ABSTRACTS
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Effect of cryoprotective agent on histological and ultrastructural changes of follicles following ovarian tissue freezing using vitrification technique

Sirivaidyapong, S; Kradangnga, K; Limpavitayakul, K and Tongtam Na Ayutaya, N

Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand.

s_sudson@yahoo.co.uk

OBJECTIVES AND METHODS: This study aimed to determine the effects of 2M Dimethyl sulfoxide, 1M Acetamide, 3M Propylene glycol (DAP 213), which is a cryoprotective agent used for canine ovarian tissue cryopreservation, on the canine ovarian tissue and to determine the histological and ultrastructural changes of follicles between the experimental groups.

Cryopreservation of many living cells has been used for genetic banking. Ovarian tissue cryopreservation is a technology that could preserve many oocytes and also many cells in the ovarian tissue which are necessary for the development of oocytes and then for grafting. Primordial follicles are the major cells survive after the cryopreservation in -196°C and the thawing process (1). These immature follicles could also be used in other technologies such as Autografting, Xenografting, In vitro maturation and In vivo fertilization (2, 3). Several methods for ovarian cryopreservation have been studied to improve the quality of cryopreserving (2, 4, 5). Meanwhile, studies in ovarian tissue cryopreservation of dogs are still limited. There was a report (6) on ovarian tissue cryopreservation for genetic banking of guide dogs. The factors to succeed in ovarian tissue cryopreservation may not depend on only the method of preservation, but the cryoprotectants in each method might also be an important factor.

Ten pairs of ovaries were collected during ovariohysterectomy from 12-24 months old healthy bitches. Each ovary was divided into three groups; fresh (I), pre-freezing (II) and frozen-thawed (III) groups. DAP 213 had been added in sample group II without freezing, while it was added to group III before freezing. Primordial, primary, secondary and tertiary follicles were assessed for the normal and abnormal of nucleus and cytoplasm by light microscope (Hematoxylin-eosin stain). Follicular density was evaluated from only primordial follicle. Two samples from each group were randomly selected for follicular ultrastructural examination evaluation by transmission electron microscope (TEM).

RESULTS: No statistically significant differences in the morphology of ovarian tissues between the three groups (P>0.05). Furthermore, there was no difference in the average number of the primordial follicles between the pre-freezing and frozen-thawed groups but a statistically significant difference was observed between the fresh and frozen-thawed groups (P<0.05). Ultrastructural study, using TEM, demonstrated vacuoles in microstructure of some oocytes and granulosa cells but majority of those and many other structures still normal.

CONCLUSION: These results indicate that although average numbers of primordial and other follicles were statistically lower in the frozen-thawed, the follicular morphology assessed from TEM remained normal. This indicated that the cryopreservation of canine ovary by vitrification using DAP 213 deteriorated the ovarian tissue quality, however, several tissues are still intact and can be employed for further canine reproductive biotechnology. Further studies on techniques and types of cryoprotectant are required to reduce the number of follicular loss during the cryopreservation.