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Influence of cadmium on buffalo oocyte and embryo development *in vitro*

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Exposure to cadmium during *in vitro* maturation negatively affects both oocyte maturation and caused chromosomal aberrations in bovine (Rodriguez Tellez *et al.* 2005) and oocyte maturation and subsequent fertilization competence in ovine (Leoni *et al.*2002). *In vivo* experiments have shown to cause increased embryo mortality and lowered the pregnancy rates in rats (Kuo *et al.*1995). It is a well known fact that the buffaloes under Indian conditions are not stall-fed and often let out for grazing. There is a greater chance that the buffalos are more prone for exposure to wide variety of chemicals and other environmental pollutants including cadmium. Therefore, the present study was designed to test the effect of cadmium on buffalo oocyte maturation and embryo development**.**

Oocytes were aspirated from ovaries retrieved from mature, non-pregnant buffaloes (*Bubalus bubalis*) collected from the corporation slaughterhouse, Bengaluru. Oocytes with more than 2 layers of cumulus cells with homogenous and evenly granular grey ooplasm were used in this study. The oocytes were transferred into 50 µl droplets (5–10 oocytes in a group/droplet) of TCM-199 supplemented with 10% FBS, 50 mM cysteamine and 0.02U pFSH in a 35 mm petridish. The evaluation of oocytes for *in vitro* maturation was based on the visual assessment of the degree of expansion of cumulus cells under steriozoom microscope as per the criteria described by Nandi *et al.* (2002). The processing of sperm and *in vitro* insemination were done as per Manjunatha *et al.* (2009).

Two experiments were performed to evaluate the influence of Cd exposure on oocyte maturation and fertilization and embryo development compared with a control group.

Experiment 1: Oocytes (8–10 ooctytes per droplet) were cultured in 7 culture media studied. TCM 199 + 0.02 U pFSH +10% FBS+50 mM of cysteamine was considered as control medium. Cadmium was added at 0.5, 1.0, 1.5, 2.5, 5 and 10 mg/ml level.

Experiment 2: The cleaved embryos (3–4 embryos per droplet) were cultured in 7 culture media studied. TCM 199 +10% FBS +cumulus cell monolayer was control considered as control medium. Cadmium was added at 0.5, 1.0, 1.5, 2.5, 5 and 10 mg/ml level. Embryos were evaluated for their development under zoom stereomicroscope.

Both experiments were replicated 12 times. The statistical software "Graph pad Prism" was used for the statistical analysis. The variations between the different groups was made by employing one way Analysis of variance followed by Dennett mean comparison test to compare treatment means with control group (significant when the probability values were less than 0.05).

After 24 h of *in vitro* culture, the COCs exposed to Cd showed abnormal expansion of cumulus cells and an extension of contacts between oocyte and cumulus cells compared to control oocytes. At 5 and 10 mg/ml cadmium most somatic cells were found free in the medium and the COCs exhibited complete degeneration. The presence of Cd in the culture media significantly affected oocyte maturation rate after 24 h of culture. The maturation rate of oocytes significantly decreased linearly with the increasing concentration of cadmium in the culture media. The mean fertilization rate although decreased progressively with the increasing concentration of cadmium but did not differ significantly at a concentration of 0.5, 1, 1.5 mg/ml when compared to control. Nevertheless, a dose dependent significant decline in fertilization rates were observed in oocytes cultured in media containing 2.5, 5, and 10 mg/ml cadmium in the culture media when compared to control. None of the oocytes which were exposed to 5 and 10 mg/ml cadmium cleaved. The mean proportion of embryos developed to morulae and blastocysts did not significantly influenced by the presence of cadmium in culture medium. Degeneration of oocytes after one day of culture was observed when oocytes were cultured in media containing 10 mg/ml cadmium. Asynchronous/ abnormal embryonic

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development was observed after three days of culture in media containing high concentration of cadmium (5 and 10 mg/ml cadmium).

The toxic properties of cadmium were associated with the alterations on oocyte maturation in cattle (Rodriguez Tellez *et al.* 2005) alterations in oocyte maturation and fertilization in sheep (Leoni *et al.* 2002) and subsequent development of cleavage of embryos in mice (Yang *et al.* 2004) were reported. The abnormal expansion of cumulus cells could be due to a decrease in the coupling between cumulus cells, probably secondary to alteration in the actin cytoskeleton and interference with cadherin–catenin complexes (Leoni *et al.* 2002). *In vitro* studies in porcine (Mlynarcikova *et al*. 2004) oocytes when exposed to cadmium resulted in abnormal cumulus, cell expansion (at lower doses of cadmium) or no cumulus expansion (at higher doses of cadmium) is due to the reduced deposition of hyaluronic acid in cumulus oophorus matrix. The other possible reasons for abnormal cumulus cell expansion in cadmium exposed oocytes could be the inhibitory effect of cadmium on the cumulus expansion that was accompanied by decreased progesterone synthesis by cumulus cells *in vitro* in porcine (Vrlanskê *et al.* 2003), cadmium induced changes in gene expression of granulosa cells affecting the synthesis of all steroid hormones in the ovary which were responsible for oocyte maturation (Henson and Chedrese 2004) and chromosomal aberrations in bovine oocytes (Rodriguez Tellez *et al.* 2005) or interaction of cadmium with trace minerals like zinc, copper (Ishitobi and Watanabe 2005) which are essential for oocyte maturation. The mean proportion of embryos developed to morulae and blastocysts were not significantly influenced by the presence of cadmium in culture medium in the present study. Two-cell embryos were remarkably resistant to cadmium, but toxicity increased with development, and morulae degenerated after cadmium exposure. Resistance to cadmium at the two-cell stage reflected a lack of uptake of this metal, whereas sensitivity to Cd at the blastocyst stage reflects the ability to accumulate cadmium as observed earlier (De *et al.*1993).

The result indicated that cadmium decreased the oocyte and embryo development at all tested doses. Cadmium significantly reduced oocyte maturation at the level of 0.5 mg/ml. No cleavage was observed in oocytes cultured in cadmium at the level of 5 mg /ml.

SUMMARY

The study was conducted to examine the influence of different concentrations of cadmium on buffalo oocyte viability, maturation and embryonic development *in vitro*. A significant decline in the maturation rate was observed as the concentration of cadmium increased in the maturation media when compared with control group. The present study demonstrated the toxicological effects of cadmium on *in vitro* oocyte maturation, fertilization and subsequent embryonic development in buffalo.

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