# EFFICACY OF *IN-VITRO* MATURATION OF BUBALINE OOCYTES IN TCM 199, TCM 199 + PMSG AND FOLLICULAR FLUID.

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## ABSTRACT

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A total number of 498 ovaries were procured from municipal slaughter house, Hyderabad. The ovaries were subjected to slicing, aspiration and post aspiration slicing in order to retrieve the oocytes. The oocyte retrieval rate was 73.68, 43.61 and 47.91 per cent in slicing, aspiration and post aspiration slicing methods, respectively. The oocyte recovery per ovary was 2.91, 1.53 and 1.89 in slicing, aspiration and post aspiration slicing, respectively.

Retrieved oocytes were graded into A, B, C and D based on cellular investment and homogencity. The yield of grade A oocytes were 53.92, 42.57 and 46.72 in slicing, aspiration and post aspiration slicing techniques, respectively. The yield of grade B oocytes was 28.87, 20.92 and 29.10, respectively. The yield of grade C and D oocytes were 15.69 and 9.52; 11.27 and 11.24; and 13.93 and 10.25 in slicing, aspiration and post aspiration slicing methods, respectively. The A, B and C graded oocytes were matured in TCM 199, buffalo follicular fluid (buFF) alone and TCM 199 supplemented with PMSG. Based on the degree of cumulus expansion, the maturation was classified as D0, D1 and D2 cumulus expansion. Significantly higher per cent of grade A and B oocytes attained D2 and D1 degree of cumulus expansion while significantly higher per cent of C grade oocytes retained in D0 degree of cumulus expansion. The per cent of grade A oocytes extruded first polar body was 70.83, 5.55 and 42.50 in PMSG, buFF and TCM 199 media, respectively Higher per cent of oocytes could be harvested by post aspiration and slicing technique and commercially available PMSG could be supplemented to TCM 199 media for invitro-maturation of follicular oocytes.

Key words: Buffaloes, Oocytes, Invitro Maturation, Follicular Fluid, TCM 199, PMSG.

## INTRODUCTION

Buffaloes are multipurpose animals providing milk, meat and draught power. Their contribution to Indian dairy industry is more than 50 per cent.

Recent advances in embryo bio-technology have made it possible to recycle the germplasm to a certain extent through the technique of in-vitro fertilization (IVF) where follicular oocytes from the ovaries of slaughter house can be retrieved, matured and fertilized in-vitro with capacitated sperm, so that these embryo's can be further transferred into the recipients. However, progress in the development of effective in-vitro fertilization protocols for buffalo has been low and very limited information is available on oocyte maturation. Hence, the present study was designed to develop suitable method for recovery of optimum number of oocytes from slaughter house ovaries and to mature bubaline oocytes in three different maturation media.

## MATERIALS AND METHODS

The ovaries were collected in pairs from individual genital organs from pluriparous buffaloes slaughtered

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at municipal abattoir, Hyderabad, soon after the slaughter. The ovaries were kept in normal saline supplemented with 50 µg of gentamycin sulphate per ml of normal saline. The ovaries were washed 5 times with sterile phosphate buffered saline (PBS) containing 50 µg of gentamycin sulphate per ml. The ovaries were exposed to 70 per cent ethyl alcohol for 30 sec and finally washed with PBS. The oocytes were retrieved aseptically by slicing, aspiration (Suresh Kumar and Mourya, 2000) and post aspiration slicing methods (Kumar *et al.* 1997). The retrieved oocytes were washed with Dulbecco's Phosphate buffer.

Based on the cellular investment and homogenicity, the oocytes were classified as Grade A, B, C and D (Chauhan *et al.* 1998). Grade A: COC's with unexpanded cumulus cells having at least 5 layers of cumulus cells with homogenous cytoplasm. Grade B: COC's with 2-4 layers of cumulus cells and with homogenous cytoplasm. Grade C: Oocytes partially denuded of cumulus cells and with irregular shrunken cytoplasm. Grade D: Oocytes completely denuded of cumulus cells with irregular shrunken cytoplasm.

The oocytes graded as A, B and C was selected for invitro maturation in the following three media.

Control : TCM 199 + 10 per cent BES (bovine estrus serum).

PMSG : TCM 199 + 10 per cent BES + PMSG 40 IU/ml.

buFF : Buffalo follicular fluid alone without any supplementation. (The follicular fluid collected from more than 5 mm follicles alone was used as a media for maturation.).

The oocytes were assessed for maturation after 24 hrs of incubation. Oocytes with moderate and fully expanded cumulus cell masses and the unexpanded oocytes with extruded first polar body in the peri vitelline space were considered as matured (Nandi *et al.*, 2001).The Cumulus expansion was evaluated by the classification scheme of degree of expansion (Kobayashi *et al.*,1994) as follows.

Degree 0: Cumulus cells were slightly adherent to the zona pellucida. (Slight or no expansion) - "D0".

Degree 1: 70 per cent of cumulus cells homogeneously expanded. (moderate cumulus cell expansion)- "D1".

Degree 2 : All cumulus cells homogeneously spreaded. (Full cumulus cells expansion) - "D2"

Oocytes with D 1 and D 2 cumulus expansion were considered as morphologically matured oocytes in the study. The oocytes were removed from the IVM drops and were transferred to a 35 mm petri dish containing TCM199 medium. The cumulus cells were stripped from the oocytes by gentle pipetting with the help of mouth pipette. The denuded oocytes were transferred to another petri dish. The oocytes were observed under phase contrast microscope for extrusion of first polar body in the perivitelline space. The per cent of oocytes extruded first polar body in different medias was recorded to compare the efficacy of media on maturation of buffalo oocytes.

## RESULTS AND DISCUSSION

A total number of 206 ovaries were subjected to slicing which yielded 599 oocytes with an average yield of 2.91 oocytes per ovary. In aspiration technique 249 oocytes were aspirated by using 163 ovaries with an average of 1.53 oocytes per ovary. In post aspiration slicing technique, 244 oocytes were retrieved from 129 ovaries with an average of 1.89 oocytes per ovary. A total of 1092 oocytes were retrieved from 498 ovaries with an average of 2.19 per ovary.

The per cent retrieval of oocytes was higher in slicing technique (73. 68) followed by post aspiration slicing (47.94) and aspiration (43.69) methods. Similar studies were reported by Suresh Kumar and Mourya (2000) and Abdul Razek Ali (2005) in buffaloes. Variation in the oocyte yield might be due to seasonal variation. The present study concluded that comparatively higher

number of oocytes retrieved by slicing method followed by post aspiration slicing and aspiration. It was reported that the recovery rates of oocytes were higher by ovum pick up technique in conjunction with stimulation of ovaries with FSH (Sait Sendeg *et al.,* 2008).

The pooled data revealed that there was significant ($p<0.01$) difference between the grades of oocytes where higher per cent of grade A oocytes were retrieved followed by grade B, C and D which is in accordance with the studies of Wang *et al.* (2007).

The degree of cumulus expansion of different grades of oocytes in 3 different medias showed.Significantly ($p<0.05$) greater per cent of oocytes developed to D2 stage of cumulus expansion in PMSG and significantly ($p<0.05$) higher per cent of oocytes retained in D0 stage of cumulus expansion in TCM199 followed by buff and PMSG medias. Whereas there was no significant difference in oocytes reached to D1 stage of cumulus expansion in all three medias.

Significantly ($p<0.05$) higher per cent of A and B grade oocytes reached to D2 and D1 degrees of cumulus expansion and significantly ($p<0.01$) higher per cent of C grade oocytes retained in D0 degree of cumulus expansion. The expansion of granulosa cells might be due to production of hyaluronic acid (Chen *et al.*, 1990) and mucification under the influence PMSG as a part of morphological maturation of oocytes. However these changes were not present in TCM199 and buff media as these media were having nil or insufficient gonadotropin to bring the morphological maturation of oocytes. FSH and LH like action of PMSG might have stimulated TCA cycle by synthesizing pyuruvate to produce sufficient ATP for the energy requirement of oocytes (Brackett and Zuelke., 1993).

Higher per cent of oocytes extruded 1st polar body in PMSG media while higher percent of oocytes did not extruded 1st polar body in buFF media. Similar studies were also carried by Gupta *et al.* (2001)[a,b], Nandi *et al.* (2001) and Ravindranath *et al.* (2003).

Lesser per cent of oocytes matured in buFF media might be due to presence of oocytes maturation inhibiting protein fraction (Coleman *et al.,* 2007) in follicular fluid of preovulatory follicles. The variation in maturation rate of oocytes might be attributed to source of follicular fluid as the follicular fluid from large follicles had lesser inhibiting affect than the follicular fluid collected from small and medium sized follicles(Ayoub and Hunter, 1993). Thus the follicular fluid from preovulatory follicles supported in-vitro maturation to certain extent but not complete development capacity of bovine oocytes (Birthe Avery *et al.,* 2003) in this study.

It is concluded that PMSG can be effectively used for in-vitro-maturation of buffalo oocytes in place of pure FSH and LH preparations making it cost effective for in-vitro maturation and fertilization studies.

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