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Prospective controlled trial of an electrophoretic method of sperm preparation for assisted reproduction: comparison with density gradient centrifugation

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BACKGROUND: A membrane-based electrophoretic filtration system, known as the Cell Sorter-10 (CS-10), that preferentially isolates spermatozoa with very low levels of DNA damage has recently been developed. However, it remains to be proven whether spermatozoa prepared in this way are capable of achieving fertilization in assisted conception. Therefore, this clinical trial was designed to answer this question. METHODS: A split-sample split-cohort study design was employed to control for differences in semen and oocyte quality between 28 couples undergoing either intracytoplasmic sperm injection (ICSI) or IVF in this clinical trial. Each semen sample was split between preparation using the CS-10 and preparation by standard density gradient centrifugation (DGC) and each cohort of oocytes was split for insemination using either CS-10 ($n = 197$) or DGC ($n = 195$) prepared spermatozoa. RESULTS: Both methods of sperm preparation yielded comparable rates of sperm recovery, motility and DNA fragmentation. There was no significant difference between the ability of CS-10 and DGC prepared spermatozoa to produce fertilization (62.4% versus 63.6%), cleavage (99.0% versus 88.5%) and high-quality embryos (27.4% versus 26.1%). CONCLUSIONS: This pilot study demonstrates that membrane-based electrophoresis is as effective as DGC in preparing sperm for IVF and ICSI, although it takes only a fraction of the time.

Keywords: electrophoresis; ICSI; IVF; spermatozoa; sperm preparation

Introduction

Human cervical mucus is known to differentially select viable spermatozoa and to act as a natural barrier to non-viable spermatozoa. However, the clinical application of assisted reproduction technology (ART) bypasses this natural selection process. In preparing and selecting sperm for ART, removal of seminal plasma is important because it contains prostaglandins that, if injected directly into the uterine cavity during intrauterine insemination (IUI), could stimulate very strong and painful uterine contractions. Furthermore, other constituents of seminal plasma stabilize the sperm membrane and prevent capacitation and hyperactivation, events that normally precede the acrosome reaction, which is necessary for successful invasion of the oocyte investments and fertilization (de Lamirande, 2007; Maxwell *et al*., 2007). Currently, the precise mechanisms by which these suppressive effects are delivered are uncertain, but protease inhibition (Nixon *et al*., 2006), membrane stabilization (Cross, 1996) and suppression of intracellular calcium (Huang *et al*., 2007) have all been implemented. Therefore, notwithstanding the important antioxidant properties of seminal plasma (Jones *et al*., 1979), efficient separation of spermatozoa from this complex fluid is a fundamental requirement for *in vitro* fertilization (IVF) and IUI (reviewed in Fleming *et al*., 1994, 1997).

Many different methods have been developed for separating human spermatozoa from seminal plasma including swim-up, self-migration sedimentation, glass wool filtration and sperm entrapment using Ficoll or Nycodenz (reviewed in Mortimer, 1994; Paasch *et al*., 2007). However, the most commonly applied method is discontinuous density gradient centrifugation (DGC) through polyvinylpyrrolidone- or silane-coated colloidal silica particles in suspension. Separation by DGC relies principally on differences in density, as spermatozoa with morphologically normal, oval heads presenting with highly compacted chromatin and little residual cytoplasm migrate to the high-density region of the gradient (Moustafa *et al*., 2004). Unfortunately, separation of spermatozoa on the basis of density alone does not ensure that the separated fraction only contains normal spermatozoa. Indeed, the possibility of inducing DNA damage within the embryo through the use of defective spermatozoa in standard ART protocols has been debated for some time now (Aitken, 1999). In this respect, sperm DNA integrity is believed to impact upon various treatment outcomes, including embryo viability, maintenance of

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pregnancy and disease in the offspring (Aitken, 1999; Loft *et al*., 2003: Lewis and Aitken, 2005).

A novel system of membrane-based electrophoretic filtration that rapidly and effectively isolates human spermatozoa exhibiting minimal DNA damage has recently been developed (Ainsworth *et al*., 2005). The latest prototype that has been developed for this purpose is known as the Cell Sorter-10 (CS-10) which is based on the principles that the highest quality spermatozoa within the ejaculate also carry the greatest net negative charge (Kirchhoff and Schroter, 2001; Giuliani *et al*., 2004; Ainsworth *et al*., 2005) and that they can be separated from other electronegative cells, such as leucocytes and immature germ cells, by virtue of their smaller cross-sectional size (Ainsworth *et al*., 2005). Consistent with this concept, a normal birth following the use of spermatozoa prepared using the CS-10 in intracytoplasmic sperm injection (ICSI) has recently been published as a case report (Ainsworth *et al*., 2007). However, there has been no prospective controlled trial to prove the suitability of this novel method in clinical practice. Moreover, it has not been demonstrated whether spermatozoa exposed to an electric current during electrophoresis are capable of achieving fertilization without the assistance of ICSI. Therefore, this prospective controlled clinical trial was designed to determine whether spermatozoa prepared using the CS-10 were as capable as spermatozoa prepared by DGC at fertilizing oocytes following IVF and ICSI, and whether the zygotes derived were equally capable of cleaving and yielding good-quality embryos.

Materials and Methods

Patient recruitment and study design

The Human Research Ethics Committee of the Sydney West Area Health Service approved this study, and informed consent was obtained from each couple. The study inclusion criteria were that the female had to be 18–38 years of age, undergoing her first, second or third cycle of IVF or ICSI, with at least 10 oocytes having been collected at transvaginal oocyte retrieval (TVOR). Each patient was only allowed to participate in the study on the one occasion. The semen sample produced on the day of TVOR had to have a minimal volume of 1 ml and a total motile count appropriate for IVF or ICSI. In total, 28 couples met these inclusion criteria and were included in the study, 17 undergoing IVF and 11 undergoing ICSI. A split-sample, split-cohort study design was followed to control for any variation there would otherwise be in sperm quality between semen samples and oocyte quality between patients, respectively. In total, 392 oocytes were retrieved from the 28 patients, 195 being allocated for insemination by DGC prepared spermatozoa and 197 by CS-10 prepared spermatozoa.

Ovarian stimulation and oocyte retrieval

Pituitary desensitization was achieved via routine long down-regulation with analogues of gonadotrophin-releasing hormone, using either subcutaneous Leuprorelin ($n = 2$; Lucrin™: Abbott Australasia Pty Ltd, Kurnell, Australia) or nasal Naferelin ($n = 26$; Synarel™: Pharmacia Australia Pty Ltd, Rydalmere, Australia). Controlled ovarian hyper-stimulation was achieved with recombinant human follicle-stimulating hormone, using either follitropin-β ($n = 15$; Puregon®; Organon Australia Pty Ltd, Lane Cove, Australia) or follitropin-α ($n = 13$; Gonal-F®; Serono Australia Pty Ltd, Frenchs Forest, Australia). Follicle growth was assessed by transvaginal ultrasound and monitored with daily serum estradiol levels throughout the stimulation period. Once a threshold ovarian response to stimulation of three or more follicles with a mean diameter of at least 18 mm was reached, 5000 IU of human chorionic gonadotrophin (Profasi™; Serono Australia Pty Ltd) was administered 36 h prior to TVOR. Follicular aspirates were examined for the presence of cumulus-enclosed oocytes, which were transported to the laboratory in Quinn's Advantage Hepes (QAH) buffered medium (SAGE In-Vitro Fertilization Inc., Trumbull, CT, USA). Each patient's cohort of oocytes were split evenly and randomly into two groups, with each group of oocytes being placed into a separate well of the same Nunc 4-well IVF dish (*In Vitro* Technologies, Melbourne, Australia) containing 900 μl Quinn's Advantage Fertilization (QAF) medium overlaid with 300 μl mineral oil (SAGE In-Vitro Fertilization Inc.). The oocytes were incubated in a Minc-1000 incubator (Cook Australia, Brisbane, Australia) at 37°C in an atmosphere of 5% CO_2, 5% O_2 and 90% N_2.

Semen analysis

Semen analysis was performed according to the World Heath Organization Guidelines using light microscopy (World Health Organisation, 1999). Following a recommended period of sexual abstinence of at least 3 days, semen samples were received in a sterile container within 1 h of collection. Following liquefaction, a portion of the sample was used for semen analysis, and at least 100 spermatozoa were analysed for each parameter assessed. Concentration was determined using a Makler chamber (Sefi Medical Instruments Ltd, Haifa, Israel), motility was determined using categories of movement a–d, and morphology was determined using the Papanicolau stain. Following analysis, the semen sample from each patient was split evenly between preparation by DGC and by membrane-based electrophoresis. In those instances where sufficient numbers of isolated spermatozoa were left over following insemination, the percentage of spermatozoa with intact DNA was also assessed.

Assessment of sperm DNA fragmentation

Assessment of sperm DNA fragmentation was performed using the terminal uridine deoxynucleotidyl transferase-mediated nick-end labelling (TUNEL) assay as described by Seli *et al*. (2004). Spermatozoa were fixed for 1 h at 15–25°C using freshly prepared 4% paraformaldehyde in phosphate buffered saline (PBS; pH 7.4) and were then washed with PBS. The spermatozoa were then permeabilized for 5 min using freshly prepared 0.1% Triton X-100 in 0.1% sodium citrate on ice. The spermatozoa were then washed twice in PBS and incubated with terminal deoxynucleotidyl transferase and fluorescein isothiocyanate-labelled deoxyuridine triphosphate provided in the *In Situ* Cell Death Detection Kit—Fluorescein (Roche, Mannheim, Germany) at 37°C for 1 h in the dark. The spermatozoa were again washed twice with PBS prior to analysis by flow cytometry (FACScalibur cytometer; Becton Dickinson). For each prepared semen sample and each control, 15 000 spermatozoa were assessed for TUNEL reactivity.

Sperm preparation by DGC

Discontinuous density gradients of silane-coated colloidal silica were prepared using ISolate™ (Irvine Scientific, Santa Ana, USA). Using a sterile 15 ml PET centrifuge tube (Corning Inc., Acton, USA), 1.5 ml of the 'upper' ISolate gradient was gently layered over 1.5 ml of the 'lower' ISolate gradient to minimize mixing of the layers in order to create a discontinuous density gradient. The portion of the semen sample allocated for preparation by DGC was

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made up to 3 ml, if necessary, using QAH medium, and this was gently layered over the discontinuous density gradient. Using our standard 'in-house' laboratory protocol (Fleming *et al*., 2007), the tube was centrifuged at 1200 rpm for 20 min to separate the spermatozoa from the seminal plasma. The supernatant was removed, leaving 0.5 ml of the 'lower' gradient containing the sperm pellet. The sperm pellet was transferred to a clean sterile centrifuge tube and washed using 5 ml QAH medium. This tube was centrifuged at 1500 rpm for 5 min, and the supernatant was removed to leave 0.5 ml QAH medium containing the sperm pellet. Again, the sperm pellet was washed using 5 ml QAH medium, the tube centrifuged at 1500 rpm for 5 min, and the supernatant was removed to leave 0.5 ml QAH medium containing the sperm pellet, which constituted the DGC prepared sperm stock.

Sperm preparation by membrane-based electrophoresis

The prototype cell-sorting electrophoretic system (Microflow® CS-10, Nusep Ltd, Frenchs Forest, Australia) has been described previously (Ainsworth *et al*., 2005) and will be referred to as the 'CS-10'. The separation unit of the CS-10 consists of a self-assembled cartridge comprising two 400 μl chambers, separated by a 5 μm polycarbonate filter and bounded by 15KDa polyacrylamide restriction membranes that allow the free transit of electrolytes but prevent cross-contamination between the semen sample and the electrophoresis buffer. The electrophoresis buffer contained 10 mM Hepes, 30 mM NaCl and 0.2 M Sucrose with an osmolarity of 310 mOsm kg^{-1}, adjusted to a pH of 7.4 using 2 M KOH. The separation cartridge was autoclaved and the electrophoresis buffer was filter-sterilized using a 0.22 μm filter (Millipore Corp., Bedford, USA) prior to use, and sperm preparation using the CS-10 was performed within a class II safety cabinet to maintain sterile conditions. A 400 μl aliquot of the portion of the semen sample allocated for preparation using the CS-10 was loaded into the 'inoculation' chamber and 400 μl electrophoresis buffer was loaded into the 'separation' chamber of the cartridge. Spermatozoa were separated from the seminal plasma at 25°C for 5 min at a constant applied current of 75 mA and a variable voltage of 18–21 V. The separated fraction of spermatozoa was removed from the separation chamber and transferred into a centrifuge tube, then washed twice using QAH medium, as described for DGC; the resulting pellet constituted the CS-10 prepared sperm stock.

Insemination, fertilization and embryo quality assessment

The DGC and CS-10 prepared sperm stocks for each patient were diluted using QAH medium to a final working concentration of ~2 million motile spermatozoa, as assessed by the same sperm prep scientist, in order to control for the subjective variation that would otherwise exist between different scientists. For the IVF inseminations, ~100 μl of each diluted sperm preparation was added to the respective well of the Nunc 4-well IVF dish containing half the cohort of oocytes in 900 μl QAF, to achieve a final insemination concentration range of 150 000–250 000 ml^{-1} motile spermatozoa. For ICSI, cumulus-enclosed oocytes were denuded using ovine hyaluronidase type III (Sigma Biosciences, Castle Hill, Australia) and injected using either DGC or CS-10 prepared spermatozoa immobilized in 5% polyvinylpyrrolidone (SAGE In-Vitro Fertilization Inc.). Fertilization was assessed 16 h post-insemination, and those zygotes having two pronuclei and two polar bodies were considered normally fertilized. Using our standard 'in-house' laboratory protocol (Fleming *et al*., 2007), morphological evaluation of all embryos was performed 42 h post-insemination, as follows: embryos were classified as either Grade 1 (irregular blastomeres with >50% fragmentation), Grade 2 (irregular blastomeres with 25–50% fragmentation), Grade 3 (regular blastomeres with 10–25% fragmentation), Grade 4 (regular blastomeres with <10% fragmentation) or Grade 5 (regular blastomeres with no fragmentation whatsoever). The number of blastomeres was also recorded. To receive a maximum score of 5, an embryo also had to be at the 4-cell stage at the time of grading.

Statistical analysis

All statistical analyses utilized Microsoft® Excel 2004 (Version 11.2 for Mac®). To identify differences in the means, a paired *t*-test was utilized, where a *P*-value of <0.05 was considered significant. A 'linear mixed-effects model fit by maximum likelihood' was used to identify any relationship above and beyond the natural regression to the mean (Byth and Cox, 2005). This model is similar to the logistic regression model but takes into account the dependence between oocytes from the same patient. It is a 'linear' model because there is a dichotomous result: an oocyte can be fertilized or not fertilized and a zygote can have cleaved or can have failed to cleave. It is a 'mixed-effects' model because there are multiple observations from different patients and within the same patient: embryo quality can be graded from 1 to 5. The statistical software package SPLUS Version 6.2 was used to analyse the fertilization data. Two-tailed tests with the significance level of 5% were used throughout. Generalized linear mixed effects models (GLMMs) were used to assess the effects of method (DGC versus CS-10) and procedure (IVF versus ICSI) on the odds of fertilization and on the odds of cleavage of normally fertilized oocytes. In the GLMMs, patient identifier and method were treated as random effects and method, procedure and their two-way interaction were treated as fixed effects.

Results

Efficacy and efficiency of DGC and CS-10 sperm preparation

The duration of abstinence prior to semen collection ranged from 2 to 5 days. No significant correlation was observed between the duration of abstinence and semen quality in the unprocessed semen sample. For a single semen sample, less time was required for sperm preparation using the CS-10 since the separation duration was just 5 minutes, which was considerably shorter than the 20 min required for DGC. Nevertheless, the proportion of spermatozoa remaining following each complete preparation method was very similar, with recovery rates of ~20% for DGC and 22% for the CS-10 (Table I). Compared with the mean sperm motility (58.1% ± 0.03) of semen samples prior to preparation for IVF, DGC resulted in a significant increase in sperm motility (71.0% ± 0.04; $P = 0.017$), whereas the CS-10 (65.0% ± 0.03; $P = 0.149$) did not. However, there was no significant difference between the DGC and CS-10 sperm preparation motilities (Table I). There was also no significant difference between the DGC and CS-10 sperm preparations in terms of the final sperm concentration and volume of the preparation used in insemination for IVF, nor in the levels of DNA fragmentation in the prepared spermatozoa (Table I). Compared with the mean sperm motility (29.0% ± 0.04) of semen samples prior to preparation for ICSI, neither DGC (41.0% ± 0.05; $P = 0.061$) nor CS-10 (28.0% ± 0.07; $P = 0.868$) sperm preparation resulted in a significant increase in sperm motility. Also, there was no significant difference between the density and motility of the DGC and CS-10 final sperm preparations used for ICSI (Table I).

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Table I. Comparison of sperm preparation quality.

	DGC Sperm Prep	CS-10 Sperm Prep	Significance
Sperm recovery	20.02%	21.72%	
IVF Sperm Preps			
Sperm motility	71.00% ± 0.04	65.00% ± 0.03	0.275
Sperm density	4.03 ± 0.46 × 10^6/ml	5.00 ± 0.58 × 10^6/ml	0.196
Insemination volume	91.11 μl ± 4.57	84.72 μl ± 5.02	0.353
DNA fragmentation	3.51% ± 2.77	2.33% ± 1.76	0.720
ICSI Sperm Preps			
Sperm motility	41.00% ± 0.05	28.00% ± 0.07	0.103
Sperm density	2.02 ± 0.92 × 10^6/ml	1.31 ± 0.43 × 10^6/ml	0.499

Values are expressed as the mean ± SEM.

Ability of DGC and CS-10 prepared spermatozoa to fertilize oocytes and produce good-quality embryos

The power of this study is reflected by the fact that ~200 oocytes were exposed to spermatozoa prepared by DGC, and ~200 oocytes were exposed to spermatozoa prepared by the CS-10 (Table II). No significant differences in normal fertilization rates, cleavage rates of normally fertilized oocytes or the percentage of top-quality embryos following insemination using the two different sperm preparation methods were observed (Table II). Likewise, there were no significant differences observed in these outcomes in either the IVF (Table III) or ICSI (Table IV) groups of patients.

Pregnancy outcomes following DGC and CS-10 sperm preparation

This study was not designed to compare pregnancy outcomes between the two different methods of sperm preparation since the embryo chosen for transfer was determined by its morphological quality alone, and not by the source of spermatozoa used to produce it and, besides, there are insufficient numbers of pregnancies from this group of patients for statistical analysis. In this study, 13 DGC-derived embryos were transferred in 11 embryo transfers, resulting in two pregnancies, no miscarriages and one normal delivery. In addition, 23 CS-10-derived embryos were transferred in 18 embryo transfers, resulting in six pregnancies, one miscarriage and three normal deliveries. Some pregnancies from both DGC- and CS-10-derived embryos are still ongoing.

Discussion

The results of this prospective controlled trial demonstrate for the first time that this method of membrane-based electrophoresis is as effective a method as DGC in the preparation of spermatozoa for both IVF and ICSI. Indeed, the CS-10 system is a particularly rapid and efficient means of preparing spermatozoa by virtue of its very rapid separation time of 5 min. The recovery rates of spermatozoa following the use of either DGC or the CS-10 were comparable at around 20%, and this is consistent with what has been reported previously (Ainsworth *et al*., 2005, 2007).

The final IVF sperm preparations following separation by DGC and the CS-10 were also comparable in terms of their density, motility, the level of sperm DNA fragmentation and the volume of the preparation used for insemination. It was not possible to compare the levels of DNA fragmentation present within the DGC and CS-10 sperm preparations for ICSI due to insufficient numbers remaining after that required for treatment, since analysis by flow cytometry requires at least 15 000 spermatozoa. Interestingly, our results are consistent with what has been reported previously, except for one subtle difference (Ainsworth *et al*., 2005). Whereas Ainsworth *et al*. (2005) found that DGC using Percoll™ resulted in significantly greater levels of motility than when using the CS-10, the difference in motility between DGC and CS-10 sperm preparations was not significantly different in this study, though DGC did result in slightly higher levels of motility. Possible explanations for this discrepancy between the report by Ainsworth *et al*. (2005) and this study are differences in donor profile, nature of the gradient used (Percoll versus ISolate; Allamaneni *et al*., 2005) and differences in the susceptibility of spermatozoa to the passage of electric current (Engelmann *et al*., 1988; Ainsworth *et al*., 2005). Whatever the reason, these small

Table II. Comparison of fertilization and cleavage rates.

	DGC Sperm Prep	CS-10 Sperm Prep	Significance
No. oocytes allocated	195	197	
No. 2PN oocytes	124 (63.59%)	123 (62.44%)	0.716
No. 2PNs frozen	20	20	
No. 2PNs cleaved	92 (88.46%)	102 (99.03%)	0.067
No. grade 4–5 embryos	24 (26.09%)	28 (27.45%)	0.725

Table III. Comparison of IVF fertilization and cleavage rates.

	DGC Sperm Prep	CS-10 Sperm Prep
No. oocytes allocated	132	130
No. GV oocytes	4	7
No. MI oocytes	7	5
No. MII oocytes	14	27
No. abnormal oocytes	1	3
No. oocytes not recovered	1	0
No. 1PN oocytes	5 (3.79%)	2 (1.54%)
No. 2PN oocytes	91 (68.94%)	80 (61.54%)
No. ≥3PN oocytes	9 (6.82%)	6 (4.62%)
No. 2PNs frozen	20	20
No. 2PNs cleaved	62 (87.32%)	59 (98.33%)
No. grade 4–5 embryos	21 (33.87%)	19 (32.20%)

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Table IV. Comparison of ICSI fertilization and cleavage rates.

	DGC Sperm Prep	CS-10 Sperm Prep
No. oocytes injected	63	67
No. MII oocytes	24	24
No. 1PN oocytes	2 (3.17%)	0
No. 2PN oocytes	33 (52.38%)	43 (64.18%)
No. ≥3PN oocytes	4 (6.35%)	0
No. 2PNs frozen	0	0
No. 2PNs cleaved	30 (90.91%)	43 (100.00%)
No. grade 4–5 embryos	3 (10.00%)	9 (20.93%)

differences in motility appear to have minimal impact upon the fertilizing capacity of the sperm preparation as a whole.

Qualitative differences between DGC and the CS-10 methods of sperm preparation may be pertinent. Repeated centrifugation, as used in DGC, has been shown to be associated with physical shearing forces that enhance the generation of reactive oxygen species as reflected by the luminol-dependent chemiluminescence generated by human spermatozoa (Aitken and Clarkson, 1988; Ainsworth *et al*., 2005; Agarwal *et al*., 2008). In contrast, membrane-based electrophoresis, as used in the CS-10, does not induce reactive oxygen species generation by human spermatozoa (Ainsworth *et al*., 2005). Also, it is likely that electrophoresis selects a different subpopulation of spermatozoa to that selected by DGC since the electro-negativity of human spermatozoa is believed to be dependent not upon sperm density but upon the sperm glycocalyx, which is rich in sialic acid residues (Kallajoki *et al*., 1986; Calzada *et al*., 1994). One such residue, known as CD52, is a highly sialated glycosylphosphatidylinositol (GPI)-anchored protein on the sperm surface, that is acquired by spermatozoa during epididymal transit (Schroter *et al*., 1999; Kirchhoff and Schroter, 2001; Giuliani *et al*., 2004). Interestingly, CD52 expression appears to be significantly correlated with normal sperm morphology and sperm capacitation (Giuliani *et al*., 2004). Therefore, it is likely that the negative charge associated with spermatozoa reflects their normal differentiation within the testis since the charge is presumably necessary for them to be capable of the massive cell–cell transfer of GPI-anchored CD52 at the sperm surface (Schroter *et al*., 1999). Hence, it has been hypothesized that the CS-10 preferentially selects human spermatozoa on the basis of charge differences between spermatozoa due to the relative presence of sialated proteins on the sperm surface (Ainsworth *et al*., 2005, 2007).

The use of a split-sample split-cohort design in this study ensured that any difference there would otherwise be in sperm quality between different semen samples, and in oocyte quality between different cohorts of oocytes, were controlled for. Of the oocytes inseminated with DGC prepared ($n = 195$) and CS-10 prepared ($n = 197$) spermatozoa, there were comparable rates of fertilization, cleavage and embryo quality. Interestingly, the cleavage rates of CS-10 derived zygotes were extremely high, approaching 100% but, nevertheless, were not significantly different to the cleavage rates of DGC derived zygotes. Although DGC prepared spermatozoa appeared to yield higher fertilization rates in the IVF group of patients, this difference was not significant. This apparent difference could be a function of the slightly higher sperm motility observed in the DGC sperm preparations, but the statistics suggest otherwise.

The fact that there have now been six pregnancies following the transfer of CS-10 derived embryos, four of these from the IVF group of patients, shows that membrane-based electrophoretic filtration is a viable method of preparing spermatozoa for both IVF and ICSI. Furthermore, the fact that these pregnancies have thus far resulted in three normal deliveries suggests that this novel method of sperm preparation is a reliable and safe alternative to DGC. Nevertheless, concerns regarding the incidence of birth defects in children conceived through ART remain (Hansen *et al*., 2002). Of equal concern, is that semen samples exhibiting high percentages of DNA fragmentation are, nevertheless, capable of achieving fertilization, especially if ICSI is the mode of insemination (Twigg *et al*., 1998; Aitken, 2004; Gandini *et al*., 2004; Lewis and Aitken, 2005). Furthermore, zygotes resulting from the use of semen with high numbers of spermatozoa with damaged DNA are capable of developing to term (Gandini *et al*., 2004). Indeed, the presence of DNA damage within spermatozoa is one of the factors believed to be responsible for the early pregnancy loss and perinatal morbidity associated with ART (Aitken, 2004; Lewis and Aitken, 2005). Therefore, there is an urgent imperative to develop and test new procedures for the preparation of human spermatozoa that can be optimized to maintain sperm DNA integrity (Zini *et al*., 2000). Although the CS-10 appeared to be slightly more effective than DGC in reducing the levels of sperm DNA fragmentation, this was not significant, which is consistent with previous findings (Ainsworth *et al*., 2005). In this respect, it is reassuring that both the DGC and the CS-10 methods of sperm preparation yield spermatozoa with comparable low rates of sperm DNA damage. However, other related methods of sperm preparation, such as magnetic-activated cell sorting using annexin V to bind to phosphatidylserine residues externalized during apoptosis (Said *et al*., 2008), may prove to be a more effective method than electrophoretic filtration with respect to reducing the level of sperm DNA fragmentation.

In conclusion, membrane-based electrophoresis is an efficient and reliable means of sperm preparation that is as effective as DGC. Since it does not require the preparation of density gradients and careful removal of a sperm pellet from a gradient, it is an intrinsically faster and simpler method of sperm preparation, involving a shorter learning curve. It also has the potential for improved risk management since different semen samples cannot be run concurrently, as opposed to DGC where different semen samples could be batch centrifuged, and it involves fewer steps where double-checking of patient identity is mandatory.

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