Comparison between chromatin condensation and morphology from testis biopsy extracted and ejaculated spermatozoa and their relationship to ICSI outcome

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A significant association between male subfertility, imperfect spermatiation and abnormal nuclear condensation has been suggested. The DNA content of spermatozoa might be responsible for inducing alterations in sperm morphology. The final nuclear shape, which is species-specific, depends on chromatin condensation during spermatogenesis as well as a precise organization of DNA within the nucleus. Many reports have described the association between disturbances in sperm chromatin condensation, morphology and male infertility. Chromatin condensation is achieved by gradual substitution of lysine-rich somatic histones by testis-specific histone and finally by protamine. In this study two groups of patients were compared: the first consisted of 63 patients who had undergone intracytoplasmic sperm injection (ICSI) with freshly ejaculated spermatozoa whereas the second included 47 patients assigned to ICSI with testes biopsy-extracted spermatozoa. In both groups chromatin condensation was assessed by aniline blue staining and morphology evaluated according to strict criteria. The condensed chromatin and morphology of spermatozoa were significantly (P < 0.0001) less in the second group compared to the first. However the fertilization, cleavage, implantation and pregnancy rates were almost the same in both investigated groups. There was no significant difference between the two groups with respect to ICSI outcome. The percentage of chromatin condensation (nuclear maturity) and morphologically-normal spermatozoa were significantly higher (P < 0.0001) in the ejaculated spermatozoa than in those from testis biopsy but the ICSI outcome (fertilization, cleavage, implantation and pregnancy rates) was the same. In view of these results the fertilization capability and the embryo quality obtained using testis biopsy extracted spermatozoa is not influenced by chromatin condensation and sperm morphology in testicular sperm extraction (TESE)–ICSI programmes. Therefore, it could be said that neither chromatin condensation nor morphology of testis extracted sperm could predict the fertilization, implantation and pregnancy rate in TESE–ICSI programmes.

Key words: chromatin condensation/ICSI/morphology/testis extracted spermatozoa

Introduction

The condensation of chromatin during spermatogenesis, epididymal transport and its decondensation at the time of fertilization are essential for successful fertilization.

The development of spermatids into mature spermatozoa is accomplished by a series of structural and chemical modifications including a gradual replacement of virtually lysine-rich histones by transition proteins and then by protamines which bind more tightly to DNA than do histones and this results in compaction of chromatin in the sperm nucleus, a process which is termed ‘sperm chromatin condensation’. Mammalian sperm DNA is the most tightly packed eukaryotic DNA, being at least six times more highly condensed than DNA in mitotic chromosomes, which allows the DNA to be compacted into a small volume (Ward and Coffey, 1991).

A number of studies have shown that spermatozoa with abnormal chromatin organization are more frequent in infertile men than in fertile men (Evenson et al., 1980; Monaco and Rasch, 1982). A significant association between male subfertility, imperfect spermatiation and abnormal nuclear condensation has been reported. Furthermore, many reports have described the association between disturbances in sperm chromatin condensation, morphology and male infertility (Bach et al., 1990; Foresta et al., 1992). Recently, intracytoplasmic sperm injection (ICSI) has led to a high success rate in fertilization and pregnancy in which the husband had undergone testicular sperm extraction procedure to recover immature spermatozoa for injection into the oocyte (Silber et al., 1995). Moreover several pregnancies have already been obtained after ICSI with testicular spermatozoa from patients with obstructive (Schoysman et al., 1993; Devroey et al., 1994) and non-obstructive azoospermia (Devroy et al., 1995; Nagy et al., 1995; Tournaye et al., 1996; Kahraman et al., 1997; Schlegel and Girardi, 1997).

Edwards et al. (1994) had already suggested that spermatids could be used as substitutes for spermatozoa to achieve fertilization of human oocytes. The round spermatid, in which meiosis has been completed and the histone–protamine transition has hardly begun, might offer the best means of transferring haploid male DNA to an oocyte. Spermatids can be collected from testicular aspirates (Blanchard et al., 1990). In humans, apparently healthy babies have been born after ICSI of spermatid nuclei (Tesarik et al., 1995).

However, in humans normal fertilization and cleavage up to 4-cell stage (Vanderzwalmen et al., 1995) and a pregnancy (Fishel et al., 1995) were reported after ICSI of an elongated spermatid retrieved from testicular tissue. Tesarik et al. (1995) and Tesarik (1996) reported the first two pregnancies followed
by delivery of healthy babies after injection of round spermatids (ROS), alternating with very severe oligozoospermia. Four other pregnancies were reported by Hannay (1995) after injection of round spermatid nuclei (ROSNI) but spontaneous miscarriages occurred in all of them.

The degree of sperm chromatin condensation (nuclear stability) can be evaluated by toluidine or aniline blue staining (Krzanowska, 1982) or by the uptake of an intercalating compound (acridine orange, ethidium bromide and propidium iodide) followed by analysis using either fluorescence microscopy (Gledhill, 1983) or flow cytometry (Spano et al., 1984; Evenson, 1989; Engh et al., 1992; Golan et al., 1997).

Terquem and Dadoune (1983) demonstrated that the acidic aniline blue staining technique selectively stains persisting lysine-rich histones and thus allows the visualization of spermatozoa with defective chromatin condensation. Due to the shift from somatic histones to specific protamines during spermatid development (Meistrich et al., 1978) and nuclear condensation, the elongated spermatids lose their ability to be stained with acidic aniline blue dye. While normal mature spermatozoa appear unstained, remaining histones in spermatozoa with insufficient chromatin condensation and in immature elongated spermatids are stained (Dadoune and Alfonsi, 1986). Dadoune et al. (1988) observed pathological aniline blue staining in 20% of morphologically-normal spermatozoa in normozoospermia specimens; these findings were confirmed by Hofmann et al. (1990).

The aim of this study was to compare the chromatin condensation and morphology of testis biopsy extracted and ejaculated spermatozoa and to determine the relationship between the chromatin condensed spermatozoa, sperm morphology and ICSI outcome.

In addition it was also to find out whether nuclear maturity (chromatin condensation) could be a useful parameter for predicting the fertilizing capability of spermatozoa after ICSI.

Materials and methods

In this prospective study 110 couples were enrolled. They were divided into two groups as follows: G1 (n = 63) consisted of patients who underwent ICSI with fresh ejaculated spermatozoa and G2 (n = 47) included patients attending ICSI therapy with spermatozoa extracted from testes biopsies (TESE). Semen samples from the first group were produced by masturbation after 3–4 days abstinence. The semen was allowed to liquefy for 20–30 min at room temperature. Thereafter the semen samples were analysed according to WHO guidelines (1992), many smears were made before and after sperm selection for chromatin condensation (aniline blue staining) and morphology (strict criteria) evaluation later. The semen was mixed with Ham’s F10 supplemented with 10% human albumin (1:1). The spermatozoa were centrifuged at 250 g for 10 min, the supernatant was discarded and the pellet was layered with 0.5 ml Ham’s F10 medium supplemented with 10% human albumin and then incubated at 37°C until ICSI was accomplished. On the other hand the testis biopsy was dissected with scissors into very small pieces under the inverted microscope in Petri dishes containing Ham’s F10 medium. The Petri dishes were incubated at 37°C for 2–3 h to give time for the spermatozoa to be released from the seminiferous tubules. Then the medium which contained the spermatozoa was centrifuged for 10 min at 250 g and prepared like the semen samples of the first group.

Aniline blue staining

In both groups chromatin condensation was assessed by aniline blue staining as previously described (Hammadeh et al., 1996). Briefly, after sperm preparation, 5 μl of the prepared spermatozoa were spread onto a glass slide, and allowed to dry. The smears were fixed in 3% buffered glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 30 min. The slides were then stained with 5% aqueous aniline blue mixed with 4% acetic acid (pH 3.5) for 5 min. A total of 100–200 sperm cells were evaluated and the percentage of stained sperm heads were calculated. Two classes of staining intensities were distinguished: unstained and completely or partially stained. The other smears were stained using a modified Papanicolaou method (WHO, 1992). The morphology was assessed by one observer based on strict criteria (Kruger et al., 1986, 1988). A total of 100 spermatozoa from each slide was evaluated under oil immersion at a magnification of ×1000 using bright field illumination.

Statistical analyses

Data storage and analyses were done at the Department of Bio-mathematics and Informatics Institute, University of Saarland. The statistical analysis was performed using the Mann–Whitney U-test and the χ² test. The possible relationship between the percentage of chromatin condensation, morphology and fertilization, cleavage and pregnancy was analysed with the Spearman rank correlation test.

Results

As shown in Table I, the effect of chromatin condensed spermatozoa (aniline blue stain) and morphology on fertilization, implantation and pregnancy rate in each group was analysed. The aniline blue staining uptake (positive stained) by the spermatozoa was significantly lower (P < 0.0001) in G1 (ejaculated spermatozoa) in comparison to G2 (spermatozoa extracted from testis biopsy) (33.1 ± 18.9% versus 70.0 ± 17.7%). The fertilization, cleavage, implantation, and pregnancy rates in G1 were 58.2 ± 22.4, 97.1, 10.1 and 27.0% respectively; the corresponding values for G2 were 30.6 ± 24.9, 99.2, 7.1 and 20.0% respectively. There were no significant differences between the two groups with respect to fertilization (P = 0.083), cleavage (P = 0.406), implantation (P = 0.462) and pregnancy rate (P = 0.495). On the other hand, the relationship between morphology and fertilization, implantation and pregnancy rate was analysed (Table I). In the first group 5.4 ± 4.7% spermatozoa showed morphologically-normal features, whereas in the second group less than 2.7 ± 3.0% of spermatozoa had morphologically-normal forms (P < 0.0001). However there were no significant differences between the two groups with respect to fertilization, cleavage, implantation and pregnancy rate.

Discussion

In this study the chromatin condensation (nuclear maturity) assessed by aniline blue staining was significantly (P < 0.0001) lower in testis biopsy extracted spermatozoa than that observed in ejaculated spermatozoa. Similarly the morphology of testicular extracted spermatozoa was significantly (P < 0.0001) lower than that of ejaculated spermatozoa. The fertilization and...
pregnancy rates of the TESE group were almost the same as observed in the first group. Moreover, the cleavage rate in the TESE group was higher than in the ejaculated group (99 versus 97%). The results did not show any relationship between the percentage of chromatin condensation (nuclear maturity) of spermatozoa and fertilization, cleavage, implantation and pregnancy rate as in ICSI programmes. A significant relationship between chromatin condensation and fertilization rate has been demonstrated in IVF programmes (Hammad et al., 1998). Haidl and Schill (1994) also found a close correlation between normal chromatin condensation and fertilization rate in an IVF programme. Nevertheless, Van Ranst et al. (1994) have shown that chromatin condensation in the spermatozoa used for ICSI failed to predict the outcome of fertilization. Moreover, Yoshida et al. (1997) have shown that structural autosomal abnormalities in the male partner do not adversely influence fertilization in ICSI and the early development of embryos. On the other hand, a high proportion of morphologically abnormal spermatozoa was shown to be associated with increased heterogeneity of chromatin structure (Evenson et al., 1984; Ballachev et al., 1987). The final nuclear shape, which is species-specific, depends on chromatin condensation during spermatogenesis, as well as a precise organization of DNA within the nucleus (Fawcett et al., 1971; Meistrich et al., 1976). Round-headed spermatozoa show chromatin condensation during morphogenesis and do not attain the same degree of maturity as do normal ones (Baccetti et al., 1977). Nevertheless, Barrovo et al. (1993) found that normal morphology does not seem to be sufficiently reliable as a sole predictor of male fertility potential. In this study, the fertilization, cleavage, implantation and pregnancy rate in both groups was similar and the quality of embryos obtained in the second group is comparable with those in the first group, albeit the percentage of morphologically-normal spermatozoa in the second group was significantly lower in comparison with that observed in the first group (P < 0.0001). These results did not confirm any relationship between fertilization and ICSI outcome on one hand and the percentage of morphologically-normal spermatozoa on the other. On the contrary, sperm morphology is widely accepted as an important factor in predicting the fertilization potential of spermatozoa in conventional IVF (Rogers et al., 1983; Kruger et al., 1986, Liu et al., 1988).

The clinical significance of sperm morphology in infertility diagnosis has been recently discussed by several authors (Aiken et al., 1995; Barratt, 1995; Oehninger and Kruger, 1995). Many studies dispute the importance of sperm morphology in predicting IVF and pregnancy (Seracchioli et al., 1995). The results from this study are in agreement with previous reports that no clear relationship exists between the percentage of spermatozoa with normal form and the fertilization, cleavage and pregnancy outcomes of ICSI (Palermo et al., 1993; Hall et al., 1995; Liu et al., 1995). Moreover, the injection of round-headed, acrosomeless spermatozoa (globozoospermia) resulted in normal fertilization (Lundin et al., 1994; Fishel et al., 1995). In addition, the feasibility of human conception with the use of spermatozoa has been suggested (Van der Zwaal et al., 1995), and has been confirmed by the first two births resulting from injection of round spermatids into human oocytes (Tesariik et al., 1995). In view of these results, the percentage of chromatin condensation and morphologically-normal spermatozoa in prepared semen for ICSI treatment was not correlated with fertilization, cleavage, implantation and pregnancy rates in an ICSI programme and supports our previous study (Hammad et al., 1996). Selva et al. (1993) reported that 76% of the penetrated oocyte which failed to progress to the pronuclear stage contained condensed sperm nuclei. Also, histone to protamine replacement occurs at the beginning of the spermatic maturation phase in humans and the distinctive imprinted pattern of the paternal genome is established by the round spermatid stage, as suggested by Kimura and Yanagimachi (1995), and the function of post-meiotic modifications of spermatozoa (maturational, activation of motility, acrosome reaction) is merely to enhance the transfer of the male genome into the oocyte as previously suggested by Yanagimachi (1994). Furthermore, it has been shown that ooplasmic factors regulate sperm head decondensation, protamine–histone exchange and pronucleate formation, and these events in turn depend on the maturity of the oocyte (Tesariik and Kopeny, 1989; Perreault, 1992). Therefore, the principal cause of failed fertilization after ICSI is the failure of oocyte activation and not ejection of the spermatozoon (Flaherty et al., 1995). In conclusion, although the percentage of chromatin condensation (nuclear...
maturity) and morphology of testicular extracted spermatozoa is significantly less than those of fresh ejaculated spermatozoa, the fertilization, cleavage, implantation and pregnancy rates were almost the same in the two groups. Therefore, neither the percentage of sperm chromatin condensation nor the percentage of morphologically-normal spermatozoa in the prepared sample has a significant prognostic value for prediction of sperm fertilizing capability and consequently cleavage, implantation and pregnancy rates in an ICSI programme.

References


Sperm morphology and TESE-ICSI outcome


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