STUDIES ON THE EFFECT OF NIDATION

XXXV. EFFECT OF REMOVAL OF CORPORA LUTEA ON OVO-IMPLANTATION AND PREGNANCY IN THE RAT

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Summary. A surgical technique for the selective extirpation of corpora lutea of pregnancy is described. Numbers of corpora lutea in the pregnant rat were reduced to three or less on the 5th day *post coitum*. Although two corpora lutea sufficed for the decidual response of nidation, three were the minimum for successful post-nidation pregnancy in a majority of rats.

Vaginal cornification usually followed reduction of the numbers of corpora lutea. If the ova implanted, no new corpora lutea were formed at this time, but if implantation was blocked oestrus was accompanied by ovulation and a prolonged period of leucocytic vaginal smears followed, presumably a pseudopregnancy. The results suggest that, even when progesterone levels from the reduced numbers of corpora lutea were still high enough to maintain pregnancy, they did not inhibit gonadotrophin secretion.

INTRODUCTION

The importance of the corpus luteum for maintenance of pregnancy (Ancel & Bouin, 1924; Corner, 1928; Ancel & Vintemberger, 1929) is attributed to its role as the secretor of progesterone (Allen & Corner, 1929; Courrier, 1945). With the availability of pure progesterone, it became customary to study the endocrinology of progestation and nidation by using ovariectomized, progesterone-injected animals (Allen & Corner, 1929). Little is known, therefore, about the response of the rat to corpora lutea reduction without ovariectomy; for this study, selective surgical extirpation of luteal tissue must be performed, leaving the other ovarian elements intact. In monovular species this can be done by extirpation of the ovary bearing the functional corpus, the other ovary containing a representative population of other ovarian elements. In polyovular species, this approach is not always feasible but it has been recorded in the guinea-pig (Dempsey, 1937). In the rabbit, two methods have proved useful: cauterization or excision of individual corpora lutea (Ancel & Bouin, 1924), or ovarian resection such that the corpora lutea are removed (Corner, 1928). McKeown & Zuckerman (1937) destroyed the corpora lutea of a small series of pregnant rats on the 4th or 5th *post coital* day of pregnancy by electrocoagulation. They noted the reappearance of oestrus 3 to 4 days after the destruction of the corpora lutea. Kelsey & Meyer (1950) removed all but one or two of

the corpora lutea on the 8th day of pregnancy, at which time the ova have already implanted. They found that two corpora lutea maintained gestation in most rats. Less luteal tissue did not suffice.

We have used high frequency electrocautery to destroy luteal tissue on the 5th day of gestation, L_4 in our terminology (L_0 is the day of positive *post coital* vaginal smear). A modification of the vital staining procedure of Long & Evans (1922) was employed to distinguish between the latest generation of corpora lutea and the older, non-functional corpora, which were not removed. The aims of this study were to gather information on the minimal amount of luteal tissue required for nidation; on the physiology of the extra-luteal ovarian tissues during gestation, especially with regard to the production of new corpora lutea over this time; and on the response of the ovary following termination of gestation by destruction of corpora lutea.

MATERIALS AND METHODS

The rats used were regularly cyclic virgins, 3 to 4 months old, second and third generation offspring of Carworth Farms stock, raised in the Institute of Biodynamics. They were mated with males of the same stock when they were in pro-oestrus and were considered pregnant if spermatozoa were found in the vaginal smear the next morning; this day was termed Day L_0 of pregnancy.

One or 2 days before mating, rats were injected intraperitoneally with 1 ml of 1% trypan blue in isotonic saline. This causes a persistent blueing of most tissues. Thus, when the corpora lutea of gestation form they are clearly distinguished by their red colour against the grey-blue of the rest of the ovary.

The pink corpora lutea of this gestation were extirpated on Day L_4 of pregnancy (see Table 1). The rats were anaesthetized by intraperitoneal injection of 1 ml/100 g body weight of tribromoethanol solution. (The anaesthetic is a fifty-fold dilution in isotonic saline of two parts of tribromoethanol dissolved in one part of tertiary amyl alcohol by weight.) Ether could not be employed because of its fire hazard. The ovaries were exposed through lumbar incisions. The ovarian bursae were opened with electrocautery or blunt dissection with forceps, care being taken to avoid opening a blood vessel. The ovary with the larger number of corpora lutea was removed. The pink corpora of the second ovary were fulgurated, using a high frequency electrocautery (Elektrotom 40, Gebr. Martin, Tuttlingen, West Germany); the electrode was a steel needle, 0.2 mm diameter. During fulguration a small gap remained between the luteal tissue and the electrode, the tissue being combusted by an electric spark. The extent of the destruction of the corpus luteum was easily followed and controlled. Usually there was little or no bleeding.

The uterus and ovary were examined by laparotomy or at autopsy when the vaginal smear deviated from the normal leucocytic pattern of pregnancy, i.e. appearance of blood, vaginal cornification, or both. Such deviations, when they were seen, occurred 2 to 4 days after operation, and at this time the implantation sites, if present, could be counted with ease. The ovary was also examined and the number of corpora lutea counted. In some of the rats, examination of the ovary at laparotomy was difficult due to massive peri-ovarian adhesions.

Some of the rats in which nidation had obviously been prevented or had been terminated very early were not killed in order that observations of the vaginal smear might be continued. Rats in which gestation was not interrupted were killed on L₁₆ or later. The uterus was studied at autopsy and the ovary was dissected from adherent tissues under a dissecting microscope and the number of corpora lutea present counted.

The adrenals, pituitary and pineal were also examined and weighed at autopsy. The results are not reported because no changes in these parameters were noted.

RESULTS

The animals are grouped according to the number of corpora lutea in the single remaining ovary at laparotomy and/or at autopsy. Some of the results are summarized in Table 1.

TABLE 1
EFFECT OF TOTAL OR PARTIAL ABLATION
OF CORPORA LUTEA ON DAY L₄ OF
PREGNANCY

Intact corpora lutea	No. of rats	Proportion of rats showing :	
		Vaginal cornification	Nidation
0	11	1.0	0
1	18	0.9	0.06
2	15	0.8	0.7
3	11	0.5	1.0

Total extirpation of corpora lutea

None of these rats had successful nidation; all showed appearance of pro-oestrous vaginal smears 2 or 3 days after removal of the corpora lutea. Examination of the uteri at this oestrus revealed either no signs of nidation or at most a few detached scraps of necrotic tissue in the uterine lumen.

The time interval until the appearance of a second oestrous vaginal smear was studied in eight rats. Only one of the eight had the 2-days leucocytic vaginal smear typical of a normal oestrous cycle. The other seven had between 9 and 15 days of leucocytic smears (mean 11.5).

The absence of corpora in the ovary was confirmed in three rats which were autopsied at the first post-surgical oestrus and in most of the other rats at laparotomy. When autopsy was postponed to the second post-surgical oestrus, many corpora lutea were found in the ovaries.

One intact corpus luteum

There was no successful implantation of ova in seventeen of eighteen rats bearing only one corpus luteum. The one rat with apparently normal nidation was killed on Day L₇ when its vaginal smear was typically pro-oestrous. Including this rat, a total of sixteen of the eighteen rats in this group had onset of

vaginal oestrus 2 or 3 days after surgery. The other two had continuously leucocytic vaginal smears, but no ovum implantation; they were killed 4 and 6 days after surgery and many young corpora lutea were found in their ovaries. This may indicate a wave of ovulation and subsequent luteinization in the ovary which, nevertheless, was not reflected in the vaginal smear.

The time interval to the second post-surgical oestrus was measured in five rats. Between 9 and 11 days of leucocytic smears were noted (mean 10.5 days).

Two intact corpora lutea

Normal ovum implantation was obtained in eleven out of fifteen rats. Six of these eleven were allowed to complete their gestations or were killed 2 days before expected parturition. All six had signs of late resorption of the products of conception in their uteri: detached placentae or bloody tissue detritus and metrial glands. Three of them also had some live young. One gave birth to ten live pups, a normal size for a litter, the other two had one live pup each.

Oestrous vaginal smears were seen in twelve rats, pro-oestrus appearing 3 or 4 days after surgery. Of these twelve rats, nine had normal-looking nidation sites at the time of vaginal cornification. In fact, one of the rats with an oestrous vaginal smear sequence had one live foetus at term.

After total corpora lutea destruction or with one intact corpus luteum remaining, the appearance of an oestrous vaginal smear marked the formation of a new generation of corpora lutea. In the present series, we found that if the oestrous vaginal smear appeared despite the continuation of gestation, no new corpora lutea formed. The failure to produce new corpora lutea under these conditions was surprising. Waves of follicular growth not leading to ovulation are known to persist throughout pregnancy (Swezy & Evans, 1930; Greenwald, 1966), and it was considered possible that luteinization of these follicles might occur from the gonadotrophins which stimulated vaginal cornification.

To summarize, two corpora lutea are sufficient for nidation, but usually not for maintenance of gestation to term. Reappearance of oestrous vaginal smears is not suppressed; if, however, pregnancy continues, the vaginal oestrus is not associated with new luteinization.

Three intact corpora lutea

All eleven rats had normal nidation. Six of the eleven had oestrous vaginal smears; the onset of the pro-oestrous smear was 3 days after surgery in all six rats. The oestrous smears were not normal since both in the pro-oestrous and cornified stages, many leucocytes were found.

Only two of the eleven rats did not have at least one live embryo at autopsy and both of these had resorbing embryos. Altogether in the eleven rats, fifty-five live embryos and forty-three resorptions were counted.

DISCUSSION

The ablation of corpora lutea by fulguration has distinct advantages over the methods which have been published. Electrocoagulation (McKeown & Zuckerman, 1937) causes a change in the colour and consistency of the tissue

by which lateral spread of the cauterization can be controlled, but control of the depth of penetration is nearly impossible. Fulguration, on the other hand, leads to complete destruction of the tissue and the progress of this destruction is marked by the appearance of a hole. Unless a major blood vessel is breached, little bleeding occurs. Although Kelsey & Meyer (1950) were able to remove the corpora lutea completely with forceps, we could not. Bleeding was severe and obscured the area under examination.

In early attempts to achieve complete removal of luteal tissue our results were extremely erratic. However, when the vital staining procedure with trypan blue was added we were able to identify and cauterize the functional corpora lutea with confidence. In preliminary tests, it was verified that the vital staining procedure described was not detrimental to the successful evolution of pregnancy.

At the time of luteal extirpation, the ovary bearing the majority of the corpora lutea was removed. This was done to minimize the amount of grossly injured ovarian tissue. Since the fulguration procedure is associated with high local temperatures, some damage to the ovarian tissue surrounding the corpus luteum is inevitable. By leaving only the ovary with fewer corpora lutea, the remaining ovarian tissue was as undamaged as possible. It is considered unlikely that the presence of only one ovary influenced the response to luteal deprivation.

The response of the progestational rat to destruction of all of the corpora lutea is interruption of pregnancy with onset of oestrus, generally followed by a prolonged period of leucocytic vaginal smears, probably a pseudopregnancy. One intact corpus luteum left in the ovary does not have any apparent effect; the response is the same as after extirpation of all the corpora. These observations illustrate the two most obvious actions of luteal progesterone, namely: maintenance of gestation, presumably by action on the uterus, and blockage of ovulation, presumably by action on the hypothalamus or pituitary (reviewed by Rothchild, 1965). Our interpretation of the vaginal cornification is that the sudden decrease of progesterone causes a surge of gonadotrophic activity. The follicular stimulation results in oestrogen secretion which, however, is not contingent on complete withdrawal of progesterone. In the presence of three corpora, cornification of the vaginal smear was seen in half of the rats. Since nidation was sustained, adequate progesterone secretion for decidualization of the nidation sites must have been present (Chambon, 1954; Chambon & Lefrein, 1955; Yochim & DeFeo, 1962).

The production of the vaginal response to oestrogen in the presence of progesterone is puzzling. Vaginal cornification in a pregnant rat is an exceedingly rare event. During a period of progesterone dominance, the vaginal response to oestrogen is mucification, rather than cornification, and true cornification is usually considered a symptom of lack of progesterone. In order to explain the cornification of the vaginal epithelium, we would propose the following hypothetical sequence. The partial extirpation of corpora lutea leads to stimulation of gonadotrophin secretion. Follicular tissue is stimulated and some oestrogen is secreted. Due to the partial extirpation of the corpora lutea, the level of available progesterone has fallen. The combination of relative decrease of progesterone and increase of oestrogen results in vaginal cornification.

It is clear from this study that less luteal tissue is required to sustain decidualization of the nidation site than to suppress oestrogen secretion. The smallest number of corpora lutea which will allow nidation is two. Two corpora are not sufficient, however, for completion of gestation. Even three corpora lutea only allow development of half of the implanted ova to term.

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