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High pregnancy rates after ICSI using sperm obtained from frozen-thawed testicular tissues

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Objectives : An introduction of intracytoplasmic sperm injection (ICSI), the breakthrough for severe male infertility in IVF-ET program, showed a high fertilization and pregnancy rates. Indication for its use was increased to obstructive azoospermia combined with MESA and TESE. Cryopreservation of testicular spermatozoa is necessary, because it may avoid a subsequent testicular biopsy for next attempts. However, the efficacy of ICSI outcome after frozen-thawed TESE is not clear. Prospective analysis of fertilization, embryonic development and pregnancy rates were carried out after ICSI between fresh and frozen-thawed TESE, and retrospective correlation between pregnancy outcomes and sperm motility before freezing were analysed.

Materials and Methods : From January 1997 to June 1998, 117 and 40 cycles undergoing TESE-ICSI and frozen-thawed TESE-ICSI programs in Jeil Women's Hospital were analyzed for this study. Frozen-thawed TESE-ICSI group was divided into good motility (n=21) and poor motility group (n=19) according to sperm motility before tissue freezing. Open testicular biopsy was performed under local anaesthesia, and testis tissues were washed and transferred to HTF containing 0.5% HSA. Spermatozoa were squeezed-out from isolated seminiferous tubule by using fine forceps and transferred into injection medium for ICSI. The remaining testis tissues were cryopreserved. After thawed on 37°C incubator and washed with HTF, the retrieved spermatozoa by squeezing out were used for ICSI. The X^2-test was used to compare fertilization, embryonic development, pregnancy and implantation rates. Mean patients age, retrieved and injected oocytes numbers were compared using student's t-test.

Results : The female patients age, the number of retrieved oocytes and fertilization rates (67.4%(820/1217) vs. 64.7% (251/388)) were not different between fresh and frozen-thawed TESE-ICSI group. The quality of oocytes was not different in both group, but embryonic development of frozen-thawed TESE-ICSI group was slightly poor than that of fresh one. However, pregnancy rates of frozen-thawed TESE-ICSI group was similar to that of fresh one(30.0% (12/40) vs. 33.4% (39/117)). In frozen-thawed TESE-ICSI, the fertilization and pregnancy rates in good motility group before freezing were not different from that in poor motility one(68.6%(120/175), 28.6%(6/21) vs. 61.5%(131/213), 31.6%(6/19)).

Conclusions : Spermatozoa from frozen-thawed testis tissues did not damaged on the ability of fertilization and further development after ICSI. Also, sperm motility before tissue freezing has no effect on the outcomes of frozen-thawed TESE-ICSI. Therefore, it is suggested that our freezing-thawing method of testis tissue has an advantage of eliminating repeated testicular biopsy and may be simple and valuable technique in TESE-ICSI.

I S—92 Possible involvement of human granulosa clls inhibin/activin α-subunit mRNA in oocyte maturation and embryogenesis.

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[Objective]To investigate if mRNA levels of inhibin/activin subunit, activin receptor(ActR) subtypes and follistatin in human granulosa cells (GCs) have any relationship with quality of oocyte and embryo derived from the same follicle. [Methods]Human GCs were obtained from IVF follicle aspirate and total RNA was extracted and reverse transcribed to cDNA using oligo dT primer. Then semi-quantitative PCR was performed with primer sets for inhibin/activin subunits (α,βA,βB), follistatin or ActR subtypes (IA,IB,IIA,IIB), respectively. After correction by β-actin mRNA level, the followings were examined: (1) correlation between targets, (2) relationship between mRNA levels of each targets with (a) oocyte maturational stage and (b) embryo quality. This study was done with informed consent. [Results]Total of 114 GCs samples were obtained from 31 patients. (1) In all combination between targets, only ActR type IA and IIA and type IB and IIB had strong positive correlation, respectively. (2) α-subunit increased from GV to M1 stage then decreased at M2 stage. (3) Embryo morphological quality had negative correlation with α-subunit and positive correlation with ActR IIA level. [Conclusions] (1) It is suggested that there are specific functional combinations between activin receptor subtypes. (2) It is supposed that α-subunit is a good candidate to predict the quality of oocyte and embryo.