Form 5

学位論文内容の要旨
Abstract of the dissertation

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学位論文題名
Improvement of the *in vitro* growth culture system for bovine oocytes derived from early antral follicles: Effect of the duration of culture, oxygen environment, and astaxanthin supplementation on the acquisition of oocyte developmental competence

(牛初期胞状卵胞由来卵子培養系の改善：培養期間、酸素濃度、アスタキサンチン添加が卵子の発生能獲得に及ぼす効果)

Embryos derived from *in vitro* procedures have lower developmental competence compared to their *in vivo*-derived counterparts. The development of an *in vitro* growth (IVG) culture system for *in vitro*-derived bovine oocytes from early antral follicles is necessary to allow their utilization by IVP procedures. Furthermore, IVG culture systems can be used as IVG models for studying *in vivo* oogenesis and folliculogenesis in mammals. In this study, I tried to improve IVG culture systems to mimic the *in vivo* growth of follicles of the early antral stage to grow to the antral follicle stage. In particular, I attempted to improve the outcome of the IVG culture systems by exploring the suitable duration of culture and the O_2 environment.

In chapter I, the effect of shortening the duration of culture to 8 days relative to 12 days of culture and increasing the O_2 availability by using the gas permeable (GP) culture device relative to the conventional gas-impermeable (GIP: control) culture device under the 20% O_2 condition was evaluated. The effect of antioxidant supplementation, astaxanthin (Ax), added to the GP device (GP + Ax) was also evaluated. Culture for 8 days promoted the oocyte-cumulus-granulosa complexes' (OCGC) viability than the 12-day culture. Culture in the control device promoted antrum formation and granulosa cell proliferation than in the GP

device regardless of Ax supplementation. However, culture in the GP + Ax condition for 8 days improved the oocyte growth and maturation competence, similar to the 12 days of culture in the control device. Further, culture in the GP + Ax and the control conditions tended to reduce the generation of the reactive oxygen species than in the GP condition. In all groups, culture for 8 days yielded blastocyst embryos even though the developmental competence was lower than the *in vivo*-derived counterparts.

In chapter II, the effects of a constant low O_2 environment or O_2 modulation from low to high on day 4 of culture (*i.e.*, 5-5% or 5-20% O_2, respectively) on the 8-day IVG culture was evaluated. In addition, the efficiency of Ax supplementation was also assessed. Ax tended to increase the viability of OCGCs cultured under 5-20% O_2 conditions. E_2 production increased during IVG culture, whereas P_4 production did not regardless of the O_2 environment or Ax supplementation. IVG oocytes cultured in the 5-20% O_2 environment with Ax supplementation showed the highest nuclear maturation rate among all experimental groups, similar to that of *in vivo*-grown oocytes. The developmental competence to blastocysts was promoted by the 5-5% O_2 environment regardless of Ax supplementation; however, under the 5-20% O_2 condition, Ax supplementation promoted blastocyst development, to a level similar to the 5-5% culture condition.

In conclusion, bovine oocytes can attain maturation and developmental competence after 8 days of culture in the gas permeable devices with astaxanthin supplementation under a 20% O_2 environment. However, O_2 modulation from 5% to 20% is the ideal condition for this culture system. This culture system can be used as the *in vitro* growth model to study mammalian folliculogenesis.