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·*Original Article*·

Ram sperm motility after intermittent scrotal insulation evaluated by manual and computer-assisted methods

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Abstract

Aim: To study whether additional measurements of motility characteristics of spermatozoa by computer assisted semen analysis (CASA) were more sensitive indicators of reduced semen quality than estimates of percentages of motile, rapid or progressive cells. **Methods:** Intermittent scrotal insulation was applied to 6 rams for 16 h per day for 21 days or to 2 of these for 12 h per day for 28 days in the following year. Semen was collected and evaluated by CASA immediately and either frozen or stored at 30ºC or 5ºC before re-evaluation. **Results:** Intermittent scrotal insulation caused falls in the percentage of motile, progressive and rapid sperm, as did freezing-thawing and storage at 30ºC or 5ºC. Motility characteristics (amplitude of lateral head displacement, mean path velocity, mean progressive velocity and curvilinear velocity), as determined by CASA fell only when the percentage of motile sperm was already reduced. Freezing and thawing or liquid storage of the semen from insulated rams caused a greater fall in the percentage of motile and rapid sperm than control semen, but only affected the motility characteristics when the percentage of motile sperm was already reduced. **Conclusion:** Intermittent scrotal insulation affected not only the motility of the freshly collected sperm, but also their ability to withstand the additional stress of storage. The additional data on motility characteristics obtained by CASA appeared to be no more a sensitive indicator than the percentage of motile cells of reductions in semen quality. *(Asian J Androl 2006 Jul; 8: 411–418)*

Keywords: testis; scrotal insulation; semen evaluation

1 Introduction

The introduction of computer assisted semen analysis (CASA) has meant that a large amount of information about the motility characteristics of motile spermatozoa can be obtained, in addition to the percentages of motile, progressive and rapid spermatozoa. However, few studies have examined if these additional parameters change when semen quality is reduced. It has been suggested that curvilinear velocity (VCL) of hamster epididymal sperm is reduced in animals treated with α-chlorhydrin, whereas percent motile sperm is not affected [1, 2].

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It has been known since the 1920's that increasing the temperature of the testes, whether by exposing the whole animal or just the testes to heat or by insulating the scrotum, causes a derangement in spermatogenesis, which leads to decreases in sperm numbers, motility and normality [3], and reduces the ability of the sperm to produce normal offspring [4, 5]. However, all previous studies with scrotal insulation, except our earlier studies [6, 7], have applied insulation continuously for at least 48 h, and usually even longer. Significant diurnal variations in scrotal as well as environmental temperatures occur in sheep in the field in southern Australia, with scrotal temperatures above 36ºC occurring only when air temperature was above 40ºC, as happens usually du-ring daylight hours [8]. The situation in humans is quite different, in that scrotal temperature is higher when the person is in bed asleep, but there is still a large diurnal variation [9]. It has also been suggested that it is a lack of cool periods, rather than periods with high scrotal temperatures that causes disruption of spermatogenesis [9, 10]. Our earlier studies used intermittent insulation for either 8 h/day or 16 h/day for prolonged periods (160 or 144 days, respectively) or ceased observations on the sperm after 21 days [6]. The longer periods of treatment produced drastic effects on sperm motility but also some decrease in sperm numbers. Therefore, we decided to re-examine the long-term effects of shorter periods of intermittent scrotal insulation to see if changes in motility could be induced without falls in sperm numbers. By reducing the duration of the scrotal insulation, it was also hoped that a threshold effect would be produced, which might also reveal if there was much variation in the response of the testes of individual rams to heat stress. As additional ways of reducing sperm motility, we have used freezing or liquid storage of the semen, as both these procedures have been reported to cause changes in the motility characteristics of ram sperm [11, 12].

Other studies using continuous insulation recorded changes after scrotal insulation in motility assessed subjectively or morphology, or studied chromatin structural changes or clusterin-positive cells [3, 13–15], but none appears to have used CASA. Therefore, we investigated whether there were changes in the motility characteristics of the surviving sperm before there was a reduction in the percentage of motile cells.

2 Materials and methods

All experiments were conducted in the Department of Animal Sciences, Waite Agricultural Research Institute, University of Adelaide, Adelaide, South Australia.

2.1 Experiment 1A

Eight adult Merino rams each weighing approximately 70 kg were kept in individual pens in a room, with temperature controlled to between 19ºC and 23ºC and artificial light for 16 h per day (05:00–21:00). In January 1990, 4 rams were subjected to scrotal insulation for 16 h per day for 21 days, beginning 4 h before the end of the light period, by the application of a bag made of one layer of aluminium foil between two layers of cotton cloth, with a covering of waterproof cotton cloth, held with tapes across the back of the animal, and 4 left as controls. Semen was collected twice weekly at 3- or 4-day intervals by artificial vagina, and sperm numbers were counted with a haemocytometer and motility characteristics assessed subjectively on all samples and with a Hamilton-Thorne Motility Analyser (HTM 1030, Version 6.03-7.2Q, Daintree Industries, Victoria, Australia) with 20 frames analyzed at 25 frames/s, using an 8-μL sample of diluted semen (1:4 with Tris-glucose-egg yolk-glycerol medium [16] and then 1:50 with phosphate-buffered saline [PBS]) placed in a10-μL Makler chamber. At least 600 sperm were assessed in three to five fields for each sample on day 49 before, and days 4, 15 and 21 after the start of insulation. Cells classed as motile (MOT) were those moving with a mean path velocity (VAP) of more than 10 μm/s and rapid (RAP) sperm were those moving with a VAP of more than 95 μm/s. Mean progressive velocity (VSL) and amplitude of lateral head displacement (ALH) were also recorded. These diluted semen samples (1:4 using a Tris-glucose-egg yolk glycerol medium) were frozen as pellets [16] and subsequently thawed for use in insemination trials, the results of which have already been reported [6], after evaluation of the motility characteristics of the sperm determined as the average from at least three pellets from each sample with three replicates per pellet after thawing in individual tubes in a water bath at 37ºC and diluting 1: 50 with PBS.

2.2 Experiment 1B

Single semen samples were collected by electroejaculation from 65 Merino rams, approximately 16 months old. The semen was evaluated fresh and following freezing and thawing, as described above, with the additional assessments of mean track speed or curvilinear velocity

(VCL) and percent progressive (PROG) sperm, defined as those moving with a mean path velocity (VAP) greater than 95 μm/s, and with straightness (STR, i.e. VSL/VAP) greater than 0.60.

2.3 Experiment 2

This experiment was conducted in two parts (A and B) in successive breeding seasons. Four rams similar to those in Experiment 1A were used under the same experimental conditions. Experiment 2A was conducted in April 1992; two rams were subjected to scrotal insulation using the same bags as in Experiment 1A for 16 h per day from 17:00 to 09:00 for 21 consecutive days, and the other two left as controls. Scrotal surface temperature was recorded using thermistor probes held on the skin with small adhesive patches. Experiment 2B was conducted in April the following year; the same two rams were subjected to scrotal insulation for 12 h per day from 09:00 to 21:00 for 28 consecutive days, so that the total time of scrotal insulation (336 h) was the same for the two parts of the experiment. The same two rams used in Experiment 2A as controls were also used as controls in Experiment 2B. Semen was collected from each ram by artificial vagina at weekly intervals for 114 days in Experiment 2A and 105 days in Experiment 2B and sperm concentrations determined with a haemocytometer following dilution of 1:500 with PBS containing 0.16% formaldehyde. Semen samples were diluted (1:4) at 30ºC with a Tris-based diluent containing hen egg yolk, citrate and glucose [16]. Motility characteristics were evaluated with the HTM after a further dilution of 1:50 with PBS and the percentages of live and morphologically normal sperm were determined on smears on at least 600 sperm per sample, after staining with nigrosin-eosin, immediately after dilution. In Experiment 2A, two samples from each collection were kept at 30ºC and two cooled to 5ºC over 2 h. The samples were then stored for a further 6 h at the respective temperatures, and the motility characteristics and percentages of live and normal sperm evaluated again at the end of that time, following rewarming 100 μL of the 5ºC samples for 5 min in a water bath at 30ºC then diluting 1:50 with PBS for CASA assessment as described above. In Experiment 2B, duplicates of each sample were cooled to 5ºC, stored at that temperature and reassessed after 96h and 168 h. At least three replicates of each sample were evaluated using three to five fields for each sample on each occasion.

2.4 Statistical analysis

The values for percentages of MOT, PROG and RAP sperm and motility characteristics were subjected to angular or arcsin transformation prior to 3-way analysis of variance (ANOVA) with a split plot design followed by a least square mean test, with treatment (control or insulated), time (days after start of insulation) and semen preparation (fresh or frozen/thawed or fresh or stored) as variables. The changes in motility resulting from freezing/thawing or storage were evaluated by paired *t*-test. The post-storage values were also expressed as percentages of the corresponding fresh value and then subjected to ANOVA and Tukey's test following angular or arcsin transformation of the percentages or subjected to the Mann–Whitney *U*-test.

3 Results

3.1 Experiment 1

3.1.1 Effect of scrotal insulation

Intermittent scrotal insulation for 16 h per day in Experiment 1A was followed by a fall in percentage of MOT sperm (Figure 1A), with a similar fall in the percentage of RAP sperm (data not shown) in frozen-thawed semen collected on day 15 and 21 of intermittent insulation ($P < 0.05$), and a small but not significant fall on day 4. A similar pattern was observed with fresh semen, as already reported [6], which overall had a higher percentage of MOT and RAP sperm than in frozen-thawed semen in controls and in insulated rams both before and during scrotal insulation. The concentration of sperm in the semen was not altered significantly during insulation [6]. Both VAP (Figure 1B) and VSL (data not shown) of sperm from fresh semen were significantly reduced by insulation on days 15 and 21, but not on day 4, when compared with the samples from the control rams on the same days. ALH was slightly but significantly reduced on day 21 of insulation. VAP (Figure 1B), VSL and ALH (data not shown) of frozen-thawed semen from rams subjected to scrotal insulation were not significantly different from the values for the control rams at any time during the experiment.

3.1.2 Effect of freezing and thawing

There were significant falls in the percent MOT, RAP and PROG spermatozoa in response to freezing and thawing in both Experiments 1A and 1B, and these falls were much greater in Experiment 1B than that in Experiment

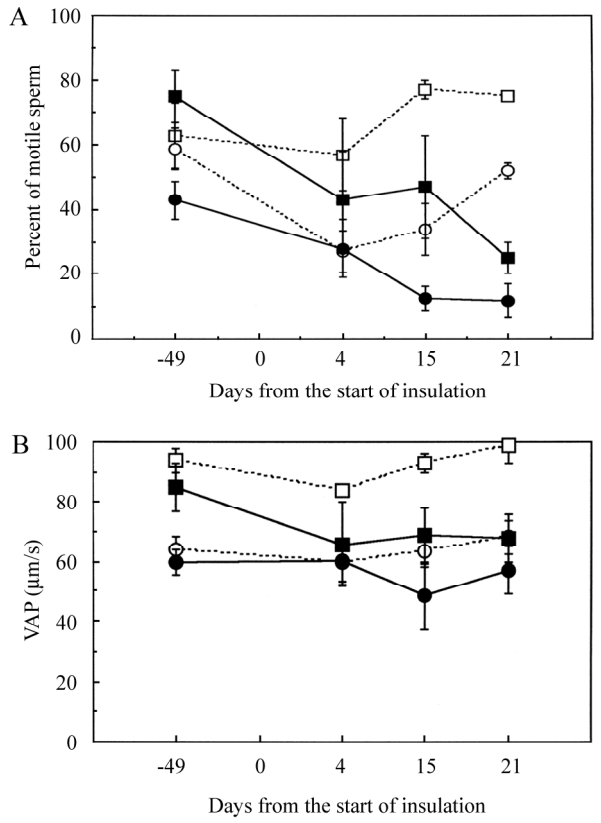

Figure 1. (A) Percent motile (MOT) sperm and (B) sperm average path velocity (VAP) in fresh semen (squares) and frozen/thawed semen (circles) from four control rams (open symbols) and from four rams subjected to scrotal insulation (solid symbols) for 16 h per day from day 0 to day 21.

1A (Table 1). The extent of the effect of freezing and thawing on the percentages of MOT and RAP sperm and the motility characteristics was determined by calculating the value after freezing and thawing as a fraction of the values when the semen was fresh. Although there were no significant differences for the individual days, the pooled data for MOT sperm from the insulated rams (Ins) on days 4, 15 and 21 showed a greater fall when compared with the pooled data for the control rams (C) and the pre-insulation data for the insulated animals (to [66.0 ± 6.7]% for C, to [45.0 ± 9.0]% for Ins, $P < 0.05$ by Mann–Whitney U-test). The percentage of RAP sperm also fell following freezing and thawing, but the fall was similar for semen from the control and insulated animals (to [47 ± 6]% for C, to [48 ± 14]% for Ins). VSL, VAP and ALH were also reduced by freezing and thawing, to a similar extent in the control rams in Experiments 1A and in Experiment 1B (Table 1). Surprisingly, the reduction as a result of freezing and thawing was less for semen collected during insulation than in the controls (VSL fell to [74.0 ± 3.7]% for C, to [86.0 ± 7.5]% for Ins, $P < 0.05$; VAP fell to [70.0 ± 3.0]% for C, to [82.0 ± 7.2]% for Ins, $P < 0.01$ by Mann-Whitney U-test). The fall in ALH was not significantly different between the insulated and control groups (to [72 ± 2.4]% for C, to [78 ± 3.4]% for Ins).

3.2 Experiment 2A

3.2.1 Effect of scrotal insulation

Scrotal surface temperature rose from 30.2 ± 1.1ºC

Table 1. The effect of freezing and thawing of semen from control rams on percent motile (MOT), progressive (PROG), rapid (RAP), live and morphologically normal (NORM) spermatozoa and the characteristics of the motility of the motile sperm, mean amplitude of lateral head displacement (ALH), mean path velocity (VAP), mean progressive velocity (VSL) and mean track velocity (VCL) as determined by a Hamilton Thorn Motility Analyzer. Experiment 1A: 22 pairs of samples from eight rams; Experiment 1B: 65 samples from 65 rams. $^{d}P < 0.001$, different from corresponding value for fresh samples.

	Experiment 1A		Experiment 1B	
	Fresh	Frozen-thawed	Fresh	Frozen-thawed
MOT (%)	68.6 ± 3.9	43.0 ± 3.8^{d}	59.0 ± 2.8	17.6 ± 1.7^{d}
PROG (%)			37.0 ± 2.5	10.2 ± 1.2^{d}
RAP (%)	31.4 ± 2.9	13.4 ± 1.6^{d}	39.9 ± 2.7	10.5 ± 1.3^{d}
LIVE (%)	87.4 ± 2.7			
NORM (%)	85.3 ± 2.0			
ALH	6.1 ± 0.2	4.3 ± 0.1^{d}	6.4 ± 0.2	4.2 ± 0.2^{d}
VAP	91.0 ± 2.4	63.0 ± 2.4^{d}	128.0 ± 4.0	105.0 ± 6.0^{d}
VSL	79.0 ± 2.4	58.0 ± 2.3^{d}	115.0 ± 4.1	99.0 ± 6.0^{d}
VCL			152.0 ± 4.1	129.0 ± 7.2^{d}

(six observations made on three occasions on the two rams at weekly intervals) by 3.6 ± 0.5ºC during the first hour after the bags had been applied. During the last hour of insulation, surface scrotal temperature was 34.2 ± 0.3ºC, and fell to pre-insulation levels within 40 min after removing the bags.

Intermittent scrotal insulation for 16 h/day for 21 days again caused a marked fall in the percentage of MOT (Figure 2A) and RAP sperm, and also in PROG sperm (data not shown), so that very low values were reached in both rams by the end of insulation on day 21. However, there was a clear difference between the two rams in the speed at which recovery occurred (Figure 2A). However, in this experiment, in contrast to Experiment 1A, there were falls in sperm concentration in the semen from day 21 after the start of insulation, and again the two rams showed different patterns of recovery (Figure 3A).

The motility characteristics measured (VAP [Figure 2C], ALH, VSL and VCL [data not shown]) also fell during and after the insulation period reaching minimum values at between 30 and 40 days after the beginning of insulation. There were also falls in the percentages of live and morphologically normal sperm up to approximately day 60 from the start of insulation (data not shown).

3.2.2 Effect of storage at 5ºC or 30ºC for 6 h

Storage for 6 h at 5ºC had comparatively little effect in control rams on the percentages of MOT, RAP or PROG sperm, or on VAP or VSL; although there was a slight reduction in ALH and VCL. With storage at 30ºC, the percentage of MOT, PROG and RAP sperm and ALH,

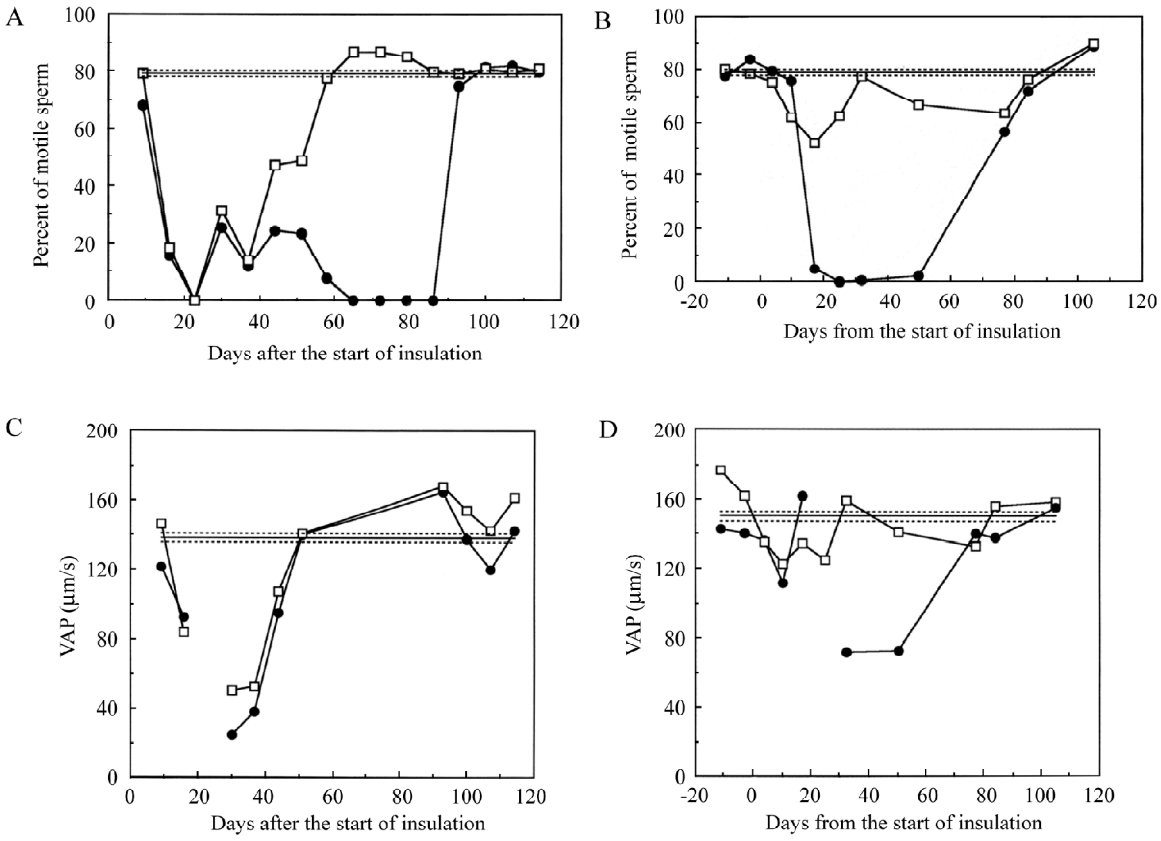

Figure 2. Percent motile (MOT) sperm (A and B) and average path velocity (VAP, C and D) in fresh semen from two rams (Ram No. 12 solid circles, Ram No. 16 open squares) subjected to scrotal insulation for 16 h per day for 21 consecutive days in Experiment 2A (A and C) or for 12 h per day for 28 consecutive days in Experiment 2B (B and D). Pooled data for fresh semen from two control rams collected over the same period are shown as an overall mean (solid horizontal line) ± SEM (dotted lines), as there were no effects of time on the values from the controls. Percent of RAP and PROG sperm showed very similar changes to percent of MOT, and ALH, VCL and VSL showed very similar changes to VAP.

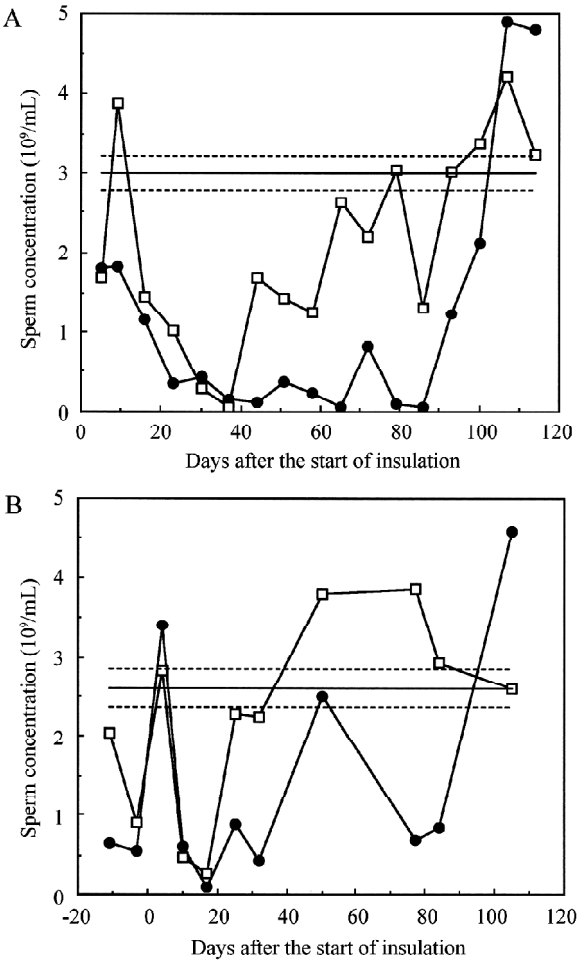

Figure 3. Sperm concentration in semen from rams No.12 and No. 16 subjected to scrotal insulation in Experiments 2A (16 h/day for 21 d, A) and 2B (12 h/day for 28 d, B). Symbols and horizontal solid and dotted lines as in Figure 2.

VAP, VSL and VCL were all significantly reduced. There were no effects on the percentages of live or morphologically normal sperm (Table 2).

Storage of sperm at 30ºC led to reductions in the percentage of MOT, PROG and RAP sperm and in ALH, VAP, VSL and VCL in the semen from insulated rams (data not shown), which were similar to those in semen from control rams (Table 2). However, following storage at 5ºC, there were greater falls in the percentage of MOT, PROG and RAP (data not shown) sperm in sperm from the insulated rams.

3.3 Experiment 2B

3.3.1 Effect of scrotal insulation

Scrotal insulation for the same total period of time, but with fewer hours each day (12 h/day *vs.* 16 h/day) over a longer period (28 days *vs.* 21 days), once more caused falls in the percentage of MOT (Figure 2B), PROG and RAP (data not shown) sperm. These falls were again more severe for ram No.12. Sperm concentrations were less clearly affected in Experiment 2B than in Experiment 2A, although the results were confounded by several low values that occurred before the start of insulation (Figure 3).

The characteristics of sperm motility (VAP [Figure 2D), ALH, VSL and VCL [data not shown]) were also reduced for one ram (No.12), as observed in Experiment 2A, which had been conducted the previous year, but not for the other ram (No.16). Ram No.12 again showed a fall in the percentage of live and morphologically normal sperm, lasting until approximately

Table 2. The effect of storage at 30ºC or 5ºC of semen from control rams on the percentage of motile (MOT), progressive (PROG), rapid (RAP), live and morphologically normal (NORM) spermatozoa and the characteristics of the motility of the motile sperm, mean amplitude of lateral head displacement (ALH), mean path velocity (VAP), mean progressive velocity (VSL) and mean track velocity (VCL) as determined by a Hamilton Thorn Motility Analyzer. Experiment 2A: 32 samples from two rams; Experiment 2B: 22 samples from the same two rams as in Experiment 2A. $^bP < 0.05$, $^cP < 0.01$, $^dP < 0.001$ respectively different from corresponding value for fresh samples.

	Experiment 2A			Experiment 2B		
	Fresh	6 h at 30ºC	6 h at 5ºC	Fresh	96 h at 5ºC	168 h at 5ºC
MOT (%)	79.0 ± 2.7	74.7 ± 3.5^d	77.6 ± 3.1	79.2 ± 1.0	62.4 ± 2.7^d	28.2 ± 4.5^d
PROG (%)	48.8 ± 2.6	43.6 ± 2.8^c	47.5 ± 2.6	56.1 ± 1.2	38.5 ± 2.3^d	13.2 ± 3.4^d
RAP (%)	62.0 ± 3.3	51.6 ± 3.6^d	59.8 ± 3.4	65.3 ± 1.4	43.5 ± 2.6^d	14.4 ± 3.8^d
LIVE (%)	79.4 ± 2.1	75.3 ± 2.1	72.5 ± 2.6	72.3 ± 2.7	66.5 ± 3.0	56.3 ± 2.8^b
NORM (%)	80.3 ± 3.9	80.9 ± 3.6	90.4 ± 3.8	90.4 ± 1.0	86.7 ± 1.4	89.3 ± 1.1
ALH	8.0 ± 0.1	6.3 ± 0.2^d	7.6 ± 0.2^b	7.4 ± 0.1	5.5 ± 0.1^d	4.7 ± 0.2^d
VAP	138.0 ± 4.0	118.0 ± 2.9^d	132.0 ± 3.1	150.0 ± 3.2	112.0 ± 2.4^d	84.0 ± 4.6^d
VSL	110 ± 4.6	98.0 ± 1.9^c	106.0 ± 3.7	126.0 ± 3.0	98.0 ± 2.1^d	73.0 ± 4.2^d
VCL	166 ± 4.0	136.0 ± 1.9^d	156.0 ± 3.3^b	173.0 ± 3.4	131.0 ± 2.6^d	98.0 ± 4.8^d

day 50 after the start of insulation, and ram No.16 showed a brief fall in the percentage of normal sperm, but no consistent change in the percentage of live sperm (data not shown).

3.3.2 Effect of storage at 5ºC for 96 or 168 h

The percentages of MOT, RAP and PROG sperm were reduced slightly after 96 h storage at 5ºC and these reductions were more obvious after storage for 168 h; all reductions were highly significant ($P < 0.001$, Table 2). The percentage of live sperm was also slightly reduced during storage, but there was no consistent effect on the proportion of morphologically normal sperm. ALH, VAP, VSL and VCL were also reduced significantly by storage ($P < 0.001$ for all differences, Table 2), but the percentage falls were smaller than for MOT.

The effect of storage on MOT, PROG and RAP were significantly greater for the rams subjected to scrotal insulation. Storage for 168 h reduced VAP, ALH, VSL and VCL (data not shown), but only when MOT was reduced.

The percentage of live sperm also fell with storage (Table 2), but the extent of the fall was similar to semen from the control and insulated rams (data not shown). The percentage of morphologically normal sperm did not change with storage in control rams (Table 2) or insulated rams (data not shown).

4 Discussion

The present results extend previous observations by showing that intermittent scrotal insulation in rams causes decreases in the percentage of MOT, RAP and PROG sperm and ALH, VAP, VSL and VCL of the motile sperm, and concurs with and extends previous observations [6, 7]. The timing of the fall in the percentages of MOT sperm is in accord with the results of earlier experiments [7], in which the intermittent scrotal insulation was for a similar fraction of each day (16 h/day), but continued for longer (145 instead of 21 consecutive days). Similar results were also obtained in experiments in which scrotal insulation was continuous for 96 h [3], a procedure that is essentially unphysiological (see Introduction). The timing of the falls in motility is also in agreement with the observations on sperm motility following local heating of the testes or of the whole ram [3]. In Experiment 2B, when the length of each insulation was decreased from 16 to 12 h per day, but the treatment continued for longer (28 *vs.* 21 days), so that the total time of exposure was the same, the effects on percentages of motile sperm were less than with 16 h per day, but more obvious than those reported earlier [7] for 8 h per day for 160 days. With 12 h per day insulation, effects on the motility characteristics were seen in only one of the two experimental rams, suggesting that the percentage of MOT, RAP or PROG sperm were more sensitive indicators of abnormality.

It was surprising to find that the falls in motility characteristics (VAP, ALH, VCL and VSL) were proportionately much smaller than the falls in the percentage of MOT, PROG and RAP, and there was not a single instance where a motility characteristic was affected when the percentage of motile sperm was not affected, whether following scrotal insulation or storage. This suggests that some sperm are rendered immotile, whereas the motile ones are only marginally affected. If this finding is extended to other causes of infertility, it means that there is little point in using highly sophisticated measurements of motility characteristics of the remaining motile sperm. However, as epididymal sperm from hamsters treated with α-chlorhydrin showed reduced VCL with no change in percentage of MOT [1, 2], this topic requires further investigation.

Perhaps the most striking finding in the present experiments was the extent of the difference in response between the two rams in Experiments 2A and 2B, and the fact that a similar difference was seen in the two experiments conducted in successive years on the same animals. This would suggest an inherent difference in the susceptibility of the testes to heat between the two animals, but unfortunately, it was not possible to determine whether this difference was genetic and could be passed on to their offspring.

The reductions in percentages of MOT, PROG and RAP, and in motility characteristics following freezing and thawing of the semen were expected, but it was surprising to see such a difference in response between the two groups of rams in Experiments 1A and 1B. Unfortunately, it is not possible to make a direct comparison between the two sets of data, as the animals were of different ages and semen was collected by artificial vagina in Experiment 1A but by electroejaculation in Experiment 1B. We have no explanation for the observation that when comparing fresh and frozen/thawed semen from insulated and control rams, the percentages of MOT, PROG and RAP sperm fell more following freezing and thawing than ALH, VAP, VCL or VSL, when these changes were expressed as percentages of the pre-freezing values, except to suggest that this treatment might

affect those sperm with good motility less than those with poor motility, causing the latter to become completely immotile, therefore increasing the average velocities that are calculated only on motile sperm. Apart from a brief report [12] of reductions in percent MOT and RAP sperm and a decrease in VCL, VAP, VSL and ALH following freezing and thawing of ram semen, there seems to be little information on the quantitative changes in motility characteristics resulting from freezing and thawing of ram semen with which to compare the results of the present study.

It has also been difficult to find data from rams with which to compare the effects on motility we have seen of storage of semen at 5ºC or 30ºC, apart for one report by Joshi *et al*. [8], who found that the percentage of MOT and RAP sperm and the ALH, VAP, VCL and VSL of the motile sperm fell significantly during storage at between 4ºC and 7ºC for 24 h. No studies comparable to ours appear to have been done on the motility of sperm in stored semen from rams with reduced motility.

In conclusion, it appears that intermittent scrotal insulation, producing only a rise in testis temperature of a few degrees Celsius for part of each day, causes not only a reduction in motility characteristics of sperm in freshly collected semen, but also these sperm are less well able to withstand the additional stress of freezing/thawing or storage at refrigerator temperature. Changes in the percentage of MOT, PROG and RAP sperm appear to be more sensitive indicators of deleterious effects of environment or storage than changes in the characteristics of the motility (VAP, ALH, VSL and VCL) of the remaining motile sperm.

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