HISTOPHYSIOLOGICAL EFFECTS OF ESTROGENIC AND ESTROGENIC-LIKE COMPOUNDS ON THE ESTRUS CYCLE OF THE BITCH

by

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INTRODUCTION

The intricate balance of several known and possibly some unknown hormones influences the onset and regression of fertility, which is referred to as the estrus or sexual cycle. The estrus cycle is essentially a rhythmic phenomenon in the females of all vertebrates. However, the males of some species, wild dogs, wild ruminants, and bird species, also exhibit a rhythmic occurrence of fertile and infertile periods.

The estrus cycle is essentially similar in all females, differing only in the time limits of the various phases of the cycle. The latter leads to the classification of the estrus cycles into polyestrus, seasonally polyestrus, and monestrus. The latter two have received the most recent attention since man has endeavored to alter the normal physiological processes to increase the productivity of the animals in this category, namely the dog and the sheep.

It should be stated clearly that physiological estrus, or "heat", is the period during which a fertile mating may be accomplished. Hence, proper preparation of the uterine mucosa and ovulation is far more essential than sexual receptivity and other outward manifestations of estrus. The manifestations of estrus are regulated by the estrogenic hormones which are produced by the cells of the ovarian follicle under the influence of the gonadotropic hormones of the anterior pituitary gland. What initiates the anterior pituitary gland to activity in producing the gonadotropic hormones is still in question.

Experimental work with sex hormones followed the suggestion of Marshall (1905) that either the interstitial cells or the follicular cells secreted

a hormone that would produce estrus. Adler (1912) produced estrus changes in virgin rats with aqueous ovarian extracts. What actually caused these changes are doubtful since the estrogenic hormones are steroid compounds and not soluble in water. There is a possibility that a crude product such as this contains a suspension of estrogenic hormones which induced the estrus changes.

The discovery of a typical estrus cycle in the guinea pig by Stockard and Papanicolaou (1917), the phases of which could be accurately determined by cellular changes in the vaginal smear, marked a great forward stride in the isolation of the estrogenic hormones. Allen and Doisy (1923) utilized this phenomenon to study the quantitative actions of estrogens, with the subsequent isolation of a comparatively pure crystalline compound obtained from follicular fluid of cows and sows, 3 mg of which would produce estrus in a rat. Doisy (1927) extracted estrone in pure form and named it "theelin". Parkes and Bellerby (1926) introduced the term "Oestrin" for the follicular hormone. This name was later modified to estrone designating the non-proprietary form of the hormone. Following these discoveries it soon became obvious that this hormone existed in numerous chemical forms with varying degrees of physiological activity.

Cook, et al., (1934) reported estrogenic activity of certain hydrocarbon compounds. Dodds et al., (1938) discovered stilbestrol, a stilbene derivative, which exhibits marked estrogenic activity similar to follicular steriods.

The discovery of estrogenic and estrogenic-like hormones led to their use in veterinary medicine to treat infertility of unknown origin; to bring anestrus animals into estrus; and more recently for obtaining another litter of pups and an early or second lamb crop each year.

Since the aforementioned usages of these compounds disagree with the known physiological actions of the estrogenic hormones, the present investigation was designed to study the actual effect of the compounds with folliculoid activity on the estrus cycle and more specifically on the ovaries of the bitch.

MATERIALS AND METHODS

A single factorial design was used in which a total of 39 animals was placed in three groups. Group one was retained as the normal, group two received diethylstilbesterol, and group three received estradiol cyclopentylpropionate (E.C.P.).

The lack of available female dogs prevented the simultaneous study of all groups. Hence the animals were placed at random in their respective groups upon procurement by the laboratory. Since sexually mature female dogs were found by preliminary experimentation to respond more readily to hormonal stimulation, and with due consideration for the inherent advantages in dealing with a more or less static physiology of monoestrus types, mature female dogs of mixed breeds were chosen as experimental animals.

All animals were maintained on the same adequate diet of "Purina Dog Kibbles" fortified with Lederle's "Aurofac" and ground horse meat obtained from the Kansas State College Veterinary Clinic.

The stage of each animal's estrus cycle was determined by the vaginal smear method of Newberry and Gier (1952) prior to any treatment. The animals in Group I were considered to have a normal estrus cycle, as they accepted the male, conceived, and developed living embryos (Table 1). The animals in Group II received varying doses of diethylstilbesterol administered either orally in tablets or intramuscularly in oil (Table 2). Animals in Group III

received varying doses of E.C.P. intramuscularly in oil (Table 3). The animals were kept in cages and were allowed to exercise freely each day. Vaginal smears were recorded on each animal throughout the experimental period. As an animal exhibited symptoms of estrus a permanent vaginal smear, stained with hematoxylin and eosin, was recorded daily. Unilateral or bilateral ovariohysterectomy was performed on each animal as she approached, or just after the termination of normal or induced estrus. The latter was determined by the vaginal smear and daily exposure to the male throughout the induced estrus period. The surgically removed ovaries were fixed in Bouins, imbedded, sectioned serially and stained with hematoxylin and eosin.

RESULTS

Case Histories

Group I

Dog 55. An 18 pound mature terrier-mix revealed a constant anestrus picture for five months. Bilateral ovariohysterectomy four months and 22 days after procurement, substantiated the absence of external symptoms of estrus. Grossly, the ovaries were small, white, smooth in contour and showed no external sign of follicler development. Microscopic examination revealed numerous developing follicles and many primary follicles. Four old corpora lutea, centrally located, were present. The periphery of the ovary was smooth and regular in outline (Plate I, Fig. 2).

Dog 66. A 14 pound mature terrier-spaniel was under observation for three months before the first outward symptoms of estrus: swelling of vulva, sanguinous discharge and vaginal cornification. When the vaginal cornification became complete three days later, she accepted the male and continued

EXPLANATION OF PLATE I

Fig. 1. Section of left ovary from dog 110, removed one day after last acceptance following treatment with 2 mg E.C.P. The corpora lutea more compact. The tertiary follicles are reduced in size and there are numerous polyovular follicles present. Primary follicles are greatly increased and the germinal epithelium is thickened and irregular (17x).

Fig. 2. Section of left ovary from dog 55, removed during anestrus. This section shows the central location of the old corpora lutea and the normal arrangement and size of primary and tertiary follicles. The germinal epithelium is smooth and thin and there are no polyovular follicles (17x).

PLATE I

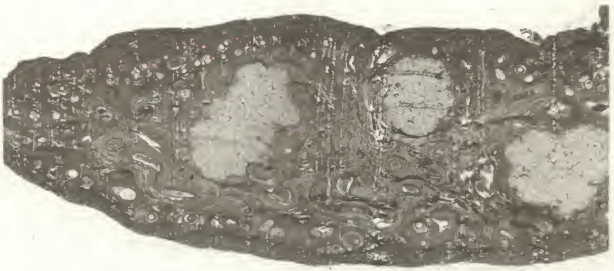


Fig. 1

Fig. 2

acceptance for the next five days. Unilateral ovariohysterectomies were performed two and four days post estrus. Two corpora lutea were present on the left ovary but no embryos were recovered. The right ovary had two corpora lutea, and one embryo (early blastocyst) was recovered, indicating ovulation and fertilization.

Dog 67. A ten pound immature mongrel, possibly four months old, was held in the laboratory four months before manifestations of estrus occurred. First acceptance of the male occurred three days after the first estrus symptoms. The acceptance period was six days, at which time, the vaginal smear also indicated the termination of estrus. Unilateral ovariohysterectomies were performed one and six days post estrus. One corpus luteum was present on each ovary and two embryos were recovered.

Dog 68. A mature 30 pound collie was under observation four months before manifestations of estrus were noticed. The vaginal smear indicated full estrus three days after the initial symptoms. She accepted the male for six days. Termination of estrus was cross checked by the post estrus picture of the vaginal smear and failure to accept the male. Surgical removal of ovaries and uterus was accomplished two and nine days after last acceptance. Two corpora lutea were observed in the left ovary and five in the right. Two embryos (8 cell stage) were recovered from the left cornu, and four embryos (morulla stage), from the right horn of the uterus.

Dog 69. A 28 pound white collie-mix exhibited first symptoms of estrus five months after procurement. She accepted the male four days later and continued acceptance for five days. Unilateral ovariohysterectomies were performed two and four days after last acceptance. Six recent corpora lutea were present on the left ovary and four embryos (8 cell stage), were recovered.

Dog 71. A 24 pound springer spaniel with a history of having pups three months prior to entering the laboratory, exhibited manifestations of estrus after approximately one month. Five days later she accepted the male, and continued acceptance for a period of ten days. Left and right ovaries and uterus were surgically removed seven and ten days respectively after last acceptance. Left and right ovaries had five and four corpora lutea respectively. Four embryos (blastocyst stage) were recovered from the left horn and three embryos (blastocyst stage) were recovered from the right horn of the uterus.

Dog 72. A 23 pound springer-collie cross was a daughter of dog 71 and had whelped about three months prior to coming into the laboratory. Symptoms of estrus were noted 20 days later. Acceptance period began eight days after first outward manifestations and continued for six days. Surgical removal of left and right ovaries was accomplished three and six days respectively after the termination of estrus. The left ovary exhibited three recent corpora lutea, and three early-cleavage embryos were recovered. The right ovary had five corpora lutea, and three embryos (early morulla), were recovered from the uterus.

Dog 73. An immature 24 pound Springer-collie was whelped by dog 72, three months prior to entering the laboratory. This female was kept in the laboratory for four months before early outward symptoms of estrus were observed. Five days elapsed before she accepted the male. This animal accepted the male for four days after which time she was kept from the male for three days and a left unilateral ovary-ohysterectomy was performed. This animal was again exposed to and accepted the male for the next three days after which time the right ovary and horn of the uterus were removed.

No ovulation points were discernable on the left ovary. The follicles were enlarged, the ovary darkened from the increased blood supply and rupture of the follicles occurred while the ovary and horn of the uterus were transferred to the Bouin solution. Grossly the ovaries were enlarged and irregular from the mature follicles. Several large mature follicles could readily be seen in cross section of the ovaries. Microscopic examination revealed two large follicles with ovulation points and luteinization of the follicular cells, a few immature follicles, and many primary follicles (Plate II, Fig. 2).

Dog 74. A 19 pound cocker-setter cross was obtained 45 days prior to the first manifestations of approaching estrus. Full estrus and subsequent acceptance of the male were noted eight days after initial symptoms. The acceptance period was eight days. Unilateral ovariohysterectomies were performed one and two days after last acceptance. The left ovary had two recent corpora lutea but there was no recovery of embryos from the reproductive tract. Examination of the right ovary revealed four corpora lutea and four embryos were recovered from the oviducts (one 4 cell and three 8 cell).

Dog 97. A normal 22 pound black cocker spaniel was approaching full estrus upon procurement. She first accepted the male two days later and continued acceptance for seven days. Left and right ovariohysterectomies were performed three days prior to and one day after the date of last acceptance. The left ovary had four large preovulation follicles. However these follicles ruptured during manipulation. The right ovary had five ovulation points and three fertilized ova (one-cell stage) were recovered from the oviduct (Plate III, Fig. 1).

Dog 101A. An 18 pound terrier-mix was held in the laboratory for two

EXPLANATION OF PLATE II

Fig. 1. Section of left ovary from dog 15, removed three days after cessation induced estrus symptoms from diethylstilbesterol treatment. The great number of multiovular follicles and thickened germinal epithelium are typical of ovaries subjected to diethylstilbesterol treatment (17x).

Fig. 2. Section of left ovary from dog 73, removed four days prior to termination of estrus. Rupture of the large follicle in the section occurred during manipulation. This section is typical of ovaries during normal estrus (8.5x).

PLATE II

Fig. 1

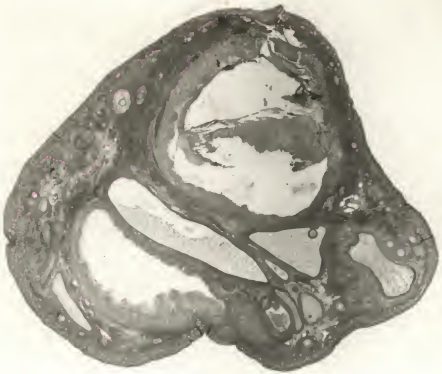


Fig. 2

EXPLANATION OF PLATE III

Fig. 1. Section of left ovary from dog 99 following treatment with 2 mg E.C.P. Increased stroma, presence of polyovular follicles and thickening of the germinal epithelium are evident even at this low manification. A normal ovary near the end of estrus should be similar to Fig. 2 (17x).

Fig. 2. Section of left ovary of dog 97, removed three days prior to termination of estrus, showing a large follicle that ruptured during manipulation (17x).

PLATE III

Fig. 1

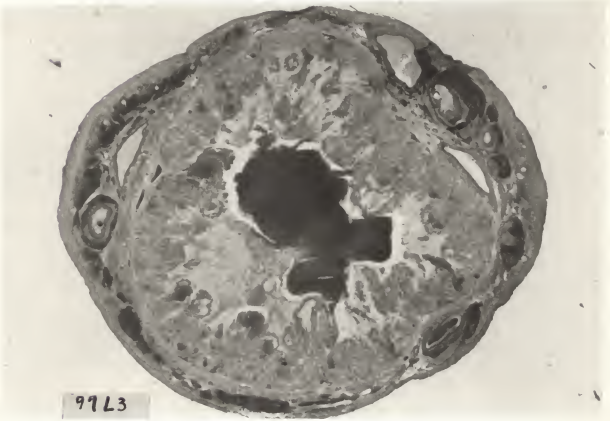


Fig. 2

months before a left unilateral ovariohysterectomy was performed. Vaginal smears were recorded periodically throughout the holding period. The smears indicated continuous anestrus. This animal was allowed to recover and was used for further studies with diethylstilbesterol.

Dog 104A. Approximately a five month old collie was under observation in the laboratory for three months before a left unilateral ovaryhysterectomy was performed. She was allowed to recover and placed in Group II. For description of the ovaries see dog 104 in Group II.

Dog 105A. A litter mate of dog 104A was under observation for three months prior to being semi-spayed. She was allowed to recover and used for further diethylstilbesterol studies.

Group II.

Dog 13. An 18 pound Springer spaniel received 1 mg. diethylstilbesterol orally on alternate days for five doses. Manifestation of estrus was apparent five days after the initial treatment. Induced estrus was maintained for five days, at which time the treatment was discontinued. The full estrus vaginal smear prevailed for one day post treatment. An exploratory laparotomy was performed two days later to determine the condition of the ovaries. This animal failed to attract and was not attracted to the males at any time during the induced estrus period. Both ovaries were small, white and had no follicular development.

Dog 14. An 18 pound cocker spaniel was treated with seven 1 mg.doses of diethylstilbesterol on alternate days. Complete estral vaginal smear symptoms were noted eight days after the initial treatment and were apparent for the next ten successive days. An exploratory laparotomy was accomplished two days after full estral vaginal smears were last obtained. The males were

not interested in this female during the induced estrus period and copulation did not occur. Both ovaries were small, white and had no follicular development.

Dog 15. A 15 pound cocker-spitz cross received six 2 mg. doses of diethylstilbesterol administered orally on alternate days. Induced estrus was noted for six days beginning in the 13th day after the initial treatment. Sexual attraction and receptivity were not present in this case. Left ovariohysterectomy was performed three days after the induced estrus period ended. The ovary was small, white and had no follicular development. Microscopic examination revealed no developing and many primary follicles. A number of the primary follicles had from two to seven multiple ova present (Plate II, Fig. 1).

Dog 17. A 21 pound white cocker-terrier cross was treated with 1 mg. diethylstilbesterol on alternate days for 11 doses. Four days elapsed and she was again treated with a single dose of 2 mg. of diethylstilbesterol. Manifestations of estrus, except for attraction and receptivity to the male, were complete from the 22nd to the 27th day after the initial treatment. Metestrus was apparent four days post treatment and a left ovariohysterectomy was performed ten days later. The ovary was small, had no follicular development and no recent corpora lutea.

Dog 59. A 22 pound multiple cross was treated with four 2 mg. doses of diethylstilbesterol on alternate days. The dosage was then decreased to 1 mg. and administered for 11 alternate days. Full estral vaginal smear was apparent from the 13th to the 19th day after treatment was initiated. The vaginal smear was typically metestrus two days post treatment and the left ovary and horn of the uterus were removed at that time. Attraction and

receptivity to the male were absent. The ovary was small, white and had no follicular development. A number of polyovular follicles were noted, but none had developed beyond secondary stage.

Dog 60. A 22 pound multiparous terrier was treated with eight 2 mg. doses of diethylstilbesterol, administered on successive days. Six days after the initial dose she exhibited a full estrus vaginal smear which continued for five days, but she did not attract or exhibit any signs of sexual receptivity toward the males. Right ovariohysterectomy was performed five days post treatment. The ovary was small, and had no follicular development. Ovulation had not occurred.

Dog 75. A 20 pound black cocker spaniel was treated with 0.5 mg. diethylstilbesterol for 28 doses, administered on consecutive days. Manifestations of estrus produced by this dosage were limited to a moderate swelling of the vulva and partial cornification of the vaginal epithelium. Left unilateral ovariohysterectomy was performed one day post treatment. This animal was allowed to recover and was then treated with 1 mg. diethylstilbesterol for 17 doses on successive days. A complete estral vaginal smear was noted 14 through 18 days after treatment was resumed. However, receptivity and attraction toward the male were absent. This animal was again allowed to recover to determine the long term effects of diethylstilbesterol. The left ovary was typical of those treated with the estrogenic compounds.

Dog 76. A 17 pound terrier cross received 0.5 mg. diethylstilbesterol on alternate days for 12 doses. The dosage was increased to 1 mg. and administered on alternate days for 16 doses. Bilateral ovariohysterectomy was performed one day after the last treatment. The 0.5 mg. doses resulted

in a slight swelling of the vulva and a slight reduction in the white cells of the vaginal smear. The 1 mg. level increased the vulvar swelling and further reduced the white cells. There was also a partial cornification of the vaginal epithelium. The 0.5 and 1 mg. dosages on alternate days were not sufficient to produce a full estral vaginal smear. The ovaries were small, white and exhibited no follicular development. The polyovular and increased numbers of primary follicles were present. The germinal epithelium was thickened and showed signs of cellular activity.

Dog 79. A 24 pound cocker spaniel received 1 mg. diethylstilbesterol daily for 14 doses. Both ovaries and horns of the uterus were removed the day following the last dose of diethylstilbesterol. The manifestations of estrus produced by the diethylstilbesterol were limited to the vulvar swelling, slight reduction of white blood cells and a partial vaginal cornification. Both ovaries were small, white and had no follicular development. However, the increase number of primary follicles and polyovular follicles were present.

Dog 80. A seven month old terrier fox hound, weighing 21 pounds, received 19,1 mg. doses of diethylstilbesterol on consecutive days. The dosage was then increased to 3 mg. and 5 mg. for three and two daily doses respectively. Complete induced estrus was noted for seven days beginning 22 days after the initial dose. Left and right unilateral ovariohysterectomies were accomplished five and 25 days respectively after treatment. Both ovaries were typical of those treated with estrogenic compounds.

Dog 101. An 18 pound terrier mix was semi-ovariohysterectomized before receiving 15 2 mg. doses of diethylstilbesterol on every third day. A complete estral vaginal smear, that was maintained for six days, was apparent 23 days after the initial dose. The remainder of the uterus and the right ovary was surgically removed four days after the induced estrus had subsided.

The ovaries resembled very closely those of dog 104.

Dog 104. A 40 pound collie was semi-spayed before being subjected to 12 2 mg. doses of diethylstilbesterol on alternate days. A complete estral vaginal smear was evident 23 days after the initial treatment and was maintained for six days. The induced estrus subsided two days after the last treatment. Right ovariohysterectomy was performed six days post treatment. The left ovary was typically anestrus and presented a number of developing folliclar antra. The periphery of the left ovary was smooth in outline and showed little cellular activity of the germinal epithelium. There were no follicles with multiple ova and no apparent increase in connective tissue stroma (Plate IV, Fig. 2). The right ovary was small, white and had undergone considerable atrophy. The developing follicles showed evidence of atresia. The periphery of the ovary was irregular and revealed evidence of cellular activity resulting in a thickening of the germinal epithelium. Several follicles with multiple ova were noted while the number of primary follicles was somewhat increased. There was an apparent increase in stroma (Plate IV, Fig. 1).

Dog 105. A 42 pound litter mate of dog 104 was semi-spayed just prior to being treated with 12 2 mg. doses of diethylstilbesterol on alternate days. A complete estral smear was maintained for five days after being first noted 23 days after the initial treatment. Right unilateral ovariohysterectomy was performed six days post treatment. The left and right ovaries resembled very closely, those of dog 104.

Group III

Dog 85. A 22 pound mature Springer-collie mix was determined to be in anestrus by the vaginal smear technic. She received 2 mg. E.C.P.

EXPLANATION OF PLATE IV

Fig. 1. Section of right ovary from dog 104 following diethylstilbesterol treatment. Repression of the tertiary follicles, presence of polyovular primary follicles, increased stroma and thickened irregular germinal epithelium, characterize ovaries following diethylstilbesterol treatment. By contrast with Fig. 2, it illustrates the typical action of estrogenic compounds on the ovaries of the bitch (17x).

Fig. 2. Section of left ovary from dog 104, removed prior to any treatment. This section is typical of normal anestrus ovaries (17x).

PLATE IV

Fig. 1

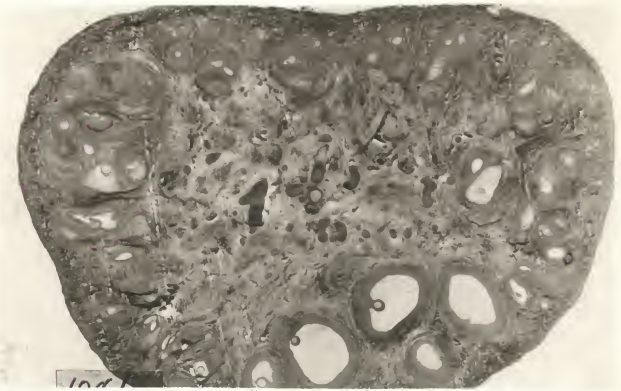


Fig. 2

intermuscularly. Vulvar swelling and pink vaginal discharge were noted four days post treatment. Exploratory laparotomy was performed four days later because vaginal smear picture was complicated by presence of large numbers of WBC's. The ovaries and uterus were anestrus and bladder was distended with urine and inflammed indicating a cystitis condition. The abdominal incision was closed and the animal was given 300,000 units of penicillin. Two days after the operation the vaginal smear indicated full estrus with the exception of the presence of many WBC's. This female was exposed to the male and coitus occurred. Acceptance period was for eight days, and profuse vaginal hemorrhage accompanied each copulation. The left ovary and left horn of the uterus were surgically removed the first day the female refused the male, which was 19 days post treatment. Right ovariohysterectomy was performed ten days later. Both ovaries were small, white and had no large follicles.

Dog 88. A 28 pound mature female coyote with anestrus vaginal smear was treated with 3 mg. E.C.P. intramuscularly 24 days after procurement by the laboratory. Early manifestations of estrus were observed four days later. This animal received an additional 4 mg. of E.C.P. 8 days after initial treatment. Full estrus vaginal smear was noted for this animal 11 days after initial treatment with E.C.P. and continued to the 13th day. Bilateral ovariohysterectomy was accomplished one day after termination of induced estrus as evidenced by vaginal smear. Grossly the ovaries were small, white with no external signs of follicular development. Sections of the ovary revealed only very small follicles.

Dog 92. A 40 pound mature setter mix, was obviously heavy with pups when received by the laboratory. She had 12 normal pups six days later

and the pups were taken from her immediately. Two months later this animal was treated with 4 mg. E.C.P. followed by 2 mg. E.C.P. nine days later. Vaginal cornification and swelling of the vulva were observed four days after initial treatment. Vaginal cornification was complete and first acceptance of the male was noted five days post treatment. The male was accepted daily for 12 days. The ovaries and uterus were removed three days after last acceptance. Both ovaries were small, white and follicles were limited to primary and early secondary stages.

Dog 93. A 29 pound mature coyote was treated with 5 mg. of E.C.P. 21 days after entering the laboratory. This animal did not respond to treatment and was destroyed 11 days after treatment. Both ovaries were small and white and had no external appearance of follicular development.

Dog 98. A mature red cocker spaniel that weighed 18 pounds, received 4 mg. of E.C.P. 18 days after procurement. She had an anestrus vaginal smear picture on the date of treatment. Progressive manifestations of estrus were noted from three days to 13 days post treatment, at which time she was receptive to and accepted the male for the next 42 days. A left unilateral ovariohysterectomy was performed 18 days after first acceptance. This female continued to accept the male for 24 days after the operation. Tearing of the vulva, excessive hemorrhage and much pain accompanied each copulation throughout the acceptance period. This animal was allowed to recover and have a normal estrus cycle in order to determine long term effects of the E.C.P. The left ovary was small, white and had no external follicular development. Microscopic examination revealed several old corpora lutea and a number of small follicles with antra present. Several primary follicles with multiple ova were noted. The periphery of the

ovary was irregular and indicated increased cellular activity of the germinal epithelium. There were no large maturing follicles and no possibility of ovulation.

Three months after last acceptance, this female exhibited symptoms of proestrus and she accepted the male 18 days later. The acceptance period was for six days and no tearing or hemorrhage occurred. Right unilateral ovariohysterectomy was performed two days after the last acceptance. Five recent corpora lutea were present and five (4 cell stage) embryos were recovered. No abnormalities of either ovary or uterus were detectable.

Dog 99. A 21 pound mature blond cocker was treated with 2 mg. of E.C.P. 18 days after entering the laboratory. Eight days later she accepted the male and continued acceptance for 14 days. Pain, vulvar laceration and excessive hemorrhage accompanied each copulation. Surgical removal of the left ovary was accomplished one day after last acceptance. This female was allowed to recover and was placed under observation to determine long term effects of E.C.P. Vaginal smears were recorded for 23 days following surgery, and partial cornification of the vaginal epithelium was still present. The left ovary was small, white and had no external signs of follicular development. Microscopic examination revealed no maturing follicles. The periphery of the ovary was irregular and the germinal epithelium indicated cellular activity. A few follicles with multiple ova were observed (Plate III, Fig. 1).

Three months after surgery this animal exhibited signs of approaching estrus. Six days later she accepted the male and continued acceptance for five days. Right unilateral ovariohysterectomy was performed four days after last acceptance. Four recent corpora lutea were present in the

ovary, and four early embryos were recovered from the oviduct.

Dog 108. A 16 pound mature terrier was under observation for 26 days before receiving 1.5 mg. of E.C.P. Full estrus was observed 13 days after treatment. She accepted the male for 16 days and tearing of the vulva, excessive hemorrhage and much pain was observed during each coitus. Left unilateral ovariohysterectomy was performed one day after last acceptance. This animal was placed under observation for approximately five months before manifestations of approaching normal estrus appeared. She accepted the male for nine days. The right ovary and remainder of the uterus were removed 15 days after last acceptance. The left ovary was small, white and no external follicular development. Microscopically, the periphery was irregular and the germinal epithelium exhibited signs of cellular activity. A number of small follicles with antra were present, but large developing follicles were absent. The right ovary had three corpora lutea. The right horn had three swellings comparable to 15 days pregnancy. This animal was allowed to recover after complete ovariohysterectomy, and was then treated with 1.6 mg. of E.C.P. Eleven days post treatment she accepted the male and continued acceptance for nine days.

Dog 109. A 14 pound mature female (litter mate of 108) was treated with 3 mg. E.C.P. after a 12 day observation period. Full estrus symptoms were observed 11 days after treatment and the acceptance period was continuous from the 11th through the 21st day post treatment. The ovaries and uterus were removed four days after last acceptance. Both ovaries were small, white and had no external signs of follicular development. Microscopically the periphery was irregular and the large maturing follicles that accompany normal estrus were absent. Several primary follicles exhibited multiple

ova. The germinal epithelium was somewhat thickened.

Dog 110. A 26 pound shepherd mix was treated with 2 mg. E.C.P. Pups had been weaned from her just prior to procurement. Vaginal cornification was apparent three days later and full estrus with subsequent copulation was noted 11 days following treatment. The acceptance period was nine days. Laceration, pain and excessive hemorrhage were apparent at each copulation. Bilateral ovariohysterectomy was accomplished one day after last acceptance. Both ovaries were small, white and had no evident follicular development. Microscopically they had irregular periphery with the germinal epithelium thickened. There were no large developing follicles. A number of small follicles with antra, a few primary follicles with multiple ova and three old corpora lutea were present. The latter were centrally located (Plate I, Fig. 1).

Dog 125. A 24 pound terrier mix was treated with 2 mg. E.C.P. Acceptance occurred six days post treatment and continued for 12 days. Left unilateral ovariohysterectomy was performed three days after last acceptance. This animal was allowed to recover for further experimentation. The left ovary was small, white and histologically resembled those treated with estrogenic compounds.

Dog 131. A 23 pound terrier-shepherd cross received 3 mg. E.C.P. 27 days after procurement. She accepted the male for 11 successive days beginning ten days post treatment. The left horn of the uterus and left ovary were surgically removed eight days after last acceptance. The ovary was small, white and resembled histologically those treated with estrogenic compounds.

Dog 142. A 15 pound multiparous mongrel was treated with 3 mg. E.C.P. She first accepted the male nine days post treatment. The acceptance period was 22 days. Left ovariohysterectomy was performed three days after the date of last acceptance. The ovary was small and white and resembled histologically those of other animals treated with estrogenic compounds.

General

The results of the control animals are illustrated in Table 1.

Table 1. Group I Control animals.

Dog	Acceptance Days	Surgery	days from last acceptance	Anestrus	Ovaries Estrus	Corpora lutea	Ovulation
55	0	L.		+	0	0	-
66	6	L.	+2			2	+
		R.	+4			2	+
67	6	L.	+1			1	+
		R.	+6			1	+
68	6	L.	+2			2	+
		R.	+9			5	+
69	5	L.	+2			4	+
		R.	+4			2	+
71	9	L.	+7			5	+
		R.	+10			4	+
72	6	L.	+3			3	+
		R.	+6			5	+
*73	7	L.	-4		+	2	-
		R.	0		-	2	+
74	8	L.	+1			2	+
		R.	+2			4	+
97	7	L.	-3		+	4	-
		R.	+1		-	5	+
**101	0	L.	-	+	-	-	-
**104	0	L.	-	+	-	-	-
**105	0	L.	-	+	-	-	-

*The left ovary had no ovulation points upon removal from the animal. Ovulation occurred, however by manipulation while the ovary was being transferred to the fixative. The cells of two follicles had already begun to luteinize.

**Unilateral ovariohysterectomy was performed on the animals to accumulate normal anestrus ovaries prior to treatment with any hormone.

Four of 13 animals were semi ovarectomized to obtain a series of normal anestrus ovaries. Three of the semi-spayed animals were transferred to Group II for treatment with diethylstilbesterol. Eight animals in this group were allowed to have a normal estrus. Each animal was bred upon exhibiting manifestation of full estrus (determined by vaginal smear). Ovariohysterectomies were performed from four days prior to ten days after the day of last acceptance. The period of acceptance varied from five to nine days with the average being 6.6 days. Recent corpora lutea were present in the ovaries, and a corresponding number of embryos was recovered. Ovulation had occurred in all eight animals. However, dog 73 had not ovulated four days previous to the last day of acceptance but ovulation occurred under manipulation of the ovary. Macroscopically, normal anestrus ovaries were small and white and had no outward appearance of follicular development, while all estrus ovaries showed large follicles or recent corpora lutea.

Diethylstilbesterol below the 1 mg. dose per day produced only manifestations of proestrus. The time required to produce full estrus with diethylstilbesterol treatment, varied from 10 days with 1 mg. on alternate days, to 19 days with 2 mg. daily. Eleven of the 13 animals treated with diethylstilbesterol exhibited symptoms of estrus between four and ten days after beginning of treatment. The symptoms of estrus regressed readily after treatment was discontinued. Three animals failed to show the manifestation of a complete estrus vaginal smear irregardless of dosage. None of the animals in this group were not receptive to the male.

Macroscopically the ovaries from the animals in this group were small, white and had no external signs of follicular development. They resemble in appearance the ovaries of normal anestrus animals. Microscopically the

periphery of the ovaries were irregular and the germinal epithelium was somewhat thickened, as compared to the normal (Plate V, Fig. 2) indicating increased cellular activity of the oogonia (Plate V, Fig. 1). There was an increase in primary oocytes accompanied by a thickening of the stroma. There was a large number of primary follicles, and several follicles with multiple ova present. The large follicles which accompany normal estrus were conspicuously absent (Plate IV, Fig. 1). There was no ovulation and in turn no opportunity for a fertile mating in this group of animals.

The effect of graded doses of diethylstilbesterol are summarized in Table 2.

Table 2. Animals treated with diethylstilbesterol.

Dog	DOSES Mg.	Total	Interval days	ESTRAL SYMPTOMS Days post treatment	Duration days	Acceptance	Ovaries	Ovulation
13	1	5	1	5	6	0	anestrus	0
14	1	7	1	8	10	0	"	0
15	2	6	1	13	6	0	"	0
17	1 3	11 2	4	22	6	0	"	0
59A	2 1	4 11	1	13	6	0	"	0
59B	2	8	daily	6	5	0	"	0
75A	0.5	28	daily		0	0	"	0
75B	1	17	daily	14	4	0	"	0
76	0.5 1	12 16	1		0	0	"	0
79	1	14	daily		0	0	"	0
80	2 3 5	19 3 2	daily	22	7	0	"	0
101	2	15	2	23	6	0	"	0
104	2	12	2	23	5	0	"	0
105	2	12	1	23	5	0	"	0

EXPLANATION OF PLATE V

Fig. 1. Section of right ovary from dog 104 following treatment with diethylstilbesterol. The proliferation and subsequent thickening of the germinal epithelium is evident and illustrate the effects of estrogenic compounds on the ovaries as compared to Fig. 2. (500x).

Fig. 2. Section of left ovary from dog 104, removed prior to treatment illustrates the germinal epithelium of normal anestrus ovaries (500x).

PLATE V

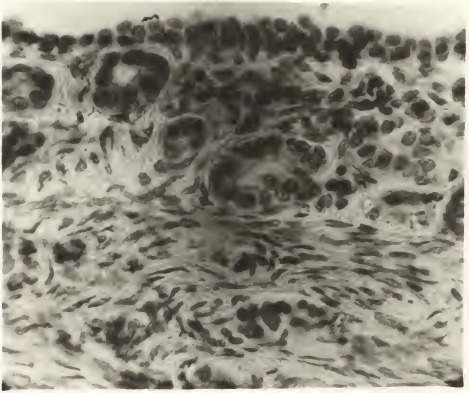


Fig. 1

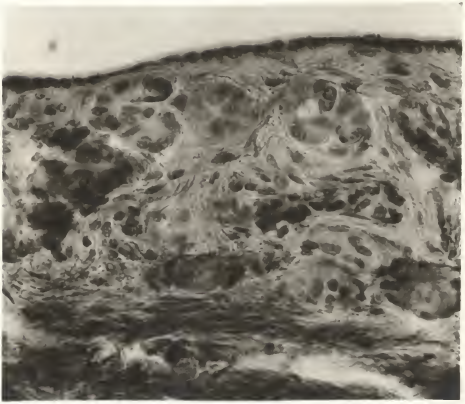


Fig. 2

The effects of graded doses of estradiol cyclopentylprotionate are summarized in Table 3.

Table 3. Animals treated with estradiol cyclopentylpropionate.

Dog	Mg.	DOSES Total	Interval days	ESTRAL SYMPTOMS Days post treatment	Acceptance days	Ovaries	Ovulation
*88	3/4	2	8	11	none	anestrus	none
85	2	1	0	10	8	"	"
92	4/2	2	9	12	12	"	"
**93	5	1	0	0	0	"	"
***98A	4	1	0	13	42	"	"
***99A	2	1	0	8	14	"	"
***108A	1.5	1	0	13	16	"	"
***109A	3	1	0	11	11	"	"
110	2	1	0	11	9	"	"
++108C	1.6	1	0	11	9		
125	2	1	0	6	12	anestrus	none
131	3	1	0	10	11	"	"
142	3	1	0	9	22	"	"

*88 coyote - vaginal smear indicated full estrus for period of 22 days.
**93 coyote - vaginal smear proestrus when received reverted to anestrus picture and would not respond to treatment.

*** These animals were kept under observation until they had a normal estrus in order to determine any damaging effects from this treatment.
++ This treatment was administered after complete ovariohysterectomy.

E.C.P. administered intramuscularly in 1.5 - 5 mg. doses produced the outward manifestations of estrus, including acceptance of the male in 11 of 13 animals. The manifestations of estrus were complete by 6 to 13 days after the initial treatment with the average being 9.5 days. The acceptance period of the 11 animals averaged 15 days with a high of 42 days and a low of 8 days. The other two animals in this group were female coyotes and they did not respond to treatment. This is an extreme variation in the period of acceptance from the normal group of animals listed in Table 1, a probable result of slow absorption of the hormones from the oil base. The tearing of the vulva and profuse hemorrhage were probably due to improper preparation of the external genitalia by the E.C.P. This is evidenced by the three animals that were allowed to recover and undergo a normal estrus, at which time they were mated with the same male and no laceration or tearing of the genitalia occurred.

Macroscopically the ovaries of this group resembled that of Group II. They were small, white and exhibited no external evidence of follicular development or ovulation. The ovaries from this group of animals were of the anestrus type.

Microscopically the periphery of ovaries was irregular and indicated increased cellular proliferation, (Plate VI, Fig. 1), beyond that observed in normal untreated ovaries (Plate VI, Fig. 2). There was an increase in primary oocytes and primary follicles, a number of which presented multiple ova (Plate I, Fig. 1). The large follicles which accompany normal estrus were absent or undergoing atresia. The connective tissue stroma was more abundant than normal.

The normal estrus ovaries were irregularly enlarged from large follicles or recent corpora lutea, and dark in color from the increased blood supply to the ovary. Ovulation points were discernable on a number of ovaries that were removed just after ovulation or ruptured by manipulation (Plate II, Fig. 2).

EXPLANATION OF PLATE VI

Fig. 1. Section of left ovary from dog 110, illustrating the cellular proliferation and irregular germinal epithelium induced with E.C.P. (500x).

Fig. 2. Section of left ovary from dog 55, illustrating the normal intact, regular layer of the germinal epithelium in contrast to Fig. 1 (500x).

PLATE VI

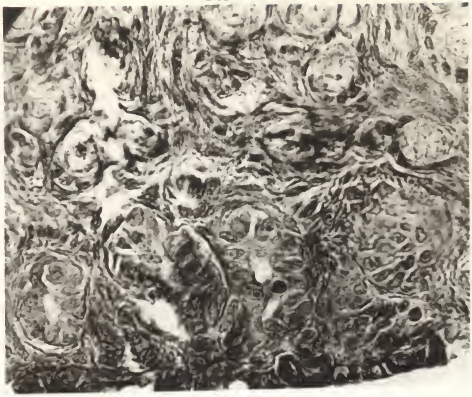


Fig. 1

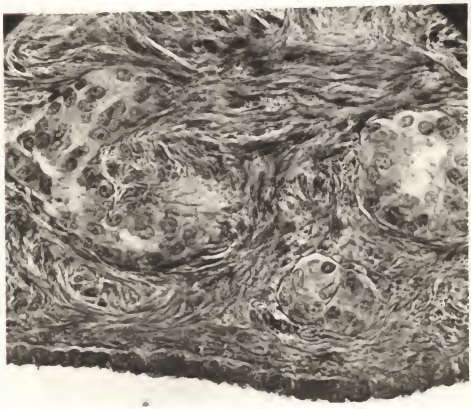


Fig. 2

Microscopically the normal anestrus ovaries exhibited in general, a smooth periphery with little cellular activity of the germinal epithelium as evidenced by the single layer of cogonia and relatively few primary follicles, (Plate I, Fig. 2). Also, old corpora lutea when present, were centrally located and in varying stages of regression. A number of larger developing follicles located toward the periphery were noted. However, the follicles with multiple ova, (Plate II, Fig. 1), were not demonstrable in most of the control animals.

The normal estrus ovaries were characterized by the large recent corpora lutea or large mature follicles and the small amount of actual ovarian tissue condensed in the area opposite to, or between the large follicles (Plate III, Fig. 2).

DISCUSSION

These data fail to agree with the work of Anderson (1938), Montgomerie and Brownlee (1941), Hammond et al (1942) and Quin and Van der Wath (1943) in which they reported full estrus in sheep when treated with various dosages of diethylstilbesterol. The above conclusions were based, in general, upon acceptance of the ram without considering reproductive success. However, Quin and Van Der Wath drew conclusions from the facts that eight of 43 ewes that accepted the male apparently ovulated. The eight animals in this case would not seem to be above the limits of the percentage of sheep that normally exhibit signs of early normal estrus. Anderson and Bugg (1942) and Brownlee et al (1942), offered similar results on their work on anestrus heifers. The former reported that first service failed to induce pregnancy. Frank and Appleby (1943) presented evidence that stilbesterol alone and in conjunction

with P.M.S. failed to produce normal estrus in ewes necessary for successful fecundation.

The results of the experiments in this report, support and extend the findings of Frank and Appleby that estrogens and compounds with estrogenic activity inhibit follicular development and will not produce the normal estrus necessary for fertilization in domestic animals.

Spencer et al (1930) and Meyer et al (1930), working with rats, and Kunde et al (1930) with immature female dogs, also reported a similar decrease in the size of the ovaries and repression of follicles in animals treated with estrogenic compounds. Gillman (1942) offered a solution to the atrophy of the ovaries when he produced extensive atresia of the larger follicles in the baboon with single or repeated injections of estrogens. The inhibiting effect and ensuing failure of the ovarian follicles to develop may best be explained by the work of Moore and Price (1932), and Cole (1946) with rats, and Sturgis and Albright (1940) human, in which they found estrogens and diethylstilbesterol prevented follicular development and ovulation by an inhibitory effect on the anterior pituitary. Foster (1942), and Finnerty and Meyer (1950) experimenting with diethylstilbesterol and natural estrogens respectively illustrated the inhibition of the anterior pituitary to be due to a decrease in the basophilic cells with a resulting reduction of F.S.H. Stevenson and Ellis (1953) demonstrated the futility of using diethylstilbesterol for successful fecundation in swine by treating gilts with 2.5 to 250 mg. daily and causing a cessation of estrus.

The present knowledge of the interaction of these hormones thus indicates that the estrogenic compounds, in addition to controlling the events of the estrus period effects a depression of the anterior pituitary. This causes a

lag in the gonad stimulating hormone and a subsequent cessation of further follicular development. A further corollary follows: overloading of the animal with sex hormones occurs only artificially or pathologically, thus eliminating the possibility of natural retardation of the anterior pituitary.

The increase in stroma noted in ovaries of dogs treated with estrogen, appeared to be due to the atresia of larger follicles with a replacement of the follicular area with connective tissue.

Connor (1951) reported that estrogens and stilbesterol produced a proliferation of the germinal epithelium and psychic reactions in the female dog similar to that of normal estrus. Connor's findings on germinal epithelial cell changes are in agreement with the present data, but the present experiment failed to secure evidence that all the psychic reactions were present. Increasing the amounts of the E.C.P. and diethylstilbesterol progressively increased the manifestations of estrus. However, the diethylstilbesterol failed to produce flirting, attraction or acceptance and both compounds inhibited ovulation. It should be stressed that the direct response to the estrogens is limited to the outward symptoms and possibly stimulation of the germinal epithelium as the treated animals failed to exhibit follicular development and ovulation. If the stimulation of the germinal epithelium is a direct effect of the estrogen produced by maturing follicles as suggested by Raps (1948), then the follicle rather than the entire ovary must be considered to be the endocrine gland. This concept is not too absurd as the corpus luteum is considered as an endocrine gland after the follicular cells are transformed into luteal cells. It is also worthy of mention that the estrogenic compounds failed to properly prepare the genitalia for copulation as evidenced by the laceration of the vulva accompanied by pain and excessive hemorrhage.

The follicles with multiple ova observed in the animals treated with the estrogenic compounds were previously reported by Evans and Swezy (1931) to be a normal physiological phenomenon in the dog. This, however, is in complete disagreement with these experiments as multiple ova were observed in the follicles of only one control animal. These same authors regarded the "egg nests" to be atretic follicles. This again is contrary to the present findings as development can be followed step by step from germinal epithelium through Pfleuger's cords to clusters of new oocytes, commonly known as "egg nests" (Plate VII). Instead of one of the cells of Pfleuger's cord differentiating into an oocyte and the remainder becoming follicular cells, excessive estrogenic action apparently causes most of the cells to differentiate into oocytes and thus appear in clusters that are enclosed in a single follicle.

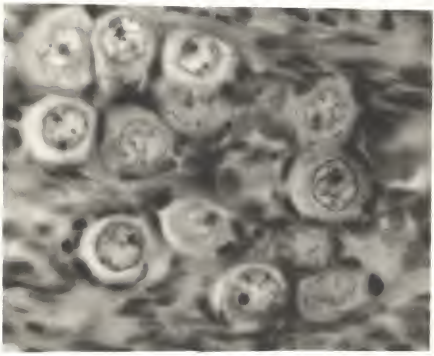
Essentially there is no difference in the actions of the estrogenic compounds in the dog, with the exception of the lack of sexual receptivity in the animals treated with diethylstilbesterol. An indirect supplying of excessive estrogenic compounds for prolonged periods caused progressive retrogressive changes in the large follicles of the ovary. These changes can be attributed to the dysfunction of the anterior pituitary with a resultant ovarian insufficiency.

The ovarian changes are apparently not of a permanent nature, as the animals that underwent unilateral ovariohysterectomy were placed under observation until normal estrus occurred three or more months after the time it would normally have occurred. This represents an actual time lag in efficiency of breeding animals. The economic losses incurred by an interruption of the normal estrus periods are insidious and are of considerable importance. It must be emphasized that ample evidence has been submitted to

EXPLANATION OF PLATE VII

Cluster of new oocytes or "egg nests" produced by treatment with estrogenic compounds. Many of these oocytes show synopsis (dog 16) and cannot be considered to be atretic (450x).

PLATE VII



prove that estrogenic compounds produce these retrogressive changes in the ovaries of domestic animals.

These estrogenic compounds, therefore, should not be used for the purpose of increasing the breeding efficiency of animals, but should be restricted to the accurately diagnosed cases of estrogen deficiency and ovarian insufficiency.

SUMMARY

Experiments were conducted on mature female dogs to determine the effect of various doses of diethylstilbesterol and E.C.P. on the estrus cycle and ovary of the dog. The results, based on vaginal smears, psychic reactions, macroscopic and microscopic examination of serially sectioned ovaries, revealed:

1. Progressive manifestations of estrus, except attraction and acceptance of the male in animals treated with adequate amounts of diethylstilbesterol.

2. Progressive manifestations of estrus (including acceptance accompanied by extreme pain and profuse hemorrhage during each coitus), in animals treated with E.C.P.

3. Both estrogenic compounds administered in adequate quantities to produce the manifestations of estrus caused a constant increase in stroma, decreased size of the ovaries, a suppression of the developing follicles, an atresia of the larger follicles and a proliferation of the germinal epithelium with a resultant increase in primary follicles and follicles with multiple ova.

4. Both compounds failed to produce the normal estrus necessary for successful fecundation.

Estrogenic compounds should be used only in cases of accurately diagnosed estrogen deficiency or ovarian insufficiency and not for the purposes of increased productivity in domestic animals.

ACKNOWLEDGMENT

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HISTOPHYSIOLOGICAL EFFECTS OF ESTROGENIC AND ESTROGENIC-LIKE COMPOUNDS ON THE ESTRUS CYCLE OF THE BITCH

by

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AN ABSTRACT OF A THESIS

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MASTER OF SCIENCE

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1954

Experiments were conducted to determine the effect of diethylstilbesterol and E.C.P. on the estrus cycle and ovaries of mature female dogs. This was accomplished to determine the feasability of the recommended use of these compounds in veterinary medicine to treat infertilities of unknown origin, bringing anestrus animals into estrus and procuring an extra litter of puppies each year.

The animals were placed at random into three groups. The control animals were considered to be normal as they accepted the male, conceived and living embryos were recovered, or the ovaries exhibited normal developing follicles during the anestrus stage. Fourteen animals received varying doses of diethylstilbesterol and 13 animals were subjected to various levels of E.C.P. The stage of each animal's estrus cycle was accurately determined by vaginal smear prior to any treatment.

Unilateral or bilateral ovariohysterectomys was performed prior to, or just after the normal or induced estrus periods. The ovaries were fixed in Bouin's, sectioned serially and stained with hematoxylin and losin for microscopic analysis.

The manifestations of estrus produced by the diethylstilbesterol were limited to vaginal cornification and swelling of the external genitalia. Coitus and ovulation did not occur. E.C.P. produced all the outward manifestations of estrus, including acceptance of the male. However, extreme pain and profuse hemorrhage regularly accompanied copulation. Microscopic analysis revealed:

1. A constant atrophy with an accompanying increase in connective tissue stroma.

2. Regression of developing follicles and an atresia of the maturing follicles.

3. Proliferation of the germinal epithelium and subsequent increase in primary and polyovular follicles.

4. Absence of maturing follicles and no opportunity for ovulation which is necessary for normal estrus and successful fecundation.

The use of the estrogenic compounds should be restricted to the accurately diagnosed estrogen deficiencies or ovarian insufficiencies, rather than being used promiscuously for treating infertilities of unknown origin or to attempt to increase the productivity of domestic animals.