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Meiotic stages during *in vitro* maturation regulating the post thaw survivability of vitrified buffalo oocytes

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ABSTRACT : The present study has been undertaken to assess the post thaw survivability of buffalo oocytes vitrified at different stages of *in vitro* maturation (IVM). Cumulus oocyte complexes (COCs) obtained from slaughter house ovaries were randomly divided into 6 different groups: control (non-vitrified oocytes were matured for 24 h in maturation medium (MM) consisted of TCM-199 supplemented with 10 per cent w/v fetal calf serum (FCS) at 38±1°C and 5 per cent $C0_2$ in a humidified atmosphere), 0 h (vitrified before the onset of maturation), 6,12,18 and 24 h groups (vitrified at 6, 12, 18 and 24 h, respectively, after the onset of maturation). Oocytes were exposed to vitrification solution (VS) consisted of 40 per cent w/v propylene glycol and 0.25 M trehalose in phosphate buffered saline (PBS) supplemented with 4 per cent w/v bovine serum albumin (BSA) for 3 min at 20-25°C. Oocytes in VS were loaded into 0.25 ml French mini straw with 1M sucrose solution separated by two airspace on either side of VS. The straws were sealed with hot forceps and plunged directly into liquid nitrogen (LN_2; -196°C). The straws were thawed after storage period of atleast 7 days by transferring them into a water bath at 37°C for 30 sec. The cryoprotectant was removed by exposing the oocytes to 1 M sucrose solution. Oocytes in 0, 6, 12, 18 and 24 h groups were further matured for additional 24, 18, 12, 6 and 0 h, respectively, to complete a total of 24 h maturation period.A total of 77, 64, 60, 51 and 62 post thawed morphologically normal oocytes in 0, 6, 12,18 and 24 groups, respectively, immediately after thawing, and 55 oocytes in control after 24 h maturation were stained with Trypan blue to assess the survivability. Survivability was significantly ($P<0.05$) higher in control (94.54%) than all five vitrification groups. Among vitrification groups, more survivability was observed in 24 h (61.29%) group as compared to 18 (56.86%), 12 (46.67%), 6 (40.62%) and 0 (37.66%) h groups. Oocytes vitrified at 24 after maturation survived significantly ($P<0.05$) better than oocytes vitrified before onset of maturation (0 h) and at 6 h after the onset of maturation. Though higher proportions of oocytes survived in 18 h group compared to 0,6 and 12 h groups, difference was significant ($P<0.05$) only with 0 h group, but not with 6 and 12 h groups. This study indicated that survivability of buffalo oocytes depends on the different developmental stages.

KEY WORDS : Vitrification, *In vitro* maturation, Post thaw survivability, Buffalo, Oocytes

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