Morphological and functional development of the ovary of the mouse

II. The development of the ovary in transplantation conditions in adult spayed hosts

by Sarah Ben-Or

From the Department of Physiology, Hebrew University—Hadassah Medical School, Jerusalem

With one plate

Grafts of ovarian tissue transplanted into adult spayed hosts 'take' well and remain functional for relatively long periods, even in homologous systems (Parkes, 1956; Harris & Eakin, 1949). Under the influence of the high endogenous gonadotrophic potential of the host, grafts of very young ovaries continue to develop and reach maturity even earlier than they would in their normal surroundings (Goodman, 1934; Dunham, Watts & Adair, 1941).

The reactivity of young ovaries to their trophic hormone(s) under transplantation conditions appear to be different from their response to an exogenous gonadotrophic stimulation in situ (Ben-Or, 1963).

The aim of the present study was to follow the morphological and functional development of young ovarian grafts in adult spayed hosts so as to (a) investigate whether the interference in follicular growth and differentiation, caused by the early exogenous stimulation in situ, was not the result of inappropriate stimulus, and (b) ascertain to what extent follicular growth may be attributed to the influence of the adult host.

Material and Methods

Transplantation of young ovaries into adult spayed hosts

The animals used were albino mice from the same local strain as those which served as subjects in the first part of this investigation (Ben-Or, 1963); thus transplanting was homologous. To obviate possible experimental error arising from genetic incompatibility, the results of the transplantations were checked...
against a series of isologous transplants for which a pure line of Swiss albino mice was employed.

The hosts, adult females, about three months old, were examined by the vaginal smear technique throughout three cycles and only animals which showed regular 4-5-day cycles were selected. They were then bilaterally ovariectomized, 2 weeks before accepting the grafts. Transplantations in a group of 12 intact hosts served as controls. The donors consisted of groups of new-born, 3-, 5-, 8-10-, 14- and 18-day-old animals.

Operative Procedure

The anterior chamber of the eye was chosen as the site of transplantation (a) as being more suitable for homologous transplantation (Woodruff, 1954; Greene, 1955), and (b) for the ease in following ovarian growth through the transparent cornea.

The donors were sacrificed by a blow on the head; their ovaries were quickly removed, freed from the capsule and kept moist by the adjacent tissue without immersion in a physiological solution. The transplantation was carried out under nembutal anaesthesia (intraperitoneal injection), the ovaries being transferred to the host's anterior chamber, which had previously been prepared to accept the graft. The 1–5-day-old ovaries were transplanted whole, while the older ones were grafted in two halves or in three parts, according to the size of the organ.

Vaginal smears were taken six days a week so as to follow hormonal activity of the grafts. The smears were fixed in ether-alcohol 1:1, and stained by the Shorr technique (Lillie 1954).

At autopsy, the animals were examined to ensure ovariectomy; the grafts were carefully removed and fixed in Zenker-formol solution, as were also parts of the host's uterus. The serial paraffin sections cut to 4 and 8 μ were stained by Mallaroy azan stain and hematoxylin-eosin respectively.

RESULTS

Immature Ovarian Homografts in Adult Spayed Hosts

Short-term experiments were carried out so as to make possible the observation of early stages of ovarian development.

Grafts from young donors (new-born, 3, 5, 8–10, 14 and 18 days old) were recovered mainly at the end of the latent interval, when the host's vagina recornified as a result of the graft's hormonal activity. In some cases the graft was maintained in the recipient for 40 days so as to follow the continued development of the grafted ovary and the nature of the renewed oestrus cycle.

Functional Activity

It was found that the earliest age at which the ovary is capable of becoming functional was 12–13 days. The rate of acceleration in hormonal activity of the
EXPLANATION OF PLATE

FIG. A. Ovarian homograft from a new-born donor at the end of the latent period. Hematoxylin-eosin.

FIG. B. Idem; from a 3-day-old donor, early lymphocytic invasion. Hematoxylin-eosin.

FIG. C. Ovarian homograft from a new-born donor after 38 transplantation days. Well developed follicles and corpora lutea. Hematoxylin-eosin.

FIG. D. Ovarian homograft from a new-born donor after 40 days in the intact host. Mallory azan.

FIGS. E & F. Ovarian homograft from 18-day-old donors after 6 days in the adult spayed host.

SARAH BEN-OR
young ovaries transplanted into adult hosts was higher the younger the donor, while the latent interval, i.e. the actual time lag between transplantation and the first signs of hormonal function, was longer. There is thus an inverse relation between the age of the donor and the duration of the latent interval. In this context two factors should be considered to account for the extended duration of the latent interval. (i) The lesser amount of tissue and the consequent smaller quantity of secreted hormones. (ii) The functional immaturity of the graft.

To investigate the relative contribution of these two factors, an experiment was performed on nine recipients, each implanted with two, three and four ovaries from 1-, 5- and 8-day-old donors of the same litter. As the latent interval was not shortened in any of the cases, the first factor may be ruled out. This suggests that the functional immaturity of the graft is the factor which determines the duration of the latent period.

Following the renewed oestrous cycles in the various groups, it was found that the vagina of all hosts was in a continuous oestrous condition. The response of the uterus, fixed at autopsy, likewise demonstrated the intense hormonal activity of the grafts.

As to the percentage of graft 'takes' in this homologous system, it was interesting to note that the proportion of successful grafts was higher in the case of older donors than with younger ones.

These observations are summarized in Table 1.

**TABLE 1**

*Immature ovarian homografts in adult spayed hosts*

<table>
<thead>
<tr>
<th>Age of donor (days)</th>
<th>Proportion of 'takes'</th>
<th>Latent Interval (days)</th>
<th>Acceleration in functional activity (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>shortest</td>
<td>longest</td>
</tr>
<tr>
<td>new-born 3</td>
<td>5/12</td>
<td>11</td>
<td>13, 18</td>
</tr>
<tr>
<td>5</td>
<td>4/8</td>
<td>10-11</td>
<td>—</td>
</tr>
<tr>
<td>8–10</td>
<td>6/10</td>
<td>7</td>
<td>9–10</td>
</tr>
<tr>
<td>14</td>
<td>6/6</td>
<td>6</td>
<td>7, 8</td>
</tr>
<tr>
<td>18</td>
<td>4/5</td>
<td>4–5</td>
<td>—</td>
</tr>
</tbody>
</table>

* Thirty-two days, the average time at which hormonal secretion is manifest *in situ.*
† Age of graft = age of donor + transplantation days.
‡ Number of hosts.

**Morphological Development**

The histological picture of grafts from new-born, 3- and 5-day-old donors, when the first signs of functional activity appear, deviates from that of the normally developing ovary. The ovary, although large in size, contains only small, disorganized primary follicles and large masses of hypertrophied interstitial cells, which take on a lutein-like appearance (Plate, Figs. A and B). Grafts left
in the host for a more extended period (say 24 days) showed primary follicles in a further advanced, but still not Graafian stage. Maturation was evident in 38-day-old grafts by the finding of healthy, fully organized corpora lutea (Plate, Fig. C).

The immune reaction set in with time. In the majority of non-functional grafts, as well as in functional ones, recovered from the host after 20–25 days, infiltration by lymphocytes obscured any characteristic ovarian features and later brought about necrotic destruction of the tissue.

The morphological development of grafts from 8–10-, 14- and 18-day-old donors, at the start of hormonal activity, differs essentially from that found in grafts from younger donors. Here, the ensuing hormonal activity correlates with morphological maturation, which is expressed by the appearance of advanced follicular stages including Graafian follicles.

The histological picture of these grafts recalls the detailed description given by Deanesly (1956) for immature homografts (7–10-day-old donors) in the adult spayed rat: early luteinization of the follicle preceding ovulation, the existence of many unovular follicles, and the egg, floating within follicular fluid, in the second miotic division (Plate, Figs. E and F).

Characteristic of these grafts is the intensity of their response; many follicles reach the Graafian stage simultaneously.

The visible signs of an immune response against grafts from 8–10- and 14-day-old donors was less than towards grafts from younger donors, despite the fact that they were recovered after an equal period of transplantation. Nonetheless, some non-functional, and even a few functional grafts, were found to be heavily invaded by lymphocytes and almost destroyed.

In the case of grafts from 18-day-old donors, observed after only 6 days of transplantation, the signs of an immune reaction were absent.

Immature Ovarian Isografts in Adult Spayed Hosts

Twelve recipients were implanted with grafts from 1-, 3- and 8-day-old donors, four hosts for each experimental group. The first signs of functional activity appeared after a time lag equal to that in the homologous transplants, except that under the present conditions, results were more constant.

Table 2 summarizes these results.

The renewed oestrous cycles (followed in three hosts) were a regular 5-day term.

As to morphological development, the results obtained in the homologous series were confirmed, except for the absence of the immune response.

Immature Ovarian Grafts in Adult Intact Hosts

Twelve hosts received ovaries from 1-, 5- and 8-day-old donors. The grafts 'took' well and re-vascularization was established. The developmental process was slow and limited. Forty days after transplantation large primary follicles
TABLE 2

<table>
<thead>
<tr>
<th>Age of donor (days)</th>
<th>Proportion of ‘takes’</th>
<th>Latent Interval (days)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>new-born</td>
<td>3/4</td>
<td>11 (2)</td>
<td>12 (1)*</td>
</tr>
<tr>
<td>3</td>
<td>4/4</td>
<td>10 (3)</td>
<td>11 (1)</td>
</tr>
<tr>
<td>8</td>
<td>3/4</td>
<td>7 (3)</td>
<td>—</td>
</tr>
</tbody>
</table>

* Number of hosts.

were found with the beginning of an antrum formation (Plate, Fig. D). The stage of Graafian follicles and corpora lutea was not reached. Under these conditions, the development of the ovary was retarded, particularly when compared to grafts in the spayed host. Even administration of 10 i.u. FSH did not accelerate graft development.

The immune response against these grafts did not differ from transplants in the spayed host.

**DISCUSSION**

The effect of endogenous gonadotrophic stimulation of the adult spayed host on the developing mouse ovary—as reflected in these transplantation experiments—while essentially similar to that of the exogenous FSH stimulation on young ovaries in situ (Ben-Or, 1963), presents some differences in timing of the events and the intensity of response.

The most significant feature, observed in grafts from infantile donors, is the divergence of functional from morphological maturity. The ovarian capacity to produce and secrete hormones is greatly enhanced whereas follicular growth is retarded. The earliest stage at which the ovary is capable of secreting hormones, under these conditions, is at 12–13 days manifested by inverse relationship between age of donor and the length of the latent interval. The earliest Graafian stage was reached only at 28–32 days. The delay might have resulted from the destructive effect of the early stimulation on the very young follicles.

Whereas endogenous stimulation interferes with the normal differentiation process at a very early stage (1–5 days), it exerts a stimulating effect on ovaries from older donors which, to begin with, are in a more advanced state of organization and differentiation. The competence of the follicles to respond to gonadotrophins is enhanced.

In consequence, the early maturation of the ovary in the adult spayed host appears to be due rather to more favourable growth conditions than to the mere availability of the trophic hormone in the system.

The nature of the ‘more favourable’ growth conditions provided by the spayed host is unknown. It could be interpreted in the light of the growth regulating
theory postulated by Weiss (1955) according to which in the spayed animal there are less (or even lack of) organ specific anti-templates which normally restrict the growth of the organ. It is suggested that the stimulatory inductive effect of the gonadotrophins becomes manifest after the ovary has been released from a presumed inhibitory control mechanism operating in the young animal in situ. This mechanism might perhaps be related to the function of the pineal gland, extracts of which have been shown to interfere with ovarian maturation, (Wurtman et al., 1963). It is interesting to mention in this connection the work of Deanesly (1956) who has shown that some immature ovarian autografts implanted subcutaneously ovulate earlier than would have been expected in the intact animal.

Breakdown of control is also apparent in the endocrine activity of the homografts, reflected in the continuous oestrous state of the host's vagina. It has been suggested that the persistent oestrous caused by ovarian grafts may be due to unopposed action of oestrogens which may be due to inadequate blood supply (Bielschowsky & Hall, 1953), to the presence of cysts within the tissue (Parkes, 1956), or to the graft's failure (Hicken & Krohn, 1960). The latter irregularity, however, was found to be limited to ovarian homografts—degenerative, as well as healthy, well-vascularized ones—as against the isografts which show a regular 5-day-cycle and may maintain this regular function for more than a year (Parkes, 1956). This fact would suggest some specificity in the control mechanism which may operate within ovarian tissue and/or in the pathways between the ovary and the pituitary gland.

Quite unexpected was the prompt immune reaction against these homografts, especially at this privileged transplantation site. This proves again what was carefully worked out by Hicken & Krohn 1960, that this endocrine tissue does not differ significantly from skin in its capability to evoke the immune response. The ovary succumbs to the immune reaction even in the anterior chamber of the eye since intimate attachments with the outer surface of the iris are established and revascularization takes place very soon after transplantation (Medawar, 1948).

The destructive effect upon the ovaries from infantile donors was the more pronounced when compared to the juvenile grafts, which explains the relatively low 'takes' of the former. The reason for this phenomenon may be the fact that the juvenile ovary is in a more advanced stage of differentiation. The follicular theca, well developed only in the juvenile ovary, forms a barrier to the lymphocytic invasion and thus protects the follicles from early destruction.

A certain degree of tissue differentiation is a prerequisite for the stimulatory effect of the gonadotrophin in situ as well as under the above mentioned transplantation conditions. Until complete organization of all follicular components with a well differentiated theca has been established, the ovary is not dependent on FSH (shown also recently in the hypophysectomized rat, Hertz, 1963). Any earlier stimulation must necessarily interfere with the normal developmental pattern.
SUMMARY

1. The morphological and functional development of immature ovaries from new-born, 3-, 5-, 8-10-, 14- and 18-day-old donors transplanted into adult spayed hosts is described. Attention is focused on the relation between age of the donor and rate of acceleration in the maturation process.

2. The effect of the endogenous gonadotrophic stimulation of the spayed host, while essentially similar to that of the exogenous FSH stimulation in situ, shows some differences in timing of the events and intensity of the response.  

3. There is an inverse relation between the age of donor and the time lag between transplantation and renewal of vaginal cornification of the host. The earliest age at which the ovary becomes functional is 13 days.

4. The development of the infantile ovaries under transplantation conditions deviates from the normal pattern. Functional maturity is accelerated and follicular growth is retarded.

5. Grafts from juvenile donors manifest an acceleration in the developmental process. Their competence to react in an organized way to gonadotrophins is enhanced.

6. The difference between the response of young ovaries to FSH in situ and to the condition provided by the adult spayed host is discussed.

RÉSUMÉ

Développement morphologique et fonctionnel de l’ovaire de Souris

II. Le développement de l’ovaire dans les conditions de transplantation dans les hôtes femelles castrés adultes

1. Description du développement morphologique et fonctionnel d’ovaires immatures prélèvés à la naissance, à 3, 5, 8-10, 14 et 18 jours et transplantés dans des hôtes femelles castrés. On s’intéresse spécialement à la relation qui existe entre l’âge du donneur et le taux d’accélération du processus de maturation.

2. L’effet de la stimulation gonadotrophique endogène de l’hôte castré, bien que parfaitement semblable à la stimulation in situ de FSH exogène, est quelque peu différent au point de vue de la chronologie des phénomènes et de l’intensité de la réponse.

3. Il existe une relation inverse entre l’âge du donneur et le laps de temps qui sépare la greffe d’une nouvelle kératinisation vaginale de l’hôte. L’ovaire devient fonctionnel à partir de 13 jours seulement.

4. Le développement de l’ovaire infantile est différent dans les conditions de transplantation et dans le développement normal. La maturité fonctionnelle est accélérée, la croissance folliculaire est retardée.

5. Des greffons provenant de donneurs juvéniles manifestent une accélération du processus de développement. Leur compétance à réagir d’une manière organisée aux gonadotrophines est augmentée.
6. La différence de réponse des jeunes ovaires à FSH in situ et aux conditions que présentent les hôtes femelles castrés adultes est discutée.

ACKNOWLEDGEMENTS

The author wishes to express her indebtedness to Prof. A. A. Moscona, who took a lively interest in the progress of this work, and to Prof. J. Magnes, for his help and criticism of the manuscript. Thanks are also due to Mrs. E. Cohen, for technical assistance, and to Mrs. J. Meron, for editorial help.

REFERENCES


(Manuscript received 17th February 1965)