Effect of supplementation of vitrification solution on post-thaw survivability rate of porcine follicular oocytes

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Oocytes cryopreservation provides greater flexibility in breeding programmes than embryo cryopreservation (Paynter and Fuller 2007), since, cryopreservation of immature oocytes obtained from abattoir ovaries offers opportunity for planned in vitro maturation and in vitro fertilization in contrast to embryo cryopreservation. Vitrification of oocytes is rapid technique with radically increased cooling and warming rates that can obviate chilling injury and ice crystal formation associated with slow freezing. Although several vitrification protocols have been applied for porcine oocytes with varying survival rates, they are still associated with severely compromised embryo development. In general, the combination of permeable cryoprotectants (CPAs) was known to be more effective for vitrification than individual CPA (Mahmoud et al. 2008, Somfai et al. 2013). Sucrose as a non-permeating agent was known to facilitate dehydration and vitrification which further reduced the toxicity of permeating cryoprotectant by decreasing its concentration. However, the best cryoprotectant or combination of cryoprotectants is yet to be determined in pig. It was suggested that addition of certain high molecular weight polymers like polyvinyl pyrrolidone (PVP) or ficol alone or in combination with sucrose had some beneficial effects on cryo-survivability of oocytes and they protected the cellular membrane and the zona pellucida from damage during cooling or warming procedures (Yang et al. 2003). In view of the beneficial effect of supplementation of vitrification solution with sucrose, PVP or their combination, present work was taken up to study the effect of supplementation of vitrification solution on post-thaw survivability rate of porcine follicular oocytes.

Porcine ovaries were collected from local abattoirs immediately after slaughter of the animal and transported to the laboratory within 1–2 h in a flask containing normal saline with antibiotic at normal environmental temperature (22–28°C). Oocytes were recovered from follicles (2–8 mm dia.) of the ovaries after washing by aspiration technique and examined on a sterile petri dish under stereo-zoom microscope. The oocytes were washed 3–4 times and classified into four grades (grade A, B, C and D) based on their gross morphology as integrity of cumulus cells (Jackowska et al. 2009). Only ‘A’ and ‘B’ grade oocytes were selected for vitrification.

The oocytes were exposed to 35% concentration vitrification solution (VS). Two types of VS, i.e. 17.5% EG + 17.5% PG and 17.5% EG + 17.5% DMSO were prepared in basic solution (BS) that contained 80 ml DPBS, 20 ml FBS and 5 mg gentamicin, pH 7.2–7.4. Both were again supplemented with three different types of supplements, i.e. sucrose (0.5 M), PVP (@50 mg/ml) and sucrose + PVP. Prior to vitrification, oocytes were first exposed to equilibration solution (cryoprotectant @ 15% v/v + sucrose 0.25 M in BS) for 5 min. Vitrification procedure was performed at room temperature (24–25°C). Within 30 sec. of exposure in vitrification solution (VS), the oocytes with little quantity of VS were loaded in the French mini straw (0.25 ml), sealed and directly plunged into LN2 tank. The thawing or warming of vitrified oocytes was done in a step-wise (1 min in 0.5 M sucrose followed by 2 min in each of 0.25 M and 0.125 sucrose in basic solution) manner at 37°C. The post-thaw vitrified oocytes were examined under stereo-zoom microscope to evaluate survivability based on morphology. Oocytes with intact zona pellucida and vitelline membrane, normal spherical shape, and a dark and evenly granulated cytoplasm were considered as viable. Oocytes with clear signs of membrane damage (brownish, fading cytoplasm) were considered as non-viable (Somfai et al. 2014). Statistical analysis of the data was done using SAS (version 4.04) software.

The mean percentage of post-thaw survivability rate of oocytes following vitrification was 85.60±5.33, 43.94±5.40 and 85.03±4.14 in EG + PG and 86.99±3.07, 41.99±1.93

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Higher concentration (Wang et al. 2008) of cryoprotective function in presence of CPA having 20% or higher addition of PVP did not bring about significant production of the first live birth following cryopreservation of PVP in the freezing solution was evidenced by successful for ice crystal formation. The efficacy of supplementation of N-vinyl-2-pyrrolidone. PVP tends to increase viscosity hydrogen bonding water, thereby decreasing the propensity of the freezing solution and also form interaction through hydrogen bonding with sucrose in EG + DMSO obtained in the present study significant, the highest post-thaw survivability rate of vitrified oocytes recorded with the addition of PVP in combination with sucrose in EG + DMSO as compared to the supplementation with PVP alone. It was concluded that addition of sucrose alone or in combination with PVP in vitrification solution significantly improved survivability of vitrified porcine follicular oocytes. Further studies are warranted on the dosage of PVP and selection of effective cryoprotectant with optimum vitrification and warming methods for efficient use of PVP in vitrification solution as supplement.

**SUMMARY**

Oocytes (510) with two or more cumulus cell layers adhered to zona pellucida obtained by aspiration from follicles (2–8 mm dia) of 127 abattoir porcine ovaries were utilized to study the effect of supplementation of 0.5 M sucrose (Suc), 50 mg/ml polyvinyl pyrrolidone (PVP) and Suc + PVP into two different vitrification solutions, i.e. 35% concentration of both ethylene glycol (EG)+ propylene glycol (PG) and EG + dimethyl sulfoxide (DMSO). Vitrified post-thaw oocytes with intact zona pellucida and vitelline membrane, normal spherical shape and dark and evenly granulated cytoplasm under a stereo-zoom microscope were considered as viable. Out of the three supplements treatments, supplementation with sucrose + PVP combination and sucrose alone resulted in significantly higher mean post-thaw survivability rate of vitrified porcine follicular oocytes. Further studies are warranted on the dosage of PVP and selection of effective cryoprotectant with optimum vitrification and warming methods for efficient use of PVP in vitrification solution as supplement.

**REFERENCES**


