The Rat Estrous Cycle Associated with Functional Corpora Lutea and the Mechanism of Its Recurrence*

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Synopsis

Pseudopregnancy (PSP)-like cycles characterized by a fully functional luteal phase and comparable in length to the period of pseudopregnancy have been induced in normally cycling rats by the isografting of anterior pituitary (AP) implants beneath the kidney capsule. Hormonal factors associated with the induction and maintenance of these spontaneously recurring PSP-like cycles were studied by measuring blood levels of prolactin, LH, FSH, estradiol-17β, progesterone and 20α-dihydroprogesterone. Transplantation of isografts comprising 1/4 anterior pituitary extended the length of the diestrous period approximately to that of normal pseudopregnancy. However, over a range of tissue mass extending from 1/4 to 2 glands, a significant correlation was observed between the duration of diestrus and the quantity of anterior pituitary tissue employed. More than 2 glands did not further extend the length of the luteal period. The secretory patterns of progesterone and 20α-dihydroprogesterone in ovarian vein blood during the PSP-like cycle were similar to those of normal pseudopregnancy. Within limits, the progesterone concentration varied directly with the quantity of transplanted pituitary tissue. Prolactin levels measured either in serum or in the in situ pituitary did not fluctuate during the luteal phase of the PSP-like cycle; however, the magnitude of the serum prolactin concentration was directly correlated with the quantity of pituitary tissue implanted possibly reflecting a direct contribution of the implant to the circulating hormone level. It is assumed that the prolactin supply from the isograft was responsible for induction and maintenance of the PSP-like cycle, since premature termination resulted from early ergocornine injection and/or removal of the grafted pituitary tissue. However, completion of the PSP-like cycle following removal of the AP-graft on or after day 5 demonstrating the capacity of the in situ pituitary to secrete adequate prolactin for maintenance. When animals were hypophysectomized during the first half of the PSP-like cycle, the isografted pituitary being left in place, progesterone secretion increased significantly providing evidence of a suppressive action on ovarian progesterone secretion by the in situ pituitary. Hypophysectomy performed during the second half of the PSP-like cycle resulted in a decreased progesterone secretion suggesting a change in luteal responsiveness to luteotrophic stimulation taking place at midcycle. The initiator of this qualitative change in luteal function probably resides in the in situ pituitary. The circulating levels of FSH and LH did not fluctuate from basal levels; however, ovarian vein estradiol-17β increased gradually during the second half of the PSP-like cycle. It is supposed that the rising estradiol level eventually triggered gonadotrophin release on the subsequent proestrus which was required for recurrence of the cycle. Finally, a statistically significant prolongation of the PSP-like cycle was induced by bilateral hysterectomy, this prolongation being of greater length when hysterectomy was performed prior to the 9th day.

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The rat has long been a favorite model animal for use in studies of reproductive endocrinology. However, because the rat, like the mouse and the hamster, lacks a fully functional luteal phase during the normal estrous cycle (incomplete estrous cycle), it is questionable to what extent information obtained in rats can be applied directly to other mammals. It has been known for some time that artificial cervical stimulation or mating with an infertile male can result in the conversion of an incomplete estrous cycle to a pseudopregnant state comparable to the condition seen in most mammals in which the estrous cycle includes a fully functional luteal phase.

It is widely accepted that prolactin is the major luteotropic hormone in the rat (Astwood, 1941; Evans et al., 1941), and it has recently been shown that pseudopregnancy and the early states of pregnancy are causally related to pulsatile diurnal and nocturnal discharges of prolactin (Butcher et al., 1972; Freeman et al., 1974; Smith and Neill, 1976). The suggestion is implicit in this relationship that if the level of prolactin secretion could be maintained experimentally, it would be possible to extend the incomplete estrous cycle to a complete estrous cycle thus making the rat a more favorable experimental model for studying the mechanisms controlling function of the mammalian corpus luteum.

Pituitary isografts have been shown to induce a pseudopregnancy (PSP)-like cycle in rats (Quilligan and Rothchild, 1960) and mice (Mühlbock and Boot, 1959; Boot et al., 1962; Montemurro and Gardner, 1963; Browning and White, 1963; Hagen and Rawlinson, 1963), and a direct relationship has been established between the number of transplanted pituitary glands and the serum prolactin concentration of recipient animals (Chen et al., 1970). The present study is concerned with the hormonal factors associated with the induction and recurrence of “complete” estrous cycles in rats.

Materials and Methods

Animals
Three hundred thirty Wistar female rats (210-240 g body weight) were selected to be pituitary graft recipients after each had presented at least two consecutive 4-day estrous cycles. Four hundred five male rats of the same strain (380-480 g body weight) were used as pituitary tissue donors. All rats were maintained in air-conditioned quarters (temperature: 22-24°C) with the lights on from 0500-1900 hr daily and were given free access to food and water.

Recipient female rats were operated on without regard to the stage of their estrous cycle. Each rat was anesthetized with pentobarbital sodium (30 mg/kg, Mintal, Tanabe Pharmaceutical Co.). The anterior pituitary gland (AP), or portions thereof, was taken up into a small glass pipette and inserted into the recipient kidney capsule with the aid of a small glass plunger. Each recipient received 1/9, 1/7, 1/4, 1/2, 1, 2, or 4 portions of AP tissue. The smaller portions, 1/9 and 1/7, were determined from the weight of the whole gland. All recipient animals were followed by daily vaginal smears. Day 1 of the resultant estrous cycle was designated as the first day of diestrus, and the cycle length was set at the total number of days of diestrus. The occurrence of ovulation was determined in a part of animals by microscopic examination of tubal ova on the day of vaginal estrus.

Pseudopregnancy
Pseudopregnancy was induced by mechanical stimulation of the cervix with a glass rod on the morning of vaginal cornification. The day following cervical stimulation was designated day 1 of pseudopregnancy.

Hypophysectomy
Hypophysectomy was performed through the parapharyngeal approach under ether anesthesia.

Removal of the AP isograft
The left kidney bearing the isografted AP tissue was removed under ether anesthesia instead of attempting to enucleate only the grafted tissue from the kidney.

Hysterectomy
Hysterectomy was performed through midline incision in the abdominal wall under ether anesthesia. By tying only the tributary vessels, the major blood
supply system was left intact when the uterine horns were removed.

Ergocornine injection
Ergocornine maleate (Forschungsabteilung, Sandoz AG) dissolved in ethanol (10 mg/ml) was given to inhibit prolactin secretion either from the grafted or the in situ pituitary. One milligram (0.1 ml) was given subcutaneously at each injection.

Blood collection
Blood for steroid analysis was collected from an ovarian vein by a modification of the method of Eto et al. (1962). Blood for pituitary hormone analysis was collected following decapitation of the animal.

Measurement of steroids
Progesterone and 20α-dihydroprogesterone (20α-OHP) were measured in extract of ovarian vein blood by gas-liquid chromatography using the method essentially the same as described by Sin et al. (1971). Steroid concentration were expressed in μg/10 ml ovarian vein blood (OVB). OVB estradiol-17β concentrations were measured by radioimmunoassay as previously described by Horikoshi and Suzuki (1974) and were expressed in pg/ml plasma.

Measurement of pituitary hormones
Pituitary gland tissue was homogenized in 0.01 M NaCl at a concentration of 1 gland/100 ml. Prolactin concentrations were determined in peripheral blood serum and in pituitary gland homogenates by radioimmunoassay according to the method of Niswender et al. (1969). Prolactin concentrations were expressed as ng/ml serum and μg/mg pituitary equated to NIAMDD Rat prolactin, RP-1, used as reference. Serum FSH concentrations were measured by radioimmunoassay using the method of Daane and Parlow (1971) and were expressed in ng/ml serum equated to NIAMDD Rat FSH, RP-1. Serum LH concentrations were measured by radioimmunoassay according to the method of Niswender et al. (1968). Results were expressed in ng/ml serum equated to NIAMDD Rat LH, RP-1.

Statistical analysis of data
All data were statistically analyzed by the Student’s t test. A probability, p<0.05, was considered to be statistically significant.

Results and Discussion

Induction of the complete estrous cycle
The effect of varying the quantity of the anterior pituitary isograft on the length of the estrous cycle is shown in Fig. 1. Data were compiled for this figure from 4070 cycles observed in 150 AP-isografted rats. No significant extension of the normal

![Graph showing correlation between diestrous days and quantity of anterior pituitary tissue](image-url)

Fig. 1. Effect of varying the quantity of the anterior pituitary isograft on the number of diestrous days. A significant correlation was observed between the number of diestrous days and the quantity of anterior pituitary tissue over a range of tissue mass extending from 1/4 to 2 glands (r=0.98). Each plotted value represents the mean±SE of prolonger diestrous days resulting from isografts of 4-AP: 4 rats, 128 cycles; 2-AP: 58 rats, 1445 cycles; 1-AP: 14 rats, 392 cycles; 1/2-AP: 59 rats, 1465 cycles; 1/4-AP: 5 rats, 140 cycles; 1/7-AP: 5 rats, 250 cycles; 1/9-AP: 5 rats, 250 cycles.
cycle resulted from implantations of 1/9th or 1/7th AP-isografts. However, when 1/4th AP or more was implanted, the number of diestrous days was increased becoming equivalent to that of normal pseudopregnancy. Following the appearance of subsequent vaginal proestrus and estrus, the prolonged pseudopregnancy-like cycles recurred spontaneously. A significant correlation was obtained between the duration of diestrus and the quantity of tissue used in the AP-isografts ($r=0.98$); the prolongation of the cycle differed statistically depending upon the size of the isograft (1/4 : 1/2, $p<0.05$; 1/2 : 1, $p<0.001$; 1 : 2, $p<0.05$). However, implantation of 4-AP isografts did not produce any additional prolongation of diestrus. Almost all the rats autopsied on the day of vaginal estrus ovulated and formed a new crop of corpora lutea.

**Ovarian progestin secretion**

The functionality of corpora lutea formed during pseudopregnancy-like cycles in mice has been established by the induction of deciduomata (Mühlbock and Boot, 1959). In the present study ovarian venous blood progestins were measured every other day from day 1 to days 12 or 15 during pseudopregnancy-like cycles resulting from isografts of 1/2-AP and 2-AP (Fig. 2). Progesterone concentration increased from day 1 to a peak at day 7 and declined thereafter. $20\alpha$-Dihydroprogesterone secretion was reciprocally related to progesterone secretion. These patterns are similar to those observed during normal pseudopregnancy (Fajer and Barraclough, 1967; Hashimoto et al., 1968). However, depending upon the quantity of the AP isograft, differences were observed in the magnitude of the progesterone secretion (Fig. 2).

Thus, the long cycle resulting from the isografting of anterior pituitary tissue did not differ qualitatively from normal pseudopregnancy. Estrus, ovulation and the formation of one crop of functional corpora lutea all occur spontaneously during each recurrence of the long cycle; therefore, we recognize that the experimental procedure of isografting sufficient anterior pituitary tissue in the rat results in the conversion

![Fig. 2. Progesterone and $20\alpha$-dihydroprogesterone ($20\alpha$-OHP) levels in ovarian venous blood during pseudopregnancy-like cycles resulting from isografts of 2-AP (○—○) and 1/2-AP (●—●). Each value represents the mean±SE of three to six animals.](image)
of a normal 4-day estrous cycle into a pseudopregnancy-like cycle comparable to the complete estrous cycles observed in many species of mammals.

The mechanism of induction of the complete estrous cycle

In order to analyze more fully the role of isografted AP, superimposed upon that of the in situ AP, in the induction of pseudopregnancy-like cycles, prolactin (PRL) concentrations were determined in both the peripheral serum and the in situ pituitary. Furthermore, the effects of ergocornine administration and removal of the grafted AP on the continuance of the long cycle were observed.

Serum and pituitary prolactin levels observed during the PSP-like cycle are shown in Figs. 3 and 4. In rats bearing 1/2-AP or 2-AP isografts, serum PRL concentrations differed in magnitude according to the quantity of isografted pituitary tissue. Prolactin levels did not vary throughout the long cycle. The daily biphasic fluctuations of PRL reported in normal pseudopregnancy (Freeman et al., 1974) were not observed as shown in Figs. 5 and 6.

The requirement for PRL support was further investigated following single ergocornine injections made on various days during the long cycle. Interruption of the cycle occurred immediately following ergocornine injection being effective even on day 12 in rats bearing 2-AP isografts. Results of ergocornine injection are given in Table 1. Removal of the grafted AP prior to day 3 resulted in failure of the

![Graph of Serum Prolactin](image)

**Fig. 3.** Serum prolactin levels during pseudopregnancy-like cycles resulting from isografts of 2-AP (○—○) and 1/2-AP (●—●). Samples were collected by decapitation of three to four animals at 0600 hr. Each value indicates the mean ± SE. The hatched columns represent the mean serum prolactin levels (± SE) during normal estrous cycle.

![Graph of Pituitary Prolactin](image)

**Fig. 4.** Prolactin concentrations of the pituitary in situ during pseudopregnancy-like cycles resulting from isografts of 2-AP (○—○) and 1/2-AP (●—●). Each value represents the mean ± SE of three to four animals.

![Graph of Serum Prolactin](image)

**Fig. 5.** Serum prolactin levels collected by decapitation at 6-hr intervals between day 3 and 4 of the pseudopregnancy-like cycle in rats bearing 2-AP isografts. Each value represents the mean ± SE of four to five animals.
Table 1. Effect of ergocornine on the maintenance of pseudopregnancy (PSP)-like cycle and pseudopregnancy

<table>
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<th>Day* of Treatment</th>
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<th>Dose (mg)</th>
<th>No. of Rats</th>
<th>Terminated**</th>
<th>Not terminated</th>
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</table>

*: The first day of appearance of leucocytes in vaginal smear is designated Day 1.
**: Termination of the cycle is defined as an appearance of vaginal proestrus within 48 hr. after ergocornine injection.
***: Vehicle alone.

long cycle's being established, and the host rat resumed its normal 4-day estrous cycle. Thus, activation of the corpora lutea required for the recurrence of the PSP-like cycle appeared to be dependent upon the continual supply of a critical level of PRL from the grafted AP (Fig. 7). Removal of the isograft after day 5 did not result in the interruption of the PSP-like cycles. After the isograft was removed on day 5,
the subsequent administration of ergocornine on day 8, however, induced the immediate interruption of the PSP-like cycle. These results suggest that the latent capacity of the in situ pituitary to secrete adequate prolactin for further maintenance of the luteal function is manifest by the removal of the isograft. Establishment of the typical rhythmic secretory pattern of prolactin during pseudopregnancy has been shown to require the presence of the ovary (Freeman et al., 1974). Also, a single injection of progesterone during a limited time interval in the morning (0700-1000 hr) of the day of estrus has been shown to induce pseudopregnancy in cycling rats (Horikoshi and Suzuki, 1974). Therefore, it is assumed that the pseudopregnancy-like pattern in the hypothalamo-hypophyseal complex in situ had already been established by the action of ovarian progesterone stimulated by prolactin secreted from the isograft prior to its removal. This assumption, however, does not appear to be compatible with our observation that daily rhythmic secretion of prolactin was not observed during the PSP-like cycle (Figs. 3 and 5). The pulsatile secretion of prolactin from the in situ pituitary was probably reduced by the high circulating level of prolactin from the isograft acting back on the hypothalamus to increase PIF production (Wolthuis and de Jongh, 1963; Chen et al., 1967; Welsch et al., 1968). Elimination of this short loop inhibition which followed removal of the isograft after day 5 appears to have permitted an augmented prolactin secretion from the in situ pituitary sufficient to maintain the long cycle.

The mechanism for the recurrence of the complete estrous cycle

Everett (1954 and 1956) and Nikitovitch-Winer and Everett (1958) have shown that hypophysectomy coupled with pituitary autografting resulted in the prolonged maintenance of corpus luteum function. By contrast, functional luteolysis certainly occurred in the present study after approximately a two-week interval in intact rats bearing pituitary isografts. A decline in the circulating prolactin level is not considered to be the cause of this functional luteolysis, since it is supposed that the grafted pituitary, being free from hypothalamic inhibition, continues to secrete PRL. It thus appears likely that the in situ pituitary plays an important role in programming the life span of the corpus luteum. In an attempt to clarify this role, the effect of hypophysectomy (removal of the in situ pituitary) upon luteal function was examined at various times throughout the PSP-like cycle by determining the progesterone concentration in ovarian vein blood collected 3 days after hypophysectomy. Rats bearing 2-AP isografts showed a marked increase in ovarian progesterone concentration when hypophysectomy was performed on days 4 and 7. Similarly, rats bearing 1/2-AP isografts showed increased progesterone concentration after hypophysectomy performed on day 4. Thus, it appeared that the in situ pituitary imposed an inhibitory effect on ovarian progesterone secretion. However, this inhibition was not manifest when hypophysectomy was performed on day 10 in rats bearing 2-AP isografts or on day 7 in rats bearing 1/2-AP isografts (see Fig. 8); under these conditions ovarian vein progesterone concentrations were lower when measured three days after hypophysectomy. A possible explanation of these findings appears to be that the in situ pituitary retained in isografted rats until day 7 or day 10, depending on whether the rats bore 1/2-AP or 2-AP isografts, induced qualitative changes in the corpora lutea rendering them unable to respond to the luteotrophic action of PRL from the isografts. The initiator of this qualitative change in luteal function may be attributable to LH and FSH. Since concentrations of serum FSH and LH
measured in both 1/2-AP and 2-AP isografted rats were detectable throughout the PSP-like cycle as seen in Figs. 9 and 10 similar to what has been shown for normal pseudopregnancy (Rabii and Kragt, 1972; Bast and Melampy, 1972) and that these basal levels of gonadotrophins are reported to play an essential role for the termination of luteal function in the rat (Takahashi et al., 1978).

The possibility that some uterine factor was operative in the recurrence of the PSP-like cycle was explored by noting the effect of hysterectomy performed at various times during the long cycle in rats bearing 2-AP and 1/2-AP isografts. These results are diagrammed in Fig. 11. A statistically significant prolongation of the cycle occurred when hysterectomy was done prior to day 11 in 2-AP grafted rats and before day 9 in 1/2-AP grafted rats (p<0.01, respectively). Similar effects of hysterectomy on the duration of normal pseudopregnancy have been described previously (Sibigier and Rothchild, 1963; Melampy et al., 1964) Following hysterectomy, PSP-like cycles recurred spontaneously with the mean cycle length being significantly longer than that

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**Fig. 8.** Effect of hypophysectomy (removal of the pituitary in situ) on progesterone concentrations in ovarian venous blood collected three days after hypophysectomy which was performed on days 4, 7 or 10 of the cycle. Values are indicated by checkered columns (2-AP) and hatched columns (1/2-AP) upon the day of blood collection. Progesterone levels in ovarian venous blood during pseudopregnancy-like cycles resulting from isografts of 2-AP and 1/2-AP are depicted from Fig. 2 for the reference of the control values.

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**Fig. 9.** Serum FSH levels during pseudopregnancy-like cycles resulting from isografts of 2-AP (○—○) and 1/2-AP (●—●). Each value represents the mean±SE of three animals. The hatched columns indicate the mean serum FSH levels during normal estrous cycle.

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**Fig. 10.** Serum LH levels during pseudopregnancy-like cycles resulting from isografts of 2-AP (○—○) and 1/2-AP (●—●). Each value represents the mean±SE of three animals. The hatched columns indicate the mean serum LH levels during normal estrous cycle.
before hysterectomy ($p<0.01$). While it was apparent that the uterine factor participated in the programming of events which determined the life span of the corpora lutea throughout the PSP-like cycle, it was also apparent that this uterine factor was not predominant for inducing luteolysis, since luteolysis occurred belatedly following hysterectomy. It should also be mentioned in this regard that this uterine factor was incapable of terminating luteal function in pituitary autografted rats.

We conclude that the mechanism regulating the recurrence of the PSP-like cycle results from the *in situ* pituitary producing a qualitative change in the trophic response mechanism of the luteal tissue which is followed spontaneously by functional and structural luteolysis. It also appears that the rise of the ovarian estradiol-17β level during the second half of the complete estrous cycle (Fig. 12) eventually triggers the release of gonadotropin required for the initiation of the next ovulation and the subsequent recurrence of the cycle. Finally, some uterine factor plays a facilitatory role toward luteolysis of the preceding crop of corpora lutea probably by modifying the action of the pituitary factor.

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**Fig. 11.** Effect of hysterectomy on the length of pseudopregnancy-like cycles. Hysterectomy was performed at various times during the cycles resulting from isografts of 2-AP (checkered column) and 1/2-AP (hatched column), and the resulting mean cycle lengths are shown according to the day of operation. The mean length of pre-operative cycles are shown at the top of the figure. Following hysterectomy, pseudopregnancy-like cycles recurred spontaneously with the mean cycle length being significantly longer than that before hysterectomy ($p<0.01$). The mean length of post-operative cycles is summarized at the bottom of the figure irrespective of the day of operation.

**Fig. 12.** Plasma estradiol-17β levels of ovarian venous blood during pseudopregnancy-like cycles resulting from isografts of 2-AP (---) and 1/2-AP (—). Each value represents the mean ± SE of three to five animals. The hatched columns represent the mean plasma estradiol-17β levels during normal estrous cycle.
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References