OVULATION IN MAMMALS, WITH SPECIAL REFERENCE TO THE MOUSE AND RAT.

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Ovulation and its connection, if any, with pairing is a subject that has long engaged the attention of mammalian embryologists. As long ago as 1828 Von Baer made the statement, seemingly based more upon theoretical than upon experimental grounds, that the Graafian follicles of mammals appear not to rupture without pairing, or some analogous stimulation of the female sexual organs. It would seem to be a simple matter to determine in a given mammal whether ovulation was, or was not dependent upon copulation, but the literature of the subject shows long controversies about this point. The matter can best be taken up by considering individually the animals thus far studied. The egg of the dog was carefully investigated by Bischoff ('45), who declared that in that animal ovulation is entirely independent of pairing. This observation has since been confirmed by Marshall and Jolly ('05), who also found that the bitch has typically two sexual seasons each year. Further confirmation has come from Ancel and Bouin ('08), who, in addition to finding ovulation occurring independently of copulation, discovered that the Graafian follicles in the dog rupture in turn.

Ovulation phenomena in the guinea-pig gave rise to a spirited controversy between Bischoff ('52 and '66), who claimed that the Graafian follicles in that animal ruptured entirely independently of pairing, and Reichert ('61), who declared that ovulation only occurred nine to ten hours after copulation had taken place. In his second paper, replying to Reichert, Bischoff states that he has experimentally demonstrated in dogs, rabbits, guinea-pigs, rats, sheep and swine that follicles mature and rupture without relation to pairing, the full data of this work being given in an earlier paper (Bischoff '44). Hensen ('76), in his paper on the early development of the guinea-pig, quotes the opposing views of Bischoff and Reichert, without expressing any opinion

of his own, and Lams and Doorme ('07) quote Reichert alone. The careful researches of Rubaschkin ('05), however, have definitely confirmed Bischoff's original contention, and it may therefore be considered as finally settled that in the guinea-pig ovulation and pairing are essentially independent phenomena.

The rabbit's egg has been studied by Barry ('39), Bischoff ('42), Coste ('47), Heap ('05), and by Dubreuil and Regaud ('08). Barry, and the last three authors named above find, contrary to Bischoff, that the rabbit cannot ovulate until a short time after pairing has occurred, but Coste claims to have induced ovulation by taking females which, isolated from males, had been in heat for a number of days, and allowing males to cover them without, however, allowing them to remain together long enough for actual insemination to take place. Heape, and Dubreuil and Regaud, on the contrary, find that if, in this animal, pairing for any reason does not occur during the oestrus period, the eggs which would naturally have been discharged all degenerate within the ovaries. Indeed, according to Heape, if the male is withheld from the doe during several consecutive periods of oestrus, most, if not all, of the older and many of the younger follicles undergo degeneration, and this may result in a more or less permanent sterility.

Marshall ('03 and '04) has studied the oestrus cycle in the sheep, and in the common ferret. He states that Scotch blackfaced ewes can, during the regular sexual season, ovulate without pairing, as claimed by Bischoff ('44) for ewes in general, but pairing, according to Marshall, even then may hasten the process of ovulation, which outside of such rutting periods may be dependent upon copulation. The common ferret, Marshall finds, behaves as recent investigators have found to be the case with the rabbit, ovulating only after pairing. The withholding of the male greatly prolongs the period of heat, and in a few exceptional cases is said to lead to the death of the female.

Benecke ('79), Eimer ('79), Fries ('79), Van Beneden and Julin ('80), and Van Beneden ('99) are all agreed that in all the various species of bats that they have studied, pairing takes place several weeks before the eggs leave the ovaries.

The condition of affairs in mice has been disputed by Sobotta ('95 and '07) and Kirkham ('07), who have claimed the inde-

pendent occurrence of ovulation and pairing, by Gerlach ('06), who denied such independence, and by Lams and Doorme ('07), who state that ovulation in the mouse is perhaps connected with pairing.

Bischoff ('44) did a little work with rats, and he came to the conclusion that in them ovulation could take place without pairing, but he confessed that his evidence was incomplete.

In view of these uncertain and conflicting statements, it has seemed desirable to submit the matter of the relation, if any, between ovulation and pairing, to a critical test in the case of the rat and the mouse, using, in some instances, individuals that had been isolated from as early a period as the sex could with certainty be externally determined. The four female white mice used were virgins from two litters, by different parents, and were kept apart from all males, including their brothers, from the time they were two weeks old. Three of these females were born in the laboratory in the middle of December, 1908, and the other early in January, 1909, and all four, until they had been weaned, were isolated with their respective mothers.

One of the virgins born the previous December was chloroformed on March 19, 1909, a day selected by chance, without any special calculation other than the general knowledge that the mice born about the same time as the test animals were beginning to show signs of pregnancy. When the body of this animal was opened, and the ovaries examined, bright red spots were found upon both these organs, a characteristic sign that ovulation had recently occurred. The ovaries and Fallopian tubes were then removed from the body, and serial sections were prepared in a manner described in a previous paper (Kirkham '07). The study of this material has revealed seven eggs in the Fallopian tubes, beside numerous eggs still in the ovaries, the latter undergoing degenerative changes after having formed the first polar body and second polar spindle. The tube eggs appear in every respect like those obtained from females which had paired, and were then killed before the spermatozoa had reached the eggs. A varying number of follicle cells are found associated with the eggs, the zona pellucida in all cases is sharply defined, all the eggs possess second polar spindles, and in two instances the first polar body is still visible, although decidedly atrophied.

The remaining three virgin mice were killed March 30, 1909, and of those born in the preceding December, the Fallopian tubes of one yielded nine eggs, similar to those mentioned above, except that seven still showed first polar bodies; the ovaries of the other animal had upon them the white scars indicative of well formed corpora lutea, and no sign of eggs was found in either Fallopian tube. Both sets of ovaries contained a number of mature eggs undergoing degeneration. The female mouse born in January showed no signs of having ovulated, no evidence of either young or old corpora lutea being found on either ovary, nor any trace of eggs in the Fallopian tubes. The ovaries of this animal, however, have in them several eggs with first polar bodies and second polar spindles, but all appear to be degenerating.

From these observations we may safely conclude that not only is ovulation in adult female white mice independent of pairing, as stated by Sobotta ('95 and '07), and by the writer ('07), but that it is an independent process from the start, maturation and ovulation occurring in the females without regard to pairing, although at present we do not know with what regularity.

These results, also, are not only of general interest, but they possess value as showing the mouse to be an eminently suitable mammal upon which to investigate the possibility of artificial parthenogenesis.

A further fact which should be noted here, is the change of opinion, on the part of the writer, regarding the fate of the first polar body formed by the mouse egg. In 1907 the writer stated that his observations led him to believe that when the first polar body was not found with the egg, it had been forced through the zona pellucida at the time of ovulation. That same year Lams and Doorme ('07) published a paper on the development of the mouse egg in which they stated that the first polar body in that animal underwent degenerative changes leading finally to its disappearance. These authors figured a few first polar bodies in an advanced state of atrophy, and the present writer has since confirmed their conclusions, using some material of his own obtained since the publication of his first statement on the subject. A fairly complete series of degenerating first polar bodies — some

belonging to eggs themselves degenerating within the ovaries, others in the process of early cleavage in the Fallopian tubes — make it practically certain that in the case of such mouse eggs as have reached or passed the stage when the second maturation spindle is formed, and yet show no trace of the first polar body, this body has broken up and disappeared; in all instances of such degeneration the chromatin granules lose their distinctive staining qualities some time before the final dissolution of the polar body cytoplasm.

The observations on rats were limited to two adult white females. These animals were kept in a large cage with males and other females until they showed signs of advanced pregnancy. They were then isolated in separate cages, and a close watch was kept for the appearance of the litters. As soon as found, the young rats were taken from their mothers, and then the latter were killed, one twenty-four, the other forty-eight hours later; it having been previously determined that the white rat, like the white mouse, during the warm months of the year is usually in heat directly after giving birth to a litter. When the bodies of these animals were opened, characteristic corpora lutea were visible on all the ovaries, and after the ovaries and Fallopian tubes had been sectioned and stained, eight tube eggs were found in one animal, and five in the other. These eggs have exactly the appearance of eggs obtained from female white rats which have been allowed to pair and then are killed just before fertilization can take place; the zona pellucida in all cases is well preserved, all of the eggs possess second polar spindles, while a majority show no trace of the first polar body, it probably having previously degenerated.

The points to be emphasized in these researches are three in number:

1. Virgin white mice mature eggs and discharge them from their ovaries at practically the same age as their sisters who have been allowed to pair, and such eggs show no differences from other unfertilized eggs.

2. Adult female white rats during the active breeding season ovulate regularly, immediately after giving birth to a litter, whether pairing is or is not allowed to take place.

3. The first polar body has degenerated in such mouse and rat eggs as show no trace of it, and yet have reached or passed the stage of the second maturation spindle.

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