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Brief communication

Distribution of follicles in canine ovary – A simple and rapid method for counting follicles-

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ABSTRACT

The distribution of follicles within canine ovarian cortex was evaluated to estimate follicular homogeneity. The analysis of follicular homogeneity prior to ovarian tissue transplantation limits the impact if follicular heterogeneity on experimental results. In this report, ovarian fragments from 14 immature bitches were embedded in OCT compound. Sections (5-μm-thick) were cut on a cryostat and stained with methylene blue. The mean number follicles ranged from 3.7 to 15.6/mm^2 in the 14 ovaries examined. The variance and distortion ranged from 2.05 to 144.30 and −2.09 and 2.01, respectively. The distribution of follicles was considered even, when the variance value was lower than 10 or between 10 and 16; and absolute value of distortion was inferior to 1. The distribution of follicles within ovarian cortex in 9 of 14 bitches was judged uneven. These results indicated that follicles were not homogeneously distributed within the ovarian cortex of the majority of bitches.

The cryopreservation of ovarian tissues is a technology with significant potential for the preservation of genetic materials of working dogs, including guide dogs for the blind. Because of the simplicity of vitrification, this technique has been applied in cryopreservation of ovarian tissues as well as embryos and oocytes as an alternative to slow freezing. Although vitrification and follicular survival of canine ovarian tissues to subsequent xenotransplantation was reported [2,9], there are suggestions that damages of ovarian grafts might have been induced by initial ischemia and/or ischemic perfusion injury rather than vitrification-associated process. This has been reported in mouse, sheep, dog, and humans [3]. Several studies have been done to prevent the follicular loss of cryopreserved ovarian tissues after transplantation [4,6,9,10]; but the degree of density and distribution of follicles in ovarian tissues seems to affect the interpretation of results after transplantation of the ovarian tissues. It has been reported that the follicles are not homogeneously distributed within the ovarian cortex in humans [7,8] and sheep [1]. However, to our knowledge, no studies have so far assessed the distribution pattern of follicles in dog ovary. In this study, we evaluated the distribution of follicles within canine ovarian cortex to estimate follicular homogeneity and limit the impact of follicular heterogeneity on experimental results.

Canine ovaries were collected from immature bitches (5 to 9-month-old) undergoing routine ovariohysterectomy, and were transported to the laboratory in a jar containing sterile saline at 37C. The fresh canine ovaries were dissected in a 60 mm diameter Petri dish (#150326, Nunc, Thermo Fisher Scientific KK, Yokohama, Japan), half-filled with TCM199 medium (#12340-030, Gibco Life Technologies, USA) containing 10% fetal calf serum (#171012, Nichirei Bioscience, Tokyo, Japan) at 37C. The cortex and medulla were separated. The cortex was further sectioned into 1.0–1.5 mm cubes. For each bitch, five samples of the ovarian fragments were embedded in OCT compound (#4583, Sakura Finetek Japan, Tokyo, Japan) in a cryostat (CM3050, Leica, Wetzlar, Germany). A section (5-μm-thick) was cut perpendicularly to the ovarian surface in each sample on a cryostat. After drying for 2 min, the sections were stained with 0.05% of methylene blue [5]. To evaluate the density and distribution of follicles, the mean number of follicles per square millimetre was calculated under an All-in-one Fluorescence Microscope (BZ-9000, KEYENCE, Osaka, Japan). Follicles that visibly

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Table 1
Follicular density and distribution in canine ovarian tissues from immature bitches assessed by frozen sections.

Specimen no.	Follicular density (no. follicles/mm^2)[a]								Variance	Distortion
	A	B	C	D	E	Mean	Median	SD		
1	7.5	10.6	10.9	11.2	11.4	10.3	10.9	1.43	2.05	−2.09
2	2.0	2.0	3.9	5.1	5.6	3.7	3.9	1.51	2.30	−0.31
3	4.8	6.2	6.8	8.9	10.5	7.4	6.8	2.03	4.15	1.09
4	2.4	4.4	6.2	6.4	8.7	5.6	6.2	2.11	4.42	−0.85
5	2.2	4.2	5.5	7.9	10.0	6.0	5.5	2.75	7.56	0.63
6	5.2	6.1	8.7	14.8	15.0	10.0	8.7	4.18	17.46	0.97
7	6.3	8.2	8.6	16.8	18.4	11.7	8.6	4.95	24.57	1.51
8	3.7	4.0	10.8	11.3	17.3	9.4	10.8	5.11	25.98	−0.55
9	11.2	10.2	11.5	21.9	5.8	12.1	11.2	5.30	28.12	1.35
10	11.9	5.1	9.2	20.7	7.0	10.8	9.2	5.46	29.85	1.61
11	2.8	3.4	3.8	12.3	21.0	8.7	3.8	7.08	49.95	1.80
12	3.0	6.2	12.6	15.0	25.6	12.5	12.6	7.84	61.36	0.42
13	10.7	8.1	8.9	21.2	29.1	15.6	10.7	8.25	67.99	1.67
14	11.7	8.0	3.2	35.8	4.1	12.6	8.0	12.01	144.30	2.01

[a] Five sections (A–E) were assessed in each specimen.

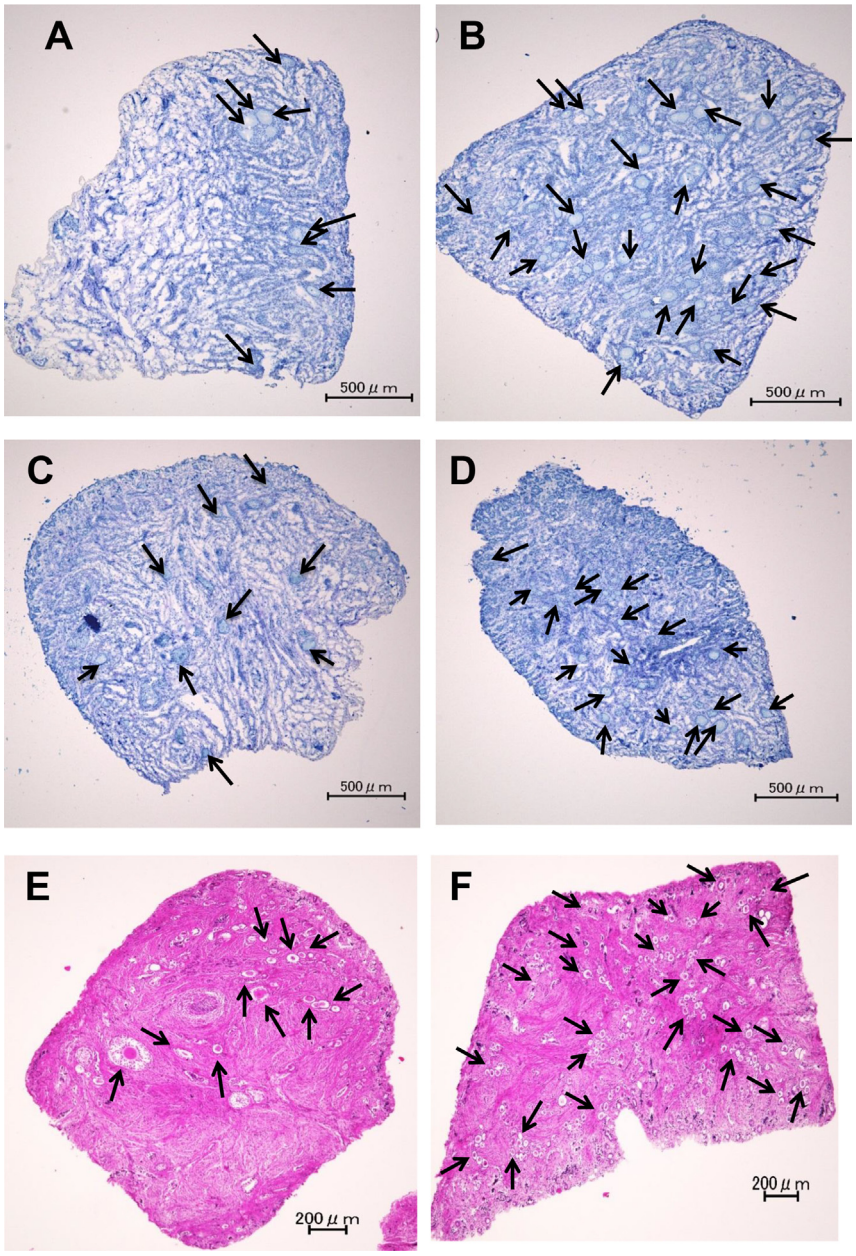

Fig. 1. Photomicrographs of frozen sections stained with methylene blue (A, B, C and D) and paraffin sections stained with hematoxylin and eosin (E and F) in canine ovarian tissues. Representative ovarian tissues with uneven distribution of follicles. A and B, C and D, and E and F are derived from an identical ovary. Follicles distribute with lower (A, C and E) and higher (B, D and F) density. Arrows indicate follicles.