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Distribution of follicles in canine ovarian tissues

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The cryopreservation of ovarian tissues is a technology with significant potential for the preservation of genetic materials of working dogs, including guide dog for the blind. It has been reported that the ovarian follicles are not homogeneously distributed within the ovarian cortex in sheep and humans. More recently, we have developed a simple and rapid method for counting follicles and proposed an index to estimate follicular homogeneity in dogs [1]. To maximize the reproducibility of experimental results, it is essential to assess the degree of density and distribution of follicles in ovarian tissues before their transplantation. In this study, follicles of ovarian fragments from immature bitches were counted and assessed their density and distribution. In addition, cryopreserved ovarian fragments from ovary judged as even distribution were transplanted to immunodeficient mice to examine the effect of desialylated erythropoietin (asialo EPO) on the follicular development after transplantation. The ovarian cortex was sectioned into 1.0-1.5 mm cubes. For each ovary, 5 samples of ovarian fragments were embedded in paraffin wax, sectioned and stained with hematoxylin and eosin. Remaining ovarian fragments were vitrified for transplantation. To evaluate the density and distribution of follicles, the mean number of follicles per square millimetre was calculated under a microscope. Follicles that visibly contained an ovum (oocyte) with a nucleus were counted. The distribution of follicles was considered even when the variance value was lower than 10 or between 10 and 16; and the absolute value of distortion was inferior to 1 [1]. The mean number of follicles ranged from 3.2 to 28.3/mm² in 21 ovaries from 11 bitches examined. The variance and distortion ranged from 3.24 to 119.64 and -1.87 to 4.40, respectively. The distribution of follicles within ovarian cortex in 11 of 21 ovaries was judged uneven. In addition, 3 of 10 bitches showed bilateral asymmetry for the density and distribution of follicles between the right and left side of ovary. Namely, one of a pair was even distribution, while other side showed uneven distribution. Furthermore, follicular density was significantly lower in ovaries showing even distribution (6.62/mm²) than in those uneven distribution (13.35/mm²). These results indicated that follicles were not homogeneously distributed within the ovarian cortex in large portion of bitches. Ovarian fragments with even distribution of follicles (variance: 14.25, distortion: 0.76) from a mixed breed were transplanted to dorsal muscle of NSG scid mice, an immunodeficient strain, with or without 400 U/kg of (asialo EPO). At 4 weeks after transplantation, the fragments were recovered from the mice and number of primordial, primary secondary and antral follicles was counted. Total number of follicles in fresh control, transplanted fragments with and without asialo EPO was 10.0, 12.3 and 3.5/mm², respectively. These results reconfirmed effect of asialo EPO on the canine ovarian xenotransplantation in our previous report [2].
