UTERINE RESPONSE TO ESTROUS SYNCHRONIZATION IN SWINE USING ICI 33828 (MATCH)

by

Richard Franklin Marshall

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
MASTER OF SCIENCE

Major Subject: Veterinary Clinical Sciences

Approved:

Signatures have been redacted for privacy

Iowa State University
Of Science and Technology
Ames, Iowa

1967

1126-106

TABLE OF CONTENTS

INTRODUCTION

Demands for more profitable pork production procedures require that efficiency of reproduction be increased. In recent years great advances in the areas of nutrition, genetics and husbandry practices have occurred. Yet reproduction remains a relatively inefficient part of the overall program. The inefficiency of swine reproduction with respect to the number of ova produced by the female in comparison to the number of pigs marketed is a well-known and documented fact.

A practical method of estrous synchronization could greatly increase the efficiency of swine production. In swine production, as in any other area of the livestock industry, financial returns depend on operating efficiency. To have the farrowing, weaning and finishing facilities used to the best advantage a regular supply of pigs is essential. A system of controlling estrus and ovulation, free of adverse effects on fertility and litter size, may furnish a solution to some of the problems associated with a breeding program.

Estrous synchronization would facilitate the utilization of artificial insemination procedures for herd improvement. The problems associated with heat detection and providing a supply of boar semen would be confined to 3 or 4 days, rather than the random occurrence of estrus of unsynchronized females spreading over 18 to 23 days.

Farrowing of these synchronized animals over a shorter period would then allow the producer to more efficiently utilize his time. Control of certain neo-natal disease problems, such as baby pig scours, could more easily be achieved.

The effects of estrus synchronizing drugs on the uterus and ovaries during synchronization are not well understood. Preliminary studies using MATCH, which is one of the more promising compounds for this purpose, have been relatively satisfactory. It could be assumed by the casual observer that the uterine condition following use of this drug and prior to implantation is normal. There is evidence, however, as conception rates and litter size indicate (Gerrits and Johnson, 1964a), that some interference with normal implantation or embryo survival may result from MATCH synchronization. Very little is actually known about the changes that might be induced in the sow's uterus following synchronization, that might interfere with embryo survival and implantation. The following study was designed to determine the differences that might occur in the endometrium of synchronized sows as compared to controls when studied by histochemical, cytological and chemical methods.

LITERATURE REVIEW

Methods of Synchronization

Several methods of estrous synchronization have been studied in swine under field conditions. Extensive use of these procedures has not developed because they have been either unsatisfactory, impractical, or unavailable for commercial use. The methods include weaning of lactating sows, progestational compounds, gonadotropins, and non-steroidal pituitary inhibiting drugs such as MATCH.

Weaning of lactating sows

Simultaneous weaning of lactating sows is a generally accepted and utilized procedure for synchronization of estrus. Sows are usually in a state of anestrus during lactation, but generally exhibit estrus 3 to 7 days after the pigs are weaned (Cole and Cupps, 1959; Nalbandov, 1964). This method is not applicable to gilts but is generally used when possible for sows, especially when large groups are available in the same stage of lactation.

Progestational drugs

Progestational compounds have been used to synchronize estrus in swine. Effective suppression of estrus has been reported following the use of 6-methyl-17-acetoxy-progesterone acetate (MAP) at a dosage of 1.1 mg/kg of body weight (Hafez et al. 1966; Nellor et al. 1961). These results were observed irrespective of the stage of the cycle in which feeding was initiated. After withdrawal of the medication most of the animals returned

to estrus in 2 to 10 days, but only 1/3 of the animals ovulated after treatment. Cystic follicles of various types and sizes accounted for most of the ovulatory failures, one of the limitations of this method of synchronization.

The incidence of cystic ovaries and the length of time required to return to estrus after withdrawal of medication may be functions of the level of MAP fed. Animals fed 1.6 mg/kg of body weight manifested progestational inhibition from which the gilts did not readily rebound after treatment. At 0.5 mg/kg of body weight daily in twice a day feeding, estrus was controlled satisfactorily, with ovulation rate and number of fetuses recovered at 35 days essentially normal (Nellor et al. 1961). This would appear to be a satisfactory method when feeding is carefully carried out at 0.5 mg/kg of body weight. However, in practice it is difficult or impossible to regulate the dosage at a critical level under farm conditions. The exactness of dosage level that is necessary with this method is one of the limitations of synchronization with MAP.

Other progestational drugs have been tested and found to be ineffective for estrous synchronization. One of these is 17-alpha-acetoxyprogesterone (Prodox) which, used at levels of 0.9 - 2.0 mg/kg of body weight, did not effectively suppress estrus and ovulation (Nellor, 1960).

Gonadotropins

Injections of gonadotropins have been used for estrous synchronization in swine. This procedure consists of a single intramuscular injection of 1200 I.U. of pregnant mare serum (PMS), followed three days later by a

single intramuscular injection of 1000 I.U. of human chorinoic gonadotropin (HCG). The result of this treatment is the concurrent induction of a new cycle for all animals treated. The life span of the induced corpora lutea is relatively constant, so that as their influence on the estrous cycle regresses the animals tend to recycle more or less simultaneously. The interval between the last gonadotropin injection and the synchronized estrus would be expected to be approximately 21 days, varying from 18 to 23 days. Ninety to ninety-five percent of the synchronized animals exhibit estrus within a 4 to 5 day period. Animals injected at a time when the natural onset of estrus is near will exhibit estrus shortly after injection, and will then return to estrus approximately 21 days later. If the injections are made in the luteal phase of the cycle, induced corpora lutea will be formed which will then exist for the normal 14 to 15 days. In those animals with functional corpora lutea already present, estrus will not be seen immediately after injections (Day et al. 1965). The use of HCG following the PMS injection appears to be an essential part of this procedure. Pregnant mare serum alone will stimulate the development of follicles irrespective of the stage of the cycle. Without the luteinizing hormone 36 - 50 percent of the treated animals failed to show estrus within 18 to 23 days of the PMS injection, because of the failure to form induced corpora lutea (Day et al. 1965).

Non-steroid pituitary inhibitor (MATCH)

A non-steroid pituitary inhibitor has been used experimentally to produce synchronization of estrus in swine. This compound is 1-alpha-

methylallylthiocarbamoyl-2-methylthiocarbamoylhydrazine, also referred to as ICI 33828 or MATCH.[1]

This compound used orally at approximately 100 mg/gilt per day has produced effective blockage of estrus and ovulation. The animals generally return to estrus on the 4th to 10th day after withdrawal, with the highest percentage exhibiting estrus on the 5th, 6th, and 7th days. The number of ova shed, fertilization, and cleavage with this method has been reported to be essentially normal (Gerrits and Johnson, 1965a; Gerrits et al. 1966a; Gerrits and Johnson, 1965b; Stratman and First, 1965; Polge, 1965; Hafez et al. 1966; and Polge, 1964). According to these reports the use of MATCH does not produce any adverse effects on embryo survival. However, one reference written on trials of 8 gilts fed 149 mg/head/day reported that the compound may have had an adverse effect on embryo survival (Gerrits and Johnson, 1964a).

Reports in the literature indicate that the effects of MATCH on the current cycle of experimental sows was dependent upon the stage of the cycle when treatment was initiated. Most of the animals in the mid-luteal or early follicular stage had synchronized ovulation after the termination of treatment. If feeding was initiated late in the follicular phase (as day 19 or 20 of the normal cycle) most of the animals exhibited estrus at the expected time, in spite of the medication. Medication started in the early luteal phase had little effect on the corpora lutea and they continued to function normally until regression on the 15th to 16th day of

---

[1]Ayerst Laboratory, New York 17, New York.

the cycle (Polge, 1965). The reason for the medication not interfering with the activity of formed corpora lutea is probably due to the fact that corpora lutea, once formed, do not depend on a pituitary stimulus for maintainence (du Mesnil du Buisson and Leglise 1963; Sammelwitz et al. 1961; Brinkley et al. 1964a; Brinkley et al. 1964b).

The recommended dosage of MATCH is approximately 100 mg/head/day for the average gilt (240-300 lbs.). Treatment should be maintained for 19 days to ensure synchronization of all the animals. It appears advisable to restrict feed intake to 4½ pounds/head/day to insure proper intake of medication (Groves, 1965).

General consideration of MATCH

The first report of the pituitary gonadotropin inhibition by MATCH was in 1961 (Paget et al. 1961). This work involved the usage of several dithiocarbamoylhydrazines in rats of which MATCH was the more active. The drug may be administered either orally or parenterally, although for practical reasons it has generally been administered orally to swine.

Mechanism of action

The exact mechanism of action of MATCH is not precisely known, but it seems to have its effect by blocking the release of gonadotropins from the pituitary. Unilateral ovariectomy will result in compensatory hypertrophy of the remaining ovary in the normal rat. Injections of MATCH will block this compensatory hypertrophy. Whether this action is specific for blockage of FSH or LH, or both, has not yet been determined (Edgren and

Peterson, 1964). MATCH injected subcutaneously into intact rats, usually caused a reduction in the weight of the uterus and occasionally the gonads. Assays of the pituitary tissue show that MATCH causes a reduction in the content of FSH, suggesting the action is one of reducing the formation of this pituitary gonadotropin (Brown, 1963a).

Laporatomies on three gilts receiving MATCH for 15 days at the level of 100 mg/head daily, revealed that the ovaries were similar in all animals, each ovary containing a few small (2 - 4 mm) follicles. The corpora lutea were pale and had regressed to about 5 mm in diameter. The uteri were small and resembled those found in prepubertal animals. The appearance of the ovaries of the treated animals suggested that the release of gonadotropins from the pituitary was almost completely inhibited. After withdrawal of the treatment, however, follicular development appeared to be initiated quickly. The fact that 90 percent of the gilts were in heat by the seventh day indicates that the return of pituitary function was rapid. In the normal cycling gilt, regression of the corpora lutea begins on about the 15th day of the cycle, and follicular maturation leading to the next estrus requires about 5 to 6 days. A close correlation existed between the interval from corpora lutea regression to the onset of estrus in the normal cycle, and the interval from withdrawal of MATCH to synchronized estrus (Polge, 1965).

Rats given MATCH during early pregnancy (at 100 mg/kg/day for 3 days) do not achieve normal implantation (Harper, 1964). The effect on implantation in rats was shown to be a depression of deciduomata formation, but was observed only when the compound was given at the initial stages

of pregnancy and in relatively high doses. Since the compound inhibits pituitary gonadotropin secretion, as earlier indicated, the production of normal ovarian steroids would be altered. The effects on fertility may thus be mediated by causing an imbalance in the progesterone:estrogen ratio or level, essential for maintaining pregnancy.

When MATCH was fed to laying hens, the blood calcium levels dropped to normal for a chicken before puberty. When exogenous estradiol was administered the blood calcium levels again increased to normal. This indicates that MATCH reduced the blood calcium level by reducing the level of estrogen formation (Sykes, 1964).

MATCH fed to pregnant mares did not appear to affect the serum levels of gonadotropins. It, therefore, could be assumed that MATCH had little effect on placental gonadotropin production in comparison to the decreased pituitary gonadotropin release (Schmidt-Elmendorff et al. 1962).

Possible side effects

The action of MATCH on the pituitary gland may not be limited to the effect seen on the gonadotropins. Some side reactions have been observed during the use of MATCH in addition to the reproductive effects. Gilts given 50, 100, or 200 mg/head/day orally showed few abnormal clinical signs. At 250 mg/day a red skin rash was reported. No apparent effect was noticed in the animal's appetites, however, the water consumption of these animals may have been increased (Polge, 1965). Lethargy and decreased activity were also observed.

MATCH given to immature hypophysectomized female rats caused an in-

hibition in growth of the tibial epiphysial cartilage. When administered with beef growth hormone, the degree of inhibition was reduced according to the amount of MATCH given. These results indicate that the MATCH is capable of affecting growth, but whether this action is a general one or an indirect action mediated through another gland, such as the adrenal, is not clear (Cargill-Thompson, 1963).

The effects of treatment on thyroid activity in humans have been studied. The drug reduced both the radioactive iodine uptake by the thyroid and the level of serum protein bound iodine in euthyroid patients. This action may be a general one or may be affecting the pituitary thyrotropic activity (Brown et al. 1963b; Tulloch et al. 1963). These reports are interesting because they call attention to the possibility that some of the action of MATCH may be through a more general action on the body rather than entirely by the effects on the pituitary gland.

Urine excretion in hens fed MATCH is increased. Continuous administration of the medication is required for this effect. A single intravenous injection of the compound will not produce the diuretic action. This tends to indicate that the diuretic action of MATCH is not directly on the kidney, but possibly by blocking the release of antidiuretic hormone from the posterior pituitary (Sykes, 1964). Gilts fed MATCH consumed more than the usual amounts of water. This diuretic effect may be involved in this change (Polge, 1965).

The effect of the compound on milk secretion has been reported in rats (Zagni and Benson, 1964) and sows (Gerrits et al. 1965). Milk production, as evaluated by litter growth, was decreased in both species

when the lactating dams were given MATCH. Production was returned to normal when exogeneous oxytocin was administered. These facts lead the authors to conclude that MATCH did not suppress lactogenic activity, but did inhibit the milk ejection reflex.

Normal Changes in Uterine Cycle

The uterus undergoes certain morphological and biochemical alterations during the estrous cycle. These changes establish definite cyclic patterns and recur with each normal cycle.

Morphological changes

The morphological changes which occur in the cycle include changes in endometrial cell height, frequency of mitosis in the endometrial cells, edema, and leucocyte number and type in the submucosa. The general structure of the uterus consists of a lining epithelium supported by a stroma of connective tissue. This stroma is surrounded by an inner circular and outer longitudinal layer of smooth muscle. The epithelium is basically simple columnar with the appearance varying greatly in different stages of the cycle. The connective tissue is areolar in nature and contains many simple tubular glands, formed by epithelium which is continuous with the epithelium lining of the lumen of the organ. Also present in the submucosa are blood and lymph vessels, various types of leucocytes and fibroblasts (Corner, 1921).

Cell height The greatest and most rapid changes in the cell height occur in the first 6 to 7 days of the cycle. At estrus the uterine epithelium is approximately 30 microns (μ) thick. It is pseudostratified colum-

nar because the cells are of only moderate height and very compact laterally, making the nuclei appear stratified. In the period of 5 to 6 days after ovulation the cell height increases to approximately 50 μ and the cells become more simple high columnar in appearance. In the period of the 6th to 7th day of the cycle (day 1 being the first day of standing heat) the overall cell height becomes rather variable and decreases, ranging from 20 - 45 μ . The surface profile is very irregular and wavy, giving the impression of an actively secreting epithelium. From 8 to 15 days of the cycle the epithelium becomes progressively more uniform and lower. The height will decrease to approximately 15 - 20 μ. The luminal surfaces will be very frayed and serrated, which may represent cellular cytoplasm being secreted into the lumen. Changes appearing after 15 days of the cycle depend on whether or not pregnancy occurs. If no pregnancy occurs, the cell height will remain low until about the 18th day of the cycle, then start to increase in height again, so that by the next estrus it will again be approximately 30 μ thick. If a pregnancy does result, the increase in cell height at day 18 does not occur. The endometrial cells will remain low throughout pregnancy, in fact the cells may become even lower (Corner, 1921; McKenzie, 1926; Green 1950). The glandular epithelium follows the same trends established by the surface cells, but without as wide a range of variations, and generally about 24 to 48 hours later in the sequence of events (Corner, 1921).

The following list of average cells heights represents the day by day cyclic changes in the uterine surface epithelium (Green, 1950).

Day	Cell Height	Day	Cell Height
1	34.3 microns	11	23.4 microns
2	33.1	12	18.5
3	29.3	13	20.0
4	37.1	14	16.8
5	46.6	15	17.1
6	41.6	16	17.8
7	41.7	17	22.8
8	27.7	18	30.0
9	26.3	19	34.6
10	21.1	20	22.4

The decrease in cell height on the 3rd day was attributed to a decrease in ovarian stimulation on the uterus, the follicles having ruptured and the corpora lutea not being developed to the stage of maximum functional capacity (Green, 1950).

The figures mentioned in the previous list represents the average cell height. The range of heights in a given individual is rather variable. One sow on the 10th day of the cycle ranged from 8 - 21 μ, while an animal in the 4th day of the cycle varied between 36 - 63 μ (McKenzie, 1926).

The epithelium of the uterus of a castrated sow was low columnar in nature, about 18 μ high. A castrate given exogenous estrogen was psuedo-stratified columnar and about 40 μ high. A castrate given estrogen followed by progesterone was found to resemble a normal animal in the 12th to 13th day of the cycle (Green, 1950).

Mitosis Mitotic figures are very numerous in the surface epithelium 1 to 2 days prior to, during, and for 3 to 4 days following estrus. Mitosis gradually disappears after estrus, so that by 5 to 6 days after

estrus it is essentially absent, not to be seen again until just prior to the next estrus. Mitosis in the glandular cells is 2 to 3 days later than in the surface cells, similiar to the sequence described for epithelial height. Mitosis will start to reappear on about the 18th to 19th day of the cycle in preparation for the oncoming estrus, if no pregnancy occurs. If pregnancy does occur, it will delay the onset of mitosis until just prior to the first post-partum estrus (Corner, 1921).

Leucocytes Leucocytes of all types are normally found in the submucosa. The relative number and the predominant type tend to vary at different stages of the cycle. At estrus large numbers of leucocytes, especially neutrophilic polymorphonuclear leucocytes, appear in the submucosa. Most of these will be located in the region of the epithelial cell basement membrane, with a few being scattered in the rest of the submucosa and some invading the epithelial layer. These neutrophils decrease greatly in number in the week just after ovulation. Eosinophils, which are almost always present to some degree, increase extensively in the basement membrane area at about 8 to 10 days of the cycle. The eosinophils do not invade the epithelium as do the neutrophils. The eosinophils remain in the submucosa in large numbers till approximately the 14th to 15th day of the cycle, when they decrease in number (Corner, 1921).

Edema in the submucosa At estrus the submucosa becomes very edematous so that the cells and fibers in the stroma appear to be widely separated. After estrus, the amount of edema steadily decreases so that by 2 to 3 days after ovulation the appearance of the connective tissue is

normal. The presence of edema will not be seen again in the normal animal until 1 to 2 days prior to the onset of the next estrus, at which time, the fluid accumulation between the cells and fibers will again produce the edematous effect (Corner, 1921). In the castrated animal the submucosa will be very dense and compact; the castrate given exogenous estrogen has a very edematous submucosa. The castrate given exogenous estrogen followed by progesterone will have a submucosa resembling that of a normal animal 12 to 13 days in a cycle (Green, 1950).

Biochemical changes

Many biochemical changes occur in a cyclic pattern in the uterus. These cyclic variations are undoubtedly due to the influence of the ovarian hormones. Two of these biochemical changes are alkaline phosphatase and glycogen.

Alkaline phosphatase The alkaline phosphatase level in swine increases when the uterus is under the influence of estrogen and decreases as the progesterone influence occurs (Austad and Garm, 1959).

The quantity of alkaline phosphatase present in the endometrium is highest at about 2 to 3 days of the cycle, reaching a maximum of about 9 Sigma Units[2] activity per gm. tissue per hour. By the 8th day of the cycle the level has decreased to 7 activity, on day 12 the level is 1.0 - 1.5 Sigma Units. Shortly before estrus, probably sometime in the 20th day of the cycle, the enzyme level starts to increase again from the low of the 19th day, so that by the 2nd to 3rd day of the next cycle the level

---

[2]Units as defined by Sigma Chemical Co., St. Louis.

is again approximately 9 Sigma Units. If pregnancy occurs, the alkaline phosphatase level continues to decrease, reaching a low of 0.5 - 1.0 Sigma Units activity on approximately the 25th day (Goode et al. 1965a).

Acid phosphatase also appears in a cyclic pattern in the endometrium. It occurs in the highest concentration when the alkaline phosphatase levels are the lowest. As the level of alkaline phosphatase increases, the acid phosphatase level decreases so that essentially an inverse relationship exists (Goode et al. 1965b).

Glycogen The occurrence of glycogen in the surface and glandular endometrium of swine has been the subject for some disagreement in the past. Several authors have reported that no glycogen could be demonstrated in the endometrium (Corner, 1921; Wislocki and Dempsey, 1946). Others have reported the occurrence of glycogen in the uterine musculature and epithelium, both surface and glandular. The glycogen was described as occurring with cyclic regularity. During estrus and for the first few days following estrus, there was no evidence of glycogen being present. The first histochemical appearance was on approximately the 9th day of the cycle (Austad and Garm, 1959). It first appeared in the cells of the deep uterine glands; the concentration gradually increased in the glands till reaching maximum concentrations on days 15 to 16 of the cycle. The appearance of glycogen in the surface cells did not occur until about the 12th day of the cycle. If no pregnancy results, the glycogen content will start to decrease on the 16th to 17th day of the cycle and will be absent by the onset of the next estrus. If pregnancy does occur, the glycogen content of the uterine or chorionic cells will be maintained for

50 to 60 days, then gradually decrease (Lovell and Garm, 1964).

Significance of Glycogen and Phosphatase

The significance of glycogen and alkaline phosphatase in uterine tissue is not well known.

Glycogen

Glycogen is the major energy and carbohydrate reserve of the body, being stored in the liver, muscle, uterus, and other body tissues. When glucose is introduced into the cell, it can be utilized immediately for energy or, in the absence of energy demands, can be stored as glycogen. The formation of this large molecule allows the cell to hold relatively large amounts of potential energy without significantly affecting the intracellular osmotic pressure (Cantarow and Shepartz, 1957). Cells with glycogen storage potential, will accumulate only a given amount. The glucose entering the cell beyond this level, and not utilized for energy, is converted to fatty acids and stored as triglycerides (Stacey, 1962).

The occurrence of glycogen in the uterus has been shown in the rat (Padykula and Richardson, 1963), bovine (Marinov, 1966), and human (Ville, 1953b) as well as swine. Bernard as early as 1859 pointed out the possibility of the placenta acting as a fetal liver in the synthesis of glycogen (Bernard, 1859). The exact function of glycogen in the placental tissues has yet to be proven. It is difficult to understand the need for placental glycogen stores since the glucose in the maternal blood is presumably available for transport across the placenta to the fetus at

all times. It is possible that this placental glycogen may exist as a food reserve, to be utilized by the embryo in the event that the maternal blood-glucose level drops precariously low (Padykula and Richardson, 1963). The placenta can produce glucose from pyruvates and store glycogen early in pregnancy, during this period the fetal liver is incapable of this function. Therefore, it is possible that the placenta acts to regulate the fetal blood glucose levels until the fetal liver is capable of assuming this responsibility (Ville, 1953b).

The synthesis of glycogen in the cell is an enzymatic process, with several possible pathways. The phosphorylases catalyze the conversion of glucose-1-phosphate into a 1:4 linked glucopyranase structure with approximately 200 glucose units in straight chains. This reaction does not occur in cells which have no glycogen present, therefore, it is believed that this method of glucose polymerization achieves the lengthening of the side chains of the glycogen molecule (Takeuchi and Kuriaki, 1955). The phosphorylases are also responsible for the initial degradation of glycogen. This enzyme causes phosphorolysis of the 1:4 linkages of the straight chains, but would not affect 1:6 branching, if the proper glucose:glycogen equilibrium exists (Cori and Cori, 1943). Phosphorylases in the presence of amylo-(1:4 - 1:6) transglucosidase converts glucose-1-phosphate into a branched polysaccharide like glycogen. The straight chain linkages of glucose molecules in glycogen are 1:4, while the branching results from 1:6 glucose linkages (Cori and Larner, 1951). Uridine diphosphoglucose-glycogen transferase is another enzyme which transfers glucose from the uridine diphosphoglucose molecule to a larger

molecule, as glycogen (Hall, 1965).

The effects of hormones on tissue glycogen can be manifest in several ways. Glucagon raises blood glucose levels by increasing the amount of glycogenolytic phosphorylase in the liver (Rall et al. 1957). Epinephrine induces glycogenolysis in a similiar fashion (Stacey, 1962). Estrogens have a stimulatory effect on uridine diphosphoglucose-glycogen transferase activity, while progesterone inhibits the action of this enzyme. Relaxin has the effect of reducing this action of progesterone inhibition of uridine diphoglucose-glycogen transferase activity in the uterus. This activity in mouse uterine musculature varies between the inner circular and outer longitudinal layers (Hall, 1960; Hall, 1965). In the ovariectomized rabbit uterus the occurrence of the uridine diphosphoglucose-glycogen transferase could not be demonstrated. It was present, however, in the tongue muscle of these animals. Different pathways of glycogen synthesis must exist between smooth and skeletal muscle, since the presence of this enzyme varies between tissues in the same animal (Bo and Smith, 1964).

The substance commonly identified as glycogen in the Periodic acid-Schiff histochemical analysis for glycogen has been identified as a highly branched, ramified polysaccharide with a structure like that generally accepted for glycogen (Kugler and Wilkinson, 1962). Differentiating glycogen from other PAS-positive substances is accomplished by the digestion of the glycogen in control slides with amylase (Stacey, 1962). The size of the glycogen particles in the rat placenta range from 300 Å to 1
(Padykula and Richardson, 1963).

Alkaline phosphatase

The alkaline phosphatases are a group of enzymes occurring in many tissues of the body, as the intestinal mucosa, renal tubules, ovarian follicles, and the mucosa-submucosa of the uterus. There is a strong likelihood that they are employed in the active transfer of organic substances across cell membranes. The alkaline phosphatases are most commonly found in cells of tissues which are growing, regenerating, secreting, or absorbing. The enzymes are important components of cells that are engaged in rapid synthesis of protein (Moog, 1946). Phosphatase activity is often associated with active solute exchange across the walls of capillaries, as in the placental transfer of solutes in swine. It is most probable that alkaline phosphatase plays an important role in the transfer of certain substances from maternal blood to fetal blood (Bradfield, 1950; Borghese, 1957). Alkaline phosphatase is not necessary for glycogenesis or glycogenolysis, but may be involved in the transfer of the metabolites essential for these processes (Atkinson and Elftman, 1947). The occurrence of alkaline phosphatase in the uterus of swine is cyclic in nature. The highest levels of activity are achieved during and immediately after estrus, followed by a steady decrease to much lower levels during the remainder of the cycle or pregnancy. The cause of the increased activity of these enzymes at the time of estrus is due to estrogen stimulation of the uterus (Goode et al. 1965a; Atkinson and Elftman, 1947; Ui and Mueller, 1956; Leathem, 1959; Steplewski and Jonek, 1965; Watanabe and Fishman, 1964). There is a direct ratio between the level of estrogen stimulation of the uterus and uterine alka-

line phosphatase activity in the mouse. This correlation suggests that activity is a part of the mechanism by which estrogen influences uterine metabolism (Atkinson and Elftman, 1947). Estrogen stimulates nucleic acid production so that the formation of intracellular enzymes is increased. Estradiol in the uterus stimulates the synthesis of certain enzymes necessary for formation of new RNA. This newly formed RNA in turn supplies the information to the ribosomes which is necessary to produce the initial hormone response (Ui and Mueller, 1956). Uterine RNA, extracted from uterine cells which were under estrogen stimulation, has been shown to evoke the estrogenic effect in untreated ovariectomized rats, with the uterus resuming its normal appearance with increasing levels of alkaline phosphatase activity (Mansour and Niu, 1965). RNA of liver cell origin from the same animals did not have this uterine stimulatory activity. The effect of estradiol on cell metabolism may be blocked by use of certain protein inhibitors, as actinomycin. Estrogen induced vaginal cornification in ovariectomized mice is blocked by intravaginal application of actinomycin D, while the uterotropic action of the estrogen is not affected. This suggests that the ability of a stimulatory hormone, as estrogen, to produce biological effects on their target organs is characterized by their ability to influence the synthesis of messenger RNA (Talwar and Segal, 1963).

Progesterone and testosterone do not induce alkaline phosphatase increases in the uterus as does estrogen (Leathem, 1959). An increase of estrogen production, induced by gonadotropin injection, in a hypothyroid mouse, causes a significant increase in uterine glycogen and small in-

creases in the amount of uterine water and protein, but the alkaline phosphatase level decreased. This does not fit the pattern considered normal for estrogen stimulation (Leathem, 1959). This effect of hypothyroidism on the uterine response to estrogen is difficult to explain in light of the present knowledge.

A significant negative correlation exists between the level of alkaline phosphatase and the number of live embryos at 25 days of gestation (Goode et al. 1965b). A positive correlation exists between the total uterine phosphatase activity (both acid and alkaline) and the size and weight of the litter in swine. Most of this activity during pregnancy is due to acid phosphatase, while the level of alkaline phosphatase has decreased (Morrissette et al. 1963). The alkaline phosphatase levels are low during pregnancy, but the presence of this enzyme does not fail to exist during this time in the maternal and fetal capillary endothelium (Borghese, 1957). A statistically sound, negative correlation between alkaline phosphatase content of the uterus and the number of implantations occurs in the rat on the 13th day of pregnancy (Bredeck and Mayer, 1955).

Uterus and Ovary Synchronization

Proper synchronization of the uterus and ovary is essential for normal embryo survival. Thirty to forty percent of the fertilized ova in swine do not survive until the normal termination of pregnancy (Wilson et al. 1949; Warnick et al. 1948; Warnick et al. 1949; Wiggins et al. 1948). Eighty percent of these die in the early stages of gestation (Morrissette et al. 1963; Goode et al. 1965a). The reason for this high percentage of

embryonic deaths is not clearly understood. In gilts tubular aberrations, blind, or missing parts of the uterine tract, or infantile uteri may not provide adequate space for all of the fertilized ova to implant normally (Wilson et al. 1949). Alterations in the physiological intrauterine environment under hormonal influence may be a factor in early embryonic death (Morrissette, 1963).

The fertilized ova and the developing blastocyst in swine are initially nourished by secretions in the oviduct and uterus. The tubular secretions, containing mucoprotein, glycogen, and phospholipids, are highest in the estrogen phase of the cycle. The ova require 3 to 3½ days to pass through the oviduct, during which time the tubular secretions are highest (Cole and Cupps, 1959). The uterine secretions, from both the surface and glandular epithelium, are composed predominately of cellular debris, including cell nuclei, and a few leucocytes. The maximum production of uterine milk occurs a few days after estrus, when the uterus is primarily influenced by progesterone. The uterine secretions are especially copious in animals with epithio-chorial type of placentation, as in swine. The uterine milk of sows probably is the source of nutrition for the developing blastocyst until implantation, and is possibly a significant nutritional factor throughout gestation.

The placenta is the region across which exchanges of nutritive and excretory products occurs between the fetal and maternal tissues. Histologically the placenta constitutes the region in which fetal and maternal circulation are in close proximity to one another (Dempsey, 1960). The placenta has some of the characteristics of the liver, intestinal

mucosa, lung, kidney, and of an endocrine organ. Many substances are present in higher concentrations in the fetal blood than in the maternal blood. These substances, such as electrolytes, fats, and nucleic acids, cannot be transferred by simple diffusion against a higher concentration gradient, but must be transferred by some active transfer process not yet completely known (Ville, 1960). The placenta has a fairly high rate of metabolism as judged by its rate of utilization of oxygen and organic substances (Ville, 1953a).

Implantation of the fetus or placentation requires that the endometrium be hormonally conditioned to a receptive state. The receptivity of the uterus in the rat depends on a progestational influence for at least 48 hours, with the addition of estrogen late in the fourth day of the cycle. This sequence produces a receptive state of the uterus on the fifth day. If either the progesterone preparation is not sufficient or the estrogen intervention is delayed, implantation will not occur. The receptive condition of the uterus is maintained for about 12 hours, after which the uterus again becomes non-receptive. Five-day-old fertilized ova, transplanted into uterine horns conditioned to the 5th day of the cycle will implant normally; if the horns are prepared as on the 3rd or 4th day of the cycle, implantation is delayed and embryonic death may result. Transplanting into uteri beyond the 5th day resulted in no implantation. In ovariectomized rats low levels of estrone given with progesterone increased the number of implantation sites, but as the level of estrone given with progesterone increased, the number of implantation sites decreased (Psychoyos, A., 1966). It has been postulated that on

the afternoon of the 5th day in rats the uterine environment changes, becoming detrimental to younger ova, but stimulating the 5 day ova in such a way that it elicits the decidual reaction and begins the process of implantation (Dickmann and Noyes, 1960).

The chances for survival of transplanted ova in rats (Noyes and Dickmann, 1960), and sheep (Averill and Rowson, 1958) depend not only on synchronization of the ova and uterus, but on the age of the ova. Ova transplanted in rats at the age of two days, even to a uterus in the 2nd day of the cycle, will not survive. Survival is best with 5 day transplants and success of the procedure decreases as time of transplantation varies either before or after this optimal time.

There is evidence in sheep to indicate that the exactness of ova-uterus synchronization is not quite as critical as in rats (Averill and Rowson, 1958). While the ova-uterus relationship is not quite as essential in sheep as rats, it still affects the percentage of success of ova transfer. Success of the ova transplant in sheep increases as the estrus of the donor ewe and recipient ewe more closely coincide.

Ova transplants in swine are accomplished by using ova recipients and donors that randomly cycle simultaneously (Hancock and Hovell, 1962) or have cycled simultaneously due to synchronization of the estrus period (Vincent et al. 1964; Dziuk et al. 1964). The amount of work done in ova transplants in swine is not as extensive as in some other species. Knowledge of the timing and coordination of the uterus and the developing conceptus that is required for normal implantation is inadequate because of this lack of specific information concerning swine.

MATERIALS AND METHODS

The tissue specimens used in the histological, biochemical, and histochemical evaluations of the uterus were collected from 24 experimental animals at necropsy. The collections were made from 18 gilts treated with MATCH for synchronization of estrus, and 6 controls.

Experimental Animals

The animals used were gilts that had reached puberty and were 7 to 10 months of age. All of the animals were examined daily, with the aid of a normal boar, previous to the treatment period in order to determine the estrous cycles. This was done by turning three sows at a time with the boar in a large outside pen. Observations as to general and sexual behavior were made and daily records kept. This pretreatment history, accompanied by breed, age and weight at slaughter is given in the following list:

Sow No.	Breed	Age at Necropsy (in days)	Weight (pounds)	No. of recorded cycles	Length of cycles
		Synchronized animals			
1	Poland China	253	278	2	19,21
2	Landrace	254	277	2	22,26
3	Yorkshire	277	294	3	23,20,22
4	Landrace	277	308	2	21,20
5	Yorkshire	259	271	2	23,20
6	Yorkshire	261	267	3	21,20,19
7	Yorkshire	283	315	2	28,21
8	Hampshire	293	306	2	23,20
9	Yorkshire	271	300	3	18,24,17
10	Yorkshire	268	311	1	20
11	Yorkshire	270	291	2	18,20
12	Landrace	271	285	2	20,22
13	Yorkshire	278	267	3	23,18,20
14	Poland China	275	304	2	23,21
15	Yorkshire	305	331	2	19,25

Sow No.	Breed	Age at Necropsy (in days)	Weight (pounds)	No. of recorded cycles	Length of cycles
(Cont.)		Synchronized animals			
16	Poland China	285	294	3	21,20,20
17	Landrace	296	313	3	23,13,22
18	Yorkshire	301	309	2	26,21
		Control animals			
19	Duroc	220	196	1	18
20	Duroc	253	252	2	20,20
21	Duroc	258	262	2	23,22
22	Poland China	276	275	3	21,19,20
23	Duroc	282	309	3	24,22,18
24	Duroc	274	296	2	25,21

Experimental Design

The animals were divided into two groups. One group of 18 was fed MATCH and another group of 6 was used as controls. The group of controls was made up of the younger individuals. Animals used for feeding MATCH all had experienced at least 2 definite standing heats, most of them having had 3 or 4. At the time feeding was started the control animals had experienced only one definite standing heat. It was thought advisable to select only animals that had demonstrated definite cycles with 2 or more heat periods for the synchronization trial. Using the younger animals as controls allowed additional time to observe the length of their cycles.

Before feeding was initiated a predetermined schedule of slaughter was established and each of the 24 gilts was randomly assigned a position in the schedule. Six of the animals were necropsied during the synchro-

nization process, 6 were sacrificed within 9 days after completion of the treatment period, during which time the synchronized estrus occurred. The last 6 were bred artificially and slaughtered 4, 14, and 20 days after the synchronized estrus. The controls were slaughtered at various time intervals in the estrous cycle to gather baseline information on the unsynchronized estrus periods.

MATCH was selected to synchronize the animals because it appeared to be the most satisfactory method of synchronization available at this time. The feeding of MATCH was carried out for the recommended period of 19 days. The dosage was 180 mg per gilt per day. Administration was accomplished by feeding the daily dose of MATCH in 1½ pounds of a balanced corn-soya ration each morning. The appropriate amount of MATCH, required for the number of gilts to be fed, was vigorously mixed for 10 minutes with the required amount of feed with a mechanical feed mixer each morning. The batch was then divided into individual feedings of 1½ pounds per gilt. An additional 1½ pounds of plain corn-soya ration was given to each gilt at a late afternoon feeding. A feeding level of three pounds of feed per day was selected to insure the proper intake of MATCH by each animal. In spite of this it was necessary in some cases to decrease the total daily feed intake to 1½ pounds because of anorexia which developed during the feeding period. Each animal was fed individually to insure that the gilts consumed only the feed intended for them. Watering of the animals was accomplished by automatic waters, making it impossible to evaluate water consumption.

The animals were artifically inseminated on each day of standing synchronized estrus. The semen was collected from the same boars used to determine the occurrence of standing estrus. Microscopic examination indicated the semen used was of normal concentration and motility. On the first day of insemination it was necessary to dilute the ejaculate with milk diluent in order to have sufficient volume. Enough semen was collected on the other days to inseminate all the animals to be bred without dilution. Fifty to seventy-five ml was used as the insemination volume. Because of the fact that the synchronized heats were prolonged most of the MATCH fed animals were inseminated on four or more successive days. The control animals were inseminated on 2 to 3 days of estrus.

On the 18th day of the treatment period blood samples were taken at random from 6 gilts to determine the total red and white cell counts, clotting time, white cell differential and hematocrit in an effort to determine any hematological alterations caused by the product.

Necropsy procedures

The animals were electrocuted at the predetermined times and the genital organs were removed as soon as possible after death. Immediately after weighing, the uterus was placed on ice until the specimens were processed and frozen so as to reduce the amount of alkaline phosphatase degradation. Sections of each uterine horn were taken near the tip and body for histological study. The pituitary, thyroid, adrenals, and ovaries were weighed and preserved for histological study.

Histological techniques

The tissues taken for histological evaluation were placed in 2 types

of preservative. Ten percent neutral formalin was used to preserve the tissues for Hematoxylin-eosin staining and the PAS histochemistry. Tissues were also placed in chilled 80 percent alcohol to preserve for the alkaline phosphatase histochemistry.

Hematoxylin-eosin The staining procedure outlined in the Armed Forces Institute of Pathology Laboratory Manual (Armed Forces Inst. of Pathol., 1960) was used for this stain. Sections stained with this procedure were used in evaluating the endometrial morphology.

Wright's stain The leucocyte differential count was obtained from blood smears stained by Wright's stain (Lillie, 1954).

Histochemical techniques

Alkaline phosphatase Demonstration of alkaline phosphatase was accomplished by using the procedure outlined by Gomori (Gomori, 1964). The procedure involves the principle that the enzyme in the tissue, incubated with glycerophosphate in the substrate, will liberate phosphate ions. These phosphate ions, when treated with cobalt nitrate and ammonium sulfide, produce the black precipitate of cobalt sulfide. This precipitate, seen microscopically, indicates the degree of action of the enzyme.

Periodic acid-Schiff (PAS) The glycogen was demonstrated histochemically by the procedure outlined by McManus and Mowry, using the Periodic acid-Schiff method (McManus and Mowry, 1965). The reaction is based on the fact that aqueous periodic acid will split and oxidize the carbon units of glycogen to aldehydes which are colored magenta or pink by the Schiff's reagent.

Biochemical technique

Precautions were taken at each step of the procedure to keep the tissues as cool as possible to keep enzymatic degradation at a minimum. The specimens collected for biochemical analysis were obtained by opening the horns of the uteri. After the uteri were opened, the endometrium and some of the submucosa were separated from the muscularis by use of curved scissors. This procedure was previously evaluated by using tissues collected from a packing house. The endometrium was separated from these uteri and examined with light microscopy with the finding of little or no smooth muscle mixed with the endometrium. The separated endometrium was placed in a Serval homogenizer and homogenized for 4 minutes, then placed in a Ten-Broeck glass homogenizer for the final homogenation. This homogenate was put into 2 gm glass vials, frozen in liquid nitrogen, and stored at -43°C until all the samples were collected and could be quantitated simutaneously. The maximum time lapse between electrocution of the animal and freezing of the tissue was 28 minutes, with a minimum of 21 minutes.

Alkaline phosphatase The levels of this group of enzymes were determined by placing 500 mg of endometrium in 10 ml cold water, blending for 2 minutes, and centrifuging lightly to pack the cellular debris (Goode et al. 1965a). A quantity of this solution (0.1 ml) was incubated in the appropriately buffered nitrophenyl phosphate substrate solution and the optical density determined at the recommended colorimeteric wave length of 410 mu. The substrate and procedures for the determination are those recommended by Sigma Chemical Co. (Sigma Chem. Co., 1961). Using the

calibration curve recommended by Sigma, the results are expressed as Sigma units, or the enzymatic activity per 10 mg tissue per hour.

Glycogen Glycogen levels were determined by separating the glycogen from the homogenate by use of alcohol precipitation (Good et al. 1933). The glycogen was then hydrolyzed to glucose with 2N H_2SO_4 (Good et al. 1933). The level of glucose was determined by the Folin-Wu procedure outlined in Henry (Henry, 1965).

Histological and histochemical evaluations

Several parameters were employed to evaluate the morphology and histochemical reactions of the endometrium.

Epithelial height Surface epithelial height was measured with a Zeiss ocular screw micrometer, at 100X magnification. Twenty-two measurements were taken at random sites from 5 different areas of the uterus, representing the tips and body of both uterine horns. These measurements were then averaged, with this figure being used to represent the cell height of that particular uterus. This method is similiar to that outlined by Green (Green, 1950). The height of the glandular epithelium was determined in a similiar manner.

Glycogen, Alkaline phosphatase The evaluation of these parameters is difficult to accurately obtain. The presence of alkaline phosphatase or glycogen is determined by the occurrence and relative amounts of the end products of the histochemical reactions described by McManus and Mowry (1965), PAS, and Gomori (1964), AP. This evaluation is subjective and the values assigned for each reaction depend on detailed examination of the tissues. These evaluations were assigned numerical

values depending on the relative amount of histochemical end-products. The numerical values assigned were as follows:

0) absent

1) slight

2) moderate

3) intense

4) very intense

Mitosis Mitosis is also difficult to accurately measure. The mitotic figures in the epithelium could be counted, however, interpretation of the number would be very difficult due to the varying size of the uterine horn sections, and the varying degree of endometrial folding seen in various uteri. Because of this the amount of mitosis is given as the total number of mitotic figures observed in a given section of uterus. These figures are represented in the following list.

0) no mitotic figures observed

1) 1 - 10 mitotic figures/section

2) 11 - 20 mitotic figures/section

3) 21 - 30 mitotic figures/section

4) 31 - 40 mitotic figures/section

Leucocytes The number of leucocytes in the endometrium and submucosa are also represented by numerical values determined by the relative concentration of leucocytes in the tissue. The concentration of leucocytes per section was recorded by the numerical values as seen in the following list:

1) very few

2) few

3) moderate numbers

4) many

5) very many

The type of leucocyte described in a particular uterus is the type which predominates in that particular specimen, realizing that in any given specimen of uterus one can identify most types of leucocytes.

RESULTS

Clinical Observations of Gilts

The animals used for this study were apparently normal before the treatment period. Daily examinations of the gilts with the boar in a large outside pen afforded an excellent opportunity to observe the behavior of the individual animals. Chart number 1 shows the days of proestrus, estrus, metestrus, and the date of slaughter of all the experimental animals. The period of treatment with MATCH is indicated on the chart for the first 18 gilts. The last 6 gilts listed received no MATCH and were used as controls.

Period during treatment

The behavior of the animals during the first several days of the treatment was essentially normal, with the exception of animal number 3. This individual started to eat less feed from the 2nd day of treatment until necropsy on the 12th day. In 5 other animals, varying degrees of anorexia were observed as shown on chart number 2. These 6 sows were fed only 1½ pounds per head per day during this period of anorexia, and often refused to eat this amount completely. The other 12 animals on the treatment consumed the prescribed amount of feed at all times. The degree of appetite depression seems to be a rather variable phenomenon since several of the animals would have readily eaten more than the prescribed 3 pounds per day. Throughout this period the controls, fed the same feed without the MATCH, continued to exhibit normal appetites.

All of the treated animals, regardless of appetite, demonstrated a profound change in general behavior. Within 4 to 5 days they became progressively depressed and lethargic. Animals that previously had eagerly awaited the opportunity to graze and root in the outside pen daily, had to be driven out. Once outside they would merely lie down and start to drowse, rather than exercise. This condition was noticable in the building also, but not to the extent that was observed outside. Throughout the treatment period the attitude and behavior of the control animals remained unchanged.

During the treatment period 3 of the 18 treated animals showed estrus. All 3 of these animals had shown obvious signs of proestrus the day before treatment was initiated. Animals number 3 and 9 were in standing heat on the first day of medication. Number 3 was in standing estrus for 5 days during treatment, in comparison to standing estrus periods of 1 or 2 days exhibited previously. Number 9 was in standing estrus for 3 days during treatment in comparison to estrus periods of 1 day before treatment. Number 4 was in proestrus on the first day of medication, with an apparently normal standing estrus, lasting 1 day, on the 2nd day of treatment. The previous standing estrus periods of this animal also were of 1 day duration. No other animals in the treatment group exhibited any outward evidence of estrus throughout the time that MATCH was administered in the feed. All of the control animals exhibited normal estrus during this time.

Blood samples were drawn from 6 of the treated animals on the 18th day of the treatment period. The clotting time, packed cell volume, total

erythrocyte count, total leucocyte count and differential count of leucocytes were determined from these blood samples. The data obtained from these samples appears in Table 1. All of the values obtained were approximately normal according to the values given in Coffin's Clinical Pathology (Coffin, 1953). It is interesting to note that with the exception of number 8, all of the animals are in the low range of the normal packed cell volume (32 - 47).

The water intake could not be measured due to the automatic waterers. Evaluation of the volume of urine excreted by these animals was impossible because fluid rapidly disappeared into floor drains in the pens and the floors were damp because the pens were washed twice daily.

Period after withdrawal

Both the depression of appetite and lethargy rapidly disappeared upon withdrawal of MATCH from the feed. These two conditions were still very evident 24 hours after withdrawal, but by 48 hours after withdrawal both appetite and behavior were near normal. All animals appeared to exhibit completely normal behavior on the 3rd day post-treatment and throughout the remainder of the trial.

The length of the interval between the end of the treatment period and the synchronized proestrus was either 3 or 4 days for all animals except numbers 13 and 16. Number 13 had a very short or imperceptible proestrus period prior to standing heat. The lack of an apparent proestrus

was also noted in this animal at the last estrus before treatment. Number 16 exhibited proestrus on the first day following withdrawal of MATCH from the feed. This proestrus lasted 4 full days in comparison to proestrous periods before treatment of 2, 2, and 1 days. It should be noted that this animal exhibited estrus for the last two days prior to initiation of MATCH treatment. The remainder of the treated animals had 1 or 2 days of proestrus which was characterized by very marked swelling and erythema of the vulva. Upon subjective evaluation this seemed to be more marked than the swelling observed during the pretreatment proestrus or those observed in the control animals.

Synchronized estrus

All of the treated animals manifested estrus on the 5th, 6th, or 7th days post-treatment. All of the animals were in standing estrus on the 7th day after withdrawal. The length of the synchronized estrus appeared to be longer than usual. Of the animals allowed to pass completely through the synchronized estrus, one had standing estrus of 3 days, 3 had 4 days of standing estrus, and 2 were in standing estrus for 5 days. Two others were in the 4th day of standing estrus on the day of necropsy. A comparison of the length of synchronized estrus to length of estrus before synchronization is as follows:

Sow no.	Length of synchronized estrus (days)	Length of previous estrus (days)
11	4	1,1
12	4	1,1,2
13	5	1,1,3

Sow no.	Length of synchronized estrus (days)	Length of previous estrus (days)
14	4	1,1,2
15	4	1,1
16	3	1,1,2
17	5	1,2
18	4	1,2,2

Five of these animals had a very thick mucopurulent vaginal discharge during the synchronized estrus as illustrated on chart number 2. Staining of smears prepared from this discharge with Wright's stain showed the cellular portion to be predominately eosinophilic neutrophils. A few squamous epithelial cells were also observed (Figure 4).

Period following estrus

Behavior and appetite following estrus were essentially normal.

Evidence at necropsy revealed that follicular development and ovulation during the synchronized estrus had been normal. Information regarding the weight of the ovaries and various ovarian structures for the experimental animals is given on Table 2. The uterus and oviducts of the animals that were inseminated were examined for evidence of pregnancy. The findings of these examinations are as follows:

Sow no.	No. of ova or conceptus	Days after last insemination
13	5	4
14	0	4
15	9	15
16	0	15
17	9	24
18	13	24
22	3	5
24	8	26

The actual number of ova or embryos recovered at necropsy was a secondary consideration in this study. The primary interest was focused on quickly obtaining endometrial specimens for chemical evaluation. Because of this fact the number of ova cited above does not necessarily represent all that might have been present, but only indicates that ovulation did occur.

Histochemical, Histological and Biochemical Results

Uterus

The results from the histochemical, histological and biochemical studies of the uterus obtained by the procedures and expressed in values outlined in the materials and methods are included in Table 3.

Size of Pituitary, Thyroid and Adrenal

Although the pituitary, thyroid and adrenal glands are not directly related to this study, they were investigated because of the possibility that they might be involved in the changes that take place in animals during the feeding of MATCH. These glands were weighed, and the results are recorded on Table 4.

DISCUSSION

Synchronization with MATCH

Effect of MATCH on experimental animals

The primary goal for which MATCH was administered to the experimental gilts was achieved, but there were some unwanted side effects that were observed.

Synchronization of estrus was satisfactorily attained in all of the treated gilts. The random occurrence of estrus in animals prior to treatment is recorded in Chart 1, as well as the anestrus of the treatment period, and the synchronized estrus that occurred after MATCH was removed from the feed. The synchronized estrus occurred in all animals within 5 or 6 days of termination of treatment.

Several obvious clinical manifestations were observed in the gilts fed MATCH that were not observed in the controls. These were anorexia and lethargy during the feeding period and tumefaction or excessive swelling of the vulva during proestrus, prolonged duration of estrus and copious amounts of mucopurulent vaginal exudate during the first 2 days of the synchronized estrus. The relationship of the anorexia and vaginal discharge to the treatment period is shown on Chart 2.

Recovery from MATCH treatment

Recovery from the clinical effects of medication occurred within the first 24 to 48 hours after withdrawal. The decreased appetite and lethargy were not apparent on the second day after withdrawal of MATCH. If this is the time that is required for the drug to be cleared from the tissues and

body fluids of the animals, one could assume that the inhibition of the pituitary would be gone at approximately the same time. There would probably be a time lag before the awakening pituitary would stimulate the ovaries. Data in Table 2 collected by observing the ovaries of gilts necropsied 2 days after withdrawal indicate that ovaries were not stimulated by the gonadotropins because there were only small follicles 3 mm in diameter. But ovaries of animals necropsied 6 days after withdrawal contained normal mature follicles 10 mm in diameter. One could speculate that gonadotropins stimulated the production of follicles between 2 and 6 days after withdrawal.

Mechanism of action of MATCH

Some investigators who have studied MATCH (Paget et al., 1961) agree that the compound reduces the gonadotropin (FSH, LH) output of the pituitary with resultant temporary suppression of certain manifestations of reproductive activity. One would expect some change to take place in the pituitary during the treatment period. A comparison of pituitary weights of the experimental and control gilts necropsied in this experiment is presented in Graph 1. Interpretation of this graph suggests that the pituitary weights were lowest in the early part of the treatment period and then increased as the treatment period progressed. There was no obvious difference between the pituitary weights of control and synchronized animals in estrus.

Selective inhibition of pituitary gonadotropins may not be the only mechanism of action of MATCH. Effects on thyroid activity, volume of urine flow, milk ejection, lethargy and depression indicate that more

than selective depression of pituitary gonadotropins results from this treatment. These facts indicate that the observed side effects of the treated animals could be due to more general alterations in the metabolic activity of the animals. It may be concluded then that other pituitary hormones, both of the anterior and posterior areas, may be affected.

Due to the fact that MATCH has been reported to affect tibial growth rates in hypophysectomized rats (Cargill-Thompson, 1963), the possibility of MATCH affecting body tissues independent of any pituitary response must be considered.

Unexpected synchronization of control animals

The control gilts all manifested estrus within 1 or 2 days of the time the feeding of MATCH was started for the experimental animals. The controls returned to estrus within 1 or 2 days of the time that the synchronized estrus occurred in the MATCH fed gilts.

This result was very unexpected and some discussion as to possible explanation of this result seems appropriate. There are several possible explanations: It was just coincidental that all of them happened to exhibit estrus at this time, some psychic or nervous stimulus associated with the handling or feeding procedures may have brought all of the controls into heat together, or the restricted diet from 5 lbs. per day to 3 lbs. per day which was initiated in the control group when MATCH was started in the others may have caused some regulatory influence on the occurrence of estrus.

It would be interesting to know what caused the synchronization in

the controls because if these factors were understood they might lead to a more satisfactory method of synchronization than feeding MATCH.

Effect of Synchronization on the Ovary

During treatment

MATCH obviously produced the desired synchronizing effect on the ovaries, as can be seen by evaluating the data compiled on the ovaries in Table 2. The ovaries of the animals necropsied during the treatment period showed no evidence of any ovarian stimulation. Animals 1 and 2 were necropsied on the 6th day of the treatment period, in the 18th and 17th days of their respective cycles. The corpora lutea were still of relatively normal size, but the occurrence of regression was evident histologically, especially in gilt 1. Regression usually starts on day 15 to 16 of the cycle, so that the findings in these ovaries are as would be expected. Gilt 3 was one of the animals in standing estrus on the first day of medication. The estrus lasted 5 days. Gross and histological examination of the ovary revealed no functional luteal tissue on the 7th day after the last signs of estrus.

According to Sammelwitz et al., (1961) and du Mesnil du Buisson et al., (1963), the influence of pituitary gonadotropins on the ruptured follicle is not necessary for the formation and maintenance of the corpora lutea. If this is true and ovulation had occurred, then gross and/or histological examinations of the ovary should have revealed the presence of luteal tissue. Because no luteal tissue was found, it is presumed that no ovulation occurred in this animal at this estrus. The fact that the follicles did not become cystic or luteinized, but rather became

atretic, may indicate that the release of the ovulatory hormone was affected by treatment. In normal animals the ovulatory stimulus occurs some time before ovulation takes place. This means that the release of the ovulatory stimulus in Gilt 3 would have occurred very near the initiation of oral treatment. Usually oral medications are considered to require a little time for absorption and the establishment of a blood level. The inhibition of the ovulatory stimulus release from the anterior pituitary is, therefore, questionable. The animal also was the very first to become anorexic. These two facts together may lead to the conclusion that this animal was very sensitive to the inhibitory effects of MATCH, although this is merely speculation. Gilt 4, also in the early stage of follicular development when the treatment was started, had 14 normal sized corpora lutea at necropsy. Ovulation had evidently occurred with normal luteal formation. Assuming that MATCH did inhibit pituitary gonadotropin release, the corpora lutea were formed and maintained from the initial stimulus of the ovulatory hormone as suggested previously.

Animals 5 and 6, necropsied on the 18th day of treatment, had relatively small ovaries with no recognizably functional structures. Both grossly and histologically no corpora lutea or follicles were seen.

From withdrawal through estrus

Ovaries from gilts 7 and 8, necropsied on the 2nd day after withdrawal of the medication resembled those found on the 18th day of the treatment period. The ovaries were small with no corpora lutea or fol-

licles visible. Gilts 9 and 10 had normal, nearly mature follicles on the 6th day after withdrawal and in the 2nd day of the synchronized estrus. These large follicles, seen 6 days after withdrawal of the treatment, indicate that the pituitary inhibition lasts only a short time after withdrawal. Gilts 11 and 12 were necropsied on the 9th day after withdrawal, or the 4th day of standing estrus. Gilt 11 had grossly and histologically normal corpora hemorrhagica present on both ovaries with progressing luteinization, indicating that ovulation had taken place. Number 12 had not yet ovulated and had 13 large, histologically normal follicles. Because of the fact that ovulation may take place at various times during the prolonged estrus, it would be difficult to determine the optimum time for insemination. Several inseminations on successive days would probably be required to insure conception.

After synchronized estrus

Gilt 13 had 11 corpora lutea about 8 mm in diameter. These structures were nearly completely luteinized. Ovulation had taken place in the synchronized estrus with relatively normal luteinization occurring. Gilt 14, necropsied on the 13th day after withdrawal or 4 days after the synchronized estrus, had 6 large luteinizing follicles and no corpora lutea. This was the only evidence of cystic follicles seen in any of the treated animals, and is not of particular significance since cystic ovaries are relatively common (2 - 3%) in swine (Wiggins et al., 1948). It should be noted that no ova were recovered from this animal at necropsy.

Gilts 15, 17 and 18 had large histologically normal corpora lutea. Number 16 had 8 corpora lutea which were large but regressing as well as 12 follicles about 6 mm in diameter. The corpora lutea were rather vascular with definite signs of cellular degeneration. This animal was necropsied on the 18th day of the cycle. Treatment was initiated on the day following standing estrus and withdrawn on the day that signs of the next proestrus first appeared. This proestrus was rather prolonged. The standing estrus that followed was rather unusual in that the animal seemed to accept the boar intermittently. On the day before necropsy the animal was showing definite signs of proestrus. Ovulation during the synchronized estrus had occurred, but no pregnancy resulted.

Condition of ovaries of control animals

Gilt 19, the youngest of all the experimental gilts, had no evidence of previous ovulation visible on the ovaries. No corpora albicans were visible grossly or histologically. Many small follicles were observed however. In light of this, no satisfactory explanation can be offered for the recording of signs of proestrus on two occasions and of standing estrus after the last proestrus. The signs of proestrus reported may indicate that some degree of follicular development occurred at these times, but ovulation did not result. Gilt 22 had large vascular corpora lutea which were histologically normal, but also had one large cystic follicle, 25 mm in diameter. The ovarian structures for the other control animals are described in Table 2. The structures on these ovaries correspond to the stage in the cycle in which the animals were necropsied.

This brief explanation of the ovarian structures present at necropsy has been made to point out the effectiveness of the synchronization procedure and to indicate the ovarian changes which have a great influence on the uterus. The preceding summary of ovarian activity indicates that the pituitary influence on the ovaries was very insignificant during the treatment period. It also points out the rapid return of ovarian function after withdrawal.

Weights of the reproductive tracts

The total weights of the reproductive tracts of the animals also tend to reflect this ovarian effect. Data from the weights of these tracts, as seen in Graph 2, show a constant decrease in total weight during the treatment period. This weight decrease continued after termination of the treatment, but rapidly increased again as the onset of estrus approached. Grossly the uteri appeared rather normal throughout the trial period. Polge (1964) states that the uteri from animals treated with MATCH were small and prepubertal in appearance. The uteri from the animals of this experiment were somewhat smaller in weight, but certainly not small enough to be considered prepubertal. This comparison may very well have been true, if the treatment period had been longer.

Evaluation of the Uterus

Histological findings

Histological evaluation of the uterus revealed that not all of the histological features studied were affected by synchronization. Some of the features were greatly affected by MATCH treatment while others were not.

Uterine epithelium height The uterus surface epithelium height during the treatment period is relatively low, as can be observed on Graph 3. This is as expected since the animals were in either the luteal phase of the cycle or without ovarian stimulation on the uterus. The outstanding feature of Graph 3 is the demonstration of the difference between the uterine epithelial height in MATCH synchronized gilts and control gilts. Treated animals studied on the 2nd and 4th day of the synchronized estrus periods had heights which were approximately in the normal range for this time (30 - 40 microns). The control animals slaughtered at a similar time in the cycle also were in this range. The medicated animals slaughtered on the 4th day after the end of standing estrus had very high surface epithelium. The controls studied at this approximate time, however, were in the normal range.

McKenzie (1926) quoted the maximum height in his studies as being 63 microns. However, one of these animals had an average of 110 uterine measurements which was 62 microns, while the other animal necropsied at this time averaged 47 microns. The maximum heights measured in these animals was 81 and 66 microns respectively. It is difficult to explain why the epithelium of these animals was so high, when the values for the control, necropsied at a similar time, were in the range considered normal. These cells were evidently either very sensitive to the ovarian steroids, or the influence of these steroids on the uterus was very high and prolonged. The sensitivity of the tissues of the reproductive tract after the treatment period was discussed in relationship to the marked vulvar edema of the synchronized proestrus. Synchronized animals necropsied two

weeks later in the trial period (pregnant animals) were well within the normal range and were similar to the control animals. Number 16, a synchronized animal that was bred but didn't conceive, was necropsied on the 16th day of the cycle. The epithelial height was much higher (see Table 2) in this animal, but it was showing initial signs of proestrus at time of necropsy.

The comparison of the glandular epithelial height of MATCH synchronized animals and controls is shown on Graph 4. Both groups conform to the cyclic variation described by Corner (1921). This is one of the features that was not obviously affected by MATCH treatment.

Frequency of observed mitosis The degree of mitosis observed in the animals is shown on Graph 5. Mitosis can be seen to occur to some degree in any given uterus at any time, although the frequency of cell division is certainly variable. Both the treated and control animals reached a maximum number of mitotic figures during the estrus period. Since mitosis is most commonly seen just prior to, during and shortly after estrus this must be considered normal for both groups. The figures cited on the graph actually represent ranges, as indicated in the materials and methods. The graph indicated that mitosis during the synchronized estrus is relatively high in frequency in comparison to the periods before and after this period of time. The control animals also showed a similar frequency of observed mitotic figures.

Submucosal edema The submucosal edema viewed histologically with representative values, which are shown in Table 3, indicate that the submucosa of the animals during treatment is rather dense and compact, like

an animal in the luteal phase of the cycle. The compactness of the submucosa decreased, after withdrawal of the medication, and follicular development occurred corresponding with the occurrence of edema. Edema seen in the submucosa is considered normal during the estrogen phase of the uterine cycle. After ovulation the edema in all the treated animals decreased. At no time did the submucosa of the animals necropsied during the treatment period appear to be more dense than those during the luteal phase following the synchronized estrus. The control animals also exhibited a normal amount of edema in the follicular phase of the cycle with more compactness of the submucosa in the luteal phase.

Numbers and types of leucocytes The relative numbers and predominate types of leucocytes in the uteri of the animals are shown in Graph 6. The total numbers and types of leucocytes seen during treatment, and in the period between withdrawal and the synchronized proestrus, were approximately normal. During estrus, however, both the treated and control animals varied somewhat from the normal described by Corner (1921). The total leucocyte count increased as expected, but the predominant type seen in both groups was not as described by Corner. According to Corner (1921), predominent leucocyte type at estrus is neutrophilic polymorphonuclear-leucocytes, with an occasional eosinophile. The occurrence of eosinophils does not become prominent then until 8 - 10 days after ovulation has occurred. In the animals used in this study the eosinophils appeared during estrus. These cells were very evident in the epithelial cell layer, contrary to indications made by Corner (1921). The most striking difference observed between the controls and the treated animals was the great

difference observed in the total numbers of eosinophils. The treated animals necropsied in estrus had a very great number of eosinophils lining the basement membrane of the epithelial cells. These leucocytes were generally tightly packed together under this membrane and often 3 or 4 layers thick. These cells were also frequently seen in the controls, but not with the consistently high frequency observed in the treated animals.

The occurrence of leucocytes, during proestrus and estrus has been reported in heifers, ewes and gilts (Nellor and Brown, 1966). These cells were described as leucocyte-like cells not of immediate blood origin, but from morphological modifications of plasma cells. The reason for the appearance of these cells was not explained. It had been postulated that they are an antibacterial mechanism of the reproductive tract.

Eosinophils of blood cell origin are thought to be associated with antigen reactions in the body. These cells may serve as highly mobile reserves of enzymes necessary for phagocytosis of the antigen particle by macrophages (Speirs, 1964). Which, if either, of these functions is performed by these eosinophilic cells in the uterine submucosa is not known. The significance of the high levels of these cells in the submucosa of the treated animals is therefore uncertain. As had been stated, a vaginal discharge which was noticed in the treated animals was composed of eosinophilic cells. The appearance of this discharge coincided with the occurrence of high numbers of similar cells in the uterine submucosa and within the epithelial layer itself. It is very possible that the source of the cells seen in this discharge originated from the uterine submucosa. The possibility remains that the discharge originated from the cervical or

vaginal portion of reproductive tract. The occurrence of these cells in the vaginal or cervical submucosal areas is not known, since no sections were studied from these areas.

Chemical and histochemical evaluation of the uterus

Alkaline phosphatase The results of the chemical determinations of alkaline phosphatase, shown in Graph 7, indicate that the level of this enzyme was very low during the treatment period and in the withdrawal-to-proestrus interval. The levels of this enzyme are normally low in the luteal phase of the cycle and increase as estrogen stimulates the uterus in its characteristic pattern. The level of these enzymes remained low through the 4th day of the standing estrus in these treated animals and increased the 8th day of the cycle. This increase, though delayed, achieved levels a little higher than the normal animals. The control animals were in the range considered near normal on the 2nd and 3rd days of estrus, but were definitely declining on the 7th day of the cycle.

It is interesting to note on this graph that the levels observed in 4 synchronized animals on the 2nd and 4th days of the cycle had alkaline phosphatase levels which were not much higher than during the medicated period. The control animals, necropsied at a similar time in the cycle, had substantially higher levels. The levels in the treated animals raised to similar levels, but 4 to 5 days later. As the progestational influence on the uteri occurred, later in the period following synchronized estrus, the levels of the controls and treated animals became more comparable. These alkaline phosphatase levels, which are stimulated by

estrogens, indicate that follicular development may have been somewhat slower in the cycle of the treated animals than in the controls, assuming that day 1 of the cycle is regarded as the 1st day of standing estrus with no relationship to actual time of ovulation. The histochemical evaluation of alkaline phosphatase activity is shown on Graph 8. The ranges shown on this graph do not demonstrate very clearly the sequence described by the chemical analysis of alkaline phosphatase. The points plotted on this graph indicate that the histochemical trends are very comparable between the treated and control groups.

Glycogen Analysis of the data obtained from the chemical determination of endometrial glycogen indicates that the glycogen levels obtained at similar stages of the cycle had a very wide range. This range observed on Graph 9 was especially great in the animals during the withdrawal to synchronized proestrus interval. These values also seem to be rather high for a tissue that is not ordinarily considered to store large reserves of glycogen. The glycogen reserves found in the liver, the most important glycogen reservoir, are only about 7 - 8% of the total wet weight. The values determined for the uterus varied from 0.75 mg to 3.5 mg%. Because of the large range found and the high levels of glycogen the accuracy of the chemical determination shown on Graph 9 for glycogen is questioned.

The histochemical values of glycogen shown on Graph 10 indicate that the animals necropsied first in the treatment period had a relatively high glycogen value. This is to be expected in histochemical observations since glycogen reaches the highest concentration in the luteal phase of

the cycle. The amounts of PAS positive material steadily decreased as the treatment period progressed and through the withdrawal to synchronized estrus interval. Values seen in both the controls and the treated animals during the first 9 days of the cycle are similar and low. These levels then start to increase at about the 8th to 10th day of the cycle, according to Graph 10. The level of glycogen increased first in the treated and then the control gilts. It should be pointed out that no control was necropsied at a period corresponding to the 18th day of the cycle as shown on the graph for a treated animal. Had a control animal been necropsied at the 18th day, the values may have been more comparable. Animal number 16 did not conceive, so the values for this animal do not apply to this graph.

General Discussion

Differences between control and treated animals

General consideration of all the parameters indicate that very little variation was observed between the treated and control animals as far as the amount of submucosal edema, glycogen and mitosis rate in the endometrium were concerned. The predominant type of leucocyte is somewhat similar for the treated and control animals. The total leucocyte number of the treated animals tended to be somewhat higher than the controls, but evaluation of leucocytes in tissue is very difficult to accurately accomplish. The peak levels of alkaline phosphatase and the epithelial height were somewhat exaggerated and delayed in comparison to the normal animals. This evidence indicates that the metabolic activities of the uteri of

synchronized swine are near normal, with the possibility that some of these cyclic changes are slightly delayed and exaggerated.

Limitations of this investigation

The results obtained in this study reveal some differences between MATCH synchronized gilts and controls which suggest some definite conclusions regarding probable consequences associated with MATCH synchronization that may decrease reproductive efficiency. The chief limitation is the small number of animals that were available for this investigation. The results and conclusions should be interpreted in the light of the limited sampling. Further work should be done to confirm or dispute the findings of this study. The results of this preliminary study should be supplemented by investigations on larger numbers of animals in the period of 2 - 10 days after withdrawal of the drug.

Consequences of MATCH induced uterine changes

The consequences of the delayed response of the uterus on the normal development of the fertilized ova are not clear. The ova should enter the uterus 3 to 4 days after ovulation in a synchronized gilt. At this time the uterine epithelial height and alkaline phosphatase will just be reaching the highest points, instead of starting to decrease as in the normal uterus. The developmental stage of the uterus will be possibly 2 - 3 days behind the development of the fertilized ova.

The significance of this delay in a normal gilt is probably not too great. (The most optimal situation exists when perfect synchrony exists between the uterus and ova.) Evidence cited earlier in studies of ova

transplants of rats (Psychoyos, 1966) indicates that the chance for survival of the ova is better if the developmental stage of the ova is slightly ahead of the uterus than if this relationship is reversed.

The failure of the uterus to be perfectly coordinated with the developing ova may be a contributing factor to some reports of adverse effects of MATCH on embryo survival (Gerrits and Johnson, 1964a). As can be noted on graphs of epithelial height and alkaline phosphatase, these factors are within the normal range by the time of implantation. This indicates that the most critical time for the ova, as far as the MATCH induced uterine variations are concerned, is in the period between the entry of the ova in the uterus and implantation. In trials where the conception rate and embryo survival have been normal, the uterus has probably been able to develop rapidly enough so that the effects of the delayed uterine response on the fertilized ova were minimized.

Gilts or sows that are not quite normal may not be able to rapidly correct the lack of unison between the fertilized ova and the uterus. The resulting uterine abnormalities then could be a contributing factor to decreased embryo survival rates or failure of the gilts to conceive at all. The rate at which the uterine conditions become normal may be delayed somewhat if pathological or other abnormal circumstances exist. The occurrence of one insult, as a pathological condition or MATCH treatment alone, may not greatly affect the survival rate. A combination of these two factors, may conceivably interfere with normal uterine development sufficiently to lower the embryo survival rate.

SUMMARY AND CONCLUSIONS

1. Genital organs and clinical manifestations were studied in 18 gilts that were synchronized with MATCH and 6 control gilts.

2. Genital organs were studied by means of gross observation, light microscopy, histochemistry and chemical analysis.

3. The occurrence of side effects due to MATCH treatment, such as lethargy and anorexia, appears to vary between individual animals.

4. The ovaries of the animals necropsied during the treatment period indicate very little or no evidence of pituitary influence.

5. The total weight of the reproductive tract decreases steadily during the treatment period, but rapidly increases with the onset of the synchronized estrus.

6. The withdrawal of the treatment brings about a rapid and apparently complete recovery from the side effects of MATCH treatment.

7. The ovarian activity after withdrawal was normal with respect to follicular development, ovulation and corpora luteal formation.

8. The time of ovulation in MATCH synchronized estrus was slower than normal, not occurring until the 3rd to 4th day.

9. MATCH synchronization appears to cause the proestrus period to be very prominent, with marked swelling of the vulva.

10. The MATCH synchronized estrus period is prolonged, lasting 4 - 5 days.

11. Treatment with MATCH causes copious amounts of mucopurulent vaginal discharge, composed primarily of eosinophils, during the first 2 days of the synchronized estrus.

12. The maximum height of the uterine surface epithelium was greater in the treated animals than in the controls. This maximum height was not achieved until approximately 4 - 5 days later in the treated animals than the highest epithelium occurred in controls.

13. The maximum levels of alkaline phosphatase activity in the MATCH treated animals occurred approximately 4 days later than in the control animals.

14. The total number of leucocytes observed in the endometrial submucosa of both the treated and control animals were similar.

15. The treated animals had a preponderance of eosinophils in the submucosa during estrus, in comparison to the control animals.

16. The determinations of glycogen, mitosis and uterine submucosal edema were similar for the treated and control animals.

17. By the approximate time of implantation, 18 - 24 days, the determinations for all the variables revealed little difference between the treated and control animals.

18. There are some differences in the uteri of MATCH synchronized animals and untreated animals during and shortly after estrus. These differences may possibly provide an explanation for the slight decrease in conception rate and litter size sometimes observed in MATCH synchronized animals.

BIBLIOGRAPHY

Armed Forces Institute of Pathology.
1960 Manual of histologic and special staining techniques. New York, The Blakiston Division, McGraw-Hill Book Co., Inc.

Atkinson, W. B. and Elftman, H.
1947 Mobilization of alkaline phosphatase in the uterus of the mouse by estrogen. Endocrinology 40: 30-36.

Austad, R. and Garm, O.
1959 Periodic acid-Schiff positive material and the alkaline phosphatase in the uterine wall of the pig during the sexual cycle. Nature 184: 999-1000.

Averill, R. L. W. and Rowson, L. E. A.
1958 Ovum transfer in the sheep. Journal of Endocrinology 16: 326.

Bernard, C.
1859 Recherches sur l'origine de la glycogenie dans la vie embryonaire; nouvelle fonction de placenta. Societe de Biologie Compte Rendus 2: 101-107.

Bo, W. J. and Smith, M. S.
1964 Glycogen synthetase in the rabbit tongue and uterus. Anatomical Record 148: 503-505.

Borghese, E.
1957 Recent histochemical results of studies on embryos of some birds and mammals. International Review of Cytology 6: 289-337.

Bradfield, J. R. G.
1950 The localization of enzymes in cells. Biological Review 25: 113-148.

Bredeck, H. E. and Mayer, D. T.
1955 Uterine phosphatase concentrations and their relationship to number and weight of embryos in the rat. Missouri Agricultural Experiment Station Research Bulletin 591: 4-19.

Brinkley, H. J., Norton, H. W. and Nalbandov, A. V.
1964a Role of a hypophysial luteotropic substance in the function of porcine corpora lutea. Endocrinology 74: 9-13.

Brinkley, H. J., Norton, H. W. and Nalbandov, A. V.
1964b Is ovulation alone sufficient to cause formation of corpora lutea? Endocrinology 74: 14-19.

Brown, P. S.
1963a Observations on a dithiocarbamoyl-hydrazine as in an inhibitor of pituitary gonadotropic activity. Journal of Endocrinology 26: 425-436.

Brown, P. S., Crooks, J., Klopper, A. I., Thorburn, A. R., and Tulloch,
1963b M. I. Some endocrinological effects of a dithiocarbamoyl-hydrazine. British Medical Journal 2: 163-165.

Cantarow, A. and Shepartz, B.
1957 Biochemistry. 2nd ed. Philadelphia, W. B. Saunders Company.

Cargill-Thompson, H. E. C.
1963 The effect of a derivative of dithiocarbamoyl-hydrazine on the tibiae of hypophysectomized rats. Journal of Endocrinology 26: 447.

Coffin, D. L.
1953 Manual of Veterinary Clinical Pathology. 3rd ed. Ithaca, Cromstock Publishing Associates, Division of Cornell University Press.

Cole, H. H. and Cupps, P. T.
1959 Reproduction in domestic animals. Vol. 1. New York, Academic Press.

Cori, C. T. and Cori, C. F.
1943 Crystalline muscle phosphorylase. Journal of Biological Chemistry 151: 57.

Cori, G. T. and Larner, J.
1951 Action of amylo-1,6-glucosidase and phosphorylase on glycogen and amylopectin. Journal of Biological Chemistry 188: 17.

Corner, G. W.
1921 Cyclic changes in the ovaries and uterus of the sow, and their relation to the mechanism of implantation. Carnegie Institute Contributions to Embryology 64: 117-145.

Day, B. N., Neill, J. D., Oxenrider, S. L., Waite, A. B., and Lasley, J. F.
1965 Use of gonadotropins to synchronize estrus in swine. Journal of Animal Science 24: 1075-1079.

Dempsey, E. W.
1960 Histophysical considerations. In Ville, C. A., ed. The placenta and fetal membranes. pp. 29-35. Baltimore, The Williams and Wilkins Co.

Dickmann, Z. and Noyes, R. W.
1960 The fate of ova transfered into the uterus of the rat. Journal of Reproduction and Fertility 1: 197-212.

du Mesnil du Buisson, F. and Leglise, P. C.
1963 Effet de l'hypophysectomie sur les corps jaunes de la Truie: resultats preliminaires. Academy de Sciences Paris Comptes Rendus 257: 261-263.

Dziuk, P. J., Polge, C., and Rowson, L. E.
1964 Intrauterine migration and mixing of embryos in swine following egg transfer. Journal of Animal Science 23: 37-42.

Edgren, R. A. and Peterson, D. L.
1964 Blockage of ovarian compensatory hypertrophy in hemicastrated rats with a dithiocarbamoylhydrazine derivative. Journal of Endocrinology 3: 399.

Gerrits, R. J. and Johnson, L. A.
1964a Synchronization of estrus by oral administration as a non-steriod and the effect on ovulation and fertility in gilts and sow. Abstract No. 117. Journal of Animal Science 23:1125.

Gerrits, R. J. and Johnson, L. A.
1964b The effect of an orally administered non-steroid on estrus, ovulation, and fertility in gilts. International Congress of Animal Reproduction and Artificial Insemination Fifth, Trento, Proceedings 3: 455.

Gerrits, R. J. and Johnson, L. A.
1965a The influence of level of ICI 33828 on synchronization of estrus and embryo survival in sow. Journal of Animal Science 24: 918.

Gerrits, R. J. and Johnson, L. A.
1965b Synchronization of estrus in gilts fed two levels of ICI 33828 and the effect on fertility, embryo survival and litter size. Journal of Animal Science 24: 917.

Gerrits, R. J., Johnson, L. A., and Kraeling, R. R.
1965 The effect of ICI 33828 on lactation in the sow. Journal of Animal Science 24: 918.

Gerrits, R. J., Krehbeil, E. V., and Kinkaid, C. M.
1966a Synchronization of estrus and reproductive performance of gilts fed ICI 33828. Abstract No. 178. Journal of Animal Science 25: 923.

Gomori, G.
1964 Microscopic histochemistry. 4th ed. Chicago, University of Chicago Press.

Good, C., Kramer, H., and Somogyi, M. M.
1933 The determination of glycogen. Journal of Biological Chemistry 100: 486-491.

Goode, L., Warnick, A. C., and Wallace, H. D.
1965a Alkaline and acid phosphatase activity in the endometrium and ovary of swine. Journal of Animal Science 24: 955-958.

Goode, L., Warnick, A. C., and Wallace, H. D.
1965b Effect of dietary energy levels upon reproduction and the relationship of endometrial phosphatase activity to embryo survival in gilts. Journal of Animal Science 24: 959-963.

Green, J. A.
1950 Studies on the histology of the female reproductive system of the domestic pig. Microfilm copy, unpublished Ph.D. thesis, University of Illinois, Urbana, Illinois. Ann Arbor, Michigan, University Microfilms, Inc.

Groves, T. W.
1965 Controlled estrus and ovulation in pigs: correspondence Veterinary Record 77: 326.

Hafez, E. S. E., Jainudeen, M. R., Kroening, G. H., and El-Banna, A. A.
1966 Use of progesterone and thiocarbamoyl-hydrazine compounds for estrus synchronization in gilts. Journal of American Veterinary Medical Association 149: 35-41.

Hall, K.
1960 Modification by relaxin of the response of the reproductive tract of mice to estradiol and progesterone. Journal of Endocrinology 20: 355-364.

Hall, K.
1965 Histochemical investigations of the effects of estrogen, progesterone, and relaxin on glycogen, amylophosphorylase, transglycoslase, and uridine diphosphate glucose-glycogen transferase in uteri of mice. Journal of Endocrinology 32: 245-257.

Hancock, J. L. and Hovell, G. J.
1962 Transfer of sheep ova. Journal of Reproduction and Fertility 4: 195-201.

Harper, M. J. K.
1964 Effects of 1-a-methyl-allylthiocarbamoyl-2-methylthiocarbamoyl-hydrazine (ICI 33828) on early pregnancy in the rat. Journal of Reproduction and Fertility 7: 211.

Henry, R. J.
1965 Clinical chemistry: principles and techniques. New York, Hoeber Medical Division, Harper and Rowe.

Kugler, J. and Wilkinson, W. J. C.
1962 The identification of the "substance" visualized by the histochemical techniques for glycogen. Journal of Histochemistry and Cytochemistry 10: 110-111.

Leathem, J. H.
1959 Some biochemical aspects of the uterus. New York Academy of Science Annals 75: 463-471.

Lillie, R. D.
1954 Histopathologic technique and practical histochemistry. New York, McGraw-Hill Book Co., Inc.

Lovell, J. E. and Garm, O.
1964 Histochemical studies of the uterus and fetal membranes of the sow during early pregnancy. International Congress of Animal Reproduction and Artificial Insemination, Fifth, Trento Proceedings pp. 170-175.

Mansour, A. M. and Niu, M. C.
1965 Functional studies with uterine RNA. National Academy of Science Proceedings 53: 764-770.

Marinov, U.
1966 Cyclical changes of the bovine endometrium and cervical mucosa. Unpublished M. S. thesis, Iowa State University of Science and Technology, Ames, Iowa.

Moog, F.
1946 The physiological significance of the phosphomonoesterases. Biological Review 2: 41-59.

Morrissette, M. C., McDonald, L. E., Whatley, J. A., and Morrison, R. D.
1963 Effect of progestins on embryonic mortality of swine. American Journal of Veterinary Research 24: 317-323.

McKenzie, F. F.
1926 The normal estrus cycle in the sow. Missouri Agricultural Experiment Station Research Bulletin 86: 41.

McManus, J. F. A. and Mowry, R. W.
1965 Staining methods, histologic and histochemical. New York, Hoeber Medical Division, Harper and Rowe.

Nalbandov, A. V.
1964 Reproductive physiology. 2nd ed. San Francisco, W. H. Freeman and Company.

Nellor, J. E., Ahrenhold, J. E., First, N. L., and Hoefer, J. A.
1961 Estrus, ovulation and fertility in gilts subsequent to oral administration of 16-methyl-17-acetoxyprogesterone. Journal of Animal Science 20: 22-30.

Nellor, J. E.
1960 Control of estrous and ovulation in gilts by orally effective progestational compounds. Journal of Animal Science 19: 412.

Nellor, J. E. and Brown, J. E.
1966 Leucocyte-like cells of the vagina and uterus, and their modification during the normal estrus cycle and by progestin and estrogen treatment. Anatomical Record 155: 591-601.

Noyes, R. W. and Dickmann, Z.
1960 Relationship of ovular age to endometrial development. Journal of Reproduction and Fertility 1: 186-196.

Padykula, J. A. and Richardson, D.
1963 A correlated histochemical and biochemical study of glycogen storage in the rat placenta. American Journal of Anatomy 112: 215-241.

Paget, G. E., Walpole, A. L. and Richardson, D. N.
1961 Non-steroid inhibitors of pituitary gonadotropin function. Nature 192: 1191-1192.

Polge, C.
1964 Synchronization of estrus in pigs by oral administration of ICI 33828. International Congress of Animal Reproduction and Artificial and Insemination, Fifth, Trento Proceedings 2: 388-393.

Polge, C.
1965 Effective synchronization of oestrus in pigs after treatment with ICI compound 33828. Veterinary Record 77: 232-235.

Psychoyos, A.
1966 Recent researches on egg implantation. Ciba Foundation Study Group 23: 4-15.

Rall, T. W., Sutherland, E. W., and Berthet, J.
1957 The relationship of epinephrine and glucagon to liver phosphorylase. Journal of Biological Chemistry 224: 463-476.

Sammelwitz, P. H., Aldred, J. P. and Nalbandov, A. V.
1961 Mechanisms of maintenance of corpora lutea in pigs and rats. Journal of Reproduction and Fertility 2: 387-393.

Schmidt-Elmendorff, H., Loraine, J. A., Bell, E. T. and Walley, J. K.
1962 An investigation into the effect of derivatives of dithiocarbamoylhydrazine on gonadotrophic activity in the body fluids of pregnant mares. Journal of Endocrinology 25: 107-114.

Sigma Chemical Company
1961 The colorimetric determination of phosphatase. Sigma Chemical Company, St. Louis, Missouri, Technical Bulletin 104.

Speirs, R. S.
1964 How cells attack antigens. Scientific American 176: 2-8.

Stacey, M.
1962 Glycogen. In Stacey, M. and Baker, S. A., eds. Carbohydrates of living tissues. pp. 1-36. London, Van Nostrand Company Ltd.

Steplewski, A. and Jonek, J.
1965 Histochemical studies on the effects of estrogens on the uterus and vagina of spayed mice. (Translated title) Endokrynologie Polska 15: 127-144. 1964. Original available but not translated; abstracted in Chemical Abstracts 63: 4612g.

Stratman, F. W. and First, N. L.
1965 Estrus inhibition of gilts fed dithiocarbamolyhydrazine (ICI 33828). Journal of Animal Science 24: 930.

Sykes, A. H.
1964 Some actions of an anti-fertility compound in the fowl. Veterinary Record 76: 393-394.

Takeuchi, T. and Kuriaki, H.
Histochemical detection of phosphorylases in animal tissue. Journal of Histochemistry and Cytochemistry 3: 153-160.

Talwar, G. P. and Segal, S. J.
1963 Prevention of hormone action by local application of actinomycin D. National Academy of Science Proceedings 50: 226-230.

Tulloch, M. I., Crooks, J. and Brown, P. S.
1963 Inhibition of thyroid function by a dithiocarbamoylhydrazine Nature 199: 288.

Ui, H. and Mueller, G. C.
1956 The role of RNA synthesis in early estrogen action. National Academy of Science Bulletin 50: 256-260.

Vincent, C. K., Robison, O. W. and Ulberg, C. C.
1964 Technique for reciprocal embryo transfer in swine. Journal of Animal Science 23: 296.

Ville, C. A.
1953a The metabolism of human placenta in vitro. Journal of Biological Chemistry 205: 113.

Ville, C. A.
1953b Regulation of blood glucose in human fetus. Journal of Applied Physiology 5: 437.

Ville, C. A.
1960 Biochemical aspects. In Ville, C. A., ed. The placenta and fetal membrane. pp. 100-108. Baltimore, The Williams and Wilkins Company.

Warnick, A. C., Grummer, R. H. and Casida, L. E.
1948 The nature of reproductive failures in sows of low fertility. Journal of Animal Science 7: 545.

Warnick, A. C., Grummer, R. H., Casida, L. E.
1949 The nature of reproductive failures in repeat-breeder sows. Journal of Animal Science 8: 569-577.

Watanabe, K. and Fishman, W. H.
1964 Changes in hydrolase enzymorphology of rat uterus and vagina after estrogens. Journal of Histochemistry and Cytochemistry 12: 908-918.

Wiggins, E. L., Casida, L. E. and Grummer, R. H.
1948 The incidence of female genital abnormalities in swine. Journal of Animal Science 7: 545.

Wilson, R. F., Nalbandov, A. V. and Kridu, J. L.
1949 A study of impaired fertility in female swine. Journal of Animal Science 558-568.

Wislocki, G. B. and Dempsey, E. W.
1946 Histochemistry of the pigs placenta. American Journal of Anatomy 78: 181-226.

Zagni, P. A. and Benson, G. K.
1964 Further studies on the inhibition of lactation in the rat with ICI compound 33828. Journal of Endocrinology 30: xi-xii.

ACKNOWLEDGEMENTS

Special appreciation is expressed to Dr. James E. Lovell for his encouragement, patience and guidance in this research project. His suggestions throughout the study are most gratefully acknowledged.

The author wishes to express appreciation to Drs. W. M. Adams, L. L. Anderson, M. J. Swenson and W. M. Wass for their guidance in the development of this investigation and the preparation of the manuscript.

Gratitude is expressed to Mr. Don Gilchrist, for the very conscientious care he gave the experimental animals; to Ione Vold, for the aid in preparing the histological specimens; and to Mrs. Virginia Marshall for her assistance in the chemical determinations.

The author wishes to acknowledge the financial support for this project from the Iowa State University College of Veterinary Medicine, specifically the Department of Veterinary Clinical Sciences and the Veterinary Medical Research Institute.

APPENDIX

Chart 1. Daily record of estrus cycles of experimental gilts.

DAILY RECORD OF ESTRUS CYCLES OF EXPERIMENTAL GILTS.

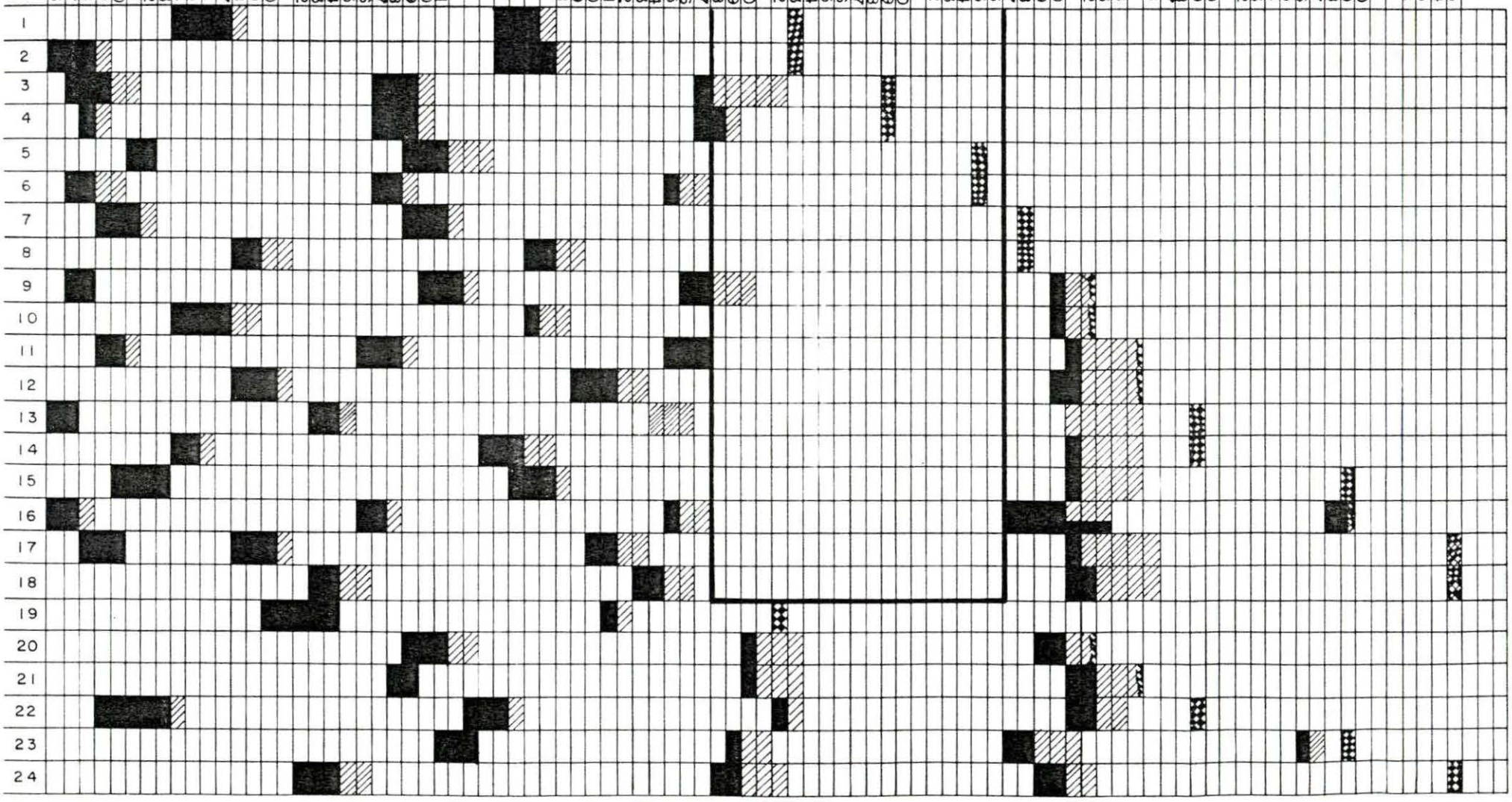


Chart 2. Anorexia and vaginal discharge of experimental gilts.

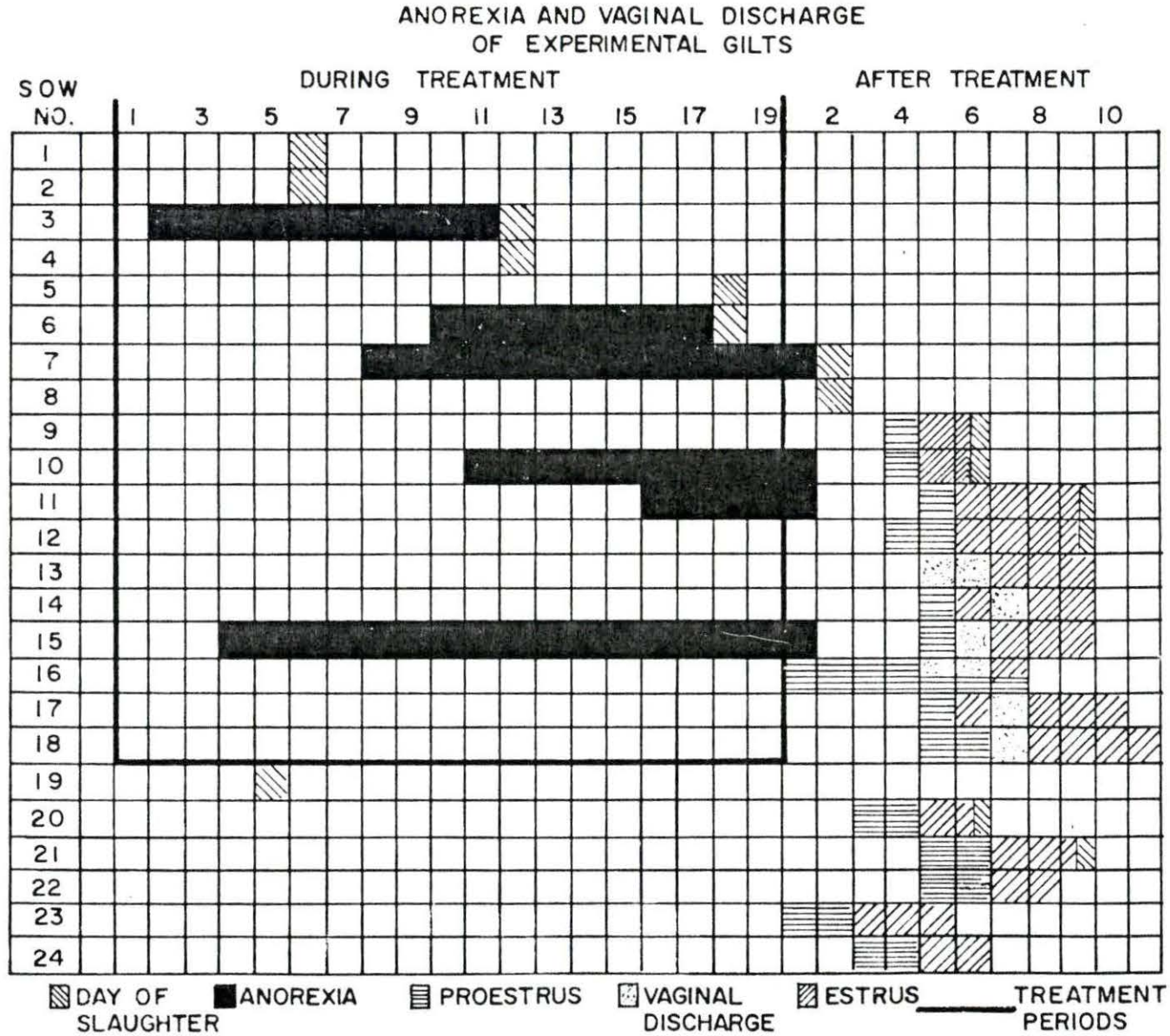

ANOREXIA AND VAGINAL DISCHARGE
OF EXPERIMENTAL GILTS
SOW NO.
DURING TREATMENT
AFTER TREATMENT
1 3 5 7 9 11 13 15 17 19
2 4 6 8 10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
DAY OF SLAUGHTER
ANOREXIA
PROESTRUS
VAGINAL DISCHARGE
ESTRUS
TREATMENT PERIODS

Table 1. Blood studies of experimental gilts

Sow no.	Total leucocytes	Total erythrocytes	Clotting time	Packed cell volume
		millions/mm^3	(min.)	(%)
5	24,297	7.97	1.55	32.0
7	20,358	9.92	4.62	34.0
8	20,267	9.33	7.64	38.0
12	12,520	8.71	6.96	28.0
16	17,321	7.96	7.83	33.5
18	19,522	8.21	6.40	33.5

Table 1. (Cont.)

	Leucocyte Differential (%)					
Sow no.	Basophiles	Bands	Segs	Lymphocytes	Monocytes	Eosinophils
5	0	2	20	59	0	19
7	1	1	34	58	0	6
8	0	2	27	67	0	4
12	0	3	17	74	0	6
16	1	9	24	64	0	2
18	0	2	12	77	2	7

Table 2. Ovarian observations on experimental gilts

Sow no.	Total weight or ovaries	Corpora Lutea size	Corpora Lutea no.	Corpora Lutea remarks	Follicles size	Follicles no.	Histological remarks
				Synchronized animals			
1	9.2	8mm	12	regression of C.L.	4mm	25	corpora lutea avascular, luteal cells degenerating, fibrosis occurring
2	11.1	10mm	14	early regression	5mm	22	still vascular, signs of cellular degeneration
3	4.6	3mm	21	c. albicans	3mm	17	no active structures
4	11.3	10mm	14	functional	3mm	16	c. lutea very vascular
5	3.7	3mm	16	c. albicans	4mm	26	appears relatively inactive
6	4.8	3mm	19	c. albicans	3mm	12	appears relatively inactive
7	5.7	2mm	19	c. albicans	3mm	33	appears relatively inactive
8	5.5	4mm	12	c. albicans	3mm	26	appears relatively inactive
9	5.9	2mm	18	c. albicans	10mm	8	histologically normal follicles
10	6.5	1mm	---	c. albicans	10mm	7	histologically normal follicles

Table 2. (Cont.)

Sow no.	Total weight of ovaries	Corpora Lutea size	Corpora Lutea no.	Corpora Lutea remarks	Follicles size	Follicles no.	Histological remarks
11	4.5	9mm	12	c. hemorrh-agicum	--	--	luteinization well progressed
12	8.7	2mm	21	c. albicans	11mm	13	follicles large, normal
13	8.8	8mm	11	c. hemorrh-agicum	3mm	18	luteinization near complete
14	18.8	2mm	11	c. albicans	20mm	2	luteinizing cystic follicles
					14mm	4	luteinizing
15	14.3	10mm	14	functional	2mm	45	C. L. histologically normal and fully functional
16	11.1	8mm	8	early regression	6mm	12	still vascular, signs of cellular degeneration
17	11.3	10mm	19	functional	4mm	40	C. L. very vascular and active
18	11.6	10mm	13	functional	5mm	27	C. L. very vascular and active

Table 2. (Cont.)

Sow no.	Total weight of ovaries	Corpora Lutea size	no.	remarks	Follicles size	no.	Histological remarks
				Controls			
19	--	--	--	------	many small follicles		no C. L. or c. albican, doubtful if animal had ovulated
20	6.4	7mm	16	c. hemorrhagicum	--	--	very early luteinization, ovulation probably only very recently occurred
21	10.8	10mm	12	c. hemorrhagicum	--	--	luteinization well progressed, not yet complete
22	21.8	12mm	8	functional	5mm	12	vascular and active C. L.
					25mm	1 cyst	cyst not luteinized
23	7.4	9mm	14	functional	2mm	few	C. L. very vascular and active
24	11.3	12mm	12	functional	4mm	27	C. L. very vascular and active

Table 3. Data from uterine studies of experimental gilts

Sow no.	Synchronized											
	1	2	3	4	5	6	7	8	9	10	11	12
	(units and values as described in materials and methods)											
Cell height in microns												
surface epithelium	24	22	28	25	23	24	20	20	43	44	33	36
glandular epithelium	15	15	14	19	11	14	12	12	16	20	15	18
Edema of submucosa*	2	2	1	1	1	2	1	1	5	3	3	5
Occurrence of mitosis	1	1	0	1	0	2	0	0	4	3	1	1
Leucocyte concentration	3	1	4	3	3	1	2	1	3	4	4	5
Predominate leucocyte type**	E	L	L	E	L	N	L	L	N	E	E	E
Histochemistry												
alkaline phosphatase	2.0	2.0	2.5	2.5	2.0	2.5	1.5	1.5	2.5	3.0	2.0	1.5
glycogen	2.5	3.0	1	2	1	1	1	1	2	1	0.5	0.5
Biochemistry												
alkaline phosphatase - 1	0.9	0.6	1.0	2.0	0.9	1.7	0.8	1.3	1.9	2.2	1.7	1.5
alkaline phosphatase - 2	0.8	0.6	1.1	2.1	0.7	1.7	0.7	1.1	1.7	2.2	1.7	1.3
glycogen - 1	2.0	2.2	0.7	1.6	1.6	1.5	1.2	3.0	1.89	1.66	1.78	1.18
glycogen - 2	1.8	2.2	0.5	1.7	1.8	1.6	1.1	3.2	2.1	-***	1.9	-***

* Evaluated according to the following standards
(1) no edema, tissue dense (2) slight edema (3) moderate edema (4) intense edema
(5) very intense edema.

** L = lymphocytes, N = neutrophils, E = eosinophils.

*** No results obtained.

Table 3. (Cont.)

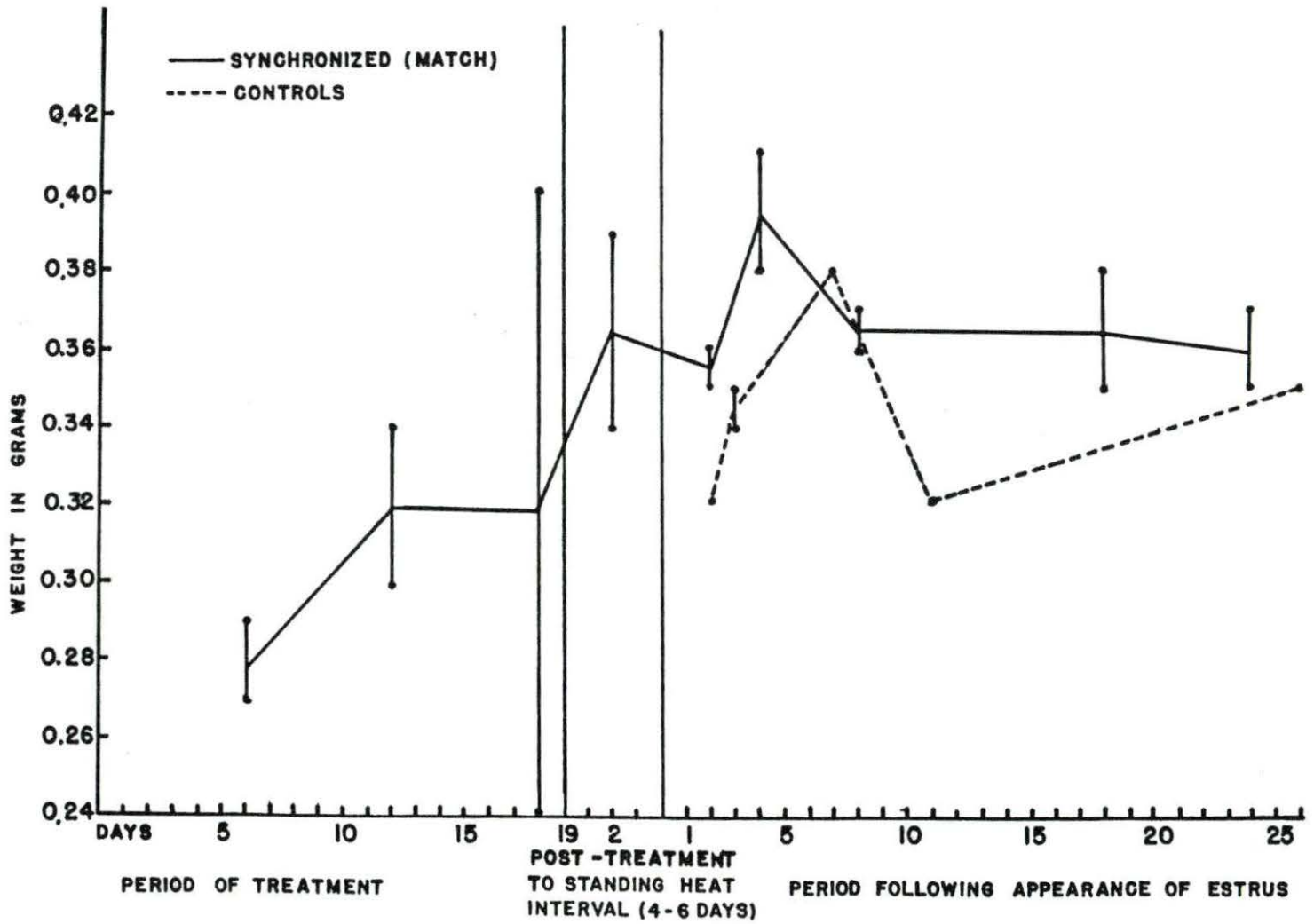
	Synchronized						Controls					
Sow no.	13	14	15	16	17	18	19	20	21	22	23	24
	(units and values as described in materials and methods)											
Cell height in microns												
surface epithelium	62	47	18	54	26	18	27	37	38	39	44	20
glandular epithelium	22	22	19	23	15	15	12	16	19	22	21	15
Edema of submucosa*	3	4	3	2	2	2	1	3	3	2	3	1
Occurrence of mitosis	1	1	0	1	0	0	1	4	3	0	4	0
Leucocyte concentration	1	1	1	2	1	1	2	4	2	2	1	1
Predominate leucocyte type**	L	N	L	L	N	E	L	N	L	E	N	L
Histochemistry												
alkaline phosphatase	3.0	5.0	1.5	3.0	1.5	2.0	1.5	4.0	3.0	3.0	2.5	1.5
glycogen	0.5	0.5	3.5	0.5	4.0	3	0.5	0.5	0.5	0.5	1.5	3.5
Biochemistry												
alkaline phosphatase - 1	8.0	10.0	1.5	6.5	0.9	0.9	4.9	7.6	7.5	4.5	5.5	0.9
alkaline phosphatase - 2	7.8	9.6	1.5	6.4	1.1	-***	5.3	7.6	7.2	4.5	5.5	0.8
glycogen - 1	0.8	1.8	3.6	2.6	3.3	2.9	1.3	2.9	2.1	1.5	3.0	2.9
glycogen - 2	****	1.9	3.6	2.8	3.0	2.7	1.6	3.0	-***	1.6	3.0	2.8

Table 4. Weights of endocrine organs of experimental animals

Sow no.	Pituitary	Thyroid	Adrenal
		(weight in grams)	
1	0.27	12.1	5.4
2	0.29	10.4	4.6
3	0.34	13.0	6.8
4	0.30	13.5	6.0
5	0.24	7.9	4.1
6	0.40	11.0	5.4
7	0.34	13.2	6.6
8	0.39	12.8	6.2
9	0.35	12.4	5.4
10	0.36	11.7	8.7
11	0.38	6.8	6.0
12	0.41	11.1	4.8
13	0.36	13.2	5.5
14	0.37	24.8	6.8
15	0.35	16.7	11.2
16	0.38	18.9	6.2
17	0.35	12.0	5.2
18	0.37	7.6	8.0
19	0.32	----	7.0
20	0.32	10.9	6.0
21	0.34	12.3	4.7
22	0.38	15.3	6.4
23	0.35	8.1	6.5
24	0.35	8.8	7.8

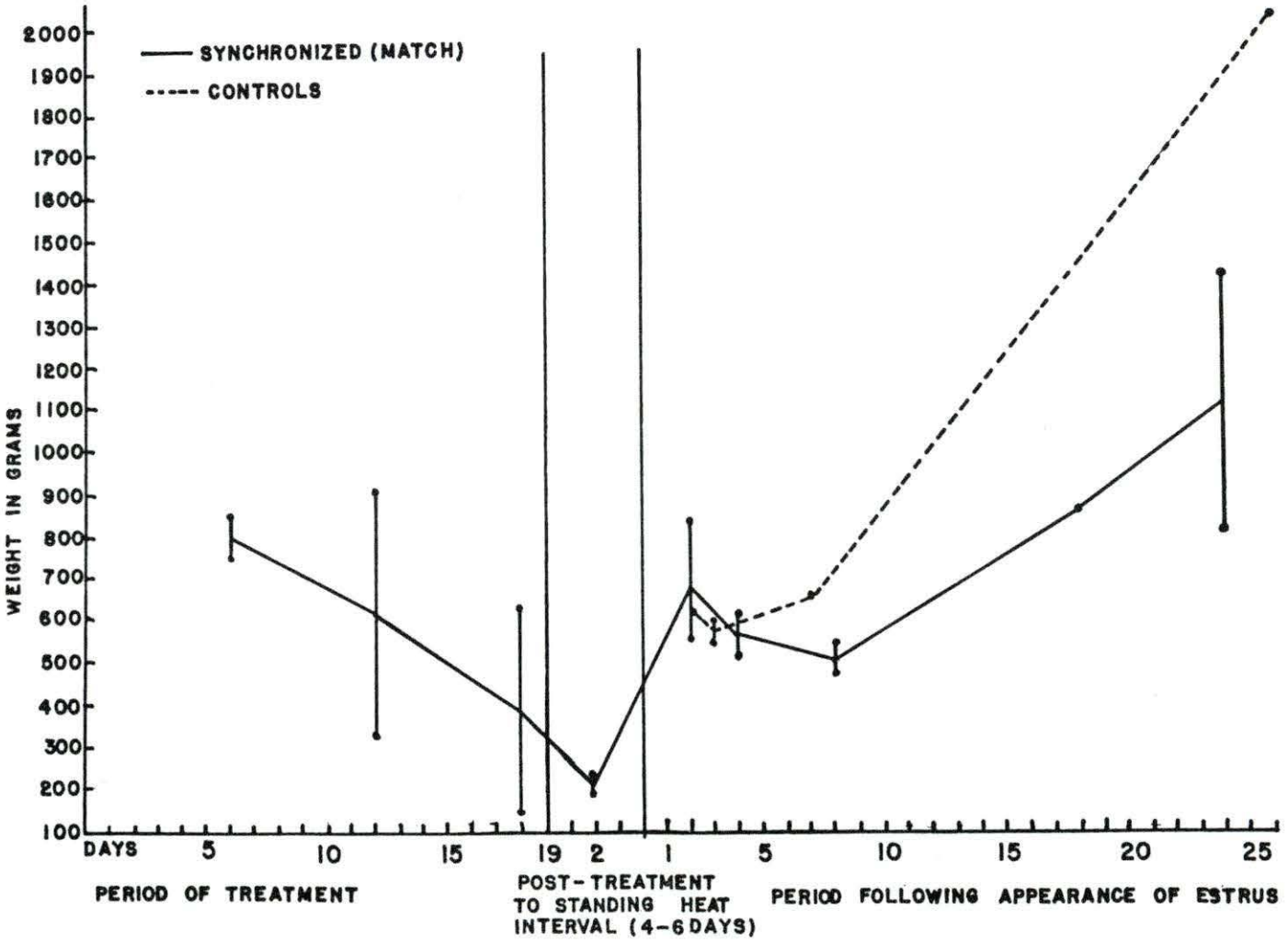
Graph 1. Pituitary weights of experimental gilts.

PITUITARY WEIGHTS OF EXPERIMENTAL GILTS



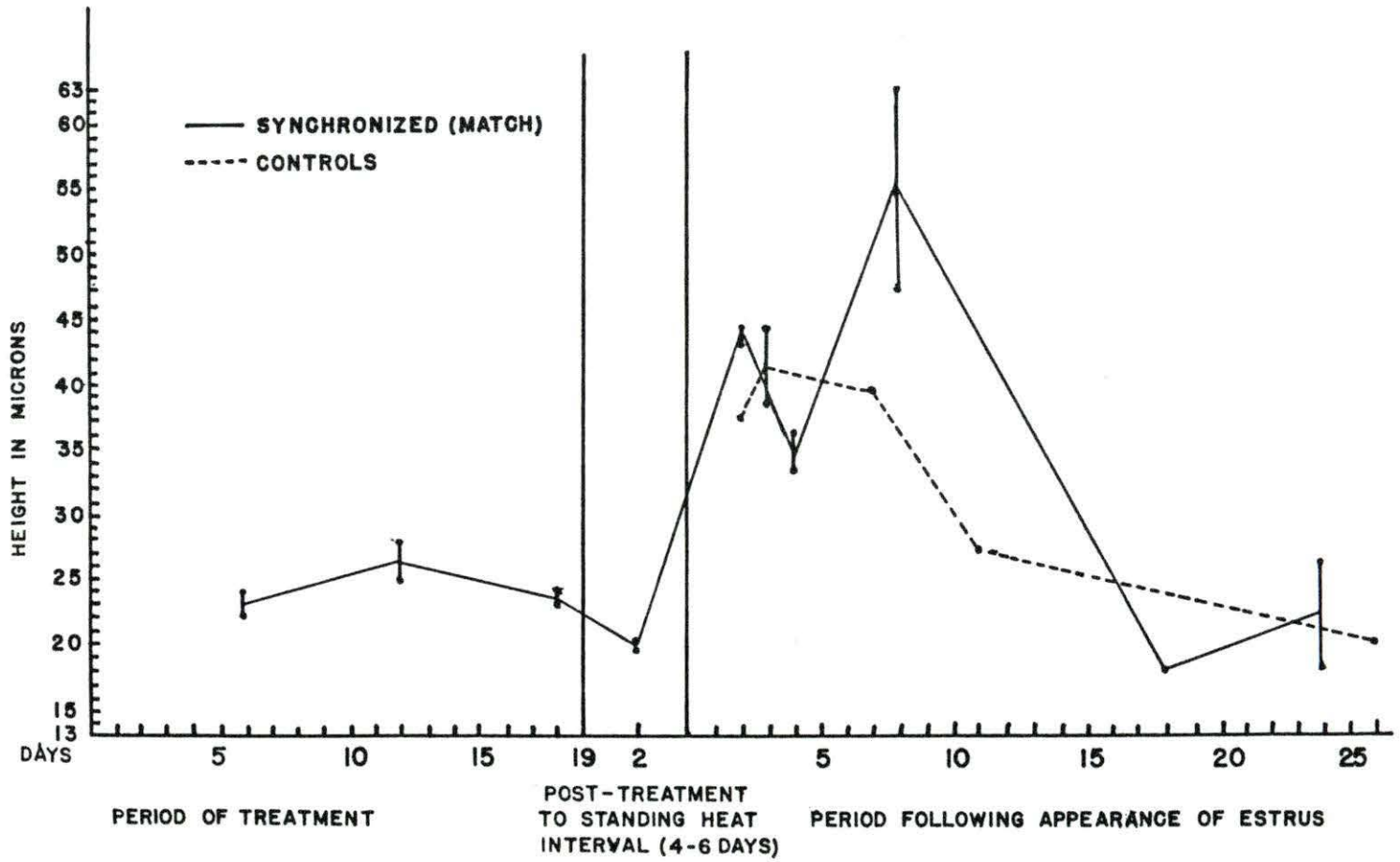
Graph 2. Weight of genital organs of experimental gilts.

WEIGHT OF GENITAL ORGANS OF EXPERIMENTAL GILTS



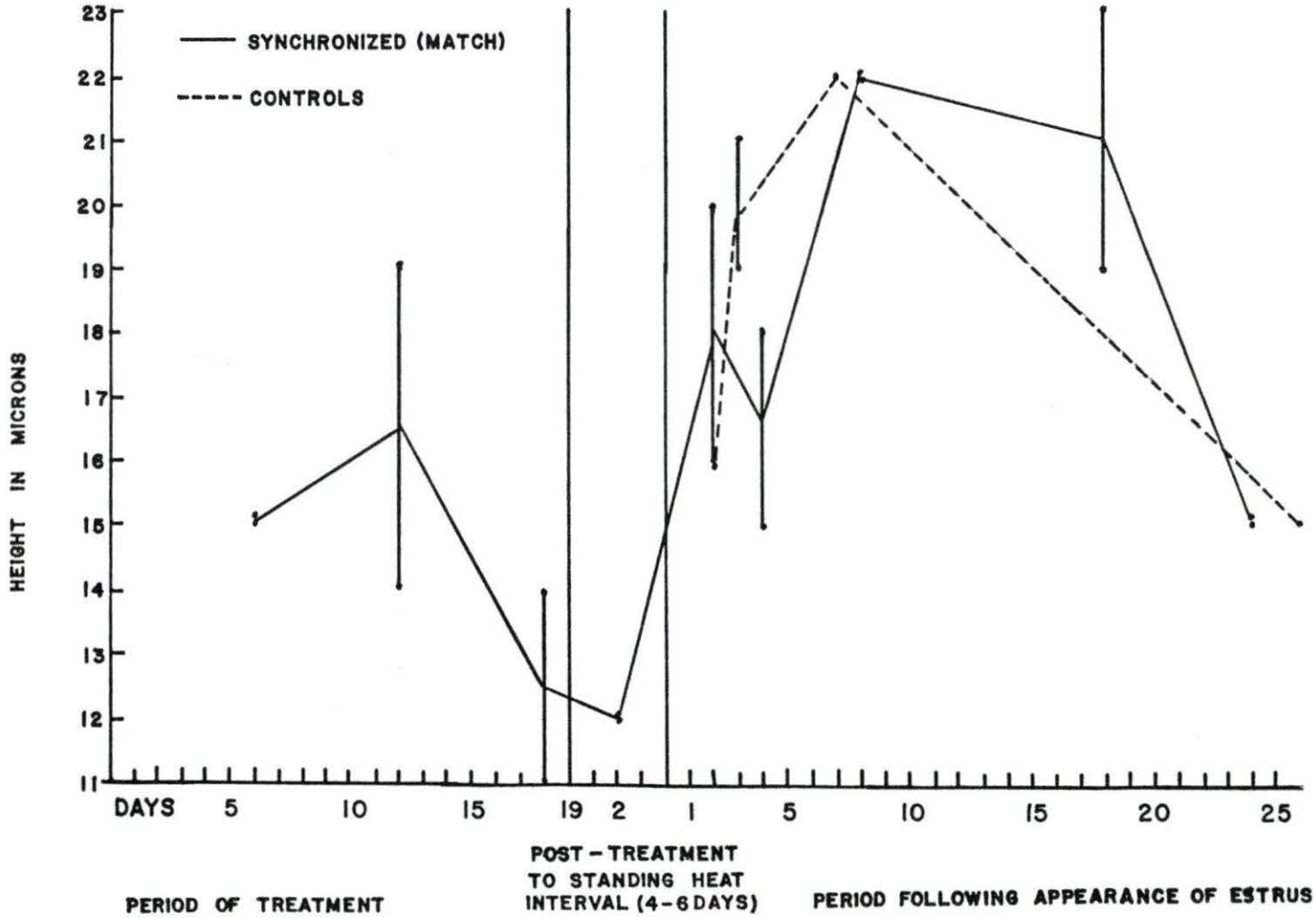
Graph 3. Height of uterine surface epithelium of gilts.

HEIGHT OF UTERINE SURFACE EPITHELIUM OF GILTS



Graph 4. Epithelial height in uterine glands of experimental gilts.

EPITHELIAL HEIGHT IN UTERINE GLANDS OF EXPERIMENTAL GILTS

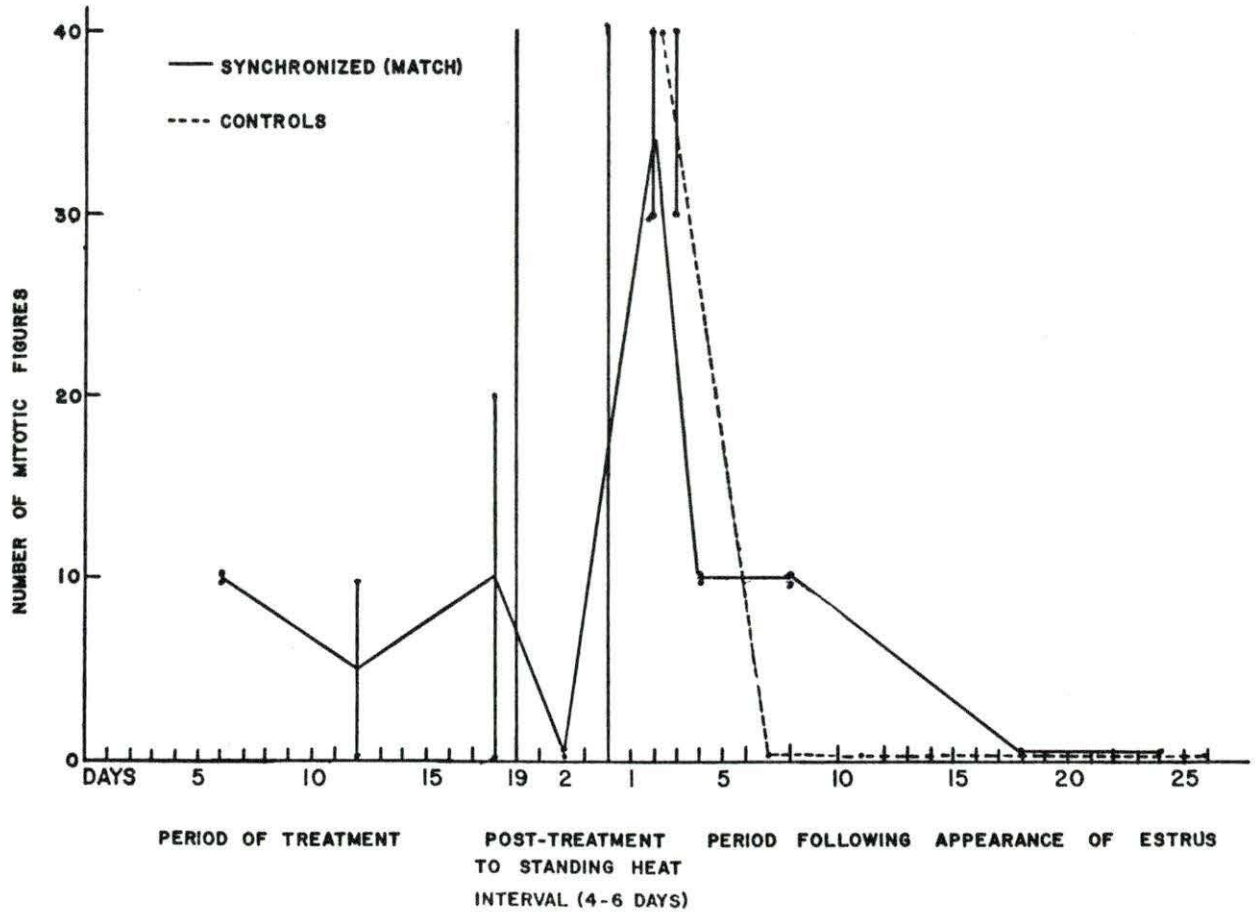


Graph 5. Comparison of observed mitosis in endometrial epithelium of experimental gilts.

Values represent number of mitotic figures estimated during detailed examination of one histological section.

0	0	no observed mitosis
10	1-10	very few mitotis figures
20	11-20	few mitotic figures
30	21-30	many mitotic figures
40	31-40	very many mitotic figures

COMPARISON OF OBSERVED MITOSIS IN ENDOMETRIAL EPITHELIUM OF EXPERIMENTAL GILTS

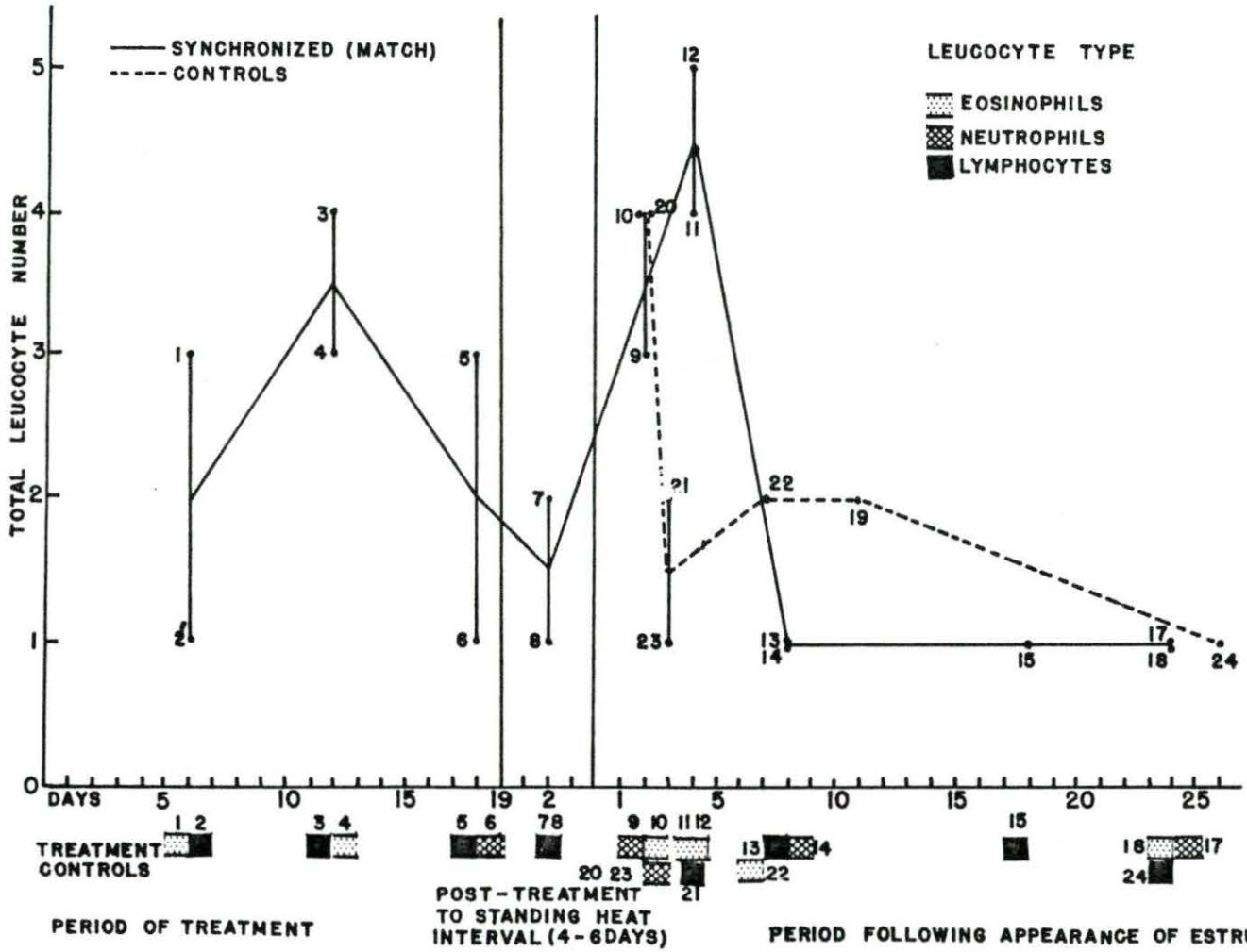


Graph 6. Relative leucocyte number and type in the uterine submucosa of experimental gilts.

Values represent the following:

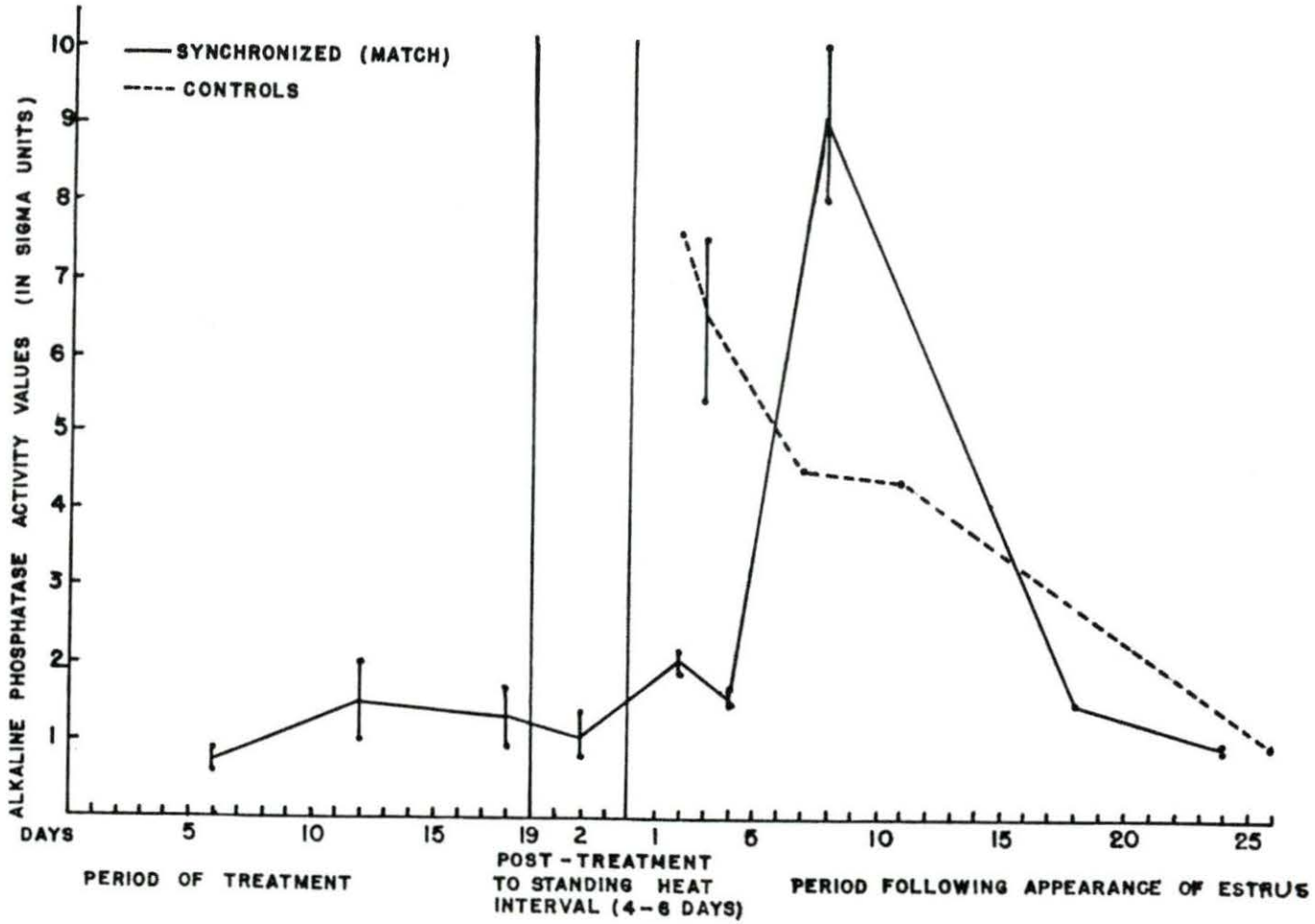
1	very few leucocytes
2	few leucocytes
3	moderate numbers of leucocytes
4	many leucocytes
5	very many leucocytes

RELATIVE LEUCOCYTE NUMBER AND TYPE IN THE UTERINE SUBMUCOSA OF EXPERIMENTAL GILTS



Graph 7. Chemical determinations of alkaline phosphatase activity in endometrium of experimental gilts.

CHEMICAL DETERMINATIONS OF ALKALINE PHOSPHATASE ACTIVITY IN ENDOMETRIUM OF EXPERIMENTAL GILTS

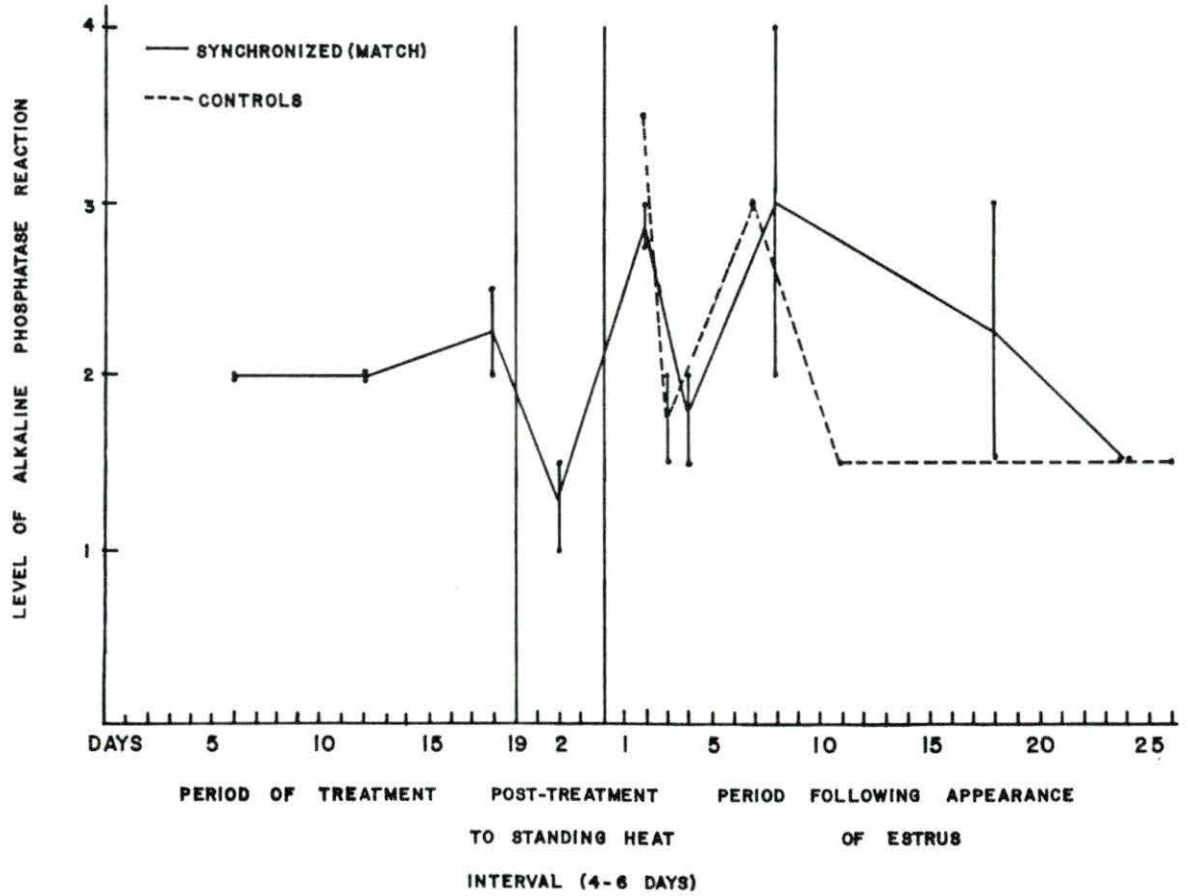


Graph 8. Histochemical estimation of alkaline phosphatase activity in endometrium of experimental gilts.

Evaluation based on the following guides:

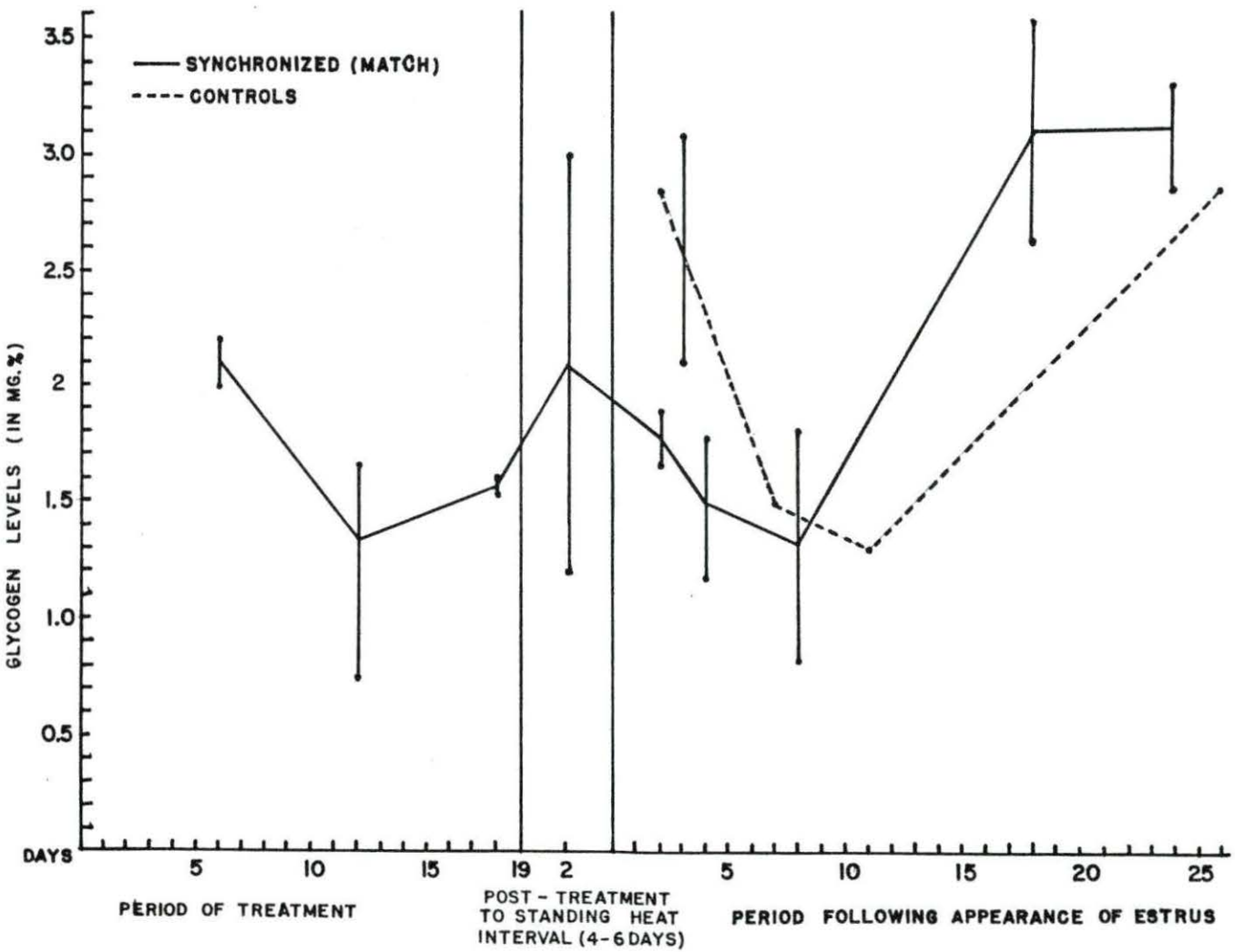
0	no activity
1	slight activity
2	moderate activity
3	intense activity
4	very intense activity

HISTOCHEMICAL ESTIMATION OF ALKALINE PHOSPHATASE ACTIVITY IN ENDOMETRIUM OF EXPERIMENTAL GILTS



Graph 9. Chemical determination of endometrial glycogen in gilts.

CHEMICAL DETERMINATION OF ENDOMETRIAL GLYCOGEN IN GILTS



Graph 10. Histochemical demonstration of glycogen in endometrium of experimental gilts (P.A.S.).

Evaluation based on the following guides:

0	no PAS reaction
1	slight PAS reaction
2	moderate PAS reaction
3	intense PAS reaction
4	very intense PAS reaction

HISTOCHEMICAL DEMONSTRATION OF GLYCOGEN IN ENDOMETRIUM OF EXPERIMENTAL GILTS (P.A.S.)

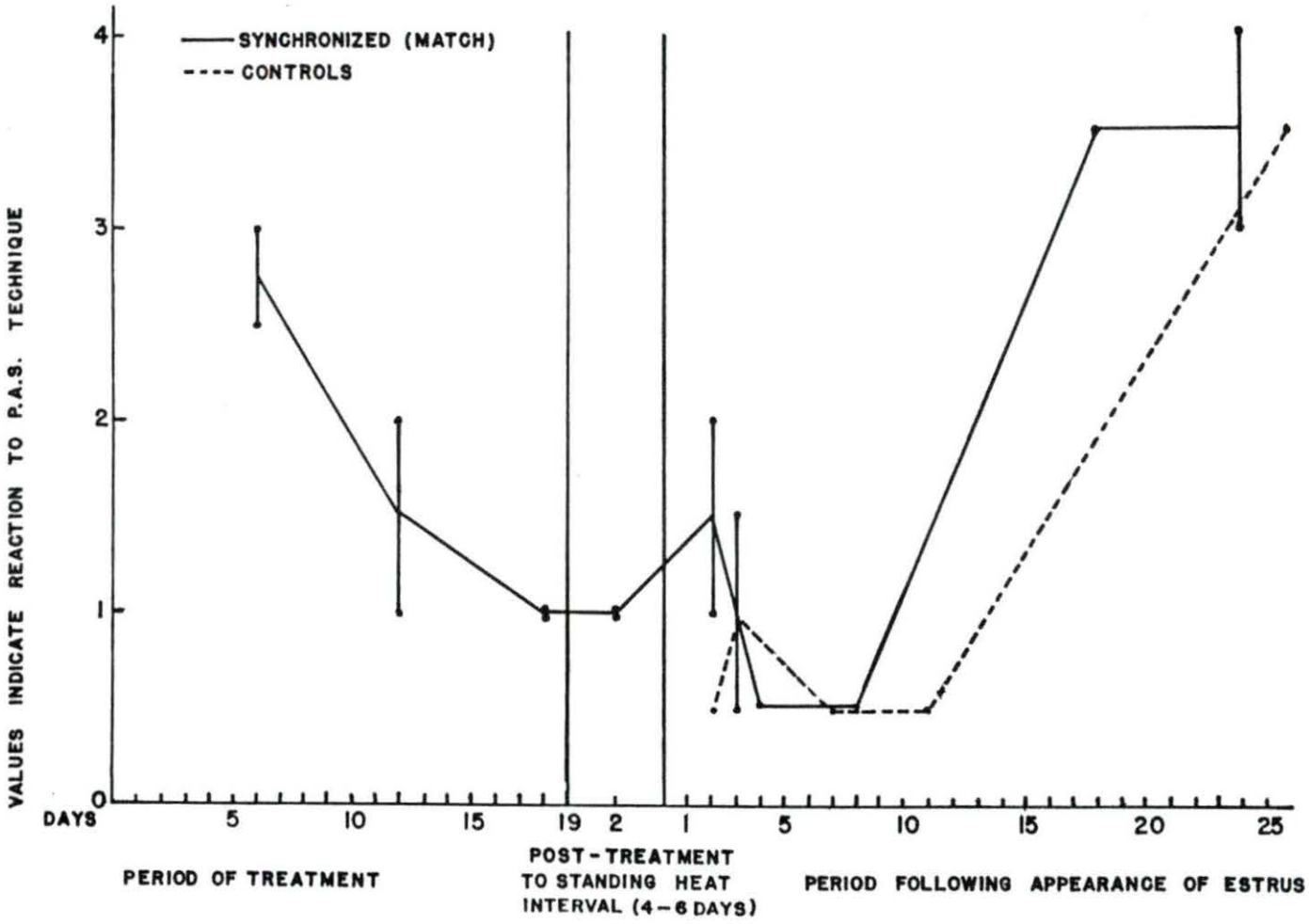
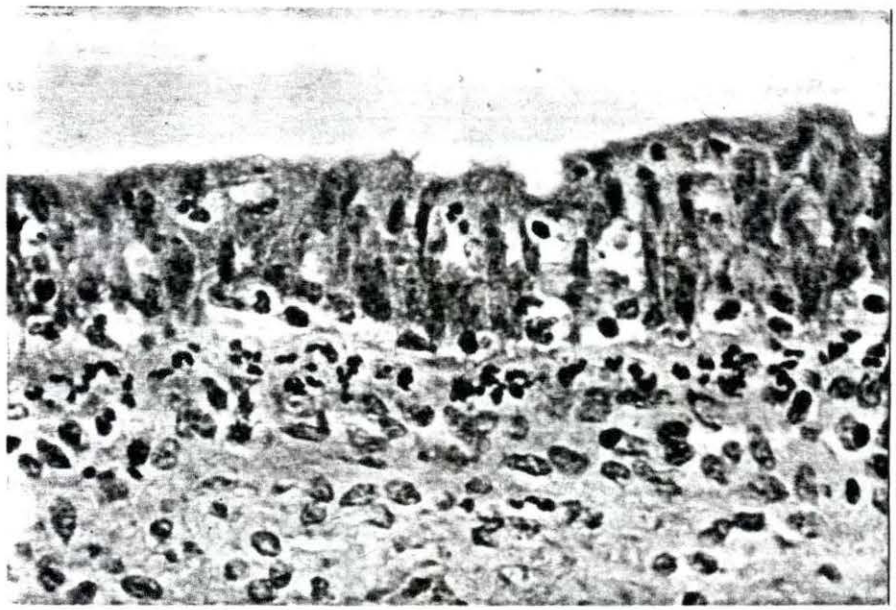


Figure 1. Eosinophils in uterine submucosa near endometrial basement membrane in synchronized gilt no. 12. Stained with hematoxylin-eosin. 420X

Figure 2. Eosinophils in uterine submucosa near endometrial basement membrane in synchronized gilt no. 12. Stained with hematoxylin-eosin. 950X



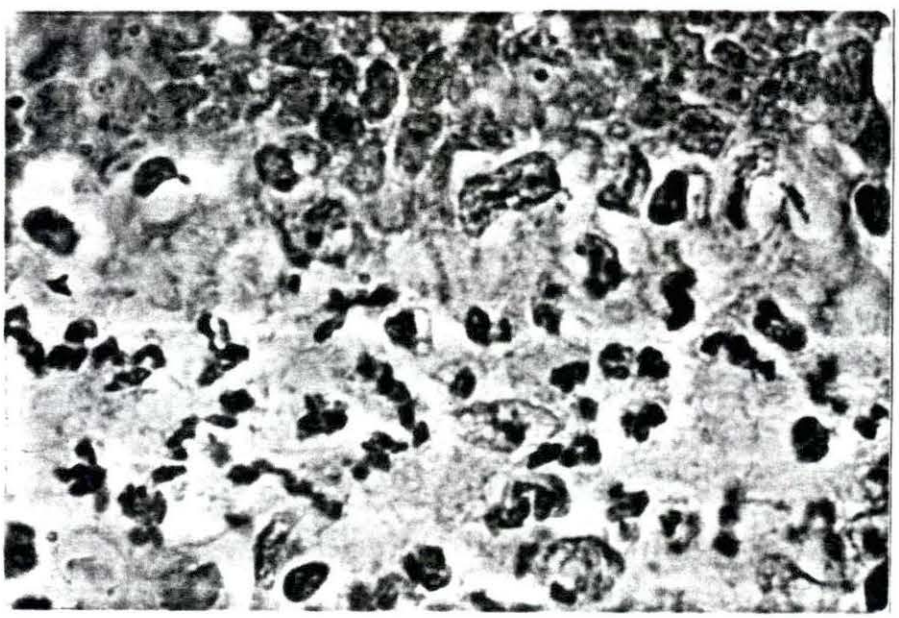
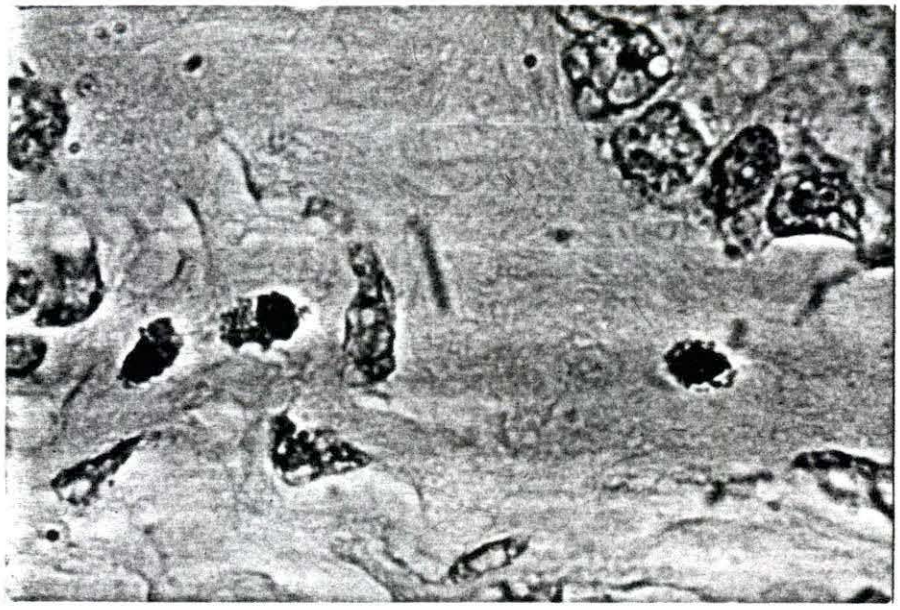


Figure 3. Eosinophils in submucosa near uterine gland (upper right corner) in synchronized gilt no. 12.
Stained with hemotoxylin-eosin stain. 950X

Figure 4. Eosinophils in vaginal discharge in gilt no. 16. Discharge occurred on 5th day after MATCH withdrawal and at 1st indication of standing estrus. Note epithelial cell in upper left corner.
Stained with Wrights stain. 950X



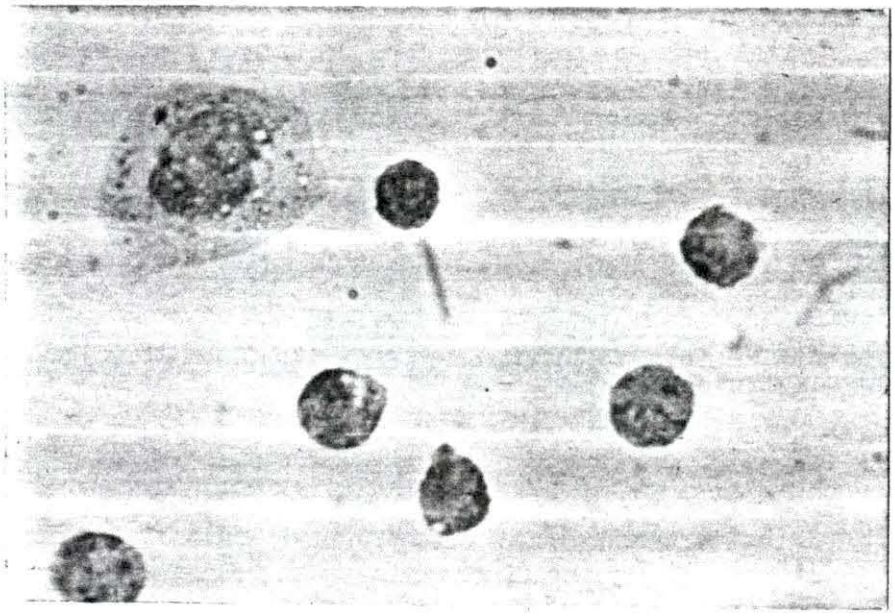
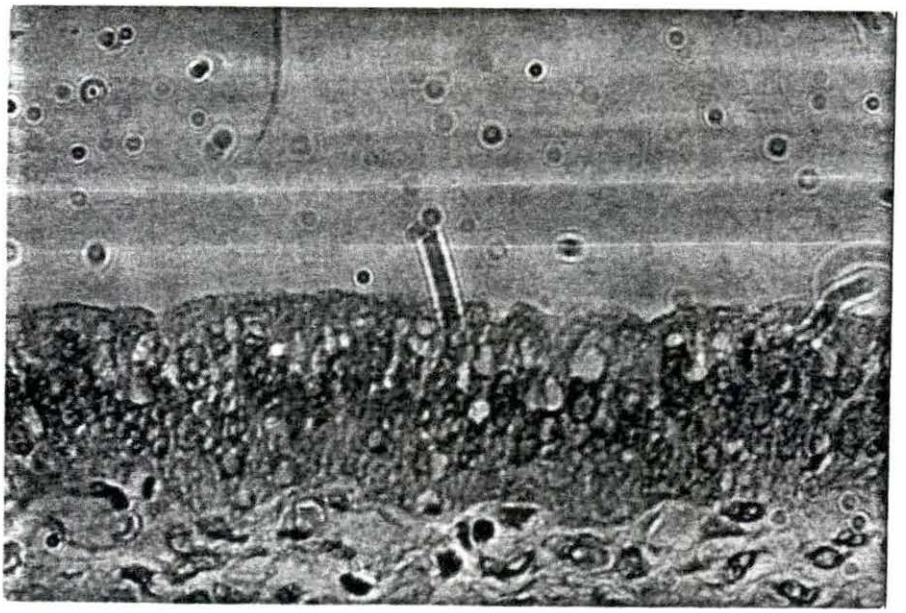


Figure 5. Uterine surface epithelium of gilt no. 22 (control) necropsied on day 7 of the estrus cycle. Compare height of this epithelium to that of gilt no. 81 seen in Figure 6.
Hematoxylin-eosin stain. 420X

Figure 6. Uterine surface epithelium of gilt no. 13 necropsied on day 9 of the synchronized estrus cycle.
Hematoxylin-eosin stain. 420X



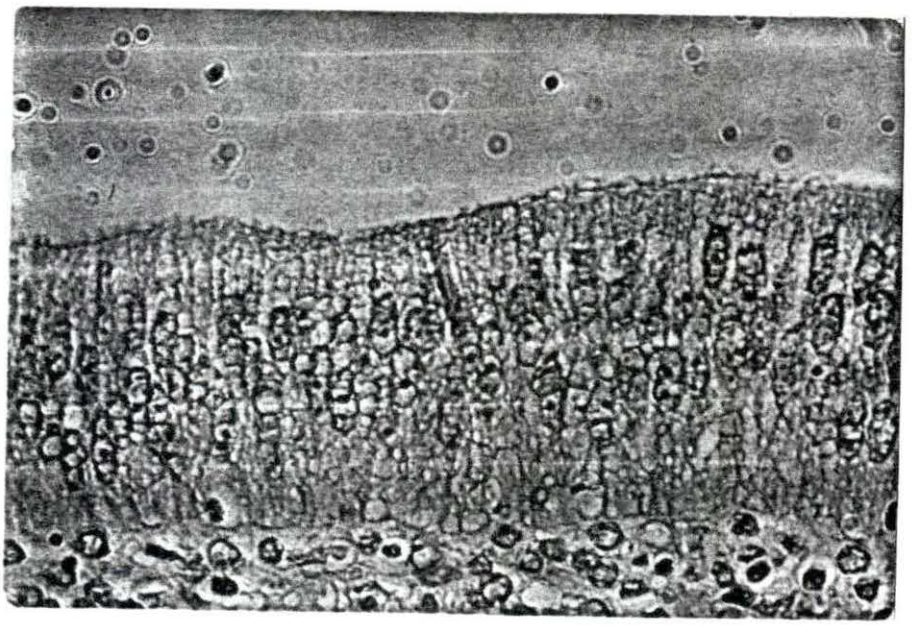


Figure 7. Alkaline phosphatase reaction in the uterus of gilt no. 22 (control) necropsied on day 7 of the estrus cycle. Compare degree of reaction in surface epithelium to that observed in gilt no. 14 seen in Figure 8.
Gomori's alkaline phosphatase technique. 420X

Figure 8. Alakaline phosphatase reaction in the uterus of gilt no. 14 necropsied on the 8th day of the synchronized estrus cycle.
Gomori's alkaline phosphatase technique. 420X