6 Developmental Capacity of Bovine Follicular Oocytes after Ultra-Rapid Freezing by Electron Microscope Grid

II. Cryopreservation of *In Vitro* Matured Bovine Oocytes

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Mature bovine oocytes were frozen ultra-rapidly with the electron microscope grid and cryoprotectant solution consisting of 30% ethylene glycol, 0.5M sucrose, 18% ficoll and 10% FBS added D-PBS (EFS30) or exposed to the freezing solution. Assessments of morphological survival, fertilization and embryonic development were recorded. Also, cytoskeletal alteration of bovine oocytes according to exposure to the freezing solution or after freezing/thawing was examined. The results obtained in this experiment were summarized as follows; After ultra-rapid freezing and thawing, a high mean percentage of oocytes (67.4%) was survived. When fertilization was identified at 18 h after insemination, the fertilization rate in control and freezing group was evaluated as total penetration (96.7, 90.0%), normal 2 pronuclei formation (74.6, 68.9%) and mean number of sperm/oocyte (1.50, 1.44). The rate of two-cell formation to freezing group was 67.2%, and was not significant different when compared to control (71.4%)and exposure (78.6%). In addition, development to the blastocyst on day 8 after fertilization of freezing group was 32.8% from the cleaved oocytes. Development to the blastocysts of frozen-thawed oocytes was very similar to that of the control (33.3%) and exposure (34.8%). These results demonstrate that developmental capacity of frozen-thawed bovine oocytes can be successfully obtained by ultra-rapid freezing method using electron microscope grid and EFS30.

7 Microtubule and chromatin organization during the first cell cycle following intracytoplasmic injection of round spermatid into porcine oocytes

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Assessment of normal fertilization and offspring production by injecting spermatid into oocytes could offer exciting prospects not only for understanding gamete physiology during fertilization but for the clinical treatment of male infertility due to defective spermiogenesis. However, little information is available on the role of paternal origin centrosome during spermatid fertilization. The objective of this study was to determine microtubule assembly and chromatin configuration in porcine oocytes during the first cell cycle following round spermatid injection into matured

porcine oocytes in the presence or absence of electrical stimulation. The oocytes with two large pronuclei and two polar bodies were classified as 'normal fertilization' at 6 to 8 h following injection. The incidence of normal fertilization following round spermatid injection with electrical stimulation was significantly higher (21/45, 47%) than that following injection alone (6/39, 15%). Although a small microtubular aster was organized near the decondensed spermatid chromatin in some oocytes (2/6, 33%, spermatid injection alone; 9/21, 29%, spermatid injection and electrical stimulation), but it did not enlarge nor fill the cytoplasm. Instead, a dense network of microtubules in the cytoplasm was organized from the cortex in normally fertilized eggs. At 12 to 15 h after injection, we classified the oocytes with closely apposed pronuclei as "normal fertilization". The electrical stimulation following spermatid injection enhanced ($P < 0.05$) the incidence of normal fertilization (18/54, 33%) as compared to spermatid injection alone (7/52, 13%). During pronuclear movement, the maternally derived microtubules filled the whole cytoplasm, which appeared to move male and female chromatin. Mitosis and 2-cell division were observed at 20 to 24 h after spermatid injection with electrical stimulation (12/41, 29%). At mitotic metaphase, the microtubular spindle had focused astral poles, and chromosomes were aligned on the spindle equator. During mitosis, asters were assembled at each spindle pole, and they filled the cytoplasm. These results suggested that round spermatid nuclei of the pig can develop into a morphologically normal pronucleus in matured porcine oocytes and are competent to participate in syngamy with the ootid chromatin. In addition, functional microtubules for the complete fertilization with spermatid were not associated with male derived centrosome, but organized solely from maternal stores.

8 생쥐 배아의 체외배양시 저농도 산소환경이 배아 발달에 미치는 영향

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포유동물의 착상전 배아를 체외에서 배양할 경우 발달의 지연 혹은 정지 현상를 보이는 것으로 알려져 있다. 이러한 현상은 초기 배아 발달에 산소유리기 (oxygen free radicals)가 관여하여 해로운 영향을 미치는 것으로 확인되었으며, 저농도 산소 환경 또는 배양액에 산소유리기 보집제를 첨가함으로써 극복되는 것으로 알려져 왔다.

본 연구에서는 과배란된 생쥐의 전핵시기 배아를 획득하여 체외에서 배양시 5% 산소 환경과 20% 산소 환경으로 나누어 각군에서 배아의 발달율을 비교하고, 2,7-dichlorodifluorescein diacetate (DCDHF-DA)를 이용하여 Quanti-cell 700 (Applied Imaging Co., UK)으로 배아내 두 군에서 발현되는 산소유리기의 농도를 비교하였다. 5% 산소 환경은 5% 산소가 공급되는 배양기 (Vision science, Korea)를 이용하였고 기본 배양액으로는 M16 배양액을 이용하였다.

1. 배양기의 산소 분압의 변화를 2시간 간격으로 48시간동안 측정한 결과 5% 배양기는 5.1±0.6%, 20% 배양기는 19.8±0.4%로 유지됨을 확인하였다.

2. 산소 분압의 차이에 따른 배양액내 산소 농도의 변화를 알아보기 위하여 배양 1일부