POLYNUCLEAR OVA, POLYOVULAR GRAAFIAN FOLLICLES AND TWINS IN MACACUS RHESUS

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The ovaries of the monkey (*Macacus rhesus*) are a pair of bean-shaped structures. Each consists of a peripheral and a medullary zone. The binuclear ova result from amitotic division of the parent nucleus and it gives rise to polyovular Graafian follicles. It is suggested that the production of the twins is a hereditary quality.

Introduction

The literature on polyovular follicles and polynuclear ova is fairly extensive. B. Schultze[1] was the first to state that double monsters and one-egg twins result from binuclear ova. Haggstrom[2] believed that binuclear ova are responsible for the production of one-egg-twins, but later, Hartman[3] stated that polynuclear ova and polyovular follicles play no part in their formation. According to Rosner[3] polyembryony in *Armadillo* is the outcome of polyovular follicles. It is now generally believed that there are two types of twins —one-egg-twins, and two-egg-twins. Conclusions regarding the origin of polynuclear ova and polyovular follicles are, however, conflicting.

Material and Technique

Monkeys (*Macacus rhesus*) which were known to give birth to twins were purchased from monkey catchers, and in one case a monkey was procured which had previously given birth to quadruplets. In all cases the animals were killed; the ovaries were immediately removed, placed in normal physiological saline and cut into longitudinal pieces. These pieces were fixed in Bouin's solution for 12 hours. Sections were stained in Mann's methyl blue eosine. Cornoy's method was also tried, but did not give satisfactory results.

Observations

The ovaries of the monkey are a pair of bean-shaped bodies bearing rounded pink-coloured prominences. They vary in size according to the age of the animal. The ovary consists of two zones, a peripheral or cortical zone and a central or medullary region. The cortical zone possesses a well-marked germinal epithelium. Small ova are visible below the germinal epithelium. The medullary region contains oocytes which are surrounded by layers of follicle cells in various stages of development. In addition to ova, stroma cells, atretic follicles and blood vessels are present.

As the oocytes increase in size, they come to lie deeper in the stroma. The smaller oocytes are without follicle cells, but, as they grow, some of the epithelial, or indifferent, cells become organised to form the first layer of the follicle.

As the oocytes undergo a further increase in size, they travel gradually towards the centre of the ovary, and by division of the cells of the original single layer a follicle of several layers is formed. The follicle cells of the outer layers grow more rapidly than those of the inner layers, so that a cavity, the antrum, is formed which becomes filled with the liquor folliculi. The rapid growth of the follicle results in the formation of an outer wall, the membrana granulosa, and in that of an inner wall, discus proligerus, which invests the ovum.

Formation of Binuclear Oocytes.—A large number o oocytes were seen to be binucleate, and a careful examination established beyond doubt that they arise through the amitotic division of the single nucleus of the early oocyte. The nucleus of the oocyte elongates, and a single large nucleolus breaks up to give rise to numerous nucleoli. This elongate nucleus undergoes constriction in its central region, and divides into two smaller nuclei (Figs. 2 and 3). The two nuclei thus formed separate, and travel to opposite poles of the oocyte (Fig. 5).

Origin of Polyovular Follicles.—Due to the division of the oocytes, follicles containing two or four eggs are frequently present. The nucleus at first becomes very active, and a large number of nucleoli are formed. It then elongates and divides across the equatorial region. The two nuclei formed in this way may remain attached for some time (Fig. 3), but finally separate and travel to opposite poles of the cell; the cell then divides into two (Figs. 1 and 5). By the further division of each ovum, four eggs may be formed within a single follicular investment.

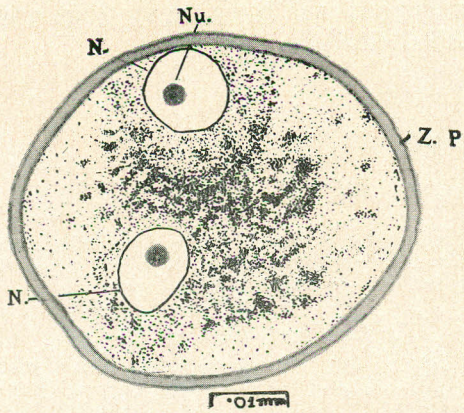

Fig. 1.

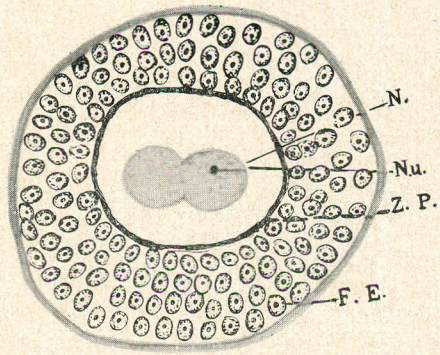

Fig. 2.

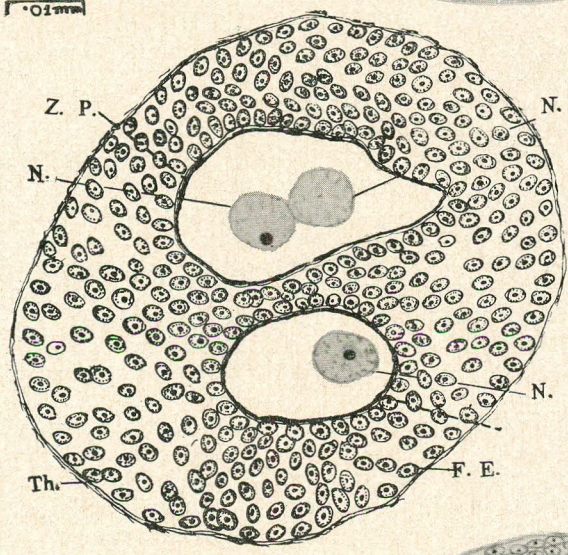

Fig. 3.

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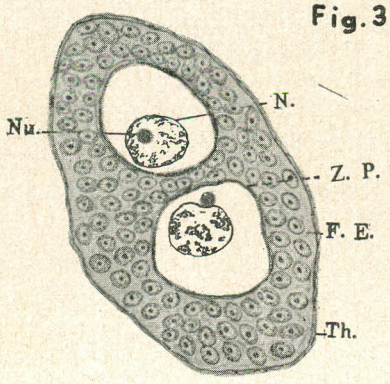

Fig. 4.

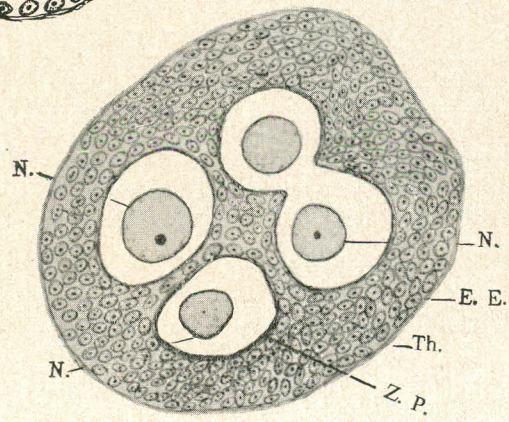

Fig. 5.

All figures were drawn with the aid of a Lucida camera. F.E., follicular epithelium; N., nucleus; Nu., nucleolus; Th., theca; Z.P., zona pellucida.

Fig. 1.—Binuclear oocyte. Fig. 2.—Graafian follicle with one oocyte. The nucleus of the oocyte is elongated and slightly constricted in the middle. Fig. 3.—Polyovular Graafian follicle. In one oocyte the single nucleus has divided into two by constriction; the daughter nuclei have not separated. Fig. 4.—Biovular Graafian follicle. Fig. 5.—A typical case in which two stages of division are seen side by side. One of the oocytes has already divided; in the other the daughter nuclei have moved apart and the cell possesses an equatorial constriction.

Discussion

According to Patterson[4] the occurrence of polynuclear follicles is rare. He examined about fifty ovaries of *Armadillo* and did not observe a single polyovular follicle. Hartman[3] reviewed the literature on polynuclear ova and polyovular follicles and stated that the occurrence of the former is a rule in the ovary of the opossum, and that hundreds were present in some of the ovaries which he examined. In the guinea-pig Beams and King[5] observed polynuclear ova and also polyovular follicles which contained 2 to 15 ova. Guthrie and Jeffers[6] identified, in the ovaries of the bat, polyovular follicles which contained as many as six ova. Haggstrom[2] observed several binuclear ova in a pair of fresh human ovaries, and Nagel[7] reported the occurrence of two binuclear human eggs. Bischoff,[8] however, denied the existence of such structures.

The occurrence of polynuclear ova is not confined to the mammalia but has been reported by various workers in other animals, e.g. snake, frog, fishes, birds and insects.

Hartman[3] studied the ovaries of two pregnant monkeys (*Macacus rhesus*) and observed binuclear ova and polyovular follicles; the number of eggs present in a compound follicle varied from 2 to 12. The present author identified binuclear ova and a large number of polyovular follicles in ovaries of monkeys but found that the maximum number of eggs in a compound follicle is four. Hartman suggested that polyovular follicles may be classified into three separate groups. In addition to the two types described by Van Beneden,[3] he recognised, in the opossum, a third type in which, by virtue of the linear arrangement of the ova, the follicles vary from the spherical to the elongate.

In the ovary of *Macacus rhesus* the author observed the following stages in the formation of polyovular follicles. After the division of the parent nucleus, the two daughter nuclei (Fig. 1) travel to opposite poles of the ovum and the follicle cells extend inwards and follow the path of the constriction (Fig. 5). The author, therefore, considers Van Beneden's and Hartman's observations to be fallacious and believes that all compound follicles either biovular or triovular are of one type. In all probability they described, as types, stages in the formation of polyovular follicles.

Three possibilities have been suggested as regards the origin of polynuclear ova. Ebner and Janosik[3] are of the opinion that the polynuclear condition is brought about by mitotic division of the nucleus. Beams and King[5] supported their findings but Goette[3] attributed the origin of binuclear ova to the fusion of eggs. The findings of Eismond[3] and those of Blanc[3] for the mouse are in agreement with Goette's views. According to Rabl [9] the binuclear condition is due to the pressure of surrounding tissue as a result of which two eggs unite. Stoeckel[10] and Falcone[3] considered that binuclear eggs arise by amitosis. Stoeckel's conclusions regarding the origin of binuclear ova in a pair of ovaries of a twenty-nine year old woman were not accepted by Hartman. The former based his conclusions on the presence of a dumb-bell shaped nucleus. Rabl also subjected Stoeckel's view to severe criticism, and pointed out that Stoeckel's specimens were poorly fixed. The author agrees with Stoeckel's observations. Hartman's contention that, on careful focussing the dumb-bell shaped nucleus is resolved into two nuclei with distinct membranes cannot be accepted, as in the monkey, prior to the appearance of the dumb-bell stage, the author observed elongate nuclei possessing central constrictions (Fig. 2). The author holds the view that the binuclear condition is preceded by amitotic division.

Stoeckel[10] believed that polyovular follicles arise by the division of polynuclear ova, and this view was supported by Rabl.[8] According to Hartman, "polyovular follicles, then, are accidents to development, doubtless due to a variable proportion of the three chief elements, germ cells, epithelium and stroma". In the case of the monkey the nucleus elongates and divides into two, the two daughter nuclei move apart, and a constriction takes place through the equatorial region. The follicles cells travel inwards and follow the path of the constriction, thus separating the two daughter eggs (Fig. 5). In view of the various stages seen in the monkey, the author believes that polynuclear ova are primarily responsible for the formation of polyovular follicles.

There are conflicting reports regarding the origin of twins. According to Haggstrom[2] binuclear ova are responsible for the formation of one-egg twins. Rosner[3] is of the opinion that polyovular follicles give rise to twins.

As polyovular follicles were observed only in monkeys, who formerly gave rise to twins, the author believes that production of twins is a hereditary quality.

The author is engaged since ten years to solve this mystery of the origin of polyovular Graafian follicles and after repeated observations is now

convinced that in *Macacus rhesus* they arise by amitosis and the phenomenon cannot be attributed to atresia.

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*Due to non-availability of original literature the views of the following authors have been taken as stated in the paper of Hartman (1926): Blanc (1892), Eismond (1898), Ebner and Janosik (1897), Falcone (1899), Goette (1863), Rosner (1901) and Van Benedon (1880).