Effect of Testicular Histopathology on Pregnancy Outcomes in Non-Obstructive Azoospermia

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비폐쇄성 무정자증 환자에서 고환의 조직병리학적 진단에 따른 체외수정시술 결과의 비교

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목 적: 비폐쇄성 무정자증 환자에서 고환의 조직병리학적 진단에 따라 고환조직내 정자채취술 (Testicular sperm extraction, TESE) 후 난자세포질내 정자주입술 (Intracytoplsmic sperm injection, ICSI)의 체외수정시술 결과를 알아보고자 하였다.

연구방법: 비폐쇄성 무정자증으로 고환조직내 정자채취술 후 난자세포질내 정자주입술을 이용하여 배아 이식을 시행한 122주기를 분석하였다. 고환의 조직병리학적 진단에 따라 Germ-cell aplasia (GA, 40주기), Maturation arrest (MA, 32주기) and severe hypospermatogenesis (S-HS, 50주기)로 구분하여 체외수정시술 결과를 비교하였으며, 이들 결과를 난자세포질내 정자주입술을 이용한 폐쇄성 무정자증 환자의 체외수정시술 결과와 비교하였다.

결 과: 고환조직내 정자채취술 후 난자세포질내 정자주입술시 수정율은 각각 58.1% in GA, 42.2% in MA and 48.0% in S-HS로 조직병리학적 진단에 따른 차이는 없었으며, 폐쇄성 무정자증 환자의 72.9%에 비해 유의하게 낮은 수정율을 보였다 ($p<0.001$). 고환조직내 정자채취술시 채취된 정자 (spermatozoa, 94주기)로 난자세포질내 정자주입술을 시행한 주기의 배아 이식 후 임신율은 각각 22.6% in GA, 29.4% in MA와 26.1% in S-HS이었으며, 출생률은 각각 16.1%, 29.4%와 19.6%로 조직병리학적 진단에 따른 차이는 없었다. 정자세포 (spermatid, 16주기)를 사용하여 난자세포질내 정자주입술을 시행한 주기의 임신율은 각각 0.0% (0/3 주기), 9.1% (1/11주기)와 0.0% (0/2주기)이었으며, 출생률은 각각 0.0%이었다. 정모세포 (spermatocyte, 12주기)를 사용한 주기의 임신율은 각각 0.0% (0/6주기), 0.0% (0/4주기)와 0.0% (0/2주기)이었으며, 출생률도 각각 0.0%이었다.

결 론: 비폐쇄성 무정자증환자의 배아이식을 시행한 주기에서 고환의 조직병리학적 진단에 따른 난자세포질내 정자주입술시 수정율은 차이가 없었으며, 폐쇄성 무정자증 환자에 비해 유의하게 낮은 수정율을 보였다. 비폐쇄성 무정자증환자에서 고환조직내 정자채취술시 정자를 채취하여 난자세포질내 정자주입술을 시행한 주기의 체외수정시술 결과는 고환의 조직병리학적 진단에 따라 차이를 보이지 않는다.

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중심단어: 비폐쇄성 무정자증, 생식세포 형성 부전증, 성숙정자, 정자형성저하증

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Non-obstructive azoospermia (NOA) is the most severe form of male infertility and is present in about 5% of all investigated infertile couples.[1] Testicular sperm extraction (TESE) combined with intracytoplasmic sperm injection (ICSI) has offered new prospects for the treatment of NOA and allowed these patients to become fathers.

Despite of successful spermatozoa extraction, there are controversies over ICSI outcomes in NOA patients compared with obstructive azoospermia (OA) patients. Some studies reported no difference in fertilization rates (FR) and clinical pregnancy rates (CPR) between the two groups.[2,3] Others reported significantly lower FR and CPR in NOA compared with OA.[4~9] Previous reported studies have defined NOA based on clinical parameters, and proper histological diagnosis has not been used.

Testicular biopsy is necessary for evaluating the etiology of azoospermia, and histology is the most reliable predictor of successful sperm retrieval with TESE. Therefore, testicular histopathology may provide information for infertility specialists when counseling NOA patients. NOA can be caused by different histopathological etiologies, and the resulting spermatozoa can produce different ICSI outcomes even though the clinical syndrome in each case is the same.

There have been reports suggesting that paternal factors influence embryo development. The *in vitro* fertilization (IVF)-ICSI cycles have shown lower blastocyst formation rates compared with those of conventional IVF cycles.[10,11] The source of the spermatozoa affects the rate of blastocyst formation and blastocyst implantation. Spermatozoa from NOA patients, when utilized for ICSI, result in embryos that progress to the blastocyst stage at a lower and slower rate.[11,12]

The impact of histopathology on sperm reproductive capacity is not well established and there are insufficient reports regarding testicular histopathology on ICSI outcomes.

The aim of this study was to evaluate TESE/ICSI outcomes of embryo-transferred cycles in NOA patients with different histopathologic subtypes and compare the results with those of a control group, OA patients.

MATERIALS AND METHODS

1. Patients

From May 1996 to February 2006, embryo-transferred TESE/ICSI cycles of azoospermic patients, NOA and OA, were analyzed retrospectively.

Each patient was evaluated with a physical examination, semen analysis and hormonal profile, and testicular biopsy was performed in NOA patients. Each seminiferous tubule in the biopsy specimen was evaluated and classification was made based on the most advanced pattern of spermatogenesis: Hypospermatogenesis (HS), reduction in the degree of normal spermatogenesis and focal spermatogenesis with spermatid stage arrest. Maturation arrest (MA), spermatocyte stage arrest with absence of the later stages of germ cells in spermatogenesis and Germ-cell aplasia (GA), absence of germ cells in the seminiferous tubules. HS was divided into mild, moderate, and severe on the degree of normal spermatogenesis. Only severe HS (S-HS) was included in this study because patients with mild and moderate HS are considered to have complete spermatogenesis.[13,14]

A total of 122 embryo-transferred TESE/ICSI cycles of NOA patients with different histopathologic subgroups were compared: GA (40 cycles), MA (32 cycles) and S-HS (50 cycles). Patients with abnormal karyotypes were excluded.

Embryo-transferred OA patients (667 cycles) who had TESE/ICSI during the same period were used as controls.

2. Multiple TESE (mTESE)

First, the diagnostic testicular biopsy was performed. Therapeutic testicular biopsy for TESE was performed

under general anesthesia at the time of ICSI or before ICSI. Using a 4 cm scrotal incision, small pieces of testicular tissue were removed through four 0.5 cm incisions on each testicle's tunica albuginea laterally. These tissues were placed in a Petri dish filled with 3 mL of HEPES [4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid] - buffered Earle's medium supplemented with 0.4% of human serum albumin. The testicular tissues were shredded with glass slides on the warmed stage of a stereomicroscope at 40 magnification. Under an inverted microscope (400× magnification) the minced tissue was then checked for the presence of spermatozoa. When any of one or more spermatocyte, spermatid or spermatozoa was found, sperm retrieval was considered a success and the tissue was cryopreserved for future cycles. If sperm retrieval failed, the same procedure was performed on the contralateral testicle.

3. Ovarian stimulation and oocyte retrieval

Controlled ovarian hyperstimulation (COH) was performed in female partners using a gonadotropin releasing hormone analogue with human menopausal gonadotropin or recombinant follicle stimulating hormone. Oocyte retrieval was performed via a transvaginal approach with sonographic guidance 36 hours after the administration of 10,000 IU of human chorionic gonadotropin (hCG) (Pregnyl®, Organon, Netherland). After oocyte retrieval, oocyte maturity was evaluated under an inverted microscope at ×400 magnification. The oocytes were incubated in human tubal fluid medium (Irvine Scientific, Irvine, CA) supplemented with 10% synthetic serum supplement (SSS; Irvine Scientific) at 37℃, 5% CO_2 in air.

A single spermatoza was inserted through the zona pellucida to the metaphase II oocyte using the ICSI procedure. The procedures have been described previously.[15]

4. Assessment of fertilization

Sixteen to 18 hours after ICSI, oocytes were observed for the presence of pronuclei and polar bodies under the inverted microscope (×200~400). The presence of two polar bodies together with two clearly visible pronuclei was considered normal fertilization (the 2 pronuclei, or 2PN stage). The cleavage and quality of embryos were observed at 40~44 hours after ICSI.

5. Embryo transfer and establishment of clinical pregnancy

The embryos were transferred into the uterine cavity on the third day after oocyte retrieval. The hCG (+) was defined as serum β-human chorionic gonadotropin (β-hCG) levels over 5 mIU/mL at 12 days after oocyte retrieval. Clinical pregnancy was defined as the presence of a fetal heartbeat using ultrasonography at approximately 6~7 weeks of pregnancy. Clinical abortion was defined as the absence of a fetal heartbeat after positive clinical pregnancy diagnosis.

6. Statistical analysis

Fisher's exact test was used to compare the FR, CPR and live birth rate (LBR) in the two groups. $P<0.05$ was considered statistically significant.

RESULTS

A total of 150 cycles were attempt for NOA group; 51 cycles for GA, 46 cycles for MA and 53 cycles for S-HS. Multiple TESE were successful in 40 cycles out of 51 attempt cycles for GA, in 34 out of 46 attempt cycles for MA and in 50 out of 53 attempt cycles for S-HS. For successful mTESE cycles, ICSI was performed with spermatids in 17 cycles and with spermatocytes in 13 cycles (Table 1). In OA mTESE were successful in all of the attempt cycles and ICSI was performed

with spermatozoa. After mTESE/ICSI procedure, embryo transfer was performed except 11 cycles; 2 cycles in NOA subgroup, MA and 9 cycles in OA (Table 1).

For embryo-transferred cycles, the mean age of male

Table 1. Demographics of NOA and OA

	NOA			OA
	GA	MA	S-HS	
No. of attempt cycles	51	46	53	676
No. of mTESE failure cycle	11	12	3	−
No. of successful mTESE cycle	40	34	50	676
Spermatozoa	31	17	46	676
Spermatids	3	12	2	0
Spermatocytes	6	5	2	0
No. of embryo transfer cancel cycles	0	2	0	9
No. of embryo transfer cycles	40	32	50	667
Male patient age (yrs)	35.4±5.1†	32.6±3.6*,†	35.1±4.1*	36.4±4.6
Female partner age (yrs)	32.7±4.7†	29.0±3.0*,†	31.7±3.0*	32.9±4.1
No. of retrieved oocytes	14.4±9.2	15.8±7.2	16.9±7.9	13.8±8.2
Fertilization rate (%)	58.1*,†	42.2*,†	48.0*	72.9

NOA, non-obstructive azoospermia, OA, obstructive azoospermia with TESE-ICSI
GA, Germ-cell aplasia; MA, Maturation arrest; S-HS, severe hypospermatogenesis
*p<0.05 compared with the value in OA group, †p<0.05 between two group

Table 2. Pregnancy outcomes of ICSI with spermatozoa in NOA subgroups and OA

	NOA			OA
	GA	MA	S-HS	
No. of ICSI cycles with spermatozoa	31	17	46	667
Female partner age (yrs)	33.5±4.9†	29.1±3.2*,†	31.6±3.1	32.9±4.1
Male patient age (yrs)	35.9±4.9	33.4±3.4*	34.9±4.2	36.4±4.6
No. of retrieved oocytes	13.0±8.8	14.5±7.3	17.3±8.1*	13.8±8.2
Fertilization rate (%)	64.0†	55.5*	49.7*,†	72.8
No. of embryos transferred	3.7±1.8	3.5±1.1	3.7±1.3	4.2±1.7
hCG(+) / embryo transfer (%)	29.0 (9/31)	41.2 (7/17)	30.4 (14/46)	37.8 (252/667)
CPR / embryo transfer (%)	22.6 (7/31)	29.4 (5/17)	26.1 (12/46)	31.8 (212/667)
LBR / embryo transfer (%)	16.1 (5/31)	29.4 (5/17)	19.6 (9/46)	26.5 (177/667)

CPR, clinical pregnancy rate; LBR, live birth rate
*p<0.05 compared with the value in OA group, †p<0.05 between two group

Table 3. Pregnancy Outcomes of ICSI in NOA according to sperm source

	NOA		
	GA	MA	S-HS
No. of ICSI cycles with Spermatozoa	31	17	46
hCG (+) / embryo transfer (%)	29.0 (9/31)	41.2 (7/17)	30.4 (14/46)
CPR / embryo transfer (%)	22.6 (7/31)	29.4 (5/17)	26.1 (12/46)
LBR / embryo transfer (%)	16.1 (5/31)	29.4 (5/17)	19.6 (9/46)
No. of ICSI cycles with Spermatid	3	12	2
hCG (+) / embryo transfer (%)	0.0 (0/3)	18.2 (2/11)	0.0 (0/2)
CPR / embryo transfer (%)	0.0 (0/3)	9.1 (1/11)	0.0 (0/2)
LBR / embryo transfer (%)	0.0 (0/3)	0.0 (0/11)	0.0 (0/2)
No. of ICSI cycles with Spermatocyte	6	5	2
hCG (+) / embryo transfer (%)	0.0 (0/6)	0.0 (0/4)	0.0 (0/2)
CPR / embryo transfer (%)	0.0 (0/6)	0.0 (0/4)	0.0 (0/2)
LBR / embryo transfer (%)	0.0 (0/6)	0.0 (0/4)	0.0 (0/2)

CPR, clinical pregnancy rate; LBR, live birth rate

patients in each NOA subgroup tended to be lower than that of OA. The mean age of female partners in each NOA subgroup was lower than that of OA, and especially MA and S-HS subgroup showed significant difference compared with OA; however, there was no significant difference in the number of retrieved oocytes between NOA subgroup and OA. After mTESE/ICSI, 2PN FR in NOA subgroups were 58.1% in GA, 42.2% in MA and 48.0% in S-HS, which were significantly lower than the 72.9% FR of OA patients ($p<0.001$) (Table 1).

For ICSI with spermatozoa cycles in NOA, the FR of GA in NOA subgroup was higher than those of other subgroups, and a significant difference was found between GA and S-HS (64.0% vs. 497.%, $p=0.02$). However, there were no significant differences in CPR and LBR with regard to embryo transfer among NOA subgroups.

The FR of OA, 72.8% was higher than that of the NOA subgroups, and significant differences were found compared with MA (55.5%, $p=0.005$) and S-HS (49.7%, $p<0.001$). The CPR and LBR per embryo transfer of OA was 31.8% and 26.5%, respectively, which was higher than that of NOA subgroups without a significant difference (Table 2).

With regard to ICSI with spermatid or spermatocyte cycles in NOA, the CPR per embryo transfer of ICSI with spermatid cycles was 0.0% (0/3) in GA, 9.1% (1/11) in MA and 0.0% (0/2) in S-HS without a live birth, respectively. For ICSI with spermatocyte cycles, there was no clinical pregnancy and live birth in any subgroup (Table 3).

DISCUSSION

The introduction of ICSI has revolutionized treatment of male factor infertility. In NOA patient, the testis is the only source of spermatozoa.[16,17] Testicular sperm extraction combined with ICSI is the treatment of choice; there-

fore, even with a single spermatozoa a pregnancy can be achieved. As sperm production is minimal and heterogeneous in patients of NOA, multiple biopsies (mTESE) are necessary to increase the success rate of sperm retrieval. Various investigators have reported successful sperm retrieval rates ranging from 48 to 86% using multiple biopsies.[18,19]

Testicular biopsy was performed prior to the IVF-ICSI cycle for histologic diagnosis of NOA. Histologic diagnosis is necessary for evaluating the etiology of azoospermia and is the most reliable parameter for prediction of successful sperm retrieval when using TESE.[20,21]

However, a few studies on testicular histological classification have been published. In the Levin classification, patients with pure germinal cell aplasia were grouped with those featuring predominant germinal cell aplasia associated with focal spermatogenesis.[22] This conventional histological classification may not provide sufficient detail or accuracy as it relates to fertility potential in men with NOA. In our study, testicular histology was subgrouped according to the most advanced pattern of spermatogenesis rather than the predominant pattern because the likelihood of sperm retrieval is dependent on the most advanced region of spermatogenesis.

Despite the potential of successful sperm retrieval, a significant proportion of NOA patients fail to have sperm retrieved with mTESE. Seo et al.[21] have reported that spermatozoa were successfully recovered in 94 of 178 (52.8%) patients with NOA. Su et al.[23] have reported that in men with pure Sertoli-cell-only syndrome, the chance of sperm retrieval is 24%, whereas those with HS have a much higher success rate (79%) and those with MA have a sperm retrieval success rate of 47%. Our study included successful mTESE cycles, not only spermatozoa but spermatid and spermatocyte retrieval cycles.

Most of the studies on ICSI outcomes in NOA patients have reported lower fertilization and pregnancy rates than OA, although these differences have not always been statistically significant.[4~9] A recent meta-analysis indicates that there was a significantly higher fertilization rate in OA patients for all analysed studies.[24]

In our study, NOA patients were divided into subgroups according to testicular histopathology. For each NOA subgroup receiving embryo-transferred mTESE/ICIS cycles, the FR was significantly lower than that of OA and there were no significant differences among NOA subgroups. For ICSI with spermatozoa cycles, the FR of each NOA subgroup was lower than that of OA. Tournaye et al.[25] reported lower FR in patients with GA and MA than in those with germ-cell hypoplasia ($p< 0.002$). However, Vernaeve et al.[26] reported no difference in FR among NOA subgroups. The significantly lower FR after ICSI with testicular spermatozoa from NOA patients may be explained by the fact that testicular spermatozoa from NOA have impaired function, possibly because of the increased incidence of genetic abnormalities.[27] In NOA patients, spermatozoa reveal higher chromosomal aneuploidy.[28~30] Moreover, ICSI with testicular spermatozoa carries an increased risk of embryo fragmentation.[31]

Higher FR were not translated into significantly higher CPR or LBR, as shown in the results of the meta-analysis of the five studies.[24] For ICSI with spermatozoa cycles, CPR and LBR per embryo transfer were higher in OA compared with NOA subgroups without significant differences and there were no difference among NOA subgroups. For ICSI with spermatocyte or spermatid, there were no live births in any subgroup.

Vernaeve et al.[26] reported significantly different CPR per embryo transfer (17.9% vs. 26.7%, $p<0.008$) between NOA and OA. However, there were no differences among the following NOA subgroups; GA with or without focal spermatogenesis, MA with or without focal spermatogenesis and tubular sclerosis/atrophy which was based on the most advanced pattern of spermatogenesis.

Schwarzer et al.[32] reported that the underlying cause of azoospermia is the most important factor determining the ICSI outcomes and in cases of NOA, patients with Sertoli-cell only syndrome have the lowest birth rate, 9% after ICSI.

A high incidence of mosaicism in embryos derived from TESE in patients with severe deficits in spermatogenesis has been observed.[27] While aneuploidy in embryos appears to increase with maternal age and is related to defects in the oocyte, mosaicism and chaotic mosaicism may be more related to defects in the spermatozoa.[33] Spermatozoa derived from mTESE in NOA patients may have a higher rate of compromised or immature centrosome structures leading to mosaicism in the embryo.[34] These mosaic embryos can not result in viable offspring.[35,36]

What is reassuring about our findings is that in NOA patients, if spermatozoa extraction from the testis is successful and embryo transfer is possible, there is no difference in ICSI outcomes among NOA subgroups. NOA histopathology may not affect pregnancy outcomes, which are the same as those of OA. Further studies with more cycles are necessary to clarify the histopathologic effect on ICSI outcomes.

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= **Abstract** =

Objective: To evaluate outcomes of patients with non-obstructive azoospermia (NOA) undergoing the testicular sperm extraction (TESE) combined with intracytoplasmic sperm injection (ICSI) with different histopathologic subgroups.

Method: A total of 122 embryo-transferred TESE/ICSI cycles were compared among NOA subgroups; Germ-cell aplasia (GA, 40 cycles), Maturation arrest (MA, 32 cycles) and severe hypospermatogenesis (S-HS, 50 cycles). Obstructive azoospermia (OA, 667 cycles) patients were served as a control. TESE/ICSI outcomes such as fertilization rate (FR), clinical pregnancy rate (CPR) and live birth rate (LBR) were evaluated.

Results: The 2PN FR of embryo-transferred TESE/ICSI cycle was 58.1% in GA, 42.2% in MA and 48.0% in S-HS, which was significantly lower than that of OA (72.9 %, $p<0.001$). For ICSI-spermatozoa cycles, there were no significant differences in CPR (22.6%, 29.4% and 26.1%) and LBR (16.1%, 29.4% and 19.6%) among NOA subgroups. The CPR of ICSI-spermatid cycles was 0.0%, 9.1% and 0.0% without a live birth. For ICSI-spermatocyte cycles, no clinical pregnancies occurred in any group.

Conclusion: There was no significant difference in the FR of embryo-transferred TESE/ICSI cycles among NOA subgroups. The FR among all NOA subgroups was significantly lower than that of OA. Testicular histopathology in NOA did not affect successful pregnancy if spermatozoa extraction from the testis is successful and embryo transfer is possible.

Key Words: Non-obstructive azoospermia, Germ cell aplasia, Maturation arrest, Hypospermatogenesis