(別紙様式第 3 号)

学 位 論 文 要 旨

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題　　目：Study on cold tolerance and developmental ability of feline oocytes from cold-stored ovaries

(低温保存したネコ卵巣由来卵母細胞の低温寛容と発育能)

論文要旨：

Animal ovaries are routinely used for practical application of in vitro fertilization for animal production. The short-term preservation for ovary transportation is especially important in the case of farm or endangered animals, when the ovarian donor is far away from specialized laboratories. The cooling of mammalian oocytes to sub-physiological temperatures is widely known to affect their viability through the induction of various abnormalities at all stages of meiosis. The duration and temperature of ovary/oocyte storage could affect to the quality of oocytes such as maturation ability, DNA fragmentation, damage of cumulus cells and/or normal development of oocytes. The objectives of this study were conducted to compare the tolerance ability to cold storage among porcine, bovine and feline oocytes after storage of ovaries at 4°C for 5 days, and to improve the developmental ability of feline oocytes collected from cold-stored ovaries.

In the first study, we conducted to compare the kinetics of nuclear status and oocyte damage in porcine, bovine and feline ovaries stored at 4°C for 5 days. The cold storage of ovaries decreased the proportions of porcine and bovine oocytes that remained at the germinal vesicle stage before maturation culture. The maturation rates of oocytes decreased with increasing storage time, independent of species. None of the porcine oocytes reached MII after 1 day of storage. In contrast, bovine and feline oocytes from ovaries that were stored for 2 days and 3 days reached MII. The proportion of DNA fragmentation in porcine oocytes from ovaries stored for 1 day was significantly higher than that in bovine and feline oocytes. The maturation competence of oocytes after the cold storage of ovaries could be related to the meiotic resumption of oocytes during storage and the occurrence of DNA fragmentation in oocytes during maturation culture. The findings demonstrate that feline oocytes maintained meiotic competence until 3 days of storage, whereas ovary storage at 4°C quickly resulted in a loss of the ability of porcine oocytes to reach MII, and bovine oocytes lost their meiotic ability after 2 days of storage. In particular, porcine oocytes were highly sensitive to chilling. The drastic loss in the maturation competence of porcine oocytes after cold storage resulted in part

(和文 2,000 字又は英文 800 語程度)

from not only the meiotic resumption of oocytes before IVM culture, but also a high occurrence of DNA fragmentation in oocytes during IVM culture.

In the second study, we investigated the effect of relaxin supplementation in maturation medium on their meiotic ability and subsequent development of feline oocytes from ovaries stored at 4 °C for one day. Relaxin is a member of the insulin-like family of hormones that promotes growth in a number of reproductive tissues, including the granulosa and theca cells. Feline oocytes collected from cold-stored ovaries remain capable of maturing in vitro, but the developmental ability of the oocytes decreases after 24 h of cold storage. Feline oocytes were collected from ovaries stored at 4 °C for one day and cultured in maturation medium supplemented with different concentrations (0, 10, 20, and 40 ng/ml) of relaxin for 24 h. They were then fertilized in vitro for 12 h with frozen-thawed spermatozoa. After in vitro fertilization, the zygotes were cultured in synthetic oviduct fluid medium for 8 days. There were no significant differences in the maturation rates and glutathione contents of oocytes among the groups, irrespective of relaxin supplementation. The rate of blastocyst formation from oocytes matured with 10 ng/ml relaxin (16.0%) was higher ($p < 0.05$) than that from oocytes matured without relaxin (5.9%). Our findings indicate that supplementation of 10 ng/ml relaxin into maturation medium may improve blastocyst formation of feline oocytes after in vitro fertilization.

The study results provide evidence that feline oocytes have an unusual tolerance to cold storage and have the ability to undergo maturation after a longer period of ovary storage than porcine and bovine oocytes. The addition of relaxin at a low concentration (10 ng/ml) to the IVM medium improved the rate of blastocyst formation of feline oocytes from ovaries stored for one day at 4 °C. The further study of mechanism of tolerance ability of feline oocyte at low storage temperature would be a key point for improvement of developmental ability of feline oocytes collected from cold-stored ovaries.

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学位論文審査の結果の要旨

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題　　目	**Study on cold tolerance and developmental ability of feline oocytes from cold-stored ovaries** 低温保存したネコ卵巣由来卵母細胞の低温寛容と発育能に関する研究

審査結果の要旨：

申請者は，低温感作に対する卵母細胞の寛容性について動物種間の違いを明らかにし、さらにネコ科野生動物の保護・再生を目的に低温保存卵巣からのネコ卵母細胞の発育能を改善するために、リラキシンの添加効果を明らかにした。

研究1では、ネコ、ウシ、ブタにおける動物種間の低温感作に対する卵母細胞の寛容性の違いを比較するために、各種卵巣を4℃の生理食塩水中に4日間保存した。保存後1日ごとに卵巣から卵母細胞を採取し、成熟培養前後の核相およびDNA損傷程度を評価した。その結果、低温保存することによりブタおよびウシ卵母細胞は、保存期間中にすでに卵核胞崩壊が卵胞内で進んでいることを明らかにした。一方、動物種に関わらず、成熟率は保存期間が長くなるに従い低下することが判明した。さらに、ブタ卵母細胞において、1日間の冷蔵保存により卵母細胞の成熟能が失われ、他の動物種と比較してDNA損傷率も有意に上昇した。一方、ウシおよびネコ卵母細胞の成熟能は、それぞれ2日間および3日間冷蔵保存しても維持されていた。以上のことから、ネコ卵母細胞は最大3日間の冷蔵保存に対しても成熟能を維持していることが示された。ブタにおいては、1日の冷蔵保存で卵母細胞の成熟能が失われ、また牛卵母細胞は最大で2日間維持されることが判明した。これらのことから、動物種により低温感作に対する卵母細胞の寛容性の違いがあり、ブタ卵母細胞は低温感作に対する障害の大きいことが示唆された。

2,000字以内

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研究２ではネコ科野生動物の保護・再生を目的に、１日間4℃で低温保存した卵巣から採取したネコ卵母細胞の発育能に及ぼすリラキシンの添加効果を検討した。リラキシンは分子量約 6000 のペプチドホルモンの一種であり、妊娠維持および分娩に関与するホルモンであるが、顆粒膜細胞の増殖を促進する作用のあることが知られている。一方、低温保存した卵巣から採取したネコ卵母細胞の成熟能は維持されているが、体外受精後の発育能は低下することが示唆されている。そこで本研究では、低温保存した卵巣由来の卵母細胞を異なる濃度のリラキシンを添加した成熟培養液で培養し、培養後の卵母細胞の GSH 濃度、成熟率さらに体外受精後の発育能を指標に最適のリラキシン濃度を検討した。その結果、リラキシン添加は成熟能に影響を及ぼさないが、10 ng/ml リラキシン添加が体外受精後の胚盤胞形成率を改善することを明らかにした。

これら成果は、国際的にも十分に評価されており、野生ネコ科動物の保護にも有用な情報を提供するものと考えられた。以上により、本論文は博士（獣医学）の学位を授与するにふさわしいと判断された。