Temperature Controlled Centrifugation: -
The Effect on Motion Characteristics of Human Sperm

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Introduction

Sperm retrieval techniques form an integral part of the assisted reproductive programme. The success of sperm separation is measured by the number of motile sperm retrieved from a given semen sample. The influence of various technical procedures and diluent media upon human spermatozoa has been tested in vitro (Jeulin et al., 1982). The percentage and velocity of motile spermatozoa are often measured objectively using laser Doppler velocimetry or Hamilton Thorne IVOS 10 computerized sperm analyzer (CASA). When spermatozoa are incubated at 37 degrees C, rapid declines in both percentage motile and in velocity were observed with incubation periods lasting more than 4-h (Jeulin et al., 1982). Human sperm incubation at room temperature does not allow capacitation, although it does not affect hFF-induced acrosome reaction in capacitated cells. The blocking effect is overcome when spermatozoa are exposed to 37°C (Maryn-Briggiler et al., 2002). Keppler et al., (2002) found significantly higher percent motility, mean average path velocity, straight line velocity, lateral head displacement, and percent hyperactivation in sperm at the 40ºC temperature (Keppler et al., 1999)

Objectives

To evaluate the effect of temperature on the sperm motion characteristics and subsequent outcome of sperm preparation.

Material & Methods

Semen samples and sperm preparation

Semen samples were obtained from normozoospermic donors and andrology referrals. Directly after collection samples were divided into 2 aliquots. One aliquot were placed in a CO_2 incubator at 34^0C while the second aliquot were left at room temperature. A period of 30 minutes was allowed to stabilize the semen temperatures before experimental procedure. Prior to the experimental onset the motion characteristics were recorded with the CASA instrument in order to obtain initial values for the measured motion characteristics.

Swim up separation

Motile sperm fractions were retrieved from the semen samples by mixing 0.5mL semen with 1mL of Quinn's Sperm Preparation medium. Two tubes were prepared for each experiment. The tubes were then placed in 2 different centrifuges, namely (i) SpermFuge SF 800 (Shivani Industries, Mumbai, India), a highly precise centrifuge with a temperature controlled chamber at 34^0C 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 culture medium and left at a 45^0 angle at 34^0C for 60 minutes. After the incubation period 0.5mL supernatant were removed from each tube and immediately analyzed for sperm motion characteristics.

Motion characteristics

Aliquots of 5μL of spermatozoa (10x106 cells/mL) from the sperm at 34ºC and 22ºC aliquots were placed in the micro chamber for analyses with the HTM-IVOS V10.9 CASA instrument (Hamilton-Thorne Research Inc., Beverley, MA, USA). The following 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); and linearity (LIN). Motion characteristics were recorded in all samples using 10 randomly selected microscopic fields. The proportion of hyperactivated spermatozoa in each experiment was determined using the SORT function of the CASA instrument. To be classified as hyperactivated, a trajectory had to meet all of the 60 Hz SORT criteria, *i.e.*, VCL≥150μm/s, LIN≤50% and ALH≥7μm (Mortimer *et al.*, 1990).

Results

Table 1 Results of semen parameters recorded in ejaculates and after preparation with SpermFuge temperature controlled vs. room temperature centrifugation

	Ejaculates				Post Swim up	
					34^0C Spermfuge	Room temperature
	Volume mL	Morphology % normal	Motile % live	Sperm Concentration Mill/mL	Sperm concentration Mill/mL	Sperm Concentration Mill/mL
Mean	3.20	10.80	58.00	80.60	12.10 a	7.30 b
SD	1.15	3.04	24.40	53.14	6.63	2.38

a vs.b Fisher's exact test p = <0.05

Significant difference obtained in post swim up sperm concentrations

Table 2 Percentage Hyperactivated sperm recorded after preparation with SpermFuge vs. Room temperature prepared

% Hyperactivated sperm			
BASELINE		SWIMUP	
34^OC	**20^OC**	**34^OC**	**20^OC**
0.8%	0.8%	1.73% a	0.35% b

Transformed ARCSIN values showed a vs.b Fisher's exact test p=<0.05

Significant difference obtained in percentage hyperactivated sperm

Table 3 Comparison of the velocity distribution of sperm cells(%) prepared by a SpermFuge temperature controlled (34°C) centrifuge vs. room temperature swim up spermatozoa

	SPERM VELOCITIES DISTRIBUTED IN 5 MOTION CATEGORIES														
	% RAPID			% MEDIUM			% SLOW			% STATIC			% HA		
	Baseline	34°C	20°C	Baseline	34°C	20°C	Baseline	34°C	20°C	Baseline	34°C	20°C	Baseline	34°C	20°C
Mean	4.2	4.5	4.8	3.2	3.5	3.6	4.0	3.6	3.3	4.0	3.6	3.9	0.4a	1.2b	0.3c
SD	0.7	0.7	1.3	0.5	0.7	0.6	0.5	0.6	1.0	0.7	0.4	0.7	0.8	1.2	0.8

Transformed ARCSIN values showed a vs.b, a vs c and b vs. c Fisher's exact test p=<0.05

Significant difference obtained in percentage hyperactivated sperm

References

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