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Effects of sauna on sperm movement characteristics of normal men measured by computer-assisted sperm analysis

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Summary

The effects of sauna exposure on sperm movement characteristics and other semen parameters were evaluated using computer-assisted sperm analysis (CASA). A significant ($p < 0.01$) decrease in average path velocity (VAP), curvilinear velocity (VCL) and amplitude of lateral head displacement (ALH) was found after exposure to sauna for 2 weeks. The altered parameters returned to their original values within 1 week after cessation of sauna exposure. Mean values for semen volume, sperm count, percentage motility, sperm morphology and sperm penetration assay (SPA) were not statistically different during and after sauna, when compared to the corresponding control values. The results suggest that increasing scrotal temperature by sauna causes a reversible decrease in sperm movement parameters.

Keywords: CASA, sauna, semen quality, SPA, sperm movement characteristics

Introduction

In numerous mammal species, including man, testicular temperature is lower than core body temperature. In man, scrotal temperature is 2–3 °C lower than rectal temperature and the optimum temperature for human spermatogenesis is considered to be 35 °C. The deleterious effect of heat on spermatogenic function of the testes has been studied by several groups of investigators. The elevation of intrascrotal temperature resulting from varicocele has been suggested as one of the causes for the deterioration of spermatogenesis in such patients (Zorgniotti & McLeod, 1973). It has been found that the higher the scrotal temperature in infertile men, the more altered are their sperm characteristics (Mieusset *et al.*, 1987c; reviewed in Mieusset & Bujan, 1995). The deleterious effect of heat on spermatogenesis has also been shown experimentally in men with normal spermatogenesis.

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Increasing testicular or scrotal temperature induces a decrease in sperm count, percentage motility and normal sperm morphology (Robinson & Rock, 1967; Mieusset *et al.*, 1985, 1987a, 1987b; reviewed in Mieusset & Bujan, 1995). Local testicular hyperthermia was induced by raising the testicles into the inguinal canal during the day in normal men. This resulted in a decrease in sperm count and motility (Mieusset *et al.*, 1987b). A daily mild increase (1–2 °C) in testicular temperature during waking hours can be used as a potential contraceptive method for men (Mieusset & Bujan, 1994; Mieusset & Bujan, 1995). Much interest has been focused on the effect of environmental heat from occupation and living habits on semen quality. Laven *et al.* (1988) reported that the proportion of good motile spermatozoa was significantly lower in 'warm' workers (seated for an average working day of 6 h or more) and 'warm' sleepers (sleeping with close-fitting underwear or using electric blankets or down-filled quilts) than in men who were exposed to a colder environment. A reversible decrease in semen quality was

also found in welders who were exposed to radiant heat with an average 1.4 °C increase in groin skin temperature (Bonde, 1992). Increasing testicular and body temperatures by means of excessive sauna use (Procope, 1965), or single sauna use (Brown-Woodman *et al.*, 1984), has been shown to disturb spermatogenesis in normal men. In contrast, no correlation was found between sperm quality and sauna exposure in men who responded to a questionnaire in a retrospective study (Oldereid *et al.*, 1992). Although saunas are popular at present, only a very limited number of studies have focused on the effects of sauna on epididymal spermatozoa, including sperm movement characteristics and fertilizing ability. Therefore, the objectives of the present study were (1) to determine the effect of sauna exposure for 14 days on epididymal sperm movement characteristics using computer-assisted sperm analysis (CASA), and (2) to determine the fertilizing ability of spermatozoa using the zona-free hamster oocyts penetration assay (SPA).

Materials and methods

Subjects

Eight healthy researchers from Mahidol University were included in this study. The mean age of the subjects was 30 years with a range of 22 to 53 years. The volunteers qualified for entry into the study by having normal semen analysis according to the criteria set by the World Health Organization (WHO, 1992). All subjects had been working in air-conditioned rooms and never used athletic supporters.

Sauna and semen samples

Subjects were exposed to sauna at a temperature of 80–90 °C in the same sauna room for 30 min per day for 2 weeks. The subjects wore loose-fitting underwear during the sauna and felt comfortable during exposure. Prior to sauna exposure, semen was collected and analysed twice, at an interval of 1 week, and the mean value served as the control reference value. After 1 and 2 weeks of sauna exposure, semen samples were collected by masturbation immediately after sauna. When sauna use had been discontinued for 1 week, semen was again collected.

Scrotal temperature, pulse rate and blood pressure measurement

Scrotal temperature was recorded as previously described (Zorgniotti & McLeod, 1973). The bulb of a digital thermometer was placed in contact with the scrotal skin between the two testes and the testes pushed together until the temperature was stable. As shown in Fig. 1, measurements was taken on subjects before, during and within 30 min after sauna exposure in a sitting position. Pulse rate and blood pressure were also recorded (arrows) at the same time as temperature measurement, as shown in Fig. 1.

Semen analysis, sperm concentration and movement characteristics

Fresh semen samples were obtained by masturbation into sterile containers after a period of sexual abstinence of at least 72 h and allowed to liquefy for 30 min at room temperature. After liquefaction, an aliquot was removed for measurement of sperm concentration and analysis of movement characteristics and morphology, and the remainder was used for SPA. One aliquot was diluted to $\approx 25 \times 10^6$ spermatozoa/mL with Tyrode's medium containing albumin, lactate and pyruvate (TALP) before analysis by CASA (Farrell *et al.*, 1996). A 10-μL diluted sample was placed in a 20-μm deep Cell-VU chamber and a coverslip was pressed down firmly to ensure uniformity of the sample. Two 20-μm Cell-VU chambers were loaded and five different fields per chamber were randomly examined to check that the number of spermatozoa was > 200 in every field of the chamber. The sperm concentration, percentage of motile spermatozoa and movement characteristics were analysed using a Hamilton Thorne integrated visual optical system (IVOS), version 10, operated at 37 °C. Sperm velocity and movement characteristics were calculated only for motile cells as mean values, and included: average path velocity (VAP), curvilinear velocity or track speed (VCL), straight line velocity or progressive velocity (VSL), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), straightness (STR), and linearity (LIN). The settings for the IVOS are shown in Table 1.

Sperm morphology

To evaluate sperm morphology, semen samples were centrifuged and smeared on a grease-free slide. Smeared semen was stained by the Diff Quick technique. After air-drying, at least 200 spermatozoa per sample were analysed and classified as previously described (Kruger *et al.*, 1995). For a cell to be classified as normal, all three parameters (size, shape and acrosome) must pass as normal. If both the size and acrosome were normal, but the shape was slightly amorphous, then the cell was classified in the subnormal category. In any other case, the cell was classified as abnormal.

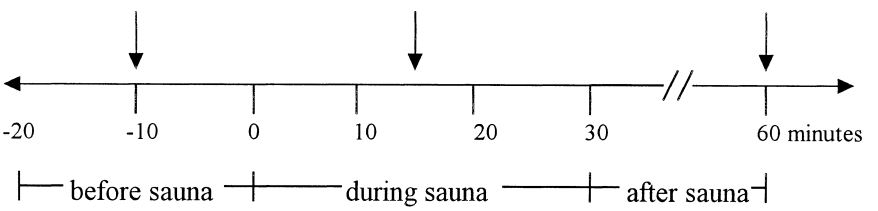

Figure 1. Measurement of temperature, pulse rate and blood pressure (arrows) before, during and after sauna exposure.

Table 1. Parameter settings used with the Hamilton Thorne IVOS Version 10 for human semen analysis

Parameter	Setting
Frames acquired	30
Frame rate	60 Hz
Minimum contrast	80
Minimum cell size	3 pixels
Minimum static contrast	30
Straightness (STR) threshold	80.0%
Low VAP cut-off	5.0 μm/sec
Medium VAP cut-off	25.0 μm/sec
Low VSL cut-off	11.0 μm/sec
Head size, non-motile	6 pixels
Head intensity, non-motile	160
Static head size	1.00–2.90
Static head intensity	0.60–1.40
Static elongation	0–80
Slow cells motile	NO
Temperature, set	37.0 °C

Sperm penetration assay (SPA)

Semen samples were poured into sterile 15-mL conical centrifuge tubes and diluted to 5 times their original volume with TALP-HEPES. The samples were washed and centrifuged at 1600 r.p.m. for 10 min. The resultant pellet was resuspended to the same volume in TALP-HEPES and recentrifuged as described above. After the second centrifugation, the final pellet was resuspended in 1 mL TALP and the sperm concentration adjusted to 1×10^6/mL. A 2–3 μL aliquot of the sperm sample was then placed in a 50-μL drop of TALP medium under mineral oil in a Petri dish. Spermatozoa were then incubated with 5% CO_2 in air at 37 °C for 3 h.

Female Golden Hamsters (6–12 weeks of age) were superovulated by an intraperitoneal injection of 30 IU pregnant mare serum gonadotrophin (PMSG) on the morning of day 1 of the oestrus cycle followed by an injection of 30 IU human chorionic gonadotrophin (hCG) 56 h later. The animals were killed 15–17 h after hCG injection. The cumulus oocyte complexes were recovered and placed into 0.1% hyaluronidase for 5 min to remove the cumulus cells. The oocytes were washed repeatedly in TALP before their zonae were removed by exposure to 0.1% trypsin for 30 sec. Zona-free oocytes were washed in TALP and 12–15 oocytes were transferred into each sperm drop and co-incubated in 5% CO_2 in air at 37 °C for 5 h. The oocytes were then washed and fixed in glutaraldehyde and stained with 1% aceto-orcein.

The stained oocytes were examined using phase-contrast microscopy at ×400 magnification to evaluate sperm penetration by observing sperm head decondensation or male pronucleus formation within the ooplasm. The percentage of oocytes penetrated was taken as the penetration rate and at least 50 oocytes were assessed per sample.

Statistical analysis

To determine the effects of sauna exposure on semen quality, the results were compared before, during and after sauna. Semen characteristics and sperm movement parameters, including SPA, were analysed using paired *t*-tests. Data are shown as means ± SEM.

Results

Scrotal temperature and vital signs

The mean values of scrotal and rectal temperatures are shown in Table 2. A significant increase in scrotal temperature ($p < 0.005$) was found during and after sauna exposure. The average increase in scrotal temperature was 2.4 °C during sauna exposure and 1.7 °C after sauna. The change in rectal temperature was similarly significant. During and after sauna the rectal temperature increased by an average of 1.1 and 0.8 °C, respectively. The means for pulse rate were significantly higher ($p < 0.005$) in all subjects during and after sauna when compared to before sauna. Pulses increased rapidly while in the sauna and slightly decreased outside the sauna, returning to baseline levels within 30 min. Blood pressure did not change significantly throughout the study period.

Semen characteristics

The results of CASA are shown in Table 3. Mean values for semen volume, sperm concentration and total sperm

Table 2. Scrotal and rectal temperature and vital signs of normal men during treatment (means ± SEM)

Parameters	Before sauna	During sauna	After sauna
Scrotal temperature (°C)	35.2 + 0.2	37.6 + 0.2*	36.9 + 0.8*
Rectal temperature (°C)	37.1 + 0.3	38.2 + 0.2*	37.9 + 0.2*
Pulses (per min)	78.5 + 3.3	103.6 + 5.6*	102.8 + 2.8*
Blood pressure (mmHg)	118 ± 0.1/82 ± 0.2	120 ± 0.1/83 ± 0.3	115 ± 0.3/80 ± 0.2

*$p < 0.005$, compared with before sauna.

Table 3. Semen characteristics of subjects before, during and after sauna exposure (means ± SEM)

Parameters	Before sauna	During sauna		After sauna
		week 1	week 2	week 1
Volume (mL)	2.9 + 1.1	2.4 + 0.7	2.9 + 1.1	3.6 + 1.5
Sperm concentration (10^6/mL)	70.8 + 20.8	55.7 + 56.6	92.8 + 36.2	73.2 + 31.1
Total sperm count (10^6)	199.8 + 86.7	106.2 + 71.0	284.9 + 164.7	297.1 + 195.2
Motility (%)	64.5 + 6.6	66.0 + 3.3	62.5 + 6.1	60.3 + 6.5
Progressive motility(%)	41.0 + 13.4	46.2 + 5.3	44.0 + 7.2	39.0 + 13.0
Normal morphology (%)	41.0 ± 12.0	37.8 + 11.8	36.5 ± 12.8	40.2 ± 7.4

count were not statistically different throughout the period of sauna and 1 week after cessation, although there was a decrease in these parameters in the first week of sauna exposure. The percentage sperm motility and percentage progressively motile spermatozoa were also not statistically different. The proportion of spermatozoa with normal form exhibited non-significant changes during the treatment period as well as post-treatment

Sperm movement characteristics and SPA

The movement characteristics of spermatozoa were studied and calculated by CASA as shown in Table 4. No significant difference was observed in any sperm movement parameter after sauna exposure for 1 week compared with values obtained before sauna, whereas analysis of sperm movement after 2 weeks of sauna showed a significant decrease ($p < 0.01$) in VAP, VCL and ALH; however, these parameters returned to baseline levels after cessation of the treatment. The mean percentage of penetration did not differ significantly during and after sauna compared with values obtained before sauna. The means ± SD for percentage penetration before, during (at 1 and 2 weeks) and after sauna cessation were 73.3 ± 4.6%, 70.7 ± 1.2%, 72.9 ± 0.8% and 71.4 ± 1.6%, respectively.

Discussion

The present study demonstrates that increasing scrotal temperature by sauna exposure reversibly affects sperm movement parameters including VAP, VCL and ALH. A significant change in sperm velocity has been reported in workers exposed to high temperatures (37 °C, 8 h/day) in the ceramics industry although sperm concentration, morphology and motility remained unchanged (Figa-Talamanca *et al.*, 1992). Procope (1965) also observed a transient but reversible decrease in sperm count in men exposed to hot sauna for 2 h and 24 min over a 2-week period. Sperm count consistently decreased and exhibited a maximum decline of 50% between 30 and 39 days after cessation of treatment. Sperm concentration and progressive motility were found to decrease significantly between a period of wearing tight-fitting underwear and a period of wearing loose-fitting underwear (Tiemessen *et al.*, 1996). However, an increase in scrotal temperature (0.8–1 °C) induced by polyester-lined athletic supporters was insufficient to cause suppression of spermatogenesis or alter sperm function (Wang *et al.*, 1997).

The changes in the pattern of sperm movement observed in our study may be due to alteration of sperm motility during the residence of these spermatozoa in the

Table 4. Sperm movement parameters and SPA of subjects before, during and after sauna exposure (means ± SEM)

Parameters	Before sauna	During sauna		After sauna
		week 1	week 2	week 1
VAP	72.4 + 8.2	66.9 + 2.8	64.2 + 6.1*	66.2 + 8.4
VSL	59.7 + 11.6	57.2 + 5.0	56.0 + 8.2	55.9 + 11.3
VCL	124.5 + 10.3	115.5 + 11.1	103.5 + 8.9*	118.5 + 1.0
ALH	4.9 + 0.4	4.8 + 0.6	4.1 + 0.3*	4.6 + 0.7
BCF	29.2 + 2.8	28.7 + 2.6	30.1 + 2.8	28.9 + 5.5
STR	82.5 + 6.6	85.0 + 3.2	8.6 + 4.8	83.0 + 6.0
LIN	51.8 + 8.4	52.8 + 6.6	56.7 + 7.4	52.3 + 10.0
SPA	73.3 ± 4.6	70.7 ± 1.2	72.9 ± 0.8	71.4 ± 1.6

*$p < 0.01$ compared with before sauna.

cauda epididymis, which is known to be thermo-dependent (Foldesy & Bedford, 1982). Temperature may affect the pattern of sperm motility by altering enzyme activity and/or calcium movement. At a few degrees above body temperature, the rate of enzyme activity decreases because heat denatures enzyme structure, and the rate of denaturation increases with increasing temperature (Rawn, 1989). Nakamura & Hall (1978) reported decreased protein synthesis by spermatids exposed to increased temperature and suggested that this was a result of disaggregation of polysomes. It has been reported that temperature change could induce modification of sperm plasma membrane properties (Holt & North, 1986) which affected cellular membrane permeability and membrane-bound enzyme activity (Tocanne *et al.*, 1989). The role of calcium and calcium channels in controlling the transition from symmetric to asymmetric flagella waveforms has also been described (Si, 1997). Moreover, a role for calmodulin (CaM), which acts as an intracellular regulator of Ca^{2+} function, has been reported. Calmodulin has been identified in both the head and flagellar parts of spermatozoa (Feinberg *et al.*, 1981; Tash & Means, 1983) and is believed to be a major signal transducer in Ca^{2+}-regulating motility. The CaM antagonist Sk1 (synthesis peptide inhibitor for CaM) caused a reduction in VCL and ALH (Ahmad *et al.*, 1996). These results suggest a regulatory role for the Ca^{2+}/CaM pathway in human sperm movement parameters, including VCL and ALH.

Although sperm movement parameters decreased following sauna exposure in the present study, fertility potential, assessed by SPA, did not change. VCL and ALH have been reported to be significantly higher for high penetrators (>20% penetration) than for low penetrators (< 20% penetration) (Fetterolf & Roger, 1990). Also, CASA measurement of fresh human ejaculate has demonstrated a positive correlation between conception outcome and VCL and ALH parameters (Marshburn *et al.*, 1992). Furthermore, there was a 40% chance of conception with a VAP of 10 μm/sec and a 90% chance of conception with 60 μm/sec (Barratt *et al.*, 1993). Nevertheless, we were unable to show that a decrease in VCL, VAP or ALH caused by sauna exposure affected sperm fertilizing ability, because hamster oocytes, which were used in this study, possess no zonae pellucidae. Assessment of fertilizing ability using human oocytes should be undertaken in future studies.

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