HORMONAL CONTROL OF DECIDUAL CELL RESPONSE IN THE RAT

by

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INTRODUCTION

Implantation and placentation are the complex processes involving endocrine sensitization of the uterus by hormones secreted by various organs, stimulation of differentiation of the maternal portion of the placenta by implanting blastocysts and the development of blastocysts in the uterus. Loeb (1907) experimenting with the guinea pig first demonstrated that the uterus was capable of responding to an artificial stimulus by rapid proliferation, a phenomenon termed decidual cell response (DCR). This proliferation provided a method of studying the capacity of the uterus for morphological changes in the absence of blastocysts. In this manner, maternal and embryonic components can be studied separately. It was later found that DCR also occurred in pseudopregnant rats as well as in other pregnant animals. It was found that maximal uterine sensitivity occurs on Day 4 of pseudopregnancy in the rat. Maximal decidualization after trauma occurs due to an increase in water content and protein synthesis. A combination of estrogen and progesterone in both absolute and balanced levels were needed to produce maximal weight and water content of deciduomata after trauma (Yochim and De Feo, 1962). The rapid growth of the traumatized pseudopregnant rat uterus appeared to be a good end point for studying the direct effect of pituitary hormones on ovarian steroidogenesis and their indirect effect on decidualization.

Antiserum to sheep luteinizing hormone (LH) produced in rabbits has been used to prevent pregnancy in mice (Munshi and Rao, 1967). In the present study, anti-LH serum was used to selectively eliminate endogenous LH in rats to determine its role in ovarian steroidogenesis.

Specific objectives of the present experiment were:

(1) To test the hypothesis that estrogen and progesterone are necessary for maximal DCR after massive uterine trauma in the pseudopregnant rat.

(2) To determine if LH is necessary for decidualization by selectively neutralizing endogenous LH with LH antiserum in the pseudopregnant rat.

(3) To determine the function of LH and/or FSH on estrogen production in the pituitary autotransplanted rat.

(4) To formulate a working hypothesis for pituitary hormone control of ovarian steroidogenesis and the resulting effect on decidualization in the mechanically traumatized pseudopregnant rat uterus.

LITERATURE REVIEW

Decidual cell response (DCR) has been widely used as a criterion for demonstrating that the rat uterus is properly prepared for implantation. The term refers to the capacity of the endometrium to proliferate and differentiate into a deciduomata in response to an embryo or an artificial stimulus (DeFeo, 1963; Yochim and DeFeo, 1962).

Loeb (1907) demonstrated that uterine irritation would produce decidual nodules during early pregnancy in the guinea pig. Daily injections of anterior hypophyseal extracts to rats in which the ovaries were present produced a decidual cell response after induced trauma to the uterine mucosa (Teel, 1926). The author concluded that the continuous presence of pituitary lutein hormones (prolactin?) conditioned the uterus for placentomata. The DCR noted after uterine trauma in the pseudopregnant rat appeared similar to the intra-uterine neoplasm containing decidual cells that occurs during pregnancy (Wrenn et al., 1966, Loeb, 1907). Endometrial proliferations by mechanical trauma in castrated monkeys treated with progesterone appeared identical with those found at normal implantations sites of fertilized ova (Hisaw, 1935, Rossman, 1940). Brouha (1928) reported that injections of anterior hypophyseal extracts to rats interrupted the estrous cycle; however, large placentomata were obtained only after the uterine mucosa had been sensitized by the corpora lutea secretions (progesterone?) then traumatized. In this experiment the largest decidual reaction was produced when the uterine mucosa was traumatized on or about the fifth day of injection. Weichert (1928) treated ovariectomized rats with corpus luteum hormones but no placentomata were formed after stimulation; however, if such rats were first brought to an estrous state by injections of follicular hormones,

placentomata were formed. He concluded that the follicular hormone is necessary to put the uterus in a proper physiological condition before it can respond to the corpus luteum hormone. This follicular hormone was thought to be absent by the seventh day of pseudopregnancy. It was later shown that prolactin was the pituitary hormone necessary for induction of pseudopregnancy and progesterone production by rat corpora lutea (Nikitovitch-Winer and Everett, 1958) and that progesterone and estrogen were necessary for maximal DCR (Yochim and DeFeo, 1962).

Pseudopregnancy, as defined by Everett (1961), is the period of sustained luteal function similar to that of the normal progestational state. This progestational state was invoked in rats by stimulation of the cervix. The pseudopregnant period is fairly uniform in the rat lasting 12 to 14 days (Everett, 1961) and is associated with the appearance of leukocytes in the vagina. It was reported that neither estrogen nor cervical stimulation was needed to induce pseudopregnancy in pituitary autotransplanted rats (Sanders and Rennels, 1957); however, hypophysectomy could not evoke a pseudopregnant situation. The transplanted pituitary was shown to secrete a luteotrophic hormone which caused pseudopregnancy in rats and enough progesterone was secreted to maintain pregnancy in hypophysectiomized rats (Everett, 1956). Everett (1961) theorized that the luteotrophic hormone from the transplanted pituitary could

stimulate corpora lutea to produce progesterone to maintain pregnancy or induce pseudopregnancy in rats. Earlier, Haterius and Pfiffner (1929) induced diestrous periods of 8-22 days (pseudopregnancy) in rats with injections of extracts of corpora lutea (progesterone?).

Pseudopregnancy induced by daily injection of corpora lutea extract could produce large deciduomata in rats by stimulation with three or four silk threads placed in the uterine horn on the third day of injections. These authors concluded that the essential conditions for placentomata formation were (1) the uterus must be prepared for the luteal stimulus by the action of the follicular hormone (estrogen?) upon the endometrium as a priming action and (2) the uterus must be sensitized by the pituitary lutein hormone (prolactin?) prior to stimulation. This stimulation must be continuous throughout the life of the deciduomata.

In a series of experiments on rats, Allen (1931) demonstrated that the changes of uterine conditions in pseudopregnancy were different from those of the normal estrous cycle. He believed these changes were preparations for implantation of the embryos and that the activity of the uterine epithelial cells at this time demonstrated that the uterus was sensitized in order that decidual reaction could take place after stimulation. The changes occurred from the fourth to the sixth day of pseudopregnancy and were similar to changes during pregnancy. Deciduomata was not

found following mechanical uterine stimulation after the seventh day.

Allen (1931) showed that the rat uterus was sensitive to trauma between the fourth and the sixth day of pseudopregnancy by producing deciduomata after mechanical uterine stimulation. Either mechanical or chemical traumatization of the endometrium on the fourth day of pseudopregnant rat resulted in massive decidualization (Shelesnyak, 1957). Maximal sensitivity was reported by Yochim and DeFeo (1962) on Day 4 of pseudopregnancy and the sensitive period appeared to be related to the last ovulation and not to the day on which the pseudopregnancy was initiated. Deciduomata was associated with an increase in both uterine weight and water content. Krehbiel (1941) suggested that ovarian activity might determine the time at which implantation would occur in rats. He showed that during early pregnancy the sensitized uterus reacted to embryonic stimulation with the formation of a deciduomata and the implantation of ova. Unilateral ovariectomy prior to implantation inhibited the attachment of the ova. Enzmann et al. (1932) found that prolonged gestation in mice was due to delayed implantation. Hamlett (1935) suggested that delayed implantation might be due to an alteration of hormonal conditions dictated primarily by the corpora lutea. The work concerning the phenomenon of delayed implantation in the rat pointed out the importance of ovarian secretions in preparing the uterus

for implantation and deciduomata formation. Yochim and DeFeo (1962) reported that in ovariectomized pseudopregnant rats injected with estrogen and progesterone (2,000-1 ratio), uterine sensitivity to mechanical trauma was first noted on Day 3, reached the peak value on Day 4 and was completely absent on Day 5; however, when the estrogen level was decreased the duration of sensitivity increased but peak values were not obtained. Humphrey (1968) used anti-estrogenic compounds to test the effect of estrogen on induction of deciduomata and delayed implantation in mice. His data showed that the antiestrogens prevented implantation that normally occurred in intact pregnant or in ovariectomized, progesterone treated mice given estrogen to induce implantation. The hormonal requirements for implantation in spayed mice were found to vary according to the time of spaying with a significant change in the uterine conditions occurring on Day 3 (Yoshinaga and Adams, 1966). Rothchild et al. (1940) used one hundred and eighty-two young adult female rats for a quantitative study of estrogen-progesterone effect in the formation of placentomata in castrated rats. In this study ovariectomy and traumatization of the uterus was carried out on the fourth day of pseudopregnancy and rats were killed on the ninth day. He showed the size of placentomata was larger with higher doses of progesterone given from Day 4 to Day 8 and the maximum size of placentomata was 5.6 to 6.12 mm in diameter with 6 Rb. U. of

progesterone daily. Rats in the same experiment given a combination of progesterone and estradiol had placentomata that reached 6.45 mm with 3 Rb. U. progesterone and 0.6 gamma of estradiol injected twice daily and 7.14 mm with 6 Rb. U. and 0.6 gamma estradiol.

Loeb and Kountz (1928) stated that the follicular hormone tended to prevent the predeciduomatous proliferation in guinea pigs on Day 3 and 4 following heat. Courrier (1950) explained this phenomenon as the result of an antagonism between estrogen and progesterone. The injection of estrogen in rabbits in the presence of progesterone made perivascular decidual sheaths over the uterus both in the myometrium and endometrium. He postulated that the uterus should be previously treated with progesterone to help the response of the uterus to estrogen. Courrier (1950) reported that an estrogen to progesterone ratio of 1:1,000 had no antagonistic effect on the endometrium of the rabbit. Antagonistic effects were noted when a level of 30 mg of estradiol was used regardless of the ratio. Rothchild et al. (1940) found a synergistic effect between estrogen and progesterone at a level of 3 mg of progesterone and 0.15 mg of estradiol and an antagonism with 3 mg of progesterone and 3 mg of estradiol on decidual reaction in rats. Hisaw et al. (1937) and Engle and Smith (1938) showed a synergism between the two hormones on uterine growth in the Rhesus monkey.

Velardo and Hisaw (1951) demonstrated the inhibitory

effects of six different estrogens on progesterone induced deciduomata in rats. The animals were evoked to pseudopregnancy by a strong faradic current applied to the cervix during estrus. Five days after cervical stimulation, animals were ovariectomized, the endometrium was traumatized by inserting a needle through the tubal sphincter beneath the oviduct down to the cervix and then treating with different doses of estrogen and progesterone. The data indicated that the ratio of estrogen:progesterone which did not modify deciduomata was different for each form of estrogen, 1:1,875 for estrone, 1:16,666 for estradiol 17-B, 1:150 for estriol. Martin (1962) found no competitive interaction between estrogen and progesterone and estrogen as well as progesterone was shown to increase uterine weight in both spayed and pregnant mice.

Finn (1965) reported that the traumatic deciduomata stimulus required only progesterone when he used arachid oil to induce deciduomata in pseudopregnant mice to imitate natural implantation. Negative reaction resulted if ovariectomized rats were treated with estrogen earlier than Day 3 or later than Day 4. Therefore, he concluded that between Day 3 and Day 4 estrogen was released by the ovary which prepared the uterus for the initiation of the oil induced deciduomata. Fin theorized that a surge of estrogen was necessary in the ovariectomized pseudopregnant mouse maintained on progesterone for decidualization to occur.

Shelesnyak and Kraicer (1961) demonstrated the role of histamine as the mediator for induction of decidualization in the rat. They showed that the optimal time for decidual induction is on the fourth day of pseudopregnancy. By injecting pyrathiazine hydrochloride as a histamine releaser at the uterine level he got very good decidual reaction. Kraicer and Shelesnyak (1958) and Humphrey (1968) used a histamine releaser to induce decidual cell reaction in the pseudopregnant sensitized mouse uterus. This compound caused the release of endogenous histamine in the uterus to act on the endometrium. Finn and Keen (1962) reported that certain sulfated polysaccharides including heparin were able to induce deciduomata when injected into the uterus, but an extensive tissue destruction or severe irritation prevented DCR (Shelesnyak, 1957). Epinephrine and ephedrine were reported to be physiological antagonists to histamine and suppress DCR (Shelesnyak, 1954).

The maintenance of optimal conditions for gestation is dependent upon adequate levels of estrogens and progestogens (Courrier, 1950; Yochim and Zarrow, 1961). A ratio of estradiol:progesterone that produced a maximal deciduomata was also capable of supporting gestation optimally. Progesterone or estrogen alone could not produce a maximal DCR. A ratio 1:2,000 or 1:4,000 of estrone to progesterone was reported to give maximal DCR (Yochim and DeFeo, 1962). Both estrogen and progesterone are requisites for decidual

growth in intact or ovariectomized pseudopregnant rats. The castrated rat bearing deciduomata responded to estrogen and progesterone in a fashion similar to that reported for the castrated pregnant rat (Yochim and Zarrow, 1961). The uterine sensitivity to oil stimuli was found on Day 4 and Day 5 (Finn, 1966) and mechanical trauma on Day 3, 4 (Yochim and DeFeo, 1962; DeFeo, 1963). After this sensitive period the uterus has a period of refractoriness.

Progesterone and estrogen play an important role in DCR; it follows then that gonadotropins should have an indirect effect on DCR, since they control ovarian secretion of these steroids (Greep, 1961; Lostroh, 1965). Secretions of FSH and LH were interrupted when the pituitary gland was placed away from the hypophyseal portal vessels (Fortier, 1951; Fortier and Selye, 1949). Under such conditions, the gland secreted the luteotrophic hormone (LTH) known to be prolactin in the rat (Everett, 1956). In his review (1954, 1956), Everett reported that LTH was secreted by adenohypophyseal transplants placed in the kidney without any special stimulation but not FSH and LH. This apparently was a result of a lack of direct hypothalamic control which was required for FSH and LH secretion from the anterior pituitary.

Luteinizing hormone has been reported to increase progestin output *in vivo* in the rabbit (Hillard *et al.*, 1964) and *in vitro* (Armstrong *et al.*, 1966; Kaltenbach *et al.*, 1967). The synergistic effect of FSH and LH on estrogen secretion

has been previously recognized (Greep et al., 1942). Prolactin was secreted at a high level when the pituitary was incubated in vitro (Talwalker et al., 1963) or when the pituitary transplant is a distance from the hypothalamus and appears to promote progesterone production from corpora lutea (Everett, 1954; Nikitovitch-Winer and Everett, 1958).

In the rat, significant production of estrogen by the follicles requires both FSH and LH (Turner, 1965). Mitchell and Yochim (1968) reported that human chorionic gonadotropins (HCG) given to female rats increased ovarian and uterine weight and caused vaginal cornification by stimulating estrogen secretion. They postulated that HCG (FSH and LH) was not a luteotrophic substance in the rat, but indirectly through estrogen induced pseudopregnancy. Biologically pure FSH did not elicit secretion of estrogen by the ovary in the hypophysectomized rat (Simpson et al., 1950; Simpson et al., 1951). However, FSH played a primary and essential role in preparing the follicular apparatus for LH action to stimulate estrogen secretion (Greep, 1961). Moon and Li (1952) demonstrated that highly purified FSH preparations evoked the secretion of estrogen in intact and immature rats and mice, but later Greep (1961) explained this secretion as a result of endogenous LH release. Gier (1963) suggested that FSH stimulates the follicle growth and LH stimulates these growing follicles to produce estrogen. Richardson (1967) concluded in his review that an FSH-LH interaction is important in

secretion of estrogen to produce a maximum uterine stimulation in rats.

It has been shown that massive deciduomata could be produced in rats made pseudopregnant by daily administration of 1 mg prolactin (Von Berswoldt-Wallrabe et al., 1964). Luteotropin release caused a pseudopregnant condition following induced ovulation in the rabbit (Everett, 1961). Mechanical stimulation of the cervix during estrus by sterile mating (Long and Evans, 1922), a glass rod (Long and Evans, 1926) or stimulation of the nipples of a non lactating rat by an active litter (selye and McKeown, 1934) initiates pseudopregnancy. Pseudopregnancy results in the rat following continual injections of estrogen (Wolfe, 1935), traumatization of the uterus on Day 4 (Peckham and Green, 1947) or when an accessory pituitary is grafted into the renal capsule (Muhlbock and Boot, 1959). Pseudopregnancy as well as pregnancy appears to result from release of luteotropin caused by neural or hormonal stimuli both on the reproductive organs and higher nervous centers.

Allen (1939) demonstrated that hypophysectomy during early pregnancy caused termination of pregnancy in several species. It seemed that luteotropin was continuously needed following ovulation. Continual prolactin injections in intact rats will lengthen cycles to 13-16 days (Lahr and Riddle, 1936; Nathanson et al., 1937), favor production of DCR after uterine trauma (Astwood, 1941) and maintain

corpora lutea of hypophysectomized rats (Lyons et al., 1943).

Greep et al. (1942) reported that hypophysectomized rats did not show a positive DCR in response to trauma. Rats bearing an autotransplanted pituitary did form a deciduomata in response to trauma presumably allowed by progesterone secretion of CL induced by prolactin secretion of the autograft (Everett, 1956, 1961).

Studying the effect of LH, Moudgal and Li (1961) reported that antiserum to sheep LH inhibited the effect of endogenous and exogenous LH on the ventral prostate gland of rats. Bourdel and Li (1963) found that antisheep ICSH serum resulted in inhibiting estrus and caused mild degeneration of the uterus, vagina and ovary. Kelly et al. (1963) demonstrated that anti-LH serum completely prevented ovulation in rats and Young et al. (1963) also showed that anti-LH serum caused cessation of estrus in rats.

Li (1962) found that antiserum to ICSH could be used to remove traces of ICSH not detectable by chemical means from FSH. Hayashida and Chino (1961) demonstrated that anti-FSH serum neutralized the stimulating effect of FSH on the weight of uterus and follicles in hypophysectomized immature rats. Hayashida (1962) also found that antiserum to sheep LH caused inhibitory effects on immature male rats in suppression of spermiogenesis and atrophy of interstitial cells in mature rats. Wakabayashi and Tamooki (1965) obtained cross reaction between antiserum to sheep LH and rat or rabbit anterior

pituitary homogenates. Ely (1956) demonstrated effective neutralization of endogenous gonadotropins by antiserum to sheep pituitary extract. Antiserum to sheep LH does not cross react with ovine FSH or sheep prolactin and is capable of neutralizing endogenous LH in rats (Marvin and Meyer, 1943; Munshi and Rao, 1967). Data from Munshi and Rao (1967) indicated that antiserum to sheep LH is capable of inhibiting blastocyst implantation and also preventing pregnancy in mice. Injections of antiserum to sheep LH on Day 1 to 4 of pregnant mature mice prevented pregnancy. These authors concluded that the antiserum effect was not one of inhibiting of the estrogen surge as hypothesized by Finn (1965), since the implantation was blocked in all female mice treated with estradiol on Day 3 of pregnancy.

MATERIALS AND METHODS

Animals

Adult, virgin female rats of the Holtzman strain weighing 220-280 g were used in this experiment. Rats were selected for experimentation only after observation of at least two complete 4 or 5 day estrous cycles. Occurrence of estrus was determined by taking daily vaginal lavages. Lavages were obtained by using a medicine dropper, the end of which was smooth to prevent irritation to the rat vagina. A clean sterile dropper was used for each rat. Approximately 0.2 cc of tap water was introduced into the rat vagina with

the dropper, then withdrawn and transferred to a clean microscopic slide. The vaginal lavage was observed microscopically before drying and the stage of estrus was recorded according to cell type as classified by Aswood (1939), Mandle (1951) and Turner (1967).

The rats were housed three per cage. Animal quarters were kept at 22-27°C with 14 hours of fluorescent illumination and 10 hours darkness per day. Midpoint of the light period was 3 pm. Purina Laboratory Chow and water were available ad libitum.

Hormones

Luteinizing hormone* (LH) and follicle stimulating hormone* (FSH) were dissolved in sterile water and kept refrigerated until injection time. Injections were given subcutaneously at intervals of 12 ± 2 hours in dosages of 0.25-0.5 ml. Progesterone and estrone were injected subcutaneously daily in 0.5 ml. of sesame oil. Rabbit antiserum to sheep LH was prepared by giving 3 biweekly injections of 1.5 mg of the antigen (LH) dissolved in 1.5 ml saline mixed with an equal volume of Freund's Complete Adjuvant (Difco, Detroit, Michigan). Two additional biweekly booster doses of 2.0 mg of antigen alone were given. The sera from bleedings were

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*NIH-LH-S-13 and NIH-FSH-S-5 were a gift of the National Institute of Health, Bethesda, Maryland, USA.

collected 1 to 2 weeks after the last injection and pooled before use. Control serum from rabbits receiving only adjuvant was also collected. The sera were frozen in small quantities until injection time. At the time of injection, the vial of serum was thawed. The uninjected portion was refrigerated until use providing the period between injections was not longer than 5 days. Antiserum to LH was tested for its ability to block ovulation in proestrous hypophysectomized rats given exogenous LH. The antigenic analyses of antiserum to sheep LH consisted of Ouchterlony gel diffusion test (Ouchterlony, 1953) and measurement of antibody titre by interfacial test (Kabat & Meyer, 1961). Antisera injections were given subcutaneously at 12 $\pm$ 2 hour intervals.

Procedures

Pseudopregnancy was induced by stimulation of the cervix with an electrical tooth brush fitted with a metal rod. Rats were given two 15 second stimulations. Pseudopregnancy was checked by microscopic examination of vaginal lavage for the presence of leukocytes throughout the experiment. Day 1 was designated as the first day of the appearance of high ratio of leukocytes in the vaginal lavage after cervical stimulation.

On day 4 of pseudopregnancy (except the groups of ovariectomized rats used to test the delayed uterine sensitivity on Day 5) uterine traumatization was performed by midventral

laparotomy at midday on rats anesthesized by ethyl ether inhalation. A sharp needle was inserted into the lumen of the right horn from cervix to oviduct to inflict trauma by scratching the antimesometrial endometrium along the horn. Unnecessary handling of the uterine horn was avoided. Care was taken to inflict uniform traumatization on each rat. Animals with nodules or incomplete decidualization on the uterine horn were discarded in the final data.

Hypophysectomy when performed was carried out on the day of estrus (day of vaginal cornification) by parapharyngeal approach under ether anesthesia. Each rat was checked to determine if ovulation had occurred before the operation by observing an excised oviduct for the presence of ova. Rats which had not ovulated were not hypophysectomized. The adenohypophysis was then autotransplanted under the left kidney capsule. All rats were observed macroscopically for completeness of hypophysectomy at sacrifice and examination for remaining hypophyseal fragments in the hypophyseal fossa was done histologically in cases where fragments were suspected. Bilateral ovariectomy was effected after traumatization on Day 4 on all ovariectomized rats, except for the groups used to test delayed sensitivity, ovariectomy and traumatization were performed on Day 5 of pseudopregnancy. The standard experimental procedure for treatment groups within the operative groups are shown in Table 1.

TABLE I. STANDARD EXPERIMENTAL PROCEDURE

Description of Treatment	Induction of pseudo-pregnancy on Day 0[a]	Check Ovulation, Pituitary Auto-trans-plantation on Day 0	1st day of Vaginal Leukocytes Day 1[b]	Traumatization		Ovariectomy on		Sacrifice on Day 9
				Day 4	Day 5	Day 4	Day 5	
Group A (intact rats)								
1. (Control serum (day 1-5 or 4-8)	+		+	+				+
2. Anti-LH serum (day 1-5)	+		+	+				+
3. Anti-LH serum (day 4-8)	+		+	+				+
4. Anti-LH serum + 3 mg progesterone (day 4-8)	+		+	+				+
5. Anti-LH serum + 1 ug estrone (day 4-8)	+		+	+				+
Group B (ovariectomized rats)								
1. 3 mg progesterone + 1 ug estrone (day 4-8)	+		+	+		+		+
2. Anti-LH serum (day 1-4) 3 mg progesterone + 1 ug estrone (day 4-8)	+		+	+		+		+
3. 1 ug estrone (day 4-8)	+		+	+		+		+
4. 3 mg progesterone (day 4-8)	+		+	+		+		+
5. 3 mg progesterone + 1 ug estrone (day 5-8)	+		+		+		+	
6. Anti-LH serum (day 1-5) 3 mg progesterone + 1 ug estrone (day 5-8)	+		+		+		+	+
Group C (pituitary auto-planted rats)								
1. Control rats		+	+	+				+
2. Anti-LH serum (day 4-8)		+	+	+				+
3. 1 ug estrone (day 4-8)		+	+	+				+
4. FSH (day 0-8)		+	+	+				+
5. LH (day 0-8)		+	+	+				+
6. FSH and LH (day 0-8)		+	+	+				+

[a]Day 0 of pseudopregnancy is the day of vaginal cornification.

[b]Day 1 of pseudopregnancy is the 1st day of appearance of vaginal leukocytes after induction of pseudopregnancy or pituitary autotransplantation.

A plus (+) means the procedure in that column was used for the treatment on that line.

Collection of data

All animals were weighed and killed on Day 9 (five days after traumatization). The uterine horns were removed, cleaned of adhering fat, separated and individually weighed to the nearest 0.1 mg on a Mettler Gram-O-Matic balance. Dry weight was determined by again weighing the horn after being placed in an oven at 150° C for 24 hours. Ovaries when present were collected, weighed and fixed for histological examination by standard procedures.

RESULTS

Antiserum tests

Antibody titre of the anti-LH serum was approximately 1:20,000 indicating that 1 ml of pooled antisera would neutralize 625 mg of LH. A heavy precipitin band was seen in a gel diffusion test of antiserum with sheep LH while light bands to FSH and TSH were noted. Eight ug of LH consistently induced ovulation in hypophysectomized proestrous rats and this ovulation could be blocked with 40 ul of antiserum but not with 10 ul. In intact proestrous rats, ovulation was blocked with 10 ul of anti-LH serum but not with 5 ul. These injections were given intravenously.

Effects of LH antiserum

Anti-LH serum (0.1 ml) given to pseudopregnant intact rats twice daily on Days 1-5 (before trauma) or Days 4-8

(after trauma) decreased the wet and dry weight of the traumatized uterine horn ($P < 0.01$) and the wet weight of non traumatized horn when antiserum was given on Days 4-8 (Table II). In pseudopregnant rats ovariectomized on Day 4 and given anti-LH serum twice daily (Days 4-8) along with daily injections of progesterone and estrone, uterine weights were not different than controls. The uterine horn weights in the ovariectomized rats receiving anti-LH serum, estrone and progesterone were similar to the group receiving only progesterone and estrone (Table III). The weight of traumatized horns in the pseudopregnant intact rats given antiserum on Days 4-8 were similar to the uterine weight of ovariectomized animals treated with only progesterone (Tables II and III) and to the pituitary autotransplanted rats (Table IV). Anti-LH serum showed a slight effect on uterine weight when given on Days 1-4 then ovariectomized and treated with progesterone and estrone (Table III). Delayed uterine sensitivity was obtained on rats given antiserum on Days 1-5 then traumatized and ovariectomized on Day 5 (Table III) with subsequent administration of progesterone and estrone. Anti-LH serum slightly lowered the ovarian weight although not statistically in the pseudopregnant intact rats. Histologically, ovaries of rats treated with anti-LH serum had fewer large vesicular follicles than animals receiving control serum.

Steroid replacement

Treatment with 1 ug estrone (days 4-8) gave maximal uterine sensitivity associated with maximal DCR in pseudopregnant intact rats given anti-LH serum (Tavle II) and in pituitary autotransplanted rats; however, treatment with progesterone failed to support maximal DCR in these cases. Combination of estrone and progesterone given to rats after ovariectomy promoted decidual growth at a normal rate (1,774.4 mg) when trauma was performed on Day 4. Progesterone or estrone alone could not support maximal DCR (1,072.9 and 339.8 mg respectively, Table IV) in ovariectomized rats. Progesterone did increase DCR in pseudopregnant intact rats (1,584.5 mg) given anti-LH serum in comparison with animals given only anti-LH serum (1,024.2 mg), but the maximum magnitude could not be obtained.

Gonadotropin replacement

FSH and LH given daily (Days 1-8) to rats with the adenohypophysis autotransplanted increased the uterine weight from 978.0 mg in the control autotransplanted group to maximal level (2,033.8 mg). Dry weight of the traumatized horn was also significantly increased ($P < 0.01$). FSH or LH alone failed to stimulate uterine growth to give maximal deciduomata (Table IV). The ovarian weight of the FSH-LH group was slightly higher than in rats given only FSH, estrone or LH although it was not statistically different.

TABLE II. THE EFFECT OF ANTI-LH SERUM OR ANTISERUM PLUS ESTRONE OR PROGESTERONE ON UTERINE WEIGHT (DAY 9) IN INTACT PSEUDOPREGNANT RATS AFTER UTERINE TRAUMA (DAY 4)

Treatment Group	No. Rats	Weight of Traumatized Uterine Horns[a]			Wet Weight of Nontraumatized Uterine Hrons	Ovaries
		Wet	Dry	% Dry		
Control Serum[b] (Day 1-5 or 4-8)	10	1838.9±67.7	293.9±14.0[c]	15.1[c]	159.0±11.8	55.9±2.2
Anti-LH Serum (Day 1-5)	5	853.0**±120.6	___	___	129.1±11.7	48.0±4.1
Anti-LH Serum (Day 4-8)	12	1024.2**±49.9	170.5**±9.8[d]	16.7[d]	120.3**±5.3	46.1**±1.1
Anti-LH Serum +3 mg Progesterone (Day 4-8)	5	1584.5*±65.0	252.1*±11.5	15.9	126.5*±7.6	46.6*±2.4
Anti-LH Serum + 1 ug estrone (Day 4-8)	5	2041.8±106.2	304.4±12.8	15.0	170.3±10.4	48.9±4.9

[a]Numbers represent the average values (mg) ± the standard error for each group.

[b]Serum injections (0.1 ml) for all groups were given twice daily and steroid injections once daily.

[c]Based on the average weight of five rats.

[d]Based on the average weight of seven rats.

* ($P < 0.05$) ** ($P < 0.01$) Significantly different from the controls.

TABLE III. EFFECT OF ESTRONE, PROGESTERONE AND ANTI-LH SERUM (ALONE OR IN COMBINATION) ON UTERINE GROWTH AFTER UTERINE TRAUMA IN OVARIECTOMIZED PSEUDOPREGNANT RATS

Treatment Group[a]	Day of Ovx[b]	Weight of Traumatized Uterine Horn[c]		
		Wet	Dry	% Dry
Progesterone + Estrone (Day 4-8)	4	1774.4±61.0	269.8±8.3	15.2
Anti-LH Serum + Estrone and Progesterone (Day 4-8)	4	1846.3±152.2	281.4+22.2	15.3
Anti-LH Serum (Day 1-4) Progesterone and Estrone (Day 4-8)	4	1658.7±131.4	251.0±19.4	15.1
Estrone (Day 4-8)	4	339.8**±43.4	59.40**±5.9	17.9
Progesterone (Day 4-8)	4	1072.9**±86.4	171.5**±13.2	16.0
Progesterone and Estrone (Day 5-8)	5	283.9**±31.1	49.1**±4.0	17.3
Anti-LH Serum (Day 1-5) Progesterone + Estrone (Day 5-8)	5	1200.8**±168.3	168.8**±22.8	14.1

[a]Estrone (1 ug) and Progesterone (3 mg) were given once daily whether alone or together; antiserum (0.1 ml) was given twice daily.

[b]Ovariectomy (ovx) and uterine trauma were performed at the same time on the indicated.

[c]Values given are average weights (mg) ± standard error for five rats killed on Day 9.

** ($P < 0.01$) Significantly different from Progesterone + Estrone (Day 4-8).

TABLE IV. THE EFFECT OF ANTI-LH SERUM, GONADOTROPINS AND ESTRONE ON UTERINE WEIGHT (DAY 9) AFTER UTERINE TRAUMATIZATION (DAY 4) IN RATS WITH AN AUTOTRANSPLANTED PITUITARY[a]

Treatment Group[b]	No. Rats	Weight of Traumatized Uterine Horn[c]			Wet Weight of Nontraumatized Uterine Horns	Ovaries
		Wet	Dry	% Dry		
Control	5	978.0±119.1	151.9±18.8	15.5	112.5±5.3	49.3±2.7
Anti-LH Serum (Day 4-8)	5	1093.3±96.6	167.4±13.4	15.4	116.6±7.5	48.2±2.0
Estrone (Day 4-8)	6	2064.9**±170.1	316.8**±25.0	15.4	170.7**±8.4	47.2±2.2
FSH (Day 0-8)	5	1338.7*±55.9	209.4*±13.1	15.6	129.2±7.7	53.3±1.8
LH (Day 0-8)	5	1360.7*±108.8	204.6±16.4	15.0	129.0±11.7	49.5±2.3
FSH and LH (Day 0-8)	5	2033.8**±174.8	295.7**±22.3	14.6	216.1**±21.2	55.4±1.3

[a]Autotransplantation was performed on Day 0; uterine trauma on Day 4 and all rats were killed on Day 9.

[b]Estrone (1 ug) was given once daily; anti-LH serum (0.1 ml), FSH (20 ug) and LH (5 ug) were given twice daily.

[c]Numbers represent the average values (mg) ± the standard error for each group.

* ($P<0.05$) ** ($P<0.01$) Significantly different from the controls.

DISCUSSION

It has previously been shown that estrogen in the presence of progesterone will induce decidual cell response after traumatic stimuli (Rotchild et al., 1940; Selye, 1936; Yochim and DeFeo, 1962) and will increase the weight and water content of the uterus (Astwood, 1938). Data from the present experiment confirm this work and in addition indicate that anti-LH serum will reduce the secretion of estrogen by neutralizing the effect of endogenous LH thereby decreasing uterine growth after massive trauma. This supports the postulation (Young, 1961) that LH plays some role in stimulating the developed theca interna of ovarian follicles to produce estrogen. The injection of 3 mg progesterone daily to rats given antiserum to LH increased the weight of traumatized uterine horns from 1,024.2 mg in intact rats treated with anti-LH serum on Days 4-8 to a level of 1,584.5 mg. This fact suggests that LH may also be involved in progesterone secretion in the rat. Luteinizing hormone (LH) has also been shown to stimulate progestin production from luteal tissue in the rat (Major et al., 1967) and within certain levels increase deciduomata weight (Velardo and Hisaw, 1951; Yochim and DeFeo, 1962). In this experiment anti-LH serum injected into intact pseudopregnant rats after trauma decreased DCR to a uterine horn weight similar to that obtained in ovariectomized rats given only progesterone and in rats with autotransplanted pituitaries. Thus, present data indicate that

antiserum to LH blocks endogenous setrogen secretion rather than progesterone. It is apparent that the action of anti-LH serum is on ovarian hormone production rather than the ability of the uterus to respond to the hormones since a maximal DCR occurred when anti-LH serum was given to ovariectomized rats receiving estrone and progesterone. Estrogen given to pituitary autotransplanted rats after trauma yielded a maximal DCR response indicating that a sufficient level of progesterone was secreted although little or no LH should be present in the pituitary autografted rat given anti-LH serum. It appears then, that prolactin from the autografted pituitary in the present experiment was sufficient stimulus for progesterone secretion and that LH was not necessary for progesterone secretion in amounts necessary for maximal DCR but was necessary for estrogen secretion. This is in agreement with Herlyn et al. (1964) who demonstrated that the lactogenic gonadotropin (prolactin) would give an adequate stimulus for the persisting corpora lutea to produce progesterone in amounts sufficient for DCR in hypophysectomized rats. Estrogen in the rat was shown to have no appreciable influence on the production of progesterone by the corpora lutea beside its effect directly on the uterus (Greenwald and Johnson, 1968). Everett (1954, 1956) demonstrated that prolactin was continuously secreted by the pituitary autotransplanted to the renal capsule and such secretion was also reported by Talwaker et al. (1963) from the in vitro incubated rat pituitary. The pituitary autograft produces very little

or no FSH and LH (Nikitovitch-Winer and Everett, 1958). Pituitary LH may be capable of promoting progesterone secretion in the rat, but it is doubtful in view of the data presented here that it is necessary for progesterone secretion at physiological levels. It is possible, of course, that LH could cause progesterone secretion from sources such as interstitial tissue rather than luteal tissue or it may work synergistically with prolactin under normal circumstances.

In the absence of estrogen in castrated females daily injections of as little as 0.25 mg progesterone supported deciduomata (Velardo and Hisaw, 1951), but very small amounts of estrogen increased the influence of progesterone (Rothchild et al., 1940). It has long been known that the ratio of estrogen to progesterone was important for a proper environment to induce deciduomata in the rat (Velardo and Hisaw, 1951). The present study is in accord with the data reported by Yochim and DeFeo (1962) and DeFeo (1963) that progesterone or estrogen alone cannot induce a maximal DCR after traumatic uterine stimuli. Maximal uterine sensitivity was also found on Day 4 in all pseudopregnant rats in the experiment and was almost lost by Day 5 which is in agreement with previous works (Yochim and DeFeo, 1962; DeFeo, 1963). In this study, uterine sensitivity was prolonged until Day 5 of pseudopregnancy in rats ovariectomized and traumatized on Day 5 and treated with anti-LH serum during the pretrauma period although the magnitude was not as high as those

traumatized on Day 4. This supplies further evidence that anti-LH serum interfered with estrogen secretion since it was previously demonstrated that the lower the level of estrogen remained during the pretrauma period, the longer the period of sensitivity lasts (Yochim and DeFeo, 1962). These workers postulated that some uterine cells could respond to progesterone alone and others could not. Delayed implantation, which probably involves similar hormonal mechanisms, was also obtained by Humphrey (1968) in mice by injecting antiestrogen compounds to the pregnant mice.

Antiserum to sheep LH prepared in the same way as that used in this experiment was shown to inhibit ascorbic acid depletion by LH in the rat (Hoppe et al., 1967) and suppressed the secretion of endogenous estrogen in the rabbit (Spies and Quadri, 1967). Munshi and Rao (1967) also reported similar anti-LH serum was capable of inhibiting blastocyst implantation and preventing pregnancy in mice. Moudgal and Li (1961) used antiserum to sheep LH and showed that the effect of endogenous and exogenous LH on the prostate gland of the rat was blocked. These authors concluded that LH antiserum neutralized LH which in turn suppressed the endogenous secretion of gonadal steroids.

Antiserum to sheep LH in this study had very little influence on DCR when given in the pretrauma period followed by ovariectomy on Day 4 and estrone and progesterone injections from Day 4-8. In this case, the deciduomata obtained

were not statistically different in weight from those observed in ovariectomized control rats. This may be interpreted to mean that estrogen levels during pretrauma may be decreased without interfering with maximal DCR if adequate levels of hormones are present post trauma. The fact that antibodies to LH were as effective in reducing uterine weight before trauma when given on Days 1-5 as when given on Days 4-8 in intact rats may be due to the fact that antibodies can remain in circulation for a prolonged period of time (Quadri, 1966). Thus, when antibody injections were given from day 1-5, the antibodies may have been present from Day 1-8 and the post trauma effect may still have been the greatest. This evidence would suggest that a high level of estrogen was not necessary for maximal DCR during the pretrauma period; however, progesterone alone given during the pretrauma period could not reproduce the degree of sensitivity displayed by intact pseudopregnant rats (Yochim and DeFeo, 1962).

The present data indicate that maximal deciduomata can be obtained in the autotransplanted rat treated with estrone on Days 4-8. Therefore, the absolute level of estrogen said to be needed by Yochim and DeFeo (1962) must be very low or secreted independently from LH and FSH during the pretrauma period since the autograft rat should have secreted very low or no LH, FSH and estrogen. A surge of estrogen about Day 4 as postulated for decidualization in intact pseudopregnant rats (Shelesnyak, 1960) might not be necessary for maximal

DCR after mechanical uterine trauma since there is no reason for postulating such an estrogen surge in pituitary auto-transplanted rats or ovariectomized rats given a constant level of estrone. The type or uterine trauma inflicted here may account for this discrepancy.

It was apparent that the gonadotropins FSH and LH were important in estrogen production in the pituitary autografted rats and were indirectly involved in decidual growth. It was found in the present study that FSH or LH alone did not support maximal DCR in hypophysectomized rats with a transplanted pituitary. A combination of FSH and LH was necessary. It has been demonstrated that LH in conjunction with prolactin will not substitute for estrogen to maintain pregnancy (Greenwald and Johnson, 1968). While FSH is necessary for follicular development (Nalbandov, 1953; Gier, 1963), LH is thought to stimulate the estrogen secretion from the growing ovarian follicles in mammals (Nalbandov, 1953; Gier, 1963). Fraenkel et al. (1943) has also shown that maximal follicle growth and estrogen secretion requires both LH and FSH. Highly purified sheep FSH or LH alone was ineffective in causing follicular secretion of estrogen (Greep, 1961; Lostroh, 1965); and FSH-LH interaction appeared to be necessary for estrogen secretion for optimal DCR in pituitary autografted rats in the present study. The FSH-LH ratio did not appear important for estrogen secretion in work by Richardson (1967).

Although the present data did not show any significant

difference in ovary weights between groups, ovarian weights did increase with FSH and LH treatment. Ovarian weights increased anytime FSH was administered to autografted rats and decreased with antiserum treatment in all intact rats.

Dry weight of the traumatized horns in FSH and LH treated, autotransplanted rats were significantly increased over control autografted, traumatized horns as well as intact or ovariectomized, progesterone and estrogen treated rats. Apparently FSH and LH was capable of causing estrogen secretion which increased in both water content and mitotic cell division (Protein synthesis) which is associated with uterine growth during decidualization (DeFeo, 1962). FSH seemed to play a role in the promotion of estrogen secretion by the ovaries by preparing the follicular apparatus on which LH can act to promote estrogen production. Since, FSH or LH was relatively ineffective alone, it may be hypothesized that without FSH the follicular apparatus necessary for estrogen production was not present, therefore, LH had no effect. On the other hand, FSH alone caused follicular growth but without LH was not capable of stimulating enough estrogen secretion for maximal decidualization.

SUMMARY

Progesterone and estrogen were shown to be necessary for maximal DCR in the rat; however, a high level of estrogen during the pretrauma period did not appear to be necessary

for DCR after massive mechanical trauma. A maximal DCR was obtained in pituitary autotransplanted rats when estrone was given during the post trauma period. Progesterone or estrogen alone was not capable of stimulating maximal decidualization.

Antiserum to sheep LH depressed uterine growth in female rats following traumatization on Day 4 of pseudopregnancy, but had no effect in rats with the adenohypophysis transplanted beneath the kidney capsule on the day of ovulation or in ovariectomized rats given estrogen and progesterone. Anti-LH serum decreased the DCR in intact pseudopregnant rats after trauma to the same weight as uteri in ovariectomized rats given only progesterone or in rats with autotransplanted pituitaries. These data indicate that antiserum to sheep LH interfered indirectly in endogenous estrogen secretion, and LH is necessary for estrogen but not progesterone secretion in amounts necessary for maximal DCR after mechanical trauma.

Simultaneous injections of FSH and LH resulted in maximal DCR in pituitary autotransplanted rats, while FSH or LH alone failed to give a significant stimulatory effect. It was suggested that FSH prepared the follicular apparatus for estrogen secretion and the action of LH actually triggered estrogen secretion. Antiserum against sheep LH given during the pretrauma period was able to prolong uterine sensitivity to trauma; this was interpreted as a further indication that LH antiserum depressed DCR by depressing estrogen secretion suring the pretrauma period which has been associated with

a prolonged period of uterine sensitivity.

A pituitary autograft was capable of promoting progesterone secretion. Apparently, prolactin secreted by the autograft was the only pituitary hormone necessary for progesterone secretion in a quantity sufficient for maximal DCR after uterine trauma in the pseudopregnant rat.

LITERATURE CITED

Allen, W. M. 1931. Cyclical alteration of the endometrium of the rat during the normal cycle, pseudopregnancy and pregnancy--production of deciduomata during pregnancy. Anat. Rec. 48:65.

Allen, E. 1939. Sex and Internal Secretions (2nd ed.) p. 931. The Williams and Wilkins Company, Baltimore, Maryland.

Armstrong, D. T. and D. L. Black. 1966. Influence of luteinizing hormone on corpus luteum metabolism and progesterone biosynthesis throughout the bovine estrous cycle. Endocr. 78:937.

Astwood, E. B. 1941. The regulation of corpus luteum function by hypophyseal luteotrophin. Endocr. 28:309.

Astwood, E. B. 1938. Time relationships in the growth and water exchange of uterus following estrogenic stimulation. Anat. Rec. Suppl. 70:5.

Bourdel, G. and C. H. Li. 1963. Effect of rabbit antiserum to sheep pituitary ICSH in adult female rats. Acta Endocr. 42:473.

Brouha, L. 1928. Production of placentoma in rats injected with anterior hypophyseal fluid. Proc. Soc. Exp. Biol. Med. 25:488.

Courrier, R. 1950. Interactions between estrogen and progesterone. Vitamins and Hormones 8:179.

DeFeo, V. J. 1963. Temporal aspect of uterine sensitivity in the pseudopregnant or pregnant rat. Endocr. 72:305.

Engle, E. T. and P. E. Smith. 1938. The endometrium of the monkey and estrone-progesterone balance. Am. J. Anat. 63:349.

Ely, C. A. 1956. Effect of antigonadotropic serum on recent intrasplenic ovarian implants of castrate mice. Endocr. 59:83.

Enzmann, E. V., N. R. Saphir and Gregory Pincus. 1932. Delayed pregnancy in mice. Anat. Rec. 54:325.

Everett, J. W. 1954. Luteotrophic function of autografts of the rat hypophysis. Endocr. 54:685.

Everett, J. W. 1956. Maintenance of pregnancy in rats by hypophyseal autografts. Anat. Rec. 124:287.

Everett, J. W. Sex and Internal Secretions (3rd ed.) W. C. Young, ed. Vol. 1, Chapter 8. The Williams and Wilkins Company, Baltimore, Maryland. 1961. pp. 497-555.

Finn, C. A. Reproduction in the Female Mammal. Edited by G. E. Lamming and E. C. Amoroso. London. Butterworths. 1967. p. 513.

Finn, C. A. 1965. Oestrogen and the decidual cell reaction of implantation in mice. J. Endocr. 32:223.

Finn, C, A. 1966. Endocrine control of endometrial sensitivity during the induction of the decidual cell reaction in the mouse. J. Endocr. 36:239.

Finn, C. A. and P. M. Keen. 1962. Studies on deciduomata formation in the rat. J. Reprod. Fert. 4:215.

Fortier, C. and H. Selye. 1949. Adrenocorticotrophic effect of stress after severance of the hypothalamo-hypophyseal pathways. Am. J. Physiol. 159:433.

Fortier, C. 1951. Dual control of adrenocorticotrophin release. Endocr. 49:782.

Fraenkel, Conrat H., C. H. Li and M. E. Simpson. 1943. Pituitary gonadotropin. In "Essays in Biology," pp. 183-191. Berkeley, Univ. of California Press.

Gier, H. T. Laboratory manual for amniote embryology, published by the author, Manhattan, Kansas. 1963. p. 36.

Greenwald, G. S. and D. C. Johnson. 1968. Gonadotropic requirements for the maintenance of pregnancy in the hypophysectomized rat. Endocr. 83:1052.

Greep, R. O., H. O. Van Dyke and B. F. Chow. 1942. Gonadotropins of the swine pituitary. Various biological effects of purified thylakentrin (FSH) and pure metakentrin (ICSH). Endocr. 30:635.

Greep, R. O. Sex and Internal Secretions. 3rd ed. Vol. I, edited by W. C. Young. The Williams and Wilkins Co. 1961. pp. 240-249.

Hamlett, G. W. D. 1935. Delayed implantation and discontinuous development in the mammals. Quart. Rev. of Biol. Vol. 10:142.

Haterius, H. O. and J. J. Pfiffner. 1929. Inhibition of oestrus in the rat by extracts of corpus luteum. Proc. Soc. Exp. Biol. Med. 26:818.

Hayashida, T. and S. Chino. 1961. Inhibition of FSH activity with antiserum to the purified hormone. Anat. Rec. 139:236.

Hayashida, T. 1962. Inhibition of spermiogenesis and prostate development in normal rats with antiserum. Anat. Rec. 142:308.

Herlyn, U., H. F. Geller and Ilse Von Berswordt Wallrabe. 1964. Failure to induce decidual cell reaction in lactogenic hormone treated and pseudopregnant rats. Acta. Endocr. 45:281.

Hillard, J., J. N. Hayward and C. H. Sawyer. 1964. Postcoital patterns of secretion of pituitary gonadotropin and ovarian progestin in the rabbit. Endocr. 75:957.

Hisaw, F. L., R. O. Greep and H. L. Fevold. 1937. The effects of oestrin-progestin combinations on the endometrium, vagina and sexual skin of monkeys. Am. J. Anat. 61:483.

Hisaw, F. L. 1935. The physiology of menstruation in Macaccus rhesus monkeys. Am. J. Obst. Gynec. 29:638.

Hoppe, P. C., S. K. Quadri and H. G. Spies. 1967. Inhibition of ovarian ascorbic acid depletion with ovine luteinizing hormone antiserum. Proc. Soc. Exp. Biol. Med. 125:1102.

Humphrey, K. W. 1968. The effect of some anti-oestrogens on the deciduoma reaction and delayed implantation in the mouse. J. Reprod. Fert. 16:200.

Kabat, E. A., Meyer, M. M. 1961. Experimental immuno-Chemistry, 2nd ed. p. 67. Charles C. Thomas, Springfield, Illinois.

Kaltenbach, C. C., B. Cook, G. D. Niswender and A. V. Nalbandov. 1967. Effect of pituitary hormones on progesterone synthesis by ovine luteal tissue <u>in vitro</u>. Endocr. 81:1407.

Kelly, W. A., H. A. Robertson and D. A. Stansfield. 1963. The suppression of ovulation in the rat by rabbit anti-ovine LH serum. J. Endocr. 27:127.

Kraicer, P. and M. C. Shelesnyak. 1958. The induction of deciduomata in the pseudopregnant rat by systematic administration of histamine and histamine releasers. J. Endocr. 17:324.

Lahr, E. L. and O. Riddle. 1936. Temporary suppression of estrous cycles in the rat by prolactin. Proc. Soc. Exp. Med. 34:880.

Li, C. H. Ciba Coll. Endocr. Vol. 14, G. E. W. Wolslenholme and M. P. Cameron, eds. London, J. & A. Churchill, 1961, p. 20.

Loeb, Leo, 1907. Wounds of the pregnant uterus. Proc. Soc. Biol. Med. 4:93.

Loeb, Leo and W. B. Kountz. 1928. The effect of injection of follicular extract on the sex organs in guinea pig and the interaction between the follicular substances and substances given off by the corpus luteum. Am. J. Physiol. 84:283.

Long, J. A. and N. M. Evans. 1922. The estrous cycle in the rat and its associated phenomena. Mem. Univ. Calif. V. 6.

Long, J. A. and N. M. Evans. 1926. On the production of the condition of pseudopregnancy by infertile coitus or mechanical stimulation of the cervical canal in the rat. Anat. Rec. 21:37.

Lostroh, A. J. 1965. Minimal amounts of ICSH required together with FSH for follicular development, uterine growth and ovulation in hypophysectomized rat. Physiologist 8:222.

Lyons, W. R., M. E. Simpson and N. M. Evans. 1943. Hormonal requirements for pregnancy and mammary development in hypophysectomized rats. Proc. Soc. Exp. Med. 52:134.

Major, P. W., D. T. Armstrong, and R. O. Greep. 1967. Effects of luteinizing hormone *in vivo* and *in vitro* on cholesterol conversion to progestins in rat corpus luteum tissue. Endocr. 81:19.

Martin, L. 1962. Interactions between oestradiol and progestogens in the uterus of the mouse. J. Endocr. 26:31.

Mandl, A. M. 1951. Cyclical changes in the vaginal smear of adult ovariectomized rats. Proc. Exp. Biol. Med. 28:585.

Marvin, H. N. and R. K. Meyer. 1943. Experimental production of deciduomata in the rat by an extract of the corpus luteum. Proc. Soc. Exp. Biol. Med. 27:863.

Mitchell, J. A. and J. M. Yochim. 1968. Relation of HCG induced ovulation to the production of prolonged diestrus in the adult rat. Endocr. 82:1142.

Moon, H. D. and C. H. Li. 1952. Effects of follicle stimulating hormones on gonads of immature C57 black mice. Proc. Soc. Exp. Biol. Med. 79:505.

Moudgal, N. R. and C. H. Li. 1961. An immunological study of a human pituitary interstitial cell stimulating hormone. Nature. 191:192.

Muhlbock, O. and L. M. Boot. 1959. Induction of mammary cancer in mice without the mammary tumor agent by isografts of hypophyses. Cancer Research 19:402.

Munshi, S. R. and Shanta S. Rao. 1967. Biological specificity of antigens present in ovine luteinizing hormone. Indian Journal of Exp. Biology. 5:135.

Nalbandov, A. V. 1953. Gonadotropic activity of the pituitary glands and the induction of ovulation. Iowa State College J. Sc. 28:45.

Nathanson, L. T., H. L. Fevold and D. B. Jennison. 1937. Inhibition of estrous cycles in the rodent with postpartum urine and commercial prolactin. Proc. Soc. Exp. Biol. Med. 36:481.

Nelson, W. O. and J. J. Pfiffner. 1930. Experimental production of deciduomata in the rat by an extract of the corpus luteum. Proc. Soc. Exp. Biol. Med. 27:863.

Nikitovitch-Winer, M. and J. W. Everett. 1958. Comparative study of luteotropin secretion by hypophyseal autotransplants in the rat. Effects of site and stages of estrus cycle. Endocr. 62:522.

Ouchterlony, O. 1953. Antigen-antibody reactions in gels; types of reactions in coordinated system of diffusion. Acta Path. et Microbiol. Scand. 32:231.

Peckham, B. M. and R. R. Greene. 1947. Attempts to produce deciduomata in the pregnant rat. Endocr. 41:273.

Quadri, S. 1966. Effect of ovine luteinizing hormone antibodies on reproduction in rabbits. M.S. thesis, KSU, Manhattan.

Ridchardson, G. S. Ovarian Physiology, 1st ed. published in Great Britain by J. and A. Churchill Ltd., London, 1967. pp. 36-40.

Rossman, I. 1940. The decidual reaction in the Rhesus monkey (Macaca mulatta). The epithelial proliferation. Am. J. Anat. 66:277.

Rothchild, I., R. K. Meyer and M. A. Spielman. 1940. A quantitative study of estrogen-progesterone interaction in the formation of placentomata in the castrate rat. Am. J. Physiol. 128:213.

Sanders, A. and E. G. Rennels. 1957. The cytology and functional capacity of the rat pituitary gland after autotransplantation. Anat. Rec. 127:360.

Selye, H. and T. McKeown. 1934. Studies on pseudopregnancy. Am. J. Physiol. 109:96.

Shelesnyak, M. C. 1957. Some experimental studies on the mechanism of ova implantation in the rat. Recent Prog. Hormone Res. 13:269.

Shelesnyak, M. C. 1960. Nidation of the fertilized ovum. Endeavour 19:81.

Shelesnyak, M. C. and P. F. Kraicer. 1961. A physiological method for inducing experimental decidualization of the rat uterus: standardization and evaluation. J. Reprod. Fertil. 2:438.

Shelesnyak, M. C. 1954. The action of selected drugs on deciduoma formation. Endocr. 55:85.

Simpson, M. E., H. M. Evans and C. H. Li. 1950. Effect of pure FSH alone or in combination with chorionic gonadotropin in hypophysectomized rats of either sex. Anat. Rec. 106:247.

Simpson, M. E., C. H. Li and H. M. Evans. 1951. Synergism between pituitary follicle stimulating hormone (FSH) and human chorionic gonadotrophin (HCG). Endocr. 48:370.

Spies, H. G. and Quadri, S. K. 1967. Regression of corpora lutea and interruption of pregnancy in rabbits following treatment with rabbit serum to ovine LH. Endocr. 80: 1127.

Talwaker, P. K., A. Ratner and J. Meites. 1963. *In vivo* inhibition of pituitary prolactin synthesis and release by hypothalamic extract. Am. J. Physiol. 205:213.

Turner, C. D. General Endocrinology, 4th ed., W. B. Saunders Co., Philadelphia. London. 1967. p. 487.

Teel, H. M. 1926. The effects of injecting anterior hypophyseal fluid on the production of placentoma in rats. Am. J. Physiol. 79:184.

Velardo, J. T. and F. L. Hisaw. 1951. Quantitative inhibition of progesterone by estrogens in development of deciduomata. Endocr. 49:530.

Von Berswordt-Wallrabe, I., H. F. Geller and V. Herlyn. 1964. Temporal aspects of decidual cell reaction. Acta Endocr. 45:349.

WaKabayashi, K. and B. Tamaoki. 1965. *In vitro* synthesis of LH in the anterior pituitary gland. Endocr. 77:262.

Weichert, C. K. 1928. Production of placentomata in normal and ovariectomized guinea pigs and albino rats. Proc. Soc. Exp. Biol. Med. 25:490.

Wolfe, J. M. 1935. Reactions of ovaries of mature female rats to injections of oestrin. Proc. Soc. Exp. Biol. Med. 32:757.

Wrenn, T. R., J. Bitman, W. R. Delander and M. L. Mench. 1966. Influence of the placenta in mammary gland growth. J. of Dairy Science. 49:183.

Yochim, J. M. and V. J. DeFeo. 1963. Hormonal control of the onset, magnitude and duration of uterine sensitivity in the rat by steroid hormones of the ovary. Endocr. 72:317.

Yochim, J. M. and V. J. DeFeo. 1962. Control of decidual growth in the rat by steroid hormones of the ovary. Endocr. 71:134.

Yoshinaga, K. and C. E. Adams. 1966. Delayed implantation in lactating mice. J. Endocr. 13:1.

Young, W. E. The mammalian ovary. In Sex and Secretions (3rd ed.). W. C. Young. The Williams and Wilkins Co., Baltimore, Maryland. 1961. V. 1, p. 456.

Young, W. P., R. Nasser, T. Hayashida. 1963. Antiserum inhibition of the estrous cycle in normal rats. Nature. 197:117.

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HORMONAL CONTROL OF DECIDUAL CELL RESPONSE IN THE RAT

by

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Adult, virgin female rats of the Holtzman strain weighing 220-280 g were divided into three operative groups: (1) intact, (2) ovariectomized and (3) pituitary autotransplanted. A daily vaginal lavage was taken to determine the stage of the estrous cycle. Pseudopregnancy was induced on the day of vaginal cornification (Day 0) by mechanical stimulation of the cervix in all rats except those with an autotransplanted pituitary. Decidual cell response (DCR) was initiated by mechanically traumatizing the right uterine horn on Day 4. Autotransplantation of the pituitary beneath the kidney capsule was performed on Day 0 after verification of ovulation by removing the left oviduct and observing ova microscopically. Ovariectomy was performed on Day 4 just prior to uterine traumatization. Uteri were removed, split at the bifurcation and dissected free of surrounding tissue when the animals were killed on Day 9. A wet and dry weight was taken on each uterine horn. Three mg progesterone and 1 ug estrone given after trauma to rats ovariectomized on Day 4 was capable of maintaining DCR of the magnitude obtained in intact rats (1774 mg vs 1837 mg). Progesterone or estrone alone in the same amounts could not support a maximal DCR (339.8 mg and 1072.9 mg, respectively). Intact pseudopregnant rats injected twice daily on Days 1-5 or Days 4-8 with 0.1 ml of rabbit antiserum to sheep LH had a decreased uterine weight as compared with the uterine weight of rats injected with control serum (853 mg and 1024 mg vs 1839 mg). Anti-LH serum given to pseudopregnant intact rats on Days 4-8 decreased

uterine weight to the magnitude obtained in ovariectomized rats receiving only progesterone on Days 4-8 or in rats with the pituitary autotransplanted beneath kidney capsule on the day of ovulation (1024 mg, 1072 mg and 978 mg, respectively). Anti-LH serum had little effect on DCR (1658 mg) when given during the pretrauma period to rats ovariectomized and traumatized on Day 4, then treated with progesterone and estrone; but caused a very pronounced effect during the post trauma period (Days 4-8) in intact rats (1024 mg). Antiserum had no effect on DCR in rats ovariectomized and given estrone and progesterone or in rats with a pituitary autograft. Simultaneous injections of FSH (20 ug) and LH 5 ug) twice daily to rats with an autotransplanted pituitary resulted in maximal decidual growth (2033 mg). Maximal DCR was also obtained when only estrone was given after trauma to rats with an autotransplanted pituitary (2064 mg). Either FSH or LH given alone had only a slight stimulatory effect on uterine weight after trauma (1338 mg and 1360 mg, respectively) in rats with an autotransplanted pituitary. Apparently, prolactin secreted by the autograft was all that was necessary for progesterone production; however, to obtain maximal DCR, FSH and LH were needed for estrogen production. The data suggest that anti-LH serum interfered with estrogen secretion. Both progesterone and estrone were necessary to give a maximal uterine growth although the amount of estrogen needed before trauma was apparently small. The hormones FSH and LH were

necessary for estrogen secretion to support maximal decidual growth. The data also indicated that LH was not necessary for progesterone production in a quantity large enough to support maximal decidual growth following mechanical uterine trauma in pseudopregnant rats, since anti-LH serum did not lower DCR in rats bearing an autografted pituitary.