DEVELOPMENT OF THE GERM CELLS IN THE OVARY OF THE MULE AND HINNY

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Summary. The ovaries of fetal and neonatal mules and hinnies were examined in order to study the development of the germ cells. Although migration of these cells into the fetal gonad and their subsequent mitotic divisions were apparently normal, most oogonia died in early neonatal life as they entered meiosis. This was thought to be due to the inability of the paternal and maternal sets of chromosomes to form homologous pairs. However, a few germ cells were able to enter meiosis and become oocytes, and possible mechanisms for this are discussed. These few surviving oocytes probably give rise to the occasional Graafian follicles found in the ovaries of adult mules, but it seems highly unlikely that the ova would ever be capable of normal fertilization and development.

INTRODUCTION

The proverbial sterility of the male and female mule has been recognized since the time of Aristotle in 350 B.C. (Platt, 1910; Thompson, 1910), and Stensen (1675) recorded the fact that follicles and corpora lutea were sometimes present in mule ovaries. The first explanation of the male mule’s sterility was provided by Wodsedalek (1916) who studied the testes of a number of mules and concluded that there was a block in meiosis due to an incompatibility between the paternal (donkey) and maternal (horse) chromosomes. Recent cytological evidence supports this idea, since it shows that the donkey has 62 chromosomes of which 38 are metacentric, whereas the horse has 64 chromosomes with 26 metacentrics; thus, the mule and its reciprocal hybrid, the hinny, both have 63 chromosomes with many unevenly matched pairs (Hsu & Benirschke, 1969). There is also a block in spermatogenesis at the pachytene stage of meiosis in the testes of zebra–horse and zebra–donkey hybrids, where the chromosomal incompatibility between the parental species is even greater (King, Short, Mutton & Hamerton, 1966).

Relatively few studies have been carried out on female mules; however, it is known that they are capable of coming into oestrus at irregular intervals (Nishikawa & Sugie, 1952; Bielanski, 1955) and that follicles and corpora lutea can frequently be found in their ovaries (Benirschke & Sullivan, 1966). From this one would suppose that the ovaries must contain oocytes, since it has

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generally been assumed that normal follicular development and ovulation do not occur in the absence of an oocyte (Franchi, Mandl & Zuckerman, 1962). However, Benirschke & Sullivan (1966) could not find a single normal oocyte in forty-seven pairs of mule ovaries that they examined; this led Ohno (1967) to conclude that the mammalian ovary must therefore be capable of giving rise to normal oestrous cycles in the complete absence of oocytes.

The purpose of this investigation was to study the fate of oogonia in the ovaries of fetal and neonatal mules and hinnies, and to see if any were capable of entering meiosis to become oocytes.

**MATERIALS AND METHODS**

During the course of another series of experiments a jack donkey was used to serve four Welsh Mountain pony mares in order to produce mules, and a Welsh Mountain stallion was used on a jenny donkey to produce a hinny. The parentage of all these inter-specific hybrid offspring was confirmed serologically and by karyotyping (Hamerton, Richardson, Gee, Allen & Short, 1971). One 60-day mule fetus was obtained by hysterotomy, and one ovary was removed from the hinny, three mules and a normal pony by laparotomy through a mid-line incision. Anaesthesia was induced by sodium thiopentone and maintained by closed-circuit halothane following intubation, and all the animals made uneventful recoveries. Ovaries were also obtained from a carthorse foal which died during a Caesarean section at term. The age of the other animals at the time of operation is shown in Table 1. The ovaries were fixed in Bouin's solution, sectioned at 8 µm, and stained with haematoxylin and eosin.

The relatively large size of the ovaries made it impossible to count the total number of germ cells in serial sections. Instead, the number of germ cells was calculated per unit area of ovarian cortex. This was done by using a microscope to project an image of the cortex on to a large sheet of paper, and the outline of the cortex was then cut out and weighed. A standard 1-mm square was similarly projected and cut out and in this way it was possible to convert weight of paper to area. The number of germ cells was counted by examining the cortex under high power; the number of sections of each ovary that were examined is shown in Table 1.

**RESULTS**

The ovaries of the 60-day mule fetus contained a large number of germ cells, many of which were actively undergoing mitosis (Pl. 1, Fig. 1).

The results for the neonatal animals are summarized in Table 1. In the 10-day-old mule, numerous nests of germ cells were present in structures like Pflüger's tubules, which lay in the deeper layers of the ovarian cortex. On closer examination (Pl. 1, Fig. 2) it could be seen that some of these germ cells were entering meiosis, and large numbers were degenerating. No primordial follicles were seen.

In the 53-day-old mule, the picture had changed. There were now far fewer germ cells present per unit area than in the 10-day-old mule. The ovarian...
Fig. 1. Oogonia in the outer regions of the ovarian cortex of a 60-day-old mule fetus. Note cells undergoing normal mitosis. × 480.

Fig. 2. A group of degenerating germ cells lying within a tubule in the ovarian cortex of a 10-day-old neonatal mule. × 400.
Fig. 3. A single oocyte surrounded by a layer of follicular cells in the ovarian cortex of a 53-day-old mule. × 640.

Fig. 4. An oocyte within a follicle in the ovarian cortex of a 93-day-old mule. × 400.

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cortex was composed almost exclusively of fibrous tissue, but it was possible to find an occasional isolated oocyte surrounded by a single flattened layer of follicular cells. A similar picture was seen in the 73-day-old hinny and in the 93-day-old mule (Pl. 2, Fig. 3), except that in the latter animal two small follicles were also present (Pl. 2, Fig. 4).

### Table 1. Numbers of germ cells in the ovarian cortex of the neonatal horse, mule and hinny

<table>
<thead>
<tr>
<th>Description of Animal</th>
<th>No. of histological sections examined</th>
<th>No. of germ cells found</th>
<th>No. of germ cells/cm² cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn horse</td>
<td>1*</td>
<td>2935</td>
<td>71,100-0</td>
</tr>
<tr>
<td>10-day-old mule</td>
<td>9</td>
<td>1066</td>
<td>342-5</td>
</tr>
<tr>
<td>53-day-old mule</td>
<td>64</td>
<td>10</td>
<td>0-8</td>
</tr>
<tr>
<td>73-day-old hinny</td>
<td>10</td>
<td>14</td>
<td>9-8</td>
</tr>
<tr>
<td>93-day-old mule</td>
<td>126</td>
<td>8</td>
<td>0-3</td>
</tr>
<tr>
<td>93-day-old horse</td>
<td>1*</td>
<td>161</td>
<td>1,000-0</td>
</tr>
</tbody>
</table>

* Due to the large number of oocytes present, only a small part of the section was scored.

In striking contrast, the ovaries of the newborn carthorse foal contained a number of macroscopically visible Graafian follicles and an enormous number of oocytes. The same was true of the 93-day-old pony, except that there were not quite so many oocytes. The ovaries of this pony were similar in size (25 × 10 × 10 mm) to those of the hybrids.

**DISCUSSION**

These results demonstrate conclusively that germ cells are able to migrate into the gonads of the hybrid fetus where they apparently undergo normal mitotic divisions. A few days after birth, when they have attempted to enter meiosis, most of them are degenerating; nevertheless a small number do survive the initial stages of meiosis and develop into oocytes. These few surviving oocytes appear to induce the development of normal follicular cells, and thus the ovary is capable of acquiring some endocrine activity in later life. It seems that these oocytes can even survive to the time of ovulation, since Bielanski & Zapletal (1968) were able to recover unfertilized eggs from the Fallopian tubes of two mules that had recently ovulated.

The fact that most of the germ cells in the hybrid’s ovary degenerate at the time of meiosis lends further support to the view that this degeneration is a direct result of the parental chromosomal incompatibility, which would make it extremely difficult to achieve pairing of homologous chromosomes. It is all the more difficult, therefore, to understand how a few germ cells manage to survive beyond pachytene to become oocytes. Since clumps of primordial germ cells form intercellular bridges so that they exist as a syncytium (Gondos, 1970; Dym & Fawcett, 1971), it may be that chromosomal rearrangement can occur between germ cells through these interconnections. There is ample evidence that ribosomes, strands of endoplasmic reticulum and even mitochondria can pass from cell to cell. In this way germ cells might even be able to exchange...
genetic material, thus making meiosis possible. Alternatively, there may be clones of tetraploid germ cells which could undergo meiosis to give diploid oocytes. There is evidence that a few germ cells can complete meiosis in the male mule and hinny, since Bratanov, Dikov & Dokov (1964) and Trujillo, Ohno, Jardine & Atkins (1969) found a few spermatozoa in semen. Meiosis even seems to be possible in the female zebra–horse hybrid, since Ewart (1899) recorded follicular development in one of these animals.

The scientific literature is full of reports of allegedly fertile female mules (Gray, 1954; Savory, 1970), but there is still not a single adequately documented case. It cannot be assumed that a mule is fertile simply because she is seen with a foal at foot, since mules can sometimes lactate and will adopt orphan foals (Tegetmeier & Sutherland, 1895). Furthermore, it is impossible to identify a mule solely on phenotypic appearance; it is essential to confirm the diagnosis by serology or karyotyping. One allegedly fertile female mule turned out to be a normal donkey on chromosomal analysis (Benirschke, Low, Sullivan & Carter, 1964).

It seems extremely unlikely that the ovulated egg of a mule could ever have a chromosomal complement that would allow normal fertilization and development. The remarkable thing about mules is not that they are sterile, but that both sexes do occasionally manage to produce gametes. How this is achieved remains to be discovered.

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REFERENCES

Ovary of the mule and hinny


