

Value of the sperm chromatin dispersion test in predicting pregnancy outcome in intrauterine insemination: a blind prospective study*

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BACKGROUND: Sperm DNA integrity has been used as a new marker of sperm quality in the prediction of pregnancy. Nevertheless, no previous study has been performed by analysing the same samples that were employed in assisted reproduction. The main objective of this work was to correlate sperm chromatin dispersion (SCD), measured by the SCD test, with semen parameters and pregnancy outcome in intrauterine insemination (IUI). **METHODS:** A total of 100 semen samples obtained from males of couples undergoing IUI were analysed by the SCD test before and after swim-up, and the results were correlated with semen parameters and pregnancy outcome. **RESULTS:** SCD was negatively correlated with sperm motility in both ejaculated and processed semen. Sperm recovered by swim-up did not show a significant improvement in DNA integrity. No correlation was found between SCD and pregnancy outcome in IUI. **CONCLUSIONS:** DNA dispersion, as measured by the SCD test, is not correlated with pregnancy outcome in IUI.

Key words: DNA/intrauterine insemination/sperm/sperm chromatin dispersion test

Introduction

Intracervical insemination with husband's sperm has been one of the most widely employed assisted reproduction techniques in the treatment of infertility, turning to intrauterine insemination (IUI) more recently. IUI is the first line of treatment in couples where pregnancy is not achieved naturally but no severe infertility problems are found on initial investigation. This initial investigation includes a semen analysis, following the World Health Organization guidelines (WHO, 1999), hormonal determinations and the assessment of tubal patency by hysterosalpingography in the female (Requena *et al.*, 2002).

There are different problems treatable by IUI, such as mild-moderate male factor, cervical factor, partial tubal permeability or ovulatory disorders (Requena *et al.*, 2002).

The best IUI results are achieved when ovaries are gently stimulated to obtain two mature follicles, without an associated increase of multiple pregnancy risk (Zuzuarregui *et al.*, 2004). Ovarian stimulation is monitored by vaginal ultrasound and estradiol (E₂) levels in blood in order to better synchronize insemination with ovulation.

The overall success of IUI varies widely, with pregnancy rates ranging between 5 and 66% per cycle (Allen *et al.*, 1985). Several prognostic factors for IUI outcome have been proposed, such as women's age (Campana *et al.*, 1996; Stone *et al.*, 2000; Hendin *et al.*, 2000), endometrial thickness, mature follicles at the time of ovulation (Tomlinson *et al.*, 2001; Stone *et al.*, 2000; Khalil *et al.*, 2001), aetiology and duration of infertility (Tomlinson *et al.*, 2001; Hendin *et al.*, 2000; Khalil *et al.*, 2001), protocols of ovarian stimulation (Khalil *et al.*, 2001), timing and number of inseminations (Silverberg *et al.*, 1992; Ragni *et al.*, 1999; Khalil *et al.*, 2001), percentage of sperm with abnormal morphology (Lindheim *et al.*, 1996; Ombelet *et al.*, 1997), type and percentage of sperm motility (Tomlinson *et al.*, 2001; Shulman *et al.*, 1998; Stone *et al.*, 2000; Hendin *et al.*, 2000) and total number of motile sperm inseminated (van der Westerlaken *et al.*, 1998; Khalil *et al.*, 2001).

However, there is a proportion of couples unable to achieve a pregnancy after several IUI attempts, even without an apparent male or female factor. This may be indicating that there is an 'occult cause' that we are not evaluating in our study previous to the treatment that may be responsible for pregnancy failure.

Sperm chromatin dispersion (SCD) could be one of these putative occult factors that are not assessed in the routine semen analysis.

There are a number of tests currently used for either direct or indirect evaluation of DNA integrity in sperm, but sperm chromatin structure assay (SCSA) (Evenson *et al.*, 1999) has been the mostly reported, and several reports have found a negative predictive value in pregnancy outcome in assisted reproduction treatment (Larson-Cook *et al.*, 2003; Virro *et al.*, 2004). However, the SCSA test is expensive, time-consuming and requires complex equipment not available in most andrology laboratories. In order to circumvent these limitations, the SCD test has recently been introduced (Fernandez *et al.*, 2003). The SCD test determines the susceptibility of sperm DNA to acid denaturation. SCD test results have been shown to be highly correlated with those obtained by DNA breakage detection–fluorescence *in situ* hybridization analysis, thus being representative of DNA breakage presence. In addition, a strong correlation has been found between the SCD and SCSA tests (J.L.Fernandez, personal communication), as would be expected, since both of these tests measure susceptibility of sperm DNA to acid denaturation *in vitro*.

The main objective of this study was to prospectively evaluate the predictive value of the SCD test on pregnancy outcome by IUI in couples with non-severe male factor, and to correlate DNA dispersion with sperm parameters.

Materials and methods

Patients

Semen samples were obtained from couples undergoing IUI (only one cycle per couple was included) for infertility treatment at the Instituto Universitario IVI, in Valencia, Spain. A total of 100 males provided 100 samples that were analysed between January 2003 and April 2004. We included in the study only couples whose males presented >25% of motile sperm, $10 \times 10^6/\text{ml}$, and a total number of inseminated sperm $>3 \times 10^6$. All males maintained 3–5 days of sexual abstinence before the sample was recovered.

All patients signed an informed consent and the study was approved by the Ethics Committee of the Instituto Universitario IVI.

Semen analysis

Semen parameters were evaluated after liquefaction at 37°C under 5% CO₂, for 10 min. The samples were examined for sperm concentration and motility in a Makler® Chamber (Sefi Laboratories, Tel Aviv, Israel) following the WHO (1999) guidelines. Sperm morphology was evaluated using Tygerberg's strict criteria (Van Waart *et al.*, 2001).

The results of the semen analysis were only considered valid when <5% of discordance in percentage motility, $5 \times 10^6/\text{ml}$ in sperm concentration and 2% in sperm morphology was found by two different observers (N.G. and M.M.) (Garrido *et al.*, 2004). When we found higher differences, we repeated our measurements.

Semen preparation

All samples were processed by swim-up. Briefly, ejaculates were diluted 1:1 (v/v) with Sperm Medium (MediCult, Jyllinge, Denmark), centrifuged at 400 g for 10 min, and the supernatant discarded. Aliquots of 0.5–1 ml of fresh medium were overlaid over the pellet and incubated at 37°C for 45 min with the tubes inclined at an angle of 45°. After this period, the upper 0.5 ml was taken. Aliquots of 10 µl were used for analysis of sperm concentration, motility, morphology

and DNA dispersion in the ejaculates and after swim-up. The remaining sample was loaded into the insemination catheter and delivered into the uterus.

Ovarian stimulation

All cycles were gently stimulated with recombinant FSH (rFSH, Gonal-F; Serono S.A., Spain; or Puregon; Organon Española, Spain) or HMG (Lepori; Farma Laboratories, Spain; or Menopur; Ferring, Spain). Stimulation was started on cycle day 3 with 75–150 IU of rFSH daily. Follicle maturation was monitored by serial vaginal ultrasound and plasma E₂ levels. When the diameter of the leading follicle(s) was >18 mm, the patients received 10000 IU of HCG (Profasi 10000 UI; Serono S.A., Spain). Two consecutive inseminations were performed ~12 and 36 h after the HCG injection.

We have previously demonstrated that the timing of IUI after HCG does not affect the cycle outcome (Vargas-Chavarría *et al.*, 2001). Luteal phase was supported by daily vaginal administration of 50 mg progesterone suppositories. Plasma β-HCG levels were measured 2 weeks after IUI to determine biochemical pregnancy. Clinical pregnancy was defined as transvaginal ultrasonographic visualization of intrauterine gestational sac(s).

SCD test

For the analysis of DNA dispersion, aliquots containing 3–5 × 10⁶ sperm were taken from both fresh and swim-up processed samples.

The samples were coded and shipped in liquid nitrogen to the Sección de Genética y Unidad de Investigación, Hospital Teresa Herrera, Complejo Hospitalario Juan Canalejo, A Coruña, Spain, where SCD test analysis was performed without the knowledge of any characteristics of either the couple or the semen sample. Prior to the initiation of the study, experiments were performed to confirm that these procedures did not change SCD values (unpublished data). After SCD test analysis, the data were sent back to the Instituto Universitario IVI for statistical analysis of the clinical data and cycle results.

The improved SCD test (Halosperm® kit, INDAS laboratories, Spain) (Fernandez *et al.*, 2003) was used for analysis. Gelled aliquots of low-melting point agarose in Eppendorf tubes were provided in the kit, each one to process a semen sample. Eppendorf tubes were placed in a water bath at 90–100°C for 5 min to fuse the agarose, and then in a water bath at 37°C. After 5 min incubation for temperature equilibration at 37°C, 60 µl of the thawed semen sample was added to the Eppendorf tube and mixed with the fused agarose. A total of 20 µl of the semen-agarose mix was pipetted onto pre-coated slides, provided in the kit, and covered with a 22 × 22 mm coverslip. The slides were placed on a cold plate in the refrigerator (4°C) for 5 min to allow the agarose to produce a microgel with the sperm cells embedded within. The coverslips were gently removed and the slides immediately immersed horizontally in an acid solution, previously prepared by mixing 80 µl of HCl (from the kit) with 10 ml of distilled water, and incubated for 7 min. The slides were horizontally immersed in 10 ml of the lysing solution for 25 min. After washing 5 min in a tray with abundant distilled water, the slides were dehydrated in increasing concentrations of ethanol (70–90–100%) for 2 min each, air-dried, and stored at room temperature in tightly closed boxes, in the dark.

For brightfield microscopy, slides were covered horizontally with a mix of Wright's staining solution (Merck, Germany) and phosphate buffer solution (Merck, Germany) (1:1) for 5–10 min with continuous airflow. Then the slides were briefly washed in tap water and allowed to dry. Strong staining is preferred in order to easily visualize the periphery of the dispersed DNA loop halos. The distilled water, ethanol, Wright staining solution (Merck 1.01383.0500) and phosphate buffer solution (Merck 1.07294.1000) are not provided in the kit. A minimum of 500 sperm per sample were scored under the ×100 objective of the microscope.

Five SCD patterns were established (Fernandez *et al.*, 2003). (i) Sperm cells with large halos (SCBH): those whose halo width is similar or higher than the minor diameter of the core. (ii) Sperm cells with medium size halos (SCMH): their halo size is between those with high and with very small halo. (iii) Sperm cells with very small size halo (SCSH): the halo width is similar or smaller than one-third of the minor diameter of the core. (iv) Sperm cells without a halo (SCWH). (v) Sperm cells without a halo-degraded (DC): similar to (iv) but weakly or irregularly stained. Sperm cells with very small halos, without halos and without halo degradation contain fragmented DNA. Finally, nucleoids that do not correspond to sperm cells are separately scored. A control microgel, containing a same sperm sample was enclosed in each slide. Figure 1 shows all patterns.

Statistical analysis

Parametric tests (*t*-test) were used for comparisons between groups when the data followed a normal distribution as demonstrated by the Kolmogorov–Smirnov test. Non-parametric tests were used to compare study parameters in fertile and infertile males when the data did not follow a normal distribution. Subsequently, in these cases, Wilcoxon tests were applied.

SCD was correlated with basic seminal parameters using regression analysis. Significance was defined as $P < 0.05$.

The statistical analysis was performed using the MedCalc Software, Ghent, Belgium.

Results

The mean age of women included in the study was 32.8 ± 0.34 years, whereas the mean age of the male partner was 33.0 ± 0.44 years. The aetiology of female factor infertility was unexplained in 79 couples (79%), tubal factor in six couples (6%), anovulation in three couples (3%), endometriosis in five couples (5%), advanced age in six couples (6%) and recurrent abortion in one couple (1%). Mean infertility duration was 2.1 years (range 1–5 years).

Of the 100 IUI cycles performed, 23 resulted in a pregnancy (23%), with a total of 25 newborns without any remarkable paediatric problem. There were 21 single pregnancies and two twins.

Three pregnancies ended in spontaneous abortion (13%). Karyotypes in these couples were normal. SCD values in semen for these three cases were 21.2, 18.7 and 50.3% of sperm with small or without halo. The swim-up process presented a correlation between abortion rate and high DNA dispersion values, although it did not achieve statistical significance.

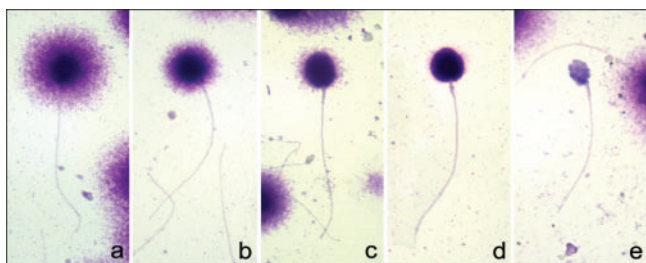


Figure 1. Sperm chromatin dispersion patterns observed after Wright staining. (a) Big halo. (b) Medium size halo. (c) Small halo. (d) Without halo. (e) Without halo and degraded.

Seminal characteristics before and after semen processing

As shown in Table I, sperm selection by swim-up results in an improvement in the percentage of sperm with progressive motility and the percentage of normal forms.

Surprisingly, sperm recovery by swim-up did not yield an enrichment of sperm with low DNA dispersion values, as reflected by a high percentage of sperm with large or medium size halo. We found that the percentage of cells with degraded chromatin was significantly higher ($P = 0.002$) in swim-up-recovered sperm. The same was found when the percentage of sperm with medium size halo was determined ($P = 0.0015$).

Semen parameters and DNA dispersion

When we analysed results of the number of cells with a + b motility and DNA dispersion, we found a negative correlation ($r = -0.22$; $P = 0.04$ for SCSH). Nevertheless, the total number of progressive sperm in the ejaculates was not correlated with any pattern of sperm DNA dispersion.

Our results indicate a negative correlation between the percentage of cells with degraded chromatin and sperm morphology in the ejaculate ($r = -0.29$, $P = 0.04$).

Also, the percentage of sperm with progressive motility in semen was negatively correlated with the percentage of cells with small halo ($r = -0.22$, $P = 0.04$), and positively correlated with the percentage of sperm cells with big halo ($r = 0.30$, $P < 0.01$), indicating a link between progressive motility and intact DNA.

These same findings were observed in sperm recovered by swim-up. A negative correlation was found between the percentage of sperm with progressive motility and the percentage of cells with big halo ($r = -0.23$, $P = 0.03$), the percentage of sperm with progressive motility ($r = 0.22$, $P = 0.04$), the total number of sperm with progressive motility ($r = -0.26$, $P = 0.01$), and the total number of sperm with normal forms and progressive motility ($r = 0.33$, $P = 0.03$).

Table I. Seminal characteristics before and after semen processing

Semen parameters	Raw semen	Swim-up	<i>P</i>
Volume (ml)	3.8 ± 0.2	–	
Concentration ($\times 10^6$ /ml)	61.7 ± 4.7	30.3 ± 2.2	< 0.0001
Forward motility (a+b%) (WHO a + b)	45.0 ± 1.4	78.0 ± 1.7	< 0.0001
TMP (million)	98.7 ± 9.8	11.7 ± 0.8	< 0.0001
Normal forms (%)	10.4 ± 0.9	12.0 ± 0.8	0.004
TMP normal (million)	13.2 ± 2.6	1.5 ± 0.2	< 0.0001
DC (%)	1.0 (0–21.0)	2.2 (0–23.9)	0.002
SCWH (%)	19.0 (0.2–60.8)	15.3 (10.0–43.5)	0.10
SCSH (%)	9.0 (1.6–26.3)	11.6 (0.4–49.8)	0.05
SCMH (%)	6.8 (1.2–18.8)	8.3 (0.2–45.0)	0.0015
SCBH (%)	63.2 (31.0–91.0)	59.3 (9.6–98.1)	0.31
TMP not fragmented (million)	46.6 (4.4–389.1)	6.56 (0.5–27.7)	< 0.0001
(SCBH + SCMH)			
TMP normal not fragmented (million)	9.1 ± 2.1	1.5 ± 0.2	< 0.0001

Results are expressed as the mean \pm SEM if they were normally distributed. We included ranges in parentheses where we had abnormally distributed data. WHO = World Health Organization; TMP = total motile progressive; DC = sperm cells without a degraded halo; SCWH = sperm cells without a halo; SCSH = sperm cells with very small halo; SCMH = sperm cells with medium size halos; SCBH = sperm cells with large halos.

This was also confirmed by the results obtained in sperm isolated by swim-up, where the total number of motile sperm and the total number of normal motile sperm are negatively correlated with DNA dispersion ($r = 0.33$, $P = 0.03$).

SCD and pregnancy outcome

Samples were divided into those that resulted in a pregnancy, and those that did not. As shown in Table II, samples were also divided into semen and sperm recovered by swim-up.

No significant differences in semen parameters were found in either semen or swim-up processed sperm from samples that did or did not result in a pregnancy. Similarly, no significant differences were found between the different halo size patterns and pregnancy outcome.

These results suggest that SCD in non-severe male factor couples does not predict pregnancy outcome in IUI.

Figure 2 demonstrates the overlapping percentage of SCD between samples that resulted in a viable pregnancy and those that did not.

Discussion

Routine semen analysis, following WHO guidelines, yields valuable information concerning testicular function, and is considered the gold standard in the evaluation of male infertility although it only measures the volume of the ejaculate, sperm concentration, motility and sperm cells with normal size and shape.

Several recent reports have focused on the male contribution to couples' infertility and other factors within sperm have been

related to male infertility. For instance, sperm mRNA subsets are now being recognized as highly relevant in early embryo development and pregnancy outcome (Ostermeier *et al.*, 2002;

Table II. Semen parameters in couples that did or did not achieve a pregnancy

Semen parameters	Pregnant	Not pregnant	P
Raw semen			
Volume (ml)	3.2 ± 1.2	4.0 ± 0.2	–
Concentration (×10 ⁶ /ml)	61.0 (13.1–260.3)	52.0 (16.2–166.3)	0.05
Forward motility (a + b%)	43.2 ± 2.7	45.3 ± 1.7	0.52
TMP (million)	74.5 (6.2–513.1)	80.7 (35.4–221.6)	0.90
Normal forms (%)	8.3 ± 0.9	10.8 ± 1.0	0.16
TMP normal (million)	4.8 (2.1–16.6)	8.9 (0.5–66.6)	0.12
TMP not fragmented (million) (SCBH + SCMH)	52.6 (23.4–172.7)	47.0 (5.3–389.1)	0.69
TMP normal not fragmented (million)	3.5 (1.4–7.5)	6.3 (0.4–47.7)	0.21
Swim-up			
Volume (ml)	–	–	–
Concentration (sperm/ml)	35.5 ± 4.6	28.6 ± 2.5	0.18
Forward motility (WHO a + b%)	77.6 ± 2.0	77.8 ± 3.5	0.95
TMP (million)	10.7 ± 0.9	13.9 ± 2.0	0.12
Normal forms (%)	12.7 ± 0.9	9.7 ± 1.4	0.12
TMP normal (million)	1.6 ± 0.2	1.2 ± 0.2	0.39
DC (%)	1.0 (0.1–21.4)	1.9 (0.2–29.4)	0.17
TMP not fragmented (million) (SCBH + SCMH)	9.4 ± 1.8	7.1 ± 0.8	0.18
TMP normal not fragmented (million)	0.7 ± 0.2	0.9 ± 0.2	0.52

Results are expressed as the mean ± SEM if they were normally distributed. We included ranges in parentheses where we had abnormally distributed data.

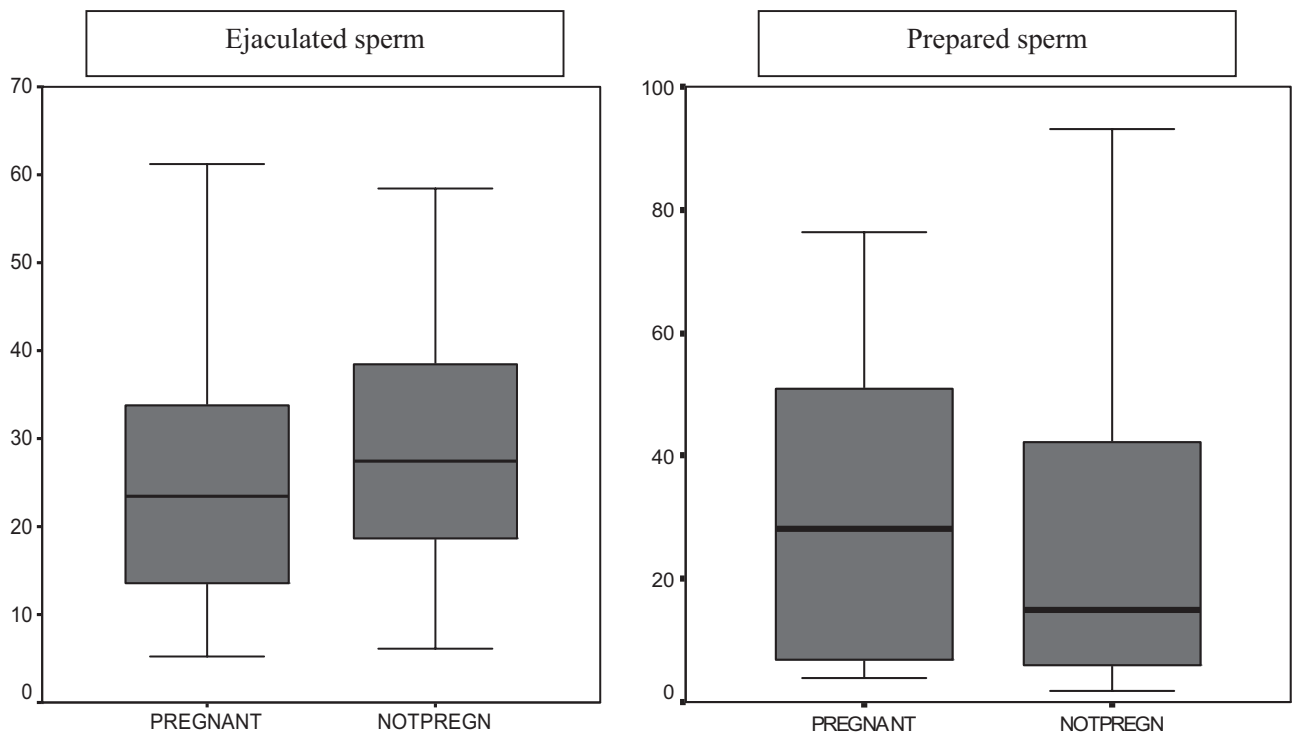


Figure 2. Box-and-whisker plot of sperm chromatin dispersion results in ejaculated sperm (expressed as percentage of sperm with fragmented DNA) in pregnant and non-pregnant women who underwent intrauterine insemination. The box represents the values from the lower to upper quartile (25th to 75th percentile). The middle line represents the median. The vertical line extends from the minimum to the maximum value.

Schatten, 2002), despite the known capacity of the oocyte to repair some sperm defects such as DNA damage.

Among the different publications about biochemical markers of male infertility (Garrido *et al.*, 2004; Meseguer *et al.*, 2004), a great deal of attention has been given to DNA dispersion.

It is widely accepted that SCD may be caused by defective chromatin packaging during spermiogenesis, apoptosis during either spermatogenesis or sperm transport through the male genital tract (Duran *et al.*, 2002), or as a consequence of oxidative stress (Garrido *et al.*, 2004; Greco *et al.*, 2005a).

Concerning these mechanisms, there is a clear correlation between defective chromatin packaging and the presence of DNA damage (Gorczyca *et al.*, 1993; Manicardi *et al.*, 1995; Sailer *et al.*, 1995). This damage results in DNA 'nicks' or single-stranded DNA breaks.

Second, there is evidence that the apoptotic Fas receptors and also caspase-3 are present in ejaculated sperm (Sakkas *et al.*, 1999; Weng *et al.*, 2002). However, apoptosis usually occurs during meiosis I and recent reports question the occurrence of true apoptosis after spermiation.

Third, recent reports strongly suggest that DNA fragmentation is induced by oxidative stress during sperm transport through the seminiferous tubules and epididymis (Ollero *et al.*, 2001; Garrido *et al.*, 2004; Greco *et al.*, 2005a) and is increased in infertile males. Treatment with antioxidants significantly decreases DNA fragmentation (Greco *et al.*, 2005b).

Recent data from our laboratory also suggest that glutathione peroxidase-4 may be a key enzyme involved in this process during spermiogenesis (Meseguer *et al.*, 2004).

To date, there have been few reports demonstrating the usefulness of SCD analysis in the prediction of pregnancy outcome in IUI. Duran *et al.* (2002) found in a prospective study using terminal deoxynucleotidyl transferase-mediated nick end labelling (TUNEL) that samples resulting in pregnancy exhibited lower DNA fragmentation values than those resulting in failed pregnancy. They compared pregnancy outcome in samples prepared by centrifugation, as well as density gradient. In addition, cycles were natural, stimulated with clomiphene citrate or rFSH, thus making it difficult to compare their results with ours. The stimulation regimen used in this study may explain the low pregnancy rates obtained (8.7% per cycle and 10.4% per patient).

In another study, Bungum *et al.* (2004), using the SCSA and the previously established threshold of <27%, found considerable differences in pregnancy rate between samples below and above this limit (20.2 versus 4% respectively), although it did not achieve statistical significance. When pregnant/not pregnant mean values of DNA fragmentation index (DFI) or highly DNA stainable (HDS) were compared, they found no statistical difference between these two groups, and only when a combination of both parameters was considered (DFI >27% and HDS >10%) was a statistically demonstrable decrease in pregnancy rate found (Bungum *et al.*, 2004). Nevertheless, this work only focused on semen samples, and did not evaluate DNA fragmentation after swim-up processing, which are the actual samples used for assisted reproduction.

Gandini *et al.* (2004) recently reported that SCSA test values well above 27% resulted in full-term pregnancies after ICSI,

further questioning the predictive value of the SCSA test in assisted reproduction treatment.

We aimed to improve the study design in several ways: (i) by analysing SCD of the same sample that was used for IUI; and (ii) by doing the analysis in a double-blind fashion, since the personnel performing the SCD had no information about the clinical outcome and the clinicians had no information about the SCD results. In addition, ovarian stimulation protocols yielded a relatively high pregnancy rate (Zuzuarregui *et al.*, 2004), and the same protocol was used in all our patients.

Several techniques are available to determine SCD, such as TUNEL, *in situ* nick translation (ISNT), COMET assay, and the SCSA (Schlegel and Paduch, in press). The COMET assay requires an electrophoretic unit and specific software for image analysis. TUNEL and ISNT assays require the use of enzymes whose activity and accessibility to DNA breaks may be limited. Therefore, some of these procedures are not well suited for routine analysis in the andrology laboratory, although they could be performed in a reference laboratory. At the present time, the SCSA is perhaps the most extensively used test for SCD determination, being the only one where a threshold for pregnancy has been reported (Evenson *et al.*, 2002). Nevertheless, this threshold has not been reproduced in recent studies (Gandini *et al.*, 2004, 2005). Moreover, its complexity and requirement of flow cytometry limits its use in a clinical setting. The SCD test is performed by conventional brightfield microscopy, and it has been shown recently that the SCD test results are highly correlated with those from the SCSA, with $r > 0.90$ (Fernández *et al.*, 2006), thus confirming the validity of SCD.

Nevertheless, the main endpoint of our study was to correlate pregnancy outcome with SCD, as measured by the SCD test. Our results indicate that, regardless of the SCD pattern observed, no correlation was found between SCD and pregnancy outcome.

Also, we must consider a limitation of our study: the possible presence of confounders regarding female characteristics or idiopathic infertility, rendering the results on predictive value of SCD on sperm negative, given that occult female factors could have been missed in the routine infertility investigation. Subsequently, our study does not allow us to conclude that SCD test is useless in the prediction of pregnancy by IUI, but the concrete group of couples for whom this test helps has not yet been determined. These drawbacks are found in all studies designed in a similar way.

The predictive value of DNA fragmentation tests depends on: (i) the test used to assess DNA fragmentation (e.g. whether the test measures real damage versus susceptibility to sperm DNA denaturation); (ii) the cause of DNA damage (apoptosis, oxidative stress, radiation therapy, single-stranded DNA nicks); (iii) the extent of DNA fragmentation per cell; (iv) whether DNA fragmentation affects introns versus exons; and (v) whether the oocyte can repair sperm DNA fragmentation after fertilization (Alvarez, 2005). In fact, in a recent study, Greco *et al.* (2005a) have shown that pregnancy rates in ICSI cycles that used sperm with DNA fragmentation values by TUNEL >15% were significantly lower than those obtained in cycles that used sperm with DNA fragmentation values <6% (44.4 versus 5.6%, $P < 0.001$).

Future studies using tests that measure primary double-stranded DNA fragmentation, such as TUNEL (as opposed to tests that measure susceptibility to DNA acid denaturation in vitro such as the SCD and SCSA tests), together with tests that measure secondary DNA damage, such as the determination of 8-OH-guanine and 8-OH-2'-deoxyguanosine, may prove to show a stronger correlation with pregnancy outcome in IUI.

Females undergoing IUI may represent a different population than those undergoing IVF/ICSI. The latter group frequently includes women with obvious fertility problems (i.e. tubal obstruction, severe endometriosis, older women, etc.) with low success probability in IUI, or with good pre-treatment prognosis that have previously undergone four failed IUI cycles. This could mean that in some cases the oocyte sperm-DNA repairing ability is compromised. Interestingly, in a recent work on IVF cycles we found a significant correlation with embryo implantation rates and embryonic features (Muriel *et al.*, 2006).

In conclusion, our study shows that chromatin dispersion in sperm, as measured by the SCD test, is not correlated with pregnancy outcome in IUI. However, a significant correlation was found between sperm motility and SCD test values.

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