FERTILISATION IN THE RABBIT

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(Received 14th February, 1932.)

(With Five Text-figures.)

In a previous paper (Pincus, 1930) it was concluded that for the fertilisation of the ovum of the rabbit it takes the spermatozoa from 2 to 3 hours after ovulation, to reach and effectively fertilise the egg. This conclusion was based on experiments in which ova, taken from the tubes at various intervals after fertile matings, were cultured in vitro. It was found that "the proportion of ova from fertile matings that divide in culture is very low for ova recovered between 11 and 12 hours after copulation, increases for ova obtained from 12 to 13 hours after copulation, and practically all ova recovered from 14 hours after copulation onward divide in culture." Since ovulation in the rabbit normally occurs at about 10 hours after copulation there is a period from ovulation to about 3 hours later when the significant events associated with sperm penetration occur. It was decided therefore to examine with some care the state of rabbit ova as recovered from the tubes during this critical period.

A number of rabbit does were accordingly mated to fertile bucks and killed from 10½ to 14 hours after copulation. Eggs were washed from the tubes according to a technique previously described (Pincus, 1930), and mounted in Ringer's solution on a glass slide. Observations were generally made under the high power of the microscope, and photographs were made of the various stages observed.

Our first effort was to find a criterion that would indicate authoritatively that sperm penetration had occurred. We were acquainted with the observations of Yamane (1930) that (1) in a doe killed 12 hours after a fertile copulation two polar bodies were present in the ova, and (2) that practically all unfertilised ova placed in Tyrode's solution with sperm developed the second polar body in 45 min. or longer, whereas ova without sperm showed no second polar body. We hesitated to accept the presence of a second polar body as an indication of sperm penetration because Yamane did not indicate that the formation of the second polar body was definitely consequent on the entrance of sperm into the egg, and even if actual sperm-entry is necessary for formation of the second polar body there is a latent period of 45 min. or longer before the appearance of the second polar body.

We therefore made a very careful examination of the recovered ova, using as our standard of comparison ovarian eggs containing one polar body, but not ovulated. Such an ovum is given in Fig. 1. It should be noted that the egg proper is firmly

1 Yamane's data indicate that formation of the second polar body is due presumably to the action of a proteolytic enzyme carried by the sperm. This enzyme may reach the egg without sperm penetration. Yamane makes no statement about actual sperm entry.
appressed against the zona pellucida. Exactly similar ova may be recovered from
the tubes (Fig. 2), and these are ordinarily well embedded in the mass of surrounding
granulosa cells. In such ova no sperm penetration has occurred. Actually two
very definite appearances in the egg precede the formation of the second polar

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Fig. 1. Drawing of rabbit ovum dissected from the ovarian follicle.
One polar body; corona radiata intact.

Fig. 2. Ovum recovered from Fallopian tubes at 12½ hours after copulation.
One polar body; corona radiata intact. No sperm penetration.
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body. First, rabbit spermatozoa easily pass the barrier of the zona pellucida, so that ova into which sperm entry has occurred contain a number of spermatozoa in the liquid-filled space between the ovum proper and the zona pellucida. Second, in these early ova which contain spermatozoa there has been a definite shrinkage of the ova, so that there is a slight but perceptible space between the egg and the zona pellucida (Fig. 3). We have observed many such ova containing often a large number of sperm swimming actively round the slightly shrunken egg. Van Beneden (1875) observed sperm swimming round the egg even in the early cleavage stages, and Pincus (1930) has noted the presence of sperm in the zona pellucida of fertilised ova.

Fig. 3. Ovum recovered from Fallopian tubes at 12½ hours after copulation. One polar body; corona radiata broken down. Sperm penetration had occurred. Note shrunken condition of egg (cf. Figs. 1 and 2).

Our data are summarised in Table I.

Table I.

<table>
<thead>
<tr>
<th>Killed, hours after copulation</th>
<th>Number of does</th>
<th>Number of ova from each</th>
<th>Number of does whose ova showed sperm and one polar body</th>
<th>Number of does whose ova showed two polar bodies and sperm</th>
<th>Number of does whose ova showed two polar bodies and pronuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>10½-10¾</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11-11¾</td>
<td>2</td>
<td>5+5</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>11½-11¾</td>
<td>2</td>
<td>4+6</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12-12¾</td>
<td>3</td>
<td>7+3+2 (?); 6+6+9+7</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>12½-12¾</td>
<td>4</td>
<td>5+5</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>13-13¾</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>14-14¾</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* The same does.
Briefly, then, the ova of only one of five does killed from $10\frac{1}{2}$-11$\frac{1}{2}$ hours after copulation (and that one killed at 11$\frac{1}{2}$ hours after copulation) gave evidence of sperm penetration; of seven does killed from 12-12$\frac{1}{2}$ hours after copulation the ova of three gave evidence of sperm penetration; the ova of all three does killed 13-14$\frac{1}{2}$ hours after copulation showed that penetration had taken place.

The youngest ova showing definite signs of sperm penetration were recovered at 11$\frac{1}{2}$-11$\frac{3}{4}$ hours after copulation or 1$\frac{1}{2}$-1$\frac{3}{4}$ hours after ovulation. Van Beneden (1875) declares that the youngest ova in which he saw actively swimming sperm were obtained from a doe killed at 11 hours after copulation.

All eggs containing swimming sperm were definitely separated from the granulosa mass (cf. Figs. 3, 4 and 5), while eggs which contained no sperm were ordinarily embedded in the granulosa mass (cf. Fig. 2). This is taken to indicate that the surrounding granulosa mass must drop away before sperm entry is possible. Once the major part of the surrounding mass is dissolved away sperm entry must be very rapid indeed, for one observes in and about separated ova numerous spermatozoa (Fig. 5).

We have never observed the fertilising spermatozoon, but have seen a slight bulge at the periphery of an ovum which probably marked the point of sperm entry. This bulging portion was clearer and less granular than the surrounding cytoplasm. In ova containing swimming sperm no penetration into the egg proper has been seen although very careful observations were made. We have seen the sperm heads strike against the egg cytoplasm and apparently rebound therefrom. This is taken to indicate that polyspermy cannot occur despite the presence of numerous sperm all round the egg. It may be impossible for these extra sperm to become attached, for purely mechanical reasons, as the space in which they swim is too narrow to allow them to collide with the egg “head on.”
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The ova of does killed at the critical time of penetration are not all in the same condition. One recovers from the same doe ova containing sperm and others without sperm; at a slightly later stage one observes ova without sperm, ova with sperm and one polar body, and ova with sperm and two polar bodies; still later, ova with sperm and one polar body, and ova with sperm and two polar bodies, are seen. If we take 10 hours after copulation as the exact time of ovulation (ovulation may occur before or after 10 hours; see Walton and Hammond, 1928), then the earliest appearance of the second polar body is 2–2½ hours after ovulation, and presumably

Fig. 5. Ovum recovered from Fallopian tube sat 12½ hours after copulation. From a photograph taken at the level of the surrounding granulosa cells to show three spermatozoa in the cell mass.

45 min. (at the earliest) after sperm penetration. We have seen no definite signs of pronuclei in eggs recovered sooner than 13 hours after copulation. It seems evident that the formation of the second polar body must precede the appearance of both pronuclei.

SUMMARY.

The series of events occurring in the Fallopian tubes of rabbit does mated to fertile bucks may be summarised as follows:

The ova liberated from the ovaries and surrounded by the follicle cells become massed together. Sperm penetrate the massed follicle cells, which fall away as the sperm pass through them. At from 1½–3 hours after ovulation the spermatozoa reach the egg. A number of spermatozoa pass through the zona pellucida, but only
one, apparently, enters the egg. At the time of sperm penetration the egg shrinks slightly but definitely. The second polar body is given off 45 min. or longer after sperm penetration. The pronuclei are formed after the formation of the second polar body and, at the earliest, 3 hours after ovulation. The critical period for sperm penetration appears to occur at 2–3 hours after ovulation (cf. Pincus, 1930).

REFERENCES.


