Article

Male and female factors that influence ICSI outcome in azoospermia or aspermia

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Abstract

Factors that influenced the clinical results of 220 first-attempt intracytoplasmic sperm injection (ICSI) cycles with testicular spermatozoa were evaluated in 107 men with non-obstructive azoospermia, 72 with obstructive azoospermia and 41 with aspermia. Linear and logistic regression analysis showed that the fertilization rate depended positively on Johnsen score (P = 0.016) and on the type of ovarian stimulation: a higher fertilization rate was observed after ovarian stimulation with agonist and recombinant FSH than after stimulation with agonist and urinary menopausal gonadotrophin (P = 0.026). Embryo development to the blastocyst stage was predicted positively by the number of injected oocytes (P = 0.016) and negatively by male FSH concentration (P = 0.019). A higher proportion of blastocysts developed after the use of frozen–thawed spermatozoa in comparison to fresh spermatozoa (P = 0.034). Embryo development to the blastocyst stage influenced pregnancy (P = 0.002) and live birth outcomes (P = 0.005); live birth was also predicted by female age (P = 0.046). Embryo culture to day 5 in comparison to day 2 did not provide higher live birth rates. In azoospermia/aspermia, the ICSI outcome depends on both male factors (FSH, Johnsen score, sperm status and motility) and female factors (age, number of injected oocytes).

Keywords: *aspermia*, *azoospermia*, *blastocyst*, *female age*, *ICSI*, *sperm quality*

Introduction

Soon after the introduction of intracytoplasmic sperm injection (ICSI) to overcome male infertility due to oligoasthenoteratozoospermia, testicular and epididymal sperm aspiration/extraction combined with ICSI was proposed to men with obstructive (OA) and non-obstructive azoospermia (NOA) (Craft and Shrivastav, 1994; Devroey *et al*., 1995).

At the University Medical Centre Ljubljana, the first percutaneous epididymal aspiration in a man with congenital bilateral absence of vas deferens was successfully performed in October 1995, resulting in a pregnancy which ended in an early miscarriage. The first successful testicular sperm extraction (TESE) and ICSI for OA that resulted in the birth of a healthy baby were performed in November 1996.

In 14 years of experience with ICSI in the treatment of azoospermia/aspermia, a number of clinical and biological male and female factors have been found to have an influence on the ICSI outcome.

After ICSI, the fertilization rate is lower in couples with male Sertoli cell-only syndrome or maturation arrest (Tournaye *et al*., 1996; Nagy *et al*., 1998), and higher if spermatogenesis is normal (Nagy *et al*., 1998). Motile testicular spermatozoa are associated with higher fertilization (Nagy *et al*., 1998) and pregnancy rates (Park *et al*., 2003). High fertilization and pregnancy rates are also obtained with viable although immotile spermatozoa (de Oliveira *et al*., 2004). On the other hand, increased sperm mitochondrial DNA deletions and nuclear DNA fragmentation result in lower pregnancy rates (Lewis *et al*., 2004). Male smoking has also been related with decreased live birth rates (Zitzmann *et al*., 2006). No report has yet considered direct effects of increased testicular and epididymal sperm aneuploidies (Levron *et al*., 2001) on the outcome of ICSI. In addition, Friedler *et al*. (2002) reported that

in patients with NOA and OA, no specific male factors influence the ICSI outcome. Furthermore, ICSI with testicular spermatozoa was reported as being less successful in men with NOA than in men with OA (Vernaeve *et al.*, 2003), although some groups have obtained identical results in NOA and OA (Ghanem *et al.*, 2005). As for OA, some authors have obtained better results in OA with congenital (Pasqualotto *et al.*, 2005) and others in OA with acquired obstruction (Mansour *et al.*, 1997).

The purpose of this retrospective study was to investigate the influence of male factors, including age, history of cryptorchidism, testicular volume, serum FSH concentration, Johnsen score, duration of sperm cryostorage and sperm status (frozen–thawed versus fresh), and sperm motility at ICSI, and female factors, including age, associated female pathology, type of ovarian stimulation, number of injected oocytes, and duration of embryo culture, on the clinical outcome of 220 first-attempt ICSI cycles in men with azoospermia or aspermia. Based on these findings, knowledge regarding the appropriate treatment modalities in these patients might improve, and provide additional information for counselling the couples.

Materials and methods

Studied population

From September 1996 to September 2006, 500 ICSI cycles with epididymal or testicular spermatozoa were performed at the Reproductive Unit, Department of Obstetrics and Gynecology, University Medical Centre Ljubljana, for couples with azoospermia/aspermia. For this analysis, only first-attempt ICSI cycles using testicular spermatozoa, i.e. 220 cycles in 107 couples with NOA, 72 couples with OA and 41 couples with aspermia, were taken into account.

Clinical and hormonal assessments

Men underwent andrological evaluation including a complete medical history, with particular attention to a history of cryptorchidism, as well as physical examination with testicular volume measurement by a Prader orchidometer. All female partners underwent clinical examination, hormonal evaluation, and pelvic ultrasound examination; 157 women (71.4%) also underwent laparoscopy and/or hysteroscopy.

Serum FSH was measured in 144 men. To June 2001 it was measured by Microparticle Enzyme Immunoassay (AxSYM System; Abbott, Chicago, IL, USA), and thereafter by Immulite 2000 (Diagnostic Products Corporation, Los Angeles, CA, USA). Serum FSH concentration was considered normal when <10 IU/l.

Testicular biopsy, histological evaluation, sperm retrieval and testicular tissue cryopreservation

Spermatozoa were retrieved by TESE or testicular aspiration (TESA) performed under local anaesthesia. In TESE procedures (n = 187), two samples of testicular tissue were taken from each testis. One sample was fixed for histopathological examination, and spermatogenesis was classified as normal or incomplete, i.e. as hypospermatogenesis, maturation arrest and/or Sertoli cell-only syndrome with focal spermatogenesis. In addition, the degree of testicular spermatogenesis was evaluated by Johnsen score, from score 1 (no germinal cell in thin sclerotic seminiferous tubules) to score 10 (spermatozoa present in thick epithelium). Spermatozoa were isolated from the second sample.

In OA and aspermia, TESA (n = 33) was performed using 16-gauge needles.

Diagnostic testicular sperm retrieval before ICSI followed by sperm cryopreservation has been the strategy of choice in this centre since 1996. Exceptions (use of fresh spermatozoa) in the series were the cases of erection failure on the day of ICSI (episodic aspermia, n = 15) and the cases with no spermatozoa retrieved on the ICSI day in the testicular tissue frozen before ICSI, or those in men with previously known histological diagnosis but without stored spermatozoa (NOA, n = 30; OA, n = 17; and chronic aspermia, n = 6), a total of 68 cases. Testicular tissue was checked for the presence of spermatozoa, then homogenized and cryopreserved in a 10% solution of glycerol in Flushing Medium (MediCult, Jyllinge, Denmark), cooled via programmed steps to –150°C in a Minicool machine (Air Liquide, Marne-La-Vallee, France) and stored in liquid nitrogen at –196°C until use. For the ICSI procedure, the sample was warmed rapidly to 30°C, and prepared by PureSperm (Nidacon International AB, Gothenburg, Sweden) density gradient centrifugation (100%/40%) (Virant-Klun *et al.*, 2003b).

The NOA group (n = 107) included men with incomplete spermatogenesis; men with hypogonadotrophic hypogonadism and Klinefelter syndrome were excluded. The OA group included men with congenital bilateral absence of vas deferens (n = 15), obstruction due to infection (n = 51) and vasectomy reversal failure (n = 6).

Chronic aspermia (n = 25) was related to anejaculation secondary to lymph node dissection for cancer treatment (n = 11), spinal cord injury (n = 8), extensive pelvic intervention for bladder extrophy (n = 1) and retrograde ejaculation for diabetes (n = 4); in one case no identifiable cause was found. Additionally, 16 men suffered from ejaculation failure (episodic aspermia group) on the day of ICSI.

The study was approved by the Slovenian National Medical Ethics Committee, Ministry of Health; informed consent for the use of data for scientific evaluation was obtained from all the patients.

ICSI procedure

Ovarian stimulation was performed using a long protocol of gonadotrophin-releasing hormone (GnRH) agonist (buserelin acetate; Suprefact; Sanofi Aventis, Frankfurt am Main, Germany) and human menopausal gonadotrophin (Pergonal/Metrodin HP-75; Serono, Vienna, Austria; and Menopur; Ferring, Kiel, Germany) (n = 151) between 1996 and October 2001, or recombinant FSH (Gonal F; Serono) (n = 57) between November 2001 and 2006; in 12 cases GnRH antagonist (Cetrotide; Serono) and recombinant FSH (Gonal F) or highly purified menopausal gonadotrophin (Menopur) were used. Human chorionic gonadotrophin (HCG; Primogonyl; Serono; and

Pregnyl; Organon, Oss, The Netherlands) was applied (10,000 IU i.m.) when follicles reached a mean diameter of ≥21 mm. After oocyte retrieval, performed 36 h after HCG injection, follicles were flushed with Flushing Medium, oocytes were identified under the stereomicroscope (SMZ-28; Nikon, Tokyo, Japan), washed and stored in Universal IVF Medium (MediCult) in a CO_2 incubator (HeraCell; Heraeus, Hanau, Germany) at 37°C and 5% CO_2 in air until denudation. Mature metaphase II oocytes were transferred using a denudation pipette (Swemed; VitroLife Sweden AB, Stockholm, Sweden) into pre-incubated hyaluronidase SynVitro Hydase (MediCult) where they were left for a short time, and then washed by passing them through 0.5 ml volumes of pre-incubated Flushing Medium three times. After denudation, each oocyte was transferred into a droplet of pre-incubated Sperm Preparation Medium (MediCult) under mineral oil (MediCult).

After TESE or TESA, each tissue sample was carefully observed under the inverted microscope for the presence of spermatozoa, and prepared by PureSperm density gradient (100%/40%) centrifugation (185 *g*, 30 min) and washing of the 100% fraction in Sperm Preparation Medium by centrifugation (252 *g*, 10 min) as described elsewhere (Virant-Klun *et al.*, 2003a). In men with poor spermatogenesis, spermatozoa can usually be found in the 40% fraction. After centrifugation, each pellet was resuspended in Sperm Preparation Medium and droplets of sperm suspension were prepared under paraffin oil. At ICSI, testicular sperm motility was classified as progressively motile versus non-progressively motile or immotile. ICSI was performed in all of the oocytes from a single woman with progressively motile spermatozoa after immobilization without polyvinylpyrrolidone. If there were no progressively motile spermatozoa, all oocytes were injected either with non-progressively motile or immotile spermatozoa. Sperm microinjection was performed about 2 h after oocyte retrieval. The oocyte polar body was oriented at the 6 or 12 o'clock position. After microinjection, each oocyte was washed in Universal IVF Medium and cultured in 0.5 ml Universal IVF Medium in a CO_2 incubator. If only a few oocytes were available, immature, metaphase I oocytes were injected only if they matured on the same day. Immature, germinal vesicle stage (prophase I) and degenerated oocytes, and oocytes with damaged or irregular zona pellucida, were not injected. Oocyte fertilization was checked the following morning and was defined as the presence of two pronuclei and the extrusion of the second polar body.

Embryo culture to day 2 or 5

Until the year 2000, embryos were routinely cultured for 2 days to the cleavage stage (190 embryos in 71 cycles), and since the year 2000, for 5 days to the blastocyst stage (381 embryos in 109 cycles). Cleavage-stage embryos were cultured in Universal IVF Medium and blastocysts in the Blast Assist sequential media system (MediCult); to reach the blastocyst stage, fertilized oocytes were transferred into fresh pre-incubated M1 medium, and on day 3 embryos were transferred into fresh pre-incubated M2 medium. Embryos on day 2 were classified as high-quality when they had at least four blastomeres of equal round shape and size, if anucleate fragments were present in less than 10% of the embryo volume, and if embryos did not show any multinucleation, damage of the zona pellucida, and any signs of degeneration (i.e. brown cytoplasm, presence of vacuoles in the cytoplasm). Blastocysts were characterized by grading the trophectoderm, inner cell mass, and blastocoele cavity. A maximum of two cleavage-stage embryos with the best morphology and no multinucleation on day 2, or best developed morulae or blastocysts on day 5, were transferred via a TDT catheter set (Prodimed, Neuilly-en-Thelle, France).

Luteal support, pregnancy and early miscarriage

The luteal phase was supported by HCG (Pregnyl) (5000 IU i.m.) and by dydrogesterone (Dabroston; Belupo, Koprivnica, Croatia) per os, 300 mg/day until the year 1998, and by progesterone (Utrogestan; Meda Pharma Gmbh, Vienna, Austria) per os and intravaginally 600 mg/day, starting on the day of embryo transfer from the year 1999. Clinical pregnancy was confirmed by ultrasound demonstration of cardiac activity at 7 weeks. Early miscarriage was defined as a pregnancy loss before 15 weeks of gestation.

Statistical analysis

Statistical analyses were conducted using SPSS software (SPSS Inc., Chicago, IL, USA) version 15.0. The serum FSH concentration was log transformed.

ANOVA, Kruskal–Wallis and chi-squared tests were used to assess differences in age, number of injected oocytes and developed embryos, and pregnancy, early miscarriage and live birth rates between the groups according to the type of male pathology (NOA, OA and aspermia groups).

The factors influencing the ICSI outcome were then tested in the entire study population regardless of the type of male pathology. Linear and logistic regression models were built with fertilization rate, embryo development to the blastocyst stage, pregnancy and live birth occurrence as dependent variables, and male age, history of cryptorchidism, testicular volume, FSH concentration, Johnsen score, duration of sperm cryostorage before ICSI (<1 year versus >1 year), status (fresh versus frozen–thawed) of spermatozoa used, and sperm motility at ICSI (motile versus immotile), and female age, number of injected oocytes, type of ovarian stimulation used (agonist and urinary menopausal gonadotrophin versus agonist and recombinant FSH), and associated female pathology as predictors.

Mann–Whitney *U*-test and chi-squared test were used to assess differences in female age, number of injected oocytes, fertilization, pregnancy and live birth rates according to duration of embryo culture (2 days versus 5 days). Statistical significance was set at $P < 0.05$.

Results

General ICSI results

Pregnancy, early miscarriage and live birth rates

Biological and clinical data and the outcome of ICSI are presented in **Table 1**.

The pregnancy rates per ICSI cycle in NOA, OA, and chronic and episodic aspermia groups, were 20.6%, 25.0%, 20.0% and 31.2%, respectively. Overall, 50 pregnancies (22.7% pregnancy

Table 1. Biological and clinical data and the outcome of ICSI according to the male pathology.

Parameter	*Male pathology*				
	NOA	*OA*	*Chronic aspermia*	*Episodic aspermia*	P-*value*
No. of ICSI cycles	107	72	25	16	
Male age (years)	34.1 ± 5.6	37.7 ± 7.9	33.8 ± 5.5	41.0 ± 7.6	<0.001
Female age (years)	31.0 ± 4.2	32.5 ± 4.7	31.5 ± 4.8	35.4 ± 5.1	<0.001
Injected oocytes	7.2 ± 3.5	6.3 ± 3.7	5.8 ± 2.9	6.2 ± 4.1	NS
Fertilized oocytes	3.0 ± 2.2	3.6 ± 2.8	2.1 ± 1.1	3.1 ± 2.5	NS
No. of embryo transfers (% per cycle)	96 (89.7)	66 (91.7)	23 (92.0)	14 (87.5)	NS
Transferred embryos	1.9 ± 0.6	1.9 ± 0.6	1.9 ± 0.7	2.1 ± 0.6	NS
No. of pregnancies	22	18	5	5	NS
Pregnancy rate/cycle (%)	20.6	25.0	20.0	31.2	NS
Pregnancy rate/embryo transfer (%)	22.9	27.3	21.7	35.7	NS
No. of early miscarriages (% of pregnancies)	4 (18.1)	4 (22.2)	3 (60.0)	0	NS
No. of live births (% of pregnancies)	17 (77.3)	14 (77.7)	2 (40.0)	5 (100.0)	NS
No. of multiple births (% of live births)	3 (17.6)	2 (14.3)	0	2 (40.0)	NS

Values are mean ± SD, unless otherwise indicated.
ICSI = intracytoplasmic sperm injection; NOA = non-obstructive azoospermia; NS = not statistically significant; OA = obstructive azoospermia.

rate) were achieved resulting in 11 early miscarriages (22.0%), one late miscarriage and 38 (76.0%) live births. Multiple pregnancies were observed in eight couples (seven pairs of twins and one set of triplets) (21.0%).

Differences in pregnancy, early miscarriage and live birth rates according to the type of male pathology (NOA, OA and aspermia)

Men with OA and couples from the episodic aspermia group were older in comparison to the other groups. No statistically significant difference was observed in pregnancy, early miscarriage and live birth rates among the groups (**Table 1**).

Factors influencing ICSI outcome

Fertilization

Fertilization showed a positive correlation with Johnsen score ($P = 0.016$) and with the type of ovarian stimulation ($P = 0.026$). The fertilization rate was 50.8% when Johnsen score was >9 and 36.9% when it was ≤8. A higher proportion of oocytes were injected (7.9, range 2–17, versus 6.3, range 1–17), a higher fertilization rate was obtained (53.8% versus 42.2%, range 0–100%), and a higher proportion of embryos was obtained (4.2, range 0–12, versus 2.5, range 0–10) after the use of agonist and recombinant FSH than after the use of agonist and urinary menopausal gonadotrophin (**Table 2**).

Development of embryos to the blastocyst stage

Blastocyst development showed a positive correlation with the number of oocytes injected ($P = 0.016$) and with the use of frozen–thawed spermatozoa ($P = 0.034$): the chance of obtaining a blastocyst was increased threefold when the number of injected oocytes was equal to or greater than five in comparison with less than five (21.5% of embryos developed to the blastocyst stage in comparison to 15.6%), and when using frozen–thawed in comparison to fresh spermatozoa (20.7% versus 15.7%). Embryo development to the blastocyst stage was also negatively predicted by male FSH concentration ($P = 0.019$): the chance of obtaining a blastocyst was decreased four-fold and the development rate dropped from 24.8% to 13.1% if FSH concentration was ≥10 in comparison to <10 IU/l. Moreover, motile spermatozoa demonstrated a strong tendency (although not reaching the level of statistical significance) to influence blastocyst development positively: three times more blastocysts (23.6% versus 6.8% development rate) were obtained when motile versus immotile spermatozoa were used (**Table 3**).

Pregnancy, early miscarriage and live birth

Embryo development to the blastocyst stage was the only positive predictor of pregnancy ($P = 0.002$) and live birth ($P = 0.005$). The pregnancy rate was 46.0% and the live birth rate 32.0%, if at least one blastocyst developed. If no blastocyst developed, the pregnancy rate was 8.3%, and the live birth rate 5.9%. Moreover, older female age was associated negatively with the occurrence of live birth. In women older than 38 years ($n = 29$), the live birth rate was significantly lower than in younger women (23.2% versus 3.6%, respectively) (**Tables 4** and **5**).

Pregnancies and live births in relation to day 2 versus day 5 embryo culture

In terms of pregnancies and live births, no difference was observed when transferring high-quality cleavage-stage embryos on day 2 or blastocysts on day 5 (pregnancy rate 35.5% versus 46.9%) in spite of a better fertilization rate in the day 5 group. However, poorer pregnancy and live birth rates were observed in the day 5 culture group when all attempts were considered, regardless of the embryo quality, but the differences did not reach statistical significance. In that group only 45% of embryos developed to the blastocyst stage in comparison to 77% of high-quality cleavage-stage embryos developed in the day 2 culture group (**Table 6**).

Duration of sperm cryostorage and associated female pathology

The mean duration of storage of testicular spermatozoa was 11.7 ± 12.9 (range 0.1–115.0) months. The ICSI outcome was not affected by the duration of sperm cryostorage.

In 69 (31.4%) female partners infertility factors were present: tubal factor (n = 20), polycystic ovaries and other endocrine abnormalities (n = 21), endometriosis (n = 4) and myoma or septate uterus (n = 24). Regression analysis revealed no influence of female pathology on the ICSI outcome.

Table 2. Factors influencing fertilization rate.

Factor	*Fertilization rate Unstandardized coefficient B*	P-*value*[a]
History of cryptorchidism	–0.061	NS
FSH level	–0.065	NS
Johnsen score	0.026	0.016
Ovarian stimulation (agonist and urinary menopausal gonadotrophin versus agonist and recombinant FSH)	0.131	0.026

[a]Linear regression.
NS = not statistically significant.

Table 3. Factors influencing embryo development to the blastocyst stage.

Predictor	B	P-*value*	*Odds ratio (95% CI)*
FSH concentration	–2.091	0.019	0.123 (0.021–0.705)
Injected oocytes	0.208	0.016	1.123 (1.039–1.457)
Sperm status (frozen–thawed versus fresh)	2.389	0.034	10.904 (1.190–99.889)
Sperm motility at ICSI (motile versus non-motile)	1.590	NS	4.906 (0.851–28.284)

B = regression coefficient; CI = confidence interval; ICSI = intracytoplasmic sperm injection; NS = not statistically significant.

Table 4. Factors influencing the occurrence of pregnancy after ICSI with testicular spermatozoa.

Predictor	B	P-*value*	*Odds ratio (95% CI)*
Johnsen score	1.296	NS	1.138 (0.821–1.577)
Female age	–0.069	NS	0.933 (0.779–1.118)
Embryo development to blastocyst	3.899	0.002	49.338 (4.409–552.087)
Sperm motility at ICSI	–2.215	NS	0.109 (0.009–1.233)
Ovarian stimulation (agonist and urinary menopausal gonadotrophin versus agonist and recombinant FSH)	0.669	NS	1.954 (0.439–8.694)

B = regression coefficient; CI = confidence interval; ICSI = intracytoplasmic sperm injection; NS = not statistically significant.

Table 5. Factors influencing the occurrence of live birth after ICSI with testicular spermatozoa.

Predictor	B	P-*value*	*Odds ratio (95% CI)*
Johnsen score	0.167	NS	1.182 (0.764–1.829)
Female age	–0.230	0.046	0.787 (0.699–0.997)
Development to blastocyst	4.883	0.005	132.097 (3.347–5229.405)
Sperm motility at ICSI (motile versus immotile)	–2.796	NS	0.061 (0.022–1.656)
Ovarian stimulation (agonist and urinary menopausal gonadotrophin versus agonist and recombinant FSH)	0.698	NS	2.010 (0.352–11.486)

B = regression coefficient; CI = confidence interval; ICSI = intracytoplasmic sperm injection; NS = not statistically significant.

Table 6. Comparison of the ICSI outcome according to the embryo quality and duration of embryo culture (2 versus 5 days).

	Embryo transfer Day 2, high-quality cleavage-stage embryo (n = 55)	*Day 5, blastocyst (n = 49)*	*Day 2, all attempts (n = 71)*	*Day 5, all attempts (n = 109)*
Female age	31.0 ± 4.4	31.1 ± 3.9	31.1 ± 4.4	32.1 ± 4.8
Injected oocytes	6.9 ± 3.6	8.1 ± 3.7	6.7 ± 3.6	6.9 ± 3.6
Fertilization rate (%)	48.1 ± 24.6[a]	63.1 ± 21.9[a]	45.0 ± 24.0[b]	52.8 ± 26.4[b]
Pregnancy rate (%)	34.5	46.9	28.2	24.8
Live birth rate (%)	29.1	32.6	23.9	16.5

Values are mean or mean ± SD.
[a,b]Values with the same superscript letter are statistically significantly different ($P < 0.05$).

Discussion

This retrospective analysis indicates that in couples with males affected by azoospermia or aspermia, the outcome of ICSI is determined by both male and female factors. Fertilization was influenced by Johnsen score and by the type of ovarian stimulation. The number of injected oocytes in the female partner, the use of frozen–thawed spermatozoa, and sperm motility at ICSI had a positive influence on the development of embryos to the blastocyst stage, whereas male FSH concentration had a negative influence. The occurrence of pregnancy and live birth was predicted positively by embryo development to the blastocyst stage and negatively by female age. However, the number of injected oocytes and developed embryos, and pregnancy and live birth rates did not differ among the NOA, OA and aspermia groups.

The men in the OA group were older in comparison to the men in other pathology groups. This group consisted predominantly of men who had a failed vasectomy reversal and those with post-infection obstruction, with an average age (48.5 ± 6.3 and 38.1 ± 6.9 years, respectively) that was higher than the age of men with NOA. In the authors' department at the University Medical Centre Ljubljana, these men first undergo surgical repair of obstruction before they are offered ICSI. The couples in the episodic aspermia group were also of an older age, and this was linked to erectile dysfunction with failure to deliver semen just before ICSI. However, male age did not affect the ICSI outcome, which is in agreement with Friedler *et al.* (2002) and Bromage *et al.* (2007).

Testicular histology and Johnsen score predicted fertilization, as previously confirmed by Tournaye *et al.* (1996). This is also in agreement with Pasqualotto *et al.* (2005) who reported that normal fertilization rates were lower in NOA in comparison with OA due to vasectomy or congenital obstruction. The decrease in fertilization is probably due to increased prevalence of genetic defects, a characteristic of men affected by maturation arrest. In order to optimize the clinical value of histology, McLachlan *et al.* (2007) have suggested that histological analysis of pathological testis should be based on a large biopsy of both testes, and that all histological components should be considered. However, this study found that testicular histology had no influence on the occurrence of pregnancy, which is consistent with the report by Tournaye *et al.* (1996) and in contrast with that by Zitzmann *et al.* (2006). Regarding the effect of the type of ovarian stimulation, it was observed that the fertilization rate was higher when using agonist and recombinant FSH in comparison with agonist and urinary gonadotrophins; this is likely to be related to a higher number of retrieved oocytes. However, in this retrospective study of a quite small series, the type of ovarian stimulation did not affect pregnancy and live birth rates. Similar observations were reported in couples with female infertility undergoing classical fertilization *in vitro* (Ziebe *et al.*, 2007); the authors postulated that poorer implantation when using agonist and recombinant FSH might be related to poorer uterine receptivity.

Concerning embryo development to the blastocyst stage, the number of injected oocytes is of prime importance regardless of the type of gonadotrophin used, as also reported by Friedler *et al.* (2002) and Greco *et al.* (2006).

As is the case with ejaculated spermatozoa (Jones *et al.*, 1998), the quality of testicular spermatozoa also affects the success of blastocyst development. A difference in blastocyst development was found when comparing fresh and frozen–thawed samples, independently of female age and number of injected oocytes. Better fertilization without influencing the occurrence of a pregnancy was also reported by Aoki *et al.* (2004). Moreover, there was no difference between the groups with regard to male FSH concentration, Johnsen score or proportion of motile versus immotile spermatozoa. A possible explanation for the favourable effect of frozen–thawed versus fresh spermatozoa on embryo development is the suggestion that cryopreservation eliminates the most deficient spermatozoa (Verheyen *et al.*, 2004). Aoki *et al.* (2004) also proposed that sperm cryopreservation could result in membrane damage, which might facilitate the membrane changes necessary for sperm pronucleus formation. In this study, it was found that the duration of sperm cryostorage had no influence on the ICSI outcome; this is consistent with the report by Huang *et al.* (2004), which addressed a possible detrimental effect of the duration of sperm cryostorage on ICSI.

The results of this study confirmed that the FSH concentration in the male has an influence on blastocyst formation (Virant-

Klun *et al*., 2003a). FSH has been related to testicular sperm morphology (Yavetz *et al*., 2001) and sperm motility at recovery (Souza *et al*., 2003); moreover, gonadal failure and high FSH concentration have been associated with increased testicular sperm aneuploidy (Levron *et al*., 2001). All these factors contribute to a negative influence on embryo development. Regression analysis showed that male FSH concentration had no effect on the occurrence of pregnancy and live birth. In cycles with male FSH ≥15 IU/l ($n = 29$), three pregnancies were achieved that resulted in three live births, of which one live birth occurred in a couple with a paternal FSH concentration of 63 IU/l. This disagrees with Zitzmann *et al*. (2006) who suggested that FSH ≥20 IU/l represents a threshold above which TESE and ICSI are contraindicated. In the current study, it was also found that more embryos developed to the blastocyst stage when motile versus immotile spermatozoa were injected, which is consistent with the reports by Liu *et al*. (1995), Nagy *et al*. (1998), Park *et al*. (2003), Verheyen *et al*. (2004) and Dafopoulos *et al*. (2005). Sperm immotility may be related to ultrastructural abnormalities of the centrosome, possibly inducing cleavage irregularities of the embryos (Nagy, 2000). Sperm motility is directly related to spermatogenesis; there are more immotile spermatozoa in NOA tissue samples than in OA (Nagy *et al*., 1998). Additionally, testicular spermatozoa from NOA may be genetically abnormal (Topping *et al*., 2006). The threat of genetic abnormalities has led some groups to contraindicate ICSI with immotile testicular spermatozoa.

In the same manner as observed in ICSI with ejaculated spermatozoa (Jones *et al*., 1998), pregnancy and live birth depend mostly on development to the blastocyst stage. However, the authors' hypothesis that a top-quality embryo cultured to day 5 in the hope of obtaining a blastocyst, will assure optimal pregnancy and live birth rates has not been confirmed in this study. A high loss of embryos between days 2 and 5 of culture is a characteristic of embryos derived from testicular spermatozoa, and is highly linked to poor sperm quality (Balaban *et al*., 2001; Nicopoullos *et al*., 2004). A negative effect on embryo development, related to suboptimal culture media (i.e. accumulation of toxic ammonium) cannot be excluded (Virant-Klun *et al*., 2006). In Department of Obstetrics and Gynecology, University Medical Centre Ljubljana, all embryos are cultured to the blastocyst stage. However, in couples with female infertility a decrease in clinical results due to prolonged embryo culture has not been observed. Moreover, in younger women with a higher number of embryos, an elective single blastocyst transfer is performed to avoid multiple pregnancy, again without decreasing clinical results. Embryos after TESE-ICSI seem to be of poorer quality and more sensitive to possible negative effects of prolonged embryo culture than embryos after fertilization with ejaculated spermatozoa.

Thirty per cent of female partners of men with azoospermia or aspermia had associated infertility factors. The presence of an associated pathology in female partners of azoospermics had no effect on the ICSI outcome. The finding that ICSI outcome was not influenced by the history of cryptorchidism is consistent with the observations of Raman and Schlegel (2003) and Vernaeve *et al*. (2004) who observed no differences in ICSI between men with the history of cryptorchidism and those without it.

Early miscarriage rates after ICSI with testicular spermatozoa have been reported as higher than those observed after ICSI with ejaculated spermatozoa (Aytoz *et al*., 1998; Ghazzawi *et al*., 1998). Increased chromosomal abnormalities affect embryos derived from testicular spermatozoa (Silber *et al*., 2003). The high early miscarriage rate observed in the chronic aspermia group may be related to the poor quality of spermatozoa in men with a history of cancer treatment or spinal cord injury (possible negative effects of chemotherapy, heat and inflammation) (Hovatta *et al*., 1993).

Although not rare, ejaculatory failure during IVF/ICSI has been seldom reported (Emery *et al*., 2004). Testicular aspiration of spermatozoa for ICSI is an effective means of rescuing an IVF cycle if erection cannot be achieved by other means (Watkins *et al*., 1996). In episodic aspermia, the authors have obtained good results with high pregnancy and low miscarriage rates, despite older maternal age. This may indicate that these men did not have a severe spermatogenesis defect.

In conclusion, this study underlines the importance of male FSH concentration, Johnsen score, frozen–thawed and motile spermatozoa at ICSI, the number of injected oocytes and female age in predicting the ICSI outcome in men with azoospermia or aspermia.

Although recombinant FSH provided more oocytes than urinary gonadotrophins in this series, its use did not improve the final ICSI outcome. In selecting viable spermatozoa and improving the embryo development to the blastocyst stage, cryopreservation of testicular spermatozoa should be encouraged. Despite the fact that blastocyst transfer improves the chances to obtain a pregnancy, embryo culture to day 5 does not guarantee a better ICSI outcome than embryo culture to day 2; these data are consistent with the Cochrane Database System Review publication (Blake *et al*., 2005), but have to be verified in prospective studies.

Bearing in mind the invasiveness of the TESE-ICSI procedures and largely yet unknown effects of pathology and treatments, efforts should be aimed at improving sperm selection, ovarian stimulation, embryo culture media and freezing techniques.

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