Meiotic Competence of Caprine Oocytes During IVM on Granulosa Cell Monolayers Developed from Small and Large Follicles in Comparison to the Granulosa Cell Coculture

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ABSTRACT : Evaluation of the granulosa cell (GC) monolayers developed from small (<5 mm) and large (>5 mm) follicles on the meiotic competence of caprine oocytes during *in vitro* maturation was done in this study in comparison to the granulosa cell coculture. Ovaries were collected from the local abattoir and follicular contents were aspirated for the monolayer culture. For IVM the oocytes were collected by puncturing the nonatretic follicles (>4 mm). Results revealed that at the same seeding rate, small follicular granulosa cell monolayer achieved confluence 24-48 h earlier than large follicular granulosa cell monolayer. GC monolayers significantly p (<0.05) improved the rate of meiotic resumption and nuclear maturation (84.76% vs 74.74%) after 27 h of culture in comparison to GC coculture. Statistically there was no significant difference in the maturation rate between the caprine oocytes matured over small or large follicular GC monolayers. It is concluded from the present study that GC monolayers support better nuclear and cytoplasmic maturation of growing caprine oocytes which is evident by better maturation rate over GC monolayer as compared to the oocytes matured with GC coculture. Granulosa cells from small and large follicles can be used for IVM with more or less in the same efficiency after conditioning them with maturation media in 18-24 h before the onset of culture. (*Asian-Aus. J. Anim. Sci. 2001. Vol. 14, No. 6 : 777-784*)

Key Words : Granulosa, Monolayer, IVM, Maturation and Coculture

INTRODUCTION

Primary oocyte in mammalian ovary remains in a state of quiescence with the nucleus arrested in prophase I (dictyate) stage of meiosis until follicle start growing concurrently (Carrol et al., 1991), either for ovulation or atretic regression (Dvofak and Tesarik, 1980). Two cell populations are involved in cytoplasmic maturation, viz. granulosa and cumulus. Thibault and Gerard (1973) described the importance of granulosa cells in the cytoplasmic maturation of rabbit oocyte. Others later supported this observation for pig (Motlik and Fulka, 1974), bovine (Lebfried-Rutledge et al., 1986), Caprine (Tyagi et al., 1997) and ovine oocytes (Moor and Trouson, 1977). Granulosa cells are reported to initiate protein synthesis, necessary for cytoplasmic competence to assume normal cooperation with the male genome (Thibault et al., 1987).

Cognie et al. (1992) reported that for IVM, coculture of granulosa cells with cumulus oocyte complexes (COCs) in maturation medium (M.M.) supplemented with FSH., LH and E_2 increased developmental capacity of *in vitro* fertilized oocytes in sheep, goat and horses. Mochzuki et al. (1991) found that addition of 1.75×10^6 granulosa cells (G.C)/ml in culture medium with Fetal Calf Serum is helpful for both nuclear and cytoplasmic maturation. Dwinford et al. (1994) indicated that potential of immature COC to be fertilized and to complete embryonic development up to the blastocyst stage *in vitro* is improved when IVM media contains Estrous Calf Serum and /or Bovine Oviductal Epithelial Cells of Granulosa cells. Herrler et al. (1992) compared to the presence of IGF-I with and without G.C in coculture during IVM and IVC. They reported that addition of IGF-I to the maturation and IVC media can increase the number of morula and blastocysts but addition of IGF-I could not replace the effect of co-culture with granulosa cells.

Fukui and Ono (1989) advised the use of G.C from large diameter follicles, where there is more active synthesis of estrogen. In the same way other authors observed that percent of maturation, normal penetration, (Foundez et al., 1988) and development up to blastocysts stages (Fassi et al., 1991) are better when oocytes are matured with granulosa cells from preovulatory follicles than from small diameter follicles. Keeping in view of the importance of granulosa cells during oocyte maturation and their use in co-culture during IVM, Tyagi et al. (1997) have developed caprine granulosa cell monolayer, and used it for IVM of caprine oocytes and they reported that meiotic competence increased in comparison to that of GC co-culture however, there was a slight delay in maturation of oocyte on granulosa cell monolayer (32-34 h) but the number of M-II stage oocyte increased significantly (86.20%).

This study was planned with the objective to study

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the meiotic competence of caprine oocytes during IVM over granulosa cell monolayer developed from small (<5 mm diameter) and large (>5 mm diameter) follicles.

MATERIALS AND METHODS

Ovaries from adult cyclic goats were collected at a local abattoir in sterile NSS (0.85%) supplemented with 50 mg/l Steptomycin 100 units/ml penicillin and antimicotics, maintained at a temperature between 30-35℃ in an isothermic container and transported to the laboratory within 2 h of slaughter.

Preparation of granulosa cell monolayers

Follicular contents were aspirated separately from large (>5 mm diameter) and small (<5 mm diameter) follicles by 22 gauge needle attached to a syringe, from the apparently nonatertic follicles. Aspirated cells were pooled in two separate centrifuge tubes, mixed with modified PBS and settled at 50 g for 10 minutes. Supernatant was discarded and cells were reconstituted with fresh PBS and again centrifuged at the same rate. The process was repeated for 6-8 times or until the cell suspension was apparently bacteria free for both the cell types i.e. from small and large follicles. Bacterial load was observed under a phase contrast microscope (400x; Olympus, Japan). Finally both the cell types were reconstituted separately in medium RPMI-1640 with 5% goat serum, penicillin 1,00000 U/l, streptomycin 50 mg/l, amphoterecin 0.25 mg/l. Cell number was adjusted around $1\text{-}2\times10^6$ live cells/ml. Live cell number was counted with Neubar chamber after assessing the cell viability with trypan blue (0.4%) dye exclusion method. Seeding was done at 0.5 ml of cell suspension per well for 24 well culture plate of 1 ml of cell suspension in the canted neck culture flask and were cultured up to confluence at 38±1℃ and 5% CO_2 in humidified air.

Oocyte collection

Oocytes were collected by puncturing the nonatretic surface follicles (>4 mm diameter) with 18 gauge needle. The oocytes surrounded by a compact cumulus mass with an evenly granulated cytoplasm were selected under a steremicroscope and washed 5-6 times in oocyte collection media followed by 3 washings in wash media (TC 199, buffered with HEPES 25 mM+10% EGS+Glutamine 0.68 mM+Pyruvate 0.25 mM and antibiotics, pH 7.2-7.4, 280 mOsm and finally washed 3 times in maturation medium (TC199+ 1 μl/ml E_2, 0.5 μg/ml FSH, 100 IU/ml LH and 10% estrous goat serum, pH 7.2-7.4, 280 mOsm). Some granulosa cell masses were also collected along with the oocytes. These cells were washed similarly and used for co-culture.

***In vitro* oocyte maturation**

Cumulus oocyte complexes were divided randomly into 3 groups of almost equal numbers. The oocytes were matured in 150 μl maturation media on granulosa cell monolayers developed from: a) Small follicles b) large follicles, and c) with granulosa cells in 100 μl microdrops of maturation medium covered with embryo tested mineral oil. Granulosa cell monolayers were preconditioned with maturation media 18 to 24 h before IVM. The COCs were matured for 27 h at 38±1℃, 5% CO_2 in humidified air.

Evaluation of oocytes post culture

To evaluate the nuclear stage after IVM, oocytes from each group were mechanically and enzymatically stripped free of cumulus cells by repeated pipetting through a narrow glass pipette and placed in the center of an area delineated by two vaseline bars on a glass slide. The oocytes were compressed gently with a cover slip to hold, and fixed for 24-48 h at 4℃ in acetic acid: methanol (1:3; V:V). Afterwards they were stained with 1% (W/V) orcein in 45% (V/V) acetic acid and examined for the evidence of oocyte nuclear maturation under a phase contrast microscope. Percentage of maturation (number of oocytes in metaphase-II stage/total number of oocytes) was assessed from the samples obtained at 27 h of culture. Statistical analysis was done by Analysis of Variance (one way Classification).

RESULTS

Monolayer culture of granulosa cells

Granulosa cells were aspirated separately from the nonatretic small (5< mm diameter) as well as large (>5 mm diameter) follicles. Total number of cells was much higher in the fluid aspirated from small follicles as compared to the fluid from large follicles. Specific characteristics of the granulosa cells from small as well as large follicles are presented in table 1. It was repeatedly noted that the cells aspirated from the large follicles had greater tendency of gel formation in the collection tube. Immediately after isolation and collection, cell viability was observed and as it is evident from the table 1 that 75-85% of the cells were found viable from both the follicle types. Cell viability was noted during washing too, after two washes in serum free media cell viability was around 80%. The percentage of the viable cells was observed to be reducing slowly with the progression of washings. Only 60-70% in small follicular cells and 50-60% in large follicular cells were found viable after 5~6 washings. Contamination load of small follicular cells reduced faster as compared to that of large follicular cells. More number of RBCs was found contaminating the small follicular cells than

Table 1. Survivable characterstics of the granulosa cells used as feeder cell monolayer

Cranulosa cells	Viable % (approx)	Viable % after 5-6 washing	Gel formation	Attachment to surface (in hours)	% of cells attached	Time of conflunce (in days)	Surivability (in months)
From small follicles	75-85	60-70	-	3	40	3-4	>2
From large follicles	75-85	50-60	+	4	25	5-6	>2

large follicular cells. In both the cell populations small vesicle like structures were observed adhering the cell surfaces, which remained there throughout the processing until seeding.

The cells from small follicles were more in aggregates. Only small proportions of them were found in smaller group of 2-4 cells. These aggregates were resistant to pippeting and withstood complete dispersion. On the other hand large follicular cell suspensions were carrying very few of such cell aggregates while majority of the cell population was in dispersed form. Cell attachment started within 4-h post seeding. While comparing the two different cell populations, cells from smaller follicles started attaching to surface earlier than the cells from larger follicles. Proportionately, higher number of cells got attached to the surface from small follicles as compared to large follicular cells (table 1). Granulosa cells from the small follicles proliferated at higher rates in comparison to the cells from larger follicles. Small follicular granulosa cell monolayers achieved confluence just within 3-4 days while it took 5-6 days to become confluent in case of large follicular granulosa cell monolayers (figure 1).

Monolayers from both the cell populations showed honeycomb like structures, but they were more prominent in small follicular cell monolayers (figure 2). After passage, honeycomb structures were not seen in either of the two monolayers (figure 3).

In vitro maturation of Caprine oocytes

Out of 3130 oocytes collected by follicle puncture method, from apparently nonatretic surface follicles of 926 ovaries, 1945 good to excellent cumulus oocyte complexes were used for IVM. Per ovary 3.38 oocytes could be collected and out of this 2.1 culturable oocytes/ovary were selected (table 2). Fully/partially denuded oocytes comprised of 38.23% of total population, some oocytes with pycnotic and partially expanded cumulus mass were also observed. Oocytes of both of these types were not included in the study.

After 27 h of incubation, a marked cumulus expansion was visually observed. There were no differences in the degree of cumulus expansion in any of the groups. Cumulus expansion was remarkably better at 39℃ than at 38℃. After completion of the maturation, when oocytes were lifted from the maturation well, it was always noted that during handling, expanded cumulus mass made a very sticky aggregate, within which most of the oocytes got trapped. Oocytes were made free from this cell mesh before incubating them with the sperms. 27 h after *in vitro* maturation, oocytes from the monolayer as well

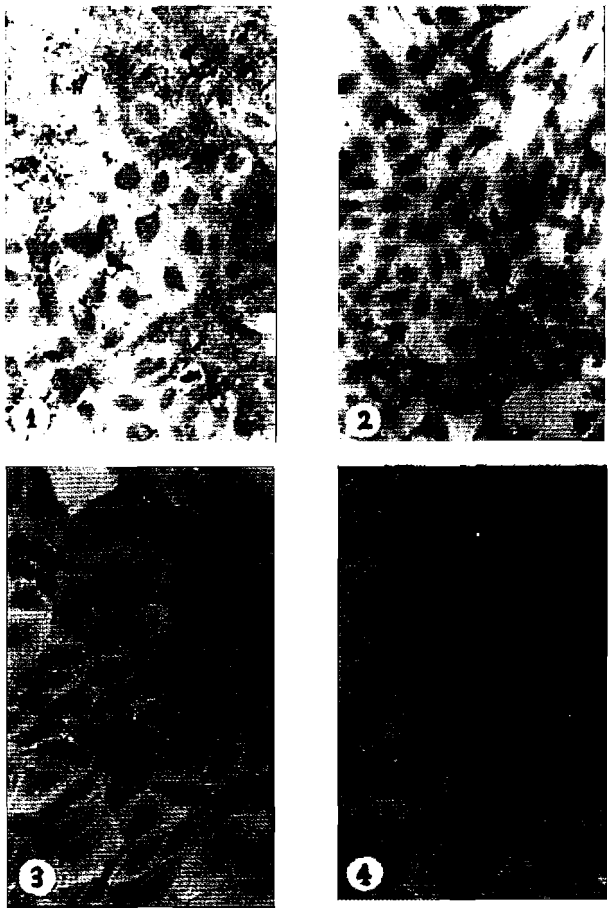

Figure 1. Photomicrographs showing monolayer of granulosa cells isolated and cultured from large and small follicles. Fixed and stained with crystal violet. 1. Confluent monolayer of granulosa cells collected from small follicles 4 days after culture. B/W (20× 10X). 2. Confluent monolayer of granulosa cells collected from small follicles 6 days after culture. B/W (20×10X). 3. Full confluent monolayer of small follicular granulosa cells after 8 days of culture. Colour (20×10X). 4. Full confluent monolayer of large follicular granulosa cells after 12 days of culture. Colour (20×10X).

as from G.C coculture were fixed and stained with aceto-orcein to determine the nuclear status of these oocytes. As it is evident from the table 3A that a total of 302 oocytes were fixed for the nuclear studies from granulosa cell monolayer group while 180 oocytes were used in the control group. After 27 h of oocyte maturation meiosis resumption was significantly ($p<0.05$) higher in the oocytes matured over granulosa cell monolayers (95.3%) than in oocytes matured with granulosa cell co culture (90.5). Significantly ($p<0.05$) higher number of oocytes reached at Metaphase-II (84.7%) stage when cultured over granulosa cell monolayer against 74.4% of the M-II stage oocytes co-cultured with granulosa cells only. In some of the oocytes M-II stage was very clearly observed without even fixation.

In the control GC cocultured group, 16.1% of the oocytes were noted at anatelophase-I while none of them was found in PM-II stage, on the other hand monolayer cultured oocytes showed a significantly less percentage of anatelophase-I oocytes (4.9%). In this group 5.6% of oocytes were found in PM-II stage, thus indicating continuity in the meiotic maturation.

While comparing the meiotic resumption in both the groups, it was observed that in the control group out of the total oocytes, in which meiotic resumption had taken place 82.2% of them, had reached to the metaphase-II stage. This figure was found 88.8% in case of the oocytes matured over granulosa cell monolayer. This comparison very clearly indicates better maturation efficiency over GC monolayers (table 3A).

With in the monolayer culture used during IVM when small and large follicular GC monolayers were compared (table 3B) it was observed that a little higher percentage (95.9%) of oocytes had resumed meiosis when matured on the monolayers prepared from small follicular cells than that of large follicular cells (94.5%) but the difference was too little and found statistically non significant. Table-3B shows that 83.9% and 85.9% of oocytes were in M-II stage when they were matured over small and large follicular GC monolayers respectively. From this it is clear that 5.7% and 6.3% of oocytes matured over small follicular granulosa cell monolayers were there in AT-I and PM-II stages oocytes against 3.9 and 4.68% for the large follicular cell group. This data indicates that there is a little slower progression of meiotic maturation in the oocytes cultured over small follicular granulosa cell monolayer compared to the larger one. But the difference is so very insignificant that it is not comparable.

DISCUSSION

Subpopulations of granulosa cells exist in ovarian follicles and play an integral role in providing proper microenvironment and cytoarchitectural support for the developing oocyte. Granulosa cells from various layers within the same follicle display morphological and biochemical heterogeneity, suggesting the existence of a differentiation gradient in the follicle (Amsterdam and Rotmensch, 1989).

While collecting the follicular aspirates frequent occurrence of gel in large follicular aspirates was noticed which was not seen in small follicular aspirates. Accidental aspiration of the atretic follicles could have been a probable reason for this. Rapid reduction in the proportions of viable cells in large follicular suspensions could be because of discrete cells or cells in small groups which are more sensitive to centrifugal shock, compared to the big groups of granulosa cells from small follicles. The pellet obtained after centrifugation was always larger in small follicular group. More number of small follicles having smaller antral space in the ovary as compared to the large follicles may be one of the main reason for that, another reasons could be that most of the cells in the small follicular suspension were in big

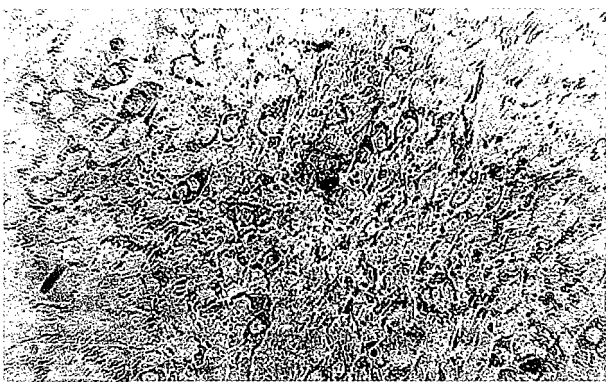

Figure 2. Monolayer developed from the small follicular cells after 4-6 days of culture showing honeycomb like structures. B/W (10 × 10X)

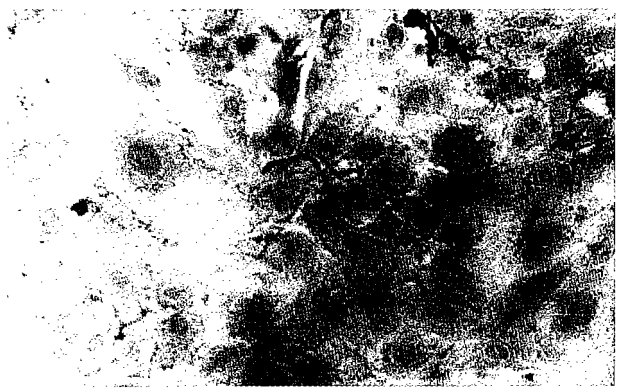

Figure 3. Granulosa cell monolayer after 6-8 days of culture. B/W (32 × 10X)

Table 2. Number of harvested and culturable oocytes from caprine ovaries

Total no. of ovaries used	Total no. of oocytes harvested	Total no. of culturable oocytes recovered	No. of oocytes/ ovary	No. of culturable oocytes/ovary
926	3130	1945	3.38	2.10

Table 3A. Nuclear status of caprine oocytes matured *in vitro* with Granulosa cell monolayers and Granulosa cell co-culture

Culture sysem	No. of Replicates	Total No. of Oocytes	GV	AT-1	PM-II	M-II	Meiotic Resumption/ Total No. of Oocytes	M. II/Meiotic resumption
G.C. co-culture	14	180	17^a (9.44)	29^a (16.11)	-	134^a (74.44)	90.55%	82.22%
G.C. MOnolayer	27	302	14^b (4.63)	15^b (4.96)	17 (5.63)	256^d (84.76)	95.36%	88.88%

In paranthesis percentage is shown. Values with different superscripts in a column differ significantly ($p<0.05$).
GV: Germinal vesicle, AT-I: Ana Telophase-I, PM-II: Prometaphase-II, M-II: Metaphase-II, G.C: Granulosa cell.

Table 3B. Nuclear status of caprine oocytes matured *in vitro* over small and large follicular Granulosa cell monolayers

Culture sysem	No. of replicates	Total no. of oocytes	GV	AT-1	PM-I	M-II	Meiotic resumption/ total no. of oocytes	M. II/meiotic resumption
Small follicular G.C	14	174	7 (4.02)	10 (5.74)	11 (6.32)	146 (83.90)	95.97%	87.42%
Large follicular G.C monolayer	13	128	7 (5.47)	5 (3.90)	6 (4.68)	110 (85.93)	94.53%	90.9%

In paranthesis percentage is shown.
GV: Germinal vesicle, AT-I: Ana Telophase-I, PM-II: Prometaphase-II, M-II: Metaphase II, G.C: Granulosa cell.

aggregates which settled easily and there was less cell wastage while discarding the supernatant as compared to large follicular cell suspension.

Small follicular cell suspensions attached to the surface of the culture wells earlier than the large follicular cell suspensions, which could be due to the dense aggregates which settled on the bottom of culture plates earlier than the discrete cells from the large follicles. Another reason for this could be the difference in viabilities of these two-cell populations. Skinner and Osteen (1988) observed that cells isolated from large follicles had a slightly lower plating efficiency as compared to those from small follicles.

Differences in the time to achieve confluence in two cell populations could be because of the differences in cell viability. After adding serum media cell viability cannot be assessed accurately as trypan blue also combines to some of the serum proteins (Griffiths, 1992). Initial viability showed high proportions of viable cells in small follicular cell populations. Differences in the plating efficiency could also be another probable reasons for that; thirdly rate of proliferation could also be higher in small follicular cell populations. In the present study, G.C survivability shown in table 1 was recorded for confluent monolayers, without passage. Gaspodarowicz and Bialecki (1978) observed that life span of cultures originating from small follicles were longer than those from large follicles were. G.C culture form 4-7 mm follicles had a life span of 11-12 generations while the life span of cultures originating form 13-15 mm follicles did not exceed seven generations.

Rouillier et al. (1996) have studied the morphological aspect of granulosa cells in culture and reported that in the absence of FSH, Bovine granulosa cells in culture were round and often in aggregates, whereas they were elongated and projected cellular sprouts in the presence of FSH. Similar observations were made in this study too. In the present experiment granulosa cells were not scraped but aspirated from the large and small follicles and used for monolayer preparation.

Evidences indicate that *in vitro* production of estradiol by bovine granulosa cells is significantly influenced by the location of cells within the follicle, size of follicle, from which cells have originated, duration of culture and incubator concentration of oxygen (Roberts and Echterkamp, 1994). Same group has reported that in culture, bovine granulosa cells from large follicles produced more estradiol than granulosa cells from small follicles but after 2-4 days E_2 was more in the small follicular cells and there was more progesterone in large follicular cells. It was

also recorded that aspirated granulosa cells produce several folds more E_2 than scrapped granulosa cells.

Isolated granulosa cells from either type of follicles i.e. Small and large were allowed to grow till confluence after isolation and then used for *in vitro* maturation. Previous study done with bovine vascular endothelial cells (Gospodarowicz et al., 1980) and vero cells have indicated that cells synthesize more peptides in their logarithmic growth phase than when confluent and resting. In contrast Savion and Gospodarowicz (1980) observed that in a monolayer culture bovine granulosa cells synthesized new peptides upon reaching confluence. Still there are no clear studies correlating peptides that are specific during *in vitro* maturation and culture. Probably in this study during IVM specific peptides were synthesized which have improved maturation rate and cleavage rate as compared to the control ones. Savion and Gospodarowicz (1980) indicated that differentiation process in granulosa cells involve a reduction in the number of proteins made by the cells or as the granulosa cells reach their final stage after terminal differentiation they become restricted in their ability to synthesize various proteins.

FSH was added in the maturation medium with which the monolayers were preincubated for 18-24 h before the onset of culture and hence there should have been an increase in the estradiol production by the granulosa cells as indicated by several workers. Increased estradiol production in response to FSH was reported in bovine granulosa cells cultured in defined media after precoating the wells with fibronectin (Saummnde, 1991) or with fetal calf serum (Alpizar and Spicer, 1993) or in plastic wells designed to enhance cell attachment (Bosndtson et al., 1995).

In the present study, it was obvserved that granulosa cells from small as well from large follicles survived for more than 2 months post culture. Though cells were passaged and kept as an alternative arrangement but preferably fresh monolayer cultures were used during IVM studies. Savion and Gospodarowicz (1980) opined that one obvious function of the granulosa cells is to provide nutrients for the oocytes, since during the initial 12 days of follicular development in mouse ovaries the oocytes quintuple in volume. Another function during the later stages of follicular development could be to contribute to the formation of liquor folliculi (from days 12-18 in mice). As mentioned earlier most of the cells express their function after getting attached to the surface (Griffiths, 1992). This is one of the possible reasons of getting a significantly higher percentage of maturation in the oocytes developed over granulosa cell monolayer as compared with the ones matured with granulosa cell co-culture.

In this study maturation of caprine COC over GC monolayer significantly ($p<0.05$) improved meiotic resumption (95.36%) as compared to those matured with GC coculture (90.55%) indicating a supportive role of GC monolayers during oocyte maturation. Tatemato and Terada (1995) suggested that newly synthesized proteins during first 8 h of culture are indispensable for including GVBD in oocytes.

It was found that chromatin condensation in bovine oocytes at GV stage was effectively inhibited by FSH stimulated cumulus cells regardless of the synthesis of new protein in the oocytes (Tatemato and Terada, 1996).

In this experiment the maturation medium used during IVM was supplemented with FSH at the rate of 0.5ug/ml and media used was same irrespective of the culture system used. So whatever FSH stimulated effects were present; they were similar for both the culture systems. This further proves a better influence of the granulosa cell monolayer system over the granulosa cell co-culture system. In a subsequent study Tatemoto and Terada (1988) indicated that FSH-stimulated cumulus cells suppress histone Hl kinase activity associated with chromatin condensation. They suggested that in meiotically incompetent oocytes specific proteins involved in resumption of meiosis were not expressed and/or those posttranslational modifications such as phosphorylation/dephosphorylation were inadequate.

Agarwal et al. (1995) matured caprine oocytes for 24 h with 20% EGS and found 54.79% M-II stage oocytes. Sharma et al. (1996) have used 20% EGS and matured caprine oocytes for 32 h and found 71.60% M-II stage oocytes. Both the groups have matured oocytes with 20% EGS without any addition of the gonadotropin in the maturation media. Although granulosa cells were used for co-culture in both the studies. Compared to these studies in the present work, the percentage of M-II stage oocytes were higher 74.44% when matured with G.C co-culture. Probable reasons could be either presence of gonadotropin in the present culture system of the follicle size which was >4 mm for this work.

In the present study most of the oocytes which did not complete meiotic maturation were blocked at AT-I stage (16.11%) of meiosis when matured with G.C co-culture. Data clearly indicates that oocytes could overcome this block when matured over GC monolayers. As the meiotic progression appeared to be continuos and there was even distribution of oocytes in the stages previous to M-II i.e. 4.96% in A.T-I and 5.62% in PM-II. Such blocks in the nuclear maturation of caprine oocytes matured with GC co-culture were also observed by Martino et al. (1995), Agarwal et al. (1995) and Sharma et al (1996). In a chronological study of nuclear maturation of caprine IVM oocytes Sharma et al. (1996) observed that such block was partially overcome by some of the oocytes after 18-20

h of culture, suggesting a minor insufficiency which was not seen with time in some oocytes.

While observing only those oocytes, which resumed meiosis it was observed that after 27 h of culture the proportions of oocytes that reached M-II were much higher (88.88%), for those matured over GC monolayers than with GC co-culture 82.22%. This is indicative of a faster meiotic progression which could probably be due to efficient overcoming of the meiotic block by the oocyes matured over GC monolayers, reflecting their superiority at cytoplasmic level. This also indicates that more number of M-II achieved is out only because of higher meiotic resumption, but also due to cytoplasmic competence of the oocytes. During this work efforts were made to study the effect of primed monolayer on IVM. Additionally all the granulosa cells in monolayers were viable but while using G.C co-culture proportions of viable cells were not exactly known. Since dead cells produce substances which can adversely affect oocyte maturation, the difference in the maturation rate amongst the oocytes matured with GC co-culture and over GC monolayer in this study may be due to the variation in the proportion of viable cells.

An important role of cumulus cells is to provide nutritional support to the developing oocyte (Buccione et al., 1990). Improvement in the IVMFC of farm animals by incorporating GC coculture during maturation could be due to the additional support in the nutritional requirement or generation of specific signals to the developing oocytes (Staigmiller and Moor, 1984). This support can be further enhanced by GC monolayers as it is well documented that many cells will express a required product more efficiently when attached to a substrate (Griffiths, 1992). Granulosa cell monolayer has been shown to produce growth factors (Satoh et al., 1994; Mulheron and Schomberg, 1992) which help in efficient maturation of oocytes.

The hormonal state of the follicles whose cells are used for IVM is important (Fukui and Ohno, 1989). Thus the development capacity of bovine oocytes is increased when they are matured with granulosa cells of preovulatory follicles instead of small follicles (Fassi et al., 1991). In ewes, it has been reported that granulosa cells from females primed with gonadotropins have a positive effect on cytoplsmic maturation, while cells from untreated females have a negative effect (Pugh et al., 1991). One more advantage of using granulosa cell monolayers for oocyte maturation is that they can be primed with the media containing gonadotropins before its use for IVM. This way it might mimic the effects of priming of females by gonadotropins.

While comparing the effect of monolayers from two different cell populations on *in vitro* maturation of caprine oocytes, it was observed that slightly more (95.97%). mumber of oocytes resumed meiosis when matured over GC monolayers derived from small follicles than those matured over GC monolayers from large follicles (94.53%). Though insignificant but proportions of oocytes reaching M-II at 27 h of culture was less (83.90) in the former than the later (85.93). Similarly out of the total oocytes which resumed meiosis, 87.42% reached to M-II at 27 h when matured over small follicular G.C monolayer. This proportion was increased to 90.90% on the large follicular GC monolayer, indicating a rapid progression of meiotic maturation in the later. It seems that granulosa cell monolayer culture originating from small or large follicles have almost same effect on maturation provided they are preincubated with hormones which are helpful in augmenting oocyte maturation.

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