THE EVALUATION OF ESTRUS INHIBITING COMPOUNDS IN SWINE

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COMPOUNDS IN SWINE

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TABLE OF CONTENTS

Chapter		Page
I.	INTRODUCTION	1
II.	REVIEW OF LITERATURE	3
	Corpus Luteum Formation in Swine	3
	Morphologic Aspects	3
	Physiologic Aspects	4
	Luteotropic Factor	4
	Effect of Estrogen on Luteal Function	9
	Effect of Uterus on Luteal Function	10
	Controlled Estrus and Ovulation in Swine	12
	Exogenous Progesterone	12
	Synthetic Steroids	13
	Gonadotropins	16
	Methallibure (I.C.I. 33828)	17
	Methallibure in Combination with Gonadotropins	21
	Conclusion	22
III.	GENERAL PROCEDURE	23
IV.	TRIAL I	29
	Materials and Methods	29
	Results and Discussion	31
V.	TRIAL II	42
	Materials and Methods	42
	Results and Discussion	44
VI.	TRIAL III	54
	Materials and Methods	54
	Results and Discussion	57
VII.	TRIAL IV	66
	Materials and Methods	66
	Results and Discussion	67

TABLE OF CONTENTS (Continued)

Chapter Page

VIII. TRIAL V. 76

Materials and Methods 76
The Method in Detail. 82

Preparation of Tissue. 82
Progesterone Extraction. 83
Thin Layer Chromatography. 84
Development of Plates. 84
Quantitation of Progesterone 85

Results and Discussion. 85

U-10,997 - 50 mg. Daily. 91
U-10,997 - 30 mg. Daily. 97
U-13,851 - 15 mg. Daily. 98
U-23,378 - 250 mg. Daily 99

IX. GENERAL RESULTS AND DISCUSSION 101

U-10,997 101
U-13,851 105
U-23,378 107
U-11,100A. 109
U-10,520A. 111
U-13,053 112

X. SUMMARY AND CONCLUSIONS. 113

LITERATURE CITED 117

APPENDIX 123

LIST OF TABLES

Table		Page
I.	Composition of the Ration Fed During This Series of Trials	25
II.	Design of Study for Trial I.	29
III.	Number of Gilts Showing Estrus During Treatment with U-13, 851, U-10,997, U-11,100A and U-13,053.	33
IV.	Effect of U-13,851, U-10,997, U-11,100A and U-13,053 on Average Daily Gain During the 20 Day Treatment Period. .	33
V.	Means and Standard Errors for Total Ovarian Weight, Number and Size of Corpora Lutea and Uterine Weight for Gilts Necropsied One Day Following Treatment with U-13,851, U-10,997 and U-11,100A	35
VI.	Design of Study for Trial II	43
VII.	Estrus Inhibiting Ability of Compounds U-11,100A, U-10,997 and U-13,851 at Various Dose Levels.	45
VIII.	Effect of Various Levels of U-11,100A, U-10,997 and U-13, 851 on Average Daily Gain During the 20 Day Treatment Period	48
IX.	Ovarian and Uterine Response of Gilts Fed Various Levels of U-11,100A, U-10,997 and U-13,851.	49
X.	Design of Study for Trial III.	56
XI.	Estrus Inhibiting Ability of Compounds U-10,520A, U-23,378 and U-10,997	57
XII.	Effect of U-10,520A, U-23,378 and U-10,997 on Average Daily Gain During the 20 Day Treatment Period.	60
XIII.	Ovarian and Uterine Response of Gilts Necropsied 1 Day after Treatment with U-23,378 and U-10,997	62
XIV.	Reproductive Performance of Gilts Following Withdrawal of Compound U-10,997.	64
XV.	Design of Study for Trial IV	68

LIST OF TABLES (Continued)

Table		Page
XVI.	Estrus Inhibiting Ability of Compounds U-23,378 and U-10,997.	69
XVII.	Effect of U-23,378 and U-10,997 on Average Daily Gain During the 20 Day Treatment Period.	69
XVIII.	Reproductive Performance of Gilts Showing Estrus Within 15 Days Following Treatment with U-10,997	71
XIX.	Reproductive Performance of Gilts Necropsied 1 Day and 15 Days Following Treatment with U-10,997.	73
XX.	Design of Study for Trial V	81
XXI.	Means and Standard Errors for Total Ovarian Weight, Number of Marked Corpora Lutea and Uterine Weight for Laparotomized Gilts Necropsied 1 Day Following Treatment with U-10,997, U-13,851 and U-23,378.	86
XXII.	Progesterone Levels in Gilts Killed 1 and 10 Days Following Treatment with U-13,851 and U-23,378.	89
XXIII.	Effect of U-10,997, U-13,851 and U-23,378 on Average Daily Gain During the 20 Day Treatment Period	92
XXIV.	Reproductive Performance of Gilts Killed 10 Days Following Last Day of Feeding with U-10,997, U-13,851 and U-23,378.	93
XXV.	Reproductive Performance of Gilts Killed Between 10 and 60 Days Following Treatment with U-10,997, U-13,851 and U-23,378.	94
XXVI.	Reproductive Performance of all Gilts Fed the Various Compounds in This Study	102
XXVII.	Structural Formula, Systematic Nomenclature and Activity of all Compounds used in This Study	123

CHAPTER I

INTRODUCTION

Development of a dependable technique for controlling estrus and ovulation in swine, free from any adverse effects on fertility and litter size, would provide distinct advantages to the swine producer. Cycle synchronization would result in a more uniform pig crop, and better use could be made of facilities and labor. Farrowings could be concentrated in a shorter period of time, and the operation of multiple farrowing programs could be facilitated. Also, to have a finishing barn in full production necessitates a regular supply of feeder pigs, which is in turn dependent on a regulated breeding program. A very important advantage is that a target date may be set and following appropriate treatment the animals can be bred without determining whether or not they are in estrus. This would permit the breeding of a large number of females during a short period of time by artificial insemination with semen from superior sires.

Ovulation can be inhibited in swine by progesterone or by orally active progestational compounds, but rather large amounts must be given in order to avoid the formation of cystic follicles. In addition, following withdrawal of the progestogens, estrus is not well synchronized and heats may occur at irregular intervals. The daily administration of methallibure (I.C.I. 33828) in the diet of pigs will inhibit estrus and ovulation and cystic follicles do not occur. Ovulation occurs spon-

taneously during the post-treatment estrus. In addition, the administration of methallibure and appropriately timed doses of pregnant mare's serum (PMS) plus human chorionic gonadotropin (HCG) permits precise control over the time of ovulation in pigs. Insemination shortly before the time of this induced ovulation results in high fertility and normal embryonic survival. The application of this latter technique could influence the practicability of controlled breeding and artificial insemination in pigs.

This study was conducted to determine the effects on normally cycling gilts of six compounds with estrus inhibiting properties. Three of these compounds were steroidal in structure and three were non-steroidal. These compounds were produced by The Upjohn Company, and their ability to inhibit estrus in species other than swine had previously been determined. This study evaluated their effectiveness in inhibiting estrus and ovulation in puberal gilts. In addition, those compounds which inhibited estrus were further evaluated for their effectiveness in synchronizing estrus and ovulation.

CHAPTER II

REVIEW OF LITERATURE

Corpus Luteum Formation in Swine

An understanding of mechanisms controlling formation of corpora lutea during the estrus cycle, and particularly factors affecting their persistence and regression is essential for developing effective methods for control of the estrus cycle in swine. Although there are still many blank spaces in the present pattern of knowledge concerning factors that determine the life of the corpus luteum in swine, the one distinctive feature is its relatively short life span. The following discussion describes some of the literature associated with the causes of luteal ephemerality.

Morphologic Aspects

Corner (1915, 1919 and 1921) described the developmental morphology of the corpus luteum in the sow. Following rupture of the Graafian follicle and escape of the ovum, the walls of the follicle usually collapse around a central blood clot and reduce the diameter to 4-6 mm. Following rupture of the Graafian follicle the granulosa layer is retained intact, except for the loss of the cumulus oophorous. The granulosa cells hypertrophy, their cytoplasm becomes laden with lipoid substances, and they form the larger luteal cells of the mature corpus luteum. On day-3 or 4 following the onset of estrus, the granulosa

layer is invaded by capillaries from the theca interna which vamify to form an extensive vascular plexus throughout the new structure. The lipoid-laden theca interna cells increase in number and pass into the corpus luteum. They become lodged between the granulosa cells and possibly persist throughout the functional life span of the gland. This process is completed at about day-6, and by day-7 the corpora lutea are usually solid and the cells are fully differentiated (Corner 1915, 1919).

Corner (1921) observed that in the pig during the week following ovulation, corpora lutea increase from a diameter of 4-6 mm. to 8-9 mm. If pregnancy follows, growth continues for 2 or 3 weeks until an average diameter of 10 to 11 mm. is reached. The corpora lutea attain complete organization on about day-7 and retain this development until day-14 or day-15. At approximately day-16 of the cycle a change takes place in the corpora lutea of animals with unfertilized ova. By day-18 the corpora lutea decrease to about 6 mm. in diameter and their pink color, indicative of active capillary circulation, changes to the white color characteristic of scar tissue, and their texture becomes tougher and firmer. By the time of ovulation, the regressed corpora lutea have diminished in diameter to 6 mm., by the midestrual period to 4 mm. and by the next ovulation to 2 mm. after which they disappear slowly. Eventually, all that remains is a small mass of scar tissue.

Physiologic Aspects

Luteotropic Factor

A definition of the luteotropic process has not been universally agreed upon. However, Rothchild (1966) defines this process as one which promotes growth of the corpus luteum and a rate of progesterone

secretion sufficient to prevent ovulation and/or to permit implantation.

According to Rothchild's definition, the secretion of prolactin is evidently an essential part of the luteotropic process in the rat. Drexel (1935) first identified the luteotropic activity of the mouse pituitary with the secretion of prolactin and Astwood (1941) and Evans et al. (1941) identified it in the rat. Within relatively recent years however, an accumulation of evidence suggests that prolactin is not luteotropic in species other than the rat or mouse. Donaldson et al. (1965) summarized the available evidence and concluded that attempts to demonstrate the luteotropic action of prolactin in guinea pigs, rabbits, cattle, sheep, goats and swine were either unsuccessful or unconvincing.

There is no experimental evidence which indicates that prolactin has luteotropic action in swine, either in vivo or in vitro. Sammelwitz and Nalbandov (1958) first reported that the daily administration of 25 or 50 mg. of prolactin failed to prevent the regression of corpora lutea in progesterone-treated-pregnant gilts. Duncan et al. (1961) reported that slices of porcine corpora lutea incubated with ovine lactogenic hormone (prolactin) showed no stimulation of progesterone synthesis. The use of prolactin obtained from sheep complicated the interpretation of these results since it is possible that corpora lutea of certain species may respond to a prolactin derived only from the same species. However, Cook et al. (1967) reported that incubation of porcine luteal slices in the presence of porcine prolactin did not stimulate progesterone synthesis. The mean progesterone concentration of 7 gilts after incubation with porcine prolactin was 196 ug./gm. corpus luteum as compared to control values of 200 ug./gm. corpus luteum. Although prolactin did not stimulate progesterone synthesis, the addi-

tion of porcine LH to the incubation medium gave a significant increase ($P < 0.001$) in progesterone concentration above control levels. The mean progesterone concentration for 7 gilts after incubation with porcine LH was 222 ug./gm. corpus luteum. This work indicates that LH is steroidogenotrophic but it does not imply that it is luteotrophic.

Even though prolactin is not luteotrophic in pigs, the prolonged maintenance of functional activity of the corpora lutea during the luteal phase of the estrous cycle, during pregnancy and after hysterectomy, suggests that a luteotropic hormone may be produced in these animals. Spies _et al._ (1959) reported that in pregnant gilts, daily injections of high levels of progesterone during early gestation caused a decrease in the average weight of corpora lutea or completely destroyed them. The daily hormone dosage was 0.4 mg. per lb. of body weight injected from day-10 to day-25 of gestation. Sammelwitz _et al._ (1961) also showed that progesterone injections cause complete or near complete degeneration of corpora lutea of pregnant pigs. This luteolytic effect of progesterone apparently is not the result of direct action of progesterone on the corpora lutea since Spies _et al._ (1960) found that local injections of 4 mg. of progesterone directly into the corpora lutea on day-18 of pregnancy produced no measurable influence on the average individual corpus luteum weight of 2 gilts at day-25 of pregnancy.

Progesterone is also luteolytic in hysterectomized pigs in which corpora lutea can persist for prolonged intervals. Spies _et al._ (1960) studied the effect of progesterone injections on maintenance of the corpora lutea in hysterectomized gilts. All animals were bred on the first day of estrus (day 0), hysterectomized on day-7 of the estrous cycle and slaughtered on day-25. The average individual corpus luteum

weight for 8 hysterectomized gilts which did not receive progesterone was 421 mg., compared to a mean value of 181 mg. for hysterectomized gilts injected with progesterone from day—10 to slaughter. From these facts, it is possible to infer that progesterone acts independently of the uterus and probably blocks the secretion of a hypophysial luteotropic substance.

In contrast to the results obtained from pregnant pigs with formed corpora lutea, progesterone injections begun at the time of ovulation and continued throughout the luteal phase of the estrous cycle will not prevent the formation and maintenance of the cyclic corpora lutea. Sammelwitz et al. (1961) found that exogenous progesterone did not induce luteal regression during the first 12 days of the cycle; however, it was effective in causing luteal regression between days 12 and 16 in previously mated pigs. Brinkley et al. (1964) reported that injecting pigs with large doses (400 mg.) of progesterone either 1 or 2 days before ovulation, on the day of ovulation, or 1 day after ovulation, did not prevent the formation and maintenance of corpora lutea for 14 days--the normal duration of the luteal phase in pigs. Considering the large dose of progesterone used; it is reasonable to assume that hypophysial blockage was established, possibly within 24 hours after the first progesterone injection. This suggests that a hypophysial block put into effect any time after ovulation is too late to block the initial release of the luteotropic substance. These workers also found that in spite of continuous hypophysial blockage for 7 to 10 days, the formed corpora lutea contained near normal amounts of progesterone at autopsy. From these experimental results, it may be postulated that progesterone is not luteolytic in the luteal phase of the cycle. Formation and maintenance

of corpora lutea in the presence of continuous progesterone treatment suggests that progesterone does not have a direct action on corpora lutea.

Anderson et al. (1967) observed that corpora lutea developed to day 12 of the cycle in pigs pituitary stalk-sectioned the day after the first day of estrus. This operation should have blocked the release of pituitary gonadotropins. The corpora lutea averaged 305 ± 32 mg. and contained 64 ± 4 ug. progesterone per gm. of tissue at day-12 which indicated that they had been subjected to a luteotropic stimulus. By day-16 a slight decline in corpora lutea weight occurred (260 mg. average) whereas the progesterone concentration showed a great reduction (3 ug./gm.). The uterine luteolytic mechanism was probably being initiated at this time. In pigs pituitary stalk-sectioned the day after mating, pregnancy continued and corpora lutea were present (average weight 431 mg.; 54 ug. progesterone/gm. tissue) at day-12. By day-16 the corpora lutea averaged 292 mg. and contained 28 ug. progesterone/gm. of tissue. The conceptus apparently prevented uterine luteolytic activity. These experimental results suggests that if a pituitary luteotropin is required during the first 12 days of the cycle or pregnancy, only initial pituitary support is essential at estrus, or even prior to estrus or ovulation.

On the basis of the data of Sammelwitz et al. (1961), Brinkley et al. (1964) and Anderson et al. (1967) it appears that in pigs, the single release of a luteotropic substance at or near the time of ovulation is sufficient to cause the corpus luteum to form and to persist and function for its normal life span during the cycle. Also, the additional luteotropic substance required to maintain the corpora lutea for the remainder of the gestation apparently is released only if conception and

implantation occurs and it is this second, possibly continuous, release of luteotropic substance that is blocked in pregnant pigs by progesterone injections. Although the porcine luteotropic hormone has not been isolated, it is possible that the luteotropic activity of the hypophysis is incorporated into one of the known hypophysial hormones--possibly LH. The work by Cook *et al*. (1967) indicated that LH was steroidogenotrophic but did not imply that it was luteotrophic. Also, stimulation of progesterone synthesis by LH *in vitro* does not imply that a continuous secretion of LH is necessary for the corpus luteum to function *in vivo*. Thus, at the present time, the evidence still does not indicate whether or not a single hypophysial substance is both luteotropic and steroidogenic, or whether these activities are the result of two different hormones.

Effect of Estrogen on Luteal Function

Results of investigations in pigs showed that exogenous estrogen influences the function of corpora lutea. Kidder *et al*. (1955) injected gilts with 3 mg. of diethylstilbestrol on either day-6, day-11 or day-16 of the cycle. Injections on day-11 lengthened the estrual cycle significantly, and this apparently resulted from lutenization of follicles since this condition occurred in all gilts slaughtered following injection at this time. The ovaries of 5 gilts injected on day-11 and killed on day-15 had from 2 to 18 corpora lutea, 10-12 mm. in diameter, which appeared to be normal. All 5 gilts had from 1 to 3 lutenized follicles which varied in size from 12 to 35 mm. Injections on day-16 varied in effect but most frequently caused a significant shortening of the cycle.

Gardner *et al*. (1963) reported that 19 of 20 gilts injected daily

with 7.5 or 15 mg. of estrone or estradiol 17β per day beginning on day 11 of the cycle maintained their corpora lutea until slaughter at 34 days postestrum. Comparison of treated gilts with control gilts showed that estrogen decreased ($P < .01$) the average corpus luteum weight and increased ($P < .01$) the progesterone concentration in the treated corpora lutea. The net effect was an insignificant difference in total progesterone content of the corpora lutea due to estrogen treatment.

The work by Gardner et al. (1963) suggested that estrogen is luteotropic in the pig. Cook et al. (1968) attempted to confirm this observation by incubating slices of porcine corpora lutea in the presence of the three classical estrogens estrone, estadiol and estriol. The progesterone measured at the end of incubation concentration showed that none of the estrogens influenced progesterone synthesis. These results offer no evidence for a steroidogenic role for estrogen in porcine luteal tissue. It is possible that estrogen could exert its luteotropic effect by maintaining the integrity of the structure of the corpus luteum, thus enabling progesterone synthesis to continue without enhancing steroid production.

The luteotropic effect of estrogen *in vivo* in pigs appears to be mediated through the pituitary gland because estrogen administration will not maintain corpora lutea in hypophysectomized animals (Denamur, 1968).

Effect of Uterus on Luteal Function

The uterus plays an active role in the control of luteal function through initiation of luteolysis during later stages of the estrous cycle. Spies et al. (1960) reported that hysterectomy prolongs the life

span of the corpora lutea of pigs. They found that corpora lutea were maintained 32 to 119 days in pigs hysterectomized at day-7 of the cycle.

The inhibition of cycling and maintenance of corpora lutea in the pig are also affected by the amount of uterus retained following partial hysterectomy. Furthermore, the quantity of uterus retained determines whether regression of corpora lutea results. The effect of different portions of one non-gravid uterine horn on the occurrence of pregnancy was investigated by Anderson et al. (1966). These workers observed that the presence of almost an entire non-gravid uterine horn reduced conception or interfered with the progress of early pregnancy. Only 4 of 25 (15%) pigs remained pregnant at least 35 days when the anterior seven-eights of one non-gravid uterine horn remained. With decreasing portions of the non-gravid horn remaining, an increased number of pigs remained pregnant. Unilateral regression of corpora lutea on the side of the non-gravid uterine horn occurred in 12 of 15 (80%) pigs by day-35 of gestation, when either seven-eights, one-half or one-fourth of one non-gravid horn remained in the animal. With complete absence of a non-gravid horn, pregnancy was maintained in 8 of 11 (73%) pigs for 35 days, and unilateral luteal regression occurred in none of these pregnant animals. These experiments demonstrated an overriding inhibitory effect of a non-gravid uterine horn on the continuation of early pregnancy in the opposite intact uterine horn. The evidence suggests that the non-gravid horn terminates pregnancy through a luteolytic action which it initiates.

The mechanism by which hysterectomy prolongs the functional life span of the corpus luteum in the pig is not known and the nature of the uterine luteolytic effect in the pig is also unknown. There is no concrete evidence that the uterus produces an active luteolytic factor.

Controlled Estrus and Ovulation in Swine

Experimental results have been reported concerning regulation of female reproduction in swine following administration of natural and synthetic steroids, as well as gonadotropins of pituitary and placental origin. Anderson (1964) stated that compounds for control of estrus must meet several requirements before they can be recommended for use under farm and range conditions. These compounds must (1) control estrus and ovulation when administered at different reproductive stages, (2) be effective at dosage levels which produce predictable results, (3) effectively synchronize estrus and ovulation, (4) not impair fertilility, (5) permit a uterine environment compatible with embryonic survival, and (6) not interfere with later reproductive potential. Of these requirements, unimpaired fertility following the treatment period would be considered the most important. Effective synchronization of the estrous cycle has been accomplished with various compounds and treatment procedures.

Exogenous Progesterone

Estrus and ovulation in gilts have been successfully inhibited by daily injections of progesterone. Ulberg (1951) reported that daily injections of 25.0, 50.0 and 100.0 mg. progesterone inhibited heat and ovulation when injections were started on day 15 of the estrous cycle and continued through day 28. However, post-treatment ovulation was consistently complete only after injections at the 100 mg. level while a high percentage of the ovaries in gilts receiving 50 mg. daily became cystic. Gilts injected with 100 mg. came into estrus an average of 6.4 days after injections were stopped but animals receiving 50 mg. showed

no heat during an observation period of 7 to 26 days post-treatment. Baker et al. (1954) obtained similar results but also found that the fertility of gilts on the 25 mg. dose was extremely low with only 1 gilt of 9 returning to estrus following injection and 7 of the 9 had cystic follicles when slaughtered. No cystic follicles developed on ovaries of 18 gilts injected with 100 mg., but the average number of normal 25 day old embryos was significantly lower than controls.

Gerrits et al. (1962) found no adverse effects on fertility from injected progesterone. Eleven gilts synchronized with 100 mg. of progesterone per day exhibited estrus on the average 6.3 days after last injections. Eleven gilts synchronized by daily injections and mated by natural service had a conception rate of 82%. Twenty gilts synchronized by 300 mg. injections once every 3 days and mated by natural service had a conception rate of 80%, and an average litter size of 12.0. Average interval to estrus was 7.4 days from last injections.

Synthetic Steroids

The high incidence of cystic follicles and the low fertility levels encountered with progesterone injections resulted in researchers shifting their attention away from progesterone when orally effective synthetic progestational compounds were developed. The oral administration of these compounds eliminated the inconvenience of daily injections, but they have not produced a consistent satisfactory response. Successful synchronization of the estrous cycle has been obtained, but again cystic follicles often occur.

Nellor (1960) reported that twice a day feeding of 1.6 mg. of MAP (6-methyl-17-acetoxyprogesterone) per lb. body weight daily for 24 days

resulted in complete inhibition of estrus and follicular growth in 8 mature gilts. Estrus occurred, on the average, 4.4 days from the end of treatment. Autopsy following the controlled estrus demonstrated that ovulation had occurred and no apparent abnormalities were noted. Oral administration of Prodox (17-α-acetoxyprogesterone) to 11 gilts during late luteal or early follicular phase inhibited estrus in only 8 animals. First et al.(1960) also reported that 66 of 68 gilts orally fed MAP for 15 days exhibited standing estrus, and 94% began estrus during a 43 hour period of 77 to 120 hours after the last hormone feeding. The animals were artifically inseminated and 25 were pregnant on day 25 of gestation.

Nellor et al. (1961) obtained a high degree of control of the time of estrus and ovulation when 36 gilts were fed 0.5 mg. of MAP per lb. body weight daily, which was administered by twice-a-day feeding for 15 days. Eighty-nine percent of the gilts came into estrus 4 to 5 days after the end of progestational treatment. The conception rate in gilts bred at the controlled estrus was 77%. Only 2 cases of follicular cysts were observed in these 36 gilts, but a very high incidence of follicular cysts occurred when 1.6 mg. of MAP per lb. of body weight were fed.

First et al. (1963) studied the factors affecting ovulation and follicular cyst formation in sows and gilts fed MAP. In 5 trails involving 193 sows and gilts, they found that doses of 100 mg. or greater were required to inhibit estrus and ovulation. One or more cystic follicles developed in 58% of the females after withdrawal of 8 different hormone doses ranging from 50 to 400 mg. per head per day. Cystic follicles developed during treatment when a low dose of 60 mg. was fed and after treatment when the dose was 240 mg. After withdrawal of a 240 mg. dose, Yorkshire sows developed cystic follicles; whereas Duroc sows had

developed preovulatory follicles. Neither frequency of feeding MAP, the energy content, nor the fiber content of the ration affected the probability of ovulation or cystic follicle formation after hormone withdrawal.

Duzik and Baker (1962) stated that cystic follicles were not found up to 18 days after hormone withdrawal in gilts that had been given a very large dose of 500 mg. MAP per day. Hafez et al. (1966) fed 31 gilts a daily dose of 1.1 mg. MAP per kg. of body weight and estrus was suppressed in all gilts during the treatment. Although 48% came into estrus 2 to 10 days after the end of treatment, only 36% of the gilts ovulated. Cystic follicles with a diameter exceeding 15 mm. (51%) and unruptured follicles with a diameter of 10 to 15 mm. (12%) accounted for most of the ovarian abnormalities encountered. Of the gilts with cystic ovaries, 63% were not detected in estrus.

Wagner and Seerly (1961) reported the results of orally administered 6-chloro. Δ^6 17 acetoxyprogesterone (CAP) to 118 breeding gilts at various levels (3.25, 16.5, 25.0, 32.5, 50.0, 97.5, and 540 mg.) for 18 days. Treatment of 25.0, 32.5, and 50 mg. inhibited both estrus and follicular growth. However, 20% of the gilts had cystic follicles 5 days after withdrawal of the hormone with greater incidence in the higher treatment levels.

Pond et al. (1965) reported that an addition of 0.66 or 1.10 mg. of AMP (17-α-acetoxy-6-methylpregna-4, 6-dien-3, 20-dione) per kg. of body weight to the ration for a 15 day period was effective in inhibiting estrus in 22 gilts. Twenty one of the gilts showed signs of estrus within 1 week after hormone withdrawal. Ten of these gilts were slaughtered 2 or 4 days following breeding and 5 had cystic ovarian follicles with the highest incidence occurring with gilts on the 0.66 mg. level.

Gonadotropins

In pigs there have been numerous experiments on the use of gonadotropic hormones in order to stimulate follicular development and induce ovulation. However, induction of ovulation occurred usually without estrus in gilts and sows given gonadotropin injections during the luteal phase of the cycle. (Tanabie et al. 1949; Spalding et al. 1955; Day et al. 1959).

Dziuk and Baker (1962) injected 275 gilts with 250 to 2,000 I.U. of HCG 5, 6, 7 or 8 days after treating them with MAP. Ovulation occurred in 94% of the animals 40 hours after injection. Four percent of the animals showed estrus at this ovulation while 71% showed heat 21, 22, or 23 days later. Dziuk and Polge (1965) obtained similar results, but also found that injections of diethylstilbestrol improved fertility. Only 20 of 45 gilts or 45% had fertilized eggs if DES was not given. In 32 gilts treated with DES, the overall conception rate was 75% even though full estrus behavior was induced in only 43% of these gilts. The DES apparently increased the number of spermatozoa transported up the oviduct which in turn increased fertilization rates. However, of 35 gilts treated with DES, only 11 were pregnant when examined at 30 days. The DES apparently created an unfavorable uterine environment and caused embryonic loss.

Mammalian eggs induced to ovulate during the luteal phase of the cycle or after progesterone injections rarely become fertilized, but the precise cause of this low incidence of fertilization is not clear. Hunter (1966) injected mature gilts with 1,500 I.U. PMS on day—5 of estrous followed by 500 I.U. HCG on day 9; ovulation occurred 40 to 42 hours later. A total of 160 eggs were recovered from 14 of 26 animals

inseminated 4 to 18 hours before ovulation; 60.6% were polyspermic and contained varying numbers of accessory male pronuclei (two to seven). It was suggested that the high incidence of polyspermic eggs found during the luteal phase may be due to an effect of the oviductal environment on the zona reaction.

Polge and Day (1967) also reported that exogenous progesterone given before ovulation reduced fertility by reducing the proportion of eggs fertilized, increasing the incidence of polyspermy, and increasing the rate of egg transport through the female tract.

The experimental evidence does suggest that eggs can be fertilized when animals are inseminated at the time of induced ovulations, but normal fertility and embryonic survival are achieved only if ovulation is induced when active corpora lutea are absent from the ovaries. Synchronization of estrus with progestational compounds followed by gonadotropins to induce ovulation is not a satisfactory method since a low fertility ensues and ovulation is rarely accompanied by estrus after such treatment. Thus, at the present time, no really effective and practical method for using steroid hormones and gonadotropins for the synchronization of estrus in swine has been developed.

Methallibure (I.C.I. 33828)

The daily administration of methallibure (a dithiocarbamoylhydrazine derivative) in the diet to mature gilts has been shown to completely inhibit the normal occurrence of estrus and ovulation. Following withdrawal of treatment, a large proportion of the animals came into estrus spontaneously between the fifth and seventh day. When mated or inseminated during this controlled estrus, fertility and litter size were

normal. Certain side effects have been observed in treated animals, but they apparently do not interfere with suppression of follicular growth.

Methallibure is a non-steroid whose estrus inhibiting properties were first observed by Paget et al. (1961) who reported that this compound inhibited the endogenous output of gonadotropins and suppressed estrus and ovulation in the rat, dog, and monkey. Sykes (1963) found this compound to be very effective in inhibiting ovulation in the fowl.

The ability of the compound to inhibit ovulation and estrus in swine was first reported by Polge (1964) and Gerrits and Johnson (1964). Working independently, these workers showed that this compound, when administered orally at a daily dose level of about 1 mg. per kg. of body weight, was very effective in suppressing normal cyclical activity in gilts. After withdrawal of treatment, normal follicular development appeared to be initiated and a large proportion of the gilts returned to estrus on day 5, 6, or 7. They found that short periods of treatment during the early luteal phase of the cycle did not affect existing corpora lutea, and treatment starting late in proestrus did not always prevent subsequent ovulation. A treatment period of 18 to 20 days was suggested in order to synchronize estrus in groups of pigs with randomly distributed cycles.

Gerrits and Johnson (1965) fed two levels of methallibure to 21 crossbred gilts. The low and high levels were, respectively, 0.90 to 1.06 and 1.39 to 2.14 mg. per kg. of body weight per day. All gilts expressed estrus from 5 to 8 days after removal from treatment; 85.7% expressed estrus within a 24 hour period. All gilts were bred on day-2 of estrus by artificial insemination. The mean number of embryos recovered and percent embryo survival for the low and high levels were

9.0, 76% and 8.8, 83%, respectively. Groves (1966) fed 100 mg. of methallibure daily to 22 gilts known to have had one estrus prior to a 20 day treatment period. Estrus was inhibited in all gilts and 19 animals came into estrus on day 4, 5, or 6 following withdrawal of treatment.

Hafez et al. (1966) stated that the mean ovulation rate was 13.2 ova per gilt fed a daily dose level of 0.8 to 1.2 mg. per kg. of body weight for 20 days. Ninety-five percent of the treated gilts returned to estrus between day 5 and 9. Groves (1966) reported trials involving 304 gilts treated daily with 100 mg. of methallibure on commercial farms in Britain. A total of 242 or 82% of the gilts came into estrus between day 4 and 5 after a 20 day treatment period. The remainder of the gilts came into estrus between 11 and 33 days after treatment.

Polge (1965) examined the effects of 4 dose levels of methallibure, 50 mg., 100 mg., 200 mg., and 250 mg. per pig per day in 110 gilts in which treatment was started between days 7 to 18 of the cycle. The animals were fed in groups of 5 to 10; therefore, it was impossible to insure complete accuracy of the dose administered. When 50 mg. was given, estrus was suppressed in only 7 of 10 gilts, and this level was considered too low to be effective under conditions of group feeding. A dose level of 100 mg. was high enough to suppress ovulation in all animals despite probably variations in food intake within the group. The average weight of gilts in the experiment was about 100 kg. Thus, it was suggested that when animals are fed in groups the effective dose of methallibure should be about 1 mg. per kg. of body weight.

The dosage level required to effectively inhibit estrus, without having any adverse side effects, appears to be rather critical. Methal-

libure has been reported to cause some depression of feed intake, but this effect seems to be associated with the central nervous system and is unconnected with palatability. Stratman and First (1965) fed 73 crossbred gilts 58, 116 and 232 mg. of methallibure in 1.816 kg. of feed per day for 16 days. As dosage level increased, body weight gains were significantly ($P < .05$) reduced during the 16-day feeding period. Significantly ($P < .05$) longer intervals from withdrawal to estrus were evident as dosage level increased (58 mg., 5.6 days; 116 mg., 7.5 days; and 232 mg., 9.1 days). Groves (1967) stated that levels greater than one mg. per kg. body weight caused occasional inappetence, with higher levels producing increased diuresis and lethargy. Red blotchy discoloration of skin was seen in some animals. Barker (1967) also observed a loss of appetite and reddish skin discoloration in gilts fed 100 mg. per day. These side effects were observed in hot weather (98° F.), and water intake was normal during this period of feeding.

Cummings (1967) reported that methallibure was not effective in mature aged sows because its appetite depressing effects in the mature animal prevented adequate daily intake. Also, a few incidences of split estrus was observed during the post-treatment period, i.e. the synchronized estrus was interrupted by one or more days of nonreceptivity to the boar. Hafez _et al._ (1966) also observed the occurrence of split estrus in gilts following treatment with methallibure.

Gerrits and Johnson (1965) reported data which indicated that the optimum level of methallibure required to control estrus in sows, per unit of body weight, is lower than that required by gilts. A level of 0.68 mg. per kg. of body weight was individually fed to 11 cycling sows and estrus was inhibited in all animals. Only one sow failed to show

estrus by 12 days post-treatment. The remaining 10 sows returned to estrus, on the average, 6.8 days after removal from treatment.

Cummings (1967) fed methallibure during the post weaning period to 6 first litter sows for either 9 or 16 days. Estrus was completely inhibited in all sows during treatment and occurred 4 to 6 days after treatment. Normal conception and farrowing rates were obtained.

Methallibure in Combination with Gonadotropins

The daily treatment of pigs with methallibure has been shown to temporarily inhibit follicular growth. This succession of events is resumed following withdrawal of treatment, but at a variable rate. The time of onset of estrus is not as predictable, or as precisely synchronized as might be expected under some circumstances. The best synchronization that has been achieved is 80 to 90% of the heats concentrated into a 3-day period with the remaining heats spread over several more days. Elimination of this spread would be desirable and has been obtained to some degree by combining the use of gonadotropins with methallibure treatment.

Polge *et al.* (1968) reported that the subcutaneous administration of 750 to 1,000 I.U. of PMS at the end of methallibure treatment reduced the variation and allowed natural service or artificial insemination within a predictable 3-day period. Intramuscular injections of 500 I.U. HCG 96 hours after the methallibure and PMS sequence induced a highly synchronized ovulation. Of 112 animals artifically inseminated without reference to estrus, but 24 hours after HCG, 90% conceived. The conception rate in 46 animals untreated with gonadotropins and inseminated conventionally 24 hours after individual heat detection was 85%. The

effects following methallibure were achieved without adverse effects on fertility or fecundity: ovulation rates were predictable and within the limits of variation shown by controls. Embryonic survival rates were not significantly different in treated and untreated groups.

Conclusion

Ovulation can be inhibited in swine by progesterone or by orally active progestational compounds, but rather large amounts must be given in order to avoid the formation of cystic follicles. In addition, following withdrawal of the progestogens, estrus is not well synchronized and heats may occur at irregular intervals. Precise synchronization of ovulation in pigs can be achieved by injecting gonadotropins after a period of treatment with progestational compounds. However, ovulation is rarely accompanied by estrus after such treatment and fertility is low. Thus, neither progesterone or the orally active progestogens can be recommended for estrus synchronization in swine.

The daily administration of methallibure in the diet of pigs showing regular estrous cycles will inhibit the normal occurrence of estrus and ovulation and cystic follicles do not occur. Ovulation occurs spontaneously during the post-treatment estrus and fertility levels are normal. In addition, the administration of methallibure and appropriately timed doses of PMS plus HCG permits precise control over the time of ovulation in pigs. Insemination shortly before the time of this induced ovulation results in high fertility and normal embryonic survival. The application of this latter technique could influence the practicability of controlled breeding and artificial insemination in pigs.

CHAPTER III

GENERAL PROCEDURE

The data in this study were collected over a two year period from five trials conducted at the Oklahoma State University swine farm. Although many different procedures were employed throughout the study, certain general procedural practices were common to all trials and they will be discussed in this section. Those procedures specific for certain groups will be described under their respective trials.

The experimental animals used in this study included a total of 102 purebred Yorkshire and 13 Hampshire gilts obtained from the Oklahoma State University swine herd. In general, all gilts were subjected to the same housing and general management regime. The gilts were maintained in small groups in pens having a maximum capacity of 10 gilts. The pens were equipped with individual stalls to permit individual feeding. The shelters provided in each pen had earth floors, were open to the south, and could be either open or closed to the north. Automatic waterers were available and located in the same position in each lot.

Six compounds provided by The Upjohn Company were evaluated for their ability to inhibit estrus and ovulation in puberal gilts. Those compounds which inhibited estrus were further evaluated for their ability to synchronize estrus and ovulation. The compounds studied were: U-10,997 and U-13,851, both 19-Nortestosterone derivatives with androgenic properties; U-13,053, a weak estrogen; U-11,100A, U-10,520A and

U-23,378, all diphenyl-dihydronapthalene derivatives with antiestrogenic properties. The structures and systematic nomenclature of these compounds are presented in the appendix, Table XXVII. The estrus inhibiting properties of these compounds, in species other than swine, had previously been determined by pilot studies carried out by the Animal Research and Development Section of The Upjohn Company. The specific dosage levels were based on the results obtained in these pilot studies. The total amount of a compound for a trial was mixed in five pounds of carrier (soybean meal) at the laboratory of The Upjohn Company, coded as to identification, and sent to Oklahoma State University. The premix was then thoroughly mixed with a standard 14 per cent protein ration in such quantities that the desired daily dose for each animal was contained in two pounds of dry feed. Composition of the ration is shown in Table I. The gilts were individually fed twice daily with the drug being included in two pounds of ration at the morning feeding. The remaining quantity of the daily feed allotment varied from three to six pounds and was offered in the afternoon.

Throughout the course of this study no attempt was made to statistically compare the ability of each compound to affect average daily gain during the 20 day treatment period. Gilts were assigned to treatment only after they had exhibited at least two estrous periods. Therefore, in each trial it was impossible to assign gilts on an equal weight basis or to start all animals on treatment at the same time. This method of assigning gilts resulted in a wide range in average initial weight of gilts on the first day of treatment and valid comparisons of weight gains could not be made. However, in evaluating the estrus inhibiting properties of these compounds it was necessary to determine

TABLE I

COMPOSITION OF THE RATION FED DURING THIS SERIES OF TRIALS

Component (% protein)	lb./ton
Milo (8%)	1592.4
Soybean meal (50%)	208.6
Tankage (60%)	50.0
Alfalfa meal (17%)	100.0
Dicalcium phosphate	25.0
Calcium carbonate	10.0
Trace mineral salt	10.0
Vitamin B_{12}	2.4
Fortafeed 2-4-9-90	1.2
Zinc sulfate	.4

whether or not they had any detrimental effects on feed intake and thereby affect weight gains during the 20 day feeding period. Thus, in each trial all gilts were weighed on the first and last day of treatment, and their average daily gain determined.

During the feeding period, careful observation was maintained for any possible side-effects (particularly any appetite depressing effects) that might be associated with the compound. Sex behavior was also noted since both testosterone and estrogen derivaties were used in this study. Sleeth et al. (1953) stated that gilts receiving testosterone injections rode each other, ranted in a typical boar-like manner, and a typical boar smell was observed in the lots where the gilts were maintained. Attention was given these factors as well as noting mammary tissue

development.

In all trials gilts were assigned to treatment groups only after they had exhibited at least two estrus periods with a normal interval. A random treatment was then assigned to the gilt. The first day that a gilt exhibited sexual receptivity was defined as day zero of the estrous cycle. A course of treatment was then started on either day—9, day—10 or day—11 following the second heat period and was continued for 20 days. Using day-zero as day—1 of heat, this would be approximately day—11 to day—13 of the estrous cycle. Daily heat observations were made throughout this 20 day feeding period to determine the effectiveness of the compounds in inhibiting estrus. Gilts which showed estrus during the treatment period were bred and continued on treatment for the remainder of the 20 day feeding period. All gilts bred during treatment were killed 30 days after the first day of breeding. Natural mating was practiced throughout the course of this study by utilizing mature boars of proven fertility from the purebred Yorkshire herd at Oklahoma State University.

The method employed for checking estrus during the pre-treatment, treatment and post-treatment periods was visual observation in the presence of a boar. A gilt was determined to be in estrus by her response to manual pressure on the back. This method is based on the results published by Signoret (1962) and reported by Nalbandov (1964). Signoret confirmed the observations of many practical pig breeders that a sow in heat will become rigidly immobile when, in the absence of a boar, a man simply applies pressure on the back. In the Yorkshire breed used by Signoret only about 50 percent of the females spontaneously responded to the signal. However, the proportion of females responding was greatly

increased if a male was present, and the role of the male in eliciting the reflex was fractionated. Ninety per cent of the females showed the reflex if they could smell the male and also hear a recording of the typical rutting call. A further 7 per cent showed the reflex if they could also see the male and if they could have physical contact with him, the remaining 3 per cent showed the reaction. These results indicated that the accuracy of heat detection would be improved if a boar was used.

All gilts involved in this study were slaughtered at the Oklahoma State University meat laboratory and their reproductive tracts recovered for detailed examination. The carcasses of all gilts were condemned for human consumption and were destroyed. Following recovery of the reproductive tracts, the ovaries were separated from the mesovarium and immediately photographed. Ovarian weights were obtained with a precision balance. The number of corpora lutea on each ovary were counted and verified by dissection and their size (mm.) determined. Gross examination of the corpora lutea was also performed to objectively determine the degree of vascularity present. Both size and degree of vascularity were then used in an attempt to ascertain their probable developmental stage as reported by Corner (1921). Corpora lutea with a diameter of 6 mm. or less and which exhibited a pale pink color were classified as regressing corpora lutea. Those corpora lutea having an extensive vascular plexus throughout their structure and a diameter greater than 6 mm. were classified as persistent corpora.

Number and size (mm.) of follicles were also determined. Follicles greater than 16 mm. in diameter were classified as cystic on the basis of data reported by First et al. (1963). An objective evaluation of the

degree of follicular development was made for ovaries with follicles less than 5 mm. in diameter.

The uteri of open gilts were dissected at the anterior extremity of the vagina and then weighed on a Harvard trip balance. In pregnant uteri an incision was made the full length of the uterine horn and the embryos removed and examined. Crown-rump measurements were made with embryos still enclosed in the amnionic sac. Hemorrhagic and partially decomposed embryos were classified as dead but such embryos were not measured.

CHAPTER IV

TRIAL I

Materials and Methods

This study was initiated in the spring, 1967, using nineteen normally cycling Yorkshire gilts, to screen four compounds for their ability to inhibit estrus and ovulation. The treatment design is given in Table II.

TABLE II

DESIGN OF STUDY FOR TRIAL I

Item	Treatment Compounds[a]			
	U-13,851 (R-142)	U-10,997 (R-143)	U-11,100A (R-144)	U-13,053 (R-145)
Daily dose level (mg.)	50	50	250	250
No. gilts allotted	5	5	5	4

[a]Figures in parenthesis refer to the individual code number used for each compound throughout this trial.

These compounds were administered to the animals for 20 days in the manner previously described for evaluating the estrus inhibiting properties of a compound. At the termination of the 20 day feeding period, all gilts which were not bred during treatment were necropsied 20 to 30

hours after the last treatment feeding and their reproductive tracts recovered and examined. Gilts bred during treatment were slaughtered 30 days after the first day of breeding. Those gilts bred during treatment, but which failed to conceive and recycled at the normal time after treatment, were bred again and slaughtered 30 days later regardless of whether or not they recycled again.

Particular attention was given to the morphology of ovaries obtained from gilts slaughtered one day post-treatment in order to evaluate the ability of these compounds to cause regression of the corpora lutea present when treatment was initiated. Ovaries with no corpora lutea present and some degree of follicular development were desired since this is the normal condition of the pig ovary when the animal comes into estrus and ovulates. The corpora lutea would have to regress completely by the time of ovulation since Hunter (1966) and Polge and Day (1967) reported a low fertilization rate for eggs induced to ovulate during the luteal phase of the cycle or when exogenous progesterone was given the day before ovulation.

Polge (1965) reported that ovaries of 3 gilts fed I.C.I. 33828 (methallibure) for 15 days contained only 2 or 3 follicles of about 5 mm. in diameter and all other follicles were only 2 to 3 mm. The corpora lutea were quite pale in color and had regressed to about 5 mm. in diameter. Since I.C.I. 33828 inhibited ovulation in these gilts and effective estrus synchronization was obtained, a similar condition was considered desirable for ovaries recovered from gilts at the end of the 20 day feeding period used in this study.

All statistical analyses were carried out according to procedures outlined by Steel and Torrie (1960). Total ovarian weight, which is

dependent on number of corpora lutea present, was analyzed by use of covariance analysis holding the independent variable constant. Other items in this study were analyzed by straight analysis of variance procedures applying the F test as a test of significance. Standard errors placed on means were based on a sample of size four to give a conservative estimate of the standard error. Treatment means were tested using Duncans Multiple Range Test.

Results and Discussion

The ability of compounds U-13,851; U-10,997; U-11,100A and U-13,053 to inhibit estrus in normally cycling Yorkshire gilts throughout a 20 day course of treatment is shown in Table III. Estrus was inhibited in all gilts receiving a daily 50 mg. dose of either U-13,851 or U-10,997. One of 5 gilts fed 250 mg. of U-11,100A daily came into estrus and was bred 5 days after treatment had started. This gilt was not pregnant when necropsied 30 days post-breeding. The non-gravid condition of the uterus could possibly have been an effect of the compound since treatment was continued for the remainder of the treatment period (15 days) following breeding. The presence of 20 normal corpora lutea (avg. size 8.45 mm.) indicated that ovulation had occurred during this period although estrus was not detected. Since one gilt did cycle during the treatment period the other 4 gilts could possibly have had a silent heat period and ovulated during treatment. However, since these 4 gilts did not exhibit estrus the assumption was made that ovulation had also been inhibited. No firm conclusion could be made regarding this point since the treatment design made it impossible to conclusively determine if ovulation had occurred during the treatment period.

All gilts fed a daily 250 mg. dose of U-13,053 returned to estrus during the treatment period and were bred. The average interval from first day of treatment to first day of breeding was 6 days. The compound apparently had no adverse effects on fertilization since three of the four gilts were pregnant at necropsy 30 days post-breeding. The other gilt recycled and was bred again 24 days after the first breeding date and was pregnant at necropsy 30 days later. An average of 15.3 corpora lutea and 13.0 embryos gave an average embryo survival rate of 85.5% for these four gilts. A fifth gilt originally scheduled for allotment to U-13,053 was eliminated from this trial because the evidence obtained from the first four gilts clearly indicated that this compound would not inhibit estrus at this level.

Daily observations made throughout the 20 day treatment period on all gilts within each treatment group did not reveal any noticeable side effects. In this trial, no outward manifestations of the treatment compounds were noted in any of the test groups. Teat development did not appear to be stimulated and there was no ranting, excessive riding of other gilts, or boar smell evident in any of the treated animals. No effect on palatability was seen in any of the treatment groups and in no case did a gilt fail to consume all of the two pounds of ration containing the compound. The effect of all treatment compounds on average daily gain during the treatment period is shown in Table IV. The average weights on both the first and last days of treatment are also included.

The average weight of gilts on day one of treatment ranged from 125 to 134 kg. During the 20 day treatment period, no detrimental effects on weight gains were observed for any of the compounds tested. The

TABLE III

NUMBER OF GILTS SHOWING ESTRUS DURING TREATMENT WITH U-13,851, U-10,997, U-11,100A AND U-13,053

Item	Treatment Compound[a]			
	U-13,851 (R-142)	U-10,997 (R-143)	U-11,100A (R-144)	U-13,053 (R-145)
Daily dose level (mg.)	50	50	250	250
No. gilts allotted	5	5	5	4
No. gilts bred during treatment	0	0	1	4

[a]Figures in parenthesis refer to the individual code number used for each compound throughout this trial.

TABLE IV

EFFECT OF U-13,851, U-10,997, U-11,100A AND U-13,053 ON AVERAGE DAILY GAIN DURING THE 20 DAY TREATMENT PERIOD

Item	Treatment Compounds[a]			
	U-13,851 (R-142)	U-10,997 (R-143)	U-11,100A (R-144)	U-13,053 (R-145)
Number gilts	5	5	5	4
Avg. initial weight (kg.)[b]	128±4.1	131±3.3	125±5.8	134±9.4
Avg. final weight (kg.)[b]	134±3.4	138±4.0	136±5.8	143±9.3
Avg. daily gain (kg.)[b]	.418±.09	.354±.03	.520±.16	.437±.04

[a]Figures in parenthesis refer to the individual code number used for each compound throughout this trial.

[b]Mean ± standard error of mean.

average daily gains were similar for all treatment groups and ranged from .354 to .520 kg. These gains were considered normal for gilts in this weight range receiving 2.5 to 3.5 kg. of feed per day. The ability of all animals to gain weight indicated that their daily energy intake was adequate and the reproductive system of these gilts should not have been impaired during the 20 day treatment period.

The results of necropsy data obtained from gilts not bred during treatment and slaughtered one day post-treatment are presented in Table V. The number and size of any corpora lutea or follicles present on the ovaries at this time were of primary importance in evaluating the effect of these compounds on the reproductive system of gilts. Since total ovarian weight is dependent on both number and size of any corpora lutea or follicles present, any affect of these compounds on either of these two variables would be reflected in the weight of the ovaries. The ability of these compounds to cause regression of the corpora lutea present on the ovaries when treatment started was considered a prerequisite for effective synchronization of estrus following the treatment period. The absence of corpora lutea and the presence of pre-ovulatory-size follicles on the ovaries at the end of the 20 day treatment period was desired since this is the normal condition of ovaries prior to ovulation.

Uterine weights were obtained because both progesterone (produced by the corpora lutea) and estrogen (produced by the follicles) can cause marked changes in the weight of the uterus. Thus, these compounds could possibly influence the weight of the uterus through any affect they may have on the ovaries or by acting directly on the uterus.

Differences in total ovarian weight were tested by covariance

TABLE V

MEANS AND STANDARD ERRORS FOR TOTAL OVARIAN WEIGHT, NUMBER AND SIZE OF CORPORA LUTEA AND UTERINE WEIGHT FOR GILTS NECROPSIED ONE DAY FOLLOWING TREATMENT WITH U-13,851, U-10,997 AND U-11,100A

Item	Treatment Compound[a]		
	U-13,851 (R-142)	U-10,997 (R-143)	U-11,100A (R-144)
Total ovarian weight (mg.)[b]	7,417.6±1,104[c]	7,481.2±1,104[c]	12,065.9±1,104[d]
No. corpora lutea	13.5±4.9	9±4.9	14.5±4.9
Size corpora lutea (mm.)	6.75±.31[c]	5.26±31[d]	8.02±.31[e]
Uterine weight (gm.)	634.3±158	559.9±158	771.3±158

[a]Figures in parenthesis refer to the individual code number used for each compound throughout this trial.

[b]Adjusted ovarian weight holding No. of corpora lutea constant.

[c,d,e]Values with different superscripts are significantly different ($P < .05$).

analysis with number of corpora lutea held constant. The combined weight of both the right and left ovaries for each gilt was used to determine the mean total ovarian weight values for each treatment. The mean total ovarian weight for gilts fed U-11,100A was significantly higher ($P<.05$) than for those gilts treated with either U-10,997 or U-13,851. The means were: 12,069.5 mg. for U-11,100A-treated gilts; 7,481.2 mg. for U-10,997-treated gilts; and 7,417.6 mg. for U-13,851-treated gilts. The latter two treatment groups had mean values which were very similar and not significantly different.

Total ovarian weight is dependent on both number and size of corpora lutea and the size of corpora lutea varies with stage of development (Corner 1919). The mean number of corpora lutea for the three treatment groups did not differ significantly. Values of 14.5, 9.0 and 13.5 were obtained for compounds U-11,100A, U-10,997 and U-13,851 respectively. Variation within treatment was large as indicated by a standard error of 4.9 corpora lutea. The ovaries of one gilt fed U-10,997 had no corpora lutea present while another had a total of only four. Size of corpora lutea differed significantly between the three groups. Corpora lutea present on ovaries of gilts fed U-11,100A (average 8.02 mm.) were significantly larger ($P < .05$) than those from gilts fed U-10,997 (average 5.26 mm.) and were also singificantly larger ($P < .05$) than the corpora lutea on ovaries of gilts treated with U-13,851 (average 6.75 mm.). Corpora lutea on the ovaries of U-13,851-treated gilts were significantly larger ($P < .05$) than those gilts fed U-10,997.

The mean total ovarian weight of 12.4 gm. reported by Erb et al. (1962) for ovaries obtained from sows at day 17 of pregnancy is similar to the 12,069.5 mg. average for gilts fed U-11,100A. The gross appear-

ance of these ovaries also indicated that they were more active than the ovaries of gilts treated with either U-10,997 or U-13,851. The corpora lutea of U-11,100A-treated gilts were a reddish-pink color characteristic of those with an active capilliary circulation present during pregnancy and the luteal phase of the estrous cycle (McKenzie 1926). These corpora lutea were also typical of those described for gilts during the luteal phase of the estrous cycle when large amounts of progesterone are being produced (Erb et al. 1962). Corner (1921) reported that the size of corpora lutea of gilts during the luteal phase was approximately 8-9 mm. This value is similar to the 8.02 mm. average corpora lutea size obtained from U-11,100A-treated gilts. Their size and appearance suggests that these corpora lutea represented those present on day one of treatment. Compound U-11,100A apparently caused both the structural and functional capacity of these corpora lutea to be maintained throughout the 20 day treatment period.

The degree of follicular development present on the ovaries of gilts fed U-11,100A was also observed. Approximately 3 to 7 follicles varying from 3 to 6 mm. in diameter were found on the ovaries of all gilts. Numerous small follicles less than 3 mm. in diameter were also observed on all ovaries. The degree of follicular development was not quantitated due to the difficulty encountered in accurately measuring follicles this small, but an objective evaluation of their development was made.

The degree of follicular development present on the ovaries of gilts fed U-11,100A was similar to that of estrogen-treated gilts reported by Foote et al. (1958). These workers reported that a single injection of 20 mg. of estradiol on day 14 of the estrous cycle suppressed the growth

of follicles in 9 gilts. An average of 3.7 follicles 3 mm. or more in diameter were found on the ovaries of these gilts when slaughtered 4 days after the single injection--or day 18 of the estrous cycle. The average number of follicles 3 mm. or more in diameter was 19.9 for 9 control gilts slaughtered on day 9 of the estrous cycle. The estradiol apparently caused the corpora lutea to persist since an average of 11 corpora lutea were present on the ovaries at this time.

The antifertility effects of U-11,100A have been described by several workers. Duncan et al. (1963) reported that daily oral administration of or above 0.025 mg./kg. of U-11,100A during proestrus or within 4 days after breeding inhibited pregnancy in rats. At effective antifertility doses it showed no estrogenic activity. However, Emmens and Martin (1965) reported that injections of 50 ug./day on days 1 to 3 or 4 to 6 of pregnancy in mice produced an antifertility effect which they attributed to its estrogenic properties. They also reported that U-11, 100A was estrogenic in vaginal smear test in rats and mice. Nelson et al. (1963) stated that the antifertility activity of this compound was believed to be exerted on the zygote. Greenwald (1965) stated that this compound caused rat ova to be transported rapidly down the oviduct and that this effect could interrupt pregnancy.

The appearance and condition of ovaries from U-11,100A-treated gilts in this study suggested a possible estrogenic effect of this compound. If ovulation was inhibited during the treatment period than this compound allowed the corpora lutea to persist throughout the 20 day treatment period. The maintenance of these corpora lutea suggests the possibility of an estrogenic effect of U-11,100A similar to those elicited by exogenous estrogens reported by Gardner et al. (1963).

However, if ovulation was not inhibited in these gilts, then all of the previous discussion regarding the possible effects of this compound would be invalid. It is possible that the gilts ovulated without manifesting any signs of estrus. This possibility is based on data reported by Duncan et al. (1963) who stated that when this compound was administered to rats concomitantly with estradiol for 10 days, the uterine response to exogenous estrogen was markedly inhibited. The compound apparently antagonizes the action of estrogen and thus exhibits anti-estrogenic properties in rats. If this same anti-estrogenic property is also manifested in gilts, then it could be postulated that the U-11,100A-treated gilts ovulated but the compound antagonized the endogenous estrogen produced by the follicles and interfered with its ability to elicit the characteristic behavioral patterns of a gilt in heat.

Although differences in mean uterine weight between the three treatment groups were not significant there was some indication of a treatment effect. The variation in uterine weights within treatment groups was large as shown by the standard error of 158 gm. However, the uteri of U-11,100A-treated gilts, were generally heavier and appeared more edematous than those from the other treatment groups. This evidence did suggest a possible uterotrophic action of U-11,100A on the uterus of gilts which again could be an expression of its estrogenicity. This possibility is based primarily on the work reported by Duncan et al. (1963) who observed a uterotrophic effect of U-11,100A in rats. Injection of the rats with this compound caused an increase in uterine weight but did not cause weight increases equaling those given by estradiol. Emmers and Martin (1965) also reported a uterotropic effect of this compound in the mouse.

In contrast to those ovaries of U-11,100A-treated gilts the smaller ovaries of gilts treated with U-10,997 (average 7481.2 mg.) had smaller corpora lutea which were pale-pink in color. The corpora lutea of these gilts were smaller than those from the other two treatment groups. Their color and smaller size (average 5.26 mm.) indicated that they were regressing and becoming non-functional. The degree of follicular development present on these ovaries was much less than that observed on ovaries from both U-11,100A and U-13,851 treated gilts. The majority of the follicles were less than 2 mm. in diameter but some were in the range of 2 to 4 mm. The ischemic condition of these ovaries indicated that this compound had a marked inhibitory effect on ovarian function. The uteri were rather small and resembled those found in prepubertal animals and this also suggested that no ovarian steroids were being produced. There were no indications that this compound failed to inhibit ovulation during the 20 day treatment period.

Ovaries from U-13,851 treated gilts were objectively compared with those from gilts fed U-10,997 and U-11,100A and they appeared to be intermediate in terms of size of corpora lutea, degree of vascularity, and follicular development. The corpora lutea averaged 6.75 mm. in diameter which was similar to the 6 mm. value reported by Corner (1921) for regressing corpora lutea on day 18 of the estrous cycle. The darker pink color observed in these corpora lutea indicated a greater degree of vascularity than that seen in corpora lutea from gilts fed U-10,997. A moderate degree of ischemia was observed in the corpora lutea of 2 gilts but the degree of vascularity in corpora lutea from the other 3 gilts was similar to that observed in U-11,100A-treated gilts. Only 2 or 3 follicles of about 5 to 6 mm. in diameter and numerous small follicles

2 to 3 mm. in diameter were found on the ovaries of 4 gilts treated with U-13,851. No follicles were present on the ovaries of the fifth gilt. The inhibitory effects of this compound on ovarian function were not as pronounced as that observed for compound U-10,997. The results suggested that compound U-13,851 did inhibit ovulation during the 20 day treatment period and maintained those corpora lutea present on the ovaries when treatment was initiated.

The results of this trial clearly showed that compound U-13,053 would not inhibit estrus in gilts. Compound U-11,100A will apparently prevent estrus from occurring when fed at a level of 250 mg. per day. However, this level may be close to the minimal effective dosage since one of five gilts fed this compound returned to estrus during the treatment period. Very good evidence was obtained which indicated that compounds U-13,851 and U-10,997 would effectively inhibit estrus in gilts when fed at a level of 50 mg. per day. The gross appearance of ovaries obtained from gilts in these latter two treatment groups suggested that ovulation had also been blocked during the 20 day treatment period. The gross morphology of the corpora lutea differed considerably between the three treatment groups in which estrus was inhibited. The degree of lutenization was sufficiently different to suggest that each compound had a different and characteristic effect on corpora lutea function.

CHAPTER V

TRIAL II

Materials and Methods

The results obtained in Trial I indicated that compounds U-11,100A, U-10,997 and U-13,851 were biologically active in puberal gilts and should be further investigated. The luteal maintenance observed with each of these compounds was undesirable and suggested an estrogenic effect of compound U-11,100A--and possibly U-10,997 and U-13,851. Although these compounds were not frank estrogens the concensus was that titrating to a lower dose might result is a more desirable situation by reducing their estrogenic activity. A decision was made to test these compounds at lower levels and determine the range of dosage where estrus could or could not be inhibited. Hopefully a level could be reached which would allow follicular development without ovulation or luteal maintenance. At this level the interval from withdrawal to first post-treatment estrus would be determined. If this level were obtained, further titration to determine the minimal effective dose would be performed.

This trial was initiated in the summer, 1967. The treatment design given in Table VI allowed for a total of 27 gilts. Each compound was titrated to three different dosage levels giving a total of nine groups with three animals allotted to each level (27 gilts). The procedure utilized in this study was to begin testing the highest levels of each

TABLE VI

DESIGN OF STUDY FOR TRIAL II

Item	Treatment Compound[a]								
	U-11,100A			U-10,997			U-13,851		
	(R-149)	(R-150)	(R-151)	(R-152)	(R-153)	(R-154)	(R-155)	(R-156)	(R-157)
Daily dose level (mg.)	50	5	.5	10	5	.5	10	5	.5
No. gilts allotted	3	3	3	3	3	3	3	3	3

[a]Figures in parenthesis refer to the code numbers used for each level of each compound.

compound. If this level was effective in inhibiting estrus the next level was tested; however, if this level was not effective further testing of the compound was discontinued. This procedure was considered feasible from an economic standpoint since the carcasses of these animals were condemned.

The compounds were administered and the data were collected in the same standard manner previously described.

Results and Discussion

The ability of compounds U-11,100A, U-10,997 and U-13,851 to inhibit estrus in gilts when fed at various dose levels is shown in Table VII.

The results obtained from feeding a daily 50 mg. dose of U-11,100A were variable but indicated that this level would not effectively inhibit estrus. Consequently, the 5 and .5 mg. levels were not tested. Two of the 3 gilts were bred 7 days after treatment was started. The gilt which did not cycle during treatment was necropsied one day post-treatment and a total of 11 normal corpora lutea (average size 8.48 mm.) and follicles 4 to 5 mm. in diameter were observed on her ovaries. The evidence suggests that this gilt ovulated during treatment without showing estrus. One of the two gilts bred during treatment was rebred 30 days after first breeding but was not pregnant at necropsy 30 days later. The other gilt did not recycle following first breeding although she too was not pregnant at necropsy. This gilt was considered to be sterile because the right uterine horn ended blindly with no connection to the uterus. This is based on a survey of anatomical and endocrine defects in swine reported by Nalbandov (1952) who observed that occlusion of one

TABLE VII

ESTRUS INHIBITING ABILITY OF COMPOUNDS U-11,100A, U-10,997 AND U-13,851 AT VARIOUS DOSE LEVELS

Item	Treatment Compounds[a]								
	U-11,100A			U-10,997			U-13,851		
	(R-149)	(R-150)	(R-151)	(R-152)	(R-153)	(R-154)	(R-155)	(R-156)	(R-157)
Daily dose level (mg.)	50	5	.5	10	5	.5	10	5	.5
No. gilts allotted	3	3	3	3	3	3	3	3	3
No. gilts bred during treatment	2	-	-	0	0	2	0	3	-

[a]Figures in parenthesis refer to the code numbers used for each level of each compound.

uterine horn in swine resulted in complete sterility. The presence of a non-gravid uterine horn in the mated pig results in early embryonic death in the opposite horn (Anderson, 1966).

Compound U-10,997 inhibited estrus when fed at a daily dose level of 10 mg. or 5 mg. but was not effective at the .5 mg. level. At the latter level, two gilts were bred during treatment and the third gilt showed signs of being in heat on day-9 of treatment but would not accept the boar. Eighteen normal corpora lutea 9 mm. in diameter were noted on her ovaries at necropsy. The evidence suggested that the animal ovulated during treatment. One of the 2 gilts bred during treatment did not cycle until day-20 of treatment and was necropsied the following day. Seven corpora hemorrhagica and four vesicular follicles were found on the ovaries which indicated that the animal had ovulated. The other gilt was necropsied 30 days post-treatment and was classified as sterile based on the presence of a blind uterine horn.

Although a blind uterine horn prevents pregnancy in the opposite intact horn, there is no evidence that this condition would effect the ability of these compounds to influence normal ovarian activity in gilts. The two gilts which exhibited this defect in this trial had normal estrous cycles prior to treatment and the number and size of corpora lutea present on both ovaries at necropsy were normal for these two animals.

Compound U-13,851 effectively inhibited estrus at the 10 mg. level but it was not effective at the 5 mg. level since all gilts returned to estrus during the treatment period and were bred. Two of the gilts conceived at first service, but one recycled and conceived at the second service. The minimal effective dose for U-13,851 appeared to be 10 mg.

per day.

The results obtained in Trial I indicated that these three compounds did not impair feed intake or weight gains during the treatment period. The results obtained in this trial appear to substantiate this observation since no detrimental effects on feed intake or weight gains were noted when these compounds were fed at different levels. The average weight on the first and last day of treatment and mean average daily gain of gilts within each treatment group are presented in Table VIII.

Although average daily gains within and between treatment groups varied considerably these gains were considered to be adequate for normal functioning of the reproductive processes to occur.

The results of necropsy data obtained from gilts not bred during treatment and slaughtered one day post-treatment are presented in Table IX. Ovaries with preovulatory size follicles and no corpora lutea were desired at this stage but were not observed in any of the treatment groups.

The ovaries of gilts fed 10 mg. of U-10,997 were essentially inactive at the end of the 20 day treatment period. There were no corpora lutea and no follicles present on any ovaries obtained from gilts within this treatment group, and they had a solid appearance and were firm to the touch. The absence of corpora lutea was desirable but some degree of follicular development was preferred. The condition of these ovaries suggested that this compound had a marked inhibitory effect on ovarian activity which could have been mediated through several possible pathways. Possibly, the complete degeneration of those corpora lutea present on day—1 of treatment resulted from either a direct luteolytic

TABLE VIII

EFFECT OF VARIOUS LEVELS OF U-11,100A, U-10,997 AND U-13,851 ON AVERAGE DAILY GAIN DURING THE 20 DAY TREATMENT PERIOD

Item	Treatment Compounds[a]					
	U-11,100A	U-10,997			U-13,851	
	(R-149)	(R-152)	(R-153)	(R-154)	(R-155)	(R-156)
Daily dose level (mg.)	50	10	5	.5	10	5
Number of gilts	3	3	3	3	3	3
Avg. initial weight (kg.)[b]	135±6.5	125±2.7	133±6.4	109±7.7	123±1.2	140±5.2
Avg. final weight (kg.)[b]	141±6.1	130±1.9	139±6.4	118±8.6	129± .9	145±4.7
Avg. daily gain (kg.)[b]	.301±.032	.212±.042	.309±.027	.487±.062	.325±.027	.252±.024

[a]Figures in parentheses refer to the code numbers used for each level of each compound.

[b]Mean ± standard error of mean.

TABLE IX

OVARIAN AND UTERINE RESPONSE OF GILTS FED VARIOUS LEVELS OF U-11,100A, U-10,997 AND U-13,851

Item	Treatment Compounds[a]			
	U-11,100A	U-10,997		U-13,851
	(R-149)	(R-152)	(R-153)	(R-155)
Daily dose level (mg.)	50	10	5	10
No. gilts[b]	1	3	3	3
No. with cystic follicles	0	0	1	0
Total ovarian weight (mg.)[c]	10,950.8	3,615.9±338	7,864.3±224	8,503.0±400
No. corpora lutea[c]	11	0	0	11.7±1.6
Size corpora lutea (mm.)[c]	8.48	0	0	7.2±.45
Uterine weight (gm.)[c]	874.4	448.1±55	495.8±9	628.56±14

[a]Figures in parenthesis refer to the code numbers used for each level of each compound.

[b]Gilts necropsied 20-30 hours post-treatment.

[c]Mean ± standard error of mean.

action of compound U-10,997 on the corpora lutea or an indirect effect by activating the uterine luteolytic factor proposed by Anderson (1967). The atrophic condition of these ovaries also suggested that the compound may have initiated a hypothalamic hypophyseal blockade and thereby inhibited secretion of the hypothalamic releasing factors and/or the gonadotrophins from the anterior hypophysis. The average weight (448.7±55 gm.) and appearance of uteri obtained from these gilts also reflected a lack of stimulation by gonadal steroids.

The ovaries of 2 of the 3 gilts fed a daily 5 mg. dose of U-10,997 contained no corpora lutea and in this respect they were similar in condition to those obtained from gilts treated with 10 mg. per day. However, numerous small follicles ranging from 2-5 mm. in diameter were present on the ovaries of these gilts and bilateral follicular cysts occurred in the third gilt. The ovaries of this gilt contained follicles greater than 16 mm. in diameter and were classified as cystic on the basis of data reported by First et al. (1963). The degree of follicular development present in this gilt was desirable although the occurrence of cystic follicles was very undesirable. These observations suggested that this level did not completely block the release of the follicle-stimulating hormone (FSH) from the anterior hypophysis.

Compound U-10,997 is a testosterone derivative and exhibits primarily androgenic activity. However, this compound apparently possesses some estrogenic activity at higher dose levels. The ability of estrogens to cause retention of corpora lutea in swine has been demonstrated by Gardner et al. (1963). In Trial I, gilts fed a daily 50 mg. dose of this compound had retained corpora lutea at the end of the 20 day treatment period. However, titrating the dosage of U-10,997 to 10 and 5 mg.

levels allowed complete degeneration of the corpora. This suggests that at the lower dose levels the estrogenic activity is reduced and the effect on the ovaries is primarily a result of the androgenic activity of the compound although some estrogenic activity is probably still present. Sleeth et al. (1953) stated that the reproductive tracts of gilts injected with both testosterone propionate and estradiol benzoate were juvenile in appearance and the ovaries were degenerated though an occasional cyst was noted. In contrast, gilts receiving only testosterone had enlarged cystic ovaries and the entire genital tracts showed marked hyperemia and hyperplasia. This description of the reproductive tract, the atrophic condition of the ovaries, and occurrence of cystic follicles for testosterone-estrogen-treated gilts is almost identical to that of gilts fed 10 and 15 mg. of U-10,997 in this study. The uteri of these animals resembled those of prepubertal gilts and the ovaries were completely atrophic, appeared white and fibrous and were very turgid.

In gilts, normal ovulation and fertilization can not occur if corpora lutea do not regress because the progesterone from the corpora prevents ovulation and may increase the incidence of polyspermy. Therefore, the inactive condition of ovaries obtained from gilts in both the 10 and 5 mg. U-10,997 treatment groups was considered desirable, and indicated that these levels would not only suppress estrus and ovulation, but may allow for full follicular development to be initiated quite quickly after withdrawal of treatment. The formation of cystic follicles in one gilt was discouraging since this ovarian abnormality is a major cause of impaired fertility and sterility in swine. The occurrence of cystic follicles was thought to be a direct effect of the compound since the natural incidence of cystic follicles in the purebred

swine population which this gilt was taken from was considered to be low. This consideration is based on data reported by Rich (1967) who used 114 gilts from this same purebred population to compare ovulation rate, fertilization rate and embryo survival of lot-mated and hand-mated gilts. All of these gilts were slaughtered following mating and cystic follicles were not observed on the ovaries of any gilt.

The effect of a daily 10 mg. dose of U-13,851 on the ovaries was almost identical to that produced by a 50 mg. dose in Trial I. Luteal maintenance occurred again which suggested that this compound still possessed some estrogenic activity at the lower 10 mg. level. The degree of follicular development varied considerably between the 3 gilts. A total of 12 follicles ranging from 5 to 8 mm. were present on the ovaries of one gilt, numerous small follicles 2 to 5 mm. in diameter were observed on the ovaries of the other 2 gilts. Those corpora lutea present on day 1 of treatment apparently persisted throughout the 20 day treatment period. An average of 11.7 corpora lutea measuring 7.2 mm. in diameter, were present one day post-treatment. A moderate degree of vascularity was observed in the majority of these corpora lutea causing them to have a light pink color and this suggests that they may have started to degenerate. Failure of these corpora lutea to regress was considered undesirable since any corpora lutea present at this time would have to regress very quickly and pituitary function would also have to be restored quickly if the animals were to ovulate soon after treatment withdrawal.

The results obtained in this trial indicated that a daily 50 mg. dose of compound U-11,100A will not prevent the normal occurrence of estrus in gilts. The minimal effective estrus inhibiting dose for com-

pound U-13,851 appears to be 10 mg. per day. However, this level will still allow corpora lutea to persist. The minimal effective dose of U-10,997 appears to be 5 mg. per day, and some ovarian activity can occur at this level. However, at the 10 mg. level this compound completely inactivates the ovaries by preventing follicular development and allowing the degeneration of any corpora lutea present. The response of the ovaries to U-10,997 was thought to be more conducive for estrus synchronization than that observed for the other two compounds.

CHAPTER VI

TRIAL III

Materials and Methods

This trial was initiated in the fall of 1967. The objectives were to evaluate the ability of U-10,997 to synchronize estrus in gilts following a 20 day treatment period and to determine the minimal dose at which this compound would effectively inhibit estrus. In addition, two new non-steroidal compounds, U-10,520A and U-23,378 were evaluated for their ability to inhibit estrus. U-10,520A is a derivative of 1,2 diphenyl-3,4,dihydronapthalene and thus possesses biological properties similar to those of compound U-11,100A used in Trials I and II. Duncan (1963) reported that U-10,520A possessed oral antifertility activity in rats by inhibiting pregnancy and antagonizing the uterine response to estrogen.

The treatment design employed in this trial utilized a total of 16 gilts and is shown in Table X. Compounds U-10,520A and U-23,378 were administered in the standard manner used throughout this study to evaluate compounds for their ability to inhibit estrus. The 4 gilts assigned to each of these 2 compounds were killed one day after treatment if they were not bred during treatment. Gilts bred during treatment were killed 30 days post-breeding. Ovarian and uterine data were obtained in the same manner previously described for open gilts, necropsied one day post-treatment and for bred gilts necropsied 30 days post-breeding.

In Trial II, compound U-10,997 had prevented estrus and ovulation by completely inactivating the ovaries during the 20 day treatment period. Complete degeneration of pre-treatment corpora lutea had occurred when this compound was administered at a rate of either 10 or 5 mg. per day. This inhibitory effect on ovarian function was considered to be more desirable than that produced by compounds U-11,100A and U-13,851. Thus, U-10,997 was considered to be an efficacious compound and a decision was made to pursue the investigation of this compound into an initial synchronization phase. The ability of U-10,997 to inhibit estrus at the 5 mg. level but not at the .5 mg. level indicated that the minimal effective dose (MED) was probably somewhere in this range. To determine if the MED was less than 5 mg., the compound was tested at the 2.5 mg. level and also retested at the 5 mg. level. Synchronization was attempted at both these levels.

Gilts employed to initiate the synchronization phase of U-10,997 at the 5 and 2.5 mg. levels received the compound for 20 days in the standard manner. Four gilts were assigned to each of the two treatment levels. Two of these gilts were necropsied one day after treatment withdrawal and their reproductive tracts were recovered and examined. The remaining two gilts were allowed to return to estrus in order to determine the interval from the last day of feeding to the first post-treatment estrus. Any gilt which returned to estrus within 15 days was bred, but any animal which failed to show estrus by day 15 was necropsied and the ovarian condition determined.

The fertilization rates of gilts bred in the synchronization phase were determined using the method described by Rich (1967) for recovering and examining fertilized ova. Gilts in heat only one day were autopsied

2 days after the first day of heat or 3 days after the first day of heat if in heat 2 or more days. Reproductive tracts were recovered and the

TABLE X

DESIGN OF STUDY FOR TRIAL III

Item	Treatment Compound[a]			
	U-10,520A	U-23,378	U-10,997	
	(R-158)	(R-159)	(R-160)	(R-161)
Daily dose level (mg.)	250	250	5	2.5
No. gilts allotted	4	4	4	4
No. necropsied one day post-treatment	4	4	2	2
No. allowed to return[b] to estrus	0	0	2	2

[a]Values in parenthesis refer to the code number used for each level of each compound.

[b]Gilts which did not return to estrus by day-15 post-treatment were necropsied on day-15.

ova were collected and examined in the following manner. The broad ligament was trimmed away from the oviduct to facilitate flushing and the oviduct was separated from the uterus one-half inch posterior to the uterotubal junction. The oviduct was flushed with 10 cc. of 0.9 per cent saline solution, and the fluid and ova were recovered in test tubes. The flushings were allowed to settle for approximately three hours. The supernatant fluid was carefully decanted to avoid disturbing the bottom 0.5 cc. in the tube. This remaining fraction was then poured into a

watch glass and examined under a microprojector for the presence of ova. As ova were located, they were removed with an eye dropper and transferred to a separate watch glass for more detailed study of clevage under high power magnification. Any normal appearing ovum that had undergone at least one cellular division was classified as fertilized. The remaining supernatant fluid was saved for further examination to insure maximum recovery of ova.

Results and Discussions

The ability of the treatment compounds to inhibit estrus during the 20 day feeding period is shown in Table XI.

TABLE XI

ESTRUS INHIBITING ABILITY OF COMPOUNDS U-10,520A, U-23,378 AND U-10,997

Item	Treatment Compounds[a]			
	U-10,520A (R-158)	U-23,378 (R-159)	U-10,997 (R-160)	U-10,997 (R-161)
Daily dose level (mg.)	250	250	5	2.5
No. gilts allotted	4	4	4	4
No. gilts bred during[b] treatment	3	0	1	1

[a]Values in parenthesis refer to the code number used for each level of each compound.

[b]Includes gilts which showed signs of estrus but would not accept boar.

Compound U-10,520A apparently will not inhibit estrus in gilts when

fed at a rate of 250 mg. per day. Three of four gilts treated returned to estrus and were bred on day-6 of treatment. Two of these bred gilts recycled 30 days post-breeding and were rebred. They were both pregnant at necropsy 30 days following the second breeding and had an average embryo survival rate of 83 percent. Although the third bred gilt did not recycle, necropsy 46 days post-breeding revealed that she had not conceived at the treatment breeding. Seventeen corpora hemorrhagica were present which indicated that she had ovulated without manifesting signs of estrus. A mistake in recording necropsy dates resulted in this gilt being killed 46 days post-breeding instead of 30 days. The fourth gilt in this treatment group did not show estrus during treatment, but the presence of 13 corpora lutea (avg. size 7.71 mm.), at the end of the feeding period suggested that she had probably ovulated during treatment without showing estrus.

The failure of U-10,520A treated gilts to return to estrus within 30 days following breeding suggests a possible antifertility effect of this compound similar to that reported by Duncan _et al._ (1963) for U-10,520A treated rats. These workers stated that this compound possessed oral antifertility activity in rats by inhibiting pregnancy. Single doses of 2.5 mg./kg. inhibited implantation when administered during proestrus or within 4 days after breeding. Comparable or higher doses administered on day-5 were not effective. Also, when this compound was administered to rats concomitantly with estradiol for 10 days, the uterine response to exogenous estrogen was markedly inhibited. Maximum observed inhibiton of uterine weight was 59 percent of the anticipated response to estradiol. This anti-estrogenic property may explain the failure of U-10,520A-treated gilts to settle on first service. Since

these gilts continued to receive this compound for 14 days following breeding, it may be postulated that U-10,520A antagonized the ability of estrogen to promote uterine growth following ovulation in these gilts. This antagonism could have a detrimental effect since estrogen is essential for both implantation and the uterine developments associated with it. This effect could be one possible explanation for the increased length of the estrous cycle (30 days) following first breeding of these gilts.

A daily 250 mg. dose of compound U-23,378 will apparently inhibit estrus in gilts. None of the 4 gilts in this treatment group exhibited any signs of estrus during the feeding period. One gilt on each of the 5 and 2.5 mg. levels of U-10,997 exhibited signs of estrus during treatment but neither would accept the boar. However, necropsy data indicated that these 2 gilts had ovulated during treatment. The data also indicated that the remaining 3 gilts on the 5 mg. level did not ovulate during treatment. The data obtained from the remaining 3 gilts on the 2.5 mg. level suggested that these animals could have ovulated during treatment. This possibility is based on the response of 2 gilts used in the synchronization phase and will be discussed later in this section.

Daily observations made throughout the 20 day treatment period on all gilts within each treatment group did not reveal any noticeable side effects. In no case did a gilt fail to consume all of the two pounds of ration containing the compound. The average weight on the first and last day of treatment and mean average daily gain of gilts within each treatment group are presented in Table XII. During the 20 day treatment period normal gains were obtained for gilts in each treatment group.

The results of necropsy data obtained from all gilts not bred

TABLE XII

EFFECT OF U-10,520A, U-23,378 AND U-10,997 ON AVERAGE DAILY GAIN DURING THE 20 DAY TREATMENT PERIOD

Item	Treatment Compounds[a]			
	U-10,520A	U-23,378	U-10,997	
	(R-158)	(R-159)	(R-160)	(R-161)
Daily dose level (mg.)	250	250	5.0	2.5
No. gilts	4	4	4	4
Avg. initial weight (kg.)[b]	138±3.9	128±4.4	132±5.1	129±3.2
Avg. final weight (kg.)[b]	150±3.4	139±6.3	141±5.6	136±3.5
Avg. daily gain (kg.)[b]	.561± .11	.521± .13	.453± .05	.444± .06

[a]Values in parenthesis refer to the code number used for each level of each compound.

[b]Mean ± standard error of mean.

during treatment and slaughtered one day post-treatment are presented in Table XIII. The ovarian condition of U-23,378-treated gilts was very similar to that observed for U-11,100A-treated gilts in Trial I. An average of 14 corpora lutea 8.72 mm. in diameter were observed, resulting in an average total ovarian weight of 13,149 mg. The size and appearance of these corpora lutea indicated that they were functional and characteristic of those present during the luteal phase of the estrous cycle. If ovulation was inhibited during the treatment period and these were persistent corpora, then U-23,378 must have some luteotrophic properties. The compound apparently maintained the structural integrity of the corpora, and although progesterone determinations were not made, their size and appearance suggested that they were functional and presumably synthesizing progesterone. Since there were no indications that these gilts had a silent ovulation during the treatment period, the assumption was made that this compound inhibited estrus and ovulation and caused maintenance of corpora lutea in gilts when fed at a daily rate of 250 mg.

The results obtained with U-10,997 indicated that the minimal effective dose for inhibiting estrus is probably 5 mg. although this level may be too low for consistent inhibition. The ovaries obtained from two gilts necropsied one day post-treatment contained no corpora lutea but a few pre-ovulatory size follicles were present. One gilt had a total of 10 follicles ranging from 5 to 7 mm. in diameter, and the other gilt had numerous follicles less than 5 mm. and one 10 mm. follicle. The absence of corpora lutea and the degree of follicular development suggested that this level inhibited estrus and ovulation, allowed regression of pre-treatment corpora lutea, and permitted follicles to develop.

TABLE XIII

OVARIAN AND UTERINE RESPONSE OF GILTS NECROPSIED ONE DAY AFTER TREATMENT WITH U-23,378 AND U-10,997

Item	Treatment Compounds[a]		
	U-23,378	U-10,997	
	(R-159)	(R-160)	(R-161)
Daily dose level (mg.)	250	5	2.5
No. gilts necropsied one day post-treatment	4	2	2
Total ovarian weight (mg.)[b]	13,149±503	6,694±595	14,428±614
No. corpora lutea[b]	14±2.05	0	18[c]
Size of corpora lutea (mm.)[b]	8.72±.75	0	7.6[c]
Uterine weight (gm.)[b]	744±82	657.3±68	511±20

[a]Values in parenthesis refer to the code number used for each level of each compound.

[b]Mean ± standard error of mean.

[c]Value refers to only one gilt which ovulated during treatment period.

The response of gilts on the 2.5 mg. level was variable but indicated that this level was too low for effective inhibition of estrus. One gilt necropsied one day post-treatment had exhibited signs of estrus during the treatment period but would not accept the boar. Eighteen corpora lutea, 7.6 mm. in diameter, were present on her ovaries at the end of treatment, indicating that she had ovulated during treatment. The ovaries of the other gilt contained no corpora, but 8 pre-ovulatory size follicles 6 to 13 mm. in diameter were present. The condition of these ovaries indicated that ovulation had been inhibited throughout the treatment period, but the suppression was apparently minimal since follicles had developed by the end of treatment.

The synchronization phase of this trial produced variable and inconclusive results. The reproductive performance of gilts used in this phase is shown in Table XIV. One gilt in the 5 mg. treatment group exhibited signs of estrus on day-10 of the treatment period but would not accept the boar. Following treatment withdrawal she returned to estrus and was bred 10 days post-treatment which was 20 days from the possible silent estrus while on treatment. However, she was not pregnant at necropsy 30 days post-breeding. Due to a mistake in breeding dates ova were not collected from this gilt. The second gilt did not show estrus by day 15 and necropsy revealed completely inactive ovaries which were very firm and fibrous with no corpora lutea or follicles present. The two gilts on the 2.5 mg. level returned to estrus 5 to 7 days following treatment withdrawal and had very good ovulation rates. The return intervals for these 2 gilts were considered adequate for estrus synchronization--if estrus and ovulation were inhibited during the treatment period. The 62.5 percent ova recovery for one gilt was low but all were

TABLE XIV

REPRODUCTIVE PERFORMANCE OF GILTS FOLLOWING WITHDRAWAL OF COMPOUND U-10,997

Gilt	Daily Dose (mg.)	Post-treatment Estrus Interval (days)	No. Corpora Lutea	% Ova Recovered	% Fertilized of Those Recovered
1[a]	5	--	0	----	----
2[b]	5	10	19	----	----
3	2.5	7	20	62.5	100
4	2.5	5	26	85.0	90.9

[a]Gilt did not cycle within 15 days post-treatment.

[b]Showed signs of estrus during treatment.

fertilized. Ninety-one percent of the ova recovered from the other gilt were fertilized. There were no indications that these two gilts cycled during treatment. However, since these gilts started treatment on days 10 to 13 of their estrous cycle, it would be possible for these gilts to have had a silent estrus approximately 7 days after treatment started. If this occurred, they could have recycled approximately 18 to 20 days later which would be 5 to 7 days post-treatment. This possibility is strengthened by the fact that one of the four gilts in this treatment group cycled during treatment, and one gilt fed 5 mg. daily ovulated during treatment.

The results of this trail indicate that U-10,520A will not inhibit estrus in gilts when administered at a daily rate of 250 mg. This compound apparently has a detrimental effect on pregnancy when administered during estrus. The evidence suggest that U-23,378 will inhibit estrus when administered at a rate of 250 mg. per day for 20 days. However, pre-treatment corpora lutea apparently persist throughout the feeding period which suggest a possible luteotropic effect of this compound. Compound U-10,997 will inhibit estrus when administered at a rate of 5 mg. per day, but the evidence suggested that 2.5 mg. per day is too low for effective inhibition. Estrus synchronization was attempted with this compound at both of these levels but variable and inconclusive results were obtained.

CHAPTER VII

TRIAL IV

Materials and Methods

This trial was initiated in the winter of 1968. The objectives were to determine the ability of U-10,997 to synchronize estrus at the 10 and 15 mg. levels and determine if U-23,378 would inhibit estrus at the 50 mg. level. The treatment design employed in this trial allowed for a total of 18 gilts and is shown in Table XV.

The three gilts assigned to U-23,378 were killed one day post-treatment if not bred, and the reproductive tracts examined. Gilts bred during treatment were killed 30 days post-breeding. Ovarian and uterine data were obtained in the same manner previously described for open gilts necropsied one day post-treatment and bred gilts necropsied 30 days post-breeding.

The compound U-10,997 apparently inhibits estrus and ovulation when administered at a rate of either 50, 10 or 5 mg. per day. Marked inhibition of ovarian activity occurs at these levels and 5 mg. per day appears to be the minimal effective dose although this level may be too low for consistent inhibition. Estrus synchronization was attempted with low levels (5 and 2.5 mg.) of this compound in Trial III but the results were inconclusive. A more extensive evaluation of the effect of U-10,997 for estrus synchronization in gilts was considered necessary and a decision was made to pursue the synchronization phase of this compound.

Fifteen gilts were used to extensively evaluate U-10,997 for estrus synchronization. The 10 and 15 mg. levels were used because data obtained in Trial II indicated that 10 mg. would effectively inhibit estrus and produced a desirable effect on the ovaries. Four gilts received 15 mg. daily for 20 days and were allowed to return to estrus following treatment. Gilts showing estrus within 15 days were bred and killed 30 days post-breeding. Gilts which did not show estrus within 15 days were killed on day-15. Eleven gilts were fed 10 mg. daily for 20 days. Three gilts in this treatment group were killed one day following treatment and their ovarian condition determined. Eight gilts were allowed to return to estrus following treatment and were bred if they showed estrus by day-15. The first one-half of the gilts bred prior to day-15 were allowed to go 30 days post-breeding before being necropsied to determine embryo survival rates. The remaining one-half of the bred gilts were killed 2 to 3 days following breeding in order to determine fertilization rates. Any gilt which failed to show estrus by day-15 following treatment was necropsied on day-15.

All compounds were administered in the standard manner used throughout this study. Gilts were checked daily for estrus during the 20 day feeding period and up to 15 days following treatment withdrawal. Gilts which exhibited signs of estrus but would not accept the boar were killed 2 to 3 days following the first indication of heat. Forced breeding was not used on any gilt. Ova were collected from bred gilts in the manner previously described.

Results and Discussion

The ability of the treatment compounds to inhibit estrus during the

20 day feeding period is shown in Table XVI. All gilts receiving a daily 50 mg. dose of U-23,378 came into estrus 6 to 9 days after treatment was initiated and all were bred. This compound apparently has no antifertility effects at this level since all gilts settled at first service and were pregnant at necropsy 30 days post-breeding. These 3 gilts had an average of 16 corpora lutea and 14 live embryos which gave

TABLE XV

DESIGN OF STUDY FOR TRIAL IV

	Treatment Compounds[a]		
	U-23,378	U-10,997	
Item	(163)	(164)	(165)
Daily dose level (mg.)	50	10	15
No. gilts allotted	3	11	4
No. necropsied one day post-treatment	3	3	0
No. allowed to return to estrus[b]	0	8	4

[a]Values in parenthesis refer to the code number used for each level of each compound.

[b]Gilts which did not return to estrus by day-15 post-treatment were necropsied on day-15.

an average embryo survival rate of 86 percent. Estrus and ovulation were inhibited in all 15 gilts fed either 10 or 15 mg. of U-10,997 daily. These results indicate that U-10,997 is a very effective estrus inhibiting compound in gilts.

The ability of U-23,378 and U-10,997-treated gilts to gain weight during the 20 day treatment period was similar to that observed for

TABLE XVI

ESTRUS INHIBITING ABILITY OF COMPOUNDS U-23,378 AND U-10,997

Item	Treatment Compounds[a] U-23,378 (R-163)	U-10,997 (R-164)	U-10,997 (R-165)
Daily dose level (mg.)	50	10	15
No. gilts allotted	3	11	4
No. gilts bred during treatment	3	0	0

[a]Values in parenthesis refer to the code number used for each level of each compound.

gilts fed these compounds in previous trials. No detrimental effects on feed intake or weight gains were noted. The average weight on the first and last day of treatment and mean average daily gain of gilts within each treatment group are presented in Table XVII.

TABLE XVII

EFFECT OF U-23,378 AND U-10,997 ON AVERAGE DAILY GAIN DURING THE 20 DAY TREATMENT PERIOD

Item	Treatment Compounds[a] U-23,378 (163)	U-10,997 (164)	U-10,997 (165)
Daily dose level	50	10	15
No. of gilts	3	11	4
Avg. initial weight (kg.)[b]	136±1.2	126±3.7	129±6.3
Avg. final weight (kg.)[b]	144± .6	134±4.1	137±7.4
Avg. daily gain (kg.)[b]	.378±.09	.424±.14	.358±.08

[a]Values in parenthesis refer to the code number used for each level of each compound.

[b]Mean ± standard error of mean.

The synchronization phase of this trial produced extremely variable results. The reproductive performance of gilts showing estrus within 15 days following treatment with U-10,997 is shown in Table XVIII. Five of the 8 gilts in the 10 mg. treated group showed external signs of estrus within a range of 8 to 15 days following treatment and 4 were bred. The fifth gilt was in standing estrus 8 and 9 days post-treatment but she would not accept the boar. Necropsy on day 11 revealed 13 ovulation sites on her right ovary, but the left ovary was completely inactive. The 4 bred gilts were to be killed 30 days post-breeding. However, 3 of these gilts recycled following the first post-treatment estrus and were rebred. The gilt which did not recycle was necropsied 30-days post-breeding and 16 normal embryos were observed. The failure of 3 gilts to settle following the first service suggested a possible antifertility effect of this compound following the 20 day feeding period. Two of the 3 gilts which recycled were killed 2 to 3 days following the second post-treatment estrus and their fertilization rates determined. Seventy percent of the ova recovered from one gilt were fertilized and 93 percent of those recovered from the other gilt were fertilized indicating that the compound did not have a permanent effect on the reproductive system of these gilts. The remaining gilt bred at the second post-treatment estrus was killed 30 days post-breeding and the percent embryos ranged from 86 to 100 percent.

Three of 4 gilts fed 15 mg. of U-10,997 daily, came into estrus and were bred 7, 9, and 10 days post-treatment. However, only one of these gilts was pregnant at necropsy 30 days post-breeding. Of the 2 open gilts, one had very cystic follicles. The gilt with cystic follicles had returned to estrus 10 days post-treatment and was bred for 8

TABLE XVIII

REPRODUCTIVE PERFORMANCE OF GILTS SHOWING ESTRUS WITHIN 15 DAYS FOLLOWING TREATMENT WITH U-10,997

	Levels (mg.) of U-10,997		
	10	15	Both
Total No. of gilts in estrus	5	3	8
Post-treatment estrus interval (days)[a]	8-15	7-10	7-15
No. of gilts bred	4	3	7
No. which ovulated	5[b]	2	7
No. with cystic follicles (>16 mm.)	0	1	1
No. killed 2-3 days post-breeding	2	-	-
No. which recycled	2	-	-
No. corpora lutea[a]	13-18	-	-
% ova recovered[a]	76-83	-	-
% ova fertilized[a]	70-93	-	-
No. killed 30 days post-breeding	2	3[c]	5
No. w;hich recycled	1	0	1
No. pregnant at necropsy	2	1	3
No. corpora lutea[a]	14-16	17	14-17
No. embryos[a]	12-16	15	12-16
% embryo survival[a]	86-100	88	86-100

[a]Range-which does not include cystic ovaries.

[b]One gilt would not accept boar but ovulated.

[c]Two were not pregnant--one had cystic follicles.

consecutive days. For this reason the cystic follicles present 30 days post-breeding were thought to represent those which developed at the first post-treatment estrus.

The reproductive performance of the 8 gilts in estrus within 15

days following treatment with either 10 or 15 mg. of U-10,997 was unsatisfactory and not acceptable for effective estrus synchronization in gilts. Only 25 percent of these gilts settled at the first post-treatment estrus. Seven of the 8 were bred, but only 2 were pregnant 30 days after the first breeding. The occurrence of cystic follicles in one gilts was considered to be a result of the treatment. The failure of one gilt to accept a boar, although she showed external signs of estrus and did ovulate, may also be a result of the treatment. The left ovary of this gilt was completely inactive which suggests that this compound may inhibit ovarian function by acting directly on the ovarian tissue and interfering with its ability to respond to pituitary gonadotropins. This is a possible explanation for the failure of 4 of 7 bred gilts to settle on first service. The compound may have impaired the ability of the ovaries to produce normal ova at the first synchronized estrus.

The reproductive performance of gilts which failed to show estrus by day-15 following withdrawal of U-10,997 is shown in Table XIX. Three of the 8 gilts fed 10 mg. per day showed no signs of estrus within 15 days and were killed on day-15. The ovaries recovered from 2 of these gilts were essentially inactive with no corpora lutea present, although a few small follicles (less than 5 mm.) were present. Both ovaries of the third gilt were cystic. The completely inactive condition of ovaries obtained from 2 of these gilts seem to provide evidence for a possible direct effect of the compound on the ovarian tissue. The ovaries could have been affected in such a way that their normal response to gonadotropin stimulation was interferred with. It seems unlikely that the compound could have continued blocking the secretion of pituitary gonadotropins for 15 days following the last day of feeding. The

TABLE XIX

REPRODUCTIVE PERFORMANCE OF GILTS NECROPSIED 1 DAY AND 15 DAYS FOLLOWING TREATMENT WITH U-10,997

	Necropsy 1 Day Post-Treatment Level (mg.)		Necropsy 15 Days Post-Treatment Level (mg.)	
	10	15	10	15
No. gilts	3	-	3	1
No. with cystic follicles (>16 mm.)	1	-	1	0
No. with vesicular follicles (6-16 mm.)	1	-	0	0
Total ovarian weight (mg.)[a]	7,302-14,250	-	6,895-6,978	29,305[b]
No. corpora lutea[a]	0 0	-	0 0	0
Uterine weight (gm.)[a]	534-568	-	463-493	973

[a]Range-which does not include cystic ovaries.

[b]Large follicles embedded in ovary--could not be measured.

condition of ovaries obtained on day-15 from one gilt fed 15 mg. of U-10,997 was somewhat normal. Large follicles were present, but they were embedded in the ovary and could not be measured. The stroma of the ovary completely engulfed the follicles and only a small portion of the follicles actually protruded from the ovary. This condition could also reflect a direct effect of U-10,997 on ovarian tissue.

The ovarian condition of gilts killed one day following treatment with 10 mg. of U-10,997 is also presented in Table XIX. The ovaries of one gilt were completely inactive with no corpora lutea or follicles present and this condition was very similar to that of gilts treated with 10 mg. in Trial II. The ovaries of a second gilt contained no corpora lutea, but very good follicular development was present. A total of 8 follicles 6 to 16 mm. in diameter were present, but the follicular fluid of most of these follicles appeared bloody. The ovaries of the 3rd gilt were cystic.

The results of this trial indicate that daily administration of 10 or 15 mg. of U-10,997 in the diet of puberal gilts will prevent the occurrence of estrus and ovulation when fed for a period of 20 days. However, following withdrawal of treatment, the length of time required for gilts to rebound and attain normal ovarian activity is too long and much too variable for effective estrus synchronization. The inactive condition of ovaries at the end of the treatment period apparently is not conducive to effective estrus synchronization. The evidence suggest that this inhibitory effect of U-10,997 is detrimental to the ovaries and the length of time required for their full functional capacity to be restored following treatment is too long and too variable. Following treatment, the fertility levels appear to be lower and the compound

apparently creates a condition favorable to development of cystic follicles since 20 percent of the treated gilts developed this abnormality. These results indicate that the 10 and 15 mg. levels of compound U-10, 997 can not be used for estrus synchronization in gilts.

CHAPTER VIII

TRIAL V

Materials and Methods

In the four previous trials compounds were considered efficacious if they inhibited estrus and also allowed pre-treatment corpora lutea to regress during the 20 day treatment period. This trial was conducted to determine if effective estrus synchronization could be obtained with compounds which would inhibit estrus, but would not allow the pre-treatment corpora to regress.

The results obtained in Trial IV indicated that the daily administration of U-10,997 to gilts at a rate of either 10 or 15 mg. per head would not permit precise control of ovulation and therefore could not be used as an effective method for synchronization of estrus. However, the evidence clearly indicated that this compound possessed certain properties which would inhibit normal ovarian activity during the period of administration and, at low levels, would allow degeneration of pre-treatment corpora lutea. The latter situation was attained by reducing the daily dosage level from 50 mg. used in Trial I, down to 10 and 15 mg. levels used in Trial IV. At these lower 10 and 15 mg. dosage levels, this compound apparently possesses certain properties not manifested at the higher 50 mg. levels. Although ovarian inhibition was observed in gilts treated with 50 mg. daily, it was not as pronounced since corpora lutea in the regressed stage were present on the last day of feeding.

The ovarian inhibition effect observed in U-10,997-treated gilts suggested that this compound had suppressed the production and/or release of pituitary gonadotrophins. However, the evidence also indicated a possible direct effect on the ovaries which interferred with their ability to recover from the suppression period and attain normal ovarian activity following withdrawal of the compound. This direct effect appeared to be more pronounced at the lower level than at the higher level. The ovaries of gilts fed 10 mg. daily were not only devoid of corpora lutea at the end of the treatment period, but they felt very turgid and had a fibrous appearance.

Corpora lutea formed during the normal estrous cycle must regress before ovulation and a new estrous cycle can be initiated. Therefore, absence of corpora lutea on the ovaries of treated gilts at the end of the 20 day feeding period employed in this study was thought to be a desirable situation which should allow, within a relatively short time, a spontaneous rebound to normal ovarian activity following the suppression period. However, the results of Trial IV suggested that at the 10 and 15 mg. levels, U-10,997 was suppressing ovarian activity to such an extent that the ovaries could not recover quickly enough to allow estrus and normal ovulation to occur within a reasonable length of time following treatment withdrawal. The evidence indicated that this degree of inhibition was too extensive and produced undesirable results. The evidence also suggested the possibility that a more desirable synchronization effect might be obtained by feeding the higher levels of U-10,997. In Trial I of this study, a daily 50 mg. dose of U-10,997 effectively inhibited ovarian activity, but the condition of ovaries obtained from gilts at the end of the 20 day feeding period did not exhibit the

marked degree of inhibition observed at the lower levels.

Although luteal maintenance occurred in gilts fed the high level of U-10,997, there was a possibility that this condition may not be detrimental for effective estrus synchronization. This possibility was based primarily on work conducted by Baker (1967) who laparotomized 3 sows which were being treated with Methallibure (ICI 33828) and found normal corpora lutea on their ovaries on the last day of a 20 day feeding period. These sows returned to estrus within 8 days following treatment withdrawal. Polge (1965) also performed laparotomies on 3 gilts which had received 100 mg. I.C.I. 33828 daily for 15 days. Corpora lutea were present although they were quite pale in color and had regressed to about 5 mm. in diameter. Gilts fed this level of Methallibure for 20 days normally return to estrus within 4 to 6 days post-treatment (Polge 1965 and Polge and Day 1968). The description of the ovaries from Methallibure-treated gilts was very similar to that of ovaries obtained from gilts treated with 50 mg. of U-10,997. This suggested that the presence of corpora lutea on the ovaries at the end of the 20 day feeding period may not prevent gilts from returning to estrus soon after treatment withdrawal.

This trial was initiated in the summer of 1968. The primary objective was to determine if estrus and ovulation could be successfully synchronized using compounds which would inhibit estrus, but would not prevent luteal maintenance during the treatment period. In addition to U-10,997, compounds U-13,851 and U-23,378 were also evaluated for their ability to synchronize estrus and ovulation. The results of Trials I and II indicated that U-13,851 would effectively inhibit estrus when fed at a rate of 50 to 10 mg. daily. Ovarian activity appeared to be inhib-

ited, but pre-treatment corpora lutea persisted throughout the 20 day treatment period. The results of Trial III indicated that U-23,378 would also inhibit estrus and ovulation when fed at a rate of 250 mg. per day, but pre-treatment corpora lutea were maintained throughout the treatment period. A second objective of this trial was to conclusively determine if these three compounds prevent ovulation from occurring during the treatment period. This was accomplished by laparotomizing gilts and marking their corpora lutea with India ink. This was done approximately 2 to 4 days prior to starting them on treatment. The presence of marked corpora lutea on the ovaries at the end of the 20 day treatment period would prove that the compound had inhibited ovulation. Conversely, unmarked corpora would indicate that ovulation had occurred.

Laparotomies were performed by veterinarians in the Department of Clinical Research, College of Veterinary Medicine, Oklahoma State University. Animals were anesthetized initially with .75 gm. of Surital (Sodium Thiamyla) injected into an ear vein. The anesthesized animals were then transferred to the operating table and placed under Halothane closed-circuit anesthesia. Standard aseptic procedures were employed throughout the operation. A mid-ventral incision approximately 4 inches in length was made and the ovaries removed from the abdominal cavity. A 28 gauge needle was used to inject a small amount of sterile India ink beneath the stromal covering of each corpora lutea. The number of corpora lutea present on each ovary was determined for comparison against the number present one day following treatment with the various compounds. Following exposure, the ovaries and the uterine horns were moistened continuously with sterile saline (10 percent) and handled as little as possible. Results of preliminary laparotomies indicated that

these precautions were necessary in order to prevent uterine and ovarian adhesions from occurring. Following the operation, the animals were left in a recovery room overnight and then returned to the experimental pens. They were injected intramuscularly with 5 ml. of penicillin for 3 consecutive days following the operation.

The treatment design employed in this trial is shown in Table XX. A total of 44 gilts were used to extensively evaluate the ability of U-10,997, U-13,851 and U-23,378 to inhibit estrus and ovulation during the treatment period and also to determine how effective they were in controlling the time of ovulation following the treatment period. U-10,997 was tested at both the 50 and 30 mg. levels; U-13,851 at the 15 mg. level; and U-23,378 was tested at the 250 mg. level. A total of 10 gilts were assigned to each of these 4 treatment groups. Four of these gilts were laparotomized in the manner just described, and their corpora lutea marked. They were killed one day following the last day of feeding in order to determine if the compound had prevented ovulation. The remaining 6 gilts that were not laparotomized were allowed to return to estrus in order to establish the length of time required for estrus and ovulation to occur following treatment. Any gilt bred prior to day 10 post-treatment was killed 2 to 3 days post-breeding in order to determine fertilization rates. However, if these gilts did not return to estrus within 10 days post-treatment, three of them were killed on day 10, and their ovarian condition determined. The remaining 3 were observed for estrus and bred for a period extending up to day 60 in order to determine the interval or length of the anestrus period.

Four sham operated control gilts were used to determine whether the stress of laparotomy would have an effect on the length of their estrous

TABLE XX

DESIGN OF STUDY FOR TRIAL V

Compound	R-No.	No. of Gilts	Daily Dose Level (mg)	Laparotomize Kill 1 Day after Last Feeding	No Surgery: Kill Day 10 after Last Feeding if No Estrus	No Surgery: Observe and Breed up to Day 60 After Last Feeding
U-10,997	R-167	10	50	4	3	3
U-10,997	R-168	10	30	4	3	3
U-13,851	R-169	10	15	4	3	3
U-23,378	R-170	10	250	4	3	3
Control	-----	4	---	4[a]		

[a]Kill on day 10 following first post-operative estrus.

cycle. These gilts were laparotomized on day 7 to 10 of their estrous cycle and checked daily until they returned to estrus. A cycle of 18 to 24 days was considered normal. They were killed on approximately day 10 of the first post-operative estrous cycle and their ovaries were recovered. Progesterone determinations were made for comparisons against ovaries obtained from treated gilts killed either one day or 10 days post-treatment.

The compounds were administered to the animals for 20 days in the same manner employed throughout this study. Daily heat observations were made during and after the feeding period in the standard manner. Fertilization rates of bred gilts were determined by killing them 2 to 3 days post-breeding and recovering their ova in the manner described in Trial III. For open gilts, ovarian weights, number and size of corpora lutea, degree of follicular development, appearance and weight of uteri, were also determined in the standard manner previously described.

Total progesterone concentration and progesterone concentration per gram of luteal tissue were determined in the following manner.

The Method in Detail

Preparation of Tissue

Ovaries obtained from treated animals were removed from solid CO_2 storage. Corpora lutea were separated from the frozen ovaries, counted, and weighed on a Federal Pacific precision balance. Pooled corpora lutea from each animal were minced and three grams were placed in a chilled Tenbroeck glass homogenizer containing isotonic KCL (0.154M). Twelve ml. of a 20 percent homogenate (w/v) were made for each sample.

Progesterone Extraction

A known quantity of progesterone-7-^{3}H was placed in the tip of each 90 ml. extraction tube to enable estimation of losses during the isolation procedure. This progesterone was evaporated to dryness under nitrogen in a water bath (45C) and a 5 ml. aliquot of the 20 percent homogenate was added. Each sample was then extracted 3 times with 2.5 volumes of dichloromethane. Extractions were carried out by inverting the tubes for one minute and centrifuging for 5 minutes.

A major difficulty in purifying progestins from tissue source lies in separating them from the lipid material. Extraction with dichloromethane, followed by partitioning between NaOH and ether as described by Stabenfeldt (1968), would not satisfactorily separate progesterone from the fat material. Thus, a procedure described by Ewing and Eik-Nes (1967) for defatting was utilized. Following evaporation of the dichloromethane, the extract of the biological sample was defatted by partitioning 5 times between petroleum ether and 70 percent methanol. This was accomplished by adding 15 ml. of petroleum ether and 5 ml. of 70 percent methanol to the sample. Partitioning was carried out on a Vortex shaker for one minute and then centrifuging for 5 minutes. The 70 percent methanol was transferred to a 90 ml. test tube and approximately two-thirds of this volume was evaporated under nitrogen. The remainder was increased to a volume of 10 ml. with glass-distilled H_2O and reextracted 3 times with dichloromethane. The solvent was evaporated and progesterone was concentrated in the tip of the tube by washing the sides of the tubes with nanograde benzene. The residue was then applied to a thin layer chromatography plate.

Thin Layer Chromatography

All TLC plates were 8 inch square plate glass and were coated with silica gel to a thickness of .25 mm. The origin was placed 1.5 cm. above the bottom of the plate, and 9 vertical lanes 2 cm. wide were inscribed on the plates, 8 for samples and 1 for standard progesterone. Spotting of plates was accomplished by adding 3, 2 and 1 drops of nanograde benzene to the tubes and transferring each sample to the plate with a fine capillary tube. The plates were then air dried for approximately 10 minutes before development in the appropriate solvent system.

Development of Plates

The TLC plates were developed in the solvent system benzene ethyl acetate (3:1, v/v). The solvent system was placed in the developing tank, and a stiff piece of absorbent paper was placed on both sides of the tank to aid in saturating the atmosphere in the tank with the solvent. The tank was allowed to equilibrate until the paper was thoroughly saturated (about 1 hour) before placing the plate in the tank. The solvent was allowed to run to the top of the plate before removal of the plate from the tank. The plate was then allowed to air dry for approximately 10 minutes.

The progesterone spot was located with a UV lamp and a 3 cm. area corresponding chromatographically to standard progesterone was eluted. A fritted disc eluter (Dependable Scientific) was used to trap the silica gel on a sintered glass filter (medium pore size) prior to elution with 10 ml. of hot methanol. Following drying, the sample was concentrated by 2 and 1 ml. rinses of benzene.

Quantitation of Progesterone

The dried eluate was then reconstructed to 1 ml. total volume with anhydrous methanol distilled one time. A .1 ml. aliquot of this elute was removed for determination of percent recovery of progesterone-7-^{3}H. The radioactivity of the eluate was determined using a Packard liquid scintillation spectrometer. For final quantitation, an additional .1 ml. aliquot was placed in a .25 ml. quartz curvette and diluted with .15 ml. anhydrous methanol. Spectrophotometric determinations were carried out on the Cary Model 15 recording spectrophotometer at a wave length of 240 millimicrons, applying the background correction suggested by Allen (1950). Absorption was measured at 225 mμ, 240 mμ and 255 mμ; the values were calculated as follows, (O.D. = optical density):

$$O.D._{240\ corrected} = O.D._{240\ observed} - \frac{O.D._{225\ observed} + O.D._{255\ observed}}{2}$$

Results and Discussion

The results obtained from feeding either 50 or 30 mg. of U-10,997, 15 mg. of U-13,851, and 250 mg. of U-23,378 indicate that at these levels, these 3 compounds will inhibit both estrus and ovulation during the 20 day feeding period. The necropsy data obtained from laparotomized gilts killed one day following treatment are shown in Table XXI.

Ovaries obtained from U-10,997-treated gilts exhibited the same degree of inactivity observed at the lower dose levels. The average number of corpora lutea marked with India ink at laparotomy was 10.7±2.1 and 13.5±.50 for the 50 and 30 mg. levels respectively. However, at

TABLE XXI

MEANS AND STANDARD ERRORS FOR TOTAL OVARIAN WEIGHT, NUMBER OF MARKED CORPORA LUTEA AND UTERINE WEIGHT FOR LAPAROTOMIZED GILTS NECROPSIED 1 DAY FOLLOWING TREATMENT WITH U-10,997, U-13,851 AND U-23,378

	Treatment Compounds[a]			
	U-10,997 (R-167)	U-10,997 (R-168)	U-13,851 (R-169)	U-23,378 (R-170)
No. of Gilts	4	4	4	4
Daily dose level (mg.)	50	30	15	250
Total ovarian weight (mg.)[b]	5474±452	4402±749	6,532±1,338	12,655±1,102
No. of corpora lutea marked at laparotomy[b]	10.7±2.1	13.5±.50	12.7±1.6	14.6±.48
No. of marked corpora lutea present at necropsy[b]	0	0	6.3±2.6	12.3±1.5
No. of gilts with unmarked corpora lutea at necropsy	0	0	0	1
Uterine weight (gm.)[b]	575±40	518±21	579±50	682±.53

[a]Values in parenthesis refer to the code number used for each level of each compound.

[b]Mean ± standard error of mean.

necropsy one day following treatment, the ovaries of all the laparotomized gilts in these 2 treatment groups were completely devoid of any corpora lutea. Ink was present throughout the stromal portion of these ovaries. This evidence clearly indicated that ovulation had been prevented and the pre-treatment corpora lutea had regressed during the treatment period. The total ovarian weights of U-10,997-treated gilts were smaller than those from U-13,851 and U-23,378-treated gilts and this was attributed to the absence of corpora lutea. The complete degeneration of the corpora lutea in gilts fed 50 mg. daily was not expected. In Trial I, the same level of this compound was fed to gilts and complete regression of corpora lutea occurred in only one of 5 gilts while the other 4 gilts had an average of 9±4.9 corpora lutea. It was not clear why gilts in Trial I and those in this trial responded differently to the 50 mg. level of U-10,997. The previous hypothesis was that this compound possessed estrogenic activity at the high level and this caused the corpora to persist. The results of this trial do not substantiate this hypothesis.

The ovaries obtained from gilts fed a daily 15 mg. dose of U-13,851 indicated that this compound would also inhibit estrus and ovulation during the 20 day feeding period. An average of 12.7±1.6 corpora lutea were marked at laparotomy and the average number of marked corpora lutea at necropsy was 6.3±2.6. The variation in number of corpora at laparotomy and at time of necropsy resulted from 2 of the 4 not having any corpora lutea present at the end of the feeding period. However, ink was present in the stromal portion of the ovaries from these 2 gilts. The ovaries from the other 2 gilts contained an average of 12.5 marked corpora lutea. Ovulation had been inhibited in all 4 of these gilts.

The degeneration of pretreatment corpora lutea in 2 of these gilts may have been a result of the laparotomy. Mild uterine and ovarian adhesions had occurred in one of these gilts and this could have interferred with the blood supply to the ovary and this could have caused the corpora to regress.

The results obtained from feeding 250 mg. of U-23,378 indicated that this may be the minimal effective dosage level of this compound since one of the gilts ovulated during treatment. This gilt had 16 corpora lutea when laparotomized but only 14 corpora were present at necropsy and none of these contained any ink. She did not exhibit any signs of estrus during the treatment period. The other 3 gilts had an average of 12.3±1.5 marked corpora lutea at necropsy. Ovulation was inhibited in these 3 gilts and pre-treatment corpora lutea were maintained throughout the feeding period.

The progesterone concentration of corpora lutea present on the ovaries of laparotomized, U-13,851 and U-23,378-treated gilts, killed one day following treatment, is presented in Table XXII. The 2 U-13,851-treated gilts in which corpora persisted had progesterone values of 10.41 and 7.59 μg per gram of C.L. tissue. The 3 U-23,378-treated gilts in which ovulation was inhibited and corpora persisted had progesterone values of 10.06, 9.34 and 9.34 μg. per gram of tissue. The progesterone values obtained from these 5 gilts were considerably less than the 29.4 μg per gram average obtained from 4 sham operated control gilts killed on day 10 of their estrous cycle. These differences were not analyzed statistically because of the small number of gilts involved in each treatment group. However, the results did indicate that the capacity of the corpora lutea of U-13,851 and U-23,378-treated gilts to produce

TABLE XXII

PROGESTERONE LEVELS IN GILTS KILLED 1 AND 10 DAYS FOLLOWING TREATMENT WITH U-13,851 AND U-23,378

	No. C.L.	Total C.L. Weight (gm.)	Total Progesterone Concentration (μg.)	Progesterone per gram of C.L. Tissue (μg.)
Gilts in Laparotomy Phase				
U-13,851 (15 mg.)				
Y-4-6	12	3.452	39.92	10.41
Y-9-3	13	5.023	38.12	7.59
U-23,378 (250 mg.)				
Y-21-4	15	6.124	61.60	10.06
Y-14-1	10	6.439	60.05	9.34
Y-14-2	12	4.341	40.54	9.34
Y-6-3[a]	14	7.711	257.00	33.42

TABLE XXII (Continued)

Gilts in Synchronization Phase				
U-13,851 (15 mg.)				
Y-9-1	14	6.250	75.87	12.14
Y-98-2	13	2.691	13.67	5.08
Y-10-3	17	6.741	79.27	11.76
U-23,378 (250 mg.)				
Y-3-4	12	5.996	46.64	7.78
Y-7-2	17	7.170	52.05	7.26
Avg. for Four Laparotomized Control Gilts[b]	14±1	6.881±.77	181±28	29.4±3.4

[a]This gilt ovulated during the treatment period.

[b]Mean ± standard error of mean.

progesterone had decreased.

The ability of U-10,997, U-13,851, and U-23,378-treated gilts to gain weight during the treatment period was similar to that observed for gilts fed these compounds in previous trials. No detrimental effects on feed intake or weight gains were noted. The average weight on the first and last day of treatment and mean average daily gain of gilts within each treatment group are presented in Table XXIII.

The synchronization phase of this trial produced extremely variable results and indicated that none of these compounds could be used effectively for synchronization of estrus in swine. A total of 24 gilts were used in this phase and none of these gilts returned to estrus prior to day 10 following treatment withdrawal. At least one incidence of cystic follicles occurred within each of the 4 treatment groups. The reproductive performance of gilts killed 10 days following the last day of feeding with the various compounds is presented in Table XXIV. Table XXV shows the reproductive performance of those gilts which were observed for estrus and bred up to day 60 after the last feeding.

U-10,997 - 50 mg. Daily

The results obtained from feeding a daily 50 mg. dose of U-10,997 were similar to those obtained in Trial IV where estrus synchronization was attempted at the 15 mg. level. The condition of ovaries from 3 gilts killed 10 days post-treatment indicated that their functional capacity had been suppressed to such an extent that they could not recover and resume normal ovarian activity within a reasonable length of time. The ovaries of one of these gilts were essentially inactive with no corpora lutea and all of the follicles present were less than 5 mm. in

TABLE XXIII

EFFECT OF U-10,997, U-13,851 AND U-23,378 ON AVERAGE DAILY GAIN DURING THE 20 DAY TREATMENT PERIOD

	Treatment Compound[a]			
	U-10,997 (R-167)	U-10,997 (R-168)	U-13,851 (R-169)	U-23,378 (R-170)
Daily dose level (mg.)	50	30	15	250
Number of gilts	10	10	10	10
Avg. initial weight (kg.)[b]	126±6.43	132±5.22	131±2.91	126±6.92
Avg. final weight (kg.)[b]	133±5.53	139±4.42	135±2.62	135±6.34
Avg. daily gain (kg.)[b]	.417±.051	.427±.043	.291±.081	.307±.069

[a]Values in parenthesis refer to code number used for each level of each compound.

[b]Mean ± standard error of mean.

TABLE XXIV

REPRODUCTIVE PERFORMANCE OF GILTS KILLED 10 DAYS FOLLOWING LAST DAY OF FEEDING WITH U-10,997, U-13,851 AND U-23,378

Compound and Daily Dose Level	Gilt Number	Total Ovarian Weight (mg.)	No. of Corpora Lutea	Avg. Size of Corpora lutea	Follicular Development		Weight of Uterus (gm.)
					No.	Size	
U-10,997	Y-50-1	5,888.7	None	-	(NSF)[a]		486
(R-167)	Y-19-4	9,437.0	None	-	22 (NSF)	6-9	844
50 mg.	Y-35-2	7,781.6	None	-	8 (NSF)	6-7	506
U-10,997	Y-94-1	5,633.4	None	-	(NSF)		665
(R-168)	Y-95-3	9,961.3	None	-	(NSF)		444
U-13,851	Y-9-1	16,022.3	14	8.65	(NSF)		791
(R-169)	Y-98-2	9,819.8	13	5.55	3	7-13	488
15 mg.	Y-10-3	12,307.0	17	7.53	(NSF)		736
U-23,378	Y-3-4	13,006.8	12	8.94	(NSF)		915
(R-170)	Y-7-2	14,507.7	17	8.48	(NSF)		838
250 mg.	Y-2-1	11,862.9	None	-	Abnormal[b]		678

[a]Numerous small follicles less than 5 mm. in diameter.

[b]Follicles were embedded in ovarian tissue and could not be measured.

TABLE XXV

REPRODUCTIVE PERFORMANCE OF GILTS KILLED BETWEEN 10 AND 60 DAYS FOLLOWING TREATMENT WITH U-10,997, U-13,851 AND U-23,378

Compound and Daily Dose Level	Gilt Number	Post-Treatment Estrus Interval (days)	Bred	Necropsy Comments
U-10,997 (R-167) 50 mg.	Y-43-3	14	Yes	Partial ovulation occurred--14 C.L. present; remaining 13 follicles were becoming cystic--8 to 22 mm. in diameter.
	Y-43-3	11	Yes	Eighteen corpora hemorrhagica present; 10 fertilized ova recovered in 2 and 4 cell stage.
	Y-40-3	43	Yes	Killed 30 days post-breeding; 16 C.L. and 14 normal embryos were present.
U-10,997 (R-167) 30 mg.	Y-28-3	10	Yes	Eighteen corpora hemorrhagica present; 12 fertilized ova in 2, 4, and 8 cell stage and 2 one cell ova recovered.
	Y-3-1	29	No	Showed heat signs for 2 days but would not accept boar; very cystic follicles had developed.
	Y-3-3	12	No	Showed heat signs for 3 days but would not accept boar; 23 preovulatory size follicles present--8 to 15 mm. in diameter--but 12 were hemorrhagic.
	Y-99-2	--	No	Became sick and emaciated; necropsy 50 days post-treatment showed a hypoplastic testicle in place of right ovary.

TABLE XXV (Continued)

U-13,851 (R-169) 15 mg.	Y-8-2	27	No	Showed heat signs for 3 days but would not accept boar; very cystic follicles had developed.
	Y-11-2	--	No	Did not show estrus within 60 days post-treatment; presence of 14 C.L. at necropsy indicated that she had ovulated within this period.
	Y-16-2	--	No	Did not show estrus within 60 days post-treatment; presence of 14 C.L. at necropsy indicated that she had ovulated within this period.
U-23,378 (R-170)	Y-89-2	14	Yes	Necropsy 3 days after first breeding revealed preovulatory size follicles (<16 mm.) and cystic follicles (>16 mm.). She did not ovulate.
	Y-86-1	12	No	Showed heat signs for 3 days but would not accept boar; necropsy revealed 22 preovulatory size follicles--8 to 14 mm.--but 8 were hemorrhagic.
	H-36-2	33	No	Showed heat signs for 3 days but would not accept boar; necropsy revealed 11 preovulatory size follicles--9 to 12 mm.

diameter. However, the degree of follicular development observed in the other 2 gilts was more extensive. One gilt had 22 pre-ovulatory size follicles ranging from 6 to 9 mm. in size and the other gilt had 8 follicles 6 to 7 mm. in diameter.

The 3 gilts observed for estrus up to day 60 following treatment returned to estrus and were bred prior to day 60. One of these gilts was bred 11 days post-treatment but only 55 percent of her ova were recovered although all were fertilized. The corpora hemorrhagica were unusually small and pale pink in color and did not appear normal. There is a possibility that they did not all produce ova and this may account for the low recovery rate. A second gilt was bred 14 days post-treatment but all follicles had not ovulated. Fourteen corpora hemorrhagica and 13 follicles ranging from 8 to 22 mm. in diameter were present which indicated that these ovaries were becoming cystic. Only 6 ova were recovered and none were fertilized. The third gilt was bred 43 days post-treatment, but ova were not recovered due to a mistake in breeding dates. Necropsy 30 days post-breeding revealed 14 embryos and 16 corpora lutea.

Compound U-10,997 does inhibit estrus and ovulation, presumably by blocking the production and/or release of FSH. The degree of follicular development observed in the 3 gilts killed 10 days post-treatment suggests that pituitary suppression is maintained for at least 10 days following treatment, or it may also be interpreted as evidence for a direct effect of U-10,997 which prevented them from responding to pituitary gonadotropins. Another alternative is that both pituitary suppression and direct inhibition of ovarian activity prevent estrus from occurring within 10 days following treatment withdrawal.

U-10,997 - 30 mg. Daily

A daily 30 mg. dose of U-10,997 produced the same variable response as did the 50 mg. level. The ovaries of 2 gilts killed on day 10 following treatment were essentially inactive with some small follicles less than 5 mm. in diameter present (Table XXIV). Only one gilt fed this level was actually bred (Table XXV). She returned to estrus 10 days post-treatment and had 18 corpora present at necropsy. Twelve fertilized ova in the 2, 4 and 8 cell stage and 2 non-fertilized ova were recovered. This was the only gilt in this trial which returned to estrus within a reasonable length of time and ovulated normally. Two other gilts returned to estrus on the 12th and 29th day but neither would accept the boar (Table XXV). Very cystic follicles had developed in one gilt and although 23 preovulatory size follicles (8 to 15 mm.) were present on the ovaries of the other gilt, 12 were hemorrhagic. A 6th gilt in this treatment group was observed for estrus up to day 50 following treatment, but she showed no signs of estrus (Table XXV). This gilt started losing weight rapidly approximately 40 days post-treatment and became emaciated. At necropsy 50 days post-treatment a large oval capsulated mass approximately 5 centimeters in diameter was observed in place of the right ovary. Microscopic examination of this mass revealed hypoplastic seminiferous tubules which were not producing spermatozoa. A large amount of interstitial tissue was observed but no vestiages of ovarian tissue were present. The diagnosis was that this was a hypoplastic testicle and the animal was a hermaphrodite. The left ovary appeared to be macroscopically normal and functional. The hermaphroditic condition of this gilt did not interfere with her ability to have a normal estrous cycle since she had cycled normally prior to being placed

on treatment.

The evidence obtained in this trial indicates that this compound, at this level, could not be used for estrus synchronization in swine.

U-13,851 - 15 mg. Daily

The response of gilts fed a daily 15 mg. dose of U-13,851 was variable and difficult to interpret. The ovaries of 3 gilts killed on day 10 following treatment had normal size corpora lutea present (Table XXIV), but they were not producing adequate amounts of progesterone. The ovaries of two gilts, Y-9-1 and Y-10-3, contained 12.14 and 11.76 μg of progesterone per gram of C.L. tissue (Table XXII). These levels are considerably below the 29.4 average for C.L. tissue obtained from laparotomized control gilts. Corpora lutea on the ovaries of gilt Y-98-2 were regressing as indicated by their size (5.55 mm.) and a low progesterone concentration of 5.08 μg. per gram of C.L. tissue. Since this compound does inhibit ovulation during the feeding period, and since these gilts did not show signs of estrus prior to day 10 following treatment, it must be assumed that these corpora lutea were the same ones present when the treatment period was initiated. This evidence indicates that U-13, 851 probably has luteotropic properties since both the structural integrity of the C.L. and their capacity to produce progesterone were maintained for a 30 day period.

Estrus was not detected in 2 of 3 gilts observed for estrus up to day 60 (Table XXV). One gilt (Y-8-2) showed signs of estrus 27 days post-treatment, but she would not accept the boar. Necropsy showed that cystic follicles had developed. The other 2 gilts were not detected in estrus prior to day 60, but the presence of 14 corpora lutea in each

gilt indicated that both had ovulated prior to necropsy.

The results of this trial indicate that U-13,851 will inhibit ovulation in gilts but will not allow corpora lutea to regress. This luteal maintenance effect continues for at least 10 days following treatment withdrawal and indicates a possible luteotropic effect of this compound. In pigs, the single release of a luteotropic substance at or near the time of ovulation is sufficient to cause corpora lutea to form and to persist and function for its normal life span during the estrous cycle. U-13,851 may cause luteal maintenance in an analogous fashion since C.L. persist in a functional manner for at least 10 days following the last day of feeding the compound. This is not a desirable situation for synchronizing estrus and ovulation because corpora lutea must regress before normal ovulation can occur. This compound could not be recommended for estrus synchronization in swine.

U-23,378 - 250 mg. Daily

The response of gilts fed 250 mg. of U-23,378 indicated that this compound also possesses luteotropic properties which are not conducive for synchronizing ovulation. The ovaries of 2 gilts killed 10 days following treatment had normal size corpora lutea (8.7 mm.) (Table XXIV) but their progesterone concentration was typical of regressing corpora. The 2 gilts, Y-3-4 and Y-7-2 had 7.78 and 7.26 μg. of progesterone per gram of C.L. tissue respectively (Table XXII). The ovaries of a 3rd gilt killed at this time contained follicles which were embedded in the ovarian tissue and could not be measured.

Only one gilt in this treatment group was bred, but she did not ovulate (Table XXV). Gilt Y-89-2 was bred 14 days post-treatment, but

necropsy 3 days after first breeding revealed both preovulatory size follicles less than 16 mm. in diameter and cystic follicles greater than 16 mm. No ovulation sites were observed. Another gilt showed signs of heat on day 12, but she would not accept the boar. Necropsy 4 days after first indications of heat revealed 22 preovulatory size follicles 8 to 14 mm. in size, but 8 were hemorrhagic. The 3rd gilt showed signs of heat on day 33, but she also would not accept the boar. Eleven follicles 9 to 12 mm. in size were found at necropsy.

The evidence suggests that U-23,378 causes pre-treatment corpora lutea to be maintained for at least 10 days following the last day of feeding. This luteal maintenance effect is similar to that observed in U-13,851-treated gilts. Administering U-23,378 to gilts will not allow ovulation to occur prior to day 10 following treatment because corpora lutea are still present on the ovaries at this time. Thus, time of ovulation following treatment withdrawal depends on the length of time required for the pretreatment corpora to regress and allow normal follicular development to occur. The results of this trial indicate that this period of time is too long and too erratic for effective estrus synchronization.

CHAPTER IX

GENERAL RESULTS AND DISCUSSION

The specific objective of this study was to evaluate various compounds for their ability to control estrus and ovulation in puberal gilts when administered orally for a 20 day treatment period. The effectiveness of these compounds in suppressing estrus and their ability to synchronize estrus are presented in Table XXVI. The results of this study indicated that 5 of the 6 compounds tested were biologically active in puberal gilts. Each compound produced its own characteristic effect on the reproductive processes. As a result, there was considerable variation in the response of gilts to each of the different compounds. The effectiveness of the various compounds in controlling the estrous cycle of gilts is best discussed in relation to their individual effects on estrus, ovulation and fertility.

U-10,997

This compound effectively inhibited estrus in 49 of 50 gilts when administered daily at a rate of either 50, 30, 15, 10 or 5 mg. per head. The 5 mg. level is probably the minimal effective dose because estrus was not inhibited consistently in gilts fed either 2.5 or .5 mg. daily. Ovarian activity was inhibited in these gilts during the 20 day treatment period and this prevented estrus and ovulation from occurring. Twenty-four of the 50 gilts were killed one day following the last day

TABLE XXVI

REPRODUCTIVE PERFORMANCE OF ALL GILTS FED THE VARIOUS COMPOUNDS IN THIS STUDY

Compounds and Dose Levels	No. of Gilts	Estrus Inhib-ited	Estrus Synchronization Attempted	No. in Heat[a]	No. Bred[b]	Post-Treatment Estrus Interval
U-10,997						
50 mg.	15	15	6	2	2	11-14
30 mg.	10	10	6	2	1	10-12
15 mg.	4	4	4	3	3	7-10
10 mg.	14	14	8	4	4	10-15
5 mg.	7	6	2	1	1	10
2.5 mg.	4	2	2	2	2	5-7
.5 mg.	3	0	-	-	-	-
U-13,851						
50 mg.	5	5	-	-	-	-----
15 mg.	10	10	6	0	-	-----
10 mg.	3	3	-	-	-	-----
5 mg.	3	0	-	-	-	-----
U-23,378						
250 mg.	14	13	6	2	1	12-14
50 mg.	3	0	-	-	-	-----
U-11,100A						
250 mg.	5	4	-	-	-	-----
50 mg.	3	1	-	-	-	-----
U-10,520A						
250 mg.	4	1	-	-	-	-----
U-13,053						
250 mg.	4	0	-	-	-	-----

[a]Includes only those gilts in estrus within 15 days following treatment withdrawal.

[b]Includes gilts which were bred but did not ovulate.

[c]Range in days for gilts in heat.

of feeding the compound, and their ovaries were either completely devoid of any corpora lutea, or small regressing corpora lutea were present. However, bilateral cystic follicles occurred in one gilt fed 10 mg. daily and one fed 5 mg. daily.

In Trial IV, eight gilts were laparotomized, and their corpora lutea marked with India ink approximately 3 days prior to initiating treatment at either the 50 or 30 mg. levels. The absence of corpora lutea and the presence of ink in the stromal portion of ovaries obtained from these gilts one day following the 20 day feeding period clearly established the fact that U-10,997, fed at the dose levels used, was very effective in causing regression of the corpora lutea as well as suppressing follicular development and estrus during treatment. Since the inactive condition of ovaries obtained from gilts fed the high levels was essentially the same as that from gilts fed the lower levels, it was assumed that ovulation had also been prevented in gilts fed the 15, 10 and 5 mg. levels in Trials II, III and IV.

The length of time required for the ovaries of U-10,997-treated gilts to recover from the suppression period and resume normal ovarian activity was established at the 50, 30, 15, 10 and 5 mg. levels. A total of 26 gilts was used in this synchronization phase. These gilts returned to estrus at very erratic and unpredictable times following treatment withdrawal. In general, 10 days appeared to be the minimal length of time required for a gilt to recover and show estrus. Five of the 26 gilts were killed on day 10 and 5 on day 15 following treatment because they had not exhibited estrus. Their ovaries were still essentially inactive with no corpora lutea and very little follicular development although one gilt had developed bilateral cystic follicles. The

condition of their ovaries was very similar to that of gilts killed one day following treatment.

Thirteen of the 26 gilts were in heat prior to day 15 following treatment and 12 were bred with ovulation occurring in 10 of the 12. The 2 gilts that did not ovulate had bilateral cystic follicles. The fertility level of the 10 gilts which ovulated was very low. Seven of the 10 were scheduled to be killed 30 days following first breeding, but 3 recycled prior to this autopsy date and only 2 of the remaining 4 that did not recycle were pregnant at necropsy. This evidence clearly indicated a detrimental effect of U-10,997 on fertility. The remaining 3 gilts used in the synchronization phase were observed for estrus up to day 60 following treatment. The earliest return date for these gilts was 29 days and this gilt had cystic follicles. Another gilt was bred 46 days post-treatment and one gilt failed to show estrus prior to day 60.

The ovarian response of gilts fed U-10,997 suggest several possible alternatives regarding the mechanism of action of this compound. It must be assumed that FSH secretion was blocked during the treatment period since follicular development did not occur. The regression of pre-treatment corpora lutea also indicated that secretion of the pituitary luteotropic substance was blocked. Sammelwitz _et al_. (1961), Brinkley _et al_. (1964) and Anderson _et al_. (1967) all reported data which indicated that in pigs, the single release of a luteotropic substance at or near the time of ovulation is the only impetus necessary to cause corpora lutea to form and to persist and function for the entire luteal phase. They further postulated that the additional luteotropic substance required to maintain corpora lutea for the remainder of the ges-

tation period is released only if conception and implantation occurs. This second release of luteotropic substance was probably blocked in the U-10,997-treated gilts, since they were started on treatment during the midluteal phase of their estrous cycle. Another possible alternative is that U-10,997 possesses luteolytic properties and acts directly on the corpora. The results obtained in this study suggested a possible direct effect of the compound on the ovaries because they were still essentially inactive in some gilts by 10 days post-treatment. In addition, those gilts which were able to return to estrus and ovulate had very low conception rates. The ovaries of gilts killed either one or 10 days following treatment were very turgid and fibrous in appearance and may not have been capable of producing normal ova. Whatever the mode of action of U-10,997, it is clear that this compound will not permit precise control of the time of estrus and ovulation in gilts.

U-13,851

This compound effectively inhibited estrus and ovulation in 18 gilts when administered daily at a rate of 50, 15 and 10 mg. The 10 mg. level is probably the minimal effective dose because estrus was not inhibited in 3 gilts fed 5 mg. daily. This compound does not cause pretreatment corpora lutea to regress. The condition of ovaries from 10 of 12 gilts fed either 50, 15 or 10 mg. and killed one day following treatment was very similar. They had an average of 12.6 corpora lutea which were 6 to 7 mm. in diameter and moderately ischemic. Their size and appearance indicated that they were not fully functional. The progesterone concentration in corpora obtained from 2 of these 10 gilts was 10.41 and 7.59 µg. per gram of luteal tissue. These levels were lower

than the 29.4 average obtained for gilts in the mid-luteal phase of their estrous cycle and was further evidence that these persistent corpora were not fully functional. The corpora lutea of 4 of these 12 gilts were marked with India ink prior to initiating treatment at the 15 mg. dose level. The presence of marked corpora lutea on the ovaries of 2 of these gilts one day post-treatment was proof that ovulation had not occurred, and that the corpora lutea represented pre-treatment corpora. The ovaries of the other 2 gilts had no corpora which indicated that they had not ovulated. Ink was present in the stromal portion of the ovaries from the marked C.L. that had regressed.

The fact that ovulation was inhibited, but pre-treatment corpora persisted throughout the treatment period, indicated that U-13,851 blocks the release of pituitary FSH but may not block release of the pituitary luteotrophin substance. A second release of luteotropic substance may be required for corpora lutea of the estrous cycle to persist if pregnancy occurs and inhibits ovulation (Sammelwitz _et al_. 1961 and Brinkley _et al_. 1964). If this hypothesis is true, U-13,851 either did not prevent this second release, or it acts as a luteotrophic substance itself. These pre-treatment corpora lutea not only persist through the 20 day treatment period, but continue to persist for at least 10 days following this period. In Trial IV, estrus synchronization was attempted with 6 gilts fed 15 mg. daily. The ovaries of 3 gilts killed 10 days post-treatment contained corpora lutea similar in size, appearance, and progesterone concentration, to those of 2 gilts killed one day following treatment. Estrus was not observed in these gilts prior to day 10, and it was assumed that ovulation had not occurred. The continuous persistence of corpora and inhibition of ovulation indicated that suppression

of pituitary FSH continued for at least 10 days following treatment. Possibly, the persistent corpora lutea were producing enough progesterone to block FSH secretion. Another alternative is that the luteotropic substance responsible for maintaining the pre-treatment corpora may have been responsible for their continuous persistence following the treatment period.

The length of time required for corpora lutea to regress following treatment with U-13,851 would determine how quickly gilts could return to estrus and ovulate. Ten days appears to be the very minimum length of time for this to occur. In Trial IV, 3 gilts were observed for estrus up to 60 days following treatment, but 27 days was the earliest return date, and this gilt had cystic follicles. The evidence obtained in this trial indicated that the luteal maintenance effect of U-13,851 is undesirable and will not allow precise control of the time of ovulation in gilts.

U-23,378

In Trials III and V, this compound inhibited estrus in 13 of 14 gilts when administered at a rate of 250 mg. per day. In Trial V, the corpora lutea of 4 gilts were marked with India ink prior to initiating treatment. The corpora lutea of 3 of these gilts were still marked with ink at the end of the treatment period, and this was evidence that ovulation had not occurred while on treatment. However, the presence of unmarked corpora lutea on both ovaries of one gilt indicated that she had ovulated during treatment. This gilt had consumed all of her daily feed allottment, and there was no reason to assume that she had not received the same daily dose as the other gilts. The evidence obtained

from these four gilts indicated that ovulation was probably blocked in the majority of the 13 gilts in which estrus was inhibited, but 250 mg. per day may be close to the minimal effective dosage level. This conclusion is strengthened by the results of Trial IV in which 3 gilts fed 50 mg. daily, returned to estrus during the treatment period and were bred.

Eight of the 14 gilts fed 250 mg. daily were killed one day following the treatment period. The remaining 6 were allowed to return to estrus in order to determine the length of time required for gilts to recover from the treatment period and ovulate. The condition of ovaries obtained from all gilts killed one day following treatment was very similar. These gilts had an average of 14 corpora lutea which were 8 to 9 mm. in size. Their size and appearance suggested that they were functional and characteristic of those present during the luteal phase of the estrous cycle. The compound apparently maintained the structural integrity of these corpora, but the concentration of progesterone in the corpora lutea of 3 of these gilts was only 9.90 μg. per gram of corpora lutea tissue as compared to 29.4 μg. per gram of C.L. tissue obtained during the mid-estrual period of control gilts. This suggested that they were not fully functional. Although one of the 8 gilts had ovulated during treatment, it was assumed that the other 7 had not. This would mean that the corpora lutea represented pre-treatment corpora which had persisted throughout the 20 day feeding period.

As with other compounds evaluated in this study, it was not possible to conclusively determine the mode of action of U-23,378, but some postulations were made based on the evidence obtained. If ovulation was truly blocked, then this compound must have prevented the secretion of

pituitary FSH, although it apparently did not block the second release of pituitary luteotrophin substance since luteal maintenance did occur. However, U-23,378 may possess luteotrophic properties itself and acted directly on the corpora causing them to persist.

In Trial V, the results obtained from 6 gilts in which estrus synchronization was attempted indicated that pre-treatment corpora lutea persisted for at least 10 days following the 20 day treatment period. Three of these gilts failed to exhibit estrus by day 10, and necropsy at this time revealed corpora lutea of the same size, appearance, and progesterone concentration of those present on ovaries of gilts killed one day following treatment. The response of the remaining 3 gilts indicated that these corpora probably would have started to regress on about day 10, and follicular development would have been initiated at this time. One gilt was bred 14 days following treatment, but she did not ovulate. Cystic follicles appeared to be developing in this gilt. A second gilt was in heat 12 days post-treatment but would not accept the boar. Preovulatory size follicles were present on her ovaries at necropsy 3 days following first day of heat. The third gilt did not show estrus until day 33. This evidence does indicate that follicular development and ovulation will not occur in U-23,378-treated gilts until the pre-treatment corpora regress. The minimum length of time required for this to occur is apparently 10 days, and this is not conducive for effective synchronization of estrus.

U-11,100A

This compound inhibited estrus in 4 of 5 gilts fed 250 mg. daily and one of 3 gilts fed 50 mg. daily. The 5 gilts in which estrus was

inhibited were killed one day following treatment. Their ovaries had corpora lutea present which were 8 to 9 mm. in size with a reddish-pink color characteristic of those with an active capillary circulation present during pregnancy and the luteal phase of the estrous cycle. The one gilt fed 50 mg. daily probably had a silent ovulation during treatment, since the other 2 gilts fed this level came into estrus and were bred during treatment. The response of the 5 gilts fed 250 mg. daily was difficult to interpret. Since one gilt was bred during treatment, each of the other 4 animals could have ovulated during treatment without showing estrus. No firm conclusion could be made regarding this point but the evidence suggested that the 250 mg. level would not consistently inhibit estrus.

The inconclusive evidence regarding the estrus inhibiting ability of this compound at the 250 mg. level made it difficult to postulate its possible mode of action in gilts. If ovulation was truly inhibited in these 5 gilts and pre-treatment corpora persisted, then a luteotrophic effect of this compound could be suggested and it would definitely be capable of suppressing FSH secretion. However, if ovulation was not inhibited a different interpretation would have to be made. It is possible that the gilts ovulated without showing estrus since Duncan (1963) stated that this compound exhibits anti-estrogenic properties in rats by inhibiting the uterine response to exogenous estrogen. If U-11,100A antagonizes the action of endogenous estrogen in gilts, then it is possible that follicular development and ovulation occurred, but U-11,100A interferred with the normal role of estrogens in eliciting the characteristic behavioral patterns of a gilt in estrus.

U-10,520A

This compound and U-11,100A are both 1,2,diphenyl-3,4-dihydronapthalene derivaties and possess similar biological properties in rats (Duncan et al. (1963). In Trial III of this study U-10,520A was administered to 4 gilts at a rate of 250 mg. daily but estrus was inhibited in only one of these gilts. Thirteen corpora lutea, which were normal in size and appearance, were present on the ovaries of this gilt at the end of the 20 day treatment period and indicated that she too had ovulated. The remaining 3 gilts were bred on day 6 of treatment and continued on treatment for the remainder of the 20 days. Two of these gilts recycled 30 days post-breeding and were rebred. The third gilt did not recycle but she was not pregnant at necropsy.

The evidence obtained in this study indicates that 250 mg. of U-10,520A will not prevent estrus in gilts, but it did suggest a possible antifertility effect similar to that reported by Duncan et al. (1963). These workers stated that this compound inhibited pregnancy in rats when administered during proestrus or within 4 days after breeding. It is not clear how this compound exerts antifertility activity but in rats, blastocysts, once in the uterine cavity, are not affected by this compound (Duncan et al. 1962). This suggests a possible direct effect on the ova during tubal transport. If this compound prevented pregnancy in gilts by acting directly on the zygote and causing its death, then the gilts should normally be expected to recycle approximately 21 days following breeding. However, these gilts did not recycle until 30 days following first service. Since these gilts continued to receive this compound for 14 days following breeding, it may be postulated that

U-10,520A interferred with the normal implantation process. Duncan et al. (1963) reported that this compound antagonizes, to some extent, the uterine weight increase caused by estradiol and thus possessed anti-estrogenic potency. Thus, U-10,520A could have had a detrimental effect on the implantation process in gilts by antagonizing the effects of en-dogenous estrogen on the endometrium. Whatever the mode of action, it is clear that this compound will not inhibit estrus in gilts at the dosage level used.

U-13,053

In Trial I, this compound was administered to 4 gilts at a rate of 250 mg. per day, but all animals returned to estrus during the treatment period and were bred. The average interval from first day of treatment to first day of breeding was 6 days. Following breeding, they were con-tinued on treatment for the remainder of the 20 day period. This com-pound apparently has no antifertility activity since 3 of the 4 gilts were pregnant at necropsy 30 days post-breeding.

CHAPTER X

SUMMARY AND CONCLUSIONS

The objective of this study was to determine the effects on normally cycling gilts of compounds with estrus inhibiting properties. The compounds studied were: U-10,997 and U-13,851, both 19-Nortestosterone derivatives with androgenic properties; U-13,053, a weak estrogen; U-11,100A, U-10,520A and U-23,378, all diphenyl-dihydronapthlene derivatives with antiestrogenic properties. These compounds were produced by The Upjohn Company and their ability to inhibit estrus in species other than swine had previously been determined. In the present study, 5 trials were conducted to evaluate their effectiveness in preventing the occurrence of estrus and ovulation in puberal gilts when administered orally for a 20 day treatment period. Compounds which inhibited estrus were further evaluated for their ability to synchronize estrus following treatment.

The results of this study indicated that 5 of the 6 compounds tested were biologically active in gilts. Each compound produced its own characteristic effect on estrus, ovulation and fertility. This resulted in considerable variation in the response of gilts to each of the different compounds.

Compound U-10,997 inhibited estrus in 49 of 50 gilts when administered at a daily rate of either 50, 30, 15, 10 or 5 mg. per head, but was not effective at 2.5 or .5 mg. levels. Ovarian activity was markedly

inhibited in those gilts in which estrus was blocked, and this prevented the occurrence of estrus and ovulation during the 20 day treatment period. The ovaries of 24 gilts necropsied one day following treatment with levels of 5 mg. or higher were essentially inactive with no corpora lutea or follicular development present in most cases. However, cystic follicles were observed in one gilt fed 10 mg. daily and one fed 5 mg. Pre-treatment corpora lutea of 8 gilts were marked with India ink prior to initiating treatment with 50 or 30 mg. daily. No corpora lutea were present on their ovaries at the end of the 20 day treatment period, and this clearly established that U-10,997 had suppressed both estrus and ovulation during this period. Regression of the pre-treatment corpora lutea indicated a possible luteolytic effect of this compound. The suppression of ovulation suggested that U-10,997 blocked the release of pituitary gonadotrophins.

Estrus synchronization was attempted with U-10,997 at 50, 30, 15, 10 and 5 mg. levels using a total of 26 gilts. The length of time required for these gilts to rebound from the treatment period and resume normal ovarian activity was very erratic and unpredictable, but 10 days appeared to be the minimal length of time. Thirteen gilts showed estrus at various times prior to day 15 following treatment. Twelve of these gilts were bred and 10 ovulated, but their fertility levels were very low. Ten gilts necropsied either 10 or 15 days post-treatment had ovaries which were essentially inactive with no corpora lutea or follicular development present. The remaining 3 gilts were observed for estrus up to day 60 following treatment. The return dates were 29 and 46 days for two gilts, but one gilt failed to show estrus by day 60. The evidence indicated that the ovarian suppression effect of U-10,997

had persisted following treatment. This effect was undesirable because precise control of time of estrus and ovulation following treatment could not be obtained.

Compound U-13,851 inhibited estrus in 18 gilts when fed at a rate of 50, 15 and 10 mg. daily, but was not effective at the 5 mg. level. Ten of 12 gilts necropsied one day following treatment with levels 10 mg. or higher had an average of 12.6 corpora lutea, 6 to 7 mm. in size. The other 2 gilts had no corpora or follicles present. The corpora lutea of 4 gilts fed 15 mg. were marked prior to treatment. The presence of only marked corpora lutea at the end of treatment proved that U-13,851 prevented both estrus and ovulation from occurring. This compound did not exhibit the luteolytic effect observed with U-10,997, but it apparently inhibited the release of pituitary gonadotrophins.

Estrus synchronization was attempted with 6 gilts fed 15 mg. but none returned to estrus within 10 days following treatment. The ovaries of 3 gilts necropsied on day 10 following treatment had C.L. present and the evidence indicated that they were pre-treatment corpora. The remaining 3 gilts were observed for estrus up to day 60 following treatment. One gilt returned to estrus on day 27 but had cystic follicles. The other 2 gilts failed to show estrus by day 60 but the presence of corpora lutea and the appearance of corpora albicans indicated that both gilts had ovulated during this 60 day period. This luteal maintenance of U-13,851 was undesirable since it prevented ovulation from occurring within a reasonable length of time following treatment.

Compound U-23,378 inhibited estrus in 13 of 14 gilts fed 250 mg. Eight gilts necropsied one day post-treatment had ovaries with an average of 14 corpora lutea. The corpora of 4 gilts were marked prior to

treatment and one gilt had unmarked corpora at the end of the treatment period, indicating that she had ovulated. The results indicated that ovulation was blocked in the majority of the gilts fed U-23,378, but 250 mg. per day may be close to the minimal effective dosage level.

The pre-treatment corpora of U-23,378 not only persisted through the treatment period, but they continued to persist for at least 10 days following treatment withdrawal and prevented ovulation from occurring. Estrus synchronization was attempted with 6 gilts fed 250 mg., but none returned to estrus within 10 days following treatment. Three gilts necropsied on day 10 because they had not exhibited estrus had corpora present on their ovaries. Only one gilt was bred (14 days post-treatment) but she did not ovulate. Of the other 2 gilts, one showed every indication of estrus 12 days post-treatment but would not accept the boar, and the other gilt did not show estrus until day 33. The results indicated that estrus could not be synchronized with U-23,378.

Compounds U-13,053 and U-10,520A failed to inhibit estrus when fed at a rate of 250 mg. daily. However, there was some indication of an antifertility effect of U-10,520A since 3 gilts bred during treatment, and continued on treatment for at least 12 days, failed to settle at the treatment breeding.

The response of gilts fed 250 mg. of U-11,100A indicated that this level would not consistently inhibit estrus.

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A P P E N D I X

TABLE XXVII

STRUCTURAL FORMULA, SYSTEMATIC NOMENCLATURE, AND ACTIVITY OF ALL COMPOUNDS USED IN THIS STUDY

U-Number	Structure	Systematic Nomenclature and Activity
U-10,997 -	[structure: OH; CH3; H; O; CH3]	17β-Hydroxy-7α,17-dimenthyl ESTR-4-EN-3-one or 7α,17-dimethyl-19-NORTESTOSTERONE [a]LD_{50} Rat (acute, oral) 302 mg./kg. a potent androgenic compound
U-13,851 -	[structure: OH; C ≡ CH; H; O; CH3]	17α-Ethynyl-17-hydroxy-7α-methyl ESTR-4-EN-3-one or 17-Ethynyl-7α-methyl-19-NORTESTOSTERONE LD_{50} Rat (acute, oral) > 3200 mg./kg. mixed hormonal activity depending on the assay, dose and endpoint
U-13,053 -	[structure: CH3; HC-CH=N-OH; HO-N]	3-Oxo PREGN-4-ENE-20β-carboxaldehyde, dioxime LD_{50} Rat (acute, IP) > 1000 mg./kg. weak estrogen

TABLE XXVI (Continued)

	Structure	Name and data
U-10,520A -	$OCH_2CH_2-N(CH_2CH_3)_2$	Triethylamine, 2-[p-(3,4-dihydro-6-methoxy-2-phenyl-1-naphthyl) phenoxy]-,hydrochloride LD_{50} Rat (acute, oral) 547 mg./kg. non-steroidal antiestrogen
U-11,100A -	OCH_2CH_2-N .HCl	Pyorolidine, 1-{2-[p-(3,4-dihydro-6-methoxy-2-phenyl-1-naphthyl) phenoxy] ethyl}-, hydrochloride LD_{50} Rat (acute, oral) 302 mg./kg. non-steroidal antiestrogen
U-23,378 -	OH OCH_2CHCH_2OH CH_3O	1,2-Propanediol, 3-[p-(3,4-dihydro-6-methoxy-2-phenyl-1-naphthyl) phenoxy]- LD_{50} Rat (acute, oral) 3419 mg./kg. non-steroidal antiestrogen

[a]LD = Lethal dose in 50 rats.

VITA

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Doctor of Philosophy

Thesis: THE EVALUATION OF ESTRUS INHIBITING COMPOUNDS IN SWINE

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