MORPHOLOGICAL CHANGES IN CULTURED GRANULOSA CELLS FROM DIFFERENT SIZED FOLLICLES OF GOAT OVARY

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ABSTRACT

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The granulosa cells (GCs) from small (<2), medium (2-5) and large (>5) mm follicles of immature and mature goat ovary were aspirated and cultured in complete culture medium (McCoy's 5A medium) for studying the morphology and size related changes. GCs formed clusters of flattened cells with typical epitheloid morphology. The number of clusters and the GCs per cluster increased with growth of the follicle as well as with the growth of ovary. A progressive increase in diameter of granulosa cells was also observed with maturation of ovary. The cell diameter almost doubled between the small and large follicles of both immature and mature ovary.

Key words: Granulosa cell, Morphology, Size, Culture, Follicles, Goat.

INTRODUCTION

Profound changes in granulosa cell morphology take place during follicular development (Bjersing 1978, Albertini 1980). The developmental stage of granulosa cells can be judged by the size of follicle in which they reside (Channing and Ledwitz-Rigby (1975) Chang and Ryan (1976)). The avascular nature of GCs necessitates intercellular contacts between neighbouring cells. During follicular development extensive gap junctions were found among GCs (Albertini and Anderson 1974). The presence of extensive gap junctions indicated that these GCs were metabolically and ionically coupled (Hsueh*et al* 1984).

Lawrence *et al.* (1979) using electron microscopy showed that the unstimulated flattened cells of rat contain bundles of microfilaments, particularly in the cortical and basal regions. Morphological studies in cultured GCs indicated the presence of smooth endoplasmic reticulum, mitochondria with tubular cristae, lipid droplets and Golgi apparatus in these cells (Amsterdam *et al* 1981, Erickson 1983). Areekijseree and Vejaratpimol (2006) suggested that the porcine GCs in the follicular fluid were round in shape and found as clusters. After culturing in *in-vitro* for 48 hrs, no change in morphology was observed. However, the GCs appeared in small clusters or were present as single cells and their sizes ranged from 6-8 µm. Quinn *et al.* (2006) reported that human GCs grown *in-vitro* showed flattened fibroblast like morphology with lipid droplets and expressed the FSH receptors. Bovine GCs when cultured alone were flattened and formed a monolayer sheet. In the present study comparison of morphological and size related changes in cultured granulosa cells from small (<2 mm), medium (2-5 mm) and large sized (>5 mm) goat follicles is done and striking changes are observed.

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MATERIALS AND METHODS

Ovaries from immature and mature goats were collected from slaughter house and brought to laboratory in Dulbecco's phosphate buffer saline (DPBS) containing 100 IU/ml penicillin and 50 µg/ml streptomycin within 1 hr of slaughter in a thermos (20-25°C). The surrounding

tissues were trimmed and ovaries were washed thrice with DPBS containing antibiotics.

The number of visible follicles were classified and counted in each ovary and their diameter was measured with a dial vernier caliper and the follicles were classified into: small (<2 mm), medium (2-5 mm) and large (>5 mm) groups. The follicles were further classified as normal or atretic based on their appearance, transparency and vascularity as suggested by the method of Kruip and Dielman (1982). Briefly, follicles with uniformly bright, transparent appearance and extensive vascularity were classified as non-atretic or normal follicles. The surface of these follicles was taut, whitish grey in colour. The atretic follicles on the other hand had a dull grey or yellowish brown colour, slightly wrinkled or turgid surface.

The GCs were aspirated from the selected follicles with the help of 1 ml syringe fitted with a 23-gauge needle. The GCs from different sized follicles were then collected and pooled separately in PBS for immature and mature animals. The cells were separated from the follicular fluid by centrifugation at 3000-4000 rpm for 10 minutes. The follicular fluid was decanted and 1 ml of PBS was added and re-centrifuged at 3000 rpm for 10 minutes to remove the traces of follicular fluid and debris. Supernatant was discarded and pellet of GCs was resuspended in 1 ml of PBS.

Granulosa cell viability was checked using trypan blue dye exclusion method. In this method one drop of trypan blue dye (0.125%) and one drop of cell suspension was placed on slide and smear was prepared. After drying the smear, it was observed under microscope. The cells which take the stain were dead and rest were live. The percentage (%age) viability was tested.

The granulosa cell with viability >80 %were dispersed, counted and plated into 35 mm petridish containing a No.1 coverslip at a density of 5×10^5 cells/ dish in a complete culture medium (McCoy's 5A medium with bicarbonate supplemented with 20 mM HEPES, 100 IU/ml penicillin, 0.1 mg/ml streptomycin, 0.3% bovine serum albumin (BSA). The cultures were incubated at 37°C in a water saturated atmosphere of 95% air and 5% CO_2. After 24 hrs, the coverslip containing cells was placed on the glass slide and another coverslip was placed over it. Changes in cell morphology were monitored by phase-contrast microscope.

RESULTS AND DISCUSSION

GCs when cultured on No.1 coverslips, formed clusters of flattened cells with typical epitheloid morphology. The GCs taken from small follicles of immature ovary showed small clusters of 2-3 cells (Plate A). Majority of cells occurred as single cells in culture. The GCs retrieved from medium follicles of immature ovary formed clusters of 9-22 cells (Plate C), whereas those obtained from large follicles formed clusters of 22-47 cells (Plate E). The number of clusters increased in growing follicles.

In mature ovary, GCs retrieved from small follicles, formed clusters of 3-10 cells during culture (Plate B). The GCs taken from medium sized follicles formed clusters of 16-42 cells (Plate D) whereas in large follicles the GCs in culture formed large clusters of 30-110 cells (Plate F).

The present results are in accordance with previous studies made by Lawrence *et al.* (1979) who have observed the epitheloid morphology of rat GCs in cultures. Also Tajima *et al.* (2006) reported that bovine GCs cultured alone were flattened and formed a monolayer sheet. Number of clusters and cells in a cluster increase in the GCs of growing follicles. In pig ovaries, GCs from large follicles exhibited epitheloid morphology containing a large number of granules (Channing 1970). Profound changes in granulosa cell morphology also take place during follicular development (Bjersing 1978, Albertini 1980). Formation of clusters of GCs is indicative of formation of gap junctions between them. These gap junctions in growing follicles also indicate that GCs are metabolically and ionically coupled (Hseuh*et al.* 1984). Lawrence *et al.* 1979 further supported this view using electron microscopy which showed that the unstimulated flattened cells contain

bundles of microfilaments particularly in cortical and basal region. The increase in cellular clusters in GCs of mature ovary may be suggestive of increased hormonal responsiveness in mature animals than immature animals. Human GCs also express FSH receptors when grown *in-vitro* (Quinn *et al.*, 2006).

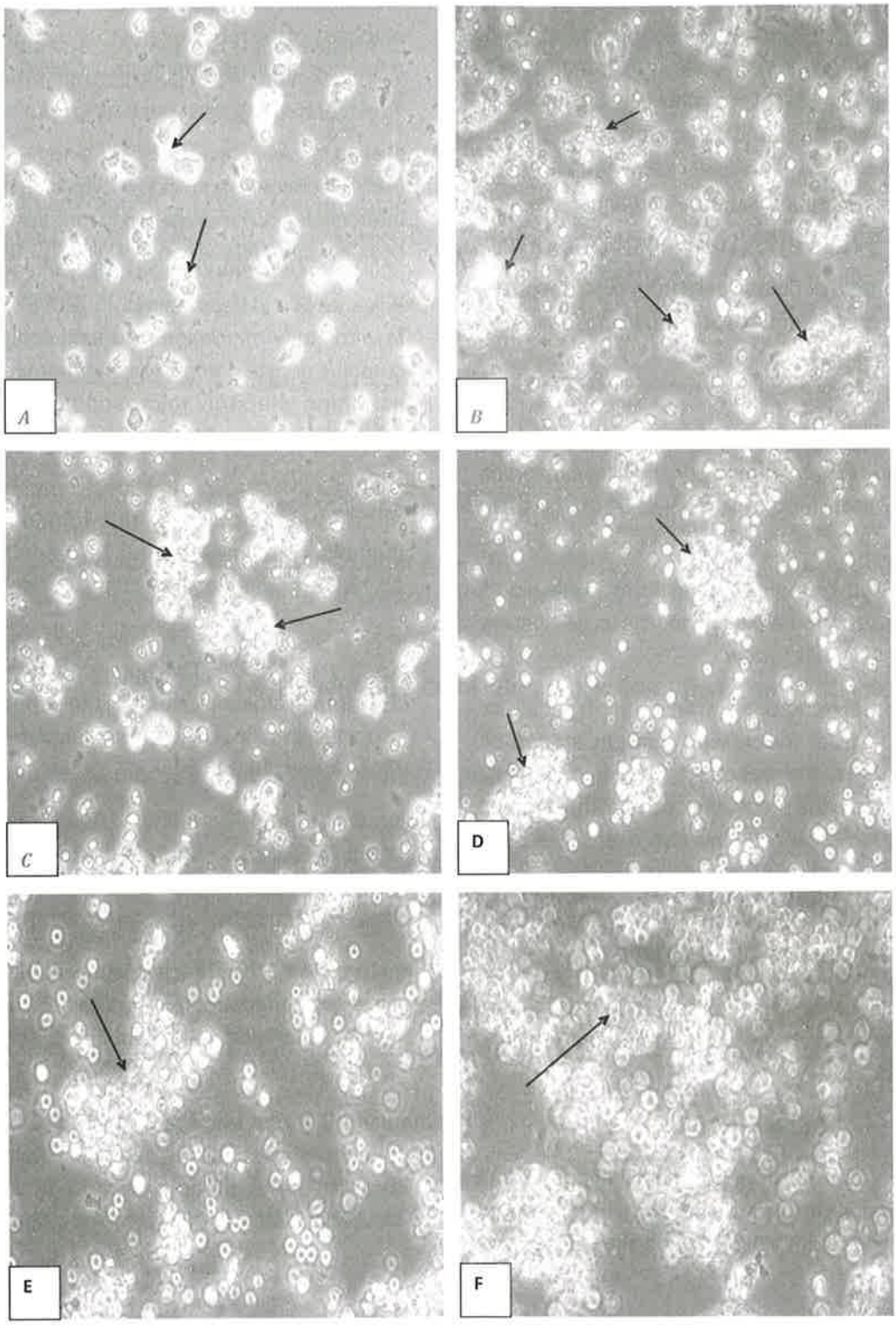

PLATE - GRANULOSA CELL MORPHOLOGY *IN VITRO*

FIG. A Cultured granulosa cells from small sized follicles of immature ovary Note small clusters of 2-3 cells (arrows).

FIG. B Cultured granulosa cells from small sized follicles of mature ovary showing clusters of 3-10 cells (arrows).

FIG. C Cultured granulosa cells from medium sized follicles of immature ovary showing clusters of 9-22 cells (arrows).

FIG. D Cultured granulosa cells from medium sized follicles of mature ovary showing clusters of 16-42 cells (arrows).

FIG. E Cultured granulosa cells from large sized follicles of immature ovary showing clustersof 22-47 cells (arrow).

FIG. F Cultured granulosa cells from large sized ollicles of mature ovary showing large clusters of 30-110 cells (arrow).

The progressive increase in diameter of granulosa cells aspirated from follicles at different stages of maturation is due to the reason that with development of the follicle before the preovulatory stage, the number of layers of granulosa cells increased and the cells become larger, primarily because of an increase in cytoplasm.(Chang *et al.,* 1977).

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REFERENCES

Albertini D F. (1980). Structural modifications of the granulosa cell plasma membrane during folliculogenesis. In: Motta PM and Hafez ESE (ed) *Biology of the ovary*. pp 138-49.MartinusNijhoff Publishers, The Hague, Boston, London.

Albertini D F and Anderson E. (1974).The appearance and structure of intercellular connections during the ontogeny of the rabbit ovarian follicle with particular reference to the gap junctions.*J. Cell. Biol.,***63:** 234-38.

Amsterdam A, Kenecht M and Catt K J. (1981).Hormonal regulation of cytodifferentiation and intercellular communication in cultured granulosa cells.*Proceed Nat .Acad.Sci.*,**78**: 300-09.

Areekijseree M and Vejaratpimol R. (2006). *In vivo* and *in vitro* study of porcine oviductal epithelial cells, cumulus oocyte complexes and granulosacells : A scanning electron microscopy and inverted microscopy study. *Micron* (Epub ahead of Print).

Bjersing L. (1978). Maturation, morphology and endocrine function of the follicular wall in mammals.In : Jones RE (ed) *The vertebrate ovary.* pp 181-214. Plenum Publishing Co, New York.

Chang S C S, Anderson W, Lewis J C , Ryan R J and Kang Y H. (1977). The porcine ovarian follicle.II .Electron microscopic study of the surface features of granulosa cells at different stages of development. *Biol.Reprod.*,**16**:349-57.

Chang S C S, and Ryan R J. (1976). A time related effect of PMSG on surface characteristics of the granulosa cells in the rat ovary. *Mayo Clinical Proceed.*,***51*:** 621-23.

Channing C P. (1970).Effects of stage of the menstrual cycle and gonadotrophins on luteinization of rhesus monkey granulosa cells in culture.*Endocrinol.*,**87**; 49-52.

Channing C P and Ledwitz-Rigby F. (1975).Methods for accessing hormone-mediated differentiation of ovarian cells in culture and in short time incubation.*Methods of Enzymology,***39(D):** 183-230.

Erickson G F. (1983). Primary cultures of ovarian cells in serum free medium as models of hormone-dependent differentiation. *Cell Endocrinol.*,**29** : 21-26.

Hsueh A J W, Adashi E Y, Jones P B C and Welsh T H. (1984). Hormonal regulation of the differentiation of cultured ovarian granulosa cells.*Endocrinol.Revi.*, **5**: 76-126.

Kruip A M and Dielman S J. (1982).Macroscopic clarification of bovine follicles and its validation by micromorphological procedures. *Reprod. Nutri. Deve.*, **22**: 465-70

Lawrence T S, Ginzberg R D, Gilula N B and Beers W H. (1979). Hormonally induced cell shape changes in cultured ovarian granulosa cells *J. of Cell.Biol.*,**80**: 21-26.

Quinn M C, McGregor S B, Stanton J L, Hessian P A, Gillet W R and Green D P. (2006). Purification of granulosa cells from human ovarian follicular fluid using granulosa cell aggregates. *Reprod.Fertil.Deve.*,**18**: 501-08.

Tajima K, Orisaka M, Yata H, Goto K, Hosokawa K and Kotsuji F. (2006). Role of granulosa and theca cell interactions in ovarian follicular maturation.*Micros Res. Tech.*,**69**: 450-58.