SUCCESSFUL PREGNANCY WITH REDUCED OVARIAN MASS IN THE RAT

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Abstract: This paper reports the successful pregnancy in rats with only 10-20% of ovarian tissue. Sprague-Dawley female rats of breeding age were divided into five groups: group 1 (completely ovariectomized), the ovaries from both the sides of the rats were removed completely; group 2 (partially ovariectomized), 80-90% of the ovary of each side was removed leaving the remaining tissue in place; group 3 (ovariectomized with flank transplant), the ovary of each side was removed from the ovarian stalk and inserted into a subcutaneous pocket made surgically in the flank on the respective side of the same rat; group 4 (partially ovariectomized with flank transplants), 80-90% of the ovarian mass was removed from the ovarian stalk and put back in the flank position in the subcutaneous pocket on the same side; group 5 (control), rats with intact ovaries. Estrous cyclicity, mating behavior and pregnancy rate were recorded in the animals. Percent rats cycled were 0.00, 80.00, 80.00, 70.00 and 100 in groups 1-5 respectively; percent cycled rats mated were 86.27, 100, 71.42 and 100 in groups 2-5 respectively; and percent mated rats reaching successful pregnancy were 36.36, 0.00, 100 and 100 in groups 2-5 respectively. Pregnancy rate in the mated rats in group 2 was lower than that of groups 4 (P<0.01) and 5 (P<0.01), whereas, it did not differ among groups 4 and 5. Gestation length in groups 2 (P<0.01) and 4 (P<0.01) was higher than that of group 5, whereas, the litter size in groups 2 (P<0.001) and 4 (P<0.01) was lower than that of group 5. The study showed that pregnancy was achieved in rats with only 10-20% of ovarian mass at the ovarian stalks. The pregnancy rate, however, was low, but it was further improved by transplanting the rest of the ovarian mass (80-90%) at the ectopic site. The gestation length was prolonged, litter size was reduced both in partially ovariectomized rats with or without extra ovarian mass at the ectopic sites.

INTRODUCTION

Extensive literature has been reported on the use of reduced ovarian mass to study various aspects of reproduction in mammals. Reduction of ovarian mass bilaterally, by tissue resection, has been used to investigate the regulation of vaginal cornification (1), menstrual cyclicity (2), and circulating hormones (3). Unilateral ovariectomy 4-6 weeks before mating or on day 8 of pregnancy, caused differential increase in placental weights at different days of pregnancy (4). Removal of one ovary and a half caused abnormal estrous cycles (5) and reduction in ovulation rate in rats (6). Orthotopic ovarian transplants from old rats to young rats caused a decline in cyclicity (7), whereas, ovulatory cycles were restored in aging mice after transplanting the ovarian grafts from young mice (8), indicating that the number of follicles plays a major role in regulating cyclicity. Compensatory ovarian hypertrophy has been shown in unilaterally ovariectomized rats (9), and in hamsters with one ovary destroyed after irradiation (10).

The relationship between ovariectomy and delayed implantation in rats has been known since the pioneer research work of Cochrane and Meyer (11), and has been used as a standard experimental procedure for more than 30 years, no study has shown the relationship of gestation length when ovariectomy is only partial. Smaller pieces of ovarian tissue can be

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retained during ovariectomy and, as shown here, can produce fertilizable ova with successful pregnancy and can influence the gestation length.

METHODS

Female Sprague-Dawley rats (body wt., 130-170 gms; age 8-12 weeks) were purchased from Charles Rivers Laboratories, Raleigh, North Carolina, USA. The rats were randomly divided into the following experimental groups: group 1 (completely ovariectomized), the ovaries from both the sides of the rats were removed completely; group 2 (partially ovariectomized), 80-90% of the ovary of each side was removed leaving the remaining tissue in place. Since the process of partial ovariectomy can not be measured exactly from intact ovaries on the stalk, a range between 80-90% was considered more appropriate to define the partial ovariectomy in this group; group 3 (ovariectomized with flank transplants), the ovary of each side was removed from the ovarian stalk and inserted into a subcutaneous pocket made surgically in the flank on the respective side of the same rat; group 4 (partially ovariectomized with flank transplants), 80-90% of the ovarian mass was removed from the ovarian stalk and put back in the flank position in the subcutaneous pocket on the same side; group 5 (controls), rats with intact ovaries.

For surgery, rats were anesthetized by intramuscular injections of 0.1 ml (10 mg) ketamine hydrochloride (Ketaset, Bristol Laboratories, Syracuse, New York, USA) followed immediately by 0.1 ml (2 mg) Xylazine (Rompun, Cutter Laboratories, Shawnee, Kansas USA). Abdominal skin was shaved with a razor blade and sterilized by wiping with 70% ethanol. A ventrolateral incision of 0.5-1 cm in length was made on either side of the abdomen with scissors. With the help of toothed forceps and fine forceps, the ovary with the oviduct and uterine horn of either side was located and pulled out partially of the abdominal incision by holding the fatty tissue surrounding the ovary and was held in position with the help of sarafine clip following the method used in mouse embryo transfer (12). The ovary with its stalk was pulled out gently from the mesovarium after making a small slit with a scalpel blade and with the help of fine forceps without disturbing the oviduct. In case of the partial ovariectomy, the ovary was removed partially as desired in the experimental groups. The mesovarium was pulled back and was closed with the silk suture (6-0). The body wall was closed with the silk suture (4-0). The skin was closed with a 9 mm Autoclip Applier (Clay Adams, Becton Dickinson and Co., Parsippany, N.J., USA). After finishing the surgery on one side, the surgery was done on the other side in the same way.

For transplantation of the ovarian tissue under the skin of flank, a small slit of 0.5-1 cm was made on the flank. Without cutting the inner body wall, a pouch was made by inserting forceps through the slit and moving under the skin for an area of 2-3 cm in diameter. Ovary of the either side was cut into small pieces of 3-4 mm in thickness and inserted in the subcutaneous pouch.

All the experimental female rats were caged for mating individually with males 9 days after surgery. Vaginal smears were checked daily. The rats with positive smears were isolated from the males and observed regularly for parturition for a period of 32 days. At parturition, the pups were counted and checked for normalcy. The gestation length was recorded considering day 1 as the day of positive smear. Positively smeared rats that did not carry pregnancy, were caged again with males repeatedly thereafter for 5 months before terminating the experiment. The rats were necropsied to confirm survivability of ovarian remnants as well as flank transplants at the end of the experiment. The data for pregnancy rate was analysed by Chi-square method using contingency tables and all the other data was analysed by analysis of variance method.

RESULTS

The results of all the groups are shown in Table I. In group 5, all the 6 female rats (100%) showed cyclicity and mated with males with positive smears and each carried pregnancy. The pregnancy rate for the rats mated, therefore, was 100% (6/6). The gestation length was recorded as 22.16±0.16 days. Five rats had 22-day gestation length and the 6th one had 23-days. The litter size ranged from 8 to 14 pups with a mean 11.66±0.84. In group 1, none of the 12 completely ovariectomized animals showed cyclicity, mated or
TABLE I: Reproductive parameters in partially and completely ovariectomized rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of female rats Studied</th>
<th>Showed cyclicity</th>
<th>Mated</th>
<th>Became pregnant</th>
<th>Gestation length days Mean±SEM</th>
<th>Range</th>
<th>Litter size Mean±SEM</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: completely ovariectomized</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2: partially ovariectomized</td>
<td>65</td>
<td>52</td>
<td>44</td>
<td>16</td>
<td>24.37±0.43*</td>
<td>22-30</td>
<td>4.31±0.91*</td>
<td>1-13</td>
</tr>
<tr>
<td>Group 3: ovariectomized with flank transplants</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 4: partially ovariectomized with flank transplants</td>
<td>10</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>24.00±0.31**</td>
<td>23-25</td>
<td>5.20±1.49*</td>
<td>1-9</td>
</tr>
<tr>
<td>Group 5: control rats with intact ovaries</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>22.16±0.16</td>
<td>22-23</td>
<td>11.66±0.84</td>
<td>8-14</td>
</tr>
</tbody>
</table>

*Gestation length in group 2 is higher than group 5 (P<0.01); **Gestation length in group 4 is higher than group 5 (P<0.001); Data analysed by analysis of variance.
Litter size in group 2 is lower than group 5 (P<0.001); Litter size in group 4 is lower than group 5 (P<0.01); Data analysed by analysis of variance.

carried pregnancy leading to 0.00% success in each parameter. In group 3, 80.00% (8/10) of the rats showed cyclicity as well as mating behavior with males, but 0.00% (0/8) of the mated rats carried pregnancy. In group 2, of the 65 rats, 80.00% (52/65) showed cyclicity. Of these, 1 rat died and of the rest, 86.27% (44/51) mated but only 36.36% (16/44) of the mated rats carried pregnancy. The gestation length for these animals ranged from 22 to 30 days with the mean of 24.37±0.43 days. Litter size varied from 1 to 13 pups with a mean of 4.31±0.91. All the neonates were normal at birth. The gestation period of the animals in this group was significantly higher than that of control animals (22.16±0.16 days; group 5, P<0.001). Litter size in this group was significantly lower as compared to that of control animals (11.66±0.84; group 5; P<0.001). In group 4, 70.00% (7/10) of the rats showed cyclicity; 71.42% of the cycled rats mated (5/7) and each mated rat carried pregnancy with the 100% pregnancy rate (5/5). The gestation length ranged from 23-25 days with the mean of 24.00±0.31 and the litter size ranged from 1-9 pups with the mean at 5.20±1.49. The litter size in this group was lower than that of control rats in group 5 (11.66±0.84; P<0.01) and the gestation length was higher than than of control rats (22.16±0.16; P<0.001).

Pregnancy rate in mated rats in group 2 (36.36%) was lower than that of groups 4 (100%; P<0.01) and 5 (100%; P<0.01), whereas, it did not differ between groups 4 and 5.

DISCUSSION

Complete absence of cyclicity and mating behavior in completely ovariectomized rats of group 1 confirmed that the ovariectomy in each animal was complete and no remnant of the ovarian tissue remained surgically on the ovarian stalk. Another group of
animals showed that the ovaries present at the ectopic location were able to maintain cyclicity and mating behavior but caused no pregnancy due the absence of ovaries on the ovarian stalks (group 3). Pregnancy was achieved in 36.36% of the mated rats with only 10-20% of ovaries (group 2) indicating that ovulation did occur and pregnancy was successfully maintained in these animals. The pregnancy rate in this group, however, was lower than that of the rats with 10-20% ovaries on the ovarian stalk along with 80-90% of the ovaries at the ectopic site (100%; P<0.01; group 4) and that of control animals with intact ovaries (100%; P<0.01; group 5). The gestation length in this group was higher than that of control animals (P<0.01). Group 4 rats showed that the extra ovarian mass (80-90%) present at the ectopic location had contributed to the partially ovariectomized mated rats in getting the pregnancy rate equal to that of control mated rats but was not helpful in bringing the prolonged gestation length down equal to that of control animals since the gestation length in group 4 was higher than that of control animals (P<0.001).

The prolonged gestation period, in this study, in partially ovariectomized rats with or without extra mass at the ectopic site (groups 2 and 4), could be either due to delayed implantation or by delayed parturition. The latter seems improbable in view of the fact that all neonates from the experimental rats were normal sized and healthy at birth. A delay of implantation in these non-lactating rats, however, could result from reduced hormonogenesis (13) and impaired ovarian vascularity (14). The method of resection of the ovarian mass was designed to minimize vascular trauma and the revascularization of the flank implants, as evidenced by their survival and function to support cycling and breeding in an ectopic site, argue against vascular failure of any consequence. Conversely, if hormonogenesis was reduced it would have limited the adequate production of the estrogens essential for inducing implantation and the associated induction of uterine protein synthesis. The reason of this prolonged gestation has yet to be investigated. Nevertheless, in the current study, some measured of compensatory ovarian activity is evident since 10-20% of the normal ovarian mass was able to sustain pregnancy in 36.36% (16/44) of the mated rats and was able to produce 36.87% (4.30/11.66) litter size as compared to that of intact control rats. Circulating hormones were not assayed in the study, however, subsequent work will be attempted to correlate the level of hormones to the different amounts of ovarian tissue resected. In conclusion, pregnancy can be achieved and maintained with reduced ovarian mass in rat model. This information encourages for the possibilities of pregnancies in human females with ovarian problems like dermoid cysts, benign endometriosis etc. Partial ovariectomy may still lead to pregnancy success in such cases.

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