LUTEOTROPHIC ACTIVITY OF THE YOUNG CONCEPTUS IN THE RAT

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(Received 11th October 1966)

Summary. Luteotrophic activity of the young conceptus was investigated on the basis of the maintenance of pregnancy produced artificially by means of egg transfer to the uteri of adult 'cycling' rats treated with progesterone for 5 to 11 days. Pregnancy rate varied according to the duration of progesterone treatment; the longer the treatment, the higher the rate of success. Luteotrophic activity of the conceptus appeared soon after implantation and increased gradually to the time of establishment of the foetal placenta.

INTRODUCTION

In the rat the corpora lutea of the oestrous cycle do not become fully functional unless a special stimulus resulting from copulation or artificial stimulation of the cervix is received. In pregnancy the corpora lutea remain functional and necessary throughout, as ovariectomy at any stage will terminate pregnancy.

Concerning the maintenance of the corpora lutea, there is abundant evidence that the placenta plays an important role during the latter half of pregnancy (Pencharz & Long, 1933; Klein, 1935; Astwood & Greep, 1938; Deanesly & Newton, 1941; Averill, Ray & Lyons, 1950). Although it has been established that the maintenance of the corpora lutea during the first half of pregnancy is dependent on pituitary luteotrophic activity, Alloiteau (1957) was able to demonstrate that the young conceptus could also maintain the corpora lutea in rats hypophysectomized just after implantation, provided that the pregnancy was supported by daily injections of progesterone.

The present work was designed not only to re-examine Alloiteau's findings but also to investigate any changes occurring in the luteotrophic activity of the conceptus from implantation to the establishment of the placenta.

For this purpose pregnancy was induced artificially in virgin rats 3 to 4 months of age, by means of progesterone treatment and egg transfer. Pregnancy maintenance after the cessation of progesterone treatment was interpreted as an indicator of luteotrophic activity by the conceptuses.

MATERIALS AND METHODS

Ninety-eight virgin, 3- to 4-months-old hooded rats from our own colony were used as recipients. They were divided into seven groups according to the

period of progesterone treatment. The oestrous cycle was traced by taking vaginal smears every morning throughout the experimental period, and only those rats showing regular oestrous cycles were used. Beginning at oestrus, all of the recipients were injected subcutaneously with 2 mg of progesterone daily, the period of treatment varying from 5 to 11 days, in different groups. On the 4th day of progesterone treatment, blastocysts collected from the uteri of donor rats on the morning of Day 5 of pregnancy were transferred to the uteri of the recipients. Four days after transfer (that is, Day 9 of pregnancy) laparotomy was performed and, in those recipients found to be pregnant, the number of implantations was recorded. A second laparotomy was performed 5 days later to record the number of living foetuses.

Apart from the egg transfer experiment, the effect of progesterone treatment on the oestrous cycle was examined in order to eliminate the possibility that progesterone treatment alone might induce pseudopregnancy. For this purpose ten cycling rats were used; they were divided into two equal groups. Beginning at oestrus all animals were given 2 mg of progesterone daily; one group for 5 days, and the other for 7 days. Vaginal smears were taken from these rats each morning in order to determine the stage of the oestrous cycle.

RESULTS

The results of the egg transfer experiment, involving the transfer of 1030 eggs to ninety-eight recipients, are summarized in Table 1. Implantation occurred

<table>
<thead>
<tr>
<th>Days of progesterone treatment</th>
<th>Total number of:</th>
<th>% survival of:</th>
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<tbody>
<tr>
<td>Eggs transferred (a)</td>
<td>Implants (b)</td>
<td>Recipients with implants (c)</td>
</tr>
<tr>
<td>Day 5</td>
<td>Day 9</td>
<td>Day 9</td>
</tr>
<tr>
<td>5</td>
<td>73</td>
<td>15</td>
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<tr>
<td>6</td>
<td>112</td>
<td>38</td>
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<td>7</td>
<td>170</td>
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<td>8</td>
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<td>113</td>
<td>28</td>
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<tr>
<td>10</td>
<td>80</td>
<td>21</td>
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<tr>
<td>11</td>
<td>78</td>
<td>31</td>
</tr>
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</table>

in sixty-seven (68%) of the recipients in which the mean proportion of transferred blastocysts surviving to implantation was 33% (range 21 to 52%). It is estimated that implantation occurred on the day following transfer (Day 6) based on the size of implants at the first laparotomy (Day 9).

The performance of each group is conveniently expressed by relating (1) the proportion of recipients with viable foetuses on Day 14 of pregnancy to that of
recipients with implants on Day 9, and (2) the proportion of viable foetuses on Day 14 to that on Day 9. It will be seen from Table 1 that the proportion of recipients maintaining the pregnancy to Day 14 increased from 0 to 100% as progesterone treatment was extended from 5 to 11 days, whilst the proportion of implants surviving to Day 14 similarly increased.

Regarding the effect of progesterone on the oestrous cycle, it was observed that the cycle was generally suppressed, that is, cornification of the vaginal epithelium was not observed during treatment. On the average, the first oestrus (fully cornified cells) appeared 4 days after the final injection, irrespective of whether progesterone treatment lasted 5 or 7 days. The interval between the oestrus that marked the beginning of progesterone treatment and the first one to occur after cessation of treatment was 8 days (5 days treatment) and 10 days (7 days treatment). Therefore, progesterone treatment did not induce pseudopregnancy, which would normally last 12 to 14 days.

**DISCUSSION**

Some pregnancies were maintained in rats in which the corpora lutea were originally 'non-functional'. As this occurred in the absence of exogenous progesterone, it appears that the corpora lutea must have become functional due to the presence of conceptuses. Our results not only confirm Alloiteau's (1957, 1958) observations that the young conceptus possesses luteotrophic activity, but they also show that this activity increases from Day 7 to 11 of pregnancy. Under our conditions the Day-8 conceptus was already sufficiently active to maintain pregnancy in some rats. It is interesting that the Day-8 placenta has also some mammotrophic activity (Ray, Averill, Lyons & Johnson, 1955). As hypophysectomy interrupts pregnancy when performed before Day 12, pregnancies maintained after the cessation of progesterone treatment in our experiment were also considered to be supported by the pituitary.

Alloiteau & Bouchours (1964, 1965a, b) showed that pregnancy was maintained by treatment with oestrogen or testosterone, or gonadotrophins which stimulate oestrogen secretion in rats hypophysectomized just after implantation. Thus, pituitary gonadotrophins other than the luteotrophic principle play an important role during the first half of pregnancy.

Concerning the identification of the component of the young conceptus responsible for luteotrophic activity, Kirby (1965) showed that the trophoblast itself was unable to stimulate corpora lutea because trophoblast or a blastocyst grafted in the kidney failed to interfere with the oestrous cycle. However, some interaction between trophoblast and uterus is necessary for the secretion of luteotrophin because blastocysts or ectypeplacental cones (the proliferative centres of trophoblast) grafted to the uterine lumen of normal cycling virgin mice at pro-oestrus stimulated the corpora lutea to pregnancy size and function (Kirby, 1966). On the other hand, the maternal component of the young conceptus, the deciduoma, interferes with the occurrence of ovulation after a normal pseudopregnancy, that is, resumption of oestrus normally occurs 13 days after cervical stimulation whereas if deciduomata are produced by traumatizing endometria on Day 5 of pseudopregnancy, resumption of oestrus
is delayed by 7 days (Ershoff & Deuel, 1943; Peckham & Greene, 1948; Velardo, Olsen, Hisaw & Dawson, 1953). Suppression of the oestrous cycle by decidualmata was also observed in progesterone treated, virgin cycling rats (non-pseudopregnant), as judged by the resumption of oestrus being delayed 7 days when decidualmata were produced by traumatization on 4th day of progesterone treatment (Yoshinaga & Short, unpublished data). Luteotrophic function of the young conceptus does not seem, therefore, to be due to a single factor but rather to a complex, foetal-maternal interaction.

Although the possibility exists of inducing pseudopregnancy by progesterone treatment during the oestrous cycle (Alloiteau & Vignal, 1958), the daily injection of 2 mg progesterone in our rats did not induce pseudopregnancy, as judged by the reappearance of cornified cells in the vagina 4 days after progesterone treatment, which lasted either 5 or 7 days. Moreover, the progesterone concentration in the plasma of ovarian venous blood collected on the day following the 7th and final injection of progesterone, was significantly lower than those found during pseudopregnancy or pregnancy (Yoshinaga & Short, unpublished data). Thus, it appears that the corpora lutea were barely functional in our progesterone treated rats.

ACKNOWLEDGMENT

One of us (K.Y.) wishes to thank the Lalor Foundation for financial support.

REFERENCES


