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Influence of Zona Pellucida Thickness of Oocyte Maturation and in Vitro Fertilization in The Cow in Iraq

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ABSTRACT

The aim of study to investigation the role of zona pellucida thickness in the oocytes maturation and in vitro fertilization in cows . This study included the collection of 200 female reproductive system from the abattoir .The ovaries collected and washing with the normal saline . Oocytes recovered via , aspiration and slicing, followed the maturation in TCM199 in Co2 incubator . Semen collected , diluents , and capacitation in order to In Vitro Fertilization . Under inverted microscopical examination the results revealed , oocytes classified in three types , complete surrounded by cumulus cells ,partial surrounded ,and denuded oocytes, synchronizing with deferent appearance of zona pellucida thickness , thin ,normal , and thick .The best thickness of zona pellucida in the oocyte ranged (15um-17um) for successfully maturation and in vitro fertilization .

KEYWORDS : Zona pellucida , IVM ,IVF , Cow.

Introduction

Cows life time many ova develop and then degenerate without ovulation, only a very few mature and then ovulate (1).The primary sperm-egg binding event has been found to involve the ZP3 subunit of the mouse zona pellucida (2).The zona pellucida (ZP) is a thick extracellular coat that surrounds all mammalian eggs. The ZP plays important roles during oogenesis, fertilization, and preimplantation development (3). Each zona pellucida glycoprotein is synthesized in growing oocytes and traffics through the endomembrane system to the cell surface, where it is released from a transmembrane domain and assembled into the insoluble zona pellucida matrix(4). The ZP is a three-dimensional network of sulfated glycoproteins (ZP2) ZP3, and ZP4 in bovine) arranged to form fibrils (5) (6). The functions of zona pellucida include physical support for the blastomeres , inhibition of inappropriate tubal implantation , and protection from the maternal immune system it acts as a barrier to sperm – oocyte interaction , poly spermy and to embryo uterine interaction prior to implantation , there are no reports to reveal whether the zona pellucida itself is essential for embryo development , zona pellucida is not an essential component of human embryo development since the absence of the zona pellucida did not inhibit embryo development (7). Therefore this study included analyze the fertilization rate ,embryo development of oocyte with abnormal zona pellucida.

Materials and Methods

Collected 200 cow genital system from Al-Shualah abattoir, during the period from the 1st of Feb 2016 to the 1st of may 2016. The reproductive status of the animals were not known. The genitalia were transported within one hour in a normal saline at 33-35 ^{0}C in cool box, to the Theriogenology Lab., Department of Surgery and Obstetrics, College of Veterinary Medicine, Baghdad University .There two methods for oocytes collection .The first, Aspirated Oocytes were obtained by postmortem follicular aspiration of ovaries from cows slaughtered at a local abattoir (8)using a 18 gauge needle attached with a sterile 3 ml disposable syringe containing 2 ml of the collecting medium. The second slicing ovaries were placed in a sterile petridish containing 10 ml of collecting medium, held with the forceps and the ovarian surface was incised with a scalpel blade. The oocytes subjected for dissecting microscope examination ,for assesses the thickness of zona pellucida by micrometry scale (9) ,figure 1. Three types of oocytes ,the oocytes complete surrounded by cumulus cells , oocytes partial surrounded by cumulus cells ,and denuded oocytes. Fresh semen was collected from testes , presented in the Al- Shualah abattoir a transported within 1 hr. to the Theriogenology Lab at 30-35 ^{0}C.Semen samples were examined under light microscope to evaluate semen quality. The mass and individual motility was assessed. The oocytes were washed twice in a maturation medium TCM-199 for maturation and in vitro fertilization . They were incubated in appropriate maturation medium at 39 ^{0}C temp, 5% CO_2 and 90% relative humidity for 27 hrs. followed by in vitro fertilization by fertile good semen .(10)

Results and discussion

A total of 200 cow genitalia from slaughterhouse were collected to obtain the oocytes .There are two methods for oocytes recovery ,the first by aspiration and the second by slicing of ovary . Denuded oocytes carried out , only oocytes with complete and partial surrounded by cumulus cells were used for maturation and fertilization . The total number of oocytes were aspirated reached 200 , while the oocytes by slicing collected 800 Table 1 – 2 . Oocytes were subjected to microscopical examination , for assessment thickness of zona pellucida . The zona pellucida thickness between 10- 25 um in the aspiration method , while the thickness of zona pellucida in the slicing gives 8-20 um . The zona pellucida thickness varies from 10-31 um with a mean of 17.5 um (11) . Thickness of zona in the slicing less than the oocytes collected by aspirated , due to earlier growing stages the oocytes in the ovaries . Oocyte growth during folliculogenesis is regulated by granulosa cell derived proteins, also before ovulation , oocyte secreted proteins signal somatic cells to initiate ovulation (12) . Therefore aspirated oocytes earlier maturation than slicing ,it is appeared first polar body figure 2 **.** The cumulus oophorus is routinely removed to assess fertilization and hence the thickness of the zona pellucida is measurable . The normal thickness of zona in the aspirated oocytes 29% while the oocytes obtained by slicing 17.25 % . The best oocytes thickness for In vitro fertilization (15um -17um) gives multiple stages of division figure 2 . The zona pellucida strongly influenced only by the embryo quality (13).The conclusion should be carried out for oocytes with thin and thick zona pellucida . Thick zona pellucida appeared low number and low fertility Table 3 -4 .

Figure 1 : a , Thick of zona pellucida .b, thin of zona pellucida .assessed by microscopical scale .

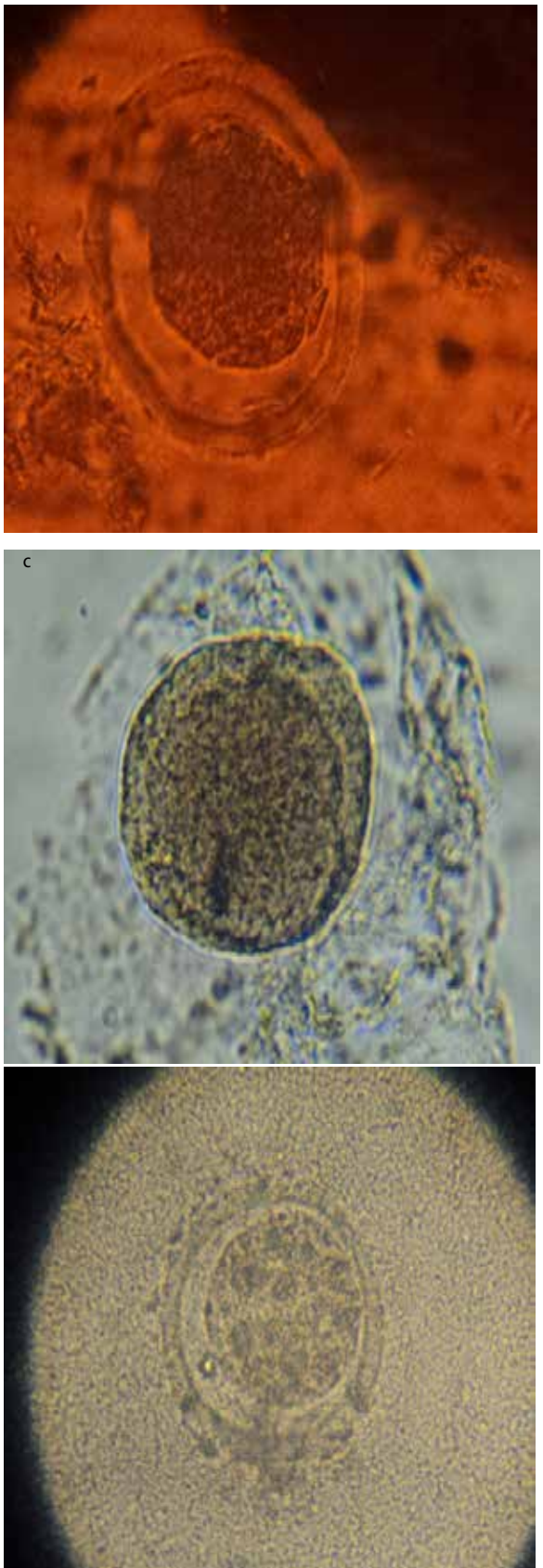

Figure 2: a, mature oocyte show the first polar body .b,-claveage fertilized oocyte in 2cell stage .c, Morella in multiple cell division .

Table 1: Shows the percentage of mature and immature oocytes with thin, thick and normal zona pellucida in aspiration .

	Number	Mature	%	Immature	%
Thin zona pellucida	87	66	33	21	10.5
Thick zona pellucida	34	13	6.5	21	10.5
Normal zona pellucida	79	58	29	21	10.5
Total	200	137	68.5	63	31.5

Table 2: Table 1: Shows the percentage of mature and immature oocytes with thin, thick and normal zona pellucida in slicing .

	Number	Mature	%	Immature	%
Thin zona pellucida	362	188	23.5	174	21.75
Thick zona pellucida	164	79	9.875	85	10.625
Normal zona pellucida	274	138	17.25	136	17
Total	800	405	50.55	395	49.37

Table 3: Shows the oocytes maturation and division collected by aspiration .

	Mature	Cleavage	Morella	Blastocysts
Thin zona pellucida	66	28	10	2
Thick zona pellucida	13	3	1	1
Normal zona pellucida	58	43	12	3
Total	137	74	23	6

Table 4: Shows the oocytes maturation and division collected by slicing.

	Mature	Cleavage	Morella	Blastocysts
Thin zona pellucida	188	45	16	6
Thick zona pellucida	79	18	4	1
Normal zona pellucida	138	86	34	14
Total	405	152	54	21

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