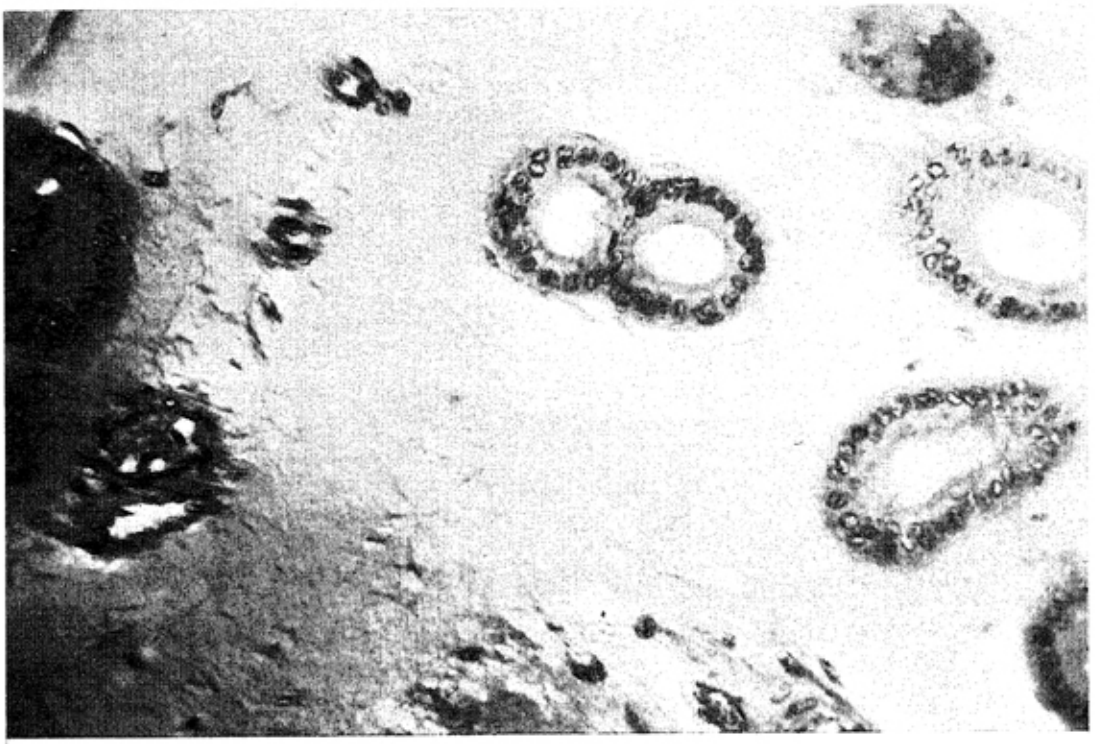
Figs. 5 and 6.—High power photomicrographs of sections of endometrium obtained from the treated gilts. Observe the endometrial glands and the heavy deposition of glycogen.



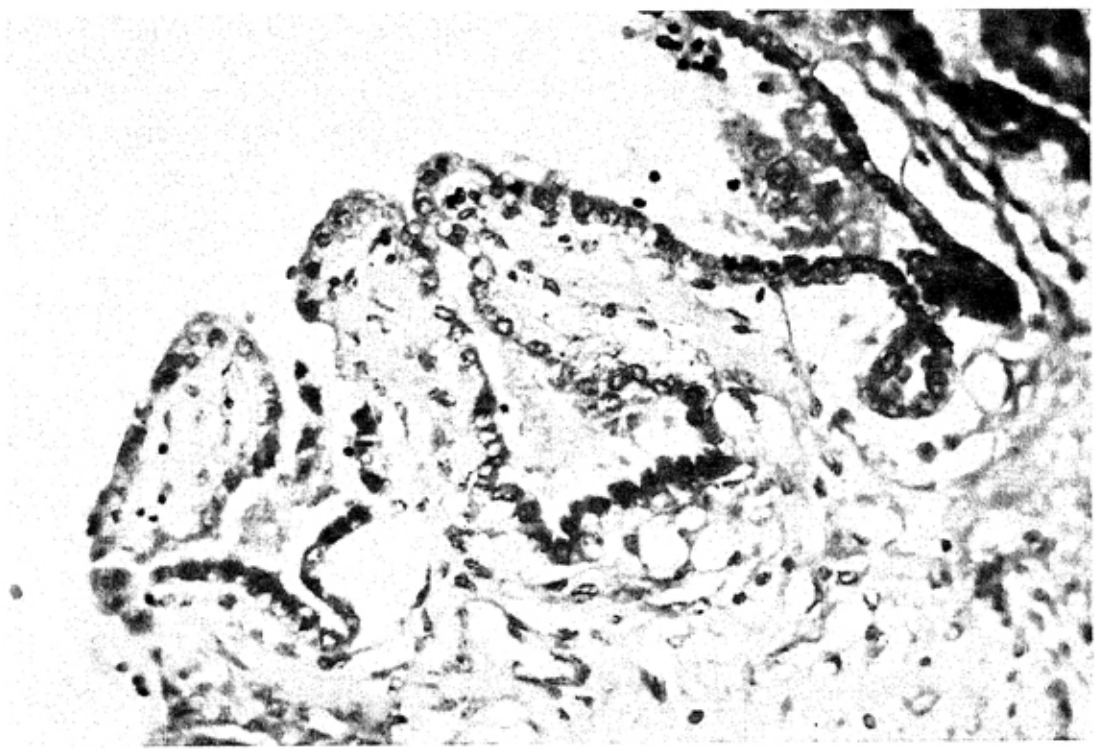


Figs. 7 and 8—High power photomicrographs of sections of endometrium from the treated gilts. Observe the stromal edema and the proliferation of the endometrium.





Figs. 9 and 10—High power photomicrographs of sections of endometrium from control gilts.



CONCLUSIONS

Results obtained in this study demonstrate that the uterus is capable of considerable enlargement due to distention by the products of conception and the great quantity of accumulated fluids in the lumen of the uterus.

The major factor influencing embryonic growth and development is an optimal uterine environment. Exogenous progesterone and estrogen in minute therapeutic doses administered at certain critical periods of pregnancy seem to exert a general beneficial effect in the uterus and alter or promote conditions conducive for subsequent demands of the products of conception. It may be conjectured that the experimental modification of the uterine environment resulted in a fuller expression of the reproductive potentialities of the animal.

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