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ARTICLE

## Influence of meiotic stages during *in vitro* maturation on the post thaw morphology of buffalo oocytes

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**Abstract :** The present experiment has been conducted to study the post thaw morphology 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) consists of TCM-199 supplemented with 10% w/v fetal calf serum (FCS) at 38±1°C and 5% CO<sub>2</sub> 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) consists of 40% w/v propylene glycol and 0.25 M trehalose in phosphate buffered saline (PBS) supplemented with 4% 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 (LIS; -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 sum of 495, 432, 457, 416 and 420 oocytes were vitrified in 0, 6, 12, 18 and 24 h groups, respectively. After thawing, 444 (89.70%), 384 (88.89%), 418 (91.47%), 381 (91.59%) and 387 (92.14%) oocytes were recovered in 0, 6, 12, 18 and 24 h groups, respectively. Recovered oocytes were examined to assess the post thaw morphology, of which, 87.16 (387), 84.38 (324), 86.60 (362), 87.40 (333) and 90.96 (352) % of oocytes vitrified at 0, 6, 12, 18 and 24 h groups, respectively, were found morphologically normal. From this study, it is clear that meiotic stages of oocytes does not influence the post thaw morphology in different vitrification groups.

**Key words :** Vitrification, *In vitro* maturation, Post thaw survivability and Buffalo oocytes

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