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Effect of Epidermal Growth Factor on *In Vitro* Maturation in Pig Immature Oocytes

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The objective of this experiment was to test the effect of EGF on nuclear maturation of pig immature oocytes *in vitro*. Basic medium used TCM-199 supplemented with 0.2 mM pyruvate, 1 μg/mℓ estradiol-17β, and 25 μg/mℓ gentamycin, this medium treated with EGF, FSH, and FBS. Experiment 1 examined to the effect according to FSH and concentration EGF (0, 1, 10, and 100 ng EGF/mℓ) in oocytes maturation. Nuclear maturation rates (MII %) of 1, 10, and 100 ng EGF/mℓ (83.0, 86.7, and 87.5%) treatments were significantly higher than those of non- and FSH-treated groups (46.0 and 60.3%, $p < 0.05, 0.001$). Experiment 2 examined to the interactive effects of EGF, FSH, and FBS during oocytes maturation. Nuclear maturation rates (MII %) of EGF alone, EGF plus FSH, EGF plus FBS, FSH plus FBS, and EGF plus FSH added FBS treatments (86.7, 90.2, 87.1, 89.6%, and 92.6%) were significantly higher than FSH and FBS alone treatment (52.2 and 42.3%, $p < 0.001$). Also, cumulus cells expansion of oocytes maturation was examined to total treatments. Normal cumulus cells expansion was shown by FSH plus FBS, EGF, or EGF with FBS combination treatment, but cumulus cells of oocyte complexes were still clumped together in EGF-treated groups although they had separated from oocytes. However, EGF showed a positive on nuclear maturation. These results conclude that EGF alone can stimulate nuclear maturation in pig immature oocytes.

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난자채취후 정자직접주입법(ICSI)에 의한 정자주입시간의 차이에 따른 수정율과 임신율에 관한 연구

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엄기붕 · 오종훈 · 윤태기 · 곽인평 김현주 · 이우식 · 고정재 · 차광열

시험관아기시술에 있어서 난자는 hCG 호르몬의 주입후 대개 36시간 전후에 채취하게된다. 채취후의 난자는 성숙도에 따라 즉시 혹은 ~8시간 정도의 전배양후에 준비된 정자와 수정시키게 된다.

과거에 난자채취후 즉시 혹은 여러시간 경과한 후에 수정을 실시하여 그 차이를 연구한 논문이 발표된바는 있으나, 난자내 정자직접주입법으로 이러한 연구를 실시한 것은 국내외를 막론하고 찾아보기 어렵다. 본원에서는 주로 남성불임이 원인이 되어 수정 및 임신이 불가능한 환자들을 대상으로 하여 난자내 정자직접주입법을 실시하면서, 난자채취후 바로(1-3시간), 혹은 4-6시간, 7-9시간 및 10-12시간 경과한 후에 정자직접주입법을 실시하여 시간 경과에 따른 수정율과 임신율을 비교함으로써 결과에 차이가 있는지 여부를 검토하였다.

총대상 535명의 환자로부터 총 5211개의 난자를 채취하여 이중 성숙된 3462개의 난자를 정자직접 주입하여 2462개 난자가 수정되었다(수정율; 71.1%). 총 535명중 511명의 환자에게

수정란 이식을 실시하였으며 이중 180명이 임신하였다 (임신율 180/511 ; 35.2%).

수정율은 난자채취 직후나 일정시간 경과한 후나 차이가 없었다. 임신율에서도 시간대 별로 통계적 유의차는 발견할 수 없었지만, 난자채취 후 3시간 이내에 정자직접주입법을 실시한 경우에는 다소 임신율이 낮아지는 경향이 관찰 되었다. 따라서 정자직접주입법을 실시할 때에도 최소한 3시간의 전배양은 필요하다고 사료된다.

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In Vitro Maturation of Human Immature Oocytes Following Exposure to Cryoprotectant and Cryopreservation : Incidence of Chromosomal Abnormalities and Limited Effect on the Second Meiotic Spindle.

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Objectives : Successful cryopreservation of human immature oocytes would be essential to establish ovum bank. Recently, survival rate and maturation rate of frozen-thawed immature oocytes have been improved progressively in mammalian species. However, there are few reports on the attempts to freeze germinal vesicle(GV) stage human oocytes. We have found that the human GV stage oocytes showed a low survival rate and a poor developmental capacity(ESHRE 1994;O-128). The reason for these phenomena is unclear.

Therefore, purposes of the present study were : (1) to investigate the incidence of chromosomal anormalities after freezing-thawing, and (2) to investigate the organization of microtubule system of the human oocytes matured in vitro after cryopreservation at GV stage.

Design : Oocytes with no treatment (Group 1), 1, 2-propanediol (PROH) treated oocytes without freezing-thawing (Group 2), and frozen-thawed oocytes (Group 3) were cultured in the medium containing gonadotropins. Gimsa or fluorescence in situ hybridization (FISH) method. Spindle structure was visualized by monoclonal anti-tubulin antibody and TRITC-conjugated second antibody.

Results : Incidences of chromosomal abnormalities were 33.3% (aneuploidy 1; polyploidy 3) in Group 1, 41.4% (aneupoidy 9; polypoidy 3) in Group 2 and 77.8% (aneupolidy 12; polyploidy 9) in group 3. There was significant difference between 33.3 % and 77.8 ($p<0.01$). Incidence of spindle 35.3 % in Group 2 (disorganized shape 5; no spindle 1), and 70.0% in Group 3 (disorganized shape 6; no spindle 8). Higher incidence of abnormalities was found in frozen-thawed oocytes($p<0.01$).

Conclusion : 1. Exposure to PROH itself at the GV-stage had no influence on the chromosomal abnormalities and organization of microtutule system in human immature oocytes. 2. Human oocytes matured in vitro after cryopreservation showed chromosomal and spindle abnormalities. 3. Increased incidence of chromosomal abnormalities in frozen-thawed oocytes cryopreservation. 4. Further studies should be addressed to reuced the incidence of chromosomal and spindle abnormalities and to find out the optimal cryopreservation method of human immature oocytes to improve rates of survival, fertilization, and development after thawing.