INTERRUPTION OF PREGNANCY,
INDUCTION OF OVULATION AND DELAYED PSEUDO-
PREGNANCY FOLLOWING SUPPRESSION
OF LUTEAL FUNCTION

By
P. F. Kraicer and M. C. Shelesnyak
with the Technical Collaboration of Ada Tandlich

ABSTRACT

The responses of rats to acute luteal insufficiency induced by ergocornine or by luteectomy (removal of the functional corpora lutea) on three different days of pregnancy were compared. Both treatments interrupted pregnancy; oestrus and ovulation recurred, followed usually by a pseudo-pregnancy. Weights of the adrenals, pituitary and pineal were not affected. Suppression of luteal secretion causes an increase of gonadotrophin secretion as well as sensitization of follicles to gonadotrophic stimulation which lead to ovulation. The corpora lutea formed at this ovulation usually become functional, suggesting the persistence of luteotrophic secretions despite the recurrence of ovulation.

Two techniques exist for specifically blocking the functional corpora lutea of pregnancy in the rat, surgical removal and pharmacological inhibition by ergocornine. Certain of the ergot alkaloids, including ergocornine, cause a decrease in available progesterone (Lindner & Shelesnyak 1967) although apparently not by direct interference with the corpora lutea (Shelesnyak 1958: Kisch & Shelesnyak, in press). Extirpation of the corpora lutea causes a decrease in available progesterone by removing its source (Kraicer, in preparation).

It is of interest to contrast the response of rats to these two treatments which differ in their mechanisms of action, yet are believed to result in the same outcome. In comparing these two treatments we have concentrated on the two major known effects of progesterone, namely, maintenance of preg-
nancy and prevention of ovulation and oestrus. In addition, other parameters of response have been sought including changes in the weights of certain endocrine glands and induction of delayed pseudopregnancy.

The aim of this study was to substantiate the efficacy of these techniques in the suppression of progesterone secretion and to seek differences in response. Although both techniques have been employed to study the effects of acute withdrawal of progesterone, there are objections to each. The handling of the ovaries and the local high temperatures incident to fulguration by electrocautery leave the ovary severely traumatized after luteectomy. Whereas the problem of physical damage of the ovary does not arise following ergocornine treatment (Lobel et al. 1966), the utilization of ergocornine, as of any drug, is complicated by the possibility of undesirable additional actions. However, in the light of the practical differences between these treatments, it is unlikely that there will be responses common to both of these treatments which are, nevertheless, unrelated to suppression of progesterone secretion. Direct comparison of the responses to these treatments would clarify the degree of specificity with respect to the induction of acute luteal insufficiency.

**MATERIALS AND METHODS**

*Experimental Design*

The response of rats to acute luteal insufficiency induced by ergocornine or by luteectomy (removal of the functional corpora lutea) on three different days of pregnancy was compared. The days of treatment chosen were L1, L4, L6 (L0 is the first day of pregnancy). On day L1 the corpora lutea are newly formed, hence this is the earliest time that surgical luteectomy can be performed. Day L4 is the day of ovum attachment to the uterus and induction of decidualization. It is considered to be the day on which progesterone preparation of the uterus is consummated and progesterone control of gestation begins. On day L6 the ovum is implanted but placental luteotrophin secretion is not yet significant; day L6 is the latest day of pregnancy on which ergocornine is still effective (Kisch & Shelesnyak 1967).

Three treatments were employed: luteectomy, sham-luteectomy and ergocornine injection after sham-luteectomy. A group of rats injected with ergocornine without surgery was not included, since the response to this treatment is well-established (Kraicer & Shelesnyak 1964), and is constantly controlled in our colony. Because there was no evidence that the sham-luteectomy affected the response to ergocornine significantly, it was superfluous to add an unoperated, ergocornine-treated group.

Following treatment these rats were observed for onset of oestrus, interruption of pregnancy and appearance of a delayed pseudopregnancy. Another small group of rats was used to establish the occurrence of ovulation after removal of the corpora lutea or injection of ergocornine. In preliminary experiments it was found that treatments on L4 or L6 were often followed by appearance of blood in the vaginal smear. This complicated the interpretation of the vaginal smear, making the day of oestrus difficult to establish. This difficulty was avoided by performing the treatments on L3.
Animal Treatment

Pro-oestrous rats from the Biodynamics Institute colony were mated with males of established fertility. When spermatozoa were found in the vagina, the next morning, the rats were assumed to be pregnant; this day was designated day L₀ of pregnancy. The rats selected for surgery, luteectomy or sham-luteectomy, were injected intraperitoneally with 1 ml of 1% trypan blue at about 9 a.m. of L₀. Animals which did not begin to turn blue within 2 hours were given a second injection of the same amount of trypan blue at about 11 a.m.

Rats were anaesthetized by intraperitoneal injection of Avertin (a fifty-fold dilution of tribromoethanol-tertiary amyl alcohol, 1:2, in isotonic saline) 1 ml per 100 g body weight. Ergocornine was injected subcutaneously as 1 mg of ergocornine methane-sulphonate dissolved in 0.25 ml of 70% ethanol.

Daily examinations of the vaginal smears were performed following treatment. The occurrence of a prolonged phase of leucocytic vaginal smears following interruption of pregnancy and onset of oestrus is well established (Kraicer & Shelesnyak 1964; Kraicer, in prep.). In order to confirm that the failure of these rats to maintain normal 4-5 day cycles was due to a spontaneous pseudopregnancy, the ability of the uteri of these rats to undergo decidualization was tested. Deciduomata were induced by intraperitoneal injection of 20 mg of pyrathiazine HCl in 1 ml of distilled water on the fourth day of the leucocytic vaginal smear (Shelesnyak & Kraicer 1961). When the rats were killed, four days later, their uteri were examined for deciduomata.

All rats, except those used for study of ovulation at the post-treatment oestrus, were killed about 14 days after coitus. The uterus and the remaining ovary were carefully examined. The ovary and the pineal, pituitary and adrenal glands were also weighed.

Luteectomy

The surgical removal of the corpora lutea consists of the following steps: (1) vital staining of previous generations of corpora lutea with trypan blue; (2) removal of the ovary containing the majority of newly formed corpora lutea; and (3) extirpation of the newly formed corpora lutea of the other ovary for fulguration (combustion by a high frequency electric spark) after stripping off the ovarian bursa (Kraicer, in prep.). Since any of these steps could conceivably affect the subsequent behaviour of the ovary, the response to luteectomy was compared to the response to a sham-operation. In sham-operated rats, the ovary with the smaller number of corpora lutea was removed, and a part of the contralateral ovary was fulgurated. Animals treated with ergocornine were given the injections immediately after sham-luteectomy.

In order to detect ovulation after luteectomy, the surgical procedure was modified. Ovaries were exposed through dorsolateral lumbar incisions. Each bursa was incised on the dorsal surface with an electrocautery needle (see Lisowski 1953 for anatomical terms); the incision ran parallel to the major bursal blood vessels, over the middle of the ovary, and extended from the border of the ovarian fat pad to the oviduct. The bursae were peeled back and the ovaries expelled by gently squeezing between the thumb and index finger. All fresh corpora lutea, unstained by trypan blue, were fulgurated. Each ovary was replaced into its bursa by spreading and retracting the bursal membrane on one side of the ovary with a pair of curved forceps, while the ovary was pushed into the empty sac with a blunt probe. By gently retracting the ovarian fat pad cephalad, it was simple to pull the second half of the bursa over the ovary. The slit in the bursa was approximated with a single stitch of 6-0 chromic catgut. If there had been bleeding into the bursa, it was flushed, through the incision, with several ml of Ringer’s solution in an attempt to remove the clots.
Detection of Ovulation

Rats were killed on the day of vaginal cornification. The oviducts were examined for ovulatory distention of the loops closest to the ovary (Long & Evans 1922). If distention was noted, these loops were excised into a drop of Ringer’s solution in a depression slide. The ova, in their cumulus masses, were expelled into the Ringer’s solution by cutting the wall of the distended loop. The Ringer’s solution was then slowly aspirated out of the well of the slide. As the level of liquid fell, the cumulus mass flattened and the individual ova were seen and counted. If no ova were found in the oviducts, the status of the follicular ova was investigated. Large follicles were cut open and the ova, with adherent follicular cells, were gently aspirated into a fine polyethylene micropipette. Follicular ova were examined by interferential contrast microscopy to determine their status: ova with germinal vesicles were classed as pre-ovulatory; ova lacking germinal vesicles were classed as ovulatory. Uteri and ovaries were weighed.

RESULTS

 Interruption of Gestation

Both ergocornine and luteectomy interrupted gestation in virtually all of the rats (Table 1). The largest difference in response was noted on L₄. However,

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day</th>
<th>No. ♀♀</th>
<th>Gestation interrupted</th>
<th>Vaginal cornification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luteectomy</td>
<td>on L₁</td>
<td>8</td>
<td>7</td>
<td>8(1)*</td>
</tr>
<tr>
<td></td>
<td>&quot; L₄</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>&quot; L₆</td>
<td>8</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23</td>
<td>22</td>
<td>21(1)*</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ergocornine</td>
<td>on L₁</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>&quot; L₄</td>
<td>8</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>&quot; L₆</td>
<td>8</td>
<td>5</td>
<td>5(1)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23</td>
<td>20</td>
<td>16(1)*</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham-luteectomy</td>
<td>on L₁</td>
<td>6</td>
<td>2</td>
<td>3(1)*</td>
</tr>
<tr>
<td></td>
<td>&quot; L₄</td>
<td>8</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&quot; L₆</td>
<td>8</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22</td>
<td>6</td>
<td>4(1)*</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Number with vaginal cornification remaining pregnant.
even at this time, ergocornine was not significantly less effective than was luteectomy ($\chi^2 = 1.65, P = 0.20$).

The sham-operation itself caused pregnancy to be interrupted in about one-quarter of the rats, but this was significantly fewer than after luteectomy or ergocornine ($\chi^2 = 26, P < 0.0001$). The effects of the sham-operation were much more severe on L1 than on L1 or L6. On L1, two of six rats had their pregnancies interrupted with onset of oestrus – a response typical of that following luteectomy or ergocornine.

In the other two groups of sham-luteectomized rats, those operated on L4 and L6, four of the rats were found to be not pregnant at autopsy. Only one of these had cornification of its vaginal smear. The other three, therefore, may never have been pregnant.

**Vaginal Cornification**

Oestrous vaginal smears were seen in 21 of 23 luteectomized rats and 16 of 23 ergocornine-treated sham-operated rats. In contrast, only 4 of 22 sham-operated, control rats had oestrous vaginal smears. In general, the appearance of oestrous vaginal smears accompanied the interruption of gestation (see Table 1). Of the luteectomized rats only 1 had vaginal cornification while remaining pregnant; two other rats showed no vaginal cornification despite interruption of gestation.

Following administration of ergocornine plus sham-luteectomy on L4, all animals whose pregnancies were interrupted showed vaginal cornification; in the L4 treatment group, 4 of 8 had cornification and in the L6 group 4 of 5 had cornification. One rat, treated on L6, had vaginal cornification despite maintenance of gestation. The overall result of 15 rats with vaginal cornification out of 20 whose pregnancies were interrupted by ergocornine is lower than that expected after ergocornine alone; it has been shown that cornification of the vaginal smear is a more sensitive index of the action of ergocornine than is interruption of gestation (Kraicer & Shelesnyak 1964).

The time interval between the experimental treatment and appearance of the pro-oestrous vaginal smear is 2 or 3 days. There was no difference between the responses to ergocornine and to luteectomy.

**Induction of Ovulation**

Except for the numbers of ova recovered, the pattern of ovulation following luteectomy and ergocornine was strikingly similar (see Table 2). One curious feature was the absence of any pro-oestrous vaginal smear in most of the rats which ovulated 21/2 days after either treatment; on the second day after treatment their vaginal smears were leucocytic, whereas the following morning they were fully cornified, and freshly ovulated ova were recovered from their oviducts.
Table 2.
The Induction of Ovulation by Ergocornine or Luteectomy.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rats with ovulation Group size</th>
<th>Number of ova per ovulation</th>
<th>Rats ovulating at various times after treatment</th>
<th>Ovarian weight</th>
<th>Uterine weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>2½</td>
<td>3½</td>
</tr>
<tr>
<td>Luteectomy</td>
<td>6/9</td>
<td>3.3</td>
<td>6*</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Ergocornine</td>
<td>12/12</td>
<td>11.0</td>
<td>5**</td>
<td>6</td>
<td>1</td>
</tr>
</tbody>
</table>

* 3/6 of these rats had no prooestrous vaginal smears.
** 4/6 of these rats had no prooestrous vaginal smears.

Ovulation was detected in only 6 of 9 rats following luteectomy. One of the 9 rats was probably pro-oestrous when it was examined, although the vaginal smear was fully cornified. Its uterus was distended by a large volume of clear fluid, which comprised 58% of the gross uterine weight, and its ovaries contained medium-sized follicles with pre-ovulatory ova. This rat was not included in Table 2. From the six rats which ovulated, only 20 ova were recovered, 18 from the oviducts and 2 from an encysted bursa ovarica. The mean recovery per oviduct containing ova was 2. The low recovery rate was due at least in part to the extensive adhesions and haematomata. Ovulatory ova were recovered from some of the large follicles in the ovaries of the two rats whose oviducts were empty.

Following ergocornine treatment, all rats ovulated and the number of ova recovered was the same as found in rats of our colony at normal cyclic ovulation. This normal ovulation stands in sharp contrast to the weaker ovulatory response of the luteectomized rats.

Delayed Pseudopregnancy

Following the onset of oestrus which accompanies the interruption of gestation by ergocornine or by luteectomy, appearance of the next oestrus is delayed for approximately 13 days. This could represent a period of ovarian inactivity or a spontaneous pseudopregnancy. In the present study, this period has been shown to be a pseudopregnancy by induction of the decidual cell reaction in the uterus. Following pyrathiazine treatment, deciduomata were found in the uteri of 4 of 6 ergocornine-treated rats and 5 of 10 luteectomized rats. The production of the decidual reaction demonstrates the presence of active corpora.
lutea. That pyrathiazine was capable of inducing the decidual reaction shows that this period of luteal activity was a true pseudopregnancy, including the phase of sensitization necessary to permit decidual induction.

**Organ Weights**

No significant differences were found in organ weights among the various groups. This is particularly interesting in the case of the ovary, since it demonstrates the recovery following luteectomy. It should be recalled that in the interval between luteectomy and autopsy, there had usually been at least one ovulation. Since much of the weight of the ovary consists of corpora lutea, the restoration of ovarian weight to normal levels is probably the outcome of formation of the new corpora lutea.

**DISCUSSION**

The responses of the progravid rat to injection of ergocornine or to luteectomy (extirpation of the active corpora lutea) are practically identical; pregnancy is interrupted, oestrus and ovulation recur and are followed, usually, by a pseudopregnancy. Both the incidence and timing of these responses are similar for the two treatments. For many purposes, therefore, ergocornine may be employed as a pharmacological blocker of luteal secretion. In contrast to surgical ablation of the corpora lutea, the ovary remains relatively undamaged (Lobel et al. 1966). In this study, for example, the recovery of ova ovulated after luteectomy was very poor: only eight of sixteen oviducts contained ova and the mean number of ova in these oviducts was only 2.5. Replacement of the bursa ovarica after luteectomy was often difficult. The extensive injury to the ovary combined with the handling of the bursa commonly led to extensive adhesions of the surface of the ovary to the inside surface of the bursa. A normal number of ova may have been ovulated and not reached the oviduct due to mechanical impediments. Therefore, without study of serial sections of the whole ovisac-oviduct region, the conclusion that the number of follicles which ovulates is decreased may not be inferred.

It will also be recalled that traumatization of ovaries on L1 did interrupt pregnancy in 2 of 6 rats, and caused cornification of the vaginal smear in 3. Since fulguration results in localized high temperatures, proximal areas of the ovary are injured during luteectomy. Thus, even sham-luteectomy alone caused some interruption of pregnancy and reappearance of oestrus.

Because both surgical luteectomy and ergocornine administration may have effects unrelated to suppression of progesterone secretion, neither can be considered the procedure of choice. In order to study the effects of acute progesterone deprivation both should be used and the results compared. This is
especially true for experiments designed to study the role of the non-luteal tissues of the ovary during a luteal phase. Surgical ablation of the corpora lutea is a more direct method of preparing ovarian tissue free of luteal tissue. But the other elements of the ovary may be damaged during the surgery. Ergocornine leaves the other ovarian elements undamaged. On the other hand, it is known to be a sympatholytic drug, and its mechanism of action on the corpora lutea is still not known. The possibility of responses to ergocornine unrelated to reduction in progesterone is, therefore, a real one. The employment of ergocornine as the sole means of depressing progesterone secretion in an experimental design is not conclusive.

It has been suggested that total or partial luteectomy results in increased secretion of gonadotrophin which, in turn, leads to oestrogen secretion and vaginal cornification. The hypothesis of increased gonadotrophin secretion is confirmed by ovulation, which is a direct response to gonadotrophin. Nevertheless, this ovulation cannot be attributed solely to the action of gonadotrophin. Follicles in the ovaries of pregnant rats are refractory to gonadotrophic stimulation (Greenwald 1966). In order to induce ovulation, it is therefore necessary to remove this inhibition of follicular responsiveness and also to provide gonadotrophic stimulation. Suppression of the corpora lutea manifestly does both.

Following the new ovulation most of the rats do not show recurring 4–5 day cycles. Rather, they become pseudopregnant, i.e. the newly-formed corpora lutea are activated and secrete progesterone. It is difficult to visualize a direct causal relationship between the spontaneous occurrence of pseudopregnancy after interruption of progravidity and withdrawal of luteal progesterone. One possibility is that luteotrophic activity persists for some days after luteectomy. Following the withdrawal of progesterone by luteal deprivation, folliculotrophic activity results in oestrus, ovulation and luteinization; however, luteotrophic activity persists. As a result, the new corpora lutea are stimulated to active function. Thus, a new luteal phase, a pseudopregnancy, follows the oestrus.

A similar sequence of events has been proposed by Zeilmaker (1965). He homotransplanted an ovary from an immature rat under the kidney capsule of an adult. After the host was made pregnant, it was ovariectomized. The grafted immature ovary, which had been quiescent, responded and the animal showed signs of oestrus, followed by a pseudopregnancy, which was interpreted as a sign of persistence of luteotrophic activity.

Unfortunately, this explanation of the spontaneous delayed pseudopregnancy is appropriate only to experiments in which the response is not mediated by inhibition of luteotrophic activity. This is definitely not the case for ergocornine. There is no evidence that ergocornine has a direct inhibitory action on corpora lutea in vivo. Rather, it has been proposed that it inhibits secretion
of luteotrophin (Shelesnyak 1958; Kisch & Shelesnyak, in press). Zeilmaker & Carlsen (1962) have found an apparent decrease in prolactin secretion following ergocornine treatment. If one attempts to explain the induction of the delayed pseudopregnancy as a persistence of luteotrophin secretion, one is led to the paradox of a drug which inhibited luteotrophin secretion nevertheless permitting persistence of luteotrophin secretion.

Treatments leading to delayed pseudopregnancy have several characteristics in common. First, by definition, the pseudopregnancy is delayed until after occurrence of ovulation and luteinization; second, the procedure which induces the delayed pseudopregnancy is instituted at a time when there are no active corpora lutea present in the ovaries or, alternatively, the procedure which induces the delayed pseudopregnancy also blocks existing corpora lutea. Since some of the treatments known to induce a delayed pseudopregnancy may not, in themselves, induce luteotrophin secretion (Everett & Quinn 1966). Everett (1967) has questioned the necessity of assuming secretion of prolactin which persists in spite of increasing levels of gonadotrophic secretion. He suggests that »the stimulus is remembered in the central nervous system« leading to prolactin secretion »in consequence to the hormonal environment of the next period of estrus«.

Further studies of the delayed pseudopregnancy phenomenon are obviously indicated. The techniques described here offer relatively uncomplicated procedures for achieving delayed pseudopregnancy, and, therefore, lend themselves to this study.

In conclusion, it is possible to inhibit the secretory activity of the corpora lutea, surgically and pharmacologically. The other tissues of the ovary remain functional. It is, therefore, possible to prepare an ovary devoid of functional corpora lutea in order to study the role of the extraluteal tissues. Because of the limitations of the techniques, it is preferable to employ both rather than either one alone. In this way, unwanted side-actions may be detected.

ACKNOWLEDGEMENTS

This work was supported by grants-in-aid from the Population Council, New York, the Ford Foundation, New York and the SABA Foundation. The ergocornine methanesulphonate was a gift from Sandoz S.A., Basle, and the pyrathiazine hydrochloride from the Upjohn Co., Kalamazoo, Michigan.

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Received on September 26th, 1967.