IMPROVED VIABILITY OF OFFSPRING WITH OESTROGEN SUPPLEMENTATION OF OVARIECTOMIZED RABBITS MAINTAINED ON PROGESTERONE

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Summary. Ovariectomized pregnant rabbits given sufficient progesterone maintained their pregnancies well but, after kindling, only a low percentage of young were found alive. Addition of oestradiol to the treatment regimen did not alter the effectiveness of progesterone in preventing abortion but increased the percentage of young found alive after parturition. The beneficial effect of oestrogen appeared to be related to a reduced incidence of prolonged pregnancies and prolonged labours, and suggested a rôle for oestrogen in the successful termination of pregnancy.

In the rabbit, the ovary is essential throughout gestation for the maintenance of pregnancy. In ovariectomized rabbits, pregnancy can be maintained by administration of progesterone and its withdrawal is followed by parturition. If, however, the objective is to simulate a normal pregnancy with the birth of viable offspring at full-term, the results so far obtained with progesterone replacement therapy appear to be poor, yielding few viable offspring. If such poor results could be confirmed, they might be shown to reflect a basic inadequacy of progesterone replacement therapy or merely an inappropriate dose level. It also seems possible that the addition of oestrogen to progesterone might influence the outcome of pregnancy, since oestrogen should also be deficient in the ovariectomized animal. In earlier studies (Courrier & Kehl, 1938; Pincus & Werthessen, 1938; Allen & Heckel, 1939) of hormone replacement in ovariectomized rabbits, progesterone treatment has on occasion been supplemented with oestrogens, but usually only briefly, early in gestation, or only in one or two animals. More recently, Csapo (1969) has reported observations in pregnant rats ovariectomized 48 hr before term. They found that none, or only a fraction of the litters of these animals were born, the remaining fetuses eventually dying in utero. Oestrogen substitution therapy, given as a single dose under various regimens, permitted the delivery of normal litters at term.

In our studies, trials were made in ovariectomized pregnant rabbits with progesterone in varying doses, with or without added oestrogen. The results
confirmed that progesterone alone gave only a low percentage of living off-
spring despite the pregnancy being maintained. The addition of oestrogen to
progesterone therapy resulted in a significantly higher percentage of living
offspring being born but the effectiveness of progesterone in preventing
abortion was not changed.

Thirty, sexually mature, female rabbits of mixed breeding and unknown
parity, weighing 3 to 4 kg, were mated and maintained in individual cages.
Purina Rabbit Chow and water were given freely. The day of mating was
counted as Day 0. On Day 20, pregnancy was confirmed by palpation, and
feed was withdrawn 24 hr before surgery. Bilateral ovariectomies were per-
formed on the 21st day of gestation under intravenous sodium pentobarbitone
anaesthesia. Postoperatively, each doe was given Combiotic (Pfizer, 400,000 U
penicillin-G and 0-5 g streptomycin) intramuscularly and assigned by random
numbers to one of six treatment groups, each with five does. Progesterone
(Schering) in sesame oil was administered intramuscularly daily to the various
groups at three dose levels (Table 1), covering the range of inadequate to fully
adequate dosage to maintain pregnancy in ovariectomized does, and at each
dose level was tested with and without oestradiol dipropionate (Ciba) in
sesame oil at an arbitrary dose of 1 μg daily to determine whether added
oestrogen altered the effectiveness of progesterone. Daily administration of
oestrogen was chosen because the interval between ovariectomy and parturition,
usually 10 to 14 days, afforded sufficient time for myometrial atrophy. Hormone
therapy was begun on Day 21 and continued to Day 29, regardless of whether
or when some or all of the fetuses were aborted.

All animals were inspected twice daily, at about 09.00 and 17.00 hours, to
Day 35. If aborted fetuses were found, they were weighed and their condition
(living or dead) recorded. The does were killed on Day 35, and their uteri were
checked for retained placentae and fetuses.

The mean length of gestation in the various groups was a function of
progesterone dosage and, for equal doses of progesterone, was not influenced
by the addition of oestradiol to the hormone regimen (Table 1). However, the
addition of oestrogen sharply increased the percentage of fetuses found alive
after birth, from 20% to 73% in the groups receiving 2.5 mg progesterone
(Groups Ib and Ia, respectively), and from 18% to 56% in the groups re-
ceiving 1.5 mg progesterone (Groups IIb and IIa, respectively).

Birth weights in the progesterone-treated groups (Ib + IIb) were compared
with those of the oestrogen-plus-progesterone treated groups (Ia + IIa) for
offspring born on Days 32 and 33; these were the only days when there were
sufficient numbers of offspring to permit comparison. The mean weight
(± S.E.M.) in Groups Ia + IIa on Day 32 was 60.9 ± 2.3 (N = 35) and in
Groups Ib + IIb, 55.3 ± 4.6 (N = 11; P = 0.25). The corresponding weights on
Day 33 were 68.6 ± 4.8 (N = 3) and 65.4 ± 2.3 (N = 10; P = 0.82). Abortuses
under 10 g (six in each of the combined groups) and two partially eaten dead
fetuses were excluded from these calculations.

The results have confirmed that ovariectomized pregnant rabbits maintained
on progesterone alone have only a low survival rate for spontaneously born
offspring. The addition of oestradiol to the treatment regimen improved
Oestrogen in ovariectomized pregnant rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>Daily treatment</th>
<th>Group Ia</th>
<th>Group Ib</th>
<th>Group IIa</th>
<th>Group IIb</th>
<th>Group IIIa</th>
<th>Group IIIb</th>
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<td>Progesterone (mg)</td>
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<td>Oestradiol (µg)</td>
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<tr>
<td></td>
<td>No. of days live fetuses</td>
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<td>31</td>
<td>23</td>
<td>24</td>
<td>23</td>
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<tr>
<td></td>
<td>No. of days dead fetuses</td>
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<td>5</td>
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<td></td>
<td>% of young found alive</td>
<td>73±7</td>
<td>20±7</td>
<td>56±9</td>
<td>18±6</td>
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<td>Mean gestation time (days)</td>
<td>31.2±0.24</td>
<td>31.9±0.40</td>
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<td>29.5±0.63</td>
<td>23.5±0.28</td>
<td>23.0±0.00</td>
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</table>

* Normal gestation period: 30 to 32 days.
survival, and further examination of the data suggests that this was related to alterations in the character of parturition. The mortality (virtually 100%) in both Groups IIIa and IIb is clearly related to a dose of progesterone inadequate to maintain the pregnancy, and these two groups are excluded in the following analysis of comparative mortality. In the remaining groups, the higher incidence of offspring found dead after birth in the non-oestrogen-treated animals as compared with the oestrogen-treated groups did not appear to reflect either poor fetal growth or the birth of a larger number of fetuses already dead for some time in utero, as shown by comparable fetal weights on Days 32 and 33 and by equal numbers of very small aborted fetuses. With comparable fetal weights and total number of offspring, it is likely that the total mass of the fetuses is also comparable, though amniotic fluid volumes were not measured. These volumes are normally very low at term in rabbits.

The high mortality rates in the non-oestrogen-treated groups (Ib and IIb) appeared to be related to prolonged gestation (delivery on Day 33 or later) and prolonged parturition (fetuses delivered over 2 or more days). One or both of these factors occurred in 80% of these ten pregnancies as compared with only 30% of the ten pregnancies in the oestradiol-treated groups (Ia and IIa). If pregnancies affected by either or both of these factors are excluded, survival rates are not significantly different in oestrogen and non-oestrogen-treated groups at corresponding progesterone dosage: 82 ± 6.7% (N = 33) and 83 ± 15.3% (N = 6) in Groups Ia and Ib and 53 ± 12.9% (N = 15) and 55 ± 16.4% (N = 9) in Groups IIa and IIb, respectively. These observations indicate that oestrogen supplementation improved the viability of offspring in these studies by influencing the time of onset and duration of parturition. The mortality associated with prolonged gestation might possibly have been avoided by earlier termination of progesterone treatment, but the mortality associated with prolonged parturition appears to reflect a rôle for oestrogen in normalizing the character of kindling.

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REFERENCES


