

Cholticha Srirungruang 2012: Effect of Cryopreservation of Canine Oocyte on Histological Characteristics of the Cumulus-oocyte complexes. Master of Sciences (Veterinary Anatomy), Major Field: Veterinary Anatomy, Department of Anatomy. Thesis Advisor: Assistant Professor Sirirak Chantakru, Ph.D. 88 pages.

The main purpose of cryopreservation of the oocytes is to preserve their qualities. Therefore, any structural changes in cumulus-oocyte complex that might be caused by vitrification will have great effects on functions and fertilization. The objective of the present study was to determine effects of solid surface vitrification (SSV) on viability and morphology of canine cumulus oocyte complexes (COCs), and also on carbohydrate distribution based on lectin-binding properties. All COCs from each dog were randomly divided into five groups. Every group except the control was equilibrated and separately introduced into different recipe of vitrification media. The vitrification medium consisting TCM 199 medium, supplemented with 0.5 M trehalose, 1.5 M ethylene glycol (EG) and 20% fetal bovine serum (FBS) was used for COCs of the groups 2 and 4, whereas the vitrification medium with consisting TCM 199 medium, supplemented with 0.5 M trehalose, 3 M EG and 20% FBS for the groups 3 and 5. Only the COC from groups 4 and 5 were vitrified with SSV. In lectin histochemistry, six biotinylated lectins including PSA, s-WGA, LEL, GSL-II, VVA and ECL were incubated with paraffin-embedded sections. Fluorescein-conjugated Avidin D and Rhodamine-conjugated Avidin D were then applied.

The result showed that the survival rates of non-vitrified and vitrified COCs were not significantly different. Morphological alterations including shrinkage, disrupted oolemma, loss of cumulus cell layer and cytoplasmic vacuolization were common features found in treatment groups. Percentages of COCs with each type of damages were not different. However, cumulus cells appeared to be the structures that were affected most from the SSV. The results from lectin histochemistry showed that cryoprotectants and/or SSV reduced PSA binding to the α -mannose on the ZP and oolemma. SSV alone caused no binding to N-acetyl glucosamine and α - and β - α -DN-acetyl glucosamine on both the ZP and oolemma. Lectin binding to carbohydrate residues on cell surface of the cumulus cells, zona pellucida and oolemma was reduced by cryoprotectants and/or SSVs. Thus, exposure of the cryoprotectant and SSV may modify structural changes of sugars in the COCs.

Student's signature

Thesis Advisor's signature