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Effect of oocyte collection techniques and maturation media on *in vitro* maturation of buffalo oocytes

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The experiment was designed to evaluate the effect of brilliant cresyl blue (BCB) stain, oocyte recovery technique and addition of hormones and sera in basic maturation media on IVM rate of buffalo oocytes.

Buffalo ovaries of unknown reproductive status were collected from the local slaughterhouse and transported within 2 h of slaughters to the laboratory at 37 to 38°C in a pre-warmed thermos flask containing 0.9 % normal saline solution supplemented with penicillin G (100 IU/ml) and streptomycin sulphate (100 µg/ml). The oocytes were collected either via aspiration of visible follicles by aspiration through 18-guage sterile needle or slicing of ovaries into small pieces with sterile surgical blades. Oocytes surrounded by 1–5 layers of cumulus cells with a full cumulus mass, un-fragmented ooplasm and intact zona pellucida were selected for further experiment. The selected cumulus oocyte complexes (COCs) were washed 3 times in Dulbecco's phosphate buffer saline (DPBS) without bovine serum albumin and exposed to 26µM of BCB diluted in DPBS for at least 90 min at 38.5°C in humidified air atmosphere at 5% CO_2. Following exposure to BCB, the COCs were washed 3 times in DPBS. Then the COCs were examined under stereo-zoom microscope, and according to their cytoplasm coloration they were divided into 2 groups, viz. oocytes with varying degree of blue coloration to the cytoplasm (BCB+, grown oocytes) and oocytes without blue cytoplasm (BCB-, growing oocytes).

The oocytes were washed serially drop-wise in maturation medium and batches of 5 to 10 oocytes were transferred to the different maturation media, viz. TCM 199 (group 1), TCM 199 + FBS + hCG (group 2), Ham's F-10 (group 3) and Ham's F-10 + FBS + hCG (group 4). Maturation of the oocytes was monitored after 24 h of culture to assess the degree of cumulus cells expansion surrounding the oocytes and graded by the methods followed by Kobayashi *et al.* (1994): Degree 0 - slight or no expansion (cumulus cells adhered to the zona pellucida), degree 1 - moderate cumulus cells expansion (cumulus cells non-homogenously spread with presence of clustered cells), degree 2 - full cumulus cells expansion (all cumulus cells homogenously spread, no clustered cells). Oocytes with degree 1 and degree 2 cumulus expansions were considered as matured. The data were suitably analyzed using SPSS statistics (version 20) software. The differences among the parameter means were performed using chi-square test.

In the present study, from among all the 4 media used for IVM, maximum maturation was observed in group 2 (54.65%), followed by group 4 (53.15%), group 1 (48.21%) and group 3 (39.08%). However, the difference was statistically nonsignificant. As like present study, Raza *et al.* (2001), Hammam *et al.* (2010) compared TCM – 199 and Ham's F – 10 media with and without different hormones and sera for *in vitro* maturation rate. In accordance with the present findings, Kumar and Maurya (2005) concluded that although the buffalo oocytes can be matured in TCM-199 and Ham's F-10 medium alone but addition of sera and hormones significantly ($P<0.05$) improves the maturation of bubaline oocytes in TCM-199 and Ham's F-10 medium.

During present investigation, higher maturation rate was observed in TCM 199 than Ham's F-10 media. Similar results were obtained by Raza *et al.* (2001) as they found better maturation rate in TCM-199 than Ham's F-10 media. Hussain *et al.* (2012) also reported highest expansion of cumulus cells in TCM-199 + additives and lowest in Ham's F-10 + additives.

Significantly ($P<0.05$) higher maturation rate was achieved in aspiration (56.77%) than slicing (42.70%) technique in the present study. The result obtained by Chandrahasan *et al.* (2012) was in agreement with the present study, as they also reported higher mean percentage of maturation of first quality oocytes to Metaphase II in aspiration than dissection methods. Mehmood *et al.* (2011) also reported that the IVM rate was better ($P<0.05$) based on expansion and GVBD with aspiration than with the slicing method in buffaloes. However contrary to the present

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findings, Hussain *et al.* (2012) reported highest expansion of cumulus cells in dissection than aspiration in TCM-199 + additives and in Ham's F-10 + additives. While, MasudulHoque *et al.* (2011) observed almost similar results in the cumulus expansion and metaphase-II stage in slicing and aspiration methods. This could be due to better quality of oocytes recovered by aspiration than slicing technique.

Nonsignificantly higher maturation rate was observed in BCB+ oocytes (53.17%) as compared to BCB– oocytes (42.96%) in present experiment. In accordance with the present study, Heleil and Fayed (2010) also observed significantly lower ($P<0.05$) metaphase II rate in BCB- than BCB+ oocytes in all follicles' diameter. Shanthi *et al.* (2012) also observed significantly higher ($P<0.05$) maturation rate in BCB+ than in BCB– oocytes. According to them this was due to more than 3 layers of COCs on oocytes.

In conclusion, staining of bubaline cumulus–oocyte complexes with Brilliant cresyl blue before IVM could be used to select developmentally competent oocytes, as BCB+ oocytes resulted in higher maturation rate in comparison BCB– oocytes. Moreover, oocyte recovered by aspiration technique and addition of sera and hormones into basic maturation media, viz. TCM-199 and Ham's F-10 may yield better IVM rate for buffalo oocytes.

SUMMARY

The study was conducted to know the effect of oocyte collection techniques and various maturation media on *in vitro* maturation of buffalo oocytes obtained from slaughterhouse derived ovaries. Highest maturation rate was observed in TCM 199 + FBS + hCG media, followed by Ham's F-10 + FBS + hCG, TCM 199 and Ham's F-10 alone. The difference between them was statistically nonsignificant. Maturation rate was significantly higher in oocytes collected by aspiration than by slicing technique. Nonsignificant difference was observed in maturation rates in BCB+ and BCB– oocytes. In conclusion, addition of sera and hormones into basic maturation media, viz. TCM-199 and Ham's F-10 resulted into better *in vitro* maturation rate.

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