EFFECTS OF PROGESTINS ON REPRODUCTION IN SWINE AND RATS

I. Effects on Embryonic Mortality of Swine

II. Effects on Embryonic Mortality and Pituitary Gonadotropins in Rats

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CHAPTER I

INTRODUCTION

It has been known for many years that the litter size of swine and other polytoccus animals at parturition is much less than the number of ova ovulated, based on the number of corpora lutea. Recently, fertilization failure has been shown to account for only a small number of missing embryos; therefore, a large number of embryos die and are absorbed during pregnancy.

Many factors contribute to embryonic mortality and it is the opinion of some investigators that a more favorable intrauterine environment, reflecting proper gonadal hormone relationships, should reduce losses.

Past attempts to enhance embryonic survival by injecting natural gonadal hormones have not been successful possibly because the optimal doses and proportions of estrogen and progesterone are not known. This is complicated by the fact that gonadal hormones have several actions and also interact with each other.

Recently chemists have been able to alter the structure of gonadal hormones, accentuating some actions while diminishing others. Some of these compounds are also active when given orally thus making their use more practical as they can be given as feed supplements rather than by hypodermic injection. Recently two orally active progestins, 17a-acetoxyprogesterone (17-AP) and 6a-methyl-17a-acetoxyprogesterone (6-M-17-AP), have been used effectively to treat repeated and threatened

abortions and menstrual disorders in women. These two progestins have also been used to control estrus in the cow, sow, and bitch.

Because modern husbandry methods make it possible for gilts and sows to rear larger litters, embryonic mortality is a greater loss to producers and consumers than ever before and new attempts to reduce these losses by hormone supplementation are therefore justified.

In view of the foregoing considerations a study, composed of four experiments, was undertaken to determine if exogenous progestins would influence embryonic mortality, fetal size, uterine enzymes, and other parameters of reproduction during early pregnancy in swine.

CHAPTER II

REVIEW OF LITERATURE

History of Embryonic Mortality in Swine. Attention was first drawn to the high incidence of embryonic mortality in swine by Hammond (39, 40) in 1914, and again in 1921, when he reported an average ovulation rate of 20 ova per sow, and an average of 12 pigs per litter at 30 to 60 days gestation. Ovulation rate was based on the number of corpora lutea. Estimation of ovulation rate based on corpora lutea counts has since been shown to be valid (79).

Fertilization failure constitutes a small part of missing embryos since it has been shown that the fertilization rate approaches 95 to 100 percent (79, 84, 89, 93). Although fertilization rates are high in swine, embryonic mortality figures as high as 30 to 40 percent have been reported, with few animals completing gestation without some losses. Perry (77) has suggested that a loss greater than 10 percent is "reproductive wastage."

In 1928 Warwick (104) observed the highest incidence of embryonic death between 20 and 30 days gestation and in 1952 Squires, et al. (93) reported that the first 25 days of gestation is the most critical period of survival. In 1954 Perry (76) conducted an extensive survey of embryonic death. The results of this survey are in agreement with the results of earlier investigations. He reported that 85 percent of embryonic loss occurs in the first half of gestation and that 75 percent of this loss

occurs before the 25th day with the greatest loss near or at nidation (10th to 20th day). Later experiments by Rathnasbapathy et al. (81) showed that 95 percent of embryonic mortality occurs before midterm.

Sorensen and Gossett (91) and Gossett and Sorensen (34) have reported that necropsy at 40 days gestation allows a more accurate estimate of early embryonic mortality. Day et al. (17) have reported embryonic mortality figures of 33 percent at 25 days and 38 percent at 50 days gestation. Their data are in close agreement with those of Gossett and Sorensen (34).

FACTORS AFFECTING EMBRYONIC MORTALITY

Breed: Duroc and Chester White breeds have larger litters than Hampshires, the latter breed having larger litters than Berkshires and Poland Chinas (50). Stewart has reported that with 10 percent inbreeding an average reduction in litter size of 0.6 of a pig occurs (96). Dickerson et al. (22) have reported only 0.2 of a pig per litter decrease with 10 percent inbreeding. Squires et al. (93) have reported 0.55 more ova ovulated, 0.33 fewer ova lost and 0.8 more embryos per gilt at 25 days gestation when 10 percent inbred lines were cross bred, thus reducing inbreeding effects within a breed. In the latter case the increase in litter size was due to increased ovulation rate and decreased embryonic mortality, but differences in litter size among breeds and inbred lines may reflect only ovulation or fertilization rates.

Age: Perry (77) has reported that in gilts the ova of the first litter have a higher rate of survival, but when some loss occurs, the percent depletion in litter size is the same as in sows. He also reported that litter size increases to the sixth litter and then declines. Stewart (96) observed that litter size increases with the age of the dam

up to the 15th month with the greatest increase from the 9th to the 12th month. Squires (93) observed an increase of 0.5 pig per litter per 10 day increase in the age of gilts from puberty to breeding. One should be reminded however, that an increase in litter size does not necessarily indicate a decrease in embryonic death.

Season: The relationship between seasons and embryonic death is not clear. Dutt (26), quoting early German researchers, stated that litters farrowed in the early spring tend to be larger. A decline in litter size and an increase in embryonic death during the warmer seasons is implied by a report that second polar body retention of fertilized ova held at high temperatures results in aberrant mitosis (70). This report is strengthened by the report of Florida investigators that a high ambient temperature of 90° F. may be more harmful to embryonic survival in gilts after 3 days post breeding than previous to that time (102). There is also some evidence that the season in which the dam is born may affect litter size. Gilts that are born in the spring reach puberty later than gilts born in the fall but produce 19 percent more ova (34).

Nutrition: Haines et al. (38) reported that restricting the energy intake of gilts delayed puberty and depressed ovulation rate but decreased embryonic mortality, whereas the opposite occurred when energy intake was unrestricted. These workers suggested that swine should be full fed for high ovulation rates, but "limited fed" during pregnancy to attain higher embryonic survival rates. Nalbandov (66) states that, "a low plane of nutrition during the first half of pregnancy does not affect ultimate fetal weight adversely provided the dietary intake is adequate during the second half." Although proper nutrition is important, embryonic death may occur in the presence of an optimal nutritional environment.

Ovulation rate: It is not always clear in the literature whether reduced litter size at farrowing is due to embryonic death or reduced rates of ovulation since fertilization failure (79) is considered to be minimal.

It has been observed that the proportion of ova lost increases as ovulation rate increases (40, 76, 93). The experiments of Casida (12) and Perry (78) revealed a slight linear correlation between ovulation rate and embryonic mortality up to 25 days gestation, although in Perry's experiment, one series of litters did not show this relationship. Casida (12) reported that in his experiments the correlation becomes greater at 70 to 105 days. He also noted that when the ovulation rate was high and the litter small the embryos were much heavier. He suggested that early embryonic death allowed more progesterone for the surviving embryos. When the ovulation rate was high and the litter size large he noted that the embryos were also larger up to the 25th day of pregnancy. He postulated that sows in good physical condition are not only able to ovulate large numbers of ova but are able to efficiently nourish and maintain more embryos during early pregnancy. From this it appears that early embryonic death is not closely related to ovulation rate.

Polyspermy, capacitation, ova ripening and late breeding: Because polyspermy, capacitation, ova ripening and late breeding are so interrelated they will be reviewed under one heading.

In 1959 Hancock (41) reported on polyspermy (multiple fertilization of a single ovum) in swine. He mated a number of sows 24, 30 and 40 hours after they first showed estrus. Later the ova were recovered at necropsy, stained and examined for pronuclei. He observed that polyspermic fertilization (excess pronuclei) increased with delayed mating. These data were offered as evidence that polyspermy contributes to embryonic death as zygotes with excess pronuclei divide erratically and die.

In 1958 Austin and Bishop (5) and Noyes et al. (69) reported that the spermatozoa of rats and rabbits must undergo a period of incubation in the tubal fluids of the female during which changes (capacitation) occur. These changes seem to involve both enzymic and structural properties of the spermatozoa. This raises the question of a relationship between polyspermy and over-capacitation. The age of the ovum involved in polyspermic fertilization must be considered too. Blandau and Young (7), working with guinea pigs, observed that aged or "over ripe" ova, though fertilized, do not develop into viable embryos.

On the basis of these reports it appears that the time of breeding after the onset of estrus and the age of the ova and spermatozoa involved may be important factors affecting early embryonic death in swine as in other species.

Sex of the embryos: In 1925 Parkes (71) discovered that the sex ratio of baby pigs at birth (secondary sex ratio) is very near equality. He also reported that at conception there are 56.8 males to 49.6 females (primary sex ratio). No explanation for this primary sex ratio was offered. It was noted that the percentage of males decreased as gestation proceeded, a result which can only be brought about by a differential mortality of males and females. Observations by Crew (14) are in agreement with those of Parkes. Crew ascribed the disparity between the two sex ratios to a "sexually selective prenatal mortality." He suggested that the reasons are genetic and that "the male type of physiology is less able to withstand the stress of functioning both pre and postnatally, than is the female."

Intrauterine migration: It has been known for many years that intrauterine migration of fertilized ova occurs in sows (13, 22, 103).

Lasley et al. (46) have shown that litters contained 1.24 more pigs when intrauterine migration was observed and 1.73 fewer corpora lutea not represented by embryos when intrauterine migration was not observed. This suggests the possibility that intrauterine migration may be important in preventing embryonic death losses, probably because of equal distribution of embryos between the uterine horns.

The experiments of du Mesnil du Buisson (23, 24) affirm the general observation that unilateral pregnancy is uncommon in swine. From their experiments it is apparent that migration of zygotes to the empty horn must occur before the 14th day of pregnancy or the empty horn will secrete a luteolytic substance that will terminate pregnancy. This suggests that intrauterine migration of at least one embryo in early unilateral pregnancy may be necessary to assure survival of the entire litter.

Intrauterine migration is under the influence of gonadal hormones. Progesterone causes rabbit uterine muscle to be highly irritable with increased ability to contract locally, but diminished ability to send waves of contraction along the length of the uterus (15). Böving (8) has related these facts to a hypothesis that early blastocyst transport is a random scattering while the blastocysts are small. As post ovulatory progesterone levels increase the blastocysts become enlarged and the uterus is simultaneously "conditioned" so that contractions move toward each end of the uterus whenever it is stimulated (local reflex) by a blastocyst above a certain size. Such contractions enable a blastocyst to cause uterine propulsion of its neighbors where the propulsive activity of the uterine muscle will not affect it. Such a mechanism in swine would not only ensure proper spacing to prevent crowding but would also promote bilateral pregnancy and thus prevent luteolysis.

Intrauterine environment and gonadal hormones: In 1946 Fekete (29) while working with an inbred strain of mice (dba) susceptible to breast cancer, noticed the development of an adrenal hyperplasia characterized by lutein-like cells. Coinciding with this, the uteri became stimulated, probably by estrogen. Mice of this strain also ovulated at a higher rate yet gave birth to fewer living young than another strain of mice (C-57) that did not develop the adrenal condition.

In 1947 Fekete (30) conducted an experiment to determine the cause of the difference in litter size between the two strains. After hundreds of inter- and intra-strain ova transfers she concluded that the ova of the two strains were of the same viability and that the "intrauterine environment" of the C-57 strain was more favorable for the development of embryos.

To support the concept of differences in intrauterine environment between the two strains she presented the following:

> The preparation of the uterus for the implantation of eggs is under the influence of estrogen and progesterone. These hormones must be present in correct proportion. Estrogen in excess or insufficient amount interferes with the development of decidual tissue. Progesterone in excess inhibits the hyperplastic effects of estrogen on the uterus. The main source of both hormones is the ovary, although the adrenal cortical hormones overlap to some extent the activities of the gonadal hormones. It may be possible that differences in the condition of uterine environment originate in the function of the ovaries or the adrenals.

Perry (77) reported that in nulliparous swine the ova of the first litter have a better chance of surviving, but when some loss occurs, the percentage of depletion in litter size is about the same as in sows. On the basis of this, one may postulate that the death of one embryo may contribute to the death of another, and that a system efficient in absorbing dead embryos and membranes might also promote a more favorable intrauterine environment and reduce embryonic death.

Because of the interaction of progesterone and estrogen, it is often difficult to identify physiologic responses as purely progestogenic or estrogenic. The relationship between progesterone and intrauterine environment (29, 30) and intrauterine migration (8, 15, 23, 24, 27, 46, 102) as related to embryonic mortality has been reviewed.

Progesterone alone or in combination with an estrogen has been shown to be essential to the maintenance of pregnancy in ovarectomized rats (52), sheep (32), and swine (17) and in cattle after ablation of the corpus luteum (58). Kendall and Hays (44) have shown that progesterone reduces embryonic death in intact rats on multiple nutrient deficient diets. There are, however, reports of increased embryonic death with parenteral administration of progesterone to intact, pregnant swine (67, 92).

In 1958 Reddy et al. (83) gave daily injections of varying proportions of estrone and progesterone to pregnant swine from the 4th to the 14th and the 14th to the 24th days of gestation. The animals were necropsied on the 55th day of gestation and the uterus and its contents were examined. Intrauterine environment appeared to be improved and an embryonic mortality of 13.50 percent occurred in the treated animals and a mortality of 23.30 percent occurred in the untreated controls. This reduction of embryonic mortality was observed only in those animals injected with 12.5 ug. of estrone and 25 mg. of progesterone (1:2000) from the 14th to the 24th day of gestation. They concluded that "exogenous progesterone and estrogen in minute therapeutic doses administerd at certain 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."

There appears to be a relationship between the gonadal hormones, uterine phosphatases, intrauterine environment and embryonic survival. In 1955, Bredeck and Mayer (10) demonstrated a positive correlation between uterine acid phosphatase activity and litter size and weights of rat embryos, whereas a negative correlation was noticed between uterine alkaline phosphatase activity and these same parameters. In 1960, Goode et al. (33) determined endometrial acid and alkaline phosphatase activity in gilts during luteal and follicular phases of the estrous cycle up to the 25th day of gestation. They found high acid phosphatase activity during the luteal phase (when progesterone blood levels are high) and high alkaline phosphatase activity during the follicular phase (when estrogen blood levels are high). Acid phosphatase is associated with modified mitochondria (lysosomes) thought to function in phagocytosis, atherocytosis, pinocytosis, intracellular digestion, and secretion (19, 20). It may be postulated that a relationship exists whereby progesterone promotes acid phosphatase activity or lysosomal activity, which in turn contributes to embryonic survival by improving intrauterine environment.

CHAPTER III

MATERIALS AND METHODS

EXPERIMENT I:

Twenty-one crossbred, second-litter sows (Duroc, Hampshire, and Landrace breeds) averaging 425 lb. body weight were bred at 4-hour intervals to each of 2 yearling Hampshire boars of proved fertility then randomly and equally allotted to a control group and 2 treatment groups. Those in one treatment group were given 1 mg. of 17-AP and 0.5 gamma of diethylstilbestrol (DES) per pound of body weight daily per os. Those in the other treatment group were given 0.5 mg. of 17-AP and 0.25 gamma of DES per pound of body weight daily per os. The control and progestin fed sows were individually penned and fed and the progestin fed sows were put on treatment at the first feeding after the second breeding. The calculated daily dose of progestins for each sow was mixed in soybean meal and fed as a divided dose twice daily in the feed throughout the experiment. The soybean meal in which the drug was mixed was part of the regular ration. The sows were fed 8 lb. per head daily of a well-balanced brood sow ration for about 10 days preceding estrus, and after breeding were fed 6 lb. of this ration per head daily until the end of the experiment.

All animals were killed on the 25th day of gestation ($\pm$ 2 days) and corpora lutea and live fetuses were counted. The difference between the two counts was recorded as embryonic mortality, since it is assumed that

each corpus luteum represented a fertilized ovum (79). The day of breeding was considered day 1. Macerating or underdeveloped embryos or embryos with avascular membranes (considered dead or dying) were recorded along with missing embryos as embryonic deaths. All corpora lutea were extirpated to ensure an accurate count.

EXPERIMENT II:

Thirty crossbred gilts (Duroc x Beltsville No. 1) averaging 290 lb. body weight were fed 8 lb. per head daily of the control ration used in experiment I for about 10 days preceding estrus. The gilts were assigned equally to control and treatment groups to make certain that the range and mean body weight in the groups were similar, since twice daily group feeding and progestin administration was practiced. Breeding was handled as in experiment I, and all groups were fed at the rate of 6 lb. of ration per head daily. One mg. of 17-AP and 0.5 gamma of DES per pound of body weight daily was included in the rations of the treated groups. This experiment was terminated by necropsy at 40 days gestation. At necropsy, right, left and total corpora lutea numbers and fetus numbers, plus intact and empty uterine weights were recorded. Intact ovary weights; right, left and total corpora lutea wet weights; fetus lengths; dry fetus weights; and dry membrane weights were determined. Maternal carcass weights were also recorded.

EXPERIMENT III:

Twenty-four Duroc gilts averaging 285 lb. body weight were used to repeat experiment I, except that the low level progestin treatment was replaced by 6-M-17-AP administered at the rate of 60 mg. per head daily. The gilts in this experiment were assigned to treatment and control groups as in experiment II and were bred, fed and individually administered

hormones as in experiment I. This experiment was terminated at 25 days gestation. At necropsy, right, left and total corpora lutea numbers and fetus numbers were recorded. Intact ovary weights; right, left and total corpora lutea wet weights; fetus lengths; dry fetus weights; and dry membrane weights were also determined. Samples of endometrium were collected and quick frozen as soon after slaughter as possible. Later, a 1% endometrial homogenate in isotonic potassium chloride (1.15%) was prepared in a Potter-Elvehjem homogenizer and centrifuged under refrigeration in an ultracentrifuge* for 45 minutes at a maximum of 105,000 g. The supernatant fluid was analyzed for alkaline and acid phosphatase content according to the methods of Bessey et al. (6) and Andersch and Szczypinski (1) with modifications outlined in Technical Bulletin #104 (Sigma)**. Tissue enzyme activities were expressed as units of enzyme activity per milligram of soluble protein. One unit of phosphatase was defined as that quantity that would liberate 1 uM (0.1391 mg.) of p-nitrophenol, from p-nitrophenol phosphate, per hour under the conditions of the assay. Protein was determined by trichoracetic acid precipitation (94).

EXPERIMENT IV:

This experiment repeated experiment I, except that third-litter Duroc sows averaging 466 lb. of body weight were used, and a total dose of 90 mg. of 6-M-17-AP per animal per day was administered to the second treatment group (Table 1).

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*Spinco Model L, manufactured by Beckman Instruments, Inc., Spinco Division, Palo Alto, California.

**Sigma Chemical Company, 3500 DeKalb St., St. Louis 18, Missouri

CHAPTER IV

RESULTS AND DISCUSSION

EXPERIMENT I:

A mean embryonic mortality of 37.90% occurred in the control group, 18.26% in the group given the lower dosage of 17-AP-DES, and 10.93% in the group given the higher dosage of 17-AP-DES. The reduction in embryonic mortality in those given the higher dosage was highly significant ($P = 0.01$) when these data were subjected to an analysis of covariance in which the covariable was the number of ova shed. The reduction in embryonic mortality in those given the lower dosage, though showing a favorable trend, was not significant. The wide range of embryonic death occurring within litters (Table 1) in this experiment indicated that the error term is characteristically of such magnitude that considerable reduction in embryonic death is necessary before significance occurs. At necropsy, a total of 6 dead embryos was found in the six control litters and 1 in the 13 litters of the treated groups. This seemed to indicate that not only did a reduction in embryonic death occur, but that treatment enhanced resorption of those embryos that did die. Embryonic death in the control group was higher than in other experiments described herein. This higher, but not unusual embryonic mortality, seemed to indicate the presence of a less favorable intrauterine environment and uteri more amenable to reinforcement by hormone supplementation. Because of the range of slaughter dates (22 to

TABLE I - EFFECT OF PROGESTINS ON EMBRYONIC MORTALITY OF SWINE

Control			1.0 mg. 17-AP / 0.5 DES/ 1b/day			0.5 mg. 17-AP / 0.25 DES/1b/day or 60 mg. 6-M-17-AP/animal/day or 90 mg. 6-M-17-AP/animal/day		
Corpora Lutea	Live Embryos	Embryonic Mortality	Corpora Lutea	Live Embryos	Embryonic Mortality	Corpora Lutea	Live Embryos	Embryonic Mortality
Experiment I - Crossbred Sows 25 Days Post Breeding								
(37.90% embryonic mortality)			(10.93% embryonic mortality)##			(18.26% embryonic mortality) (0.5 mg. 17-AP / 0.25 DES/1b/day)		
16	4	12	17	13	4	18	17	1
19	12	7	18	17	1	20	16	4
26	14	12	19	18	1	23	18	5
23	18	5	15	13	2	17	9	8
20	17	3	18	14	4	18	16	2
20	12	8	18	18	0	19	18	1
0* non-pregnant-cystic ovaries			23	21	2	0* non-pregnant-cystic ovaries		
TOTAL 124	77	47	128	114	14	115	94	21
MEAN 20.66	12.83	7.83	18.29	16.28	2.0	19.17	15.67	3.50
SD 3.4	5.00	3.7	2.4	3.0	1.5	2.1	3.4	2.7
Experiment II - Crossbred Gilts 40 Days Post Breeding								
(25.67% embryonic mortality)			(23.00% embryonic mortality)					
15	10	5	13	13	0			
14	12	2	17	14	3			
16	14	2	17	10	7			
14	8	6	19	16	3			
22	8	14	15	12	3			
16	12	4	17	15	2			
18	14	4	13	9	4			
22	15	7	15	11	4			
24	18	6	21	11	10			
16	16	0	14	12	2			
23	12	11	17	13	4			
12	11	1	17	11	6			
9	8	1	14	10	4			
16	15	1	17	17	0			
16	15	1	0* non-pregnant					
TOTAL 253	188	65	226	174	52			
MEAN 16.87	12.53	4.33	16.14	12.42	3.71			
SD 4.2	3.1	4.0	2.3	2.4	2.6			
Experiment III - Duroc Gilts 25 Days Post Breeding								
(18.92% embryonic mortality)			(12.76% embryonic mortality)			(27.60% embryonic mortality) (60 mg. 6-M-17-AP/animal/day)		
15	15	0	15	15	0	17	8	9
11	9	2	20	17	3	21	15	6
13	13	0	17	16	1	15	12	3
19	15	4	14	14	0	17	13	4
15	12	3	13	12	1	18	16	2
22	16	6	15	8	7	17	12	5
16	10	6	0* non-pregnant-cystic ovaries			0* non-pregnant - cystic ovaries		
0* anestrus-inactive ovaries			0* anestrus-inactive ovaries			0* non-pregnant - cystic ovaries		
TOTAL 111	90	21	94	82	12	105	76	29
MEAN 15.86	12.86	3.0	15.67	13.67	2.0	17.50	12.67	4.83
SD 3.7	2.7	2.5	2.5	3.3	2.7	1.97	2.8	2.5
Experiment IV - Duroc Sows 25 Days Post Breeding								
(27.55% embryonic mortality)			(29.33% embryonic mortality)			(19.31% embryonic mortality) (90 mg. 6-M-17-AP/animal/day)		
23	8	15	21	13	8	20	15	5
23	18	5	19	11	8	19	18	1
21	15	6	21	17	4	17	13	4
15	13	2	17	15	2	14	13	1
19	14	5	18	8	10	22	18	4
19	16	3	20	18	2	22	15	7
18	16	2	0* non-pregnant-cystic ovaries			0* non-pregnant-cystic ovaries		
0* anestrus-inactive ovaries			0* non-pregnant-cystic ovaries			0* non-pregnant-cystic ovaries		
						0* non-pregnant-cystic ovaries		
TOTAL 138	100	38	116	82	34	114	92	22
MEAN 19.71	14.28	5.43	19.33	13.67	5.67	19.0	15.33	3.67
SD 2.9	3.2	4.5	1.6	3.8	3.4	3.1	2.2	2.3
Summary								
35 litters			33 litters					
(27.32% embryonic mortality)			(19.86% embryonic mortality)#					
TOTAL 626	455	171	564	452	112			
MEAN 17.89	13.00	4.89	17.09	13.70	3.39			
SD 4.04	3.34	3.97	2.58	3.18	2.84			

##P = .01
#P = .17
*Animal discarded from experiment

27 days) and rapid growth of tissues at this time, data such as fetus and membrane weights were not analyzed. One control sow and 1 low-level treatment sow had cystic ovaries at necropsy and were discarded from the experiment.

EXPERIMENT II:

The apparent favorable reduction in embryonic mortality in experiment I prompted another experiment to determine if such reduction could be repeated in crossbred gilts. A mean embryonic mortality of 25.67% occurred in the control group and 23.00% in the group treated with 17-AP-DES (Table I). This difference, though indicating a slight reduction of embryonic death in the treated group, was not significant as indicated by analysis of covariance. Embryonic death in the control group was surprisingly low even though there were 15 experimental animals. One treated animal was not pregnant and had developing follicles at necropsy; consequently, these data represent 14 pregnancies.

Analysis of the ovarian and uterine data showed no significant differences. Two dead embryos were found in the control litters and none were found in the treated litters.

Although these gilts were group fed, and probably some animals consumed more than the intended amount of the hormone-containing feed, evidence of toxicity was not observed during the 40 days of the experiment. There was no evidence that the presence of the hormones in the feed interfered with feed consumption or rate of gain.

EXPERIMENT III:

In this experiment, an unusually low mean embryonic mortality of 18.92% occurred in the control group, 27.60% in the 6-M-17-AP treated group, and 12.76% in the 17-AP-DES treated group (Table 1). Although it

appeared that embryonic mortality was increased in the 6-M-17-AP treated group and decreased in the 17-AP-DES treated group, the differences were not statistically significant as indicated by analysis of covariance. Four dead embryos were found in the seven control litters, 7 in the six litters treated with 6-M-17-AP and 3 in the six litters treated with 17-AP-DES. Analysis of the ovarian and uterine data revealed no significant differences, this being in accord with the findings in experiment II. Edema of the uterine mucosa was evident in 4 animals given 6-M-17-AP, indicating that the dosage of this hormone may have exceeded physiologic limits. Two gilts given 6-M-17-AP and 1 gilt given 17-AP-DES had cystic ovaries and were not pregnant at necropsy. One animal originally assigned to the control group and 1 animal assigned to the 17-AP-DES group never mated and necropsy revealed underdeveloped uteri and inactive ovaries in both gilts. Only pregnant animals were considered when analyzing data.

EXPERIMENT IV:

This experiment was designed to repeat experiment I, although Duroc sows had to be substituted for crossbred sows, and the lower dosage of 17-AP-DES was replaced with 6-M-17-AP. A mean embryonic mortality of 27.55% occurred in the control group, 19.31% in the 6-M-17-AP treated group, and 29.33% in the 17-AP-DES treated group (Table 1). None of these differences were statistically significant. Five dead embryos were found in the seven control litters, 3 in the six litters treated with 6-M-17-AP and 3 in the six litters treated with 17-AP-DES. Analysis of the ovarian and uterine data revealed no significant differences, which is in accord with experiments II and III.

Edema of the uterine mucosa was observed in 2 sows given 6-M-17-AP and 2 sows given 17-AP-DES had cystic ovaries. None of these sows were

pregnant and consequently were discarded from the experiment. Because of the high incidence of cystic ovaries in the 6-M-17-AP treated group, an extra sow was bred and added to the group.

The cysts in all ovaries from all experiments were 1 to 4 cm. in diameter and the number of cysts per ovary did not exceed the number of expected corpora lutea. Possibly luteinizing hormone release in the gilts and sows which ovulated late in estrus or luteotropic hormone effects (11, 85, 86) were inhibited by the progestins thus contributing to cyst development. There may be a breed difference in susceptibility to cyst formation. When crossbreds and Durocs are compared, the latter appears to be more susceptible. The incidence of cystic ovaries (Table 2) was 2.6% in the control animals, 8.9% in the 17-AP-DES treated animals, and 29.4% in the 6-M-17-AP treated animals disregarding parity, breed or hormonoid dosages. The incidence was higher in sows than in gilts. According to Nalbandov's classification (64), the cysts could best be classified as "multiple, bilateral, large," except that luteinization was absent or scanty and the uterine endometrium was not progestational. A few cysts from the ovaries of all groups were filled with sanguineous fluid.

TABLE II. OCCURRENCE OF CYSTIC OVARIES IN SWINE AT NECROPSY

	Control	17-AP-DES Treated	6-M-17-AP Treated
Sows	1/15 (6.7%)	3/22 (13.6%)	3/9 (33.3%)
Gilts	0/23 (0%)	1/23 (4.3%)	2/8 (25.0%)
Total	1/38 (2.6%)	4/45 (8.9%)	5/17 (29.4%)

In order to evaluate uterine acid and alkaline phosphatase data, experiments III and IV were pooled. Significant differences in phospha-

tase activity did not occur and there were no significant relationships between enzyme activity and litter size. These results are quite different from those reported by Bredeck and Mayer (10) for the pregnant rat; however, different techniques were used. In Bredeck and Mayer's experiment, a whole-tissue homogenate of the rat uterus was used for the enzyme determinations and therefore measured both endometrial and myometrial enzyme activity. In the swine experiment reported herein, supernatant fluid from the centrifuged homogenate of selected endometrial samples in apposition to the fetal membranes was assayed for enzyme activity. Because assay conditions favor lysis of lysosomes, the supernatant fluid should be high in free acid phosphatase in spite of some loss due to secondary adsorption to sedimentable particles (19). Although no significant differences in enzyme activities were observed in pregnant animals, the comparison of uterine enzyme activities of pregnant animals with nonpregnant animals having cystic ovaries is worthy of comment. In all pregnant animals, acid phosphatase activity was greater than alkaline phosphatase activity, whereas in nonpregnant animals with cystic follicles, the opposite was true. In 38 pregnant gilts and sows, the mean acid phosphatase and alkaline phosphatase activity was 34.06 and 6.33 units/mg. of soluble protein, respectively; whereas, in 8 nonpregnant gilts and sows with cystic ovaries, the mean acid phosphatase and alkaline phosphatase activity was 6.66 and 37.11 units/mg. of soluble protein, respectively. There was a large degree of individual variation within the groups. These findings agree with Goode et al., (33) in that a high acid phosphatase activity is associated with luteal dominance and a high alkaline phosphatase activity is associated with follicular dominance. Lesser effects due to hormone administration, if present, were not detected.

After completing the four experiments, it was apparent that the higher dose of 17-AP-DES had reduced embryonic mortality to some degree in three of four groups of gilts and sows while probably inducing a low incidence of cystic ovaries. Because the gilts and sows in the four experiments were of different ages, parity and breed, they represented a population cross section. To better evaluate the results due to treatment by the higher dose of 17-AP-DES in the four experiments, the four experiments, including the low level 17-AP-DES treated group, and the 6-M-17-AP treated groups, were analyzed as a randomized block design (each of the experiments composing a block), and occurrence of embryonic death in the treated animals was tested by an analysis of covariance (Table 1). Ovulation rates in the 35 control and 33 treated litters in the four blocks, were adjusted to a common mean. In the pooled control litters, the mean embryonic mortality was 4.89 pigs per litter compared to 3.39 pigs per litter in the pooled treated groups. This difference was significant at the probability level of 0.17. The regression coefficient (corpora lutea to embryonic mortality) was 0.6 for the control groups and 0.32 for the treated groups. The correlation coefficient for the control groups was 0.89 and for the treated groups, 0.5.

CHAPTER V

SUMMARY AND CONCLUSIONS

Four experiments involving 100 gilts and sows were conducted to test the hypothesis that the daily administration of orally active progestins, from breeding until slaughter at 25 or 40 days gestation, would reduce embryonic mortality by enhancing intrauterine environment.

In these experiments, significant reduction in embryonic mortality occurred only when the embryonic mortality in the control animals approached the 30 to 35% level reported by previous investigators. There was an unusually low embryonic mortality in some control groups and a high degree of individual variation.

Analysis of pooled data, wherein 33 pregnant animals, given 1 mg. of 17-AP and 0.5 ug. of DES per pound of body weight daily were compared with 35 pregnant control animals, indicated a reduction in embryonic mortality (3.39 vs. 4.89 dead embryos) significant at the probability level of 0.17. There was no reduction of embryonic mortality in the 12 pregnant animals given 6-M-17-AP compared with 14 pregnant control animals.

Significant differences in endometrial alkaline and acid phosphatase activities did not occur between groups except when pregnant and nonpregnant animals were compared.

The incidence of cystic ovaries at necropsy was 5 of 17 (29.4%) in the 6-M-17-AP treated groups, 4 of 45 (8.90%) in the 17-AP-DES treated

groups, and 1 of 38 (2.60%) in the control groups. From these results and a recent report (11), it appears that some animals were put on treatment before ovulation and that ovulation was blocked in those animals sensitive to the antiovulatory effects of progestins. Sammelwitz et al. (87) and Sammelwitz and Nalbandov (86) have reported that a luteotropic hormone (LTH) sufficient for formation of corpora lutea (essential for maintenance of pregnancy) occurs during, or a few hours after, ovulatory LH release and that this LTH can be blocked by progesterone. Brinkley et al. (11) have suggested that ovulation per se (LH release) is essential to formation and function of corpora lutea and that progestins given after ovulation will not interfere with luteal development and function. If LH and/or LTH was blocked by progestins in the experiments described in this thesis, the mature follicles would not have ruptured (ovulation) or become luteinized. These follicles would have continued to secrete antral fluid thus becoming cystic. One can conclude that the gilts and sows were put on treatment too early in estrus causing an increase in the incidence of cystic ovaries at necropsy.

Although the benefits of hormone supplementation during early pregnancy were not profound in these experiments and there was an incidence of ovarian cystic disease, the results are encouraging. These findings also lend support to a hypothesis that embryonic mortality may be due to multiple causes, one of which may be removed in part or in full by correct hormone administration.

Part II. Effect of 17α-acetoxyprogesterone on Embryonic Mortality and Pituitary Gonadotropins in Rats.

CHAPTER I

INTRODUCTION

In the experiments discussed in Part I of this dissertation the daily administration of synthetic progestins to swine reduced embryonic mortality but induced follicular type ovarian cysts in 14.51% of 62 animals compared to 2.60% of 38 untreated animals. Swine given 6a-methyl-17a-acetoxyprogesterone (6-M-17-AP) had a higher incidence of ovarian cysts than those given 17a-acetoxyprogesterone (17-AP) (Table II, Part I). Sows and gilts with ovarian cysts were not pregnant. These induced cysts were similar to many of the cysts observed in commercial animals at the abattoir.

Ovarian cystic disease of swine is listed as second only to embryonic mortality in economic importance, and is one of the most interesting yet least understood problems of reproductive physiology (66, 80).

Ovarian cysts occur in most species but the incidence is highest in swine and apparently lowest in sheep and rats. The literature indicates that progesterone-induced and spontaneously occurring cysts are due to gonadotropin alteration. It appears from a review of the literature that the incidence of spontaneous ovarian cysts is highest in those species in which cysts are induced most easily. Swine have the highest incidence of spontaneous ovarian cysts and cysts are induced very easily in swine, whereas rats apparently have few spontaneous cysts and are insensitive to large doses of progesterone. Although the literature indicates that swine are very susceptible to the adverse effects of progesterone *per se*

(follicular cysts, luteal regression, or embryonic death), earlier experiments (Part I) showed that they are less sensitive to the adverse effects of 17-AP than to 6-M-17-AP. There was a reduction in embryonic death and no observable luteal regression in pregnant animals receiving 17-AP even though ovarian cysts were found in animals that were not pregnant at necropsy.

Because of these observations the question was raised whether pregnant rats would experience the beneficial effects of 17-AP and not the adverse effects, or show a complete lack of response as they do to progesterone. Another question raised was whether the response of a species insensitive to large doses of progesterone and characterized by a low incidence of spontaneous cysts might provide basic information concerning the cause of, and the species susceptibilities to, follicular cysts.

In an attempt to answer these questions a study was undertaken, using pregnant rats, to determine if 17-AP would influence embryonic mortality, fetal size, uterine phosphatase content, pituitary gonadotropin amounts, the occurrence of follicular cysts and other parameters of reproduction during the first 13 days of pregnancy.

CHAPTER II

REVIEW OF LITERATURE

Ovarian cystic disease: Pomeroy (80) listed the ovarian cystic disease of swine as second only to embryonic mortality in importance. Nalbandov (66) states that "the formation of cysts is one of the most interesting yet least understood topics in the physiology of reproduction." Both progesterone induced and spontaneously occurring cysts are thought to be due to an imbalance of pituitary gonadotropins (64, 99). From a review of the literature one can conclude that there is a direct relationship between the incidence of spontaneously occurring cysts and ovarian responsiveness to the adverse effects of exogenous progesterone (luteal regression or follicular cysts) in swine (86, 99), cattle (49, 100, 109), sheep (108), and rats (67, 87). Ovarian cysts are a common cause of sterility in swine and dairy cattle but are only rarely observed in sheep (66). Apparently the incidence of spontaneously occurring follicular cysts is low in the rat since a search of the literature does not reveal a record of such a condition.

Since human chorionic gonadotropin (principally luteinizing hormone) is often effective in treating animals with cystic follicles it is possible that the ovarian cystic disease is caused by a malfunction of mechanisms associated with ovulation and/or luteinization.

History of embryonic mortality in rats: The factors contributing to embryonic mortality are reviewed in Part I and, though embryonic death in

swine is emphasized, the factors discussed also apply to rats.

The earliest reported figures on embryonic mortality in the albino rat are those of Long and Evans (48). They reported mean values of 10.40 ova ovulated, 9.16 implanted but only 6.40 young born. In the wild rat Perry (75) found that only 5 to 15 percent of the fetuses were lost between ovulation and implantation which is in contrast to the 38 percent loss reported by Long and Evans in the albino rat. Tyler and Chapman (98) have reported 10.9 ova and 8.6 young per litter or 21 percent missing fetuses. The determination of embryonic mortality on the basis of corpora lutea counts assumes a 100 percent fertilization rate which introduces some error since Braden (9) reported a fertilization rate of 92 percent for albino rats. He did not count corpora lutea but counted the fertilized and unfertilized ova in uterine washings. He states that "all estimates of embryonic mortality include the loss due to non-fertilization of eggs, which normally is less than 10 percent, although under some circumstances it may be considerably higher."

Bredeck and Mayer (10) have observed that 60% of the embryonic mortality in rats occurs before placental function which probably begins on or after the 14th day (48). They (10) also observed a relationship between litter size and acid and alkaline phosphatase which implies a relationship between uterine enzymes and embryonic survival. The details of their experiments and the possible relationship between the gonadal hormones, uterine phosphatases, intrauterine environment and embryonic survival are discussed in Part I under Intrauterine environment and gonadal hormones.

Pituitary gonadotropins: There is substantial evidence that in mammals the anterior hypophysis secretes three trophic substances which stimulate and govern gonadal activity (37). These trophic hormones are follicle

stimulating hormone (FSH), luteinizing hormone (LH) and prolactin or lactogenic hormone, which in some species has luteotropic properties (68). Luteotropins other than prolactin have been designated as LTH (86). In the remainder of this dissertation if the luteotropic hormone referred to is prolactin it will be indicated in parenthesis.

Follicle stimulating hormone (FSH): Follicle stimulating hormone determines, to a large degree, the growth of the ovary by promoting follicular enlargement though the ovum itself is not affected by this hormone (53). In hypophysectomized animals (free of LH), FSH stimulated ovaries appear as a mass of translucent cystic follicles. Follicular enlargement, which is mostly due to the accumulation of antral liquor, occurs at a constant rate for any given species. In intact animals a rapid preovulatory enlargement of the follicle occurs due to a rapid increase in the secretion of follicular liquor. There may be marked hyperemia (43). The effects of FSH as an entity absolutely free of LH are unknown but when FSH acts with minute amounts of LH, estrogen and progesterone secretion by the ovaries is enhanced (31). There is complete agreement that estrogen in moderate to high dosage inhibits FSH synthesis and liberation (37). This inhibition of FSH secretion is controlled by the "negative feed back" of at least one gonadal hormone. Progesterone is thought to prevent the release but not the secretion of FSH by the pituitary gland and to prevent the reduction of pituitary FSH caused by estrogen (101). Because of the difficulty in assaying circulating FSH it is not known if pituitary FSH levels reflect circulating levels.

The earlier assays for FSH were indirect and based on the weight of the uterus in FSH-injected, intact, immature mice (45). In recent years the ability of FSH to synergize human chorionic gonadotropin (HCG) to increase the weight of ovaries from immature rats has been used (95).

Recently a more sensitive assay for FSH has been described by Payne et al. (73). The assay is based on the sensitization of the ovaries of hypophysectomized immature rats to FSH with an estrogen, diethylstilbestrol (DES). This assay, like that of Steelman and Pohley (95), is based on ovarian weight but the weight increases are primarily due to follicular development rather than interstitial tissue growth. Payne's method is claimed to be more sensitive and quantitative.

Luteinizing Hormone (LH): Luteinizing hormone has been shown to cause repair of atrophic ovarian interstitial tissue cells in long-term hypophysectomized immature female animals (90). When LH is given to intact immature female animals the ovarian manifestations are inconspicuous (37), but if given with FSH there is evidence of estrogen and progesterone secretion by the follicle (36). The effect of LH on the ovaries of intact adult rats are macroscopically evident. The rupture of mature follicles occurs (ovulation) and there is widespread luteinization of both ruptured and unruptured follicles. Dempsey (21) suggested that sexual periodicity was due to fluctuation in LH release, with consequent ovulation and corpus luteum development, accounting for the estrous cycle. This concept is well substantiated (36, 43, 54).

It has been shown that both pituitary and circulating LH are diminished by exogenous progesterone which lends credence to the idea that the secretion and release of LH is regulated by the "negative feed back" of progesterone (101). Recently McCann (56) has shown that there is a hypothalamic luteinizing hormone releasing factor (LH-RF). This factor is blocked by both progesterone (57) and estrogen (97) which lends further credence to the "negative feed back" control of pituitary and circulating LH.

Robinson and Nalbandov (85) have postulated that the bio-assay of pituitary gonadotropins does measure the rate at which gonadotropins are secreted and released into the blood stream. Recent research (57, 96), and more refined assay techniques, indicate that this postulation may be correct for LH.

The most popular assay for LH has been that of Greep et al. (35). In this assay hypophysectomized immature male rats are injected with the LH-containing material for several days; the LH causes the gonadal interstitial tissue to secrete androgen which in turn causes the ventral prostate to increase in weight. Ventral prostate weights of the assay animals are used as an indication of LH content of the material. This assay lacks sensitivity.

Recently a very sensitive method for the assay of LH has been developed (72) and evaluated (88) and has opened the way for a more critical evaluation of the relationship between LH and the gonadal hormones. This assay is the ovarian ascorbic acid depletion (OAAD) method and is based on the fact that LH depletes the ascorbic acid content of the luteal tissue of immature pseudopregnant rats. The nature of this depletion is not known. The degree of depletion is easily measurable four hours after LH has been injected. Less than 1 ug. of LH can be measured by this assay. A detailed evaluation of this technique (88) has revealed that the proper strain of rats (Holtzman) must be used, that larger quantities of LH are not always accurately measured and that serial dilutions of assay material do not always parallel the standard curve. In spite of these problems this assay is by far the most sensitive and accurate if conducted properly.

Luteotropic and associated phenomena: Reproductive physiologists are vitally interested in the mechanisms of development, maintenance and

function of corpora lutea because of the relationship between progesterone and embryonic mortality. It appears that when corpora lutea are not formed or maintained properly, the danger of cystic follicle development is greater. Several luteotropic mechanisms have been described for mammals but which mechanism applies in each species is not clear. Some of these mechanisms will be reviewed from a comparative approach.

Astwood (4) has shown that an anterior pituitary hormone, luteotropin (prolactin), directly maintains the functional activity of corpora lutea in rats. This observation has since been confirmed by other investigators (16, 28, 51). Nalbandov (68) states that "it is becoming increasingly more certain that prolactin is either completely nonluteotropic or, at best, only partially luteotropic in such animals as sheep, pigs, cows, guinea pigs, rabbits and women." He suggests that the question of hormonal species specificity is yet unanswered. Prolactin may be partially luteotropic in sheep (62).

It is evident that a luteotropic substance(s) other than prolactin is present in some species (11, 87) including the rat (3). Sammelwitz et al. (87) and Brinkley et al. (11) found evidence that in swine the release of luteotropic hormone(s) sufficient for formation and maintenance of corpora lutea in cycling gilts occurs during or a few hours after ovulatory LH release. They also reported that if pregnancy occurs, another surge of LTH is released between the 10th and the 16th day of pregnancy. The latter surge of LTH is sufficient to maintain the corpora lutea through pregnancy. These surges of LTH are said (86) to be blocked by progesterone. Although pituitary prolactin content increases with luteal development and function in swine (18) it is not thought to have luteotropic activity (68). This increase in pituitary prolactin content during luteal development in swine is probably due to the same mechanism as that described in

rats (57). Pituitary (59, 82) and circulating (107) prolactin have been shown to increase when LH is blocked by progesterone and other steroids. McCann (57) has found that when LH-RF is inhibited by gonadal hormones, pituitary prolactin is secreted and released in greater quantity. In the rat, pituitary LTH (prolactin) is essential to the maintenance and function of corpora lutea for the first 10 days of pregnancy because hypophysectomy after that time does not terminate pregnancy (74). The source of LTH after that time has been shown to be the placenta (3).

The presence of a foreign body (glass beads) in the uterus of the ewe increases the life span of the corpus luteum and denervation of the uterine segment containing the foreign body nullifies the response (61, 65). It has been shown in the rat that the endometrium of the empty uterus manifests a luteolytic effect that is nullified by section of the sacral parasympathetics (42). This observation implies that a neural mechanism is involved in the release of a luteolytic substance from the empty uterus of the rat and offers some explanation for the retention of corpora lutea in hysterectomized animals (47). French scientists have shown that in swine a luteolytic hormone is apparently released by the endometrium of the empty uterus or by the endometrium of the empty uterine horn in unilateral pregnancy on about the 16th day after ovulation (23, 24). If the uterine horns are pregnant, the luteolytic substance is not released. This could not be a neurohumoral mechanism since empty uterine grafts were also luteolytic. The endometrial luteolytic substance of swine is inhibited by estrogen. The luteolytic mechanism in swine described by the French workers is compatible with the luteotropic mechanism in swine described by Sammelwitz and Nalbandov and Brinkley et al. (86, 11).

There is no assay for LTH or the LTH activity of prolactin. Pro-

lactin is classically assayed by the pigeon crop method. Wolthuis (105, 106) has suggested a new assay for prolactin based on corpora lutea nuclei numbers (CLNN) in pseudopregnant rats. Perhaps this assay expresses some measurement of LTH activity.

CHAPTER III

MATERIALS AND METHODS

Forty nulliparous rats of the Holtzman strain averaging 197 gm. body weight and identified as sperm positive in the morning (day 1) were randomly and equally allotted to a control group and three treatment groups. Those in the control group were given 0.2 ml. of sesame oil per 200 gm. of body weight daily *per os*. Those in treatment groups 1, 2, and 3 were given 1, 3, and 6 mg. of 17-AP in 0.2 ml. of sesame oil per 200 gm. of body weight daily *per os*, respectively. Each animal was individually dosed each day beginning on day one until necropsy. The ration consisted of a commercial laboratory chow and tap water *ad lib*. All animals were weighed on the 13th day of pregnancy and killed by decapitation. At necropsy intact ovary weights, total corpora lutea numbers, and fetus numbers were recorded. The difference between the number of corpora lutea and live fetuses was recorded as embryonic mortality. Obviously, some missing fetuses could result from fertilization failure (9) and persistent corpora lutea from a previous ovulation could also affect the difference. All corpora lutea were extirpated to ensure an accurate count. The average weight of the implants (the fetus and its membranes) in each litter was determined by weighing the pregnant and empty uterus and dividing the difference by the number of live fetuses. A sample of whole uterus from a locus where a fetus had been implanted was collected and quick frozen as soon after decapitation as possible.

Later, a 1% homogenate of the frozen specimen was prepared in isotonic potassium chloride (1.15%) in a Potter-Elvehjem homogenizer. The homogenate was then analyzed for acid and alkaline phosphatase content according to the methods of Bessey et al. (6) and Andersch and Szczypinski (1) with modifications outlined in Technical Bulletin #104 (Sigma)*. Tissue enzyme activities were expressed as units of enzyme per gram of wet uterine tissue. One unit of phosphatase activity was defined as that quantity that would liberate 1.0 uM of p-nitrophenol from p-nitrophenol phosphate under the conditions of the assay.

The pituitary glands were removed, defatted and desiccated in acetone and pooled within each treatment group thereby providing four samples of pituitary tissue. The pooled, dried pituitary glands of each group were assayed for FSH using hypophysectomized diethylstilbestrol (DES) treated rats as described by Payne et al. (73). This assay is based on ovarian weight and histological changes. The assay consisted of injecting 0.5 mg. of desiccated pituitary tissue in 0.2 ml. of physiological saline daily for 4 days into each assay animal. Because of the small amount of tissue available, only three hypophysectomized animals were injected from each sample. As this number of assay animals is inadequate for statistical analysis, the assay only provided an estimate of pituitary FSH content. At necropsy, 48 hours after the last injection, the ovaries of the assay animals were fixed in buffered formalin and stained with hematoxylin and eosin to prepare them for histological examination. A series of DES treated assay animals were injected with 0, 30, 60, and 90 ug. of lyophylized standard sheep NIH-FSH reconstituted in physiological saline to provide a standard curve.

The pooled, dried pituitary tissue was also assayed for LH content

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*Sigma Chemical Company, 3500 DeKalb St., St. Louis 18, Missouri.

using Parlow's ovarian ascorbic acid depletion (OAAD) method (72) with modifications according to Schmidt-Elmendorff and Loraine (88). To find a quantity of desiccated tissue with an LH content within the range of the standard curve for LH, each of 4 assay animals (intact pseudopregnant) was injected with 0.1 mg. of each sample of desiccated tissue while another series of animals was injected with 0.4 mg. of each sample. Again, the number of animals used was limited by the quantity of material available for assay but in this case was adequate for statistical analysis. A series of immature pseudopregnant rats was injected with 0, 0.4, and 1.6 ug. of lyophylized standard sheep NIH-LH reconstituted in physiological saline to provide a standard curve. Necropsy was performed 4 hours after the last injection and ovarian ascorbic acid was determined using the method of Mindlin and Butler (60) and McCann (55). Immature female rats of the Holtzman strain were used for both assays. Pseudopregnancy was induced by subcutaneous injection of 50 international units of pregnant mare serum (PMS) and a subcutaneous injection of 25 international units of human chorionic gonadotropin (HCG) 55 to 65 hours later. Bioassay was performed the 8th day after the injection of HCG.

CHAPTER IV

RESULTS AND DISCUSSION

At necropsy a mean embryonic mortality of 48.13% had occurred in the control group and 43.32%, 49.71%, and 32.88% mortality had occurred in treatments 1, 2, and 3 respectively (Table 1). These differences in embryonic mortality between groups were not significant as indicated by an analysis of covariance in which the covariable was the number of corpora lutea. No dead embryo fragments were found in the control or treatment groups. One rat in treatment 2 was not pregnant and was removed from the experiment. Fertilization failure may have contributed to the apparent high embryonic mortality although a fertilization rate of 92 percent of the ova ovulated has been observed in albino rats (9). The lower embryonic mortality in treatment 1 and treatment 4 compares with the 38 percent embryonic mortality reported by Long and Evans (48). An embryonic mortality of 21 percent has been reported (98). Although litter size in this strain of rats was comparable to some previous reports (9, 98) the ovulation rate based on corpora lutea numbers, was higher than reports in the literature. Embryonic mortality in this experiment is characterized by considerable variation and a large error term. The fact that no dead fetuses were found on the 13th day of pregnancy indicated fertilization failure or early death and resorption.

Analysis of covariance in which the covariable was the number of corpora lutea revealed no significant differences in acid phosphatase activity, alkaline phosphatase activity or average implant weight, nor

were these differences significant when the covariable was the number of live fetuses per litter. Correlation coefficients for these measurements are shown in Table 2. Though not significant, a slight positive correlation between litter size and acid phosphatase activity was observed in all rats which is in agreement with Bredek and Mayer (10); however the negative correlation between alkaline phosphatase and litter size reported by these workers was not observed.

Ovarian weights and empty and pregnant uterine weights were not affected by treatment. No ovarian follicles were found that could be identified as cystic on the basis of size and all corpora appeared normal. Histologic study of the ovaries was not possible since they were dissected to ensure an accurate count of corpora lutea. The absence of follicular cysts in these 17-AP treated rats is in contrast to the number of follicular cysts found in swine treated with 17-AP as discussed in Part I. From these results it is obvious that the apparent insensitiveness of pregnant rats to high doses of progesterone previously reported (67) was observed in this experiment using a progesterone congener. This insensitiveness suggests that either the pituitary gonadotropin levels of these rats were not altered or that luteotropic hormone release and/or activity was not impaired. The assay of pituitary LH indicated an altered level; therefore, the suggestion that luteotropic hormone release or activity was not impaired seems more logical.

The assay of pituitary LH, based on the OAAD method (Fig. 1) indicated that the anterior pituitary glands of the control group and treatment 1 contained 14 and 12 ug. of LH per mg. of desiccated tissue. The LH content of the anterior pituitary glands from treatment 2 was not measurable, whereas the LH content of pituitary tissue from treatment 3 was approximately 1 ug. per mg. of desiccated tissue. Injections of

TABLE I

SOME EFFECTS OF 17a-ACETOXYPROGESTERONE (17-AP) IN PREGNANT RATS*

	Control		Treatment 1** 1.0 mg. 17-AP/200 gm. body wt/day		Treatment 2 3.0 mg. 17-AP/200 gm. body wt/day		Treatment 3 6.0 mg. 17-AP/200 gm. body wt/day	
	48.13% Embryonic Mortality		43.32% Embryonic Mortality		49.71% Embryonic Mortality		32.88% Embryonic Mortality	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
Body wt. day 1 (gm.)	188.30	17.10	213.00	39.90	199.00	23.30	201.10	28.20
Body wt. day 13 (gm.)	230.80	24.50	250.00	38.80	236.20	23.90	250.80	36.30
Ovary wt. (mg.)	107.20	9.70	106.10	5.40	109.90	5.30	108.30	8.00
Corpora Lutea	18.70	5.30	16.90	3.30	17.50	3.60	14.60	3.50
Live Fetuses	9.70	2.90	9.50	2.90	8.80	1.60	9.80	5.30
Missing Fetuses	9.00	4.20	7.40	3.50	8.70	3.60	4.80	5.80
Pregnant Uterine wt. (gm.)	3.39	1.06	3.10	1.13	3.21	0.63	3.52	0.59
Empty Uterine wt. (gm.)	1.59	0.05	1.43	0.67	1.52	0.26	1.91	0.33
Ave. Implant wt. (mg.)	186.50	12.10	167.00	45.70	191.20	32.40	176.30	48.70
Units Uterine Alkaline Phosphatase/gm.	359.80	81.00	269.00	147.60	310.60	42.50	298.70	80.30
Units Uterine Acid Phosphatase/gm.	519.00	60.70	512.00	85.90	474.10	74.90	458.30	109.20

* 17-AP given orally; necropsy on day 13 of pregnancy
** 9 rats this treatment; all other treatments ten rats each

TABLE II

CORRELATION COEFFICIENTS OF SIX MEASUREMENTS OF REPRODUCTION IN UNTREATED AND 17a-ACETOXYPROGESTERONE (17-AP) TREATED PREGNANT RATS AT 13 DAYS GESTATION

	Treatments	Live Fetuses	Missing Fetuses	Alkaline Phosphatase	Acid Phosphatase	Average Implant wt.
	Control	.604	.837**	.311	.380	.223
	1	.348	.633	.456	.340	-.121
Corpora Lutea	2	.237	.907**	-.164	.498	.282
	3	.398	.870**	.553	.576	-.648
	Control		.072	.059	.277	.075
Live	1		.504	-.068	.272	-.795*
Fetuses	2		-.191	.388	.024	.071
	3		-.105	.319	.109	-.256
	Control			.349	.286	.228
Missing	1			.477	-.193	.545
Fetuses	2			-.333	.493	.254
	3			.427	.566	-.565
	Control				.650*	-.091
Alkaline	1				-.128	-.122
Phosphatase	2				.357	-.377
	3				.361	-.757*
	Control					.363
Acid	1					-.091
Phosphatase	2					-.460
	3					-.099

* P = .05; ** P = .01

The control rats received 0.2 ml. of sesame oil each day per os; whereas the rats in treatments 1, 2, and 3 received 1, 3, and 6 mg. of 17a-acetoxyprogesterone per os per day in 0.2 ml. of sesame oil. There were ten rats in each treatment except treatment 1 which was composed of 9 rats.

0.1 mg. of desiccated tissue into the assay animals gave values applicable to the working range of the standard curve. Statistical analysis by Duncan's (25) multiple range test indicated that the LH content of the anterior pituitary glands of the control and treatment 1 pregnant rats was not significantly different, nor was the LH content of the glands from treatments 2 and 3 significantly different. The difference between the LH levels in the two populations (control / treatment 1 versus treatment 2 / treatment 3) was significant ($P = .05$). The reduction of pituitary LH by progesterone is well documented (57, 101).

The assay of pituitary FSH from the pregnant rats, based on the method described by Payne et al. (73) (Table III) indicated that the anterior pituitary glands of the rats in the control group and treatment 1 contained approximately 48.5 and 45.5 ug. of FSH per 0.5 mg. of desiccated tissue respectively, whereas the values for treatments 2 and 3 were much higher and beyond the range of the standard curve. The assays were based on ovarian weight changes of rats injected with the desiccated pituitary tissue compared with ovarian weight changes of similar rats injected with standard sheep FSH. Histological examination of the ovaries of the hypophysectomized rats injected with pituitary tissue (Figs. 2, 3, 4, 5) revealed a progressive increase in luteinization and a decrease in follicle numbers as the dose of 17-AP was increased in the treated groups. The ovaries of rats injected with standard sheep FSH (Figs. 6, 7, 8, 9) showed a progressive increase in follicle development with some luteinization at the 90 ug. dose.

If the ovarian weight of hypophysectomized immature assay rats is used as the only criterion of pituitary FSH content, then the pituitary FSH content of the 17-AP treated rats appeared to increase as the dose of progestin was increased. However, when one compares the degree of

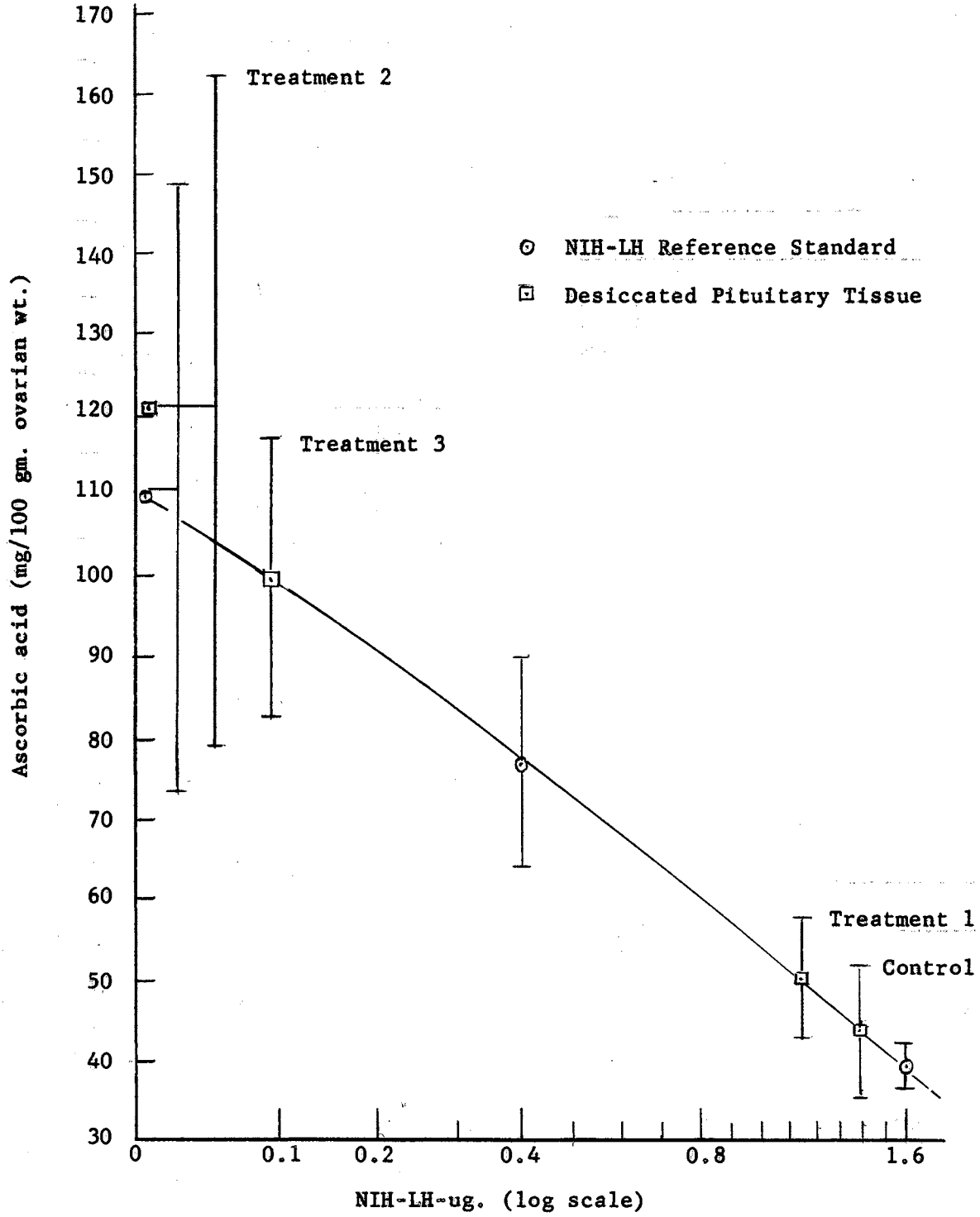

FIG. 1. The Effect of NIH-LH or Desiccated Pituitary Tissue from Progestin Treated and Untreated (Control) Rats on the Ovarian Ascorbic Acid Content of Immature Pseudopregnant Rats.

TABLE III

EFFECT OF STANDARD NIH-FSH OR DESICCATED PITUITARY TISSUE FROM PROGESTIN TREATED PREGNANT RATS* ON THE OVARIAN WEIGHT OF IMMATURE HYPOPHYSECTOMIZED DIETHYLSTILBESTROL (DES) TREATED RATS**

	Material Injected	Ovarian wt. (in mg/100 gm. body wt.) MEAN	SD
Reference Standard Assay	DES only	22.43	1.40
	DES plus 30 ug. NIH-FSH	57.33	5.90
	DES plus 60 ug. NIH-FSH	103.59	9.04
	DES plus 90 ug. NIH-FSH	129.42	17.86
Pituitary Tissue Assay	DES plus 0.5 mg. of pituitary tissue from controls	82.81 /	12.79
	DES plus 0.5 mg. of pituitary tissue from treatment 1	78.88 //	10.10
	DES plus 0.5 mg. of pituitary tissue from treatment 2	140.32	20.21
	DES plus 0.5 mg. of pituitary tissue from treatment 3	188.96	29.25

* 17a-acetoxyprogesterone (17-AP) given daily in oral doses of 0, 1, 3 and 6 mg. (control, treatment 1, treatment 2, and treatment 3 respectively); pituitaries were collected and desiccated at necropsy on day 13 of pregnancy.

** An assay for pituitary FSH using estrogen-treated hypophysectomized immature rats (Payne et al., 1959); 3 assay rats injected per assay.

/ Indicates 48.5 ug. FSH per 0.5 mg. of desiccated tissue, disregarding histology.

// Indicates 45.5 ug. FSH per 0.5 mg. of desiccated tissue, disregarding histology.

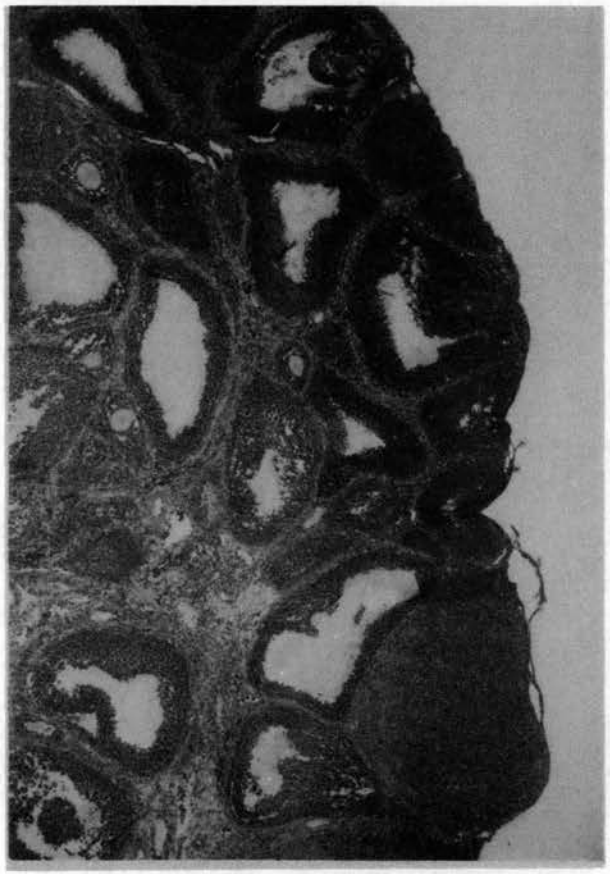

FIG. 2. Ovary from an Immature Hypophysectomized Rat Injected with 1 mg. of DES and 0.5 mg. of Desiccated Pituitary from a 13 Day Pregnant Rat (control).

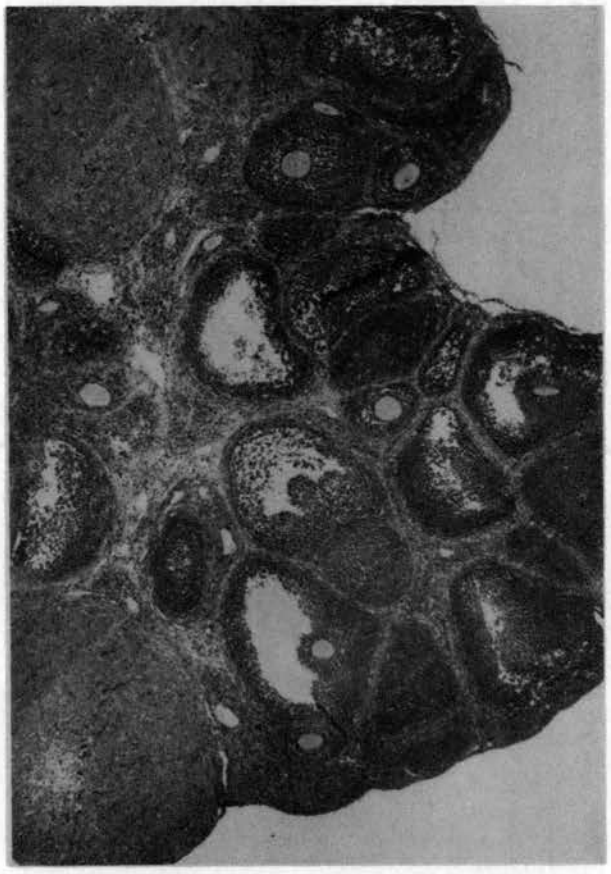

FIG. 3. Ovary from an Immature Hypophysectomized Rat Injected with 1 mg. DES and 0.5 mg. of Desiccated Pituitary from a 13 Day Pregnant Rat Treated Daily with 1 mg. 17-AP. (Treat. 1).

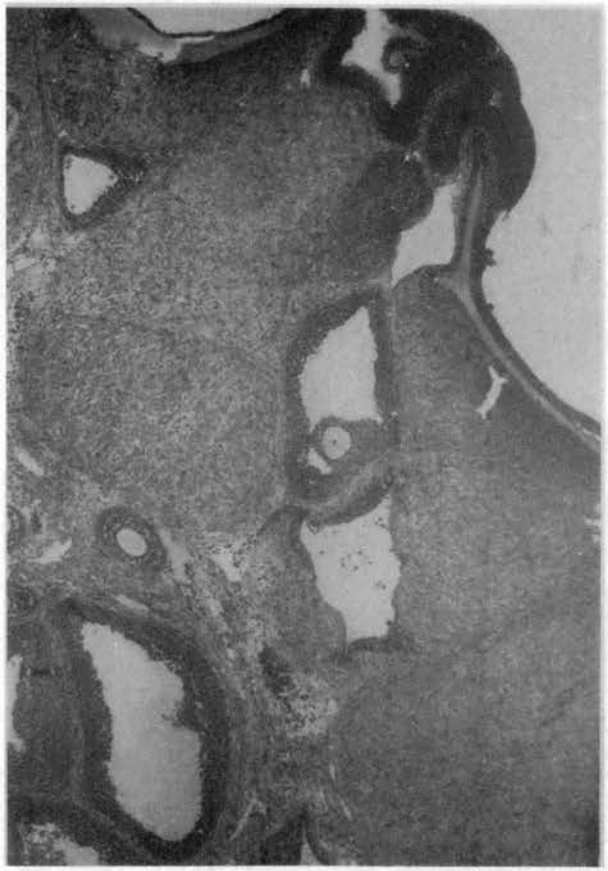

FIG. 4. Ovary from an Immature Hypophysectomized Rat Injected with 1 mg. DES and 0.5 mg. of Desiccated Pituitary from a 13 Day Pregnant Rat Treated Daily with 3 mg. 17-AP. (Treat. 2)

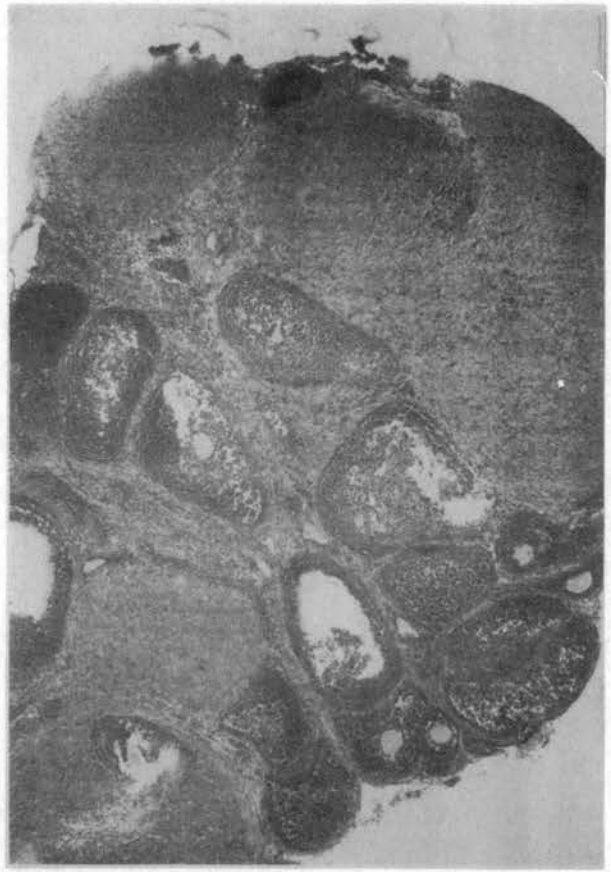

FIG. 5. Ovary from an Immature Hypophysectomized Rat Injected with 1 mg. DES and 0.5 mg. of Desiccated Pituitary from a 13 Day Pregnant Rat Treated Daily with 6 mg. 17-AP. (Treat. 3)

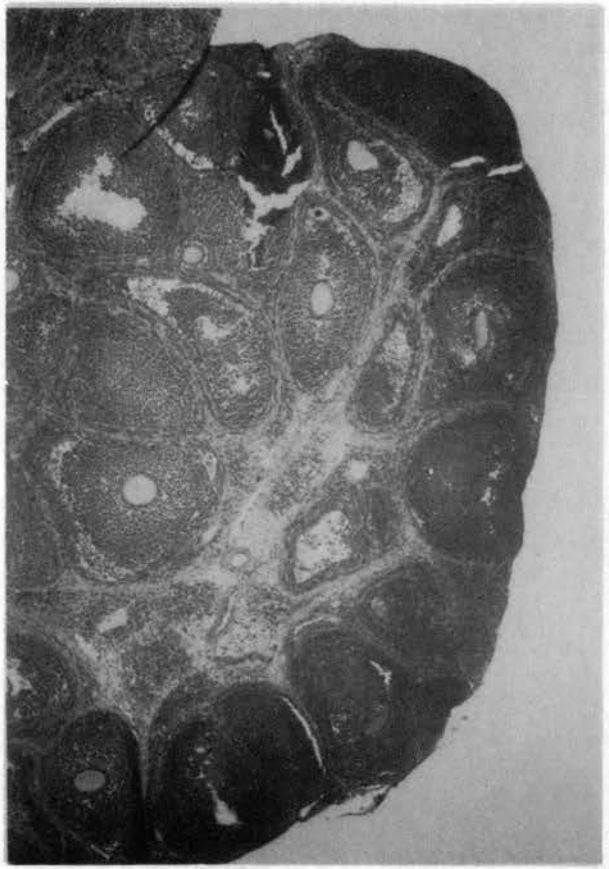

FIG. 6. Ovary from an immature Hypophysectomized Rat Injected with 1 mg. of DES to Provide a Base for a Standard Curve.

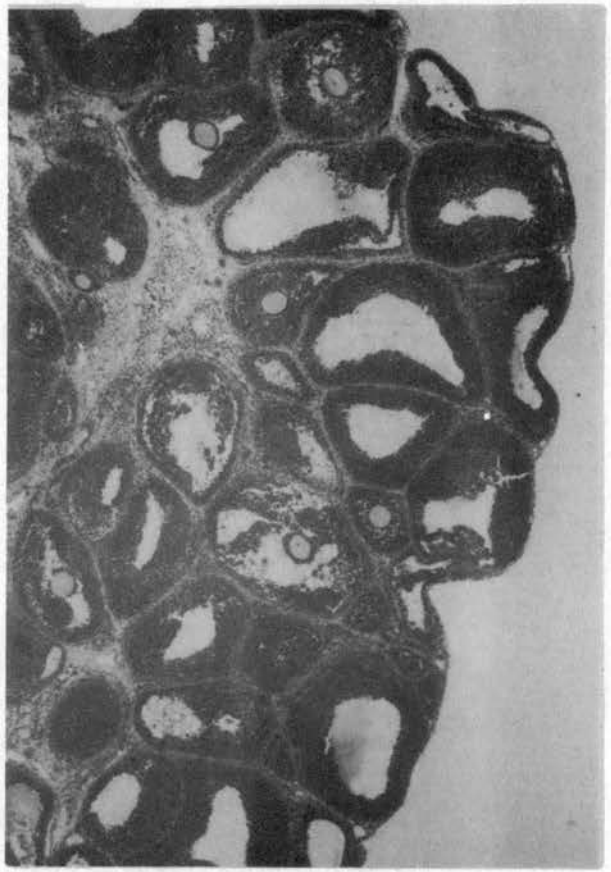

FIG. 7. Ovary from an Immature Hypophysectomized Rat Injected with 1 mg. of DES and 30 ug. of Standard Sheep FSH.

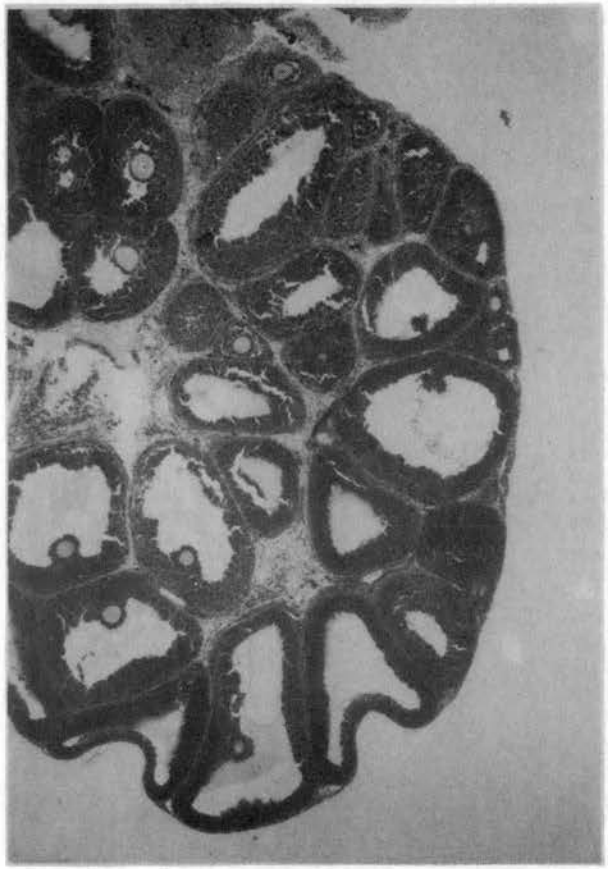

FIG. 8. Ovary from an Immature Hypophysectomized Rat Injected with 1 mg. of DES and 60 ug. of Standard Sheep FSH.

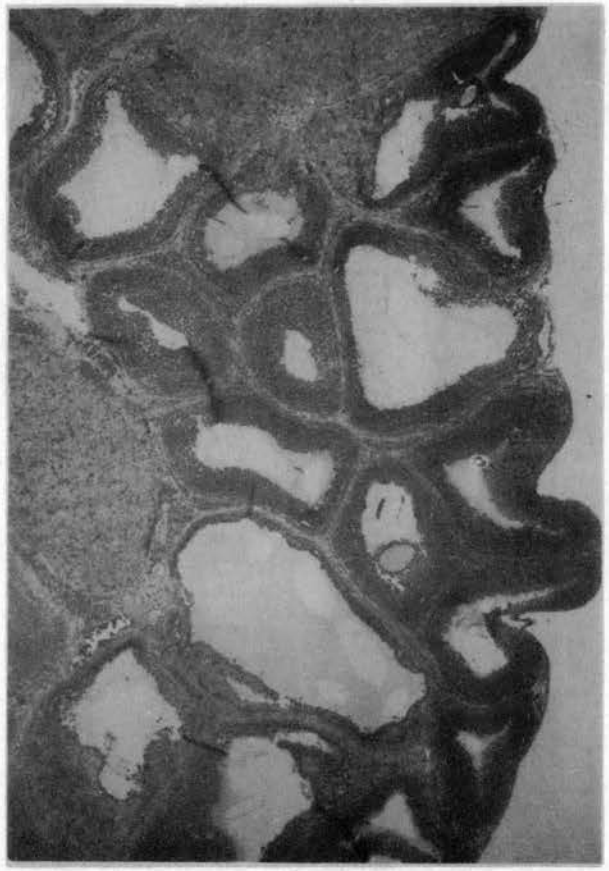

FIG. 9. Ovary from an Immature Hypophysectomized Rat Injected with 1 mg. of DES and 90 ug. of Standard Sheep FSH.

luteinization of the ovaries of the hypophysectomized assay animals, injected with desiccated pituitary tissue from progesterone treated pregnant rats (Figs. 2, 3, 4, 5) with those of comparable ovarian weight from hypophysectomized animals injected with standard sheep FSH (Figs. 6, 7, 8, 9), it is apparent that the increase in ovarian weight was due to progressive luteinization and not progressive follicle growth. It appears that FSH was not measured, although some FSH may have been present. Possibly the luteinization associated with the higher doses of standard sheep FSH was due to LH or LTH (prolactin) contamination even though this standard hormone is relatively pure. Because there was a progressive decrease in pituitary LH content with 17-AP administration in this experiment, the luteinization should not have been due to LH but due to a luteotropic substance, probably prolactin, since pituitary (82) and circulating prolactin (57, 105) has been shown to increase in the rat when LH is blocked by progesterone or other steroids. McCann (57) found that a luteinizing hormone releasing factor (LH-RF) is inhibited by the negative feed back of gonadal hormones thus preventing secretion and release of LH. He proposed that it is inhibition of the LH-RF that allows prolactin secretion.

These observations provide the basis for a postulation that pregnant rats are insensitive to the adverse effects of exogenous progestins because LTH (prolactin) is not blocked but released in greater quantity. On the other hand the luteotropic hormone(s) in swine is blocked by exogenous progesterone or progestins thus making the ovaries of swine more subject to luteal regression (11, 86, 87). This postulation is strengthened by the fact that prolactin is not luteotropic in swine (68) and could not afford any protection even though it appears to be released as in rats (18). One can postulate further that rats do not develop cystic ovarian follicles like swine when treated with progesterone or progestins because in the rat,

luteinization of unruptured follicles is influenced by prolactin. Such a mechanism may function spontaneously and could explain the low incidence of follicular cysts in rats and possibly sheep since there is some evidence that prolactin may prolong the life of corpora lutea of the estrous cycle for a short time in some sheep, and cause some luteinization of large follicles in anestrus ewes (62).

CHAPTER V

SUMMARY AND CONCLUSIONS

Previous experiments with pregnant swine (Part I) indicated that 17-AP given orally reduced embryonic mortality. The only adverse effect noted was that some treated animals which were not pregnant developed follicular type ovarian cysts.

From a review of the literature one can conclude that species most susceptible to spontaneous ovarian cystic disease are most susceptible to the adverse effects (follicular cysts and luteal regression) of exogenous progesterone. Of the species reported, swine are most susceptible and rats are least susceptible. Spontaneous and hormone-induced cysts are similar and apparently related in etiology (alteration of gonadotropins). It was felt, therefore, that a study of a species insensitive to large doses of progesterone or other progestins might provide basic information concerning the cause of ovarian cystic disease or species susceptibilities to the disease.

To test this postulation, and other assumptions set forth in the literature, a study was designed using 1 control group and 3 treatment groups of nulliparous sperm positive rats of the Holtzman strain. Those in the control group were given 0.2 ml. of sesame oil per 200 grams of body weight daily per os. Those in treatment groups 1, 2, and 3 received 1, 3 and 6 mg. of 17-AP in 0.2 ml. sesame oil per 200 gm. of body weight daily per os respectively. Necropsy was performed on the 13th day of

pregnancy. At necropsy, ovary weights, corpora lutea numbers, fetus numbers, implant weights and full and empty uterus weights were recorded. Uterine acid and alkaline phosphatase activities were also determined. The pituitary glands were pooled within each group and assayed for LH and FSH.

A mean embryonic mortality of 48.13% occurred in the control group and 43.32%, 49.71%, and 32.88% mortality occurred in treatments 1, 2, and 3 respectively. These differences were not significant as indicated by an analysis of covariance. The 17-AP did not affect ovary, uterus or implant weights or uterine acid and alkaline phosphatase activities. No follicular cysts were observed in these rats which is in contrast to the incidence of follicular cysts observed in swine fed the same progestin in similar experiments (Part I). Attempts to measure the FSH content of the pituitaries of the treated groups were not successful because the levels of a pituitary luteotropic substance (probably prolactin) were increased as the dose of 17-AP was increased. This luteotropic substance caused luteinization of any follicles that the pituitary FSH from the treated rats may have induced.

These observations provide the basis for a postulation that the ovaries of pregnant rats are less responsive to exogenous progestins than swine because prolactin is luteotropic in rats and is released in greater quantity when LH-RF is blocked by progestins. The luteotropic hormone(s) in swine is not prolactin and is blocked by exogenous progesterone or progestins. Such a mechanism may function spontaneously, if the function of the LH-RF is impeded, and could explain the low incidence of ovarian follicular cysts in rats and possibly sheep, and the higher incidence in other species, especially swine.

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A P P E N D I X

Part I

EXPERIMENT I
(Sows, 22-27 days gestation)

CORPORA LUTEA, LIVE EMBRYOS AND DEAD OR MISSING EMBRYOS (EMBRYONIC MORTALITY) IN RIGHT AND LEFT CORNUA OF UNTREATED (CONTROL) AND 17-AP-DES FED MULTIPAROUS PREGNANT SWINE*

CONTROL

Corpora Lutea		Live Embryos		Embryonic Mortality	
Left	Right	Left	Right	Left	Right
4	12	3	1	1	11
10	9	6	6	4	3
18	8	8	6	10	2
12	11	10	8	2	3
12	8	10	7	2	1
11	9	6	6	5	3
MEAN 11.16	9.50	7.16	5.66	4.00	3.83
SD 4.49	1.64	2.71	2.42	3.28	3.60

TREATMENT 1 (0.5 mg. 17-AP and 0.25 mg. DES/1b/day)

Corpora Lutea		Live Embryos		Embryonic Mortality	
Left	Right	Left	Right	Left	Right
12	6	5	12	7	⁄6**
7	13	9	7	⁄2**	6
9	14	10	8	⁄1**	1
4	13	4	5	0	8
9	9	7	9	2	0
10	9	9	9	1	0
MEAN 8.50	10.67	7.43	8.33	1.16	1.50
SD 2.73	3.14	2.42	2.33	1.41	1.22

TREATMENT 2 (1.0 mg. 17-AP and 0.5 mg. DES/1b/day)

Corpora Lutea		Live Embryos		Embryonic Mortality	
Left	Right	Left	Right	Left	Right
8	9	7	6	1	3
9	9	9	8	0	1
9	10	9	9	0	1
8	7	7	6	1	1
10	8	7	7	3	1
9	9	9	9	0	0
13	10	9	12	4	⁄2**
MEAN 9.42	8.85	8.14	8.28	1.28	1.00
SD 1.71	1.06	1.06	2.07	1.60	1.41

* Individually fed ** Intrauterine migration

EXPERIMENT II
(Gilts 40 days gestation)

CORPORA LUTEA, LIVE EMBRYOS AND DEAD OR MISSING EMBRYOS (EMBRYONIC MORTALITY) IN RIGHT AND LEFT CORNUA OF UNTREATED (CONTROL) AND 17-AP-DES FED NULLIPAROUS PREGNANT SWINE*

CONTROL					
Corpora Lutea		Live Embryos		Embryonic Mortality	
Left	Right	Left	Right	Left	Right
6	7	7	6	⌿1**	1
6	11	6	8	0	3
12	7	9	7	3	0
10	11	8	3	2	8
8	9	10	7	⌿2**	2
5	12	5	5	0	7
7	7	6	4	1	3
11	4	6	6	5	⌿2**
4	10	6	6	⌿2**	4
9	8	6	5	3	3
7	10	6	7	1	3
10	5	4	7	6	⌿2**
9	4	5	4	4	0
10	7	7	8	3	⌿1**
7	9	7	8	0	1
MEAN 8.06	8.06	6.53	6.06	1.86	2.33
SD 2.31	2.51	1.55	1.57	2.18	2.64

* Group fed
** Intrauterine migration

Experiment II (continued)

TREATED (1.0 mg. 17-AP and 0.5 ug. DES/1b/day)					
Corpora Lutea		Live Embryos		Embryonic Mortality	
Left	Right	Left	Right	Left	Right
10	6	6	8	4	⁄2**
7	9	7	8	0	1
4	11	5	5	⁄1**	6
5	9	7	5	⁄2**	4
10	4	6	2	4	2
0	9	0	8	0	1
8	8	8	8	0	0
16	7	7	5	9	2
11	11	8	7	3	4
14	10	9	9	5	1
9	13	3	5	6	8
8	4	6	5	2	⁄1**
10	6	8	4	2	2
11	7	7	7	4	0
MEAN 8.78	8.14	6.21	6.14	2.78	2.21
SD 4.04	2.65	2.32	1.99	2.81	2.53

* Group fed
** Intrauterine migration

EXPERIMENT II
(Gilts, 40 days gestation)

GROSS ANATOMICAL DATA FROM UNTREATED (CONTROL) AND 17-AP-DES TREATED NULLIPAROUS PREGNANT SWINE*

CONTROL

	Corpora Lutea wt. (gm.)		Ovary wt. (gm.)		Uterus wt. (kg.)	
	Left	Right	Left	Right	Left	Right
	2.8	4.5	8.0	10.0	3.86	1.95
	2.1	3.1	11.8	6.4	4.99	2.27
	3.0	3.9	8.3	9.5	3.85	1.95
	2.2	5.8	8.2	11.6	2.50	1.36
	4.8	2.7	7.5	10.1	2.04	0.90
	0.0	5.3	6.1	13.0	2.27	1.36
	5.7	2.3	11.9	7.0	2.04	1.02
	3.2	3.4	8.4	8.1	3.86	2.00
	8.0	3.8	14.9	9.5	2.95	1.47
	4.8	4.7	11.0	10.5	3.52	1.77
	7.1	5.0	15.1	11.1	4.77	2.37
	3.2	4.7	8.4	10.2	2.72	1.25
	1.4	4.0	8.5	4.9	3.06	1.25
	6.1	3.7	13.2	10.1	3.40	1.81
	5.3	3.3	7.6	8.6	3.23	1.81
MEAN	3.86	4.01	9.92	9.37	3.27	1.60
SD	2.22	0.97	2.83	2.09	0.90	0.43

* Group fed

Experiment II (continued)

TREATED (1.0 mg. 17-AP and 0.5 ug. DES/1b/day)

	Corpora Lutea wt. (gm.)		Ovary wt. (gm.)		Uterus wt. (kg.)	
	Left	Right	Left	Right	Left	Right
	3.6	4.7	8.3	11.0	3.86	2.27
	3.3	5.7	8.6	12.4	3.63	1.81
	6.3	4.0	13.3	10.5	5.00	2.78
	4.2	4.8	11.1	10.6	2.84	1.25
	5.2	5.3	10.3	10.6	4.54	2.27
	3.3	5.9	8.2	11.4	2.50	1.36
	3.4	3.9	7.6	7.3	2.72	1.25
	7.1	2.7	13.0	6.9	3.06	1.59
	2.0	4.6	5.6	9.0	2.50	1.36
	5.2	4.2	12.4	11.4	3.40	1.81
	3.6	4.3	8.9	9.1	2.84	1.48
	4.8	2.1	6.2	9.1	2.50	1.13
	4.1	2.1	10.3	7.5	1.81	1.13
	4.8	3.5	8.8	6.8	4.08	2.04
MEAN	4.35	4.12	9.47	9.54	3.23	1.68
SD	1.33	1.20	2.37	1.85	0.88	0.50

* Group fed

EXPERIMENT II
(Gilts, 40 days gestation)

WEIGHTS AND MEASUREMENTS OF EMBRYOS FROM UNTREATED (CONTROL) AND 17-AP-DES GROUP FED NULLIPAROUS PREGNANT SWINE

CONTROL*

	Embryo Length (mm.)	Wet Embryo wt. (gm.)	Dry Embryo wt. (gm.)	Dry Membrane wt. (mg.)
MEAN	55.25	11.07	1.13	3557.07
SD	3.90	1.61	0.19	921.60
RANGE	45 - 62	7.02 - 15.07	0.73 - 1.64	1370 - 6400

* 15 litters; 188 embryos

TREATED (1.0 mg. 17-AP and 0.5 ug. DES/lb/day)

	Embryo Length (mm.)	Wet Embryo wt. (gm.)	Dry Embryo wt. (gm.)	Dry Membrane wt. (mg.)
MEAN	54.51	10.94	1.11	3415.00
SD	4.34	1.98	0.25	864.37
RANGE	42 - 63	7.20 - 14.24	0.69 - 1.48	700 - 4870

* 14 litters; 174 embryos

EXPERIMENT III
(Gilts, 25 days gestation)

CORPORA LUTEA, LIVE EMBRYOS AND DEAD OR MISSING EMBRYOS (EMBRYONIC MORTALITY) IN RIGHT AND LEFT CORNUA AT UNTREATED (CONTROL) AND 6-M-17-AP FED NULLIPAROUS PREGNANT SWINE*

CONTROL

	Corpora Lutea		Live Embryos		Embryonic Mortality	
	Left	Right	Left	Right	Left	Right
	10	6	6	4	4	2
	12	10	10	8	2	2
	6	9	5	7	1	2
	12	7	9	6	3	1
	5	8	7	6	∤2**	2
	10	1	5	4	5	∤3**
	10	5	9	6	1	∤1**
MEAN	9.28	6.57	7.28	5.85	2.29	1.28
SD	2.62	2.99	2.05	1.46	1.97	3.92

TREATMENT 1 (1.0 mg. 17-AP and 0.5 ug. DES/lb/day)

	Corpora Lutea		Live Embryos		Embryonic Mortality	
	Left	Right	Left	Right	Left	Right
	14	1	8	7	6	∤6**
	16	4	9	8	7	∤4**
	9	8	8	8	1	0
	5	9	7	7	∤2**	2
	8	5	6	6	2	∤1**
	7	8	5	4	2	4
MEAN	9.83	5.83	7.17	6.67	3.00	1.00
SD	4.26	3.06	1.47	1.50	2.96	3.66

TREATMENT 2 (60 mg. 6-M-17-AP/animal/day)

	Corpora Lutea		Live Embryos		Embryonic Mortality	
	Left	Right	Left	Right	Left	Right
	12	5	4	4	8	1
	15	6	8	7	7	∤1**
	10	5	7	5	3	0
	7	10	7	6	0	4
	6	12	7	9	∤1**	3
	6	11	5	7	1	4
MEAN	9.33	8.17	6.33	6.33	3.16	2.00
SD	3.66	3.18	1.50	1.75	3.58	1.34

* Individually fed
** Intrauterine migration

EXPERIMENT III
(Gilts, 25 days gestation)

GROSS ANATOMICAL DATA FROM UNTREATED (CONTROL) AND 17-AP-DES TREATED NULLIPAROUS PREGNANT SWINE*

CONTROL

	Corpora Lutea wt. (gm.)		Ovary wt. (gm.)		Uterus wt. (kg.)	
	Left	Right	Left	Right	Left	Right
	3.54	2.27	9.38	4.50	2.24	0.92
	3.69	3.12	7.80	7.58	2.30	1.25
	2.63	4.07	6.78	8.39	2.52	1.23
	4.51	2.43	10.34	7.32	3.60	1.52
	1.68	2.42	4.35	6.43	2.24	0.90
	4.15	0.45	8.91	3.20	2.23	0.85
	4.20	2.55	9.20	6.30	3.11	1.61
MEAN	3.48	2.47	8.11	6.24	2.60	1.18
SD	1.00	1.08	2.01	1.81	0.54	0.30

TREATMENT 1 (1.0 mg. 17-AP and 0.5 DES/lb/day)

	Corpora Lutea wt. (gm.)		Ovary wt. (gm.)		Uterus wt. (kg.)	
	Left	Right	Left	Right	Left	Right
	6.24	0.38	12.22	4.38	2.40	1.03
	5.00	0.90	11.80	4.85	3.68	1.40
	3.46	2.72	7.67	7.09	2.76	1.31
	1.41	2.67	3.85	6.55	2.71	0.98
	2.40	1.90	6.30	8.28	2.18	0.91
	1.80	1.95	4.40	4.70	0.97	0.68
MEAN	3.38	1.75	7.70	5.97	2.45	1.05
SD	1.90	0.94	3.60	1.57	0.89	0.26

TREATMENT 2 (60 mg. 6-M-17-AP/animal/day)

	Corpora Lutea wt. (gm.)		Ovary wt. (gm.)		Uterus wt. (kg.)	
	Left	Right	Left	Right	Left	Right
	3.00	1.26	9.07	6.58	1.60	0.95
	4.44	1.41	9.72	5.47	2.23	1.07
	3.31	1.92	8.98	5.55	2.10	1.06
	1.90	2.84	4.30	5.97	2.57	1.34
	2.20	4.22	6.47	10.46	2.51	0.89
	2.35	3.75	5.80	8.40	2.40	1.21
MEAN	2.86	2.56	7.39	7.07	2.23	1.08
SD	0.93	1.24	2.18	1.98	0.35	0.14

* Individually fed

EXPERIMENT III
(Gilts, 25 days gestation)

WEIGHTS AND MEASUREMENTS OF EMBRYOS FROM UNTREATED (CONTROL) AND 17-AP-DES AND 6-M-17-AP INDIVIDUALLY FED NULLIPAROUS SWINE

CONTROL*

	Embryo Length (mm.)	Wet Embryo wt. (gm.)	Dry Embryo wt. (mg.)	Dry Membrane wt. (mg.)
MEAN	17.90	488.49	41.09	349.34
SD	1.80	105.46	10.21	108.00
RANGE	15 - 20	251 - 694	21 - 57	176 - 560

* 7 litters; 90 embryos

TREATMENT 1 (1.0 mg. 17-AP and 0.5 ug. DES/lb/day)*

	Embryo Length (mm.)	Wet Embryo wt. (gm.)	Dry Embryo wt. (mg.)	Dry Membrane wt. (mg.)
MEAN	18.22	460.21	39.27	360.27
SD	1.66	91.46	9.08	125.98
RANGE	10 - 21	281 - 596	16 - 57	118 - 579

* 6 litters; 82 embryos

TREATMENT 2 (60 mg. 6-M-17-AP/animal/day)*

	Embryo Length (mm.)	Wet Embryo wt. (gm.)	Dry Embryo wt. (mg.)	Dry Membrane wt. (mg.)
MEAN	17.80	407.07	34.34	302.20
SD	1.72	91.70	7.86	107.80
RANGE	13 - 22	157 - 578	15 - 52	44 - 474

* 6 litters; 76 embryos

EXPERIMENT III
(Gilts, 25 days gestation)

ENDOMETRIAL PHOSPHATASE ACTIVITY*OF UNTREATED (CONTROL)
17-AP-DES, AND 6-M-17-AP FED NULLIPAROUS PREGNANT SWINE

	CONTROL		TREATMENT 1 (1.0 mg. 17-AP and 0.5 ug. DES/lb/day)		TREATMENT 2 (60 mg. 6-M-17-AP/ animal/day)	
	Acid Phosphatase	Alkaline Phosphatase	Acid Phosphatase	Alkaline Phosphatase	Acid Phosphatase	Alkaline Phosphatase
	40.77	3.34	55.61	3.15	49.18	7.09
	24.18	6.98	39.65	1.35	44.64	6.54
	38.42	4.32	29.03	8.70	75.26	3.32
	11.83	8.38	2.18	8.52	13.38	3.49
	67.38	6.99	27.22	18.40	79.74	38.57
	115.62	7.70	12.57	10.41	14.62	7.64
	15.88	8.37				
MEAN	44.86	6.58	27.71	8.42	46.13	11.11
SD	36.40	1.98	19.00	5.50	28.48	13.58

* Units per mg. of soluble protein

EXPERIMENT IV
(Sows, 25 days gestation)

CORPORA LUTEA, LIVE EMBRYOS AND DEAD OR MISSING EMBRYOS (EMBRYONIC MORTALITY) IN RIGHT AND LEFT CORNUA OF UNTREATED (CONTROL) 17-AP-DES AND 6-M-17-AP FED MULTIPAROUS PREGNANT SWINE*

CONTROL					
Corpora Lutea		Live Embryos		Embryonic Mortality	
Left	Right	Left	Right	Left	Right
10	13	7	11	3	2
12	9	9	6	3	3
11	4	7	6	4	⌿2**
13	6	8	6	5	0
10	9	8	8	2	1
9	9	6	10	3	⌿1**
13	10	4	4	9	6
MEAN 11.14	8.57	7.00	7.28	4.14	1.85
SD 1.57	2.88	1.63	2.50	2.34	2.27

TREATMENT 1 (1.0 mg. 17-AP and 0.5 ug. DES/lb/day)					
Corpora Lutea		Live Embryos		Embryonic Mortality	
Left	Right	Left	Right	Left	Right
20	1	7	6	13	⌿5**
11	8	7	4	4	4
7	14	9	8	⌿2**	6
13	4	5	10	8	⌿6**
10	8	4	4	6	4
11	9	8	10	3	⌿1**
MEAN 12.00	7.33	6.67	7.00	5.67	2.33
SD 4.38	4.46	1.90	2.76	4.60	4.41

TREATMENT 2 (90 mg. 6-M-17-AP/animal/day)					
Corpora Lutea		Live Embryos		Embryonic Mortality	
Left	Right	Left	Right	Left	Right
16	6	8	7	8	⌿1**
12	8	8	7	4	1
11	8	9	9	2	⌿1**
11	6	7	6	4	0
7	7	8	5	⌿1**	2
11	11	12	6	⌿1**	5
MEAN 11.33	7.67	8.67	6.67	3.00	1.17
SD 2.88	1.86	1.75	1.36	3.10	2.14

* Individually fed
** Intrauterine migration

EXPERIMENT IV
(Sows, 25 days gestation)

GROSS ANATOMICAL DATA FROM UNTREATED (CONTROL) 17-AP-DES AND 6-M-17-AP TREATED MULTIPAROUS PREGNANT SWINE*

CONTROL

	Corpora Lutea wt. (gm.)		Ovary wt. (gm.)		Uterus wt. (kg.)	
	Left	Right	Left	Right	Pregnant	Full
	3.00	3.20	6.56	7.22	4.19	1.19
	5.16	4.23	11.40	9.10	4.45	1.94
	3.50	1.47	7.80	4.37	2.31	1.38
	4.27	1.96	10.68	6.69	3.35	1.61
	2.88	2.53	8.02	6.81	3.38	1.94
	3.84	3.67	9.20	8.43	2.70	1.58
	5.40	4.00	11.43	15.82	2.11	1.30
MEAN	4.00	3.01	9.32	8.34	3.21	1.56
SD	0.99	1.05	1.91	3.62	0.89	0.30

TREATMENT 1 (1.0 mg. 17-AP and 0.5 ug. DES/lb/day)

	Corpora Lutea wt. (gm.)		Ovary wt. (gm.)		Uterus wt. (kg.)	
	Left	Right	Left	Right	Pregnant	Full
	7.97	0.46	16.96	5.35	2.54	1.28
	2.93	2.08	7.75	5.62	2.39	1.40
	2.20	4.00	9.30	8.60	2.90	1.40
	5.70	1.62	10.32	5.61	2.82	1.55
	3.63	2.45	8.54	8.00	2.23	1.23
	5.00	2.65	10.00	9.58	3.75	1.45
MEAN	4.57	2.21	10.47	7.12	2.77	1.38
SD	2.11	1.17	3.31	1.83	0.54	0.10

TREATMENT 2 (90 mg. 6-M-17-AP/animal/day)

	Corpora Lutea wt. (gm.)		Ovary wt. (gm.)		Uterus wt. (kg.)	
	Left	Right	Left	Right	Pregnant	Full
	5.58	1.97	12.88	7.12	3.68	1.64
	4.10	2.78	10.50	6.68	2.82	1.29
	4.20	2.49	8.15	7.09	3.37	1.53
	6.84	2.26	18.31	7.64	2.92	1.70
	2.63	2.32	9.55	7.65	2.37	1.38
	4.15	3.80	8.25	7.75	4.20	2.00
MEAN	4.58	2.60	11.27	7.32	3.22	1.59
SD	1.45	0.64	3.86	0.42	0.66	0.25

* Individually fed

EXPERIMENT IV
(Sows, 25 days gestation)

WEIGHTS AND MEASUREMENTS OF EMBRYOS FROM UNTREATED (CONTROL) 17-AP-DES AND 6-M-17-AP INDIVIDUALLY FED MULTIPAROUS SWINE

CONTROL*

	Embryo Length (mm.)	Wet Embryo wt. (gm.)	Dry Embryo wt. (mg.)	Dry Membrane wt. (mg.)
MEAN	17.63	413.72	32.97	328.30
SD	1.60	85.44	8.15	109.99
RANGE	12 - 21	150 - 639	17 - 54	119 - 595

* 7 litters; 100 embryos

TREATMENT 1 (1.0 mg. 17-AP and 0.5 ug. DES/lb/day)*

	Embryo Length (mm.)	Wet Embryo wt. (gm.)	Dry Embryo wt. (gm.)	Dry Membrane wt. (mg.)
MEAN	18.20	457.32	36.48	333.06
SD	1.64	77.45	8.93	77.39
RANGE	14 - 21	258 - 622	18 - 64	164 - 537

* 6 litters; 82 embryos

TREATMENT 2 (90 mg. 6-M-17-AP/animal/day)*

	Embryo Length (mm.)	Wet Embryo wt. (gm.)	Dry Embryo wt. (mg.)	Dry Membrane wt. (mg.)
MEAN	17.98	460.86	36.69	333.46
SD	1.42	91.69	9.17	93.81
RANGE	14 - 21	222 - 607	15 - 61	131 - 570

* 6 litters; 92 embryos

A P P E N D I X

PART II

EXPERIMENT IV
(Sows, 25 days gestation)

ENDOMETRIAL PHOSPHATASE ACTIVITY*OF UNTREATED (CONTROL) 17-AP-DES, AND 6-M-17-AP FED MULTIPAROUS PREGNANT SWINE

	CONTROL		TREATMENT 1 (1.0 mg. 17-AP and 0.5 ug. DES/lb/day)		TREATMENT 2 (90 mg. 6-M-17-AP/ animal/day)	
	Acid Phosphatase	Alkaline Phosphatase	Acid Phosphatase	Alkaline Phosphatase	Acid Phosphatase	Alkaline Phosphatase
	15.62	1.46	1.77	4.57	10.57	3.32
	20.49	1.93	24.61	10.36	25.21	8.53
	16.74	4.92	37.90	8.07	69.09	5.28
	27.20	3.19	21.29	0.00	16.63	0.00
	77.39	6.15	25.81	2.51	91.07	5.76
	7.73	0.00	10.77	6.04	17.43	5.06
	19.06	0.00			10.57	3.32
MEAN	26.31	2.52	20.35	5.25	34.36	4.46
SD	23.26	2.36	12.60	3.75	32.25	2.64

* Units per mg. of soluble protein

GROSS ANATOMICAL AND PHYSIOLOGICAL DATA FOR UNTREATED (CONTROL) PREGNANT RATS AT 13 DAYS GESTATION

	Body wt. gm. Day 1	Body wt. gm. Day 13	Ovary wt. mg.	Corpora Lutea	Live Embryos	Missing Embryos	Pregnant Uterus wt. gm.	Empty Uterus wt. gm.	Implant wt. mg.	Alkaline Phospha-tase *	Acid Phospha-tase *
	172	224	92	12	9	3	3.17	1.58	177	329	429
	177	203	110	18	5	13	1.83	0.90	186	354	343
	178	217	107	24	10	14	3.84	1.90	194	571	981
	206	258	114	28	16	12	5.14	2.68	154	350	503
	220	253	116	22	10	12	4.28	1.67	261	314	576
	185	229	104	23	11	12	3.62	1.39	203	352	394
	172	214	114	17	7	10	2.14	1.10	149	287	463
	193	238	89	13	8	5	2.25	1.20	144	368	370
	174	225	109	16	11	5	4.27	1.76	228	287	675
	206	257	117	14	10	4	3.40	1.72	169	386	466
MEAN	188.3	230.8	107.2	18.7	9.7	9.0	3.39	1.59	186.5	359.8	519.0
SD	17.1	24.5	9.7	5.3	2.9	4.2	1.06	0.05	12.1	81.0	60.7

* Units per gm. of wet uterine tissue

GROSS ANATOMICAL AND PHYSIOLOGICAL DATA FOR TREATMENT 1 PREGNANT RATS*
AT 13 DAYS GESTATION

	Body wt. gm. Day 1	Body wt. gm. Day 13	Ovary wt. mg.	Corpora Lutea	Live Embryos	Missing Embryos	Pregnant Uterus wt. gm.	Empty Uterus wt. gm.	Implant wt. mg.	Alkaline Phospha-tase **	Acid Phospha-tase **
	297	335	106	16	7	9	1.00	0.51	70	152	438
	246	282	111	16	3	13	1.68	0.95	243	396	497
	163	208	93	12	10	2	3.25	0.44	181	124	462
	188	230	108	17	11	6	3.51	1.60	174	490	593
	197	243	103	21	13	8	4.60	2.41	168	325	563
	221	252	113	20	11	9	3.70	1.94	160	364	369
	203	239	104	12	10	2	3.45	1.92	153	128	559
	181	212	105	19	10	9	2.99	1.42	157	362	479
	222	250	112	19	11	8	3.88	1.71	197	87	646
MEAN	213	250	106.1	16.9	9.5	7.4	3.10	1.43	167	269	512
SD	39.9	38.8	5.4	3.3	2.9	3.5	1.13	0.67	45.7	147.6	85.9

* 1 mg. 17a-acetoxyprogesterone orally each day
** Units per mg. of wet uterine tissue

GROSS ANATOMICAL AND PHYSIOLOGICAL DATE FOR TREATMENT 2 PREGNANT RATS* AT 13 DAYS GESTATION

	Body wt. gm. Day 1	Body wt. gm. Day 13	Ovary wt. mg.	Corpora Lutea	Live Embryos	Missing Embryos	Pregnant Uterus wt. gm.	Empty Uterus wt. gm.	Implant wt. mg.	Alkaline Phospha-tase **	Acid Phospha-tase **
	165	200	103	14	8	6	3.23	1.47	220	295	462
	206	241	110	15	10	5	3.78	1.90	188	595	464
	218	260	114	22	10	12	3.33	1.72	161	330	628
	179	217	103	13	11	2	3.35	1.52	166	350	405
	180	221	116	20	9	11	3.36	1.40	218	149	442
	207	239	104	16	8	8	2.61	1.24	171	364	504
	222	255	114	21	10	11	4.34	1.87	247	179	375
	238	277	109	16	7	9	2.40	1.24	166	249	450
	197	240	109	15	6	9	2.31	1.19	187	182	444
	178	212	117	23	9	14	3.42	1.64	198	413	567
MEAN	199.0	236.2	109.9	17.5	8.8	8.7	3.21	1.52	191.2	310.6	474.1
SD	23.3	23.9	5.3	3.6	1.6	3.6	0.63	0.26	32.4	42.5	74.9

* 3 mg. 17α-acetoxyprogesterone orally each day

** Units per gm. of wet uterine tissue

GROSS ANATOMICAL AND PHYSIOLOGICAL DATA FOR TREATMENT 3 PREGNANT RATS* AT 13 DAYS GESTATION

	Body wt. gm. Day 1	Body wt. gm. Day 13	Ovary wt. mg.	Corpora Lutea	Live Embryos	Missing Embryos	Pregnant Uterus wt. gm.	Empty Uterus wt. gm.	Implant wt. mg.	Alkaline Phospha-tase **	Acid Phospha-tase**
	181	225	102	18	9	9	2.82	1.73	121	293	436
	210	252	112	14	7	7	2.99	1.70	184	292	480
	218	244	114	15	11	4	3.71	2.06	150	376	389
	196	245	103	11	10	1	3.68	2.18	150	281	322
	188	221	98	10	9	1	4.05	1.84	246	197	398
	183	205	111	13	9	4	3.00	1.56	272	409	455
	216	258	118	18	13	5	4.46	2.33	164	372	645
	266	312	114	18	12	6	4.13	2.44	141	285	378
	166	214	116	19	9	10	2.84	1.47	152	333	651
	187	232	95	10	9	1	3.48	1.83	183	149	429
MEAN	201.1	250.8	108.3	14.6	9.8	4.8	3.52	1.91	176.3	298.7	458.3
SD	28.2	36.3	8.0	3.5	5.3	5.8	0.59	0.33	48.7	80.3	109.2

* 6 mg. 17a-acetoxyprogesterone orally each day

** Units per gm. of wet uterine tissue

GROSS ANATOMICAL DATA FROM IMMATURE HYPOPHYSECTOMIZED RATS INJECTED WITH DIETHYLSTILBESTROL (DES) AND STANDARD SHEEP FSH TO ESTABLISH A STANDARD CURVE FOR FSH*

	Body wt. of Assay Rats gm.	wt. of Ovaries of Assay Rats gm.	wt. of Ovaries of Assay Rats mg. %	Dose of Standard FSH plus 1 mg. of DES**
	51.00	11.00	21.56	
	59.00	12.00	30.33	
	63.00	16.00	25.39	0 ug. NIH-FSH
MEAN	57.66	13.00	22.43	
SD	37.50	7.00	1.40	
	58.00	35.00	60.34	
	60.50	30.80	30.90	
	56.50	34.90	61.76	30 ug. NIH-FSH
MEAN	58.33	33.53	57.33	
SD	4.00	11.47	5.90	
	51.50	53.00	102.91	
	55.50	58.00	104.50	
	58.50	67.00	114.52	60 ug.
	53.00	49.00	92.45	NIH-FSH
MEAN	54.62	56.75	103.59	
	9.39	60.25	9.04	
	57.50	73.40	127.65	
	53.00	78.50	148.11	
	61.50	69.20	112.52	90 ug. NIH-FSH
MEAN	57.33	73.70	129.42	
SD	18.08	29.05	17.86	

* FSH assay (Payne et al. Endo. 65:389, 1959)

** 1 mg. of DES was injected with the designated dose of standard FSH each day for 4 days beginning on the day of hypophysectomy. The control assay rats were injected with DES only. Necropsy was performed 48 hours after the last injection.

GROSS ANATOMICAL DATA FROM IMMATURE HYPOPHYSECTOMIZED RATS INJECTED WITH DESICCATED PITUITARY TISSUE FROM 17a-ACETOXYPROGESTERONE (17-AP) TREATED PREGNANT RATS PLUS DIETHYLSTILBESTROL (DES)*

	Body wt. of Assay Rats gm.	wt. of Ovaries of Assay Rats gm.	wt. of Ovaries of Assay Rats mg. %	Source of Material Assayed**
	51.45	40.10	77.93	
	52.90	51.50	97.35	
	58.50	42.80	73.16	Control (No 17-AP)
MEAN	54.28	44.80	82.81	
SD	13.86	35.49	12.79	
	66.50	50.80	76.39	
	49.00	44.10	90.00	
	54.50	38.30	70.27	TREATMENT 1 (2 mg. 17-AP)
MEAN	50.66	44.40	78.88	
SD	80.08	38.13	10.10	
	55.00	78.50	142.72	
	52.00	82.80	159.23	
	61.50	73.20	119.02	TREATMENT 2 (3 mg. 17-AP)
MEAN	56.16	78.16	140.32	
SD	23.58	23.08	20.21	
	44.00	98.00	222.72	
	46.50	80.50	173.11	
	47.00	80.40	171.06	TREATMENT 3 (6 mg. 17-AP)
MEAN	45.83	86.30	188.96	
SD	5.17	10.13	29.25	

* FSH assay (Payne et al., Endo. 65:389, 1959).

** Desiccated pituitary tissue from control and 17-AP treated rats. The 17-AP was given orally at the dose indicated each day till necropsy on the 13th day of pregnancy. The desiccated tissue (0.5 mg.) was injected with 1 mg. DES each day for four days. Necropsy was performed 48 hours after the last injection.

GROSS DATA FROM PSEUDOPREGNANT RATS DEPLETED OF OVARIAN ASCORBIC ACID FOUR HOURS AFTER INJECTION WITH STANDARD SHEEP NIH-LH*

	wt. of Ovaries of Assay Rats (mg.)	Ovarian Ascorbic Acid ug. per Ovary	Ovarian Ascorbic Acid in mg. per 100 gm. of Ovarian Tissue	Dose of Standard NIH-LH
	231.00	129.50	56.06	
	181.00	275.50	143.90	
	240.00	315.50	131.50	
	235.00	260.00	110.63	0 ug. of NIH-LH
MEAN	221.75	245.25	110.49	
SD	27.41	80.52	38.86	
	222.80	206.70	92.77	
	289.20	230.40	79.66	
	194.00	120.00	61.85	
	184.00	131.40	71.25	0.4 ug. of NIH-LH
MEAN	222.50	172.12	76.38	
SD	47.42	54.66	13.13	
	195.80	71.80	36.67	
	204.20	95.00	46.52	
	188.00	56.00	29.78	
	204.40	92.00	45.00	1.6 ug. of NIH-LH
MEAN	198.10	78.70	39.49	
SD	7.82	18.31	2.77	

* LH assay (Parlow, Fed. Proc. 17:402, 1958; Schmidt-Elmendorff and Loraine, Endocrinol. 23:413, 1962).

GROSS DATA FROM PSEUDOPREGNANT RATS DEPLETED OF OVARIAN ASCORBIC ACID FOUR HOURS AFTER INJECTION WITH DESICCATED PITUITARY TISSUE FROM 17a-ACETOXYPROGESTERONE (17-AP) TREATED PREGNANT RATS*

	wt. of Ovaries of Assay Rats (mg.)	Ovarian Ascorbic Acid ug. per Ovary	Ovarian Ascorbic Acid in mg. per 100 gm. of Ovarian Tissue	Source of Desiccated Pituitary Tissue**
	175.40	67.80	38.65	
	198.40	74.60	68.81	
	286.00	74.00	25.87	
	250.20	104.40	41.72	CONTROL (No 17-AP)
MEAN	227.50	80.20	43.74	
SD	49.99	16.43	18.09	
	165.40	67.60	40.87	
	185.40	90.60	48.86	
	198.10	99.00	49.97	
	146.80	88.50	60.28	TREATMENT 1 (1 mg. 17-AP)
MEAN	173.92	86.42	49.99	
SD	22.54	13.34	7.97	
	203.80	332.00	162.90	
	178.40	185.00	103.69	
	207.20	145.00	69.98	
	184.00	270.00	146.73	TREATMENT 2 (3 mg. 17-AP)
MEAN	194.35	233.00	120.82	
SD	14.28	84.00	42.10	
	196.20	195.00	99.38	
	236.80	208.50	88.04	
	202.40	205.50	101.53	
	150.80	166.50	110.41	
	197.80	198.00	100.00	TREATMENT 3 (6 mg. 17-AP)
MEAN	196.80	194.60	95.87	
SD	30.62	16.67	17.71	

* LH assay (Parlow, Fed. Proc. 17:402, 1958; Schmidt-Elmendorff and Loraine, Endocrinol. 23:413, 1962).

** 0.1 mg. of desiccated pituitary tissue from 17-AP treated pregnant rats was dissolved in physiological saline and injected into the tail vein of the assay rats. The 17-AP was given orally each day. The pituitary tissue was collected on the 13th day of pregnancy.

VITA

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Candidate for the Degree of

Doctor of Philosophy

Thesis: EFFECTS OF PROGESTINS ON REPRODUCTION IN SWINE AND RATS. I. EFFECTS ON EMBRYONIC MORTALITY OF SWINE. II. EFFECTS ON EMBRYONIC MORTALITY AND PITUITARY GONADOTROPINS IN RATS

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