The effect of chromatin condensation (Aniline Blue staining) and morphology (strict criteria) of human spermatozoa on fertilization, cleavage and pregnancy rates in an intracytoplasmic sperm injection programme

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The main purpose of this study was to determine the possible relationship between chromatin condensation (Aniline Blue staining), the morphology of spermatozoa according to strict criteria, and the fertilization, cleavage and pregnancy rate in an intracytoplasmic sperm injection (ICSI) programme. A total of 60 patients were divided into two groups (27 versus 34) according to sperm stainability by Aniline Blue. The first group involved patients having a positive Aniline Blue staining test with 0–29% stained. The fertilization rate in this group was 60.8%, cleavage rate 54.4% and pregnancy rate 18.5%. In the second group in which >29% spermatozoa were positively stained, the fertilization rate was 62.1%, cleavage rate 62.0% and pregnancy rate 35.3%. There was no statistically significant difference between the two groups. Furthermore, the influence of morphology according to strict criteria after Papanicolaou staining on successful fertilization, cleavage and pregnancy was studied in 85 patients who were divided into two groups according to the percentage of morphologically normal sperm. The fertilization, cleavage and pregnancy rates were 44.21, 63.37, and 39.47% respectively in the first group (<4%), the corresponding values for the second group (>4%) were 56.50, 46.04 and 21.21%. There was no significant correlation between the fertilization (P = 0.722), cleavage (P = 0.519) and pregnancy (P = 0.096) rates in either group. This study demonstrates that neither chromatin condensation (Aniline Blue staining) nor morphology could assess the fertilization potential, cleavage and pregnancy rate in an ICSI programme.

Key words: chromatin condensation/ICSI/morphology

Introduction

Several reports from different groups have shown that sperm morphology analysed using the criteria (Kruger et al., 1986, 1988; Oehninger et al., 1988, 1992; Menkveld et al., 1990; Hinting et al., 1990, Enginsu et al., 1991) could be a useful predictor of the fertilization rate in standard in-vitro fertilization (IVF) programmes. Similar results were obtained by Mahadevan and Trounson (1984), David et al. (1975), Jeulian et al. (1986), Yovich and Stanger (1984), Liu and Baker (1988), and Chan et al. (1989) using other morphology criteria.

Abnormal sperm morphology is attributed to abnormalities of the sperm head, and may involve several parts of the cell, including the acrosome, nucleus and post-acrosomal region (Zamboni, 1987). Fawcett et al. (1971) suggested that a low DNA content of spermatozoa might be responsible for inducing alteration in sperm morphology. Round headed human spermatozoa lacking an acrosome have been observed to show variable condensation and shaping of the nucleus during morphogenesis. This is said to result in poor DNA protection through incomplete protamine packing (Silvestroni et al., 1976; Bianchi et al., 1993, 1996). A significant association between male subfertility, imperfect spermiation and abnormal nuclear condensation has been suggested. 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).

The degree of sperm nuclear condensation or maturation can be assessed by Aniline Blue staining, which discriminates between lysine-rich histones on the one hand, and arginine and cysteine-rich protamines on the other (Auger et al., 1990; Hofmann and Hilscher, 1991). The histone-rich nuclei of immature spermatozoa contain abundant lysine and will react positively by taking up the Aniline Blue stain, whereas the protamine-rich nuclei of mature spermatozoa with abundant arginine and cysteine react negatively and remain unstained. According to Dadoune et al. (1988) and Hofmann et al. (1990), a normal ejaculate should contain at least 75% unstained spermatozoa, which indicates a normal nuclear maturation of ejaculated spermatozoa.

The aim of this study was to determine the relationship between the percentage of chromatin condensed spermatozoa, sperm morphology, and fertilization, cleavage and pregnancy rates in patients undergoing intracytoplasmic sperm injection (ICSI).

Materials and methods

In this prospectively designed study, 146 infertile couples were investigated before undergoing ICSI, male factor infertility being the indicator and the criterion for inclusion. Of these, 85 were studied for sperm morphology as a possible prognostic parameter of ICSI outcome. These were divided into two groups based on Kruger criteria with 4% as the cut-off point. Group A (n = 38) consisted of patients with <4% spermatozoa with normal morphology, and group B...
Intravaginally administered progesterone (300 mg/day, vaginal capsule; Roland Pharmaceutical, Essen, Germany) was used for luteal phase support which was started on the day of ovulation induction and continued until the 12th week of pregnancy.

Following oocyte retrieval, the cells of the cumulus oophorus and corona radiata were removed by incubation for 30 s in HEPES-buffered Ham's F10 medium with 80 IU hyaluronidase/ml (Type VIII, specific activity 320 IU/mg; Sigma Chemical Co., Deisenhofen, Germany). Afterwards, each cell was rinsed several times in Ham's F10 medium supplemented with 10% patient's serum. ICSI was carried out only on oocytes that had already reached the metaphase II (MII) stage by 3–4 h after retrieval. Each oocyte was placed in a droplet of 5 µl of Ham's F10 medium surrounding a central drop containing a sperm suspension mixed with 10% polyvinylpyrrolidone (PVP) solution. In all cases, the sperm sample which was used to assess either sperm morphology or chromatin condensation was also the one that was used for the ICSI procedure.

The statistical analysis was performed using the Mann–Whitney U test and the chi² test (Tables I and II). A ROC analysis was used in order to define the optimal cut-off point for the Aniline Blue staining test.
Results

Table I shows the fertilization, cleavage and pregnancy rates in a total of 61 patients undergoing ICSI. They were divided into two groups according to chromatin condensation. Group I showed 0–29% spermatozoa stained with Aniline Blue, and group II had >29% spermatozoa stained. The fertilization rate was almost the same in the two groups and there was no significant difference in the cleavage and pregnancy rates.

Regarding sperm morphology (Table II), 85 patients were divided into two groups according to the percentage of morphologically normal spermatozoa. In the first group (A), <4% spermatozoa showed morphologically normal features according to strict criteria Kruger et al. (1988), whereas in group B >4% of the spermatozoa had morphologically normal forms. The fertilization, cleavage and pregnancy rates were not significantly different between the two groups.

Discussion

The fertilization rate after ICSI is related to sperm characteristics, normal spermatozoa achieving fertilization in 63% of attempts in comparison with 47% where spermatozoa suffered from triple defects (sperm concentration, motility and morphology) (Van Steirteghem, 1993a,b).

Poor fertilization rates (0–5%) sometimes occur, despite successful sperm injection into oocytes (Payne et al., 1994). Flaherty et al. (1995a) showed a complete failure of sperm head decondensation occurred in ~11% of the unfertilized metaphase oocytes, whereas Dozortsev et al. (1994) reported a much higher corresponding percentage (38%). In the current study the fertilization rate with respect to chromatin condensation was almost the same in the two groups. The cleavage rate was also the same, whereas the pregnancy rate unexpectedly appeared to be higher (35.3 versus 18.5%) in the group with more immature spermatozoa (group 2, >29% spermatozoa positively stained) but this was not significant (Table I).

These results did not show any relationship between chromatin condensation and fertilization, cleavage and pregnancy rates. On the other hand, a significant relationship ($P < 0.0001$) between chromatin condensation, morphologically normal spermatozoa and fertilization rate has been demonstrated in standard IVF (Hammadeh and Haidl, 1995). Haidl and Schill (1994) also found a close correlation ($r = 0.825$) between normal chromatin condensation and fertilization rate in a IVF programme. A relationship between sperm morphology and fertilization rate after subzonal insemination (SUZI) has also been reported (Payne et al., 1994).

Van Ranst et al. (1994) have, like us, shown that chromatin condensation in the spermatozoa used for ICSI failed to predict the outcome of fertilization. Although Aniline Blue staining is a highly predictive test, its value is apparently restricted to conventional IVF procedures since no relationship was found between chromatin condensation (Aniline Blue staining) and outcome of ICSI.

Selva et al. (1993) reported that 76% of the penetrated oocytes which failed to progress to the pronuclear stage contained condensed sperm nuclei. The failure of sperm decondensation in the oocytes may be a consequence of a subtle sperm abnormality that is unrecognizable by conventional analysis (Bedford and Kim, 1993), such as a structural or biochemical defect associated with chromatin packaging or organization during spermatogenesis (Aitken, 1994; Zamboni, 1994). Umer et al. (1993) suggested that certain patients with male factor infertility may have intrinsic abnormalities in membrane structure or chromatin organization that could be associated with the failure of the male pronucleus to develop.

Concerning sperm morphology, the fertilization rate in both groups was similar, whereas the pregnancy rate appeared to be higher in group A (<4% normal morphology) (Table II). However, there was no significant difference between the groups with regard to fertilization, cleavage and pregnancy rates. The quality of the embryos obtained in both groups is comparable with the quality of embryos obtained in conventional IVF programmes; this is reflected in the mean pregnancy rate of ~30% and the mean implantation rate per embryo of ~9% (data not shown).

These results confirm previous reports that no clear relationship exists between the percentage of spermatozoa with normal morphology, and fertilization rates and the pregnancy outcome of ICSI (Palermo, 1993; Hall et al., 1995; Liu et al., 1995a,b).

Recently, fertilization has occurred in some patients after ICSI where only round headed spermatozoa (globozoospermia) were injected (Lundin et al., 1994; Liu et al., 1995a,b).

On the other hand, it has been shown that ooplasmic factors regulate sperm head decondensation, protamine–histone exchange and pronucleus formation, and these events in turn depend on the maturity of the oocyte (Tesarik and Kopecky, 1989a,b; Perreault, 1992). Furthermore, the principle cause of failed fertilization after ICSI is the failure of oocyte activation and not ejection of the spermatozoon (Flaherty, 1995b).

In conclusion, this study demonstrates that neither chromatin condensation (Aniline Blue staining) nor sperm morphology has a significant prognostic advantage concerning the prediction of fertilization, cleavage and pregnancy outcome in an ICSI programme.

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References


