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Scrotal subcutaneous temperature is increased by scrotal insulation or whole-body heating, but not by scrotal neck insulation; however, all three heat-stress models decrease sperm quality in bulls and rams

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ABSTRACT

Ruminant testes are ~2–6 °C below body temperature; increased testicular temperature reduces sperm motility and morphology. Our objective was to serially monitor scrotal subcutaneous temperatures during testicular heat stress and relate those to sperm quality. Two experiments were conducted, with temperature sensors surgically implanted in scrotal subcutaneous tissues recording temperatures every 15 min and semen collected and evaluated weekly. After an initial control interval, testicular temperature was increased. In Experiment 1, in two Angus bulls, whole-scrotum insulation for 96 h increased scrotal subcutaneous temperatures by ~2.0–2.5 °C ($P < 0.05$). Total and progressive motility decreased ($P < 0.05$) and reached a nadir at Week 3 (~20 and 10%, respectively). Furthermore, morphologically normal sperm and acrosome integrity also decreased significantly, reaching nadirs at Weeks 3 (15%) and 4 (34%). In Experiment 2, 10 Dorset rams were allocated randomly into two equal groups and either: 1) exposed to 28 °C ambient temperature and 30–34% RH for 8 h/d for 4 d; or 2) subjected to scrotal neck insulation that was applied and removed at the same time as the start and completion, respectively, of heat exposures in the other rams. Scrotal subcutaneous temperatures (monitored in three rams per group) were increased in response to whole-body heating (~0.8 °C, $P < 0.05$), but not significantly changed by scrotal neck insulation. Decreases in sperm quality were generally similar between treatments, with the most profound changes evident 4 wk after heat stress, with ~10 percentage point reductions in both total and progressive motility and ~10 and 20 percentage point decreases in morphologically normal sperm in rams with whole-body heating versus scrotal neck insulation, respectively. In conclusion, scrotal subcutaneous temperature was significantly increased by scrotal insulation or whole-body heating, but not by scrotal neck insulation; however, all three heat-stress models decreased sperm motility and morphology in bulls and rams.

1. Introduction

Heat stress (HS) has deleterious effects on animal health and reproduction. As a consequence, climate change and frequent peaks of high ambient temperature in many countries are likely to reduce food-animal productivity (Kastelic et al., 2018; Shahat et al., 2020a). Heat stress occurs when an animal is subjected to temperatures that exceed its physiological range and compensatory ability. Testes are especially vulnerable to HS because spermatogenesis requires mammalian testes to be ~2–6 °C below body temperature (Pérez-Crespo et al., 2008). Consequently, increasing testicular temperature, either acute or chronic, reduces sperm concentration, motility, viability, normal morphology, acrosome integrity, and chromatin stability, resulting in transitory infertility (Setchell, 1998).

Numerous scrotal insulation studies have been done (Boe-Hansen et al., 2020; Brito et al., 2003; Pereira et al., 2020; Rahman et al., 2011), but none monitored intrascrotal temperature. Scrotal or testicular temperatures have been monitored to assess effects of heat load on scrotal thermoregulation; however, most of these studies were one-time measurements, or at most, two or more times over a short interval, e.g., 1 d (Brito et al., 2004; Kastelic et al.,1995, 2000; Waites, 1970). Only two studies used small, surgically implanted data loggers (DLs) to serially monitor intrascrotal temperatures in nonrestrained bulls (Wallage et al., 2017) and rams (Shahat et al., 2020b). The latter study involved

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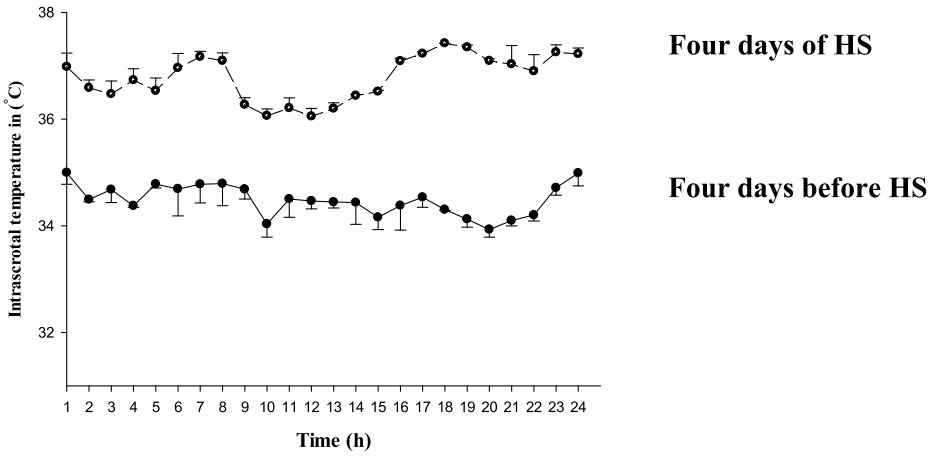

Fig. 1. Mean and SEM scrotal subcutaneous temperatures in two bulls before and during whole-scrotum insulation. Difference ($p < 0.05$) during HS compared to before HS.

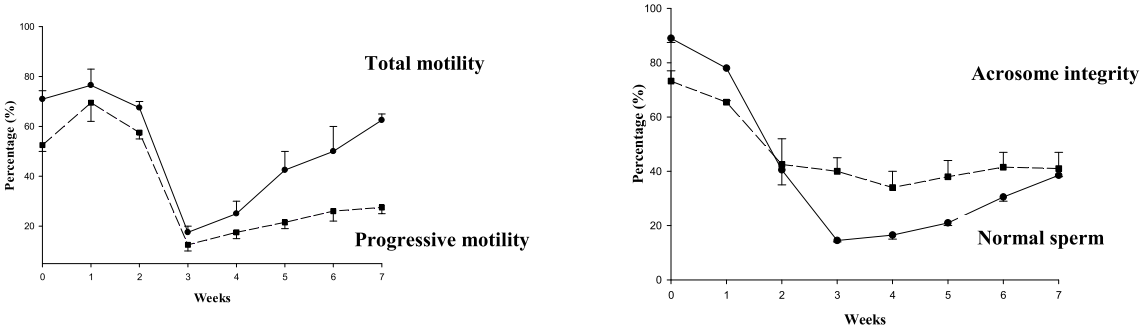

Fig. 2. Mean and SEM sperm motility and sperm characteristics before and after whole-scrotum insulation in two bulls. Difference ($p < 0.05$) during HS compared to before HS.

whole-scrotum insulation and was done to validate the measurement of scrotal subcutaneous temperature measurements with DLs, although semen collection and evaluation were not performed.

In general, models of testicular HS use one of two methods: heating the whole body or heating only the testes. Perhaps the most realistic model of HS is to expose animals to a warm environment. However, there are two crucial factors. First, the body reacts to heat stress in a variety of ways; important physiological, metabolic, and endocrinological changes may have indirect effects on the testis. Second, an animal's ability to produce sweat is influenced by its previous exposure to heat, so testicular temperature can vary significantly in response to given heat exposure (Setchell, 1998).

Local testes heating is usually achieved in one of three ways: induced cryptorchidism, scrotal insulation, or short-term heating (the latter usually by immersion in a water bath). Scrotal insulation is the most common method in farm animals to simulate increased testicular temperature, both by retaining heat and interfering with scrotal sweating (Rahman et al., 2018). Although this model has been widely used, the extent of testicular warming was usually not reported, making it difficult to compare results across studies (Shahat et al., 2020a). Scrotal neck insulation has been occasionally used and appears to cause a smaller increase in testicular temperature and lesser reductions in sperm quality (Brito et al., 2003; Kastelic et al., 1996).

Our objective was to continuously monitor scrotal subcutaneous temperatures during HS and relate those temperatures to changes in sperm quality. In Experiment 1, we applied whole-scrotum insulation (considered substantial HS) to bulls, whereas in Experiment 2, our objective was to compare two models of moderate HS in rams, namely scrotal neck insulation and whole-body heating. In both studies, DLs were used to serially monitor scrotal subcutaneous temperature and sperm quality and motility were assessed once weekly.

2. Materials and methods

2.1. General

Unless otherwise indicated, all chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). These experiments were approved by the University of Calgary Veterinary Sciences Animal Care Committee (AC19-0106) and conducted in accordance with Canadian Council on Animal Care guidelines.

Temperature DL (DST Micro-T, Star-Oddi, Gardabaer, Iceland), 8.3 × 25.4 mm and 3.3 g and designed to be implanted, were used to continuously monitor scrotal subcutaneous temperatures. The DLs were activated (using the manufacturer's communication box and Mercury® software), set to record temperature every 10 min, and placed in a waterbath (28.0–40.5 °C) to verify function. Thereafter, they were set to record at 15-min intervals, disinfected (2% glutaraldehyde for 10 h), rinsed with sterile water and surgically implanted.

2.2. Experiment 1: bulls

Two adult Angus bulls (4–5 y), approximately 600 kg, were housed together in a small outdoor paddock, with *ad libitum* access to hay, water and salt and they were used during the breeding season. Immediately prior to surgical implantation of DLs, bulls were given 1 ml/10 kg

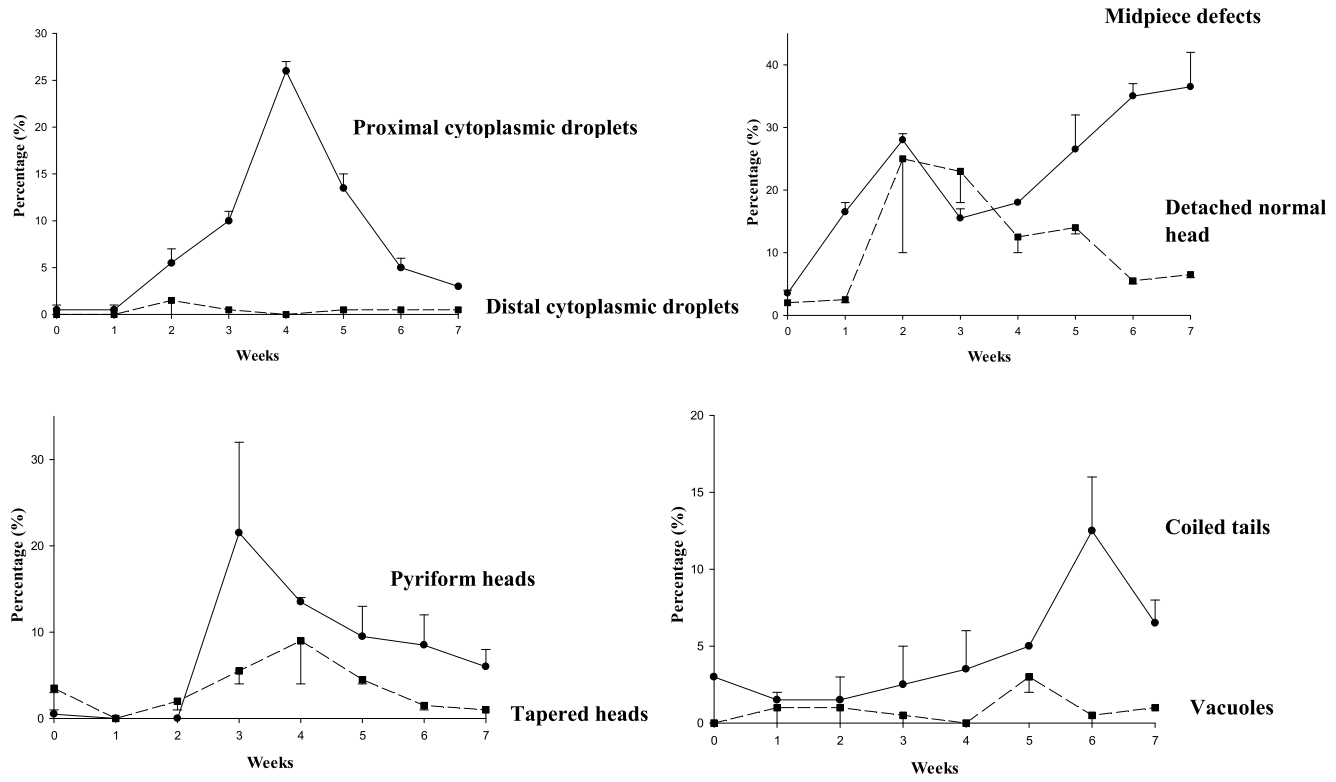

Fig. 3. Mean and SEM of various sperm morphological abnormalities before and after whole-scrotum insulation in two bulls. Difference ($p < 0.05$) during HS compared to before HS.

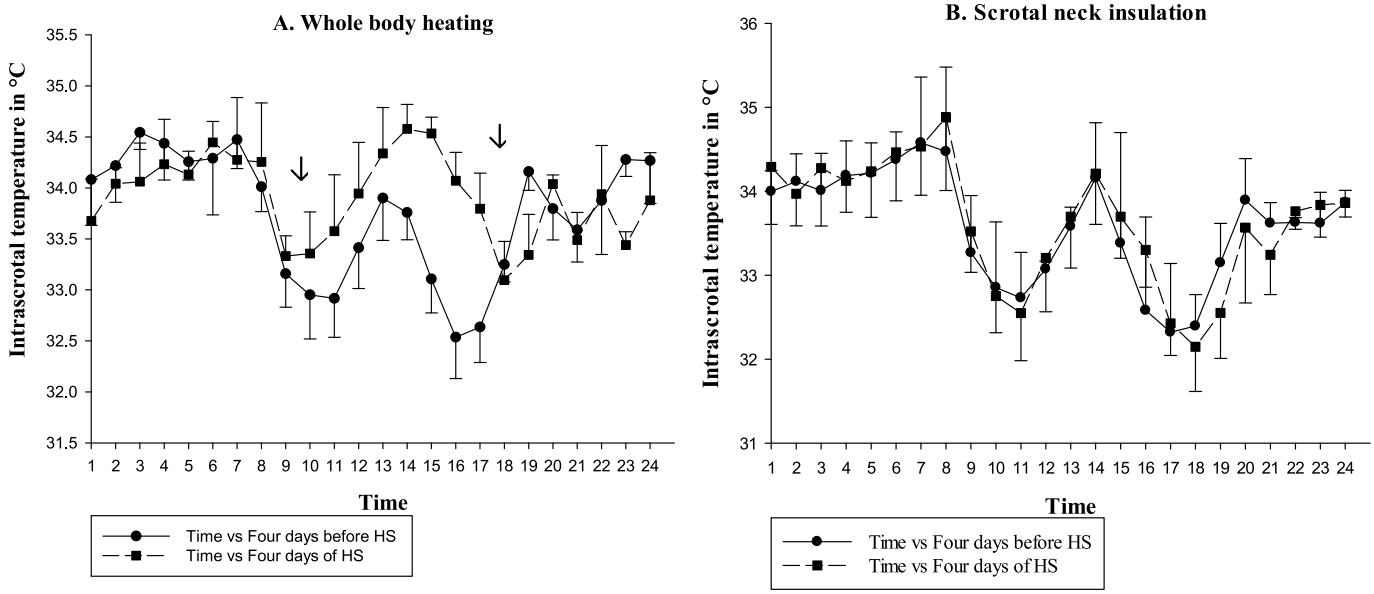

Fig. 4. A and 4B. Mean and SEM scrotal subcutaneous temperatures in rams before and during whole-body (WB; 5 rams) heating and scrotal neck insulation (SI; 5 rams). Arrows indicate the start and cessation of whole-body heating. There were effects of effects of treatment ($p < 0.015$), time period (9:00 a.m.-4:45 p.m.; $p < 0.01$) and group ($p < 0.001$), and interactions of time period*group ($p < 0.038$), treatment*time period ($p < 0.001$), and treatment*time period*group ($p < 0.003$).

oxytetracyline SQ (Bio-Mycin 200 LA, Boehringer Ingelheim, Burlington, ON, Canada), 0.5 mg/kg meloxicam SQ (Metacam, Boehringer Ingelheim), caudal epidural anesthesia (0.07 mg/kg xylazine in ~5 mL saline; Rompun, Bayer, Mississauga, ON, Canada) in the 1st intercoccygeal intervertebral space and local subcutaneous blocks with bupivacaine (Bupivacaine, 2.5 mg/ml, Hospira Inc., Lake Forest, IL, USA) at incision sites. Two skin incisions (~2 cm) were made on the posterior scrotum, one near the top of the testis and the second near the bottom (above the epididymis), lateral to the midline, with blunt dissection used to create a subcutaneous 'pocket' ventral to the surgery site, a DL placed in each site and skin closed with staples (Supplementary Fig. 1).

Starting 10 d after DLs were implanted, semen was collected weekly using an electro-ejaculator. After both bulls were collected for 2 wk as

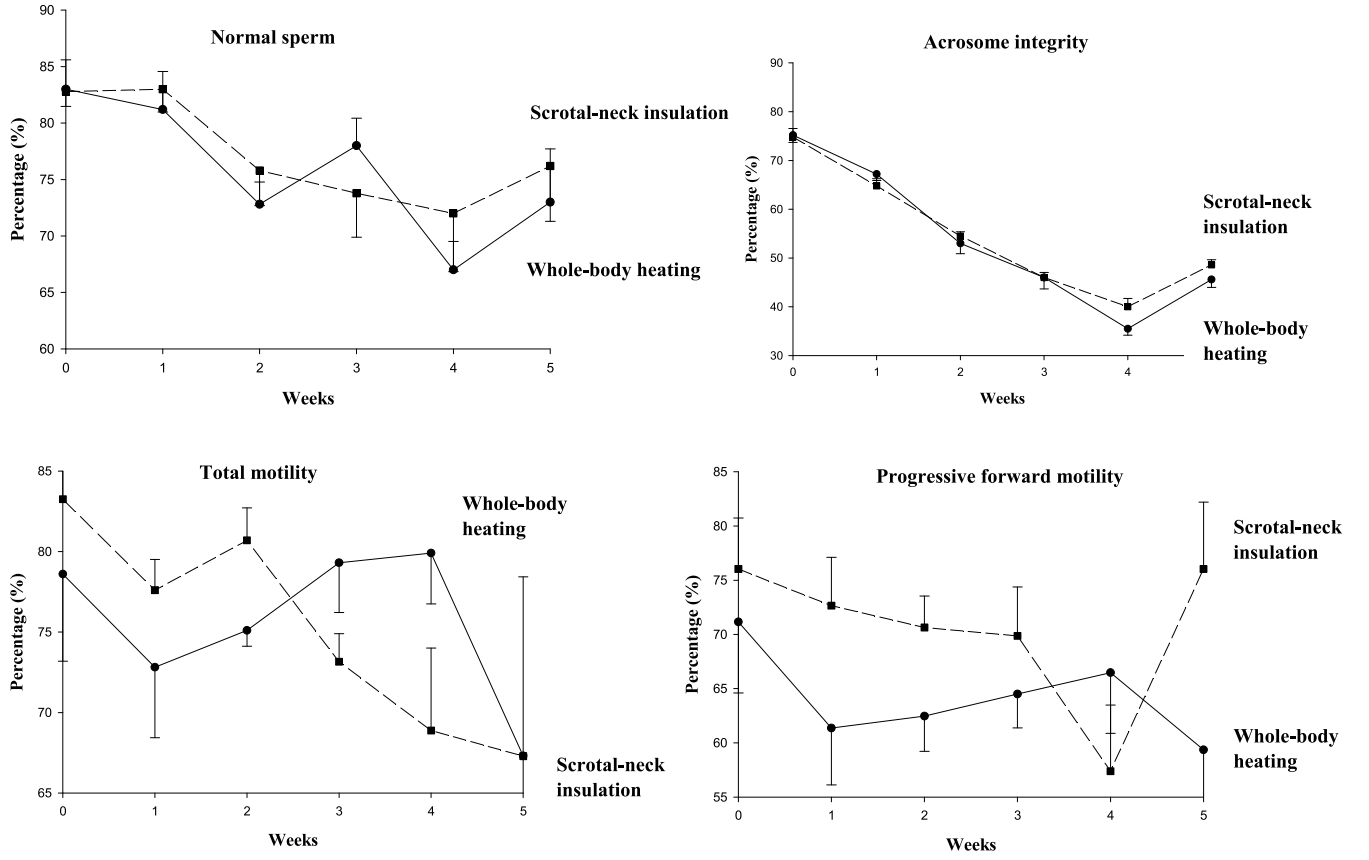

Fig. 5. Mean and SEM sperm characteristics in rams before and during scrotal neck insulation and whole-body heating (five rams per group). For acrosome integrity and total motility, there were effects of week ($p < 0.001$ and $p < 0.05$, respectively), whereas for total motility, there was an effect of group ($p < 0.03$) and for progressive motility, only an effect of group ($p < 0.003$).

control samples, both bulls were subjected to whole-scrotum insulation (one layer each of disposable baby diapers and cloth taped around the scrotum; Supplementary Fig. 2) for 4 d. After heat treatment, semen was collected once weekly for 7 wk. Semen was transported to the laboratory and evaluated using CASA (Sperm Vision®), morphology using eosin-nigrosin, and acrosome integrity using FITC-PSA.

Once semen collection was completed, to enable retrieval of the DLs, small incisions were made in the scrotal skin using identical procedures as described above. Temperature data were subsequently retrieved using the Mercury software. All DLs were working, there were no missing data and no signs of inflammation or reactions where DLs were located.

2.3. Experiment 2: rams

Ten Dorset rams, 3 adults (3–4 y; 90–100 kg) and 7 juveniles (1–1.5 y; 65–80 kg) were housed together indoors, at ~18–20 °C, with lights going on at 7:00 a.m. and going off at 4.30 p.m. At approximately 8:00 a.m. and 3:00 p.m., they were fed pellets and grass hay, with *ad libitum* access to water and salt.

Six of the juvenile rams were given 8 μg/kg dexmedetomidine IM (Dexdomitor, Zoetis, Parsippany-Troy Hills, NJ, USA), lumbo-sacral epidural anesthesia (0.07 mg/kg xylazine in ~4 mL saline; Rompun, Bayer, Mississauga, ON, Canada), and local blocks with bupivacaine (Bupivacaine, 2.5 mg/ml, Hospira Inc.) at incision sites. A skin incision (~2 cm) was made on the anterior scrotum, lateral to the midline and ~6 cm from the bottom of the scrotum, blunt dissection used to create a subcutaneous 'pocket' ventral to the surgery site, a DL placed and the incision closed with staples (Supplementary Fig. 3). Implanting DLs on the anterior scrotum put the scrotal incision in apposition with the caudal abdomen during recumbency, to promote cleanliness (Shahat et al., 2020b). Rams were given 1 ml/10 kg oxytetracyline SQ (Bio--Mycin 200 LA), plus 0.5 mg/kg meloxicam SQ (Metacam) before surgery.

Semen samples were collected weekly using a mount ewe in estrus or, if that was not successful, an eletroejaculator. At 10 d after implanting DLs, semen was collected and evaluated from the 10 rams for 1 wk as a control sample. Then, rams were allocated randomly into two groups, each with five rams; one group was exposed to whole-body heating at 28 °C ambient temperature and 30–34% relative humidity for 8 h daily (from 8:00 a.m. to 4:00 p.m.) for four consecutive days and we recorded rectal temperature of those rams before and after heating time (Supplementary Table 1). For the other group, scrotal neck insulation (using approximately six layers of flannel cloth encircling the scrotal neck and held in place with tape; Supplementary Fig. 4) was started concurrent with placement of the other group in the warm room for the first time and removed after 3.5 d, concurrent with removal of the other rams from the heated room on the last day of heat exposure. With the exception of when rams were exposed to whole-body heating, all rams were housed together in a single group.

After heat treatment, semen was collected once weekly from all rams for 5 wk, with evaluations for motility using CASA (Sperm Vision®), morphology using eosin-nigrosin, and acrosome integrity using FITC-PSA. Thereafter, DLs removal and data retrieval were as done in Experiment 1. All DLs were working, with no missing data and no signs of inflammation or swelling at the sites where DLs were implanted.

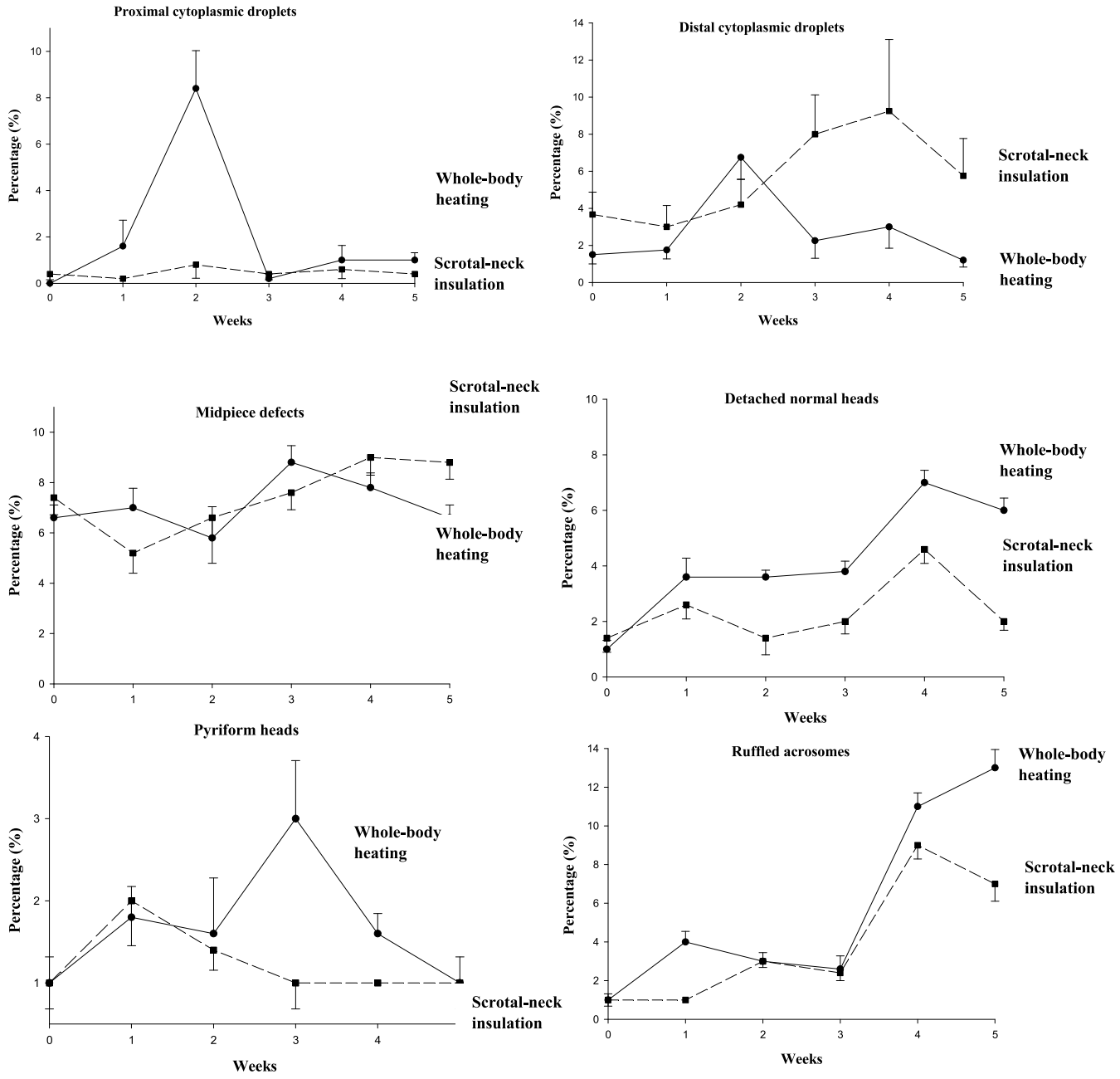

Fig. 6. Mean and SEM of various sperm morphological abnormalities in rams before and during scrotal neck insulation and whole-body heating (five rams per group). Difference ($p < 0.05$) between whole-body heating group compared to scrotal neck insulation group.

2.4. Statistical analyses

A Mixed Linear Model was done to compare scrotal subcutaneous temperatures before and during heat treatment in both experiments. In addition, the same statistical model was used to compare the two groups (whole body heating and scrotal neck insulation) in Experiment 2. A General Linear Model (univariate analysis) was done to compare corresponding semen evaluation end points among control and whole-scrotum insulation in Experiment 1 and whole-body heating and scrotal neck insulation groups in Experiment 2, with a *post-hoc* Bonferroni test used to locate differences.

3. Results

3.1. Experiment 1: bulls

Scrotal subcutaneous temperatures were ~2–2.5 °C warmer ($p < 0.05$) during HS compared to before HS (Fig. 1). Total and progressive motility decreased after whole-scrotum insulation, reaching a nadir at Week 3 (~20 and 10%, respectively); thereafter, both began to increase starting from Week 5 but had not returned to pre-treatment at the end of the experiment (Week 7, Fig. 2). Similarly, morphologically normal sperm and acrosome integrity also decreased dramatically after whole-scrotum insulation, reaching nadirs at Weeks 3 (15%) and 4 (34%), respectively (Fig. 2). All morphological abnormalities (Fig. 3) began to increase after HS, with most peaking 3 or 4 wk after HS, then started to decrease, reaching baselines at Weeks 6 or 7. However, midpiece defects

increased until Week 2, with a minor reduction at Week 3 then a peak at Week 7 (end of the experiment).

3.2. Experiment 2: rams

For scrotal subcutaneous temperatures, there were effects of treatment, time period (9:00 a.m.-4:45 p.m.) and group, and interactions of time period*group, treatment*time period, and treatment*time period*group. In the whole-body heating group, there was a rise in temperature during HS (~0.8 °C, $P < 0.05$) compared to the same time frame prior to HS (Fig. 4A). However, in rams with scrotal neck insulation, scrotal subcutaneous temperatures were not significantly different between prior to versus during HS (Fig. 4B).

For acrosome integrity and total motility (Fig. 5), there were effects of week ($p < 0.001$ and $p < 0.05$, respectively), whereas for total motility, there was an effect of group ($p < 0.03$) and for progressive motility, only an effect of group ($p < 0.003$). Regarding morphological abnormalities (Fig. 6), proximal cytoplasmic droplets were increased in the whole-body heating group ($p < 0.05$), peaking at Week 2 (8%) compared to scrotum-neck insulation (3%). Distal cytoplasmic droplets were increased ($p < 0.05$) in scrotum-neck insulation, peaking at Week 4 (9%) compared to 7% at Week 2 for the whole-body heating group. Furthermore, midpiece defects peaked (9%) at Week 3 for whole-body heating and at Week 4 for the scrotum-neck insulation group. Moreover, ruffled acrosomes peaked (12%) at Week 5 in the whole-body heating group, whereas in the scrotum-neck insulation group, they peaked (8%) at Week 4.

4. Discussion

In this study, whole-scrotum insulation in bulls profoundly increased scrotal subcutaneous temperature; this decreased sperm quality parameters (reductions in total and progressive motility and in acrosome integrity, and increases in various sperm morphological abnormalities). Our results were consistent with previous reports in which whole-scrotum insulation decreased sperm production and semen quality in bulls (Brito et al., 2003; Januskauskas et al., 1995; Pereira et al., 2020). To our knowledge, this was the first report to describe serial recording of intrascrotal temperature in the bull during scrotal insulation, with concurrent collection of semen and assessment of sperm quality. Previous studies that involved serial monitoring of intrascrotal temperatures in bulls (Wallage et al., 2017) and rams (Shahat et al., 2020b) did not concurrently assess sperm quality.

The proportion of total and progressive motile sperm in bulls declined starting from Week 2 and reach a nadir at Week 4, consistent with previous reports (Barth and Bowman, 1994; Brito et al., 2003; Vogler et al., 1993). However, in the present study, it took ~50 d to reach pre-treatment values, compared to 30–42 d in those previous studies. Furthermore, although acrosome integrity reached a nadir at Week 4, it was not fully restored by the end of the experiment (Week 7). In addition, morphologically normal sperm reached the lowest level at Week 3, but had not returned to pre-treatment levels at the end of the experiment. These differences between studies on the effect of HS on sperm quality or interval to recovery were attributed to differences in extent and duration of the HS model used (Shahat et al., 2020a). An important limitation of this study was the limited sample size (only two bulls). Consequently, these preliminary results should be verified by additional studies involving more bulls.

Whole-body heating as a moderate HS model increased scrotal subcutaneous temperature during exposure to the warm room, with expected decreases in sperm quality after HS. However, for scrotal neck insulation, despite no changes in scrotal subcutaneous temperature, there was a similar decrease in sperm quality compared to whole-body heating. In a previous report (Kastelic et al., 1996), scrotal neck insulation in bulls did not change scrotal surface temperature but did increase scrotal subcutaneous and testicular temperatures. This difference could be attributed to species differences, as sheep are less sensitive to HS compared to cattle due to higher thermoregulatory capacity (Aziz, 2010; Joy et al., 2020). Furthermore, it could be due to differences in materials used to insulate the scrotal neck and as a consequence, differences in the extent of the HS and body response. Another possibility was that the scrotal neck insulation model used in our study did not impair testicular thermoregulatory ability. In that regard, in a study in bulls on fat accumulation around testicular vascular cones that resembled scrotal neck insulation, there were no significant correlations between vascular cone fat thickness and scrotal temperature, sperm quality, or sperm production (Brito et al., 2012). Finally, perhaps scrotal neck insulation was stressful, increasing cortisol concentrations, decreasing LH and testosterone concentrations, and reducing sperm quality despite no changes in scrotal subcutaneous temperature, consistent with findings in bulls (Barth and Bowman, 1994).

Both moderate HS models used in our ram study altered sperm quality parameters (total and progressive forward motility, morphological abnormalities, and acrosome integrity) with slight differences between the two methods, except for progressive forward motility that was significantly lower for whole-body warming compared to the scrotal neck insulation model.

5. Conclusion

In conclusion, scrotal subcutaneous temperature was significantly increased by scrotal insulation or whole-body heating, but not by scrotal neck insulation. Regardless, all three HS models decreased sperm motility and morphology in bulls and rams, with the extent of the decrease commensurate with the degree of testicular HS.

Author contributions

Abdallah Shahat and John Kastelic designed the experiment; performed the experiment and Abdallah collected the data. Jacob Thundathil and John Kastelic revised the drafted manuscript. All authors provided intellectual feedbacks on the manuscript and approved the final version of the manuscript.

Declarations of competing interest

The authors declare that they have no conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2021.103064.

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