Results of cytogenetic analysis in men with severe subfertility prior to intracytoplasmic sperm injection

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Intracytoplasmic sperm injection (ICSI) is increasingly becoming the treatment of choice for severe male subfertility. Cytogenetic evaluation of men with andrological subfertility reveals an increased incidence of chromosomal abnormalities when compared with the normal population. We performed chromosomal analysis on the male partners of 32 couples referred for andrological subfertility. In two of these men, constitutional chromosomal translocations were diagnosed prior to ICSI [(45,XY,t(21;22)(p11;q11) and 46,XY,t(22;Y)(p11;q11)]. Since ICSI bypasses many potential barriers of fertilization, successful pregnancy can be achieved despite the presence of severely impaired spermatozoa in a population at high risk for chromosomal aberrations. It is well known that the presence of a chromosomal aberration plays a significant role in partial or complete spermatogenic arrest. ICSI does not seem to increase the risk of fetal chromosomal abnormalities when a spermatozoon from a chromosomally normal male is used. To exclude a higher risk for spontaneous abortion and fetal chromosomal abnormalities, we advocate cytogenetic screening of males with severe male subfertility who opt for ICSI.

Key words: chromosomal abnormalities/cytogenetic analysis/ICSI/subfertility

Introduction

Intracytoplasmic microinjection of a spermatozoon (ICSI) into the oocyte bypasses many potential barriers to fertilization caused by malfunction of the male gamete (Uehara and Yanagimachi, 1976). The success of this technique in achieving fertilization with subsequent embryo development and the birth of live offspring in animal experiments led to its clinical application to human gametes (Lanzendorf et al., 1988). The report of the first human pregnancies and births after ICSI has been followed by its increasing clinical use. In particular, couples with severe male subfertility who have failed to achieve fertilization in vitro, or those patients with too few (or only immotile) spermatozoa to attempt in-vitro fertilization (IVF) have been candidates for ICSI (Palermo et al., 1992). Today, ICSI is the treatment of choice for andrological subfertility, allowing fertilization and pregnancy rates close to those of natural conception, even in the presence of severely compromised semen parameters (Van Steirteghem et al., 1993; Joris et al., 1994; Nagy et al., 1994). The presence of living spermatozoa is the only semen parameter limiting this form of assisted reproduction, as fertilization and pregnancy rates are independent of either sperm motility or morphology. Furthermore, various procedures used to enhance the acrosomal reaction, for example the incubation of spermatozoa in a medium with metabolic stimulants such as pentoxifylline and 2-desoxyadenosine, do not influence pregnancy rates (Tournaye et al., 1994). Indeed, the most successful approach is mechanical immobilization of the spermatozoon before aspiration into the microinjector. ICSI has revolutionized infertility treatment to such an extent that some advocate that it should be treatment for all forms of infertility.

Since many of the sperm selection processes that have developed during the evolution of the fertilization mechanism are circumvented, concerns have been raised as to potential adverse effects on the offspring. Children born after ICSI or conventional IVF do not differ in paediatric follow-up from age-matched control patients. Results of 268 prenatal karyotypes and prospective follow-up of 235 children born after ICSI do not indicate an increase in congenital abnormalities (Bonaudelle et al., 1994). However, it is evident that significant medical and genetic pathology can be uncovered in subfertile men by comprehensive evaluation (Honig et al., 1994). More than half of all men with bilateral congenital absence of the vas deferens, for example, can be identified as having a mild defect in the transmembrane conductance regulator (CFTR) gene, and are mildly affected with cystic fibrosis (Anguiano et al., 1992).

The incidence of chromosomal anomalies can be expected to be significantly increased to 2.2% in a non-selected group of males with subfertility (Chandley et al., 1975) when compared with the general male population (0.7–1%; Lange and Engel, 1991). Severity of spermatogenic impairment and incidence of chromosomal anomalies seem to be positively correlated, as the latter increases to 7–14% in the presence of a sperm count of <10×10⁶ or azoospermia (Retief et al., 1984). Previous studies of male and female IVF patients have demonstrated an increase of translocations and mosaics (Lange et al., 1993). Thus, infertility treatment with ICSI is aimed at a male population at particular risk for chromosomal anomalies. We present our results of cytogenetic analyses performed in men with andrological subfertility prior to ICSI.

Materials and methods

Since its introduction in the Division of Assisted Reproduction of the Medical University of Lübeck, we have used the ICSI procedure
in 271 treatment cycles. The average age of the patients was 29 years. Many of these couples had already undergone repeated attempts of intrauterine insemination and IVF without success. As the subject of this study was the incidence of chromosomal aberrations in males of subfertility prior to ICSI, only couples with referral for this reason were included. Causes of female subfertility had been excluded prior to ICSI and female partners all had normal tubal, ovarian and endocrine functions. Consequently, no cytogenetic analyses were performed on the female partner. The data presented here represent the results of cytogenetic analyses of the first 32 males treated for andrological subfertility at the Division of Assisted Reproduction in the Department of Obstetrics and Gynaecology at the Medical University of Lübeck. Statistical analysis was not performed due to the small sample size.

Ovarian stimulation and aspiration of follicles

Ovarian stimulation was achieved by administration of human menopausal gonadotrophin after initial pituitary suppression with a gonadotrophin-releasing hormone agonist. Oocyte maturation was induced by administration of 10 000 IU of human chorionic gonadotrophin (HCG) at an average leading follicle size of 20–22 mm and an adequate concentration of oestradiol. Oocyte retrieval was carried out under general anaesthesia if required, using vaginal ultrasound-guided puncture of follicles 36 h after HCG.

Evaluation and preparation of the gametes—the ‘mini-swim-up’ method

Diagnosis of andrological subfertility was verified using standard World Health Organization criteria (WHO, 1993) on semen samples obtained after masturbation. At our laboratory, sperm preparation is carried out using the ‘mini-swim-up’ method. We use this technique of sperm preparation in preference to Percoll because it has proved effective for the isolation of spermatozoa even in the presence of severely impaired semen parameters (Al-Hasani et al., 1995).

Oocytes were treated with 0.5% hyaluronidase (Sigma Co., Hamburg, Germany) for 30 s for enzymatic lysis of the cumulus oophorus cells. Cells of the corona radiata were removed mechanically with a Pasteur pipette under stereo microscopic guidance (magnification = ×50). Subsequently, the maturity of the oocytes was determined and only oocytes in metaphase II were used for the ICSI procedure.

Microinjection

The choice of apparatus and the manufacturing of the pipettes have been described previously (Van Steirteghem et al., 1993). The inverted microscope with Hoffmann phase-contrast objective, hydraulic micro-manipulators and injection devices were manufactured by the Narishghi Company (Tokyo, Japan). The microelectrode puller for the borosilicate glass capillary tubes used in the manufacturing process of the injection pipettes was supplied by the Sutter Company (Novato, CA, USA). Injection pipettes were manufactured in the laboratory before the procedure and measured 4.5–5 μm in inner diameter.

ICSI was carried out using standard procedures described previously (Van Steirteghem et al., 1993). As a rule, 3–4 oocytes were injected and then suspended in Ham’s F-10 medium. If more oocytes were available, these were injected at a later stage. The oocytes were examined 16–18 h after injection for the presence of two or more pronuclei. If more than three oocytes had been fertilized, the surplus were cryopreserved. Prior to transfer, embryos were assessed morphologically for regularity of cell size, degree of fragmentation and growth rate relative to insemination time. A maximum of three embryos were transferred into the uterine cavity after an additional 24 h.

Microinjection

Cytogenetic analysis

Only males with impaired semen parameters by standard WHO criteria underwent cytogenetic analysis. Cytogenetic studies were performed on GTG-banded chromosomes using standard techniques after culture of lymphocytes obtained from peripheral blood.

Results

Two of the 32 men examined were found to have chromosomal anomalies (6.45%). One patient was diagnosed as a carrier of the Robertsonian translocation 45,XY,t(21;22)(p11;q11). A second patient was diagnosed as having the translocation 46,X,t(22;Y)(p11;q12). Both patients had grade III oligoasthenoteratozoospermia syndrome but did not show any other clinical abnormalities. The incidence of chromosomal aberration among the patients with oligoasthenoteratozoospermia was 10% (two out of 20).

Semen analysis revealed grade III oligoasthenoteratozoospermia in 20 patients. Five patients had oligozoospermia, two had cryptozoospermia, three had teratozoospermia. Oligospermia was diagnosed in two cases (Table I). Sperm counts ranged between 2 and 10×10⁶ spermatozoa/ml. Normal sperm morphology was in the range 0–40%.

A total of 313 metaphase oocytes were retrieved in 47 treatment cycles, in the group of patients examined (Table II). Of these oocytes, 168 were fertilized after ICSI (53.7% fertilization rate). The fertilization rate in the presence of grade III oligoasthenoteratozoospermia was 36.9% (Table II). After 47 embryo transfers, using three embryos on average, we have had six pregnancies (12.8% pregnancy rate/treatment cycle). In the two cases with paternal translocation, the fertilization rate was 57.6% (19 fertilized oocytes/33 oocytes retrieved). All pregnancies are still ongoing. ICSI was unsuccessful in the first couple with paternal translocation [45,XY,t(21;22)(p11;q11)]. In the second case [46,X,t(22;Y)(p11;q12)], ICSI resulted in a twin pregnancy. Cytogenetic analysis carried out
at 14 weeks gestation after amniocentesis revealed a normal female karyotype in one twin. The second twin had a male karyotype with an unbalanced 22,Y translocation. The pregnancy is still ongoing.

Discussion

In 40% of infertile couples the male factor can be identified as the cause of childlessness (Schirren et al., 1989). Chromosomal anomalies are a well-documented cause of male subfertility. Robertsonian translocation of chromosomes 13 and 14, for example, are known to result in oligoasthenoteratozoospermia (Johannisson et al., 1993). Mean sperm counts are significantly lower in chromosomally abnormal men than in men with a normal karyotype (Chandley et al., 1975). Although the exact mechanisms are not clear, it has been suggested that a wide variety of chromosomal anomalies exert an adverse effect on spermatogenesis, resulting in oligo- or azoospermia (Lange and Engel, 1991). Consequently, the incidence of chromosomal anomalies in the male subfertile population is significantly increased (Chandley et al., 1975; Retief et al., 1984). Furthermore, centrosomes in the early human embryo are paternally inherited and may determine the mitotic potential of the human zygote (Palermo et al., 1994). Thus, chromosomal aberrations may exert their adverse effect on fertility by various mechanisms. However, it is not known how many men with chromosomal anomalies in the general population are also infertile. Association of karyotype anomalies with male subfertility has mainly been studied in the patient population presenting to infertility clinics rather than in the general population. Spermatogenic arrest as a result of a chromosomal abnormality in these males is inconsistent and the genetic make-up of the gamete is equally unpredictable. At the current time, routine analysis of the sperm karyotype of spermatozoa is not possible in clinical practice. Disadvantages using heterospecific fertilization of zona-free hamster oocytes are time and labour intensity and the small number of cells analysed. Fluorescent in-situ hybridization of interphase spermatozoa offers an accurate and reliable method for analysis of a large number of spermatozoa, even in the presence of a low sperm count (Han et al., 1992; Holmes and Martin, 1993; Robbins et al., 1993; Guttenbach et al., 1994a,b; Miharu et al., 1994). However, the estimated incidence of a pathological sperm karyotype in the presence of a paternal chromosome abnormality has mostly been studied in individual patients rather than in a large population. Consequently, the incidence of impaired sperm parameters and pathological gamete karyotypes is not known precisely.

Although our small sample size may not warrant generalization, the incidence of chromosomal aberrations reported here correlates with that found in larger patient populations. Assisted reproductive technologies have revolutionized the treatment of male subfertility and have caused euphoria, as well as critical views about possible adverse effects on the offspring (Seamark and Robinson, 1995).

Our results illustrate that pregnancy can be achieved using spermatozoa from a male partner carrying a karyotype abnormality. Furthermore, the offspring has been shown to carry a major chromosomal abnormality propagated using ICSI.

In the first male (21:22 carrier) presenting with grade III oligoasthenoteratozoospermia, impaired spermatogenesis may be a result of an X-trivalent association during the pachytene stage, when pairing of homologous chromosomes is completed (Johannisson et al., 1993). However, as male carriers of Robertsonian translocations are a heterogeneous group, behaviour of the meiotic trivalent is inconsistent, making prediction of the percentage of chromosomally unbalanced spermatozoa difficult.

The second male (Y:22 carrier) had an unusual presentation. Translocations between an acrocentric autosome and the Y chromosome usually do not affect spermatogenesis, as there is no loss of genetic information (Chandley, 1991). In this case, we suggest meiotic pairing failure of the X chromosome and the Y segment of the translocation chromosome to be responsible for the spermatogenic deficiency (Burgoyne and Baker, 1984).

In view of the significant risk of inheritance of chromosomal aberrations for the offspring of the male population treated with ICSI, knowledge of the paternal karyotype seems desirable in order to offer some prediction of the genetic risk for the offspring. In the presence of a paternal chromosomal abnormality, there is the danger of propagation into the next generation by performing ICSI. With knowledge of the paternal karyotype, genetic counselling can be carried out in a more directed fashion to select couples who should be offered prenatal diagnosis after successful pregnancy following ICSI. Currently, prediction of the karyotype after fertilization of oocyte is only possible by preimplantation diagnosis of the embryo. However, employment of this technique without any clear indication of the genetic risk involved seems unwarranted. It is desirable to select an embryo with a normal karyotype for transfer. The likelihood of this being the case is higher if spermatozoa with a normal karyotype are used for ICSI. While the genetic make-up of the male gamete cannot be predicted from the paternal karyotype, other parameters are equally unhelpful. Sperm morphology alone does not correlate with the chromosomal constitution (Cummins and Jequier, 1994; Mortimer, 1994), and morphological parameters of embryo quality prior to implantation show no correlation with karyotype. In one study, 30% of apparently ‘normally fertilized’ human embryos after IVF carried a major chromosome abnormality when examined by preimplantation diagnosis (Jamieson et al., 1994). In this study, it was estimated that in the case of two embryos being transferred, both would have a genetically normal complement in only 49% of patients.

Ultrasound measurement after successful implantation suggests that smaller size of chorionic sac diameter and embryonic crown–rump length, with respect to reference values for gestational age, correlate positively with anembryonic and embryonic abortion and abnormal karyotypes (Dickey et al., 1994a,b).

A definitive diagnosis of the embryonic/fetal karyotype after pregnancy can only be obtained by prenatal diagnosis. The knowledge that the rate of karyotype anomalies is not increased after ICSI is insufficient in our eyes. A diagnosis of andrological
subfertility alone also seems imprecise in view of the many possible causal factors. Chromosomal aberrations being such a prominent cause should be excluded as part of the diagnostic work-up. Screening should be done for deletions and translocations, as they constitute major causes for male subfertility (Lange and Engel, 1991). The knowledge of the paternal chromosome complement in the presence of andrological subfertility will significantly improve our risk assessment for the occurrence of chromosomal aberrations in the offspring after ICSI. We need to know the incidence of chromosome anomalies in the offspring when spermatozoa from men with a pathological karyotype are used for ICSI. This can only be achieved by controlled prospective studies. We advocate routine cytogenetic analysis of the male partner prior to ICSI in cases of documented andrological subfertility. If the result is abnormal, genetic counselling should be performed: Examination of the embryo karyotype can be carried out by preimplantation diagnosis. Alternatively, classical methods of prenatal diagnosis may be employed after the establishment of a successful pregnancy.

In conclusion, it seems that ICSI using spermatozoa from chromosomally normal males does not increase the risk of fetal chromosomal abnormalities. Therefore, prenatal diagnosis on the basis of ICSI alone is not recommended, unless the woman carries an independently increased risk. In view of the increased incidence of chromosomal translocations and deletions in the presence of andrological subfertility, screening for karyotype abnormalities should be part of the diagnostic work-up carried out in the male partners prior to ICSI. At a time where assessment of the genetic complement of sperm is not yet possible, we strongly advocate prenatal diagnosis and genetic counselling after the establishment of a successful pregnancy due to the likelihood of a pathological result.

References


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