IN VITRO MATURATION OF Bos indicus OOCYTES - EFFECT OF CUMULUS OOCYTE COMPLEX MORPHOLOGY

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India is having large number of cattle breeds and most of them are on the edge of extinction. Since cow slaughter is banned in India except in state of Kerala and West Bengal much work has not been carried out in Bos indicus cattle in the field of IVM and IVF; which is a very powerful tool for conservation of breeds and species. This experiment was designed to carry out a study on Cumulus Oocyte Complex morphology on in vitro maturation potential of Bos indicus oocytes.

Key words: Oocyte, in vitro Maturation, Cumulus Oocyte Complex.

Slaughterhouse derived ovaries of south Indian breeds were used in this study. Ovaries were dissected out from animals within 30-60 minutes of slaughter and transported to the laboratory in normal saline fortified with antibiotics and maintained at 36-38°C. Oocytes were retrieved from ovaries by any one of the retrieval methods namely aspiration, slicing and puncture, carried out in HEPES buffered Tyrode’s lactate medium enriched with BSA @ 0.6% and maintained at 37°C. Heparin was supplemented to this medium @ 0.1 mg/ml.

Based on number of layers of cumulus cells and ooplasm character, the oocytes were graded into four classes (deWit and Kruip 2001). Class A with more than 5 complete layers of cumulus cells and uniform granulation of ooplasm, Class B with 3-5 complete layers of cumulus cells and uniform granulation of ooplasm, Class C with 1-2 complete layers of cumulus cells and uniform granulation of ooplasm, Class D as denuded oocytes with uniform granulation of ooplasm.

Oocyte maturation medium containing TCM-199 enriched with FSH 0.5 μg/ml (Folltropin – V, Vetrepharm Canada Inc.), LH – 5 μg/ml (Lutropin - V, Vetrepharm Canada Inc), oestradiol 1 μg/ml, sodium pyruvate 0.2 mM and Foetal Calf Serum (FCS) 10% was freshly prepared. Culture condition set for this study was 38.5°C temperature, 5% carbon dioxide tension and maximum humidity. Standard water-jacketed type CO₂ incubator (Lab line instruments inc, USA) was used to achieve this culture environment. After 24h of culture, all oocytes in the culture drops were examined under zoom stereomicroscope (Leica MZ-6, Leica micro systems, Germany) for maturation associated changes such as expansion and mucification of
cumulus cells. The oocytes showing cumulus expansion were denuded by vortexing, stained with one per cent aceto-orcein (with 1% orcein in 45% acetic acid), (Li et al., 2002) and then examined for nuclear changes associated with maturation.

Data was analysed with Chi-square analysis and those data showing significant difference was subjected to pair wise data analysis with Chi-square test. All chemicals and media used in this study were from Sigma Chemicals St. Louis, USA, unless mentioned otherwise.

On analysis, it was found that class A oocytes were having no significant difference in maturation rate than class B oocytes, except in slicing method. But significantly lower maturation rate was observed for class C oocytes in comparison to class A and B (P=0.05). Class D oocytes failed to mature in all methods of retrieval. Results are expressed in detail in table 1 and 2.

Results of the study clearly indicated that the morphology of COCs have significant effect on maturation of bovine oocytes. Greater the number of cumulus layers greater was the maturation rate. Oocytes with more than 3 layers of cumulus cells exhibited significantly higher maturation rate than the denuded oocytes and oocytes with partial cumulus layers. These results agreed with the results of Leibfried and First (1979) and Konishi et al. (1996).

Cumulus cells communicate to the oocyte across zona pellucida through corona radiata cells, which penetrate the zona pellucida and form gap junctions with oolemma. These intercellular communications allow metabolic transfer as molecules of small molecular weight and help in nutrition of oocytes, which ultimately plays a vital role in oocyte growth and maturation (Buccione et al., 1990; Armstrong et al., 1996). Staigmiller and Moor (1984) reported that granulosa cells provide energy substrate, some amino acids, nucleotides and phospholipids precursors to the oocyte, that generate some interactional signals which influence the nucleus and direct the synthesis of certain structural proteins and maturation specific proteins.

Lorenzo et al. (1994) reported both EGF and IGF-I alone or together stimulated nuclear maturation in immature bovine oocytes and opined that their beneficial effect was mediated through cumulus cells. IGF II with FSH synergistically enhanced DNA synthesis, protein synthesis and steroidogenesis in the presence of granulosa cells.(Pawshe et al., 1998).

So the result of this experiment is in confirmation with the view that the cumulus oocyte complex morphology have definite role in the in vitro maturation of Bos indicus oocytes. Oocytes with multiple layers of cumulus cells (Three or more layers) matured better than the denuded oocytes or oocytes with lesser number of cumulus cells (Class C and D). Best quality oocytes for in vitro maturation purpose in Bos indicus cattle are oocytes with more than three complete layers of cumulus cells (Class A and B).

REFERENCES


