Yonsei Medical Journal
Volume 12, 1971

The Thickness and Volume Change of the Zona Pellucida Following Ovulation and Fertilization

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ABSTRACT

Since the volume gives a better comparison of size than thickness, the volume of the zona pellucida was also calculated by substraction of the volume of the inner zonal cavity from the volume of the total egg and compared with the zona pellucida thickness. All calculations were made by computor (CEIR timesharing computor).

The zona pellucida of the tubal ova is thicker than that of the oocyte, with the zona pellucida of the fertilized egg being definitely thinner when compared with unfertilized eggs. This phenomenon of decreased thickness in the fertilized egg may be associated with the zona reaction.

The entry of the first sperm into the egg initiates a reaction in the zona pellucida the effect of which is to preclude the entry of additional sperm. Braden, Austin and David (1954) defined this phenomenon as the zona reaction. The results of anatomical changes in the zona pellucida may also be a phenomenon such as a "zona reaction".

*This Study has done in The Johns Hopkins University School of Hygiene and Public Health Department of Population, Dynamics Laboratory of Reproductive Biology

INTRODUCTION

The physiology and biochemistry of the fertilization of the mammalian egg has, in recent years, attracted considerable interest. Nevertheless little quantitative work has as yet been done on this stage. The topic of this study is the volume changes of the zona pellucida which occur in ovulation and fertilization of mouse eggs. The mouse was chosen for study because of its frequent use as an experimental animal by investigators of metabolism of early developmental embryos, and because large numbers of mouse eggs can be obtained with relative ease. Moreover, the laboratory technique for the manipulation of these eggs is well developed. Changes in thickness and volume of the zona pellucida of the mouse egg during the initial 18 hours of development following ovulation and fertilization were observed.

Previous reports on the size of each of these anatomical structures of egg have shown many different values, even for those measurements made on the ova of the same species at the same stage of development by different investigators. The

cause of these inconsistencies is partially due to the different equipment used in some studies and to the failure on the part of many investigators to state the age of the egg when measured and the number of blastomeres present.

Because of the inconsistencies of these reports and the small amount of anatomical information on zona pellucida changes available, a study was carried out following ovulation and fertilization of the mouse egg to determine the changes in volume and thickness of the zona pellucida.

METHODS AND MATERIALS

Mice of a randomly bred Swiss strain colony derived from the institute for cancer research were obtained locally (Hazelton-Carbia, Burtonsville, Maryland), at 7-8 weeks of age. Male mice were kept in individual cages. For mating, one female was placed in a cage with one male. The mice were maintained in an air conditioned environment subject to a light cycle of 14 hours of light (6:00 A. M. to 8 P. M.) and 10 hours of darkness (8:00 P. M. to 6:00 A. M.) and at a temperature of 70-75°F.

Twenty-seven mated female mice were randomly distributed into nine groups of three mice each after the intraperitoneal injection of five IU PMS. Then, after 48 hours, five IU HCG were injected. Twenty-seven unmated females with the same hormonal treatment were also randomly distributed into nine groups.

Mice were killed at 3 hour intervals over a 24 hour period beginning 12 hours after the injection of HCG. Since the first cleavage division was completed in this study by 21 hours after ovulation and fertilization, the last 2 groups were discarded.

Unfertilized and fertilized ova were recovered from the ampulla of the tubes after mice were killed as designed above. The fallopian tubes were removed and placed in a drop of medium in a plastic petri dish (60×15 mm style) (Falcon plastics #1007). The ova surrounded by cumulus cells were released by rupturing the distal portion of the ampulla of the tube with a fine hypodermic needle. The ova and cumulus were transfered to 1 ml of medium in an embryological watch glass and 1 ml of the hyaluronidase solution (evine testes, Sigma Corp. stored below 0°C) was added. Within two to three minutes the cumulus cells fell away from the ova, at which time the ova were transfered to 1 ml of the medium under 2 ml of mineral oil in a second watch glass and washed.

Cumulus free ova were then transfered to a microdrop under oil, and then were photographed through a wild M 40 inverted biological microscope with a 40×objective and a 10×photo eye piece, using tungsten illumination and a green filter. The photographic system included a wild microphotoautomat and a wild photomicrographic camera I. Kodak 120 Panatomic X film was used in this study. Magnification on the film was determined by photographing under identical optical conditions a fine lined scale mounted on a microscope slide.

Additional magnification was obtained by projection of the negative image of the ova and microscale on paper using a photographic enlarger. The size of the ova was determined by calibration of the size of the projected image of the microscale.

One micron on the scale was magnified to one millimeter under a photographic enlarger and measurement was made directly on the projected images.

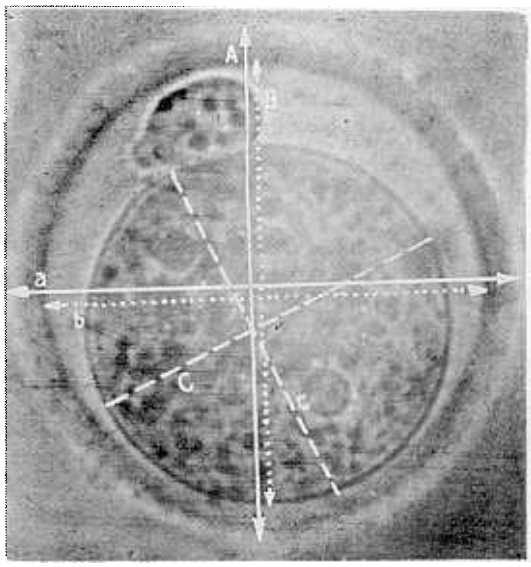

Figure 1. Illustration of the method used to obtain the measurements

A: Largest outer diameter of zona pellucida (do max)
a: Smallest outer diameter of zona pellucida (do min)
B: Largest inner diameter of zona pellucida (di max)
b: Smallest inner diameter of zona pellucida (di min)
C: Largest diameter of vitellus (dv max)
c: Smallest diameter of vitellus (dv min)

The data on the largest and smallest diameters of the inner and outer surface of the zona pellucida on unfertilized and fertilized tubal mouse ova were analyzed on a CEIR time sharing computor. The thickness of the zona pellucida (t) was calculated as follow:

$t=1/4(do^{max}+do^{min})-(di^{max}+di^{min})$

do^{max}: Largest outer diameter of zona pellucida

do^{min}: Smallest outer diameter of zona pellucida

di^{max}: Largest inner of zona pellucida

di^{min}: Smallest inner diameter of zona pellucida

The volume of zona pellucida was calculated by subtraction of the volume of inner zonal cavity from the volume of the total egg. Two geometric models have been assumed to represent the form of the ovum. These are the obulate spheroid and the prolate spheroid. The volumes of these forms are:

$$\text{Obulate spheroid}=\frac{\pi}{\sigma}d^{max}.d^{min}$$

$$\text{Prolate spheroid}=\frac{\pi}{\sigma}d^{max2}.d^{min}$$

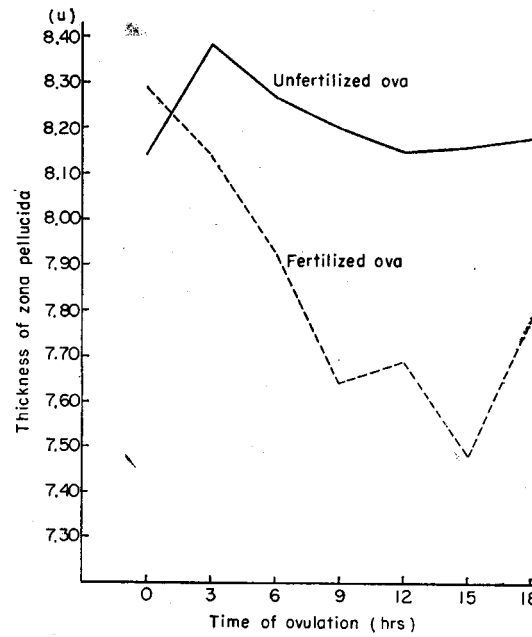

Figure 2. The thickness (u) of zona pellucida during the initial 18 hours of development following ovulation and fertilization.

Table 1. Thickness(u) of the zona pellucida of the mouse EGG, during the initial 18 hours following ovulation and fertilization

GROUP	STAGE	NO.OF OVA	THICKNESS ZONA	SE
1	FERT.	47	8.29	0.87
(0 HR.)*	UNFERT.	55	8.14	0.57
2	FERT.	48	8.14	0.74
(3 HR.)	UNFERT.	57	8.38	0.53
3	FERT.	61	7.93	0.62
(6 HR.)	UNFERT.	51	8.27	0.93
4	FERT.	57	7.64	0.46
(9 HR.)	UNFERT.	55	8.20	0.75
5	FERT.	64	7.69	0.88
(12 HR.)	UNFERT.	53	8.15	0.77
6	FERT.	64	7.48	0.22
(15 HR.)	UNFERT.	60	8.16	0.75
7	FERT.	48	7.79	0.77
(18 HR.)	UNFERT.	52	8.18	0.68

*0 HR. = Estimated time of ovulation = 12 hr. after HCG injection.

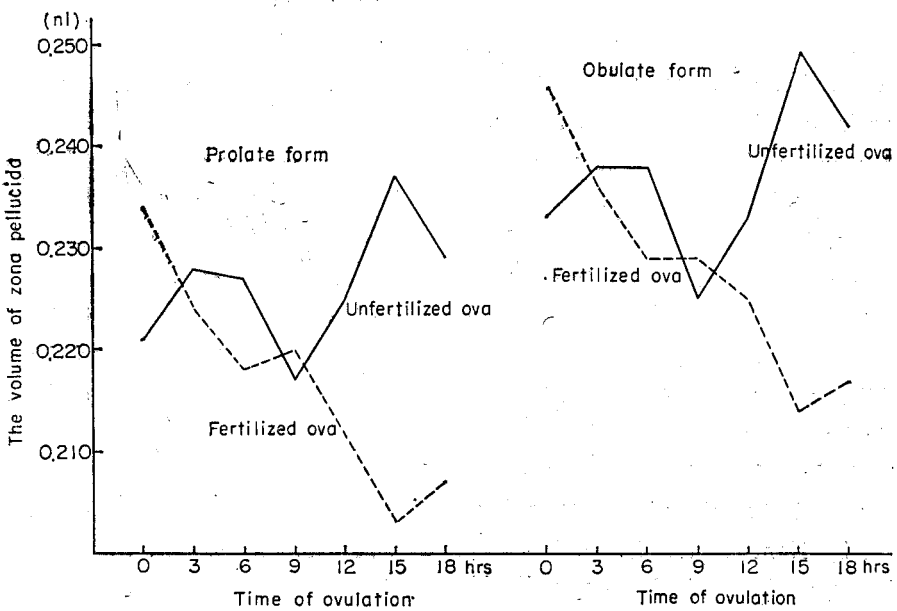

Figure 3. The volume (nl) of the zona pellucida during the initial 18 hours of development following ovulation and fertilization.

Table 2. Volume (nl) of the zona pellucida of the mouse EGG during the initial 18 hours following ovulatin and fertilization.

GROUP	EGG	OBULATE	SE	PROLATE	SE
1	FERT.	0.246	0.0036	0.234	0.004
(0 HR.)*	UNFERT.	0.233	0.0030	0.221	0.0031
2	FERT.	0.236	0.0028	0.224	0.0029
(3 HR.)	UNFERT.	0.238	0.0028	0.228	0.0026
3	FERT.	0.229	0.0034	0.218	0.0029
(6 HR.)	UNFERT.	0.238	0.0037	0.227	0.368
4	FERT.	0.229	0.0032	0.220	0.0033
(9 HR.)	UNFERT.	0.225	0.0026	0.217	0.0028
5	FERT.	0.225	0.0039	0.212	0.0035
(12 HR.)	UNFERT.	0.246	0.0030	0.236	0.0032
6	FERT.	0.214	0.0040	0.203	0.0040
(15 HR.)	UNFERT.	0.249	0.0032	0.237	0.0031
7	FERT.	0.217	0.0033	0.207	0.0038
(18 HR.)	UNFERT.	0.242	0.0035	0.229	0.0032

*0 HR. = Estimated time of ovulation = 12 hr. after HEG injection.

RESULT AND DISCUSSION

The second experiment was repeated one week following the initial experiment. The results of the two experiments were similar and the data were combined for statistical analysis.

The data for the thickness of the zona pellucida are summarized in Table I and in Figure I. A decrease in the thickness of the zona pellucida of 0.36 u (4.3%) occurred in the 1-cell fertilized tubal ova between the time the first observations were made shortly after ovulation and those made 6 hours after ovulation by which time the second polar body is extruded. The rate of decrease in the thickness of the zona pellucida was approximately constant during the first 15 hours after ovulation. There was no change in the zona pellucida of the unfertilized tubal ova during this period.

In the fertilized tubal egg, the volume of the zona pellucida also undergoes a reduction in volume of approximately 0.017 nl by six hours after ovulation and fertilization. In the unfertilized tubal egg, there are no changes in the volume of the zona pellucida until nine hours after ovulation. There after there is a sudden expansion. The expension of the egg may be due to degenerative

change of the egg. Table II and Figure II.

The zona pellucida of mammalian eggs consists of an homogenous, thick, transparent, elastic matrix with vague irregular areas of varying density, a smooth inner layer and a relatively rough-showing outer layer (Austin, 1968; Sotelo and Porter, 1959; Odor, 1960; Yamada et al, 1967). In electron-microscopic studies the zona pellucida appears to have two concentric layers in both rabbit and sheep eggs, and three concentric layers in big eggs(Dickman and Dziuk, 1964) but the existence of layers in the zona pellucida of the pig egg has been questioned by Hancock (1965).

The inner surface of the zona pellucida of the rat and mouse appears smooth. In oocytes it is in close contact with the plasma membrane of the vitellus whereas in ovulated eggs it is separated from the vitellus by a filled space of variable width. The outer surface of the zona appears granular and relatively rough, showing no clear-cut boundary (Austin, 1965, 1968; Dickman, 1963, 1964; Odor, 1960; Yamada et al, 1957).

The zona pellucida changes following ovulation and fertilization. Light microscopic observations have shown that both the microvilli and the follicle cell processes of rat ova are retracted or break up, leaving fragments within the zona pellucida. No discernible passages are left by these bodies suggesting that the substance of the zona is elastic (Austin, 1968). In rabbit, sheep, pig and guinea pig, the spermatozoa leave a channel in the zona by traversing it and these slits have a characteristic shape referred to as the "penetration curve"(Austin and Bishop, 1958; Dickman, 1964; Dickman and Dziuk, 1964, 1965).

Some workers could not observe sperm penetration through the zona pellucida immediately after ovulation in the rat and mouse. The zona pellucida may first undergo some change allowing subsequent penetration by spermatozoa (Austin and Braden, 1954). It has been suggested that the physical properties of the zona pellucida in the dog, hamster, and sheep are altered after the first sperm passes through it and enteres the vitellus. It is postulated that a substance is secreted by the vitellus which "tans" the zona so that additional sperm cannot penetrate it. Braden, Austin and David (19 54) described it as zona reaction.

In this experiment, the result shows that the entry of the sperm into the egg initiates a reduction of volume and thickness of zona pellucida. Therefore, the anatomical change of the zona pellucida is also a phenomenons of zona reaction of the mouse.

ACKNOWLEDGEMENT

I am indebted to Professor John D. Biggers for his invaluable advice and guidance throughout the investigation. This work was supported in part by grants from the National Institutes of Child Health (5RO1-HDO-3077-02), the Ford Foundation, the Poulation Council and the Jessie Smith Noyes Foundation.

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