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CHROMATIN DECONDENSATION OF HUMAN SPERM IN VITRO AND ITS RELATION TO FERTILIZATION RATE AFTER ICSI

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The inability of sperm chromatin to decondense has been implicated in the failure of fertilization, This study was undertaken to identify the relationship between sperm chromatin decondensation in vitro after incubation with follicular fluid at various points in time and fertilization or pregnancy rates after intracytoplasmic sperm injection. Moreover, an attempt was made to determine whether this test could be used as a predictive test for the outcome of ICSI. Thirty-two infertile couples undergoing ICSI therapy were included in this prospective study. One milliter of semen from each sample was mixed with 1 mL of follicular fluid obtained from ICSI patients at the time of oocyte retrieval and incubated for 24 h. Many smears were made directly after semen liquefaction at the following time intervals: 30, 60, and 120 min and 24 h. Chromatin decondensation was evaluated with acridine orange staining. The mean percentage of uncondensed chromatin of spermatozoa in the native semen samples was 25 ± 18.3%, which increased within 24 h to 91 ± 9.5%. On the other hand, the fertilization and ongoing pregnancy rates were 64 ± 21.7% and 20%, respectively. However, no correlations were found between chromatin decondensation at various point of time (30, 60, and 120 min and 24 h) and fertilization rate. No correlation was shown between the chromatin decondensation and sperm counts in the ejaculate, morphology, or the percentage of condensed chromatin. In light of this study, chromatin decondensation in vitro cannot be recommended for predicting the fertilization potential of spermatozoa and pregnancy rates in the ICSI program. Further research is necessary, especially in cases where ICSI is being considered as a therapeutic option.

Keywords chromatin decondensation, follicular fluid, ICSI, sperm

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Regular condensation of sperm chromatin occurs during spermatogenesis. A sequence of events ending ultimately in the decondensation of the sperm chromatin after penetration or injection into the oocyte is the prerequisite for successful fertilization [10, 13, 25]. Flaherty et al. [8] showed a complete failure of sperm head decondensation in ~11% of the unfertilized metaphase oocytes, whereas Dozortzev et al. [6] reported a much higher corresponding percentage (38%). Selva et al. [18] reported that 76% of the penetrated oocytes that failed to progress to the pronuclear stage contained 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 [3], such as a structural or biochemical defect associated with chromatin packaging or organization during spermatogenesis [2, 24]. Intrinsic abnormalities in membrane structure or chromatin organization could be associated with the failure of the male pronucleus to develop [22]. Nevertheless, development in pronuclear decondensation and centrosome reconstruction in vitro suggest novel diagnosis assay for these previously undetectable types of male infertility [7, 19]. The issue of decondensation of chromatin at the light microscopy level, however, is not addressed in the WHO laboratory manual [23] although it is important in assessing the fertility potential of man. Moreover, studies investigating the relationship between sperm chromatin decondensation and semen parameters are inconclusive [15, 16].

The aim of this study was (1) to find out the relationship between chromatin decondensation in vitro after semen incubation with follicular fluid obtained at the day of oocyte retrieval of patients undergoing ovarian stimulation for ICSI therapy and the fertilization rate after ICSI, and (2) to determine whether this test could be used to predict the fertilization rate in the ICSI program.

MATERIAL AND METHODS

Thirty-two couples were included in this prospective study with male factor infertility being the mean indication for inclusion. After liquefaction each semen sample was assessed according to WHO guidelines, except for morphology which was assessed according to strict criteria [12]. The samples were then divided into two aliquots. The first aliquot were processed by means of mini-swim up technique [1] and after 3 hours capacitation the obtained spermatozoa were used for performing ICSI according to our standard methods as previously described [11].

The second aliquot was then further studied for sperm decondensation in vitro as follows: the semen sample was washed (5 min centrifugation at 250g) three times with Ham F-10 in order to remove the seminal plasma. Thereafter, 1 mL of follicular fluid obtained from patients who underwent oocyte retrieval for ICSI therapy was added to the washed spermatozoa. The mixture was then incubated for 24 hours at 37°C, 5% CO_2 in air and 99% humidity. Many smears were made in the following time intervals: directly within 1-10 min, after 30 min incubation with follicular fluid, after 60 min, after 120 min and after 24 hours. The reaction was immediately stopped by spraying the smears with 3% glutaraldehyde in phosphate buffer saline. The chromatin decondensation was evaluated after staining with acridine orange according to the method described by Tejada et al. [20]. The female partners underwent ovarian stimulation for ICSI therapy as described earlier [11]. The percentage of chromatin decondensation was evaluated by analyzing 100-200 spermatozoa at each time period (30, 60, 160 min and 24

Table 1. Spermatozoa chromatin decondensation in vitro after incubation with follicular fluid at various points of time

Parameter	Follicular fluid (FF)
Number of samples	32
Age of patients (M ± SD) (years)	34 ± 4.2
Sperm concentration (mill/mL)	36 ± 28.9
Morphology (M ± SD)	10.3 ± 6.9
Native	25.7 ± 18.3
10 min	65 ± 26.5
30 min	74 ± 21.7
60 min	84 ± 14.1
120 min	88 ± 13.1
24 h	92 ± 9.5

h) and the results correlated with fertilization rate using the Spearman rank correlation coefficient test.

RESULTS

The mean sperm concentration was 36 ± 28 million/mL and the mean normal morphology was 10 ± 6.9%. However, 25 ± 18% of spermatozoa was uncondensed in the native semen samples, and this percentage increased after sperm incubation in follicular fluid to 65 ± 26% within the first 10 min, 74 ± 21% after 30 min, 84 ± 14.1% after 60 min, 88 ± 13.1% after 120 min, and 91 ± 9.5% after 24 h (Table 1). The number of retrieved and injected (MII) oocytes was 8.6 ± 4.6, 7.6 ± 4.2, respectively, and the fertilization rate was 64 ± 21%, whereas the ongoing pregnancy rate was 19.4% (Table 2).

No significant correlations were observed between the chromatin decondensation at various time intervals and fertilization or pregnancy rate. In addition, no correlation was found between the rate (velocity) of chromatin decondensation in vitro at various points of time and the fertilization rate. The chromatin decondensed very rapidly from 25.7 ± 18.3% in the native semen sample to 65 ± 3.5%, within 10 min of incubation with follicular fluid and to 88 ± 13% after 120 min.

Table 2. Intracytoplasmic sperm injection (ICSI) outcome in the investigated group

Paramater	Follicular fluid (n = 32)
Age of the patients (M ± SD) (years)	33.5 ± 4.0
Number of retrieved oocytes	8.6 ± 4.6
Number of mature oocytes (MII)	7.6 ± 4.2
Number of fertilized oocytes (M ± SD)	3.1 ± 2.5
Fertilization rates (%)	64 ± 21.7
Number of pregnancies	6
Pregnancy rate (%)	19.0

DISCUSSION

Using microscopy, several other groups have suggested that defective chromatin decondensation can be found in some cases of infertility [4, 5, 14, 17]. Asthenozoospermic men also displayed a high percentage of spermatozoa with nuclear abnormality, and their sperm chromatin decondensed slowly and partially compared to that of normozoospermic men [9]. According to Gopalkrishnan et al. [10], semen samples showing a decondensation of >70% (SDS/EDTA) can fertilize ova but no fertilization took place when the decondensation was less than 70%. In the present study, the chromatin decondensation increased from 25.7 ± 18.3% in the native semen sample to 74.5 ± 21.7% and to 88.6 ± 13.1% after incubation with FF for 30 and 120 min, respectively, and to 91.6 ± 9.5 after 24 h. However, no correlation was found between chromatin decondensation at different time points (10, 30, 60, and 120 min and 24 h), the rate of decondensation between various time points (0–10 min, 10–30 min, 30–60 min, 60–120 min, 120 min to 24 h) and fertilization. Furthermore, there is no correlation between chromatin decondensation and sperm concentration, morphologically normal spermatozoa as well as the percentage of spermatozoa with condensed chromatin in the native ejaculates. These results are in accordance with these of Rosenborg et al. [16] and Liu et al. [15]. The principal cause of failed fertilization after ICSI is the failure of oocyte activation and not ejection of the spermatozoa [8]. Therefore, penetration of spermatozoa into the oocyte without decondensation may indicate an oocyte deficiency related to the inability of the cytoplasma to promote decondensation [21].

In conclusion, chromatin decondensation in vitro after incubation with follicular fluid could not be used as a predictive factor for ICSI outcome, while the percentage of chromatin decondensation at various time intervals showed no correlation with fertilization rate in patients undergoing ICSI treatment. Additional studies with regard to chromatin decondensation in vitro, using SDS detergent and other S-S reducing agents, such as glutathione, are under investigation in our laboratory.

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