EFFECT OF MATERNAL AGE ON VIABILITY OF OVA AND UTERINE SUPPORT OF PREGNANCY IN MICE

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Summary. The technique of ova transplantation was used to study the causes of the decline in litter size in ageing mice. Only 14% of morulae and blastocysts obtained from the uteri of young donors survived to term when transplanted into the uteri of old hosts (13 to 24 months of age), whereas 48% survived in young hosts (2 to 7 months of age). A slightly higher percentage of grossly abnormal ova was recovered on the 4th day of pregnancy from the uteri of old mice than from young mice (13% compared with 5%). However, normal-appearing morulae and blastulae from old mice survived as well as those from young mice when transferred into uteri of young hosts. It was concluded that the initial decline in litter size in aged mice is probably due to an unfavourable uterine environment.

INTRODUCTION

A decline in reproductive capacity with increasing age can be demonstrated by the reduction in litter size in such polytocous animals as mice (Thung, Boot & Mühlbock, 1954), rats (Asdell, Bogart & Sperling, 1941) and hamsters (Soderwall, Kent, Turbyfill & Britenbaker, 1960). It has been shown in mice by Jones & Krohn (1961) and Biggers, Finn & McLaren (1962a) that the change precedes any reduction in the number of Graafian follicles, ovulated eggs or newly formed corpora lutea. Attention has therefore been focused on changes in uterine morphology and physiology as the most likely cause of the decline in fecundity with age (Biggers, Finn & McLaren, 1962b; Finn, 1963).

Krohn (1962) reported that orthotopic ovarian grafts from young into old mice developed satisfactorily and in due course ovulated. The eggs were fertilized and implanted, but no litters came to term. This observation further tended to implicate the uterus as the immediate cause of the relative infertility of old mice but did not rule out the possibility that the oocytes in the transplanted young ovary had become less viable as a result of exposure to circulating extra-ovarian factors peculiar to old mice. Furthermore, the possibility still existed that such ova were influenced adversely during their passage through the uterine tubes.

An obvious, but neglected, approach to the problem is to use the technique

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of egg transfer between animals of different ages. As long ago as 1954, Boot & Mühlbock transferred a few fertilized ova from young mice into the uteri of old pseudopregnant mice without success and concluded that the uterus or its hormonal support was incompetent in old mice. The present study was designed to amplify this method of attack on the problem and to determine the effect of maternal age on the viability of ova and on the ability of the uterus to maintain pregnancy. A short abstract of this work has appeared elsewhere (Talbert & Krohn, 1965).

Since the investigation began, Blaha (1964a) has reported a similar study in the hamster, the results of which differ substantially from those presented here.

MATERIALS AND METHODS

Female mice from CBA, A and C57Bl inbred strains and from hybrid offspring of these strains (CBA×A, CBA×C57, C57×A) were used in this study. The animals were bred in the Birmingham colony and maintained under identical conditions. Unlimited food and water were provided.

Three experimental groups were employed. Blastocysts and morulae (hereafter referred to as ‘ova’) were transferred from old donors to young hosts, from young donors to old hosts and from young donors to young hosts. The old mice were 13 to 24 months old and the young mice 2 to 7 months of age.

Seventeen of the thirty old donors were virgins; the remaining thirteen donors had had previous litters, but eight had failed to carry any young to term in their last pregnancy before being included in this investigation. All hosts had borne at least one previous litter.

The donors were placed after 17.00 hours with normal CBA×A males and the hosts with vasectomized CBA×A males. Both groups were checked for vaginal plugs between 09.00 hours and 10.00 hours the following morning. Females which had mated were considered to be in Day 1 of pregnancy or pseudopregnancy.

All transplantations were done between 10.00 hours and 15.00 hours, using donors on the 4th day of pregnancy and hosts on the 3rd day of pseudopregnancy. This timing has been shown to be optimal by McLaren & Michie (1956).

The donor mice were killed with ether and the newly formed corpora lutea in each ovary were counted. The uterine horns were then flushed with a medium consisting of equal parts of horse serum (Horse Serum No. 2; Burroughs Wellcome) and normal saline. The lower one-third of each uterine tube was searched if insufficient ova were recovered from the uterus. Fertilized ova from a single donor were marshalled together by a current of fluid blown from an orally-controlled micropipette. The stage of development of the ova was noted. Grossly abnormal ova or ova which had already lost their zona pellucida were not transferred. The ova were kept at room temperature and were shaded from light while the host was being prepared.

The hosts were anaesthetized with Avertin (Winthrop Laboratories, New York). One ovary and the cranial end of the uterine horn were through a dorsolateral incision. Two to eight ova in less than 0.005 ml fluid were then
Viability of ova in pregnancy in mice

drawn by oral suction into the micropipette below an air bubble. The uterotubal junction was grasped with the tip of fine pointed forceps and the pipette inserted into a needle puncture about 1 mm from the cranial end of the uterine horn. The eggs were expelled by gentle orally-controlled pressure with movement of the air bubble into the uterus indicating that the eggs had left the pipette. In this way the eggs were transferred with very little air and fluid, an excess of either of which appears to interfere with implantation. All the ova were transplanted unilaterally. The pipette was then flushed to be sure that no ova remained in the capillary tube. Examination of the ovary and transfer of ova were carried out under a Zeiss operating microscope at a magnification of ×6. The micropipette was similar in design to that employed by Noyes & Dickmann (1960).

The hosts were laparotomized under Avertin anaesthesia on the 8th day of gestation, and the number of implantation sites was determined. At term the number of fully developed young was counted. If the pregnant animal was unable to deliver at normal term, as frequently occurs in old mice, she was killed and the number of normal foetuses was recorded.

Seven young and ten old mice were used as hosts a second time, the second transplantation being made into the opposite uterine horn from that used initially. A minimum of 2 weeks intervened between transplantations into the same host. The mice used a second time included animals which had had varying degrees of success in supporting pregnancy following the first transplantation. There was no evidence that this procedure influenced the outcome of the study.

RESULTS

The average number of corpora lutea in the ovaries and the average number of ova recovered from the uterus and tubes of the old mice were less than those found in the young mice (Table 1). A significantly higher percentage of the ova from the young mice had reached the blastocyst stage of development at the time of recovery. Furthermore, a significantly higher percentage of grossly abnormal ova was recovered from the old mice (i.e. 13% as compared with 5%).

Table 2 and Text-fig. 1 show clearly that apparently normal ova obtained from old mice survived as well as those from young animals, when transplanted into young hosts. The much higher percentage of morulae transferred in the old-young group (Table 3) compared with the young-young group apparently did not affect the proportion surviving. There is some slight evidence that ova recovered from donors which were over 600 days of age were less viable than those recovered from mice which were 400 to 600 days old, but this difference is not statistically significant (Table 4).

Six of the eight old donors which had failed to maintain pregnancy within a 3-month period before they were included in this study provided ova which developed into term foetuses in as high a percentage as did those from the other old donors following transplantation into young hosts.

The uteri of old mice were less able to support pregnancy than were the uteri of young adult mice (Table 2). Only four old hosts maintained more than two-thirds of the transplanted ova until the 8th day of gestation and in none
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**Table 1**

STAGE OF DEVELOPMENT AND CONDITION OF FERTILIZED OVA RECOVERED FROM OLD AND YOUNG MICE

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of mice*</th>
<th>No. of corpora lutea</th>
<th>No. of ova recovered</th>
<th>No. of ova recovered/mouse</th>
<th>Blastocysts (%)</th>
<th>Morulae (%)</th>
<th>Abnormal ova (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Old</td>
<td>76</td>
<td>482</td>
<td>6-3</td>
<td>252</td>
<td>3-3</td>
<td>58</td>
<td>29</td>
</tr>
<tr>
<td>Young</td>
<td>108</td>
<td>881</td>
<td>8-2</td>
<td>656</td>
<td>6-1</td>
<td>81†</td>
<td>14</td>
</tr>
</tbody>
</table>

* Includes some mice not used as donors in data in Table 2.
† Significantly higher percentage blastocysts recovered from young mice ($P<0.01$).
‡ Significantly higher percentage abnormal ova recovered from old mice ($P<0.05$).

**Table 2**

EFFECT OF AGE OF DONOR AND HOST ON SURVIVAL OF TRANSPLANTED OVA

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Hosts</th>
<th>No. of ova transplanted</th>
<th>No. of ova transplanted/mouse</th>
<th>No. implanted at 8 days</th>
<th>Survival at 8 days (%)</th>
<th>No. of term foetuses</th>
<th>Survival at term (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young to young</td>
<td>30</td>
<td>157</td>
<td>5-1</td>
<td>95</td>
<td>61</td>
<td>75</td>
<td>48</td>
</tr>
<tr>
<td>Old to young</td>
<td>30</td>
<td>144</td>
<td>4-8</td>
<td>91</td>
<td>63</td>
<td>78</td>
<td>54</td>
</tr>
<tr>
<td>Young to old</td>
<td>38</td>
<td>222</td>
<td>5-8</td>
<td>64</td>
<td>29*</td>
<td>33</td>
<td>14*</td>
</tr>
</tbody>
</table>

* Significantly lower percentage survival than in young–young group ($P<0.01$)

**Table 3**

STAGE OF DEVELOPMENT OF TRANSPLANTED OVA

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of ova transplanted</th>
<th>No. of blastocysts transplanted</th>
<th>No. of morulae transplanted</th>
<th>Morulae (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young to young</td>
<td>157</td>
<td>143</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Old to young</td>
<td>144</td>
<td>88</td>
<td>56</td>
<td>39*</td>
</tr>
<tr>
<td>Young to old</td>
<td>222</td>
<td>184</td>
<td>38</td>
<td>17</td>
</tr>
</tbody>
</table>

* Significantly higher percentage of ova were morulae than in young–young or young–old groups ($P<0.01$).

**Table 4**

EFFECT OF AGE OF 'OLD' HOSTS AND DONORS ON PERCENTAGE SURVIVAL OF TRANSPLANTED OVA

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (days)</th>
<th>No. of animals</th>
<th>No. of ova transplanted</th>
<th>Survival at 8 days (%)</th>
<th>Survival at term (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hosts</td>
<td>400 to 500</td>
<td>12</td>
<td>73</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>501 to 600</td>
<td>20</td>
<td>110</td>
<td>34</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>601 to 750</td>
<td>6</td>
<td>39</td>
<td>33</td>
<td>18</td>
</tr>
<tr>
<td>Donors</td>
<td>400 to 500</td>
<td>10</td>
<td>51</td>
<td>69</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>501 to 600</td>
<td>15</td>
<td>77</td>
<td>61</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>601 to 750</td>
<td>5</td>
<td>16</td>
<td>56</td>
<td>38</td>
</tr>
</tbody>
</table>
of these hosts did over two-thirds of the ova develop into term foetuses (Text-fig. 1). Resorbing embryos were rarely observed on the 8th day of gestation indicating that most of the losses prior to this age were either due to failure of implantation or to very early death following implantation. The oldest hosts (over 600 days of age) appeared to be just as capable of maintaining pregnancy as were 400- to 600-day-old animals (Table 4).

![Percentage survival of transplanted embryos](image.png)

**Text-fig. 1.** Percentage survival of transplanted embryos: (a) 8th day, and (b) term. White columns, young to young; cross-hatched, old to young; solid, young to old.

There was no clear evidence that the strain of the donors or hosts influenced the survival rate. However, only hybrid animals were used in sufficient numbers for useful comparisons to be made.

Within the range studied, the number of ova which was transferred did not appear to affect the success of transplantation into young or old mice.

**DISCUSSION**

Many of the old donor mice used in this study appear to have passed the initial stage of reproductive decline since the number of ovulations, as judged by the number of newly formed corpora lutea, had already decreased (Jones & Krohn, 1961; Biggers *et al.*, 1962a). In untreated old mice this change would necessarily contribute to the decline in litter size, but could not be a factor in the present study where the number of eggs transplanted, and thus available for implantation, was controlled.

Apparently normal ova from old mice were found to be just as viable as those recovered from young adults. It is noteworthy that eight of these old donors had failed to deliver young following their last mating before being included in this study. Since six of these eight mice provided ova which developed to term following transplantation into young hosts, their previous reproductive failure cannot reasonably be ascribed to loss of viability of the ova.

The percentage of donor ova which had reached the blastocyst stage of development was much higher in the young–young than in the old–young transfers. Consequently it appears that morulae and blastocysts are equally viable when transferred into a host on the 3rd day of pseudopregnancy.

It has been clearly demonstrated in the mouse by McLaren & Michie (1956) and in the rat by Dickmann & Noyes (1960) that timing of the transfer of ova
is most important and that ova that are delayed in development in relation to the uterus rarely implant successfully. Conversely, these same investigators found that ova which are more advanced in development than the uterus are able to wait for the uterus to reach a receptive condition for implantation. Numerous studies on delayed implantation also demonstrate this property of blastocysts. Shelesnyak & Kraicer (1961) and DeFeo (1963) have further shown that, in the rat, a maximal decidual response to mechanical or chemical stimuli is confined to a period of only a few hours. It is, therefore, conceivable that in untreated animals the morulae, which appear to be more prevalent in old mice than in young mice on the 4th day of pregnancy, might not be as closely synchronized with uterine development as the blastocysts and would therefore be less likely to implant successfully.

We have no direct evidence to account for the higher percentage of morulae recovered from the old mice. Either a later mean time of fertilization or a slower rate of development of the zygote are obvious possibilities. Jones & Krohn (1961) have suggested that delayed fertilization could occur in old mice as a result of changes in the connective tissue and stroma of the vagina and uterus which might interfere with the rate of ascent of spermatozoa.

The higher percentage of grossly abnormal ova which were recovered from the old mice confirms earlier observations in mice (Boot & Mühlbock, 1954) and in hamsters (Blaha, 1964b). However, since the figures rose only from 5% in young mice to 13% in the old mice, it is unlikely to be a major factor in bringing about the initial reduction in litter size. The cause of this small but significant increase in abnormal ova in old mice is not known, but delay in fertilizing the egg is again an obvious possibility. Blandau & Jordan (1941) have shown in the rat and Chang & Fernandez-Cano (1958) in the hamster that eggs which are not exposed to spermatozoa until several hours after ovulation often develop abnormally. It is therefore possible that the higher percentage of morulae and the higher percentage of abnormal ova recovered from old mice may be due to a common factor.

In mice the uterus clearly becomes less able to maintain pregnancy with increasing age. Similar results have been obtained by Blaha (1964a) in the hamster, but the specific cause or causes for this change in the uterus of old animals are not known. Since the ova used in the experiments were recovered from the uterus or terminal portion of the uterine tube it can be inferred that the uterine tube environment of old mice is not harmful. The uterine environment of aged animals may reduce the viability of eggs prior to implantation but it seems more likely that the uterine wall is less receptive to implantation and is also less able to maintain the conceptus after implantation. These results are in agreement with those reported by Biggers et al. (1962a) and Blaha (1964b) who found increased pre- and post-implantation loss in aged mice and hamsters. It is clear that the implantation rate in the young–young group and presumably also in the young–old group is considerably lower than would be expected in untreated pregnant animals, but there is no obvious reason to believe that this technical loss associated with transplantation was higher in the young–old than in the young–young group.

There is no definite evidence at the present time that changes in hormonal
or nutritional factors acting on the uterus are responsible for the decline in reproductive capacity. Corpora lutea were not counted in the old host animals in this study. However, it is likely that the number was somewhat reduced from that found in young adults since we know this to be true of the old donor mice (Table 1) which came from the same stock. Such a drop in the number of corpora lutea is unlikely, in itself, to interfere with the maintenance of pregnancy, but it could be accompanied or even preceded by reduced production of hormone by the corpora lutea without any morphological changes. It is also possible that old uteri become less sensitive to hormonal stimulation. Biggers et al. (1962a), however, tend to support the theory that the ageing changes originate in the uterus and may be associated with increased collagen deposition in the uterine wall.

It is interesting that there was no apparent progressive decline in reproductive capacity of the uterus in mice over 400 days of age (Table 4). These limited data suggest that although uterine changes may well be instrumental in the initial decline in litter size, the further decline and ultimate loss of reproductive ability may be the result of other factors, such as a decline in the number of eggs ovulated or in their viability.

The high survival rate of ova from old mice was in sharp contrast to the results obtained by Blaha (1964a), who found that ova transferred from the uteri of old to young hamsters rarely survived until term. Part of the difference is undoubtedly due to the fact that one-sixth of the ova which were transferred in the hamster were delayed in development or were clearly malformed, whereas all the ova transferred in the mouse appeared normal. But apparently normal ova from old hamsters, with few exceptions, also failed to survive, so a major difference between the two studies still seems to exist.

A possible explanation of these conflicting results is that the old mice used in the present study may not have been in as late a stage of reproductive decline as were the hamsters at the time they were investigated. Indeed there is some evidence that the ova obtained from mice over 600 days of age were somewhat less viable than those from younger animals (Table 4). It is also conceivable, however, that egg viability declines at a relatively earlier age in the hamster than in the mouse.

Little can be said concerning the nature of the change in ova from old mothers which may reduce their viability. Perhaps the best documented change of this type is the striking increase in mongolism in children of older women which appears to be largely the result of defects in the later stages of meiosis (Jacobs, Baikie, Court Brown & Strong, 1959). There is, however, no evidence of comparable conditions in mice born of old mothers. Therefore the damage to the egg must be so severe that the zygote is not viable or it does not happen at all. The latter possibility is supported to some extent by Goodlin (1965), who failed to find any evidence of aneuploidy in the offspring of elderly hybrid mice.

ACKNOWLEDGMENTS

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