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学位論文の題目	Characterization of gilt's ovaries and attempts to achieve a successful in vitro maturation of oocytes from small and medium follicles (未経産豚卵巢の特徴とその小中卵胞由来卵母細胞の体外成熟能改善の試み)
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### 学位論文内容の要旨

Currently, female gametes are easily collected from slaughtered or live ovaries and used for various reproductive biotechnologies to improve the efficiency and value of mammalian embryos. Pigs have been utilized as a model in biomedical research, whereas the gametes and embryos are quite unique from other species. In the developed countries, although pigs are usually slaughtered for pork production at 5-6 months old when those gilts have neared or still not reached to the puberty, ovarian morphologies have not been considered for the resource of cumulus-oocyte complexes (COCs). Furthermore, the effect of interrupting communication between oocyte and the surrounding cumulus cells has not been examined in porcine COCs derived from small follicles (SF; < 3 mm in diameter). In the present studies, firstly, gilt's ovaries with different follicular morphologies were characterized and the meiotic competence of oocytes was compared among the criteria. Porcine ovaries were able to be classified into three categories; ovaries with smooth (SSO), bubbled (BSO) or mixed (uneven) surface (MSO). A significantly higher number of SF was observed in MSO than SSO and BSO and the number of middle follicles (MF; 3-6 mm in diameter) per ovary was significantly higher in BSO and MSO than SSO. Since MSO showed a significantly higher percentage of metaphase-II stage oocytes than SSO in SF and MF, it was concluded that MSO could suppose a good source of oocytes for improving the efficiency of the actual reproductive technologies. In the second experiments, the apoptotic status and meiotic progression of oocytes from SF and MF were examined when the oocytes were denuded during culture for in-vitro maturation (IVM). Apoptotic status of the oocytes was only affected by the time when the oocytes were denuded (0, 20 and 44 h after the start of IVM). Although the percentage of mature oocytes was higher in MF, maturation rates were significantly higher when oocytes were denuded at 20 h, as compared with 0 and 44 h after the start of IVM. Therefore, it was concluded that removing CCs at 20 h after the start of IVM seemed to promote meiotic progression of the oocytes to the metaphase-II stage even when the COCs were collected from SF. In the last experiments, the effect of the timing of removing cumulus cells during IVM on developmental competences of oocytes was assessed, and it was also examined if the addition of GDF9 or VEGF could further improve IVM rates of oocytes denuded during maturation culture. Regardless of the presence of GDF9 or VEGF, denuding oocytes 20 h after the start of IVM did not affect their developmental competence to the blastocyst stage, concluding that denuding the oocytes 20 h after the start of IVM could be a good method to promote the meiotic resumption of oocytes, without any reduction in the developmental competence.

Results from our current experiments demonstrate that using COCs from MSO or by removing the cumulus cells of COCs from SF and MF 20 h after the start of IVM can promote the meiotic resumption of oocytes, without any reduction in the developmental competence. This information may contribute to improve the efficiency to prepare female resources for in-vitro embryo production.

## 論文審査結果の要旨

本提出論文は、その個体が誕生前後から急速に数を減少させ極めて限りある生殖資源である哺乳動物の雌性生殖細胞の利用効率の向上を目指して、ブタ卵巢の小中卵胞由来卵母細胞を用いた体外成熟能を格段に改善するための試みについて成果をまとめたものである。

先ず、これまで検討されてこなかった春季発動前ブタ卵巢の形態的特徴とその小中卵胞内の卵母細胞の体外成熟能の関係性について検討し、体外成熟能の高い卵母細胞を比較的多く得ることが出来る卵巢の形態を明らかにした。次に、卵巢内に比較的多数存在するが体外成熟能が低いことが明らかな小卵胞由来卵母細胞の卵丘細胞とのコミュニケーションに着目し、卵丘細胞の除去のタイミングがその体外成熟能の改善に需要であることを明らかにした。また、卵母細胞および卵丘細胞分泌因子の存否に関わらず、体外成熟培養開始20時間目での卵母細胞の裸化が、小卵胞由来卵母細胞の体外成熟能だけでなく初期発生能をも向上させることを明らかにした。これらの成果は、種々の生殖補助技術で利用される哺乳動物雌性生殖細胞の効率的な準備と利用に極めて有益な情報を提供する。

以上の知見は、実用的かつ効率的なブタ小中卵胞由来卵母細胞の利用技術として、さらにそれに係わる基礎資料として、高く評価できる。また、本提出論文が明らかにした知見は、人類にとって重要な肉資源の一つであるブタの改良増殖効率の改善に役立ち、産業上も極めて意義深い。

以上のことから、本学位審査委員会は、これらの成果をまとめた本論文の内容および参考文献を総合的に審査し、本論文は、博士（農学）の学位に値すると判断した。