THE IMPLANTATION AND EARLY SEGMENTATION OF THE OVUM OF DIDELPHIS VIRGINIANA

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FIFTEEN FIGURES

Since few investigators have described the development of the opossum and since there is rather incomplete information at hand concerning the early stages of its development, it seems desirable that the subject be re-investigated. The following results will serve as a preliminary account of a more extensive work which will appear later. During the last five years we have collected and examined about fifty female opossums for embryos. We were unable to find any early stages of development until March 10, 1914.

November 2, 1913, six opossums, three males and three females were collected at Graydon Springs, Missouri. They were taken to Springfield, Missouri, and kept in a cage, made of chicken-wire netting, in which a large box was placed which served as a bed and hiding place. During February, 1914, the females were examined from time to time to see if the external genitals and marsupium, including the mammary glands, showed signs of oestrus such as congestion and enlargement of the external genitals and mammary glands and the moistening of the marsupium.

These conditions were observed February 12, 1914, in one large, adult female. This specimen was killed and the ovaries and the uteri were examined. The uteri were not enlarged and ovulation had not taken place as sections made later of the ovaries showed. On February 23, 1914 another large female was killed and examined when the above mentioned conditions

were observed and the uteri were not enlarged and ovulation had not taken place.

On March 10, 1914 a young female opossum was killed and examined about four days after the beginning of the oestrual period. The remainder of this paper will deal principally with the material furnished by this specimen. The uteri were found enlarged at least six times the size of those of the two preceding specimens. Ovulation had taken place as the ruptured Graafian vesicles were visible to the unaided eye. The enlarged uteri were very soft, delicate and much congested. When an incision was made into the cavity of the uteri it was difficult to distinguish the cut surfaces of the uterine walls from their mucous lining. The walls of the uteri of this specimen are made up of a very delicate, homogeneous appearing, loosely constructed tissue which when sectioned was found to be almost entirely glandular as is shown in figures 12, 13 and 14.

The left uterus was cut open first and the walls were laid back with considerable difficulty. One might well have expected to find the large and distended uterus in an advanced stage of pregnancy but a careful examination of it revealed merely the presence of many, small, glistening, globular bodies scattered irregularly over the uterine mucosa in depressions. Osborn ('87) states that, "the inner wall of the uterus at the mid-period (of gestation) presented one or two long parallel furrows faintly defined upon its ventral surface, in which the embryos were ranged in a single row." At first these globular bodies which afterwards were found to be segmenting ova were scarcely visible to the unaided eye, due to the facts that they were more or less embedded in the uterine mucosa, that the mucosa was much congested and that they were more or less stained with blood. The left uterus with its segmenting ova intact was placed in toto in Alexander Petrunkewitch's sublimate mixture[1] for a few minutes. The globular bodies or segmenting ova rolled out of their cups or depressions in the uterine mucosa on slight

[1] The sublimate mixture of Alexander Petrunkewitch is made as follows: 40 per cent alcohol, 500 cc.; fuming nitric acid, 10 cc.; glacial acetic acid, 90 cc.; bichloride of mercury to make a saturate solution (about 10 grams).

disturbance after they had been placed in the killer. From the action of the killer the delicate ova suffered more contraction than did the uterine lining. This shrinking tended to separate the ova from their uterine cups.

A longitudinal incision was made the full length of the right uterus. When the walls were laid back many segmenting ova were found more or less embedded in the uterine mucosa as in the left uterus. Blocks of uterine tissue containing the ova were cut out with a razor and placed in the killer. Twenty-five ova were recovered from both uteri. We were able to carry twenty-two of the twenty-five ova through the histological process. The tissue was embedded in paraffin and cut into sections eight micra in thickness. It was stained in Heidenhain's iron-hematoxylin and hematoxylin and eosin. The iron-hematoxylin gave the better results.

Some of the tissue was sectioned through the ova perpendicular and some horizontal to the surface of the uterine mucosa. The perpendicular sections show that the ova are embedded about one-half their diameter and sometimes more deeply in cups in the uterine mucosa. According to Selenka ('85), the blastodermic vesicles lie at first quite free and at random in the uterus; on the fourth day (after the beginning of segmentation) the blastodermic vesicle over the germinal area becomes loosely adherent to the uterine epithelium. We find that the segmenting ova and even a one-celled ovum are embedded from the first in cups in the uterine mucosa as is well illustrated in figures 12, 13, 14 and 15. The horizontal sections show that the ova are in cups and not in straight grooves as Osborn ('87) found the embryos. The uterine mucosa is composed of three or four layers of cells but it is much flattened out where it comes in contact with the ova. The ova still have many follicular cells adhering to them.

These segmenting ova with their envelopes vary in diameter in the fresh state from about 0.75 to 1.50 mm. The ovum proper makes up relatively a small amount of the complete egg as can be seen in figures 12 and 14. The cytoplasm of the ovum is coarse, granular and much vacuolated. The egg envelopes are divided into two more or less distinct areas. The central one

which immediately surrounds the egg is composed of many concentric, coarse, reticular layers (fig. 11). The peripheral area is finely reticular and the layers are not well defined. The egg envelopes seem to be a coagulated white in the sections. The egg envelopes are so loosely constructed that the segmenting ova in some of the specimens have subsided and thus become eccentric in the envelopes during the histological process as can be seen in figures 13 and 14.

Selenka ('85) stated that the ovum of the opossum is intermediate in type between the meroblastic and holoblastic egg. It is difficult to understand the significance of this statement unless it refers to the yolk extrusions and the egg envelopes which do not segment. There are extrusions of yolk given off before the egg begins to segment. The egg proper is holoblastic. What we call the egg envelopes probably correspond to the accessory envelopes of the hen's egg.

Before or during the process of segmentation the ova have given off fragments or extrusions of cytoplasm or yolk. These

Fig. 1 A section of an ovum in the process of fertilization showing the fusion of the male and female pronuclei. The lower pronucleus which is probably male is more deeply stained than the upper female pronucleus. Correlated with the relatively numerous yolk extrusions is the small size of the ovum.

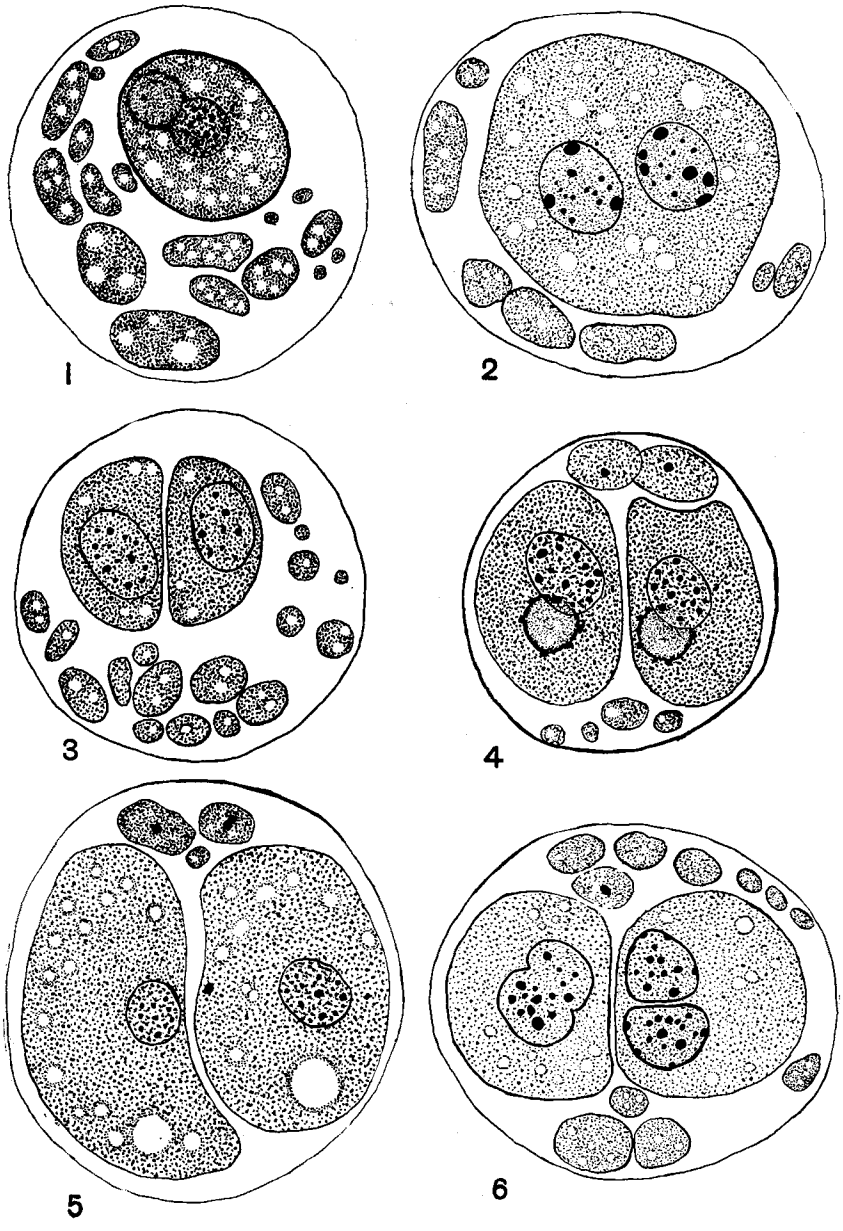
Fig. 2 An ovum in which the nucleus has divided into two nuclei but the cytoplasm shows no signs of division. There are relatively few yolk extrusions and the ovum is large.

Fig. 3 A two-celled stage of segmentation. The elongated nuclei indicate that the next division will be an equatorial one. The blastomeres are relatively small due to the large number of yolk extrusions.

Fig. 4 A two-celled stage of segmentation with the polar bodies. There are few yolk extrusions and the blastomeres are relatively large. Each blastomere has two oval nuclei; one of them is generally seen as a peculiar round body with large chromatin granules around its periphery. This may be the maternal derivative. It has been found that the pronuclei may segment before they fuse. These nuclear-like structures may be divided and unfused pronuclei. Professor E. G. Conklin has called such structures 'gonomeres.' These conditions resemble those in figure 1. It is very difficult sometimes to determine whether nuclei are dividing or fusing.

Fig. 5 A two-cell stage of segmentation with the polar bodies. There are very few yolk extrusions and the blastomeres are very large.

Fig. 6 A two-cell stage of segmentation in which the nucleus of one of the blastomeres has divided and in the other it has begun to divide. One polar body is noticeable.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fragments vary in size and number. Some of the ova have four or five fragments while others have as many as a dozen or more. Such extrusions lie around the blastomeres and are not scattered throughout the reticular egg envelopes (figs. 2, 4, 6 and 9). The ova and the blastomeres are smaller when they are surrounded by a large number of these fragments; conversely the blastomeres are larger when the fragments are fewer in number as can be seen in figures 1 and 3. The ova in the advanced stages of segmentation have fewer of these fragments than the one and two-celled stages. They have probably been reabsorbed as food. These extrusions do not contain nuclear or chromatic material; at least they do not take a nuclear stain. It is probable that these extrusions represent a normal condition. Judging from their constancy they would seem to be of significance. The fact that they resemble the cytoplasmic material would indicate that they had once been a portion of that material. According to Selenka ('87) these fragments of cytoplasm are enveloped by the blastomeres of the blastula and thus lie in the segmentation cavity.

The ovum of the opossum has marked polarity which is noticeable at the beginning of segmentation. The first and second polar bodies are sometimes evident in these ova. The egg envelopes which are probably nutritive do not segment. The first segmentation plane which is meridional divides the egg into two equal blastomeres as is shown in figures 3, 4 and 5. The second plane of division which is an equatorial one divides each of the first two blastomeres into two unequal cells; the smaller ones being at the animal pole. The second divisions are not simultaneous as is well shown in figures 6, 8 and 9. The division of the nucleus precedes by a long time that of the cytoplasm and of the cell wall as is illustrated by figures 2, 7 and 8. The third plane of division is meridional and divides the first four blastomeres into two cells each, thus giving the eight-celled stage which is shown in figure 10. The blastomeres of the early stages of segmentation do not adhere closely together.

The ova of this last described specimen varied in their development from one to eight cells. Two of the ova are in the one-

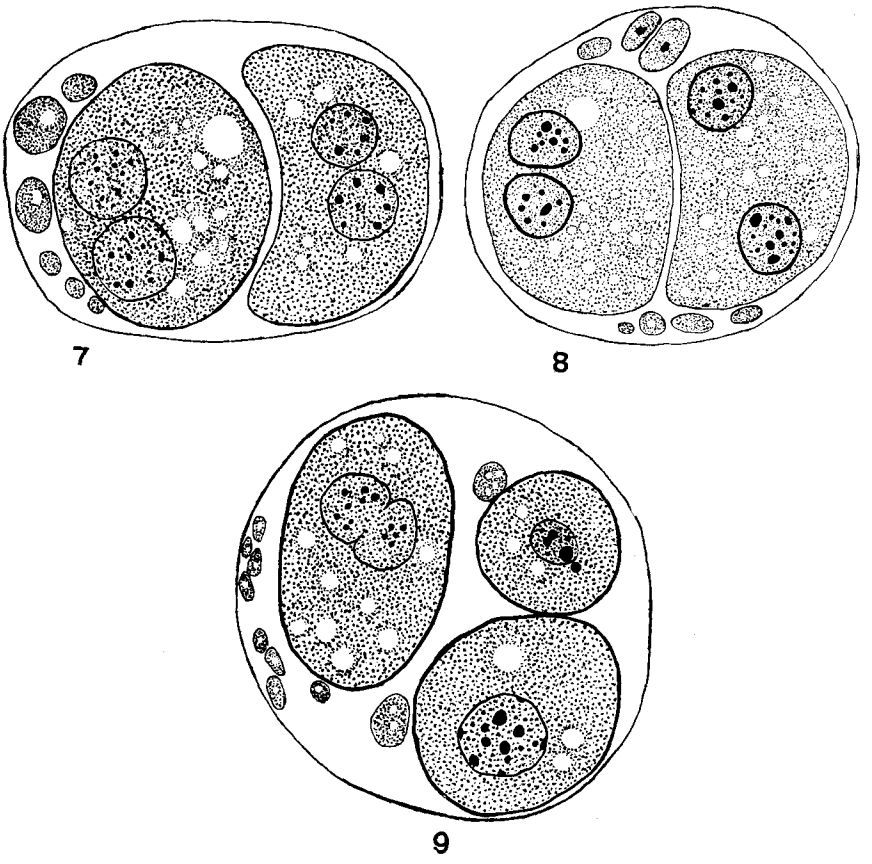
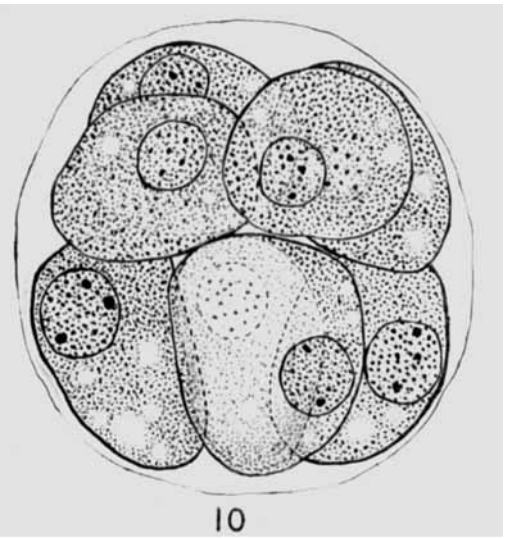


Fig. 7 A two-cell stage of segmentation in which the nuclei of both blastomeres have divided.

Fig. 8 A two-cell stage of segmentation with the polar bodies. The nuclei of both blastomeres have divided. The nuclei of the right blastomere have moved to opposite ends of the cell preparatory to the division of the cytoplasm. It will be noted in figures 6, 7 and 8 that the second plane of division is an equatorial one.

Fig. 9 A three-celled stage of segmentation in which the nucleus of the left blastomere is almost divided preparatory to the formation of the four-celled stage. Note the unequal distribution of the cytoplasm in the resultant right blastomeres.



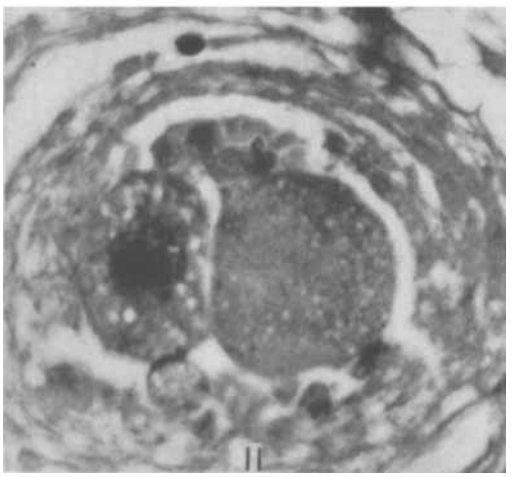


Fig. 10 An eight-cell stage of segmentation. The four blastomeres at th animal pole are smaller than those at the vegetative pole.

Fig. 11 Photomicrograph through the center of an egg to show the appearance of the central part of the egg envelopes. The egg is in the two-cell stage of segmentation with the polar bodies and many yolk extrusions.

cell stage, five in the two-cell stage, six in the two-cell stage with four nuclei, three in the eight-cell stage, two probably in the blastula stage and the other four are in the four-cell stage of segmentation.

Selenka ('85) found from nine to twenty-seven ova in the uteri. There are never as many embryos in the marsupium as there are ova in the uteri. Selenka never observed more than six embryos in the marsupium. We found one specimen with eight embryos in the marsupium. Most opossums, however, bring to maturity a smaller number owing to the fact that there is always a high rate of mortality for the developing ova. The difference in the number of the developing ova in the uteri and the number of the embryos in the marsupium is probably due in part to the difficulties encountered in the transfer because they are transferred at a very immature stage of development. According to Selenka gestation lasts exactly eight days. Taking into consideration the variation in the rate of segmentation of the ova of this opossum there would be considerable difference in the stages of maturity of the embryos at the end of eight days if the weaker ones did not die or become abnormal.

The sections from which the photomicrographs are taken are magnified about one hundred times. The drawings were made with the aid of a camera lucida and are magnified about six hundred times. The heavy line surrounding the ova in the drawings is not as uniform and continuous as it is illustrated. It is more of a compressed net than it is a continuous line. It appears to be a product of coagulation. It is not the zona which is a distinct membrane in the ova of most mammals. The drawings cover only the egg proper. The cytoplasm is more vacuolated than is shown in the drawings. If the egg envelopes were included in the drawings and drawn to the same scale the drawings would have an additional radius of from four to five inches. Figures 1 to 10 are drawings from sections. Figures 11 to 15 are photomicrographs.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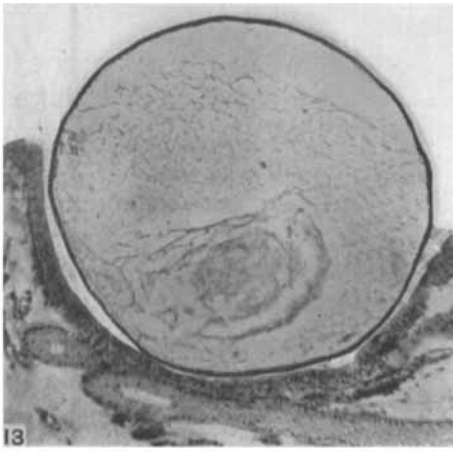
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Figs. 12, 13 and 14 Photomicrographs from sections through or near the center of the egg, perpendicular to the uterine mucosa which show the implantation of the eggs in the uterine cups. The cups are cut in cross section. The cups are formed by circular folds of the uterine mucosa which extend well above its surface. Figures 12 and 14 illustrate very well the gross structure of the ovum. On the outside is seen the thick limiting membrane. Beneath this is a thick layer of a coagulated albuminous material which is limited internally by a sharply staining surface which is probably a coagulation product. This has been arbitrarily indicated by a solid line enclosing the ova in figures 1 to 10. Inside this demarcation is a less dense substance in which the blastomeres are suspended.

Fig. 15 Photomicrograph of a section through a uterine cup, horizontal to the uterine mucosa. The section is a little below the center of the egg.