THE EFFECT OF CENTRIFUGATION TEMPERATURE ON SPERM RETRIEVAL, MORPHOLOGY AND MOTILITY PARAMETERS

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Introduction

For the majority of couples seeking treatment in assisted reproduction, the choice of most appropriate treatment is based on the quality of the ejaculate (sperm count, motility or morphology) or the source of the gametes (Tournaye, 2005). If the quality of the ejaculate can be further improved through existing preparation techniques, a greater variety of treatment options may become available to people who usually fall outside the requirement bracket for a certain line of treatment.

Intrauterine insemination (IUI) is usually the first approach when treating female infertility or mild male factor infertility. For this procedure, a sample with good motility, concentration and forward progression is required; generally a pregnancy rate of 15-20% is achieved with a count of at least $1x10^6$ motile spermatozoa per insemination.

In vitro fertilization is the next treatment approach and is indicated for female factor infertility. Although fertilization takes place outside of the body, good sperm motility and morphology is still needed for normal fertilization of the ova in vitro. The number of spermatozoa added to each mature oocyte depends on the sperm morphology: ≤4% (up to $2x10^6$ sperm/ovum); >4 ≤ 14% (500 000 sperm/ovum); >14% (100 000 sperm/ovum). Pregnancy rates of 35-40% can be achieved with this method. With gamete intrafallopian transfer (GIFT) fertilization takes place inside the female, but the required semen parameters and general success rates are the same (Windt et al., 2007).

For the treatment of severe male factor fertility, intracytoplasmic sperm injection (ICSI) has been very successful. Here the quality of the sperm sample is of very little importance and only dead or severely abnormal sperm will have a negative influence on fertilization rates. However, the application of ICSI preceded a full understanding of the underlying etiology of the male factor infertility, which may be of a transmissible genetic basis. Since the injection of sperm into the oocyte bypasses the oocyte's selection of viable sperm via the zona pelucida, questions were raised as to the genetic consequences of selecting sperm for ICSI from males with severe oligozoospermia and azoospermia. As

the application of ICSI became more popular, Johnson (1998) conducted a study to review the most significant genetic disorders associated with oligospermia and azoospermia and recommended genetic counseling and screening prior to ICSI due to the risks associated with this line of treatment.

Both sperm motility parameters and percent normal morphology are significant factors in predicting fertilization and pregnancy rates in IVF. A low post wash total progressively motile count can, in general, only result in successful IVF if the female patient responds well to ovarian hyperstimulation (Rhemrev et al., 2001; Donnelly et al., 1998). The most frequent male factor parameters relating to the success of IUI have been reported as progressive motility and the number of spermatozoa in the inseminate, while morphology by strict criteria has also been noted (Duran et al., 2002; Morshedi et al., 2003). Also, a study by Dickinson et al. (2002) reported that the single largest predictor of IVF outcome was total progressive motility in the prepared sperm specimen. Consequently, if the total progressively motile count is not within required parameters for IUI or IVF, ICSI is the recommended treatment. Although there has been much success with ICSI, the consequences of this type of assisted reproduction is not yet fully comprehended and therefore IVF and IUI still remain the best techniques for the natural selection of optimal genetic material.

Consequently, a simple, cost-effective method to improve parameters of prepared sperm to the extent that IUI and IVF would be possible and successful in the greater majority of patients is needed.

A prospective randomized study by Morshedi et al. (2003) found that the influence of various semen parameters on the pregnancy with IUI outcome is only reduced when the number of sperm in the inseminate is greater than 22×10^6, emphasizing the need for an efficient preparation technique with optimal yield. This is especially true for samples from sub fertile males since these semen parameters will be poor. Obtaining the maximum amount of progressively motile spermatozoa from a sample in the most efficient and cost effective way, is therefore vital to the success of ART, specifically IUI.

In general sperm has to be separated from seminal plasma quickly and efficiently to be potentially functional. Prolonged exposure to seminal fluid components results in a decline in both sperm motility and vitality (Mortimer, 1994). Sperm separation techniques form an integral part of the assisted reproductive programme and the success of these methods is measured by the yield of morphologically normal and motile progressive sperm and the percentage of these sperm in the final sample (Sanz et al., 2003).

There are two methods of semen preparation in the laboratory which are most widely used to generate post processing samples containing a high concentration of morphologically normal sperm with good progressive motility. Swim-up (method of self-migration of spermatozoa) select the subpopulation with the best motion characteristics, while the recovery rate is understandably low. With discontinuous density gradient centrifugation (DGC), sperm is selected on the basis of their density, making it possible to select a highly motile sub-population with optimal morphology although the yield is also low (Morshedi et al., 2003). Simple wash and filtration are also used, though less effective. The effect of the non-motile fraction on the motile sperm during preparation is another concern, which demands efficient techniques to minimize preparation time and possible damage to the spermatozoa used in ART.

Several studies have been done to optimize sperm retrieval rates. Keppler et al. (1999) conducted a study with fertile donors and found significantly higher percentages of motility, mean average path velocity, straight line velocity, lateral head displacement, and percentage hyperactivation in sperm at 40ºC. Su et al., (2002) found that the optimal culture conditions for testicular sperm were at 37ºC.

A study by Petrella et al. (2003) to determine the optimal conditions for maintaining sperm motility and viability after processing, concluded that motility and viability declined in general 5-10% every hour for the first 6 hours, without any significant statistical difference between the groups. After 6 hours, conditions for best preservation of motility and viability were in the order 20ºC>37ºC>4ºC. During that study the

processed samples were incubated and examined at 4ºC, 20ºC (Room temperature) and 37ºC (5% CO_2) respectively until the motility and viability measures reached zero.

In close agreement, Marín-Briggiler et al. (2002) found that after 4 hours all the kinematic characteristics evaluated in normozoospermic samples (VAP, VSL, VCL, ALH, BCF STR and LIN) were higher for sperm incubated at 37ºC than those at 20ºC.

These results were supported by other studies (Agarwal, 2003) which also found that the levels of ROS generated at 37ºC were significantly lower than those of the samples incubated at 25ºC and 4ºC respectively. Hirano et al. (2001) conducted a study with 136 fresh samples from 99 infertile subjects treated with IVF-ET and found that although there were significant correlations between fertilization rates and post swim-up sperm concentration, motility, normal morphology and STR, no significant correlations were found between fertilization rates and the other post swim-up sperm kinematic characteristics (ALH, BCF, VCL, VSL, VAP, LIN) and %Rapid [illegible]

Given that sperm motion characteristics are influenced by temperature, it can be assumed that morphology and as a consequence of this possibly DNA integrity, will also be influenced by temperature. One of the first studies to provide evidence of the relationship between sperm morphology and motion characteristics in fertile donors was conducted by Katz et al. (1982). They found that, in the same specimen, sperm with normal head morphology swam significantly faster than those with abnormally shaped heads. They also found that although there were differences in straight line velocity (mainly a result of the significantly lower flagellar beat frequencies of the cells with abnormal morphology) the amplitude and wavelength of the normal and abnormal cells did not differ significantly. When only the abnormal cells were analysed, those with amorphous and pyriform tapering heads were the slowest.

In the light of these findings, a related study was conducted by Oehninger et al. (1990). They found that, although the motility, velocity and percentage of fast swimming cells increased significantly in the both the G (good morphology, 5-14% normal forms) and P (poor morphology, 0-4% normal forms) groups after swim-up, the improvement in motility was significantly higher for the G samples. Also, the recovery rate after swim-up in the P group was substantially lower than that of the G group, suggesting that specimens with poor morphology have a functional impairment after capacitating conditions.

Concerning chromatin packaging, it has been found that disturbances in chromatin decondensation and centrosome abnormalities are the major cause of fertilization failure after ICSI. A study on the effect of sperm preparation techniques on chromatin packing and morphology (Hammadeh et al., 2000) reported that swim-up does not necessarily select sperm with well-condensed chromatin structure, indicating that there might be no association between sperm morphology and chromatin condensation. Esterhuizen et al. (2002) concluded that the quality of chromatin packaging, in combination with sperm morphology assessments, can be a useful indicator of fertilization failure.

Our study was designed in pursuit of contributing to experimental knowledge that could lead to the improvement of existing sperm preparation techniques. The objective was to evaluate the effect of temperature during sperm preparation centrifugation steps on:

(i) Sperm retrieval rate

(ii) Chromatin packaging quality and morphometric parameters

The method of sperm preparation used in this study was swim-up, since it significantly improves the percentage of sperm with normal morphology (Hammadeh et al., 2000). In contrast with others, Younglai et al. (2001) suggested that with normal spermatozoa the double swim-up procedure does not impair the fertilizing ability of the recovered sperm. Also, the level of DNA damage does not increase with separation of motile sperm by swim-up (with or without centrifugation).

Material & Methods

a) Semen samples and sperm preparation

Thirty semen samples were obtained from 10 normozoospermic donors from our sperm donor programme (sperm donors are recruited on an annual basis using advertisements in the Faculty of Health Sciences). Directly after collection, samples were divided into 2 aliquots. One aliquot was placed in an incubator at 34ºC while the second aliquot was left at room temperature (RT: 25ºC). The samples were left for 30 minutes to stabilize at the given temperatures, before starting with the experimental procedure. At this point (prior to experimental onset) motion characteristics were recorded with the CASA instrument, serving as initial or baseline values for the measured motion characteristics.

b) Swim up separation

Motile sperm fractions were retrieved from the semen samples with a swim-up procedure. Four test tubes were prepared for each sample (two from the RT aliquot, and two from the 34ºC aliquot): 1ml of Quinn's Advantage© Flush Medium (supplemented with 0.3% bovine serum albumin, Sigma chemical company, St Louis, USA) added to 0.5ml semen. The test tubes were then placed in two different centrifuges, namely (i) Spermfuge SF 800 (Shivani Industries, Mumbai, India), a highly precise centrifuge with a temperature controlled chamber at 34ºC and (ii) Sigma bench top with no temperature control facilities. Both centrifuges were set at 1500rpm (428xG) for 5 minutes. Following the second washing procedure, both sperm pellets were layered with 0.5ml Quinn's Advantage© Flush Medium (supplemented with 0.3% bovine serum albumin). These test tubes with layered pellets were then left at a 45º angle at 34ºC and RT respectively, for 60 minutes to allow motile spermatozoa to swim up into the layered medium. After the incubation period, 0.5ml supernatant was removed from each tube and immediately analyzed with the CASA instrument for sperm motion characteristics.

c) Motion characteristics

Aliquots (5µl) from swim-up-prepared spermatozoa (±$10x10^6$ cells/ml) from all four of the experiments (Spermfuge RT and 34ºC; Sigma RT and 34ºC) were placed in the

micro chamber for analyses with the HTM-IVOS V10.9 CASA instrument (Hamilton-Thorne Research Inc., Beverley, MA, USA). These are the standard set-up parameters: 30 frames/60 Hz; minimum contrast, 80; minimum cell size, 2; minimum static contrast, 30; low VAP cut-off, 5 µm/s; low VSL cut-off, 11 µm/s; head size, non-motile, 3; head intensity, non-motile, 160; static head size, 1.01-2.91; static head intensity, 0.60-1.40; slow cells, non-motile; magnification, 2.01; and temperature at 34ºC.

The following parameters were evaluated: sperm concentration; motile and progressively motile concentrations; percentage motile and progressively motile; path velocity (VAP); straight-line velocity (VSL); curvilinear velocity (VCL); amplitude of lateral head displacement (ALH); beat cross frequency (BCF); straightness (STR); linearity (LIN), percentage with rapid motility; percentage with medium motility; percentage slow; and percentage static.

Table 1 Definitions of the parameters investigated

Characteristic	*Description*
Percent motility	Percent of sperm moving in a manner fitting motility determination parameters
Velocity curvilinear (VCL)	Point to point velocity (total distance traveled) per second.
Velocity average path (VAP)	Point to point velocity on a path constructed using a roaming average. The number of points in the roaming average is 1/6th of the frame rate of video used.
Velocity straight line (VSL)	Velocity measured using the first point and the average path and the point reached that is furthest from this origin during the measured time period.
Linearity (LIN) VSL/VAP	Describes path curvature
Beat cross frequency (BCF)	This value is determined through detection of the frequency at which VCL crosses VAP. In cases where a sperm has a highly circular path, the values generated are not valid

Motion characteristics were recorded in all samples using 10 randomly selected microscopic fields.

d) Sperm function

The following sperm functions were evaluated for the different semen preparations;

(i) Chromatin packaging quality (CMA3); and

(ii) Sperm morphology (both manually and with the metrix system of the CASA instrument)

i) Chromomycin A3 fluorescent staining (CMA3)

Semen smears were fixed in methanol/glacial acetic acid 3:1 at 4ºC, for 10 minutes. For CMA3 staining, 40µl of McIlvane's solution (pH 7.0 containing 10mM $MgCl_2$) was added to the 5µl 10mg/ml aliquot of CMA3 (Sigma chemical company, catalogue C2659) to make up the working solution. Each slide was covered with 45µl working solution and left to develop in a light-sealed container for 20 min. Afterwards the slides were washed once with PBS, air dried and mounted with mounting medium (Sigma chemical company, St.Louis, USA).

ii) Diff-Quick staining for morphology evaluation

Semen smears were fixed in methanol/glacial acetic acid 3:1 at 4ºC, for 10 minutes. Each slide was then treated with the three solutions in consecutive order: Hemacolor solution 1 (Merck, Darmstadt, Germany) for 10s; Hemacolor solution 2 for 20s; Hemacolor solution 3 for 15s. Afterwards the slides were washed with water, air dried en mounted with mounting medium (Sigma chemical company, St.Louis, USA).

Data analysis

All above mentioned sperm motion characteristics were transformed to ARCSIN values and results of the two centrifugation methods were compared with Mann-Whitney test for independent samples and the Student's t-test.

Results

Table 2 Sperm retrieved after swim-up

	34°C	*RT*	*P-values*
Sperm concentration retrieved from Spermfuge centrifugation ($1x10^6$/ml)	38.1 ± 47.7	30.9 ± 33.3	$p > 0.05$ Not significant
Sperm concentration retrieved from Sigma centrifugation ($1x10^6$/ml)	32.7 ± 31.5	30.6 ± 37.2	$p > 0.05$ Not significant
%Motile sperm after Spermfuge centrifugation	**64.0 ± 19.9[a]**	**46.7 ± 29.0**	**p = 0.003 Significant**
%Motile sperm after Sigma centrifugation	**57.2 ± 21.3**	**46.5 ± 24.2**	**p = 0.04 Significant**

a: there was a significant difference ($p = 0.03$) between these values and the baseline values before swim-up

Although the heated samples yielded higher sperm concentrations, no significant increase was seen in the concentration of sperm retrieved after swim-up. However, the percentage motile sperm retrieved from the heated samples in both centrifuges were significantly higher than that of the samples at room temperature. Also, the percentage motile sperm in the heated sample after Spermfuge centrifugation was the only one to show a significant increase from the baseline values.

Table 3 Comparison between baseline and experimental values presented as average values and standard deviations for percentage rapid, medium, slow and static moving cells after double swim-up

	% RAPID		%MEDIUM		%SLOW		%STATIC	
	AVE	*SD*	*AVE*	*SD*	*AVE*	*SD*	*AVE*	*SD*
Baseline	43.9	18.4	7.7	6.4	11.3	7.8	37.4	17.0
Spermfuge34ºC	40.4	22.8	**24.2**[a]	11.6	13.2	11.0	**22.2**[b]	16.4
SpermfugeRT	**28.3**[c]	28.2	**17.4**[d]	16.1	16.2	16.4	38.0	39.3
Sigma34ºC	**36.1**[e]	23.8	**21.5**[f]	14.0	14.5	8.6	27.9	18.8
SigmaRT	**26.3**[g]	22.6	**20.4**[h]	13.3	**17.0**[i]	12.0	35.9	22.2

Significant p-values, $p < 0.001$ (a, f, g, h)
b: 0.006; c: 0.003; d: 0.009; e: 0.032; i: 0.033

The increase in percentage cells from baseline with medium movement characteristics according to the CASA parameters was significant for all the groups. However, the two highest values were measured in the 34ºC groups (Spermfuge 34ºC and Sigma 34ºC respectively).

An important observation was that all the groups except for Spermfuge 34ºC showed a significant decrease in percentage rapid moving cells from baseline, while the only significant decrease in percentage static cells was found in the Spermfuge 34ºC group.

Table 4. Mean and standard deviations for motion characteristics after double swim-up (path velocity (VAP)µms^{-1}; straight-line velocity (VSL))µms^{-1}; curvilinear velocity (VCL))µms^{-1}; amplitude of lateral head displacement (ALH))µm; beat cross frequency (BCF) Hz; straightness (STR) %; linearity (LIN) %

	VAP	**VSL**	**VCL**	**ALH**	**BCF**	**STR**	**LIN**
Baseline	39.7±19	34.4±17	58.2±28	3.04±1	23.76±7	83.74±17	56.6±14
Spermfuge34ºC	42.47±17	36.85±14	60.89±21	2.93±1	25.51±5	87.59±5	61.33±8
Spermfuge RT	39.71±19	34.42±17	58.20±26	3.04±1	23.76±7	83.74±17	56.62±14
Sigma 34ºC	45.56±18	39.87±17	64.92±22	3.29±1	25.78±5	87.59±	59.40±7
Sigma RT	35.37±15	30.54±14	53.54±18	3.15±1	26.88±5	87.74±4	57.11±8

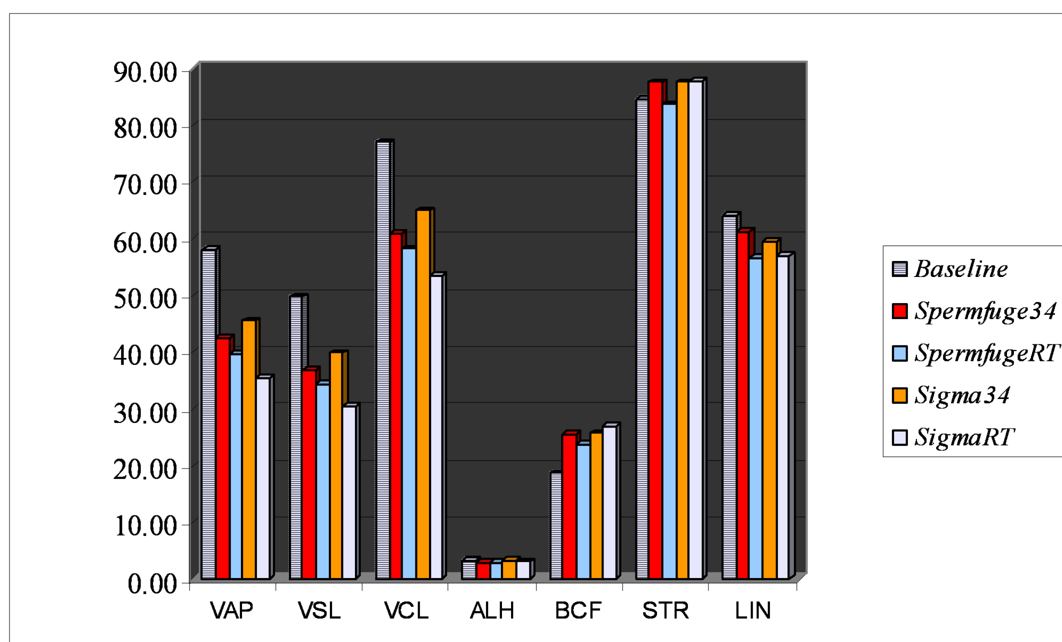

Figure 1 Mean values for motion characteristics before and after swim-up.

Figure 1 illustrates the data in Table 4 and shows that the heated samples (34ºC) consistently contributed the two highest values post swim-up for all the parameters, except the BCF, where they were second and third highest. There were no significant differences between the heated samples and those at room temperature for these parameters, but when the pre and post swim-up values for these parameters were compared, the STR values for the heated samples were significantly higher than baseline.

Table 5 Percentage normal sperm morphology according to Tygerberg criteria

	Spermfuge RT	*Spermfuge 34ºC*	*Sigma RT*	*Sigma 34ºC*
Average (%)	11	12	11	13
SD	3.9	3.4	3.0	3.4

Table 6 Percentage spermatozoa with positive CMA3 post swim-up

	Spermfuge RT	*Spermfuge 34ºC*	*Sigma RT*	*Sigma 34ºC*
Average (%)	32	32	29	29
SD	4.4	3.8	6.8	6.1

Sperm morphology values recorded for baseline and experimental procedures showed no significant difference in the percentage normal cells before and after centrifugation. This observation is in accordance with the results recorded with the CMA3 tests, where again no differences could be seen.

Table 7 Morphometry dimensions obtained with HTM-IVOS V10.9 CASA (metrix) after double swim-up procedure and Diff-Quick staining

	Major axis (µm) Length	*Minor axis (µm) Width*	*Area (µm²)*	*Perimeter (µm)*	*Acrosome % of head area*
SPERMFUGE ROOM TEMPERATURE					
Average	5.50	3.73	15.43	14.75	21.68
SD	0.36	0.40	1.49	0.94	11.59
SIGMA ROOM TEMPERATURE					
Average	5.46	3.50	14.75	14.33	24.06
SD	0.31	0.29	1.71	0.77	13.20
SPERMFUGE 34ºC					
Average	5.23	3.62	14.62	14.18	23.90
SD	0.71	0.63	2.83	1.97	15.53

SIGMA 34ºC					
Average	4.03	3.05	11.53	11.53	19.80
SD	0.59	0.55	2.48	1.74	8.53

The only significant difference (Mann-Whitney test for independent samples, $p<0.05$) found between the head morphometric results from the different samples was between the major axis values for Sigma and Spermfuge samples at room temperature.

In Table 7 we can see that the average values for head area and major axis were smaller in the heated samples (Spermfuge 34ºC and Sigma 34ºC). However, none of these values differed significantly between the samples, except for the major axis values from Sigma RT and Spermfuge RT.

Table 8 Interquartile ranges for average morphometric parameters

	Major axis (µm) Length	*Minor axis (µm) Width*	*Area (µm²)*	*Perimeter (µm)*	*Acrosome % of head area*
Spermfuge RT	0.30	0.33	1.28	0.95	7.18
Spermfuge 34ºC	0.48	0.55	1.68	1.08	23.33
Sigma RT	0.60	0.43	2.68	1.30	10.95
Sigma 34ºC	1.23	1.03	5.00	2.28	23.50

Aziz et al. (1998) conducted a prospective blind clinical trial on the distribution of head size of live sperm in semen and sperm preparations as a predictor of fertility. The participants in the study (couples undergoing IVF treatment) were divided into two groups (fertilizers and non-fertilizers) according to the amount of oocytes fertilized per cycle. They found significant differences in interquartile ranges of head area and major axis between the fertilizing and non-fertilizing groups (fertilizers having smaller values).

When we compare the interquartile ranges from the different samples in Table 9, we see that the smallest interquartile ranges are found in the samples prepared in the Spermfuge

centrifuge (both RT and 34ºC). Also, the interquartile ranges for sperm head area were again smaller for the Spermfuge samples (both RT and 34ºC). When using the Student's t-test ($p \leq 0.05$), no significant differences were found between either the interquartile ranges from the samples at 34ºC and RT, or the Spermfuge and Sigma samples respectively.

Discussion

The aim of this study was to evaluate the effect of temperature during sperm preparation centrifugation on retrieved motile sperm and chromatin packaging quality. Motion characteristics were measured before and after swim-up and these values were compared. In Table 2 we see no effect of temperature on the concentration of sperm retrieved after centrifugation. This could be expected since only normozoospermic donors were used in this study, with an average concentration and motility before swim-up of 110×10^6/ml and 54% respectively. Since swim-up yields a fraction that consists of the sperm with best motility in a given sample, we do not expect the swim-up procedure to increase the concentration dramatically in samples with good baseline motility and concentration. One could argue that when you have good motility and low concentration at baseline, swim-up would drastically increase the yield of spermatozoa, making it a better sample with which to potentially illustrate the effect of temperature during centrifugation.

We also found significant temperature effects on the yield of motile sperm after centrifugation from both the centrifuges. This is the parameter we would expect to increase to a greater extent than concentration in normozoospermic samples. The heated samples showed significantly greater percentage motility than the samples kept at RT. Also, the heated samples from the Spermfuge centrifuge showed significant increase in percentage motility from baseline. In normoozoospermic samples, where the effect of swim-up on motility and concentration is not often marked, significant differences were seen at 34ºC as apposed to RT. Also, heated samples (with or without heating during centrifugation) show greater yield in motile spermatozoa than those kept at RT. We could

infer that keeping samples at 34ºC during the entire swim-up procedure (including centrifugation) could significantly increase the yield in motile sperm.

When we look more closely at the distribution of static to rapid cells (Table 3), we see that the greatest decrease in percentage static cells from baseline were seen in the heated samples, with the Spermfuge 34ºC values significantly lower than baseline. This is an important finding since it testifies to the quality of the sperm retrieved – the heated samples therefore not only yielded significantly higher percentage motile sperm, but also less static sperm. This is what we would expect from a swim-up since the purpose is to retrieve a motile complement from the given sample. All the samples showed a significant increase in percentage cells with medium movement, the heated samples generating nearly a threefold increase from baseline. Temperature gradients i.e. 34ºC and RT therefore did not play a role in the percentage medium moving cells, since values in both gradients were significantly increased.

The rapid cells however, decreased significantly from baseline in 3 of the 4 samples. The reason for this could be that a large part of the rapid moving cells in the fresh samples were hyperactivated and that, after swim-up, these cells' energy sources were depleted beyond which could be restored by the supplemented medium. This however is not a predictor of fertilization rates in vitro (Hirano et al., 2001), and therefore inconsequential.

The tendency observed in the heated samples to significantly increase the yield of medium moving cells might either be due to the fact that the movement of the slow moving cells are enhanced due to the supplemented medium, increase in temperature or both; or that the rapid moving cells become medium moving cells as hyperactivation wears off, or energy resources become depleted; or both. The fact that there was a significant increase in percentage slow moving cells from the Sigma RT sample is irrelevant because the medium and static cells also increased significantly in this sample.

Although significant differences between the kinematic characteristics (VAP, VSL, VCL, ALH, BCF, STR, LIN) have been recorded by, Keppler et al. (1999) and Marín-Briggiler

et al. (2002) at 40ºC and 37ºC respectively, no significant differences were found between the heated and room temperature samples after swim-up. The heated samples did however produce consistently higher results than those at room temperature, for all the parameters. The fact that these differences were not significant could be due to a host of factors; the most apparent being the sperm separation and centrifugation techniques as well as supplementation of media used. When we look at the difference between pre and post swim-up values for these parameters, the STR values for the heated samples are significantly higher than baseline. This, according to Hirano et al. (2001) is a good predictor of fertilization rates in vitro.

Since a relationship between morphology and motility has been established (Katz et al., 1982) we would have expected to find significantly higher percentage normal morphology in the heated samples (which showed significantly higher motility) than the samples at room temperature. Although the differences in average percentage normal morphology between the samples were not significant, the averages for the heated samples were higher than those of the samples at room temperature. Results from the CMA3 staining showed no significant differences or trends which is expected since chromatin packaging is closely related to morphology. However, these findings are in agreement with Hammadeh et al. (2000) who found no relationship between the two.

Had a baseline morphology before swim-up been recorded, a better picture could be formed as to the effect of temperature during swim-up. This might be a subject for future study, since the other parameters relating to morphology were influenced significantly.

Among the head morphometric results in Table 8, we see that the interquartile ranges from the Spermfuge centrifuge for these parameters were smaller than that of the Sigma centrifuge for all the parameters except %Acrosome. Although the smaller sperm head area and major axis interquartile ranges in the Spermfuge samples could be predictors of fertilizing ability (Aziz et al., 1998), the fact the no significant differences were found between the different centrifuges or temperatures, complicates the interpretation. If there were significant differences between the centrifuges for these parameters, it would negate

any differences in temperature for the combined samples, and vice versa. Therefore, it could be deduced that the results recorded confirm that there is no relationship between centrifugation temperature and morphometric parameters.

One of the shortcomings of this study is that it only included normozoospermic samples, making the trends very slight and hard to interpret. However, these trends (such as increased motile yield, % normal morphology, and motion characteristics in heated samples), should be indicative of larger tendencies in the greater population of samples processed for ART. Furthermore, not all the parameters evaluated were measured at baseline (morphology and CMA3), making the supposition of trends indiscriminate.

It would seem that temperature significantly increases the yield of motile sperm and may have a positive effect on the improvement of prepared sperm parameters for use in ART. As such, these findings demand further investigation and research.

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