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Review

# Thermoregulation and reproductive responses of rams under heat stress. Review

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**Abstract:**

The high temperatures recorded during the summer season in hot regions compromise the reproductive capacity of domestic animals. In rams, heat stress (HS) causes in the body a series of physiological, metabolic, endocrine and molecular adjustments in order to maintain normothermia and survive; however, several of these changes are negatively associated with their fertility, mainly endocrine ones. HS in rams causes a decrease in blood testosterone concentrations through different mechanisms, and this is negatively reflected on the process of spermatogenesis and sexual behavior. Consequently, heat-stressed rams exhibit low seminal quality and libido; at the sperm level, structural and DNA damage has been observed. Given this situation, the use of HS mitigation strategies during the summer in sheep farms in hot regions is recommended, such as the use of shades in pens, administration of antioxidants or modifications in the diet. Therefore, the objective of this document is to review the current knowledge regarding the effect of HS on the thermoregulation and reproductive capacity of rams, as well as the application of strategies for its mitigation.

**Key words:** Ram, Male sheep, Libido, Seminal quality, Sperm damage.

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# Introduction

Regions with hot climates are characterized by high ambient temperatures (Ta) and relative humidity (RH) in summer, which generally exceeds the upper limit of the thermoneutral zone for production animals ($\leq$ 30 °C), causing them the presence of environmental conditions of heat stress (HS)[1,2]. The productive and reproductive impact generated by HS in animals varies among species, with small ruminants showing the best adaptation to these environmental conditions[3]. Some reviews have described the thermoregulation mechanisms used by sheep to avoid hyperthermia under HS[1,2,4,5], but little attention is paid to the effect it has on ram reproduction. In hot climates, the reproductive success of the flock depends largely on the adaptation and proper reproductive functioning of the rams.

The organism of heat-stressed rams presents a series of changes to avoid hyperthermia[1,6-8]. Thus, the reproductive capacity of rams decreases while making physiological, metabolic and endocrinological efforts to stay in normothermia[3,7,9-11]. HS can negatively affect the reproduction of the ram by different mechanisms, the main ones being: 1) decrease in testosterone concentrations, and 2) direct damage in the morphometry and content of genetic material of the sperm(12,13). This is reflected in failures in the process of spermatogenesis, as well as in low seminal quality, reproductive behavior and fertility(7,14-16). However, the implementation of HS mitigation strategies improves the reproductive capacity of the ram in these climatic conditions(1,8,17).

It is worth mentioning that the results of the effects of HS on ram reproduction are not consistent among studies. Differences between breeds in the level of adaptation to HS largely explain these discrepancies(3). Therefore, this review aims to describe the current knowledge that exists in relation to the effect of HS on the thermoregulation and reproductive capacity of rams, as well as the application of strategies for its mitigation.

## Sheep in hot climates

In recent decades, the excessive accumulation of greenhouse gases (GHG) in the atmosphere is causing an increase in the Ta of the earth's surface(1), so climate change worldwide is eminent, mainly with tendencies to promote a greater presence of hot climates and, consequently, the desertification of more regions of the terrestrial globe(11). Sheep exposed to high environmental Ta, as well as any other production animal, experience HS(2), which represents a physiological-metabolic challenge for the organism to stay in conditions of homeothermy(6).

In the search for strategies that help to maintain the production of food of animal origin under this adverse climate scenario, some authors propose sheep production as an alternative(1,10,18), mainly because they are able to maintain their productive performance under HS conditions(3). Adaptability characteristics possessed by sheep include resistance to parasites, diseases and scarcity of drinking water(15,19); also ability to take advantage of poor quality agricultural fodder and wastes, and maintenance of the reproductive capacity of the flock and lamb growth under HS scenarios(1,3,5). It is worth mentioning that the adaptation level of sheep varies widely among breeds, since there is a great diversity of them developed from cold to hot climatic conditions.

## Heat stress and sheep production

Stress is generated by the presence of an external event causing alterations in a biological system[20]. In production animals, there is stress when some external factor alters their health, basal metabolism and productive capacity[3]. In this sense, sheep can develop symptoms of stress from facing drastic changes in climatic conditions, and in fact, they develop HS when the combination of environmental factors cause an increase in the Ta above the upper limit of their thermoneutral zone[1].

Climatic variables that can promote the HS environment are Ta, RH, solar radiation, wind speed and precipitation; however, Ta and RH are the main factors associated with presence of HS[5], and consequently, both are used to construct the temperature-humidity index ($THI = Ta - [(0.31 - 0.31*RH)(Ta - 14.4)]$)[4]. It should be clarified that this index was not developed for sheep, however, it is currently widely used to define the degree of HS in this species, since to date there is no specific one for them. Based on that THI, sheep are considered to begin experiencing HS at 22.2 units, being of moderate type between 22.2 and <23.3 units, severe between 23.3 and <25.6 units, and extreme severe at THI ≥25.6 units[4].

The thermoneutral zone for most sheep breeds is between 5 and 25 °C[1], however, there are adapted breeds that begin to experience HS above 30 °C[5,15]. This suggests that, despite being homeotherms, sheep's tolerance to HS varies widely among breeds, and specific studies for each breed should be conducted to assess their tolerance to high Ta. In the world, there are more than 1,000 sheep breeds, which vary in their ability to thermoregulate in hyperthermia environments, and this is due to their climatic origin[3]. In Mexico, there are both wool and hair breeds, but the latter are more tolerant to HS, since they originated in hot climates, while wool breeds originated in cold or temperate climates[5]. This does not mean that there are no HS-tolerant wool breeds in other countries; in Australia, the Merino breed shows great adaptability to warm regions[1].

The thermoregulatory response of sheep to HS also varies with sex, and within sex with age, physiological state and reproductive activity[5,21]. While negative effects of HS are more noticeable in offspring and pregnant and lactating ewes[21,22], rams seem to be less noticeable since their metabolic heat production is lower compared to ewes, and even more so when they are in reproductive rest[1]. The latter could be the cause that most studies are developed in ewes and consider that the topic is of little relevance for research in rams. However, testicular reproductive processes are very sensitive to changes in Ta, which is associated with low fertility in rams during the hot season. In this sense, the rest of the literature review will focus on analyzing the effects of HS on ram reproduction.

## Heat stress and thermoregulation of the ram

The thermoregulation of rams under thermoneutral conditions is essentially due to the activation of non-evaporative mechanisms, without this implying metabolic, endocrine or maintenance energy alterations[1,3]. However, under HS conditions, rams activate a series of thermoregulation mechanisms that favor homeothermy in the face of thermal challenge.

The HS in warm regions increases the average values of physiological variables, such as rectal temperature (RT), respiratory rate (RR), heart rate, and sweat rate (Table 1)[10,18,23]. Thus, rams maintain their normothermia, although it is important to note that the increase in the number of breaths is the main mechanism used by rams to lose the body heat load[21]. In fact, sheep under HS can eliminate between 60 and 90 % of the thermal load through the respiratory system[4]. Another activated physiological mechanism, which is more evident in hair-breed rams subject to HS, is the redistribution of blood flow to peripheral tissues to dissipate body heat by radiation through the skin[5,15]. As the temperature gradient between the skin and the environment decreases, RR increases until it becomes the main route of body heat dissipation[24].

**Table 1**: Changes in the physiological variables of heat-stressed rams

| Source | Breed | Treatment / season | Air temperature (°C) | Findings during the season/treatment with higher temperature |
|---|---|---|---|---|
| (52) | Suffolk | Winter<br>Summer | 14.5<br>28.2 | ↑ RT, ST |
| (45) | Najdi | Winter<br>Summer | 19.8 ± 0.4<br>38.4 ± 0.3 | ↑ RT, RR and HR |
| (72) | Malpura | Winter<br>Summer | 7.0 - 25.5<br>23.0 - 40.0 | ↑ RR and HR |
| (15) | Santa Inés | Winter<br>Summer | ~ 12.5 - 28.0<br>~ 18.0 - 32.0 | ↑ RT, HR and SR |
| | Morada Nova | Winter<br>Summer | ~ 12.5 - 28.0<br>~ 18.0 - 32.0 | ↑ RT, HR and SR |
| (73) | Santa Inés | Winter<br>Summer | ~ 12.5 - 27.0<br>~ 19.0 - 31.0 | No changes |
| | Morada Nova | Winter<br>Summer | ~ 12.5 - 27.0<br>~ 19.0 - 31.0 | ↓ RR and ↑ TMT |
| | Texel | Winter<br>Summer | ~ 12.5 - 27.0<br>~ 19.0 - 31.0 | No changes |
| | Dorper | Winter<br>Summer | ~ 12.5 - 27.0<br>~ 19.0 - 31.0 | ↓ RR and ↑ TMT |

| | | | | |
|---|---|---|---|---|
| (18) | Small-tailed Han | Thermoneutral<br>Heat stress | ~ 22.0 - 23.0<br>~ 30.0 - 35.0 | ↑ RR |
| (74) | Polish Merino | Thermoneutral<br>Heat stress | 16.5 ± 1.0<br>50.0 ± 1.0 | ↑ RT and RR |
| (10) | Merino | Thermoneutral<br>Heat stress | 20.1 - 20.9<br>28.6 - 30.6 | ↑ RR and HR |
| (24) | Malpura x Malpura x Garole | Thermoneutral<br>Heat stress | 33.6 ± 0.7<br>44.2 ± 0.2 | ↑ RT and RR |

RT= rectal temperature, RR= respiratory rate, HR= heart rate, ST= scrotal temperature, TMT= testicular mean temperature; SR= sweat rate.

In rams, the scrotum functions as a thermoregulation organ under both thermoneutral and HS conditions[1]. In hot summer environmental conditions, the scrotum is one of the body regions that most dissipates heat load due to the large vascularization (pampiniform plexus) on the testicular surface, and the large number of sweat glands[15,16]. There is a high correlation between body core and scrotal temperatures, so knowing the variability of scrotal temperature allows evaluating the thermoregulation efficiency in rams[15].

The activation of evaporative mechanisms demands a large amount of body water, so water intake can increase between 19 and 25% in rams during the summer[25]. In consequence, feed intake is decreased by a substitution effect[26]. However, in hair sheep, it was shown that feed intake remained similar in summer and spring, regardless of the increase in water intake recorded during summer[5]. This suggests that the substitution effect of water intake for feed intake occurs mainly in rams with less tolerance to HS. Thus, the reduction in feed consumption is the result of the ram's effort to reduce endogenous heat production, by partially suppressing metabolic and rumen activity[1,4,27].

All the physiological adjustments presented by the rams as a result of HS cause an increase in the maintenance energy requirements, while the reduction in the feed intake alters its availability[5]. Consequently, high summer Ta alter the metabolism of rams, firstly to distribute energy to thermoregulation processes, and secondly to reduce endogenous heat production, while making the use of energy substrates more efficient[28,29]. However, the results of the effect of HS on serum concentrations of metabolites and metabolic hormones are not consistent among studies (Table 2).

**Table 2**: Changes in blood metabolites of heat-stressed rams

| Source | Breed | Treatment / season | Air temperature (°C) | Findings during the season/treatment with higher temperature |
|---|---|---|---|---|
| (44) | Ossimi | Winter | 24.1 | ↑ GLU<br>↓CHOL and LIPT |
| | | Summer | 33.7 | |
| (45) | Najdi | Winter | 19.8 ± 0.4 | ↑ GLU and PROT |
| | | Summer | 38.3 ± 0.3 | |
| (24) | Malpura x Malpura x Garole | Thermoneutral | 33.6 ± 0.7 | ↓ PROT and $T_3$<br>↑ COR |
| | | Heat stress | 44.2 ± 0.2 | |
| (10) | Merino | Thermoneutral | 20.1 - 20.9 | ↑ COR |
| | | Heat stress | 28.6 - 30.6 | |
| (25) | Fat-tailed Iranian | Thermoneutral | 21.0 | ↓ GLU, TRIG, $T_3$ and $T_4$<br>↑ PROT and COR |
| | | Heat stress | 40.0 | |
| (74) | Polish Merino | Thermoneutral | 16.5 ± 1.0 | ↓ GLU<br>↑ COR |
| | | Heat stress | 50.0 ± 1.0 | |
| (18) | Small-tailed Han | Thermoneutral | ~ 22.0 - 23.0 | ↓ TRIG, PROT |
| | | Heat stress | ~ 30.0 - 35.0 | |

GLU= glucose, CHOL= cholesterol, TRIG= triglycerides, PROT= total protein, LIPT= total lipids, COR= cortisol, $T_3$= triiodothyronine, $T_4$ =thyroxine.

The high RR observed in heat-stressed rams demands an excessive amount of glucose as an energy source for the functioning of the muscles of the respiratory system(3). Consequently, the rams in summer increase blood glucose concentrations compared to thermoneutral seasons, which is because cortisol concentrations also increase in response to HS(1,3). Cortisol promotes gluconeogenesis and hepatic glycolysis(28). According to this, Ossimi(30) and Najdi(31) breed rams registered higher blood cortisol and glucose concentrations in summer than in winter. Nevertheless, there are studies where serum glucose concentrations decreased(18,32) or did not change(10,23) due to HS in rams. This could be associated with an increase in plasma insulin concentrations(28).

In sheep exposed to chronic HS conditions, mainly those of breeds adapted to hot climates, blood insulin concentrations increase as an adaptive mechanism to maintain proper metabolic functioning, improve energy use efficiency and reduce fatty tissue catabolism(28,29).

Particularly, high insulin levels allow heat-stressed rams to: 1) avoid apoptosis of pancreatic β cells by increasing the production of non-esterified fatty acids; 2) promote the circulating glucose cellular uptake for its metabolism; and 3) maintain anabolism and prevent catabolism, mainly of fatty tissue[3,28]. This last point has been associated with tower serum concentrations of triglycerides, cholesterol and total lipids in rams subjected to chronic HS[18,30]. Additionally, a reduction in blood concentrations of these lipid metabolites is partially associated with the mobilization of fatty acids to meet energy requirements when the glucose-saving system is activated[1,28]. Macías-Cruz *et al*[33] mention that, in sheep, serum concentrations of glucose, cholesterol, triglycerides, total protein and urea vary according to the type of HS. Chronic HS reduces serum metabolite concentrations associated with energy metabolism (i.e., glucose, cholesterol, and triglyceride), but increases metabolite concentrations associated with protein metabolism (i.e., total protein and urea). In the case of acute HS, variations in blood concentrations of these metabolites show an effect contrary to that observed in chronic HS, which is due to the fact that energy metabolism changes to ensure greater availability of energy substrates when making physiological adjustments[3].

Finally, the thyroid gland also plays an important role in the thermoregulation of all species, including rams[13]. The HS causes a reduction in the release of thyroid hormones, which favors lower metabolic heat production and body heat load[23,32]. Notoriously, triiodothyronine has a shorter half-life and is more thermo-sensitive than thyroxine, as demonstrated in a study of Malpura breed rams[23].

## Heat stress and reproductive endocrinology of the ram

Environmental factors play an important role in controlling the reproductive capacity of rams. An inadequate environment can cause stress to the ram and this triggers alterations in the neuroendocrine function of the reproductive axis[34]. In hot regions, high summer Tas generate a HS environment for rams, leading them to prioritize activities associated with thermoregulation processes rather than reproductive functions[13]. In fact, their reproductive capacity could be totally inhibited in breeds susceptible to HS, while such inhibition could be partial or non-existent in adapted breeds[1,15,23].

Rams, in response to HS conditions, activate the sympatho-adrenal-medullary (SAM) system and the hypothalamic-pituitary-adrenal (HHA) axis[12]. The SAM system stimulates the release of catecholamines (adrenaline and noradrenaline) in the medulla of the adrenal glands[9], which induce peripheral vasodilation and increase energy availability through gluconeogenesis and lipolysis[1,13]. For its part, the HHA axis begins its activation with the hypothalamic secretion of corticotropin-releasing hormones (CRH), which in turn stimulate the secretion of adrenocorticotropic hormone (ACTH) in the adenohypophysis[12,13,17]. Additionally, catecholamines together with CRH cause the hypothalamic release of β-endorphin, whose precursor is the proopiomelanocortin polypeptide, which is also a precursor of ACTH[17,34]. ACTH via endocrine stimulates the synthesis of glucocorticoids (cortisol and corticosterone) and mineralocorticoid (aldosterone) in the adrenal cortex from cholesterol[13,17,32]. The release of cortisol is the main mechanism through which the HHA axis inhibits the functioning of the hypothalamic-pituitary-gonadal (HHG) axis[9,11], and consequently, the degree of reproductive activity in rams exposed to HS[34].

The activity levels of HHA and HHG axes are negatively related, in such a way that lower testosterone concentrations and, consequently, reproductive activity are commonly observed in heat-stressed rams[1]. The increase in cortisol in the blood causes the levels of testosterone available in the seminiferous tubules to decrease, which in turn reduces sperm production and quality due to low activity in the process of spermatogenesis[35,36]. Also, libido and mounting ability is reduced due to low testosterone concentrations[37-39].

Testosterone is synthesized and released by Leydig testicular cells, which respond to the stimulation of luteinizing hormone (LH) for such action[9,40]. Sertoli cells, in response to follicle-stimulating hormone (FSH) stimuli, synthesize and release the androgen-binding protein, which is responsible for binding with circulating testosterone to introduce it to the seminiferous tubules[40]. Once inside the seminiferous tubules, testosterone is responsible for synchronizing the entire process of spermatogenesis[12]. However, the activation of the HHA axis in response to HS may negatively compromise the correct functioning of this mechanism at different points. It has been widely documented that cortisol generates negative feedback on GnRH at the hypothalamus level[1,34], a situation that in turn prevents the adenohypophysis from synthesizing and releasing gonadotropin hormones (FSH and LH)[12,13]; both essential to ensure the presence of sufficient testosterone concentrations within the seminiferous tubules, to carry out spermatogenesis. Some studies also indicate that testosterone concentrations may decrease by different mechanisms than those associated with the functioning of the hypothalamus and hypophysis in heat-stressed rams[9,12,41].

Testosterone concentrations may decrease because glucocorticoids reduce the expression of receptors for LH into Leydig cells[41-43]. It has also been reported that Leydig cells require certain cytokinins such as IL-1 and IL-6 for the testosterone release, however, an increase in glucocorticoid synthesis showed to decrease the immune response and, therefore, the production of these cytokinins[12,44]. Other evidence indicates that the production of androgen-binding protein in Sertoli cells may decrease due to a low production of thyroid hormones[22,45]. Similarly, germ cell damages and low expresion of the protein Conexin-43 (responsible for the union among Sertoli cells) are attributed to the direct effect of testicular hyperthermia[46]. These alterations at the level of Sertoli cells could lead to a low availability of testosterone within the seminiferous tubules[12]. Note that some of these studies were not done in rams, so they may be the reason for future lines of research.

# Heat stress and reproductive capacity of the ram

## Effects on seminal quality

Seminal quality in rams decreases under HS conditions due to the activation of neuroendocrine, physiological and metabolic mechanisms, as well as the increase in maintenance energy expenditure to preserve normothermia conditions[1]. Generally, the damage caused to the sperm by HS becomes visible between 14 and 21 d after the start of exposure of the rams to high Tas[47], therefore, a decrease in seminal quality is detected until then.

The most affected seminal characteristics are progressive motility, sperm abnormalities, plasma membrane integrity, sperm concentration, and ejaculate volume (Table 3)[1,48]. Progressive and mass motility decrease between 5 and 25 %[49,50], which is associated with an increase in the percentage of abnormal sperm[16]. The sperm abnormalities predominating due to HS are head and acrosomal defects[51]. It is worth mentioning that these abnormalities are less frequent in native breed rams of warm regions, in such a way that these breeds adapted to HS present between 1 and 5 % of abnormal sperm[49,52].

**Table 3**: Changes in semen characteristics of heat-stressed rams

| Source | Breed | Treatment / season | Air temperature (°C) | Findings during the season/treatment with higher temperature |
|---|---|---|---|---|
| (49) | Chios | Autumn | 9.7 - 18.3 | ↓ MOT and CON |
| | | Summer | 19.1 - 30.6 | ↑ SA |
| | Friesian | Autumn | 9.7 - 18.3 | ↓ MOT and CON |
| | | Summer | 19.1 - 30.6 | ↑ MP and SA |
| (54) | Persian | Winter | 5.8 ± 3.8 | ↓ VOL |
| | Karakul | Summer | 26.0 ± 4.8 | ↑ VIT and TES |
| (52) | Suffolk | Winter | 14.5 | ↓ SC, MM, VIT and CON |
| | | Summer | 28.2 | ↑ seminal pH, SA and acrosomal damage |

| | | | | |
|---|---|---|---|---|
| (55) | Hamari (not sheared) | Winter<br>Summer | 14.1 - 32.4<br>22.9 - 43.3 | ↓ VOL, VIT, MM and MP<br>↑ SA |
| | Hamari (sheared) | Winter<br>Summer | 14.1 - 32.4<br>22.9 - 43.3 | ↓ VOL, MM and VIT<br>↑ SA |
| (50) | Dorper | Winter<br>Summer | 18.0 - 26.0<br>26.0 - 32.0 | ↓ SC, CON, MM and MP<br>↑ SA |
| (27) | Zulu | Winter<br>Summer | 23.3<br>28.3 | ↓ VOL, CON and PMI<br>↑ SC |
| (15) | Morada Nova | Winter<br>Summer | ~ 12.5 - 28.0<br>~ 18.0 - 32.0 | ↑ CON and SSA |
| | Santa Inés | Winter<br>Summer | ~ 12.5 - 28.0<br>~ 18.0 - 32.0 | ↓ PMI<br>↑ CON |
| (14) | Pelibuey | Winter<br>Autumn | --<br>26.0 - 27.8 | ↓ SC and CON<br>↑ SA |
| (56) | Ouled Djellal | Spring<br>Summer | --<br>33.0 - 40.0 | ↓ SC, VIT and TES |
| (53) | Malpura | Thermoneutral<br>Heat stress | --<br>42.0 | ↓ SC, VOL, MM, CON and TES |
| (24) | Malpura x Malpura x Garole | Thermoneutral<br>Heat stress | 33.6 ± 0.7<br>44.2 ± 0.2 | ↓ MOT |

SC= scrotal circumference, MOT= sperm motility, MM= mass motility, PM= progressive motility, CON= sperm concentration, VOL= ejaculate volume, VIT= sperm vitality, PMI= plasma membrane integrity, SA= sperm abnormalities, SSA= secondary sperm abnormalities, TES= serum testosterone.

The scrotal perimeter and sperm concentration have also shown to decrease due to HS[16], which is possibly related to less sperm cell proliferation and greater apoptosis of cells of testicular parenchyma[47]. Some studies indicate a decrease of 2 to 7 cm in the scrotal perimeter and 3,000 million sperm per milliliter of ejaculate, after subjecting the rams to HS conditions[52,53].

On the other hand, the secretory activity of the accessory glands decreases in heat-stressed rams, which is directly reflected in lower ejaculate volume[36,53-55]. The lower secretion of seminal plasma in the accessory glands is associated with serum testosterone concentrations[12,56]. Additionally, the composition of seminal plasma is modified by HS conditions, mainly at the level of electrolyte and protein concentrations, compounds that maintain the seminal pH between neutral and slightly alkaline (7.0 to 7.3)[11]. In general, HS increases the seminal pH of rams[52,57], which reduces the number of sperm per ejaculate and increases the percentage of abnormalities[36].

In summary, elevated environmental Tas negatively affects ram fertility, essentially because they decrease sperm production, as well as the seminal plasma quantity and quality. This ends up having a negative impact on the microscopic characteristics of the semen. Note that heat-stressed rams do not immediately regain their optimal fertility when switching to a thermoneutral environment; in fact, they require staying between 9 and 11 wk in this environment to ejaculate a semen of normal quality[47].

## Effects on sexual behavior

The sexual behavior of rams has been little evaluated under HS conditions, and the results are contradictory so far. Considering that the service of females is mostly given by natural mounting in the different production systems, it is imperative to elucidate in future research the impact of HS on the mounting capacity of rams.

In Malpura breed rams (adapted to hot climates), the HS induced in thermo-environmental chamber (42 °C) reduced libido and mounting capacity, which was deduced because heat-stressed rams took more time to perform a mount with ejaculation, as well as a higher number of mounting attempts to the first and second ejaculate[53]. Likewise, Rembi breed rams exhibited lower libido during the summer season in an arid region[38]. The reduction in sexual behavior shown by rams exposed to HS was associated with a lower ability to secrete testosterone. However, there are other studies conducted on purebreed[23] or crossed[7] rams from Malpura genotype, where the effects of HS on sexual behavior were minimal without any difference in serum testosterone concentrations. In hair breed rams used in Mexico, one study reported only an increase in the reaction time of mounting by the effect of the dry and hot season compared to the cool-humid season of a tropical climate[39].

Discrepancies between results could be due to the fact that in those studies where there were no effects[7,23], the differences in Ta were not so marked. Other important factors to consider are body condition (CC) and reproductive seasonality. Rams with optimal CC (3.0 on a 1-5 scale) have better sexual behavior than rams with low (≤ 2 points) or high (≥ 4 points) CC under HS conditions[37]. On the other hand, the summer season represents a transition period between the end of the anoestrus period and the beginning of the natural reproductive period[58]. Therefore, rams of breeds with greater sensitivity to reproductive seasonality could present a reduction in sexual behavior during the summer in hot regions, not only because of high temperatures, but also because of their natural reproductive circannual rhythm. In the case of Mexican hair sheep breeds, which are characterized by low reproductive seasonality but high adaptation to hot climates[5], the expected negative effects of HS on their sexual behavior could be minimal, as demonstrated in tropical conditions[39]. However, little research has been done on this topic in hair breed rams and existing studies are still superficial. Hair breeds in Mexico have great relevance for meat production in warm climates, so it is necessary to investigate in depth the impact that HS has on the behavior of these rams.

## Effects on sperm damage

Sperm damage due to HS begins to be generated from sperm cells that are in differentiation within the seminiferous tubules until sperm that are in transit in the epididymis. Ram sperm last between 13 and 15 d in epididymal maturation, so they are the first to show damage from hyperthermia[59]. Previous studies report that rams exposed to Ta greater than 35 °C can cause 17.5 % of pyriform heads[60], 18.5 % of abnormalities in acrosome[61] and about 30 % of tailless sperm[35]. Overall, chronic HS (> 60 d) is estimated to cause 43.4 % of minor abnormalities in sperm (e.g., presence of distal cytoplasmic droplet, coiled tip or fully coiled tail, and free normal heads) and 3.6 % of major abnormalities (e.g., proximal cytoplasmic droplet and microcephalic sperm)[57]. Another study indicated that head ellipticity appears in the ejaculates of rams from d 42 after testicular hyperthermia, which is associated with direct damage from HS to sperm in the spermiogenesis phase, although the mechanism that leads to this head malformation is unclear[62]. It is worth mentioning that the sperm damage generated by HS becomes constant while such environmental conditions remain, and is usually projected for several more weeks after the thermal challenge ends[1,50].

The testicles must remain between 2 and 8 °C below body temperature for proper functioning, otherwise testicular hyperthermia causes damage to testicular somatic and germ cells[63]. Spermatocytes and spermatids are considered more susceptible to apoptosis due to the effect of HS because of their high meiotic rate[1,47], although degeneration can also occur in spermatogonia, and Leydig and Sertoli cells[23,64]. Chronic HS, but not acute, appears to affect sperm that have already completed their formation and are in the epididymis[63,64]. Sperm located in the epididymis increase their level of oxidative stress and decrease their antioxidant capacity in response to continuous and prolonged exposure to HS[63]. The latter has been widely demonstrated in mice, so research is required in rams.

On the other hand, blood flow in the testicles of heat-stressed rams is insufficient, which causes testicular hypoxia and, together with direct hyperthermia, it promotes oxidative stress conditions due to an increase in reactive oxygen species (ROS)[63]. Excessive testicular production of ROS leads to peroxidation of sperm membrane phospholipids, triggering direct damage at the level of membrane integrity (20 % degradation) and DNA[23]. These damages can decrease the expression of the PH-20 protein in the membrane, which is associated with the activity of binding of the sperm with zona pellucida[65]. In addition, they make sperm from rams more susceptible to damage to chromatin conformation[11], and to the presence of DNA fragmentation[47,65]. This damage to sperm DNA can cause subfertility or infertility in rams[11], as well as a decrease in sperm resistance when used in artificial insemination and *in vitro* fertilization programs[51].

## Mitigation of heat stress in rams

The use of HS mitigation strategies is a necessity to improve the reproductive capacity of rams in warm climates. There is a wide variety of strategies that can be implemented; however, they are not equally efficient in all climates and production systems. For example, in wool breeds, shearing in the summer months is a widely used strategy to improve thermoregulation capacity in rams, however, in Argentina, it was reported that the incidence of elliptical sperm heads increased (76 %) in Australian Merino rams for shearing them completely in an HS environment[62]. Similarly, shearing in Desert Hamari hair breed rams was shown to be effective in improving thermoregulation, but counterproductive to seminal quality during the summer season[60]. For his part, Rathore[61] found 16 % for sperm abnormalities when testicular wool was sheared in rams. These findings suggest the need for further studies that determine the effectiveness of this HS mitigation strategy in improving ram fertility.

In Morada Nova and Santa Inés rams, thermoregulation capacity and scrotal circumference and testicular firmness improved, in addition, sperm abnormalities decreased below 4% due to the shade installation[66]. Similarly, the implementation of asbesto shade improved the maintenance of normothermia in Barki breed rams[67]. On the other hand, an increase in airflow under high Ta provided advantages in physiological variables of rams[10]. In addition, the use of straw beds in rams' housing pens improved body heat loss by conduction[1]; however, the effect of cooling systems or the use of different bedding materials on ram reproductive activity is not known.

Dietary supplementation with proteins, lipids, antioxidants and minerals has been shown to improve the ability of adult sheep and lambs to cope with HS[1,68,69]. Nevertheless, in rams, there is only information on the use of antioxidants as a strategy to mitigate the effects of HS. Dietary supplementation of the antioxidant γ-oryzanol in rams decreased ROS production by

26 % and increased the percentage of sperm with intact membrane after testicular hyperthermia; however, there was also an increase in sperm abnormalities[59]. Parenteral administration of vitamin E or vitamin E plus selenium improved the seminal quality and libido of Awassi rams subjected to Ta from 43 to 54 °C[70]. Research needs to be developed on some nutritional strategies that can help minimize the negative effects of HS on the reproduction of rams.

On the other hand, with the intention of improving the thermoregulation capacity in the offspring, it has been chosen to select progenitors of autochthonous breeds that show thermoresistance capacity and adaptation to the environment in which they have developed[27]. In this way, interest in the identification of genetic markers, such as the Booroola fertility gene (FecB), is growing, which apart from increasing the prolificacy of ewes, also positively influences the ability to produce semen of desirable quality under conditions of hot semi-arid climate in purebred Garole rams or crosses with Malpura[23,71].

# Conclusions

Despite the characteristics of resistance and natural rusticity having sheep, HS causes a series of physiological and metabolic changes in the ram that modify energy and reproductive hormonal balance, which finally has a negative impact on blood testosterone concentrations and, therefore, on seminal quality and sexual behavior. In addition, hyperthermia causes direct damage at the level of the membrane and DNA of the sperm, decreasing its fertilizing capacity. Therefore, the use of HS mitigation strategies in rams is a necessity to maintain fertility in the flock, particularly in the hot season of the year of hot climates. The HS mitigation strategy to be used will depend on HS type (acute or chronic) and intensity (moderate or severe) to which the ram is exposed, as well as its degree of adaptation, so it could be used from a simple shaded area with or without fans, to the supplementation of additives such as antioxidants.

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