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The relationships among the morphological and histological features of bovine follicles and the estimated developmental competence of oocytes

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The purpose of this study was to investigate the relationship between the morphological and histological features of bovine follicles and the estimated developmental competence of oocytes based on their morphological features.

Bovine follicles derived from ovaries with corpora lutea were initially classified into two categories based on the presence of blood vessels on the surface of follicles (Group A: with clear blood vessels, Group B: with unclear blood vessels), and subsequently grouped into three categories based on their follicular diameters (a: <2.0mm, b: 2.1–6.0mm, c: >6.1mm).

Histological examination was performed by using the following criteria: the rate of granulosa cells undergoing pyknosis and cell division, the presence of macrophage invading into the follicular antrum or theca folliculi and the presence of atretic bodies in the follicular antrum. The histological feature was affected by the presence of blood vessels ($p < 0.05$). Follicles in Group B

had a higher rate of granulosa cells undergoing pyknosis and were highly invaded with macrophage compared to follicles in Group A. However, the follicular diameter did not affect the histological feature.

In the next experiment, cumulus-oocyte complexes were isolated from the follicles classified as above and then their developmental competence were estimated from the morphological features of cumulus and ooplasm. The larger sized follicles showed a higher rate of vascularized follicles ($a < b < c$, $p < 0.05$). However, oocytes with high developmental competence were present in vascularized follicles having diameters between 2.1 and 6.0mm.

The results suggest that vascularized follicles might be healthy and contain oocytes with high developmental ability. Under the present criteria for histological examination, the correlation between follicular regression and follicular diameters could not be established

Effects of ovarian status, oocyte morphology and hormone supplementation on *in vitro* maturation of domestic cat oocytes

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The improvement of *in vitro* maturation systems for oocytes is needed to establish a

reliable *in vitro* fertilization (IVF) technique for the domestic cat. The present study examined

the effects of the ovarian status, oocyte morphology and hormone supplementation on *in vitro* oocyte maturation in domestic cats.

Pairs of ovaries recovered through ovariectomy were categorized into three phases (inactive, follicular and luteal) based on the presence or absence of follicles and corpora lutea. Oocytes from each phase were classified into five groups (class A; oocytes with dark ooplasm and compact cumulus cells, class B; oocytes with dark ooplasm and less compact cumulus cells, class C; almost naked oocytes with dark ooplasm and a part of the corona radiata, class D; oocytes with pale ooplasm, class E; oocytes with irregular ooplasm).

In the first experiment, all oocytes, except those of class E, were cultured in HEPES-buffered TCM199 supplemented with fetal calf serum (FCS), sodium pyruvate, FSH and 17β -estradiol (E_2) at 39°C for 48–49 hours. In class A and B oocytes, the proportion of oocytes that achieved metaphase II (M II) was significantly higher ($p < 0.05$) than those in classes C and D. There were no significant differences in the proportion of oocytes reaching M II among oocytes collected from inactive, follicular and luteal phase donors. Class A and B oocytes, with high meiotic competence, were collected from the follicular phase donors in higher proportion than from the inactive phase donors ($p <$

0.05). The proportion of oocytes from the luteal phase donors was not significantly different from the other two phases.

In the next experiment, the effect of hormone supplementation to the maturation medium was examined. Class A and B oocytes from luteal phase donors were matured in TCM199 + FCS with or without FSH, FSH and E_2 . The supplementation of FSH to the maturation medium increased the proportion of oocytes that reached M II ($p < 0.05$) compared to the control group. Adding E_2 with FSH did not result to a significantly different proportion of oocytes that achieved M II compared to that supplemented with FSH alone or those matured in the absence of hormones.

These data indicate that the *in vitro* maturation of domestic cat oocytes is affected by oocyte morphology. Namely, oocytes surrounded by cumulus cells show high meiotic competence. These oocytes with high meiotic competence were collected from follicular phase donors in higher proportion than those collected from inactive phase donors. The addition of FSH to the maturation medium promoted *in vitro* maturation of domestic cat oocytes. However, the variation of individuals was large, suggesting that other factors affect domestic cat oocyte maturation *in vitro*.

Histopathological study on pancreatic acinar atrophy in the dog

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The purpose of this study was twofold: (1) to evaluate the microscopic changes of the early stages of pancreatic acinar atrophy (PAA) and (2)

to investigate related changes of pancreatic islet cells in dogs with PAA by histopathological and immunohistochemical examinations.