



## **New Data on the Human Embryo Developed *In Vivo* at the Stage of Two Pronuclei**

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### **ABSTRACT**

*The article is aimed at assessing morphological features of the primary human embryo at the stage of two pronuclei, developed in vivo. The human embryo at the stage of two pronuclei was washed out from the uterus of a woman approximately 20-25 hours after the coitus. The embryo was placed into a microchamber with a micropipette and fixed with 10% neutral buffered formalin. Having been embedded in paraffin, histological sections of 7 µm thick were stained with Mayer's hematoxylin & eosin. The dimensions of the pronuclei and the cone of fertilization were measured. A cone or tubercle of fertilization with a clearly defined crater-like depression, which is a site of penetration of the spermatozoon, was revealed in the zona pellucida of the fertilized egg. The measurements showed that the size of the inlet was half the diameter of the male pronucleus (6 µm). The larger (female) pronucleus (8 µm in size) was located closer to the site of penetration. The ooplasm around pronuclei was more basophilic. Both pronuclei retained their nuclear envelopes; chromatin was dispersed; there were no nucleoli. The zona pellucida is thickened in the area of the spermatozoon penetration. Not all is known about fertilization and the first hours of human embryo development under natural conditions. It is extremely important to study this process, which allows to understand better the events unfolding during artificial fertilization and to prevent undesirable consequences.*

**Key words:** human embryo, pronuclear stage of embryogenesis.

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### **INTRODUCTION**

An analysis of the literature shows that today are described only seven primary human embryo formed as a result of fertilization under natural conditions. This explains the value of studying the morphological features of such objects, fertilization and development of which was carried out under natural conditions. Besides, we had not found any reports in the available literature that have information about the place of penetration of the oocyte by the spermatozoon either in case of in vitro or in vivo fertilization, which occurred in situ in a woman's organism.

The purpose of the article is to estimate morphological features of the primary human embryo at the stage of two pronuclei, developed under natural conditions.

### **MATERIAL AND METHODS**

The human embryo at the stage of two pronuclei was obtained as a result of irrigation with a physiological solution of the uterine cavity of a woman approximately 20-25 hours after the coitus. The embryo was discovered in an instillate under a binocular magnifying glass, placed into a special microchamber with a micropipette and fixed with 10% neutral buffered formalin. Further study of the object was carried out in the laboratory of the Department of Histology and Embryology of V.I. Vernadsky Crimean Federal University.

The primary embryo was embedded in paraffin; histological sections of 7 microns thick were made afterward. Two pronuclei were revealed in the cytoplasm in one of the sections, contrasted with Mayer's hematoxylin & eosin, when compared. The dimensions of the pronuclei and the cone of fertilization were measured using an eyepiece micrometer. The description and main morphometric parameters of this object were published [12].

## RESULTS

A micrograph of the above-mentioned section of the embryo, where a kind of "defect" is clearly visible in the zona pellucida of the ovum, is presented in the current paper (Fig. 1). This defect is in the zone of a very small elevation at one of the poles of the egg, which is a cone or tubercle of fertilization. The crater-like depression in the form of a kind of "gate", which is the site of sperm penetration through the zona pellucida, is clearly defined here. The diameter of this defect is about 3 microns, its edges are clearly rounded and hyperchromic in comparison with the rest of the zona pellucida (see Fig. 1). In morphometric studies, it was established that the size of the inlet was twice as small as the diameter of the male pronucleus (6  $\mu\text{m}$ ). It should be assumed that during the time elapsed since the spermatozoon penetrated the ooplasm (about 20 hours [2], its pronucleus enlarged as a result of swelling, characteristic of this stage of development. No less interesting is the fact that the larger (female) pronucleus (8  $\mu\text{m}$  in size) was located closer to the place of penetration in the plane of the section.

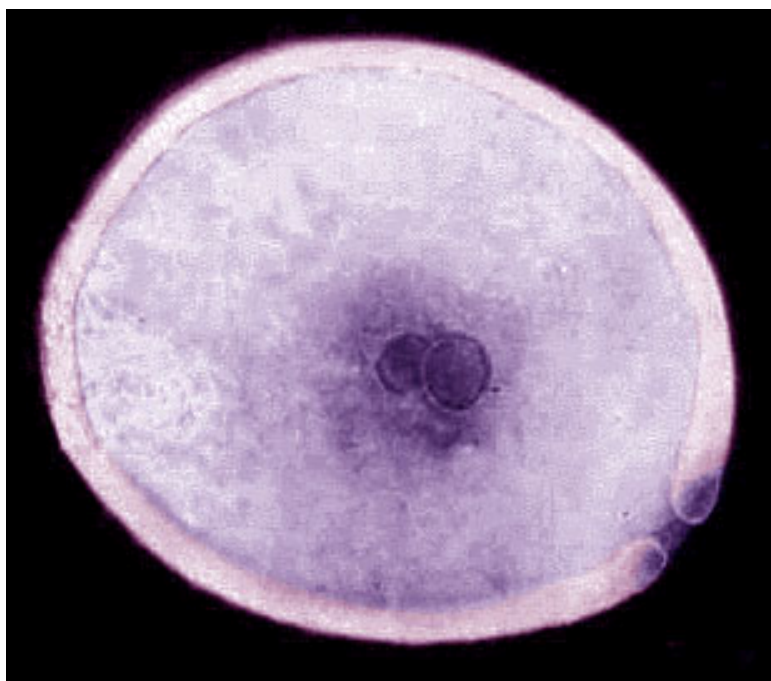


Figure 1. Primary human embryo at the pronuclear stage, discovered by P.L. Velikij in the instillate during irrigation of the uterus. Staining: Mayer's hematoxylin and eosin. Magnification: objective 40x, ocular 10x.

The ooplasm, located directly around the pronuclei, reveals a more pronounced basophilia than that located at the periphery of the oocyte, showing a relatively heterogeneous distribution of the basophilic substrate. One can see it especially well at the pole of the oocyte opposite to the inlet. At the same time, that part of the ooplasm that is directly adjacent to the inlet is colored weaker and relatively homogeneous. We consider that this is probably due to the lytic effect of hyaluronidase introduced here by the spermatozoon.

Both pronuclei preserve a clearly expressed nuclear envelope, chromatin in them being in a dispersed state, and therefore, the contents of pronuclei, showing moderate basophilia, are stained homogeneously. Against the background of pronuclei, we were unable to detect the nucleoli. The polar bodies are also absent in the plane of this section, and the perivitelline space is not visualized.

The zona pellucida surrounding the embryo has visually approximately the same thickness over a greater extent, and only in the zone of its penetration does it somewhat thicken. Tinctorial features of the zona pellucida include the fact that it is intact to hematoxylin, but shows a weakly positive reaction to eosin.

## DISCUSSION

It should be assumed that when we were preparing sections, quite accidentally the orientation of the object of study turned out to be such when both pronuclei and the place of penetration of the zona pellucida by the spermatozoon appeared simultaneously in the plane of the section.

In an extensive review of the early stages of human embryonic development, embryos at the initial stage of embryogenesis, preceding the zygote stage, are called ootids and belong to Carnegie Stage I of human embryogenesis [10]. Only five samples studied by Velikij P.L. and Shapovalov Yu.N., [12], B.P. Hvatov (2 objects) [7], Z. Dickmann et al. and R.W. Noyes et al. [5, 9], L. Zamboni *et al.* [13] showed no doubts about presence of two pronuclei in their ooplasm. A number of human embryos at the pronuclear stage were obtained in vitro fertilization [8, 11]. The publications present excellent micrographs, including those in phase contrast. The male and female pronuclei in them have almost converged, are of approximately the same size and located in the ooplasm in a somewhat eccentric way. It is mentioned that as the male pronucleus approaches the female one, it swells, acquires a perfectly rounded shape and the “chromatin bodies” become clearly visible in it, as well as in the female pronucleus by this time [11].

Comparing the published data on the morphometric parameters of the ootids, obtained in vitro and developing *in situ*, it should be pointed out that their sizes and the sizes of pronuclei contained in them differ noticeably. This, apparently, can be explained by the fact that some objects were measured before and some after fixation. It should also be assumed that the density of the medium into which the objects were enclosed could have been of no less importance.

Particularly noteworthy is discussion of the fact that the embryo we described stayed freely in the uterus and was obtained as a result of its instillation, which was successfully used in the 1970s to obtain ova non-surgically [3].

In this regard, [2] formulated a statement that in humans, sexual cell fusion can take place in any part of the fallopian tubes, while in mammals this occurs in the ampullae of the oviduct only. Perhaps, this can explain the phenomenon of discovery of this embryo in the uterus.

According to [10], three successive stages are distinguished during the fertilization process: 1) contact of the spermatozoa with the zona pellucida, penetration of one or more of them into the ooplasm, and swelling of the sperm head; 2) formation of the pronucleus; 3) the beginning of the first mitotic division.

Analyzing the morphological features of the human embryo we described, it should be assumed that it is at the end of the 2nd stage of this process. This is confirmed by the fact that both pronuclei have already come into contact with each other, but still retain their nuclear envelopes. This fact and our discovery for the first time of the place of penetration of the sperm cell through zona pellucida (cone or tubercle of fertilization) *in vivo*, may be of undoubted interest for scientists involved in embryology and in dealing with problems of in vitro fertilization.

## CONCLUSION

Observations on fertilization in humans in vitro helps to uncover the unknown of the conception of a new organism, but there is no certainty that this process develops in the same way as in vivo. Thus, recently there have been reports that multinucleated blastomeres develop quite frequently in human embryos cultured in vitro. However, most multinucleated embryos appear to have the ability to correct themselves during early cleavage divisions and can develop into euploid blastocysts, resulting in healthy newborns [1]. Multinuclearity after the first cleavage division does not affect adversely the subsequent development of embryos, if they show normal cytokinesis at this stage. Poor development of multinucleated embryos is mainly caused by the abnormal first cleavage division [6]. At the same time, it is indicated [4] that multinuclearity at the two-cell stage in both blastomeres have a significant negative effect on the potential of birth. We have not come across descriptions of human multinucleated blastomeres under natural conditions of development in the available literature. Therefore, new findings are needed to further discuss the process of fertilization in humans *in situ*.

## Conflicts of interest

The authors declare no conflicts of interest.

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