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

터 5일까지 배양액내 산소 농도를 측정한 결과, 5% 산소환경의 배양액은 4.3±0.4 mg/l, 20% 산소환경의 배양액은 6.89±0.3 mg/l로 유의한 차이를 보였다 ($p<0.05$).

3. 생쥐의 전핵시기 배아를 배양하면서 24시간 간격으로 120시간까지 관찰한 결과 포배기까지의 발달율은 5% 산소 환경에서 59.1% (78/132), 20% 산소환경에서 34% (49/144)였으며, 부화율은 각각 40.9% (54/132), 24.3% (35/144)로 5% 산소환경에서 높은 발달율을 보였다 ($p<0.05$). 또한 포배시기 배아의 할구수는 5% 산소환경에서 97±11.9, 20% 산소환경에서는 72±9.4로 5% 산소환경에서 유의하게 높았다($p<0.05$).

4. Quanti-cell 700 (Applied Imaging Co., UK)으로 측정한 배아내 산소유리기의 상대적 강도는 5% 산소 환경에서 28.9±1.5, 20% 산소환경에서 53.2±3.7로 5% 산소환경에서 유의하게 낮았다 ($p<0.05$).

9 Application of Chromosome Microdissection and Fluorscence in-situ Hybridization (Micro-FISH)

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Meltzer et al. (1992) and Guan et al. (1994) have developed a novel procedure for the rapid generation of region-specific genomic clones from dissected chromosomal DNA using a degenerate oligonucleotide primed polymerase chain reaction (DOP-PCR). Their simplified method also offered the advantage that region-specific painting probes could be used for cytogenetic alnalysis. Micro-FISH, a term they introduced, made it possible to extend the limits of conventional cytogenetic analysis by providing band-specific probes for the analysis of unknown chromosomal regions such as marker chromosomes. Recently, Speicher et al. (1996) have developed epifluorescence filter sets and computer software for the detection and discrimination of 27 different DNA probes hybridized simultaneously, and their DNA probes were generated by microdissection and subsequent PCR amplification and labelled by nick translation.

Although the FISH fields has been extensivery used in human cytogenetics and cancer biology studies, its use is rather limited. Hence, it is important to develop an efficient and rapid method for the generation of whole chromosome painting probes. (WCPs) or chromosome arm painting probes (CAPs). However, we describe here the applicable method of a strategy for the rapid construction of the painting probe and their corresponding DNA libraries were developed by chromosome microdissection, FISH, PCR amplification, microcloning and DNA sequencing.