（学位第３号様式）

学 位 論 文 要 旨

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題　目	Study on improvement of in vitro embryo production system using ovum pick-up （経膣採卵を用いたウシ体外胚生産系の高度化に関する研究）

In the present study, the following three experiments were conducted to improve *in vitro* embryo production system using ovum pick-up（OPU） technology.

First, we addressed the low conception rate during hot season in cows and conducted to clarify the seasonal difference in the quality of cumulus-oocyte complexes（COCs）. The COCs were collected from Japanese black cows by the OPU method during the hot and cool seasons. The number and morphological grade of COCs did not differ between the hot and cool seasons, although the rectal temperature and respiration rate of cows were significantly increased during hot season. On the other hand, number of apoptotic cells in cumulus cells was significantly higher in COCs obtained during hot season than that of cool season. Likewise, the rate of normal mitochondrial distribution observed in oocytes was significantly decreased in hot season compared with that of cool season. These findings indicate that heat stress would decrease the quality of COCs in Japanese Black cows.

Second, we examined whether the use of *in vivo*-matured oocytes collected by OPU from superstimulated Japanese black cows can improve the productivity and quality of *in vitro* produced embryos. Normal distribution rate of cortical granules in the superstimulated group was significantly higher than that in non-stimulated group. The normal cleavage rate and freezable embryo rate were significantly higher in the superstimulated group than in the non-stimulated group. Among the transferable embryos, the ratio of embryos from normal cleavage was also significantly higher in the superstimulated group than in the non-stimulated group. Pregnancy rate was significantly higher in normally cleaved embryos than in abnormally cleaved embryos in the superstimulated group. These results suggest that high quality embryos in terms of developmental kinetics can efficiently be produced with the use of *in vivo*-matured oocytes collected by OPU from superstimulated Japanese black cows, and thus contribute to improving the pregnancy rate of *in vitro*-produced embryos.

Third, the developmental kinetics of *in vitro* produced embryos from *in vivo*- and *in vitro*-matured oocytes were observed using time-lapse cinematography, and pregnancy rate after transfer was investigated. Regardless of the maturation method, there was no difference the duration of the first cell cycle between *in vivo*- and *in vitro*-matured oocytes. On the other hand, the first cell cycle was influenced by bulls which were collected semen for *in vitro*-fertilization. Pregnancy rate tended to be higher in normally cleaved embryos compared to in abnormally cleaved embryos produced with the use of *in vivo*-matured oocytes. These results suggest that the first cleavage patterns would be an useful indicator for objective and simple embryo selection, which contributes to efficient calf production by *in vitro* embryo production system.