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In vitro maturation, fertilization and embryonic development of morphologically classified goat oocytes*

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ABSTRACT

This study was designed to examine the effect of compactness of cumulus cells on i*n vitro* maturation, fertilization and embryonic development. The morphologically classified goat oocytes were isolated and classified into 3 categories, i.e. good, fair and poor quality oocytes according to the compactness of the cumulus cells and were assigned for *in vitro*, maturation, fertilization and embryonic development. Maturation rates (M II) in good quality oocytes were significantly higher (85.06%) as compared to fair (58.33%) and (42.65%) poor quality oocytes. Total and normal fertilization rates were also higher in good quality oocytes than fair and poor quality oocytes. The cleavage (65.32%), morula (13.70%) and blastocyst (23.38%) development of good quality oocytes was found to be significantly higher than fair and poor quality oocytes. Thus, it is concluded that the selection of good oocytes according to cumulus cells compactness is an important criteria for *in vitro* maturation, fertilization, and embryonic development.

Key words: Embryo development, Fertilization, Goat oocytes, *In vitro* maturation

The cumulus cells play a very important role during oocytes growth and maturation. Oocytes are deficient in their ability to transport certain amino acid and carrying out glycolysis and cholesterol biosynthesis, the cumulus cell provides some specific amino acid and the product required for oocyte maturation (Su *et al*. 2009). They are known to supply nutrients and messenger molecules and to mediate the effects of hormones on the cumulus-oocytes complexes (Suzuki *et al.* 2000).

Nagai (1994) reported that after collection of oocytes, selection of cumulus oocytes complexes (COCs) is extremely important for successful maturation and fertilization *in vitro*. The appearance of the oocytes and investments around them was used to estimate or assess the development potential of the oocytes. Oocytes surround by tight and complete multi-layered cumulus investments and containing ooplasm with a sandy appearance are most likely to be developmental competent (Staigmiller and Moor 1984).

Oocytes do not have receptors for gonadotrophin and estradiol, receptors for both types of hormones are present only in adjacent follicle cells (Lawrence *et al.* 1980). Therefore, signals induced by estrogen or gonadotrophin in follicle cell are transduced into the oocytes via gap junction or extracellular (Downs *et al.* 1988).The question then arise, whether the surrounding follicular cells are needed during maturation for better fertilization and embryonic development *in vitro*. Basic information has not been sufficiently available in the literature about effect of intact cumulus cells surrounding on *in vitro* maturation, subsequent fertilization and embryonic development in goat, therefore the present study was undertaken to study the influence of cumulus cells support *in vitro* maturation, fertilization and embryonic development of goat oocytes *in vitro*.

MATERIALS AND METHODS

In vitro *maturation:* Unless specified, the reagents used in this study were cells culture tested. Goat ovaries were collected from a local slaughter house and were transported to the laboratory in normal saline (30°C) within 1 to 2 hr of slaughter. Ovaries were washed thoroughly in warm water. The small follicles of about 2 to 6 mm in diameter were aspirated using a sterile disposable 18 G needle and 10 ml syringe. The oocytes were recovered from the follicular fluid aspirate, scanned under stereo zoom microscope. The collected oocytes categorized as good, fair and poor quality based on their gross morphology and integrity of cumulus cells: (i) oocytes with more than 4 layers of compact cumulus cells (COC) with evenly distributed cytoplasm as good quality; (ii) the oocytes with one to two layers or with partial

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cumulus cell with uniform cytoplasm were classified as fair oocytes; (iii) oocytes without cumulus cells with uniform cytoplasm were classified as poor quality oocytes.

The oocytes maturation, fertilization and embryonic development were carried out as described previously (Pawshe *et al.*1996). Oocytes were washed in HEPES buffered tyrode's medium (TL-HEPES) followed by 2 washing in maturation medium containing TCM–199 supplemented with 10% fetal calf serum, 5 µg LH / ml, 0.5 µg of FSH / ml and 1 µg / ml estrodiol 17ß. The oocytes were cultured in 100 µl drops of maturation medium covered with 10 ml sterile mineral oil at 38°C in humidified atmosphere of 5% CO_2 in air for 24 h. For the study of the maturation rate of the oocytes, after 24 h of maturation some of the oocytes were fixed in acetic acid and ethanol (1: 3) and stained with aceto-orcein and classified as germinal vesicle (GV), Metaphase I (M-I) and Metaphase II (M-II).

In vitro *fertilization:* Goat spermatozoa collected from caudal epididymis and motile spermatozoa were seperated by using the percoll gradient method, the matured oocytes were washed with TL-HEPES and the final washing were given in Tyrode's fertilization medium (Pawshe *et al.*1996). Ten matured oocytes were distributed in each of the 48 µl drops of fertilization medium containing 10 µg/ml heparin. A 2 µl aliquot of sperm suspension was added to every single drop of oocytes to yield final concentration 2×10^6 spermatozoa / millilitre. Oocytes and spermatozoa were co-incubated for 20 h at 38°C under 5% CO_2 in humidified air. For the study of the fertilization rate, some of the oocytes after 20 hr of fertilization were fixed in acetic acid and ethanol (1: 3) stain with aceto-orcein and evaluated for presence of male and female pronucleus.

Embryo culture: After 20 h of sperm-oocyte co incubation, oocytes were freed from cumulus cells and spermatozoa by repeated pipetting and presumptive zygote were randomly distributed to an individual drop of 100 µl TCM–199 containing 10% FCS and co-culture with goat oviduct epithelial cells for 5 to 7 days. The cleavage and development of embryos was monitored every 48 h.

The effect of *in vitro* maturation, fertilization, and embryonic development of morphologically classified goat oocytes were analysis using a 2×2 fractional design of chi square test (Snedecor and Cochran 1967).

RESULT AND DISCUSSION

In this experiment, rate of maturation, fertilization and embryonic development of morphologically classified goat oocytes was investigated. Oocytes collected from goat ovaries were classified according to morphologically character. All the oocytes were then matured *in vitro* in medium (TCM–199), supplemented with sera (10% FCS) and hormones (FSH, LH and E_2). Data reflecting the percentage maturation rate of morphologically classified goat oocytes are summarized in Table 1

In the present experiment morphologically classified oocytes were used for *in vitro* maturation. The proportion of poor quality oocytes arrested at GV stage 11.88% which was higher than that of good and fair quality oocytes. However, 3.24 and 6.25% oocyte in good and fair quality does not go beyond GV stage. The proportion of oocytes arrested at M-I differ significantly in good, fair and poor quality and per cent oocytes arrested at M-I exhibited 8.44, 24.30 and 20.97 per cent respectively. The percentage nuclear maturation rate reach to M-II of good, fair and poor quality oocytes were 85.06, 58.33 and 42.65% respectively. Thus the per cent maturation rate of good quality oocytes was significantly higher ($P<0.01$) as compared to fair and poor quality oocytes. The per cent degeneration was higher in poor (24.47) as compared to good (3.24) and fair (11.11) quality oocyte.

In the present study, oocytes were selected on the basis of their morphological characteristics. The per cent maturation rate of oocytes which was more than four layers of cumulus cells (i.e. good quality oocytes) was 85.06%. Maturation was lower in oocytes partially invested with cumulus cells. The maturation rate was found to further decrease to a significant extent when the oocytes were without cumulus cells (i.e. poor quality oocytes). In the present investigation, the maturation rate of good quality oocytes were similar to that reported previously (Pawshe *et al.*1994). Our result coincide with the finding of Younis *et al.* (1991) who have reported a reduced maturation percentage in unselected fair quality and denude oocytes maturation *in vitro* as compared to selected oocytes which have a compact cumulus cells investment. The completion of nuclear maturation does not necessarily identify normal cytoplasmic maturation. There are several

Table 1. *In vitro* maturation of morphologically classified goat oocytes (r=5)

Morphological classification of goat oocytes	Total number of oocytes	Maturation (%)			
		Germinal vesicle	Metaphase I	Metaphase II	Degeneration
Good	154	5 (3.24)	13 (0 8.44)	131 (85.06)[a]	5 (3.24)
Fair	144	9 (6.25)	35 (24.30)	84 (58.33)[b]	16 (11.11)
Poor	143	17(11.88)	30 (20.97)	61 (42.65)[c]	35 (24.47)

a, b, c mean value in the same column with different superscripts differ significantly ($P<0.01$).

essential components required for completion of both nuclear and cytoplasmic maturation; the most important being the presence of cumulus cells (Trounson 1992).

After 24 h of incubation oocytes with spermatozoa were fixed and evaluated for evidence of total fertilization, normal fertilization and abnormal fertilization. The total fertilization rate was 73.83, 60.00, and 39.56% in good, fair and poor quality oocytes. The normal fertilization rate with oocytes having two pronuclei was higher in good quality oocytes (67.28) than fair (55.00%) and (36.26) in poor quality oocytes. There was a significant (P<0.05) difference in total and normal fertilization rate between different categories of oocytes. In abnormal percentage of fertilization was higher in fair (5.55%) than good (4.67%) and poor (3.29%) respectively. Data reflecting the percent fertilization rate of morphologically classified goat oocytes are summarized in Table 2.

The per cent total fertilization and normal fertilization rate in oocytes containing more than four layers of cumulus cells, i.e. good quality oocytes, were 73.8% and 67.2% respectively. This observation is in consonance with the finding of Younis *et al*. (1991) who reported 60–68% normal fertilization in goat oocytes. However, the normal fertilization rate in the present investigation was higher than that observed by Mogas *et al*. (1993). The normal fertilization rate obtained with fair quality oocytes (1–2 layers of cumulus cells) was 55% and the fertilization rate obtained for poor quality oocytes was 36.26% in our study. These results are in agreement with the finding of Downs *et al*. (1986) who reported 41% fertilization rate in the cumulus free or denuded oocytes in mouse.

Suss *et al*. (1988) reported that completion of nuclear maturation of bovine oocytes is independent of medium supplementation with serum and gonodotrophin, but its effect on cytoplasmic maturation is necessary for fertilization and embryonic development. The addition of hormone, growth factor or presence of both these within the serum is used to supplement the culture medium which acts on the receptors present on the cumulus cells but not on the oocytes. This effect is mediated through the cumulus cells and exerts a beneficial effect on the oocytes. This is supported by the observation that the fertilization and development capacity of cumulus cell enclosed oocytes is improved when goat oocytes are matured in the presence of FSH, LH and E_2 (Pawshe *et al*.1996).

Cleavage rate of 65.32%, 55.00% and 32.11% in good, fair and poor quality oocytes were observed. The per cent cleavage rate was significantly higher in good and fair then poor. The cleavage rate and development rate of fertilized oocytes to morula and blastocyst stage was significantly higher in good quality oocytes (13.70% and 23.38%) as compared to fair (7.5% and 10.83%) and poor quality oocytes (4.37% and 9.48%), respectively. Data reflecting the per cent embryonic development rate of morphologically classified goat oocytes are summarized in Table 3.

Oocytes having more than four layers of cumulus cells, i.e. good quality oocytes cleaved at the rate of 65.32% and the development rate of morula and blastocyst formation from cleaved oocytes were 13.70% and 23.38%, respectively. However, with fair quality oocytes the percentage of cleavage formation was 55.00%. The percent of morula formation from cleaved oocytes was 7.5% and that of blastocyst formation was 10.83%. The difference was insignificant between good and fair quality oocytes for cleavage and morula development; nevertheless, the difference in blastocyst development was significant (P>0.05). In the present study 65.32% cleavage rate obtained in good quality oocytes was higher than that reported earlier by Younis *et al*. (1991).

Table 2. *In vitro* fertilization of morphologically classified goat oocytes (r=4)

Morphological classification of goat oocytes	Total number of oocytes	Number of ova fertilized (%)		
		Total	Normal	Abnormal
Good	107	79 (73.83) [a]	72 (67.28)[a]	5 (4.67)
Fair	100	60 (60.00) [b]	55 (55.00)[b]	5 (5.00)
Poor	91	36 (39.56) [c]	33 (36.26)[c]	3 (3.29)

a, b, c mean value in the same column with different superscripts differ significantly (P<0.05).

Table 3. *In vitro* cleavage and embryonic development of morphologically classified goat oocytes (r=4)

Morphological classification of goat oocytes	Total number of oocytes	Development %		
		Cleavage	Morula	Blastocyst
Good	124	81 (65.32)[a]	17 (13.70)[a]	29 (23.38)[a]
Fair	120	66 (55.00)[b]	9 (7.50)[b]	13 (10.83)[b]
Poor	137	44 (32.11)[b]	6 (4.37)[b]	13 (9.48)[b]

a, b mean value in the same column with different superscripts differ significantly (P<0.05).

Similarly, Fukui (1990) reported that a higher percentage of cleavage, morula and blastocyst development in good quality oocytes as compared to the fair and poor quality oocytes in cattle.

Buccione *et al*. (1990) suggested that the beneficial effect of cumulus cells on *in vitro* development could be either due to general conditioning of the medium or due to production of specific substances by cumulus and granulose cells. Sirard *et al*. (1988) have demonstrated that the follicle cells, especially the cumulus cells surrounding immature oocytes, play a crucial role in the developmental competence of embryos. The fertilization abnormality and low development of *in vitro* matured and fertilized bovine oocytes (Pavlok *et al*. 1992) are likely to be due to deficiencies in the cytoplasmic maturation and may be related to the type of oocytes and the size of follicles from which the oocytes are collected. This finding may be explained on the basis of incomplete synthesis of leading to the lack of synthesis of some essential protein for maturation (Sirard *et al*. 1989). In the present investigation, low cleavage and embryonic development was observed in denuded oocytes. It's may be because denude oocytes degeneration before maturation and fertilization. Staigmiller and Moor (1984) have demonstrated that an adequate number of cumulus cells and direct cell-oocyte contact is necessary for completion of meiosis and cytoplasmic maturation. Therefore, for successful *in vitro* maturation and fertilization, oocytes having compact cumulus cells should be selected which effectively undergo normal embryonic development.

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