Article

Factors affecting outcome after ICSI with spermatozoa retrieved from cryopreserved testicular tissue in non-obstructive azoospermia

Dr Konstantinos Dafopoulos was born in 1968 in Greece. He studied medicine at Athens University in Greece and graduated in 1991. After obtaining his speciality degree in Obstetrics and Gynaecology in Greece he worked as a postgraduate fellow in reproductive medicine and endoscopic surgery at the Medical University of Lübeck under Professor Klaus Diedrich. He is currently a lecturer in Obstetrics and Gynaecology at the University of Thessalia in Greece under Professor Ioannis E Messinis. His special interests lie in the field of evidence-based reproductive medicine. He has published more than 15 papers in international peer-reviewed journals.

Dr Konstantinos Dafopoulos

Konstantinos Dafopoulos, Georg Griesinger, Askan Schultze-Mosgau, Yasser Orief, Beate Schöpper, Nikos Nikolettos, Klaus Diedrich, Safaa Al-Hasani[1]
[1]Correspondence: Department of Obstetrics and Gynaecology, University of Schleswig-Holstein, Campus Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany; e-mail: sf_alhasani@hotmail.com

Abstract

There is a lack of data regarding variables affecting the treatment outcome for non-obstructive azoospermia when spermatozoa from cryopreserved testicular specimens are utilized for ICSI. The objective of the present retrospective analysis was to investigate the effect of various parameters on treatment outcome in such cases. One hundred and sixty-five couples with non-obstructive azoospermic males undergoing a total of 297 cycles were included. In all cases the testicular tissue retrieved by multiple open-biopsy testicular sperm extraction was stored in liquid nitrogen and, after thawing, only mature spermatozoa were used for ICSI. When no motile spermatozoa were recovered, immotile spermatozoa were used. In 159 cycles, motile spermatozoa were utilized for ICSI, while in 138 cycles immotile spermatozoa were utilized. Higher normal fertilization rate (60.4 ± 3.1 versus 51.3 ± 1.6%, $P < 0.05$), number of embryos transferred (2.8 ± 0.06 versus 2.6 ± 0.04, $P < 0.05$), modified cumulative embryo score (31.2 ± 1.6 versus 23.9 ± 0.8, $P < 0.001$), and proportion of motile spermatozoa injected (67.8 versus 49.8%, $P < 0.05$) were observed in cycles that resulted in clinical pregnancies. Binary logistic regression analysis showed that sperm motility (odds ratio 2.06, 95% CI 1.1–3.9, $P < 0.05$), but not woman's age, number of treatment cycle, type of GnRH-analogue used for pituitary suppression, number of oocytes retrieved or number of embryos transferred was a significant determinant of the likelihood of clinical pregnancy. In conclusion, sperm motility after freeze/thawing of testicular tissue is the major determinant of the success of ICSI in non-obstructive azoospermia.

Keywords: *ICSI*, *non-obstructive azoospermia*, *sperm motility, TESE*

Introduction

Non-obstructive azoospermia (defective spermatogenesis mainly) accounts for 60% of azoospermic patients (Matsumiya *et al*., 1994). After the first reports of using spermatozoa from testicular tissue derived by testicular sperm extraction (TESE) for intracytoplasmic sperm injection (ICSI) (Schoysman *et al*., 1993; Devroey *et al*., 1994), this method has become an established technique for assisted reproduction in cases of non-obstructive azoospermia. The recovered spermatozoa may be used freshly for ICSI or the tissue may be cryopreserved for future ICSI.

There is a lack of data regarding variables affecting the treatment outcome for non-obstructive azoospermia when spermatozoa from cryopreserved testicular specimens are utilized for ICSI. The aim of the present retrospective analysis was to investigate the effect of sperm motility and other parameters on treatment outcome in such cases.

Materials and methods

Sample size calculation

The number of cycles needed to detect a difference in clinical

pregnancy rate of 10% between the two groups was calculated. Assuming that a clinical pregnancy rate of 25% would be feasible and higher in the group in which motile spermatozoa were utilized, a one-sided z-test with continuity correction was used. Sample sizes of 206 cycles in each group would be needed to achieve 80% power at a significance level (alpha) of 0.05. The significance level and power were calculated for 10 sequential steps (looks) using the O'Brien–Fleming spending function.

Patients

In the present retrospective study, 165 non-obstructive azoospermic males pursuing assisted conception in the Department of Obstetrics and Gynaecology at the University of Schleswig-Holstein, Germany from December 1995 to December 2002, were included. In all cases the testicular tissue retrieved by open multiple-biopsy TESE was cryopreserved and only mature spermatozoa (no early spermatids) were used for ICSI after thawing. The age (mean ± SEM) of the male and female partners was 34.2 ± 0.6 and 32.2 ± 0.2, respectively. Histologic diagnoses of the testicular biopsies showed some form of spermatogenesis defect in all cases, such as germ cell aplasia, maturation arrest and tubular sclerosis, and in the majority of cases mixed forms were diagnosed. All patients had a normal karyotype. Clinical pregnancy was defined as fetal heart activity in transvaginal ultrasound at 5–6 weeks after embryo transfer, although this definition is somewhat early and might overestimate the clinical pregnancy rate.

Retrieval of testicular tissue

Testicular tissue was obtained under local or general anaesthesia using an open multiple-biopsy technique (one cranial and one caudal tissue section in each testis). From each part a sample was fixed for the histopathological examination. Testicular specimens in Ham's F10 medium were examined under 40× objective phase-contrast microscopy to confirm the presence of spermatozoa, and were subsequently frozen in up to 10 fractions.

Freezing method of testicular tissue

Spermatozoa were not extracted before freezing, and tissue the size of a rice grain was frozen, although there are reports about successfully freezing few spermatozoa in empty zona pellucida (Cohen *et al*., 1997; Montag *et al*., 1999). From the present authors' experience, the difficult isolation of a very small number of spermatozoa in non-obstructive azoospermia cases and the action of testicular tissue as a cryoprotectant, reducing the freezing shock to spermatozoa, advocates freezing of testicular tissue. The testicular tissue suspension samples were placed in 0.5 ml HEPES-buffered medium, consisting of modified Earle's balanced salt solution with 0.4% human serum albumin and 15% glycerol as a cryoprotectant (Sperm Freeze, Medicult, Hamburg, Germany). The samples in 2 ml vials (Greiner, Frickenhausen, Germany) were placed on the top of a styrofoam box filled with liquid nitrogen and left there for 20–30 min. Afterwards they were immersed in liquid nitrogen and stored until required for ICSI.

Ovarian stimulation

Ovarian stimulation was performed using urinary or recombinant gonadotrophins [Menogon (Ferring Arzneimittel GmbH, Kiel, Germany) or Gonal-F (Serono International S.A., Geneva, Switzerland); Puregon (Organon, Oss, The Netherlands) respectively] and either gonadotrophin-releasing hormone (GnRH) agonists [Enantone Gyn (Takeda Pharma GmbH, Aachen, Germany) or Decapeptyl-Gyn Depot (Ferring Arzneimittel GmbH)] (205 cycles) or antagonists [(Cetrotide (Serono International S.A.) or Orgalutran (Organon)] (90 cycles) for pituitary suppression in various protocols, while in two cycles there was no pituitary suppression. Ovulation was induced by administration of either urinary or recombinant human chorionic gonadotrophin (HCG) [Choragon (Ferring Arzneimittel GmbH) or Ovitrelle (Serono International S.A.) respectively]. Oocyte retrieval was performed by transvaginal ultrasound guided puncture of follicles 36 h after the HCG injection with or without general anaesthesia. For luteal-phase support, progesterone [Utrogest (Dr Kade, Berlin, Germany) or Crinone (Serono International S.A.)] with or without additional HCG was utilized.

Thawing procedure

After oocyte retrieval, testicular tissue samples containing spermatozoa were thawed in a 37°C water bath for 3–5 min and prepared by using the mechanical method (Baukloh, 2002) for ICSI. The minced tissue was incubated in Ham's F-10 medium up to 5 h before being used for the ICSI procedure.

ICSI

ICSI of spermatozoa was performed as previously described (Al-Hasani *et al*., 1995). The supernatant medium was centrifuged for 1–2 min in an Eppendorf tube. One microlitre from the pellet was added to one or two drops of medium under oil to be used for injection. In cases where no motile spermatozoa could be recovered, immotile spermatozoa with otherwise normal morphology were injected. If at least slight movements of the head or tail were observed, spermatozoa were considered motile, which is suggestive of viability. Retrieved oocytes were incubated for 3–4 h before ICSI, and this practice has been shown to be associated with improved maturation of oocytes, fertilization and cleavage rates and embryo quality (Isiklar *et al*., 2004).

Statistical analysis

Statistical analysis was performed using Student's *t*-test, chi-squared test, Pearson correlation and binary logistic regression analysis. Numeric data were normally distributed (one sample Kolmogorov–Smirnov test). Descriptive statistics are presented as mean ± SEM. The statistical software package used was SPSS (Statistical Package for Social Sciences, Chicago, IL, USA) for Windows version 11.0 and NCSS (Number Cruncher Statistical Systems, Kaysville, UT, USA) 2001.

Results

The present study ended once the significance level and power boundaries of the chi-squared test, in terms of difference in clinical pregnancy rate between the two groups (motile and immotile sperm), were fulfilled. In particular, for 288 cycles in both groups a significance level of 0.024 and a power of 52.2% would suffice to establish a statistically significant difference and interrupt the study. The 297 cycles analysed showed clinical pregnancy rates of 26.4 and 14.5% in the motile and immotile