경우 포배기로 발달하지 못하고 대부분 2 세포기에서 발생이 정지된다. 이러한 현상을 일으키는 원인중의 하나로 oxygen free radicals의 증가가 관련있는 것으로 알려지고 있다. 이러한 배아내 상승된 oxygen free radicals가 세포막, 단백질, 핵산과 같은 biological system에 상해를 주어 배아 발달에 영향을 미치는 것으로 알려져 있으며, 최근 연구에서는 세포내 oxygen free radicals 증가는 DNA fragmentation을 일으키는 것으로 밝혀져 있다.

본 연구에서는 전핵과 2세포기 시기에 획득된 배아를 체외에서 배양하여 발달 시기에 따른 oxygen free radicals level을 측정하였고, 포배기까지의 발달률을 조사하고 포배내 할구수를 계산하였다. 또한 전핵과 2세포기 시기에 획득된 배아를 free radical scavenger의 하나인 superoxide dismutase (SOD)를 배양액에 첨가하여 각 발달 시기별로 free radicals level 및 발달율과 포배내 할구수를 측정하여 SOD가 배아 발달에 미치는 영향을 알아 보았다. oxygen free radical 측정은 2, 7-dichlorodihydroflurescein diacetate(DCDHF-DA)를 이용하여 440nm의 "excitation"과 510nm의 "emission"하에서 Quanti-cell 500(Applied imaging Co.UK)으로 단일 배아 세포내에서 발생하는 oxygen free radical level을 정량적으로 측정하였다. 또한 포배기의 배아에서 ombined nucler and terminal transferase mediated DNA end labelling(TUNEL) method를 이용하여 할구의 DNA fragmentation의 여부를 in situ detection함으로써 초기 배아내 증가된 oxygen free radicals와 apoptosis와의 연관성을 알아보았다.

P-24

Expression of c-kit in the Ovary and Testis of Fetal and Adult Human and Subhuman Primates

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김학순, Collin B. Smikle, Robert B. Jaffe

Gametogenesis in the mature ovary and testis is regulated primarily by follicle stimulating hormone secreted by the anterior pituitary. Gonadotropin-regulated autocrine and paracrine factors may mediate communication between the various cell types within the microenvironment of the gonads, and these coordinated events may influence follicular development, egg maturation and cyclic release of ova from selected follicles during each menstrual cycle in the ovary and spermatogenesis in the testis. Recently, stem cell factor(SCF) and its receptor, c-kit, have been proposed as critical elements not only in gametogenesis, but also in the migration of germ cells from the hindgut to the gonadal ridge in embryonic development. Although the ontogeny of c-kit has been studied in the reproductive tract of the rodent, it has not been studied in the human and subhuman primates. Therefore, we examined the ontogeny of c-kit in the testis and ovary of the human and subhuman primate.

We determined the localization of c-kit in the testes of 4 human fetuses(15-22 weeks' gestation) and 7 adults(35-82 years old), and in the ovaries of 6 human(13-23 weeks' gestation) and 2 rhesus monkey(90-165 days; model for late gestation human fetus) fetuses and 5 adult human(35-62 years old) and 1 adult rhesus monkey by immunocytochemistry using a highly specific polyclonal antibody to peptides 145-158 of the extracellular portion of the c-kit molecule. In the testes, c-kit was expressed by the spermatogonia in the fetus and by the spermatids and spermatozoa in adults. The level of c-kit expression did not appear to change in the different age groups studied. In the ovaries, staining for c-kit was limited to the oogonia and oocytes in human and rhesus monkey. Furthermore, during fetal life, there was abundant staining for c-kit in the oocytes which diminished after birth and was seen only rarely in the ovaries of postmenopausal women. This decline in staining mirrors the age-related decline in the number of oocytes that occurs with aging. Interestingly, c-kit staining in

naked oocytes was less than in oocytes enclosed within a layer of granulosa cells.

This is the unique report suggesting a difference in expression of c-kit in the different population of oocytes of human and subhuman primates. This report also provides the first ontogenic evaluation of c-kit expression in the germ cells of human and subhuman primates. As SCF has been found in rodent granulosa and sertoli cells, we conclude that c-kit and its cognate ligand, SCF, may play a role in oocyte and spermatozoal development and in the communication between these germ cells and the surrounding mesenchymal cells.

Group 5, discussion : 15:30~16:00

P-25

Technical Advance of Intracytoplasmic Sperm Injection (ICSI) : Multisperm Loadind ICSI Procedure (MSLIP)

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Routine ICSI procedure (ROIP) have been performed that only one immobilized spermatozoon was loaded into the injection pipette and then injected into one oocyte. It is a time consuming procedure in cases of sizable number of ICSI are performed daily. Thus, we tried to shortening the processing time of ICSI, applied a time saving procedure. In this modified method, multisperm loading ICSI procedure (MSLIP), we loaded three immobilized spermatozoa into an injection pipette at once and then injected into three oocytes, one by one. According to sperm quality, we used side migration technique (SMT) or direct PVP technique (DPT) to select normal motile spermatozoa. In our hands, ROIP took average 145 seconds (n=22) and 113 seconds (n=50) per injected oocyte in SMT and DPT, respectively. MSLIP took average 104 seconds (n=110) and 89 seconds (n=45), respectively. This MSLIP saved 41 seconds in SMT and 24 seconds in DPT per injected oocyte. There were no difference in normal fertilization rate between ROIP (70.8%, 51/72) and MSLIP (71.0%, 110/155). In ICSI procedure, saving time have some advantages, shortened the exposure time out of incubator condition and gave the allowance time of other procedures. For these reasons, we propose a time saving ICSI procedure, namely MSLIP.

P-26

삼성제일병원의 TESE-ICSI 200주기 결과에 대한 분석

삼성제일병원 불임연구실, 비뇨기과[1], 산부인과 불임크리닉[2]

전진현, 이호준, 임천규, 박용석, 서주태[1], 이유식[1], 손일표[2], 강인수[2], 전종영[2]

여러 가지 원인에 의한 무정자증 환자에서 고환 정자 채취술(TESE)과 세포질내 정자주입술(ICSI)을 이용하여 성공적인 체외수정과 임신이 보고되고 있다. 본 연구에서는 1994년 11월부터 1996년 8월까지 157 명의 환자에서 시행한 연속된 200 주기의 TESE-ICSI 결과를 분석하여 체외수정 및 임신 결과에 영향을 미치는 요인을 알아보고, TESE-ICSI의 유용성에 대해 검증하고자 한다. 그 결과는 다음과 같다.

1. 폐쇄성 무정자증인 118 명 156주기에서 성숙 정자를 회수하지 못한 경우는 3 주기(1.9%)였으며, 156 주기의 배아이식에서 54 주기(34.6%)에서 임신이 확인되었다. 비폐쇄성 무정자증인 39 명 44 주기에서 성숙 정자를 회수하지 못한 경우는 24 주기(54.5%)였으며, 33 주기의 배아이식에서 11 주기