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Sperm head ellipticity as a heat stress indicator in Australian Merino rams (*Ovis aries*) in Northern Patagonia, Argentina

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ABSTRACT

In Northern Patagonia, Argentina, the ovine mating season starts on March 15, which is the time when rams are submitted to summer temperatures. This study assessed the adaptability of 12 Australian Merino rams, six unshorn and six shorn, half of which were treated in a heat chamber for five days (09.00 hours to 17.00 hours) that gradually reached 40 °C. In an attempt to quantify the effects of heat stress on sperm head morphology, ellipticity was analyzed to establish the relationship between the distributions of subpopulations, light hours, temperature and humidity. Ellipticity was measured on 9224 sperm heads that were obtained over 12 weeks starting in the summer time. Four sperm head subpopulations (S) were identified by comparison with a sperm head population of ejaculates obtained in the late breeding season without the effect of heat stress (S1 = heads with ellipticity ≥ 2.00; S2 = sperm head with range of ellipticity between 1.80 and 1.99; S3 = sperm head with range of ellipticity from 1.60 to 1.79; and S4 = sperm head with range of ellipticity from 1.30 to 1.59). The variable sperm head ellipticity for each ejaculate was expressed as the means and frequencies of subpopulation. The results demonstrate changes in ram sperm head ellipticity in different conditions (control/treated, unshorn/shorn) throughout the experiment ($P < 0.05$). Treated shorn rams had a higher mean ellipticity and frequency of elliptical heads (mean ellipticity value = 2.06 and S1 frequency = 76.35%), peaking in the seventh week posttreatment (on the basis of the action of heat stress on seminiferous tubules). According to this study, unshorn rams were better adapted to heat stress than the shorn ones.

1. Introduction

Sheep production is the primary livestock activity in Argentinean Patagonia [1]. The mating season in Northern Patagonia begins on March 15, which is the time when rams are exposed to summer temperatures.

In temperate regions, mean temperatures are expected to increase because of climate change. This increased temperature could have negative effects on agriculture, biodiversity, the energy sector, hydrology, and human and animal health [2].

Climate is one of the most important factors in animal adaptation to its surrounding environment [3]. The

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variation in climatic variables such as temperature, humidity, air movement, photoperiod, and solar radiation may impose stress on the productive and reproductive performance traits of sheep. However, the effect of ambient temperature is aggravated in the presence of high relative humidity [4,5]. Such effects evoke a series of drastic changes in the animals' biological functions that include depressed food intake efficiency and utilization; disturbances in the metabolism of water, protein, energy, and mineral balances; enzymatic reactions; hormonal secretions; blood flow; and metabolites [6,7]. Such changes result in depressed reproduction including a decline in male semen quality or fertility [7–9], failure of the ewe to exhibit estrus, failure of ova to be fertilized in the ewe, loss of fertilized ova shortly after mating, and fetal dwarfing [10–13].

Optimal climatic conditions for sheep and goats would be approximately an air temperature of 13 °C to 20 °C, wind velocity of 5 to 18 km/h, relative humidity of 55% to 65%, and a moderate level of sunshine [14–17]. However, these factors are interrelated [7].

The coexistence of different sperm morphometric subpopulations within the mammalian ejaculate is widely accepted [18,19]. Sperm dimensions and shapes could be related to its fertilizing ability [20]. Ellipticity is a shape parameter that is influenced by season and most likely by exogenous environmental factors [19].

In the Argentinean Northern Patagonia, traditional flock shearing is in November to December (late spring); thus, the rams spend the summer with fleece less than 1-in long. Opinions regarding whether wool protects individuals from heat are divided. Dutt and Hamm [21] concluded that for Southdown rams, shearing before exposure in a heat chamber reduced heat stress as measured by changes in body temperature, pulse rate, and respiration rate compared with the responses in unshorn rams under similar treatment. Al-Ramamneh et al. [22] determined that under temperate conditions, shearing significantly reduced core body temperature, water intake, and respiratory rate, indicating heat stress in fleeced animals. On the other hand, there is scientific evidence that wool protects sheep from extreme heat. A thick fleece mostly guards against temperature changes because of its insulating properties. According to research studies, sheep with a 1-in fleece are more comfortable than sheep with less wool because wool fibers dissipate heat more rapidly. Sheep with long wool were reportedly less sensitive to solar heating than newly shorn animals [23,24].

Thus, we considered it important to perform a study that would enable us to analyze the adaptation capacity of unshorn and shorn Australian Merino rams in Northern Patagonia to high temperatures and to quantify the effects of heat stress and recovery capacity through mean head ellipticity of different sperm subpopulations.

2. Materials and methods

2.1. Experimental location and climate

The experiment was conducted for 16 weeks during the summer season from 24 January to 9 May 2011 at Facultad de Ciencias Agrarias (FCA), Universidad Nacional del Comahue, near Cinco Saltos (Province of Río Negro, Patagonia, Argentina). The meteorologic data were collected daily from the thermohygrograph located 100 m from open pens at FCA (38° 51′ S, 68° 04′ W, 281 MASL) in the Neuquén river valley that is surrounded by the Patagonian arid plateau. The region has an arid environment with an annual average rainfall of 186.24 mm (range = 90.7 mm–357.4 mm) and annual average temperatures of 14.91 °C (range = −1.4 °C to 33.7 °C; 2001–2010 data). Climatologic information regarding this location during the course of the study is summarized in Table 1.

2.2. Experimental animals

In total, 12 healthy mature Australian Merino rams randomly selected from sheep farm, according to physical condition and clinical examination of the reproductive system, were evaluated; six unshorn (fleece 4.3-in long = Us) and six shorn (fleece 0.7-in long = S), aged 3.5 to 4 years, with live body weight ranging from 51 to 64 kg. The rams were previously acclimatized for 3 months. The shearing was applied before the adaptation period and to the whole body; the remaining fleece was 0.2-in long after shearing and was 0.7-in long at the beginning of the heat chamber experience. Food was offered once a day at 09.00 hours (1.350 g of Lucerne dry matter and oat energy supplement). Water was available *ad libitum*. The whole experiment was performed considering animal welfare conditions in accordance with the statements at FCA, Universidad Nacional del Comahue.

2.3. Experimental design

The rams were randomly divided into two groups: one group (n = 6, three Us and three S) was subjected to outdoor conditions (Table 1), whereas the other group (n = 6, three Us and three S) was kept indoors (Table 2). The first

Table 1
Climatologic information of experiment location.

Time period	Temperature (°C)		Relative humidity (%)		THI		Rainfall (mm)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
December 21–31, 2010	23.44	7.27	48.07	21.61	20.64	5.02	7.70	0.21
January 1–31, 2011	22.00	6.75	48.49	20.27	19.52	4.66	5.70	1.25
February 1–28, 2011	22.00	6.77	49.54	19.73	19.83	4.61	19.00	5.83
March 1–31, 2011	16.77	7.75	50.87	21.03	16.02	5.37	5.00	2.40
April 1–30, 2011	12.44	7.24	53.82	22.10	13.05	4.95	4.30	1.06
May 1–9, 2011	10.41	8.00	54.47	23.00	11.65	5.00	0.00	0.00

Mean day temperature, relative humidity, temperature–humidity index (THI), and rainfall from December 21, 2010 to May 9, 2011.
Abbreviation: SD, standard deviation.

Table 2
Microclimatic heat chamber conditions over 5 days (from 09.00 to 17.00 hours, totally 40 hours) of the experiment.

Days of treatment	Temperature (°C)		Relative humidity (%)		THI	
	Mean	SD	Mean	SD	Mean	SD
February 14	38.2	2.8	42.4	3.2	37.84	2.77
February 15	38.6	3.7	38.0	3.6	38.20	3.60
February 16	39.6	3.4	37.0	4.3	39.24	3.36
February 17	39.4	3.2	41.0	2.2	39.04	3.17
February 18	37.2	4.0	43.2	3.2	36.92	3.87

Mean temperature, relative humidity, and temperature-humidity index (THI).
Abbreviation: SD, standard deviation.

experimental group served as the control group (C) and was housed in open pens. The second group, the treated group (T), was exposed to heat stress for 8 hours (from 09.00 hours to 17.00 hours) over five days (14–18 February 2011) followed by housing in open pens. Both groups were exposed to same duration of natural photoperiods throughout the experiment. Three weeks before the experiment (24 January 2011), the six rams of the treated group were transferred to a place that had two spaces, one open connected to another closed (heat chamber) where they kept on circulating freely to ambient temperature. During five days of treatment (14–18 February), they entered to the heat chamber at 9 AM, were fed and subjected to heat, and 5 PM went into open space, where they spent the night. During the eight hours that the rams remained in the heat chamber, they were subjected to a gradual increase in temperature of 25 °C to 40 °C, guaranteeing four hours at 40 °C daily. During the remaining 12 weeks, called posttreatment (PT, 19 February to 9 May), all the rams were kept in an open-sided shelter-type barn. Those weeks were analyzed grouped in five periods: period 1 (P1 = weeks 1 and 2; Days 1–14 PT); period 2 (P2 = weeks 4–6; Days 22–42 PT); period 3 (P3 = week 7; Days 43–49 PT); period 4 (P4 = weeks 9–10; Days 57–70 PT), and period 5 (P5 = weeks 11 and 12; Days 71–84 PT). According to the spermatogenic cycle (approximately 52 days) [25,26], those periods are coincident with heat influence on epididymal maturation (P1); seminiferous tubules (spermatogenesis and meiosis = P2), seminiferous tubules (part of spermatocytogenesis = P3), and recovery of the normal semen production period (P4 and P5), respectively. Sperm parameter values of 107 ejaculates (concentration $> 500 \times 10^6$ cell/mL and volume > 0.3 mL) were included in the Appendix. Future fertility of these types of heat-exposed animals is out of the scope of the present study.

2.4. Estimation of heat stress severity and photoperiod

A means of estimating the severity of heat stress was proposed using both ambient temperature and relative humidity, termed the temperature–humidity index (THI). The equation used was

$$\text{THI} = \text{Tdb} - [(0.31 - 0.31\ \text{RH})(\text{Tdb} - 14.4)]$$ [27–29]

where Tdb is the dry bulb temperature (°C) and RH is the relative humidity (RH%)/100. Because heat stress was analyzed and the maximum values of THI in summer in studied area were registered between 13.00 and 17.00 hours, Tdb and RH at 15.00 hours every day were used as representative of the maximum THI (THI/15.00) for both groups, control and treated. In the special case of the treated rams, Tdb and RH corresponding to 15.00 hours inside the heat camera were used; in this case, the temperature was 40 °C. Marai et al. [29] used this index to Egyptian Suffolk rams, and the values obtained indicate the following scores: less than 22.2 = absence of heat stress; 22.2 to <23.3 = moderate heat stress; 23.3 to <25.6 = severe heat stress; and 25.6 and more = extreme severe heat stress. Climatic factors were recorded from 31 December 2010 to 9 May 2011, and the photoperiod was expressed as light hours per day. Temperature and humidity at 15.00 hours were obtained from climatologic records.

As a measure of the photoperiod and heat load received by the epidydimes and testes during spermatogenesis, climatic factors were expressed as a 52-day mean before each weekly extraction PT. Climatic factors per period (1–5) were calculated, averaging climatic factors per week (eg, for P1, weeks 1 and 2 were averaged). Additionally, from 52 THI/15.00 values corresponding to spermatogenesis of weekly extractions, the number of days (d) for each of the four THI heat stress scores was recorded [29].

2.5. Sperm head ellipticity

The study was performed using 81 ejaculates PT: P1, 11; P2, 22; P3, 9; P4, 18; and P5, 21. Ejaculates were collected at 9.00 hours weekly; the first semen collections after treatment were 23 February (C) and 28 February (T) by means of an electroejaculator for sheep and goats (Bailey, Western Instrumental Company, Denver, CO, USA). One ejaculate was collected each time per ram.

From eosin-nigrosin–stained smears, at least 100 spermatozoa (n = 9224, average 115 heads) were photographed by a camera that was mounted on an inverted microscope Nikon Eclipse Ti-S. The sperm head length (L) along the main axis and the width (W) along the smaller axis were measured using ImageJ 1.46r [30]. The shape parameter ellipticity was calculated as L/W with high values corresponding to more elliptical sperm heads. As a reference to a sperm head population, three ejaculates were obtained in July 2013 from rams of normal reproductive performance present in the laboratory. The mean sperm head ellipticity of the reference rams (in the late breeding season without the effect of heat stress) was 1.69, and sperm head subpopulation frequencies were S1 = 0.00%; S2 = 23.80 ± 9.35%; S3 = 72.17 ± 7.66%; and S4 = 4.03 ± 1.89%. Comparison of the ellipticity values for these ejaculates with those obtained in the experiment identified ellipticity ranges that characterized sperm head subpopulations. According to this, four subpopulations (S) were recognized: S1 = heads with ellipticity ≥ 2.00; S2 = sperm head with range of ellipticity between 1.80 and 1.99; S3 = sperm head with range of ellipticity from 1.60 to 1.79; and S4 = sperm head with range of ellipticity from 1.30 to 1.59 (figure with representative sperm heads of subpopulations 1 to 4 was included as Supplementary Material). The variable sperm head ellipticity for each ejaculate was expressed as the means and frequencies of each subpopulation.

2.6. Statistical analyses

An initial descriptive analysis was realized which included combinations of factors control/treated and unshorn/shorn (CUs, CS, TUs, and TS), named rams' conditions, in five periods studied, averages of response variables studied (mean ellipticity, subpopulation frequencies of sperm heads) and averages of predicting variables (photoperiod and THI/15.00). A biplot was applied to obtain a plot which aims to represent both the observations (rams' conditions) and variables of a matrix of multivariate data on the same plot.

The effects of treatment/wool/period of mean ellipticity were analyzed on 81 ejaculates. At first, a mixed model was applied to analyze the random structure of rams. Structure of variance–covariance was adjusted for each ram. A likelihood ratio test was applied to compare the fit of linear mixed and random models using Akaike and Bayesian information criterion. As no significant differences were found between the models, we decided to apply a more reduced and parsimonious linear analysis with factorial structure of treatment (ANOVA). A Tukey test was used when the effects were significant. In all cases the level of significance used was 5%. To analyze the relationships between response variables (mean ellipticity and subpopulation frequencies) and predicting variables (climatic factors), Pearson correlation for 81 ejaculates was calculated.

The statistical analysis was performed with Infostat and statistical language R version 3.0.2 [31].

3. Results

In Figure 1, the relationship between the ellipticity expression of the rams' conditions and climatic factors per period are observed. An initial analysis demonstrated that the TS rams in P3 were isolated and had a high mean ellipticity value (2.06) and a high S1 frequency (76.35%). This result was consistent with the ANOVA where the TS rams in P3 had a significant difference for mean ellipticity values ($P < 0.05$).

In Figure 1, the remaining rams' conditions are arranged in a chronologic order from left to right beginning with the TUs in P1 and ending with the CUs in P5. These rams' conditions had a mean ellipticity of 1.83 and 1.64; frequencies of S1, 16.01% and 0.51%; and S3 of 23.45% and 77.93%, respectively. According to the photoperiod, the order was from 13.20 hours of light to 10.12 hours of light. Temperature and humidity conditions registered at 15.00 hours through THI went from highest (25.09) to lowest (19.53).

Figure 2 illustrates the mean ellipticity of the different rams' conditions within each period. It is noteworthy that the mean ellipticity in P1 and P2 were not significantly different. In P3, there was a significant difference in TS; in P4, the CUs were different from treated ones; and in P5, the TS were different from the control ones.

From the Tukey test applied as a comparative analysis between periods, we noted two groups of rams' conditions that were well separated: group 1 consisting of all the P1, P2, and P3 rams and the treated P4 and P5 not including P5 TUs (highest mean ellipticity values); and group 2 consisting of the control rams in P4 and P5 (lowest mean ellipticity values). Here, it is necessary to emphasize that the P5 TUs were included in the latter group.

Table 3 demonstrates Pearson correlation coefficients between the ellipticity expressions and climatic factors. It is important to note that the correlations between mean sperm head ellipticity with photoperiod, THI/15.00, and number of days with severe and extreme severe heat stress are positive,

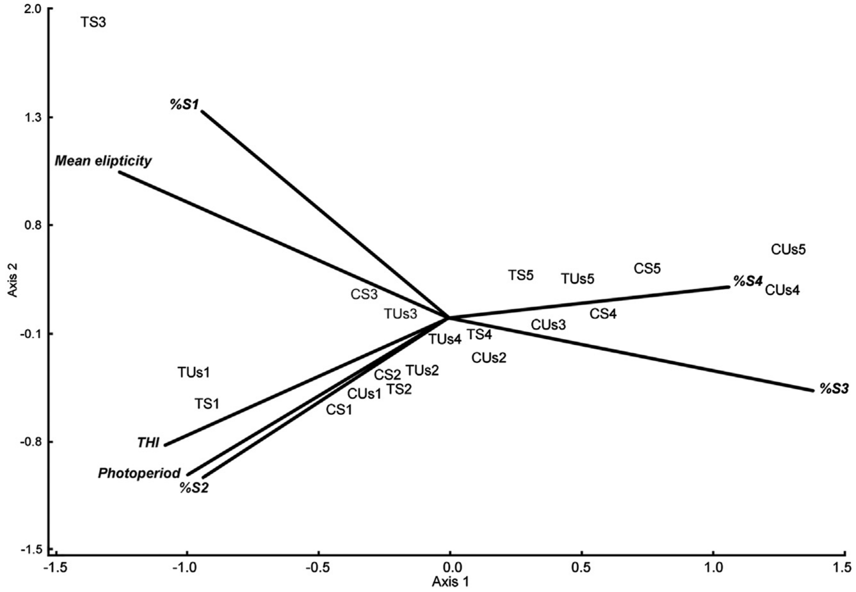

Fig. 1. Biplot demonstrates relations between rams' conditions according to the averages of mean sperm head ellipticity, subpopulation frequencies (%S1 to %S4), photoperiod, and temperature–humidity index (THI) at 15.00 hours during the experiment (period 1–5). It is important to emphasize that the TS rams from P3 are isolated which had high mean ellipticity value = 2.06 and a high S1 frequency = 76.35%; the remaining rams' conditions are arranged from left to right beginning with the TUs from P1, which had high mean ellipticity and S1 frequencies values and ending with the CUs from P5 which had low mean ellipticity and S1 frequencies values. CS, control shorn; CUs, control unshorn; P, period; TS, treated shorn; TUs, treated unshorn, frequencies of sperm head subpopulations (%S) 1 to 4.

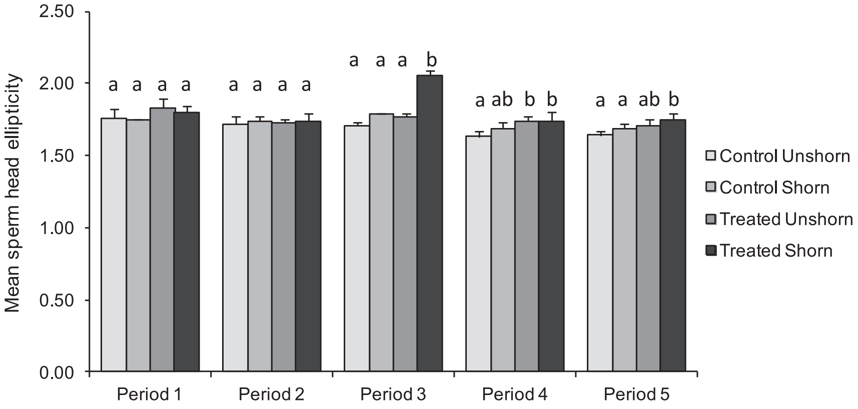

Fig. 2. The bar graph illustrates the mean ellipticity of the different rams' conditions within each period. Period 1: 1 to 14 days posttreatment (PT); period 2: 22 to 42 days PT; period 3: 43 to 49 days PT; period 4: 57 to 70 days PT; and period 5: 71 to 84 days PT. Different letters indicate statistical differences ($P \leq 0.05$) according to the Tukey test.

whereas mean sperm head ellipticity with number of days of absence of heat stress and moderate heat stress are negative.

4. Discussion

Heat stress was evident during P3 (Days 43–49 after treatment) in TS ejaculates that demonstrated more elliptical sperm heads with a mean ellipticity (2.06) in S1 and S2 at high frequencies (76.35% and 21.17%, respectively) and a low frequency of less elliptical heads S3 (2.48%; Fig. 1). These data illustrate that heat chamber conditions (40 hours, 8 hours in 5 days under extreme severe heat stress; THI > 25.6; Table 2) affected spermatocytogenesis at seminiferous tubules and head shape changes occurred as a response (more elliptical heads appeared), as well as frequency of sperm head subpopulations (increased S1 and decreased S3 frequencies). These results are in accordance with the spermatogenesis rhythm in rams, which takes approximately 7 weeks [32,33]. Changes occur at the level of the seminiferous tubules; therefore, they are primary sperm abnormalities according to the classification by their site of origin. These abnormalities are extremely important because the sperm head contains a nucleus (which bears genetic material) and acrosomal enzymes for fertilization [34].

Table 3
Correlations among the ellipticity of 81 ejaculates and climatic factors 52 days before extraction.

Climatic variables	Mean ellipticity	%S1	%S2	%S3	%S4
Photoperiod	0.40*	0.23*	0.42*	−0.45*	−0.26*
THI/15.00	0.41*	0.28*	0.39*	−0.45*	−0.23*
N absence of heat stress	−0.37*	−0.24*	−0.36*	0.41*	0.22*
N moderate heat stress	−0.25*	−0.08	−0.37*	0.32*	0.23*
N severe heat stress	0.25*	0.11	0.28*	−0.26*	−0.23*
N extreme severe heat stress	0.39*	0.25*	0.39*	−0.44*	−0.23*

Frequencies of sperm head subpopulations (%S) 1 to 4, photoperiod (light hours), temperature–humidity index (THI) at 15.00 hours, and number (N) of days THI heat stress scores.
* $P < 0.05$ significance.

Histologic studies reveal that *in vitro* and *in vivo* exposure of testis to high thermal stress impairs spermatogenesis by spermatogonial germ cell elimination in the seminiferous tubules and Sertoli and Leydig cell degeneration [35] in addition to reducing sperm fertility [36]. Elevated ambient temperatures for 90 days significantly reduced the number of young spermatids in the yearling boar seminiferous tubules without any effect on the number of type A spermatogonia or spermatocytes [37]. A possible explanation for the histologic changes mentioned previously may be that heat sufficient to cause spermatogenic damage results in hypoxia in the testis but does not consistently alter the blood flow or the glucose supply [6].

There were no significant differences in mean sperm head ellipticity between the rams' conditions within and between P1 and P2 (Days 1–42 after treatment, Fig. 2). This result indicates that in the epididymides, changes of sperm head shape in response to heat stress do not occur. This is in agreement with Chemineau [33], who stated that long-term heat stress is needed for the emergence of alterations in the ejaculated semen, explaining that its effect is limited to sex cell production and the relative insensitivity of sperm cells within the epydidymes. Howarth [38], Braden and Mattner [39], and Williamson [40] concluded that spermatozoa undergoing epididymal passage were not affected by short-term temperature elevation.

Within P4 and P5 (Days 50–84 after treatment), significant differences in mean ellipticity among the rams' conditions were observed; it was also observed that CUs in P4 were different from treated and TS in P5 was different from the control (Fig. 2). The results demonstrated evidence that recovery is differential according to the rams' conditions. Recovery of the control rams began on P4 and continued in P5 to reach a mean ellipticity value of 1.63. In those that were treated, the recovery was delayed, with an exception of the TUs, which recovered more quickly than the others and reached the mean ellipticity value of 1.71 in P5; according to the Tukey test, this rams' condition was included in group 2 with control rams in P4 and P5. To summarize, the rams' conditions ranked according to its capacity of recovery would be CUs, CS, TUs, and TS (Fig. 1).

During P4 and P5, control ram semen would apparently recover earlier from the intense heat of January and February, than treated ram semen. These findings are consistent with those from other authors. Ortavant and Loir [41] argued that the duration and intensity of exposure to thermal stress determined the return to normal semen quality that takes 40 to 60 days. Shelton [42] claimed that six weeks are required for ram recovery after environmental conditions return to normal.

The relationship between sperm head ellipticity, photoperiod, and THI/15.00 averages was analyzed (Table 3), interestingly, most elliptical head subpopulations (S1 and S2) were present in ejaculates that were subjected to more hours of light, high THI/15.00 values, and more days with an extreme THI score in extreme and severe heat stress, coincident with summer heat and treatment. Less elliptical heads (S3 and S4) demonstrated higher frequencies in those ejaculates that were subjected to fewer hours of light, low THI/15.00 values, and absent or moderate heat stress, coinciding with the autumn months and time of semen recovery.

The location of TS3 on the top and left in the biplot (Fig. 1) had similar ellipticity expressions (high value of mean ellipticity, S1, S2 and low S3) as TS1, and the delayed recovery of the treated rams during P4 and P5 with respect to the control is evidence of the effect of rising temperature and photoperiod. High temperatures suppressed the beneficial effect of decreased daylight hours on reproduction. This result is consistent with Dehghan et al. [43], who stated that a high ambient temperature during summer months increased the effect of photoperiod-mediated suppression of testicular function in rams.

To comparatively analyze unshorn and shorn conditions, the control rams were right to shorn ones in the biplot (Fig. 1). The unshorn and shorn rams had very marked differences (high mean ellipticity, high S1 frequency and low S3 in shorn rams) in P3 that would indicate better adaptability as assessed through ellipticity in the unshorn rams. For these reasons, the shorn rams suffer more heat stress that those that are unshorn.

Our findings are consistent with data from other authors, which stated that wool protects sheep from extreme heat and extreme cold. A thick fleece is mostly protective against temperature changes because of its insulating properties. According to this research, sheep with a 1-in fleece are more comfortable than sheep with less wool as wool fibers dissipate heat more rapidly. Sheep with long wool were reportedly less sensitive to solar heating than newly shorn animals [44,45]. Piccione et al. [24] confirmed that shearing induces adaptive responses in the organism. Both shorn and unshorn ewes were subjected to heat stress, but different sensitivity to heat stress in shorn ewes compared with unshorn ewes is evident.

Woolly and hairy animals should be sheared before the onset of hot weather. Spring shearing allows sheep to have adequate wool growth to keep them cool in the summer (and avoid sunburn) and a full wool coat in the winter to keep them warm [46,47].

In conclusion, heat stress between Days 43 to 49 after treatment is evident in treated shorn rams, which had more elliptical sperm heads in their ejaculates. These changes occur at the level of the seminiferous tubules and therefore are primary sperm abnormalities. Recovery of semen quality is evidenced by a decrease in most elliptical sperm head subpopulations (S1) and increased less elliptical subpopulations (S3) starting approximately 60 days post-treatment. Recovery is influenced by the severity of heat stress that was observed for each ram condition: unshorn are in better condition than shorn. The sperm head ellipticity must be considered an excellent indicator of thermal stress in Australian Merino rams. This indicator is also a useful tool for sheep farmers to suggest the beginning of mating season, which will vary depending on the summer environmental conditions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.theriogenology.2014.10.020.

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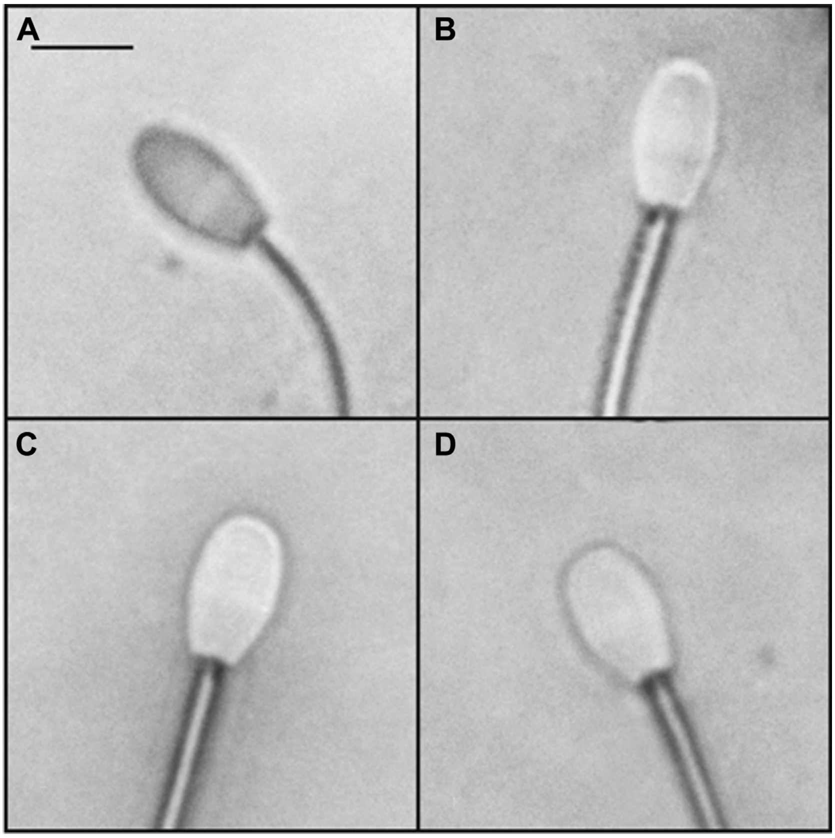

Supplementary Fig. 1. Representative sperm heads of subpopulations 1 to 4: (A) subpopulation 1 (L/W = 2.00); (B) subpopulation 2 (L/W = 1.84); (C) subpopulation 3 (L/W = 1.65); and (D) subpopulation 4 (L/W = 1.53). Scale bar = 5 μm.

Appendix

Period	Rams' conditions	Number of ejaculates	Volume (mL)		Mass motility		Sperm concentration ($\times 10^6$ spz/mL)		Total number of spermatozoa ($\times 10^6$ spz/mL)		Sperm cell motility (%)		Vigor		Cell viability (%)		Acrosome integrity (%)		HOST (%)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PT	CUs	6	0.46	0.14	3.42	1.07	1533.33	1365.89	707.50	724.35	77.50	29.11	3.00	0.55	84.17	3.19	99.17	0.98	52.00	28.93
	CS	3	0.60	0.20	2.83	0.58	2008.33	1139.17	1171.67	742.57	78.33	17.56	3.33	1.04	84.33	6.66	98.67	0.58	39.33	39.51
	TUs	7	0.49	0.23	3.36	0.48	2564.29	1171.44	1421.43	1244.84	87.14	6.36	3.36	0.63	83.57	8.04	95.29	7.23	61.14	15.43
	TS	6	0.57	0.23	3.00	0.71	1656.67	888.99	1075.00	920.25	83.33	8.76	3.42	0.38	79.67	12.42	87.83	17.03	55.20	29.42
P1	CUs	4	0.60	0.12	3.00	0.82	1166.67	702.38	690.00	307.90	85.00	13.23	3.67	0.29	60.25	30.12	88.00	18.71	28.00	1.41
	CS	2	0.45	0.31	2.58	1.20	1304.76	754.40	800.72	768.96	64.56	35.26	2.49	1.55	69.34	30.49	74.68	37.64	17.35	11.74
	TUs	4	0.48	0.13	2.75	0.50	4100.00	500.00	1930.00	773.24	61.25	39.66	3.00	1.35	79.25	10.18	97.00	6.00	34.25	33.90
	TS	5	0.68	0.17	4.00	0.00	1575.00	1056.33	1147.50	856.44	93.75	2.50	3.75	0.50	80.75	8.10	100.00	0.00	21.75	26.44
P2	CUs	8	0.70	0.26	2.06	0.32	1100.00	—	—	—	57.50	38.70	3.25	0.76	72.20	31.19	58.57	25.48	7.50	4.95
	CS	8	0.86	0.31	2.88	0.35	1275.00	955.09	1108.25	1016.30	72.86	28.70	3.07	0.98	45.86	32.26	73.00	20.06	8.29	4.03
	TUs	7	0.65	0.29	3.71	0.76	3635.71	2346.05	2398.33	1403.20	63.57	22.86	3.00	0.71	46.14	24.19	64.29	32.22	16.14	26.49
	TS	10	0.91	0.48	3.35	0.63	1583.00	623.16	1300.00	713.43	74.44	24.93	3.67	0.56	38.22	31.08	60.00	26.29	12.89	19.79
P3	CUs	3	0.90	0.14	2.00	0.00	—	—	—	—	70.00	28.28	2.25	0.35	19.50	3.54	42.00	2.83	6.00	2.83
	CS	2	0.85	0.07	3.50	0.71	1750.00	919.24	1455.00	657.61	90.00	0.00	3.00	0.00	31.50	2.12	77.50	23.33	18.00	15.56
	TUs	3	0.98	0.45	2.83	1.04	2353.33	2476.94	2008.00	1656.44	81.67	7.64	3.50	0.50	54.00	16.09	82.67	8.50	2.67	2.08
	TS	2	0.75	0.49	3.50	0.71	2775.00	1873.83	1617.50	31.82	80.00	14.14	4.00	0.71	53.00	9.90	93.00	1.41	5.50	0.71
P4	CUs	6	0.92	0.52	2.42	0.38	800.00	494.22	730.33	531.16	55.83	32.00	2.67	0.82	45.83	15.17	74.60	23.38	10.00	5.48
	CS	5	0.77	0.49	3.00	0.35	1587.00	1002.11	1342.00	1422.61	66.25	27.50	3.50	0.41	43.80	19.88	57.80	35.49	46.00	49.50
	TUs	4	0.90	0.22	3.00	0.82	2866.67	1750.24	2810.00	1555.22	62.50	9.57	3.13	0.75	56.00	18.24	65.50	42.30	3.00	2.45
	TS	3	0.55	0.13	3.33	0.76	1150.00	672.68	658.33	499.51	76.67	15.28	3.50	0.00	38.67	9.45	49.00	49.50	8.00	—
P5	CUs	3	0.73	0.29	2.33	0.58	1208.33	545.63	440.00	—	43.33	30.55	3.00	1.00	52.00	9.00	60.33	13.05	9.00	1.41
	CS	6	0.75	0.34	3.17	0.93	2120.00	1266.20	1395.00	727.99	71.67	26.20	3.58	0.66	55.00	24.73	71.50	30.78	20.50	21.53
	TUs	5	0.73	0.26	3.30	0.97	2725.00	1887.46	1233.33	758.84	67.00	17.18	3.50	0.71	45.80	25.69	86.60	15.95	13.80	16.04
	TS	5	1.12	0.56	3.20	0.45	1800.00	1181.10	2236.00	1978.71	74.00	20.74	3.10	0.74	50.20	18.25	76.00	20.37	16.20	15.55

Sperm parameter values of 107 ejaculates (concentration $> 500 \times 10^6$ cell/mL and volume > 0.3 mL).
Abbreviations: CS, control shorn; CUs, control unshorn; HOST, hypo-osmotic swelling test; P, period; SD, standard deviation; TS, treated shorn; TUs, treated unshorn.