Animal (2018), **12:S1**, pp s133–s141 © The Animal Consortium 2018
doi:10.1017/S1751731118001167

animal

Review: Testicular vascular cone development and its association with scrotal thermoregulation, semen quality and sperm production in bulls

J. P. Kastelic[†], G. Rizzoto and J. Thundathil

Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary, 3330 Hospital Drive NW, Calgary, AB, Canada T2N 4NT

(Received 21 November 2017; Accepted 17 April 2018)

Several structural and functional features keep bull testes 2°C to 6°C below body temperature, essential for the production of morphologically normal, motile and fertile sperm. The testicular vascular cone (TVC), located above the testis, consists of a highly coiled testicular artery surrounded by a complex network of small veins (pampiniform plexus). The TVC functions as a counter-current heat exchanger to transfer heat from the testicular artery to the testicular vein, cooling blood before it enters the testis. Bulls with increased TVC diameter or decreased distance between arterial and venous blood, have a greater percentage of morphologically normal sperm. Both the scrotum and testes are warmest at the origin of their blood supply (top of scrotum and bottom of testis), but they are cooler distal to that point. In situ, *these opposing temperature gradients result in a nearly uniform testicular temperature (top to bottom), cooler than body temperature. The major source of testicular heat is blood flow, not testicular metabolism. High ambient temperatures have less deleterious effects on spermatogenesis in* Bos indicus v. Bos taurus *bulls; differences in TVC morphology in* B. indicus *bulls confer a better testicular blood supply and promote heat transfer. There is a long-standing paradigm that testes operate on the brink of hypoxia, increased testicular temperature does not increase blood flow, and the resulting hypoxia reduces morphologically normal and motile sperm following testicular hyperthermia. However, in recent studies in rams, either systemic hypoxia or increased testicular temperature increased testicular blood flow and there were sufficient increases in oxygen uptake to prevent tissue hypoxia. Therefore, effects of increased testicular temperature were attributed to testicular temperature* per se *and not to secondary hypoxia. There are many causes of increased testicular temperature, including high ambient temperatures, fever, increased recumbency, high-energy diets, or experimental insulation of the scrotum or the scrotal neck. It is well known that increased testicular temperatures have adverse effects on spermatogenesis. Heat affects all germ cells and all stages of spermatogenesis, with substantial increases in temperature and/or extended intervals of increased testicular temperature having the most profound effects. Increased testicular temperature has adverse effects on percentages of motile, live and morphologically normal sperm. In particular, increased testicular temperature increases the percentage of sperm with abnormal morphology, particularly head defects. Despite differences among bulls in the kind and percentage of abnormal sperm, the interval from increased testicular temperature to the emergence of specific sperm defects is consistent and predictable. Scrotal surface temperatures and structural characteristics of the testis and TVC can be assessed with IR thermography and ultrasonography, respectively.*

Keywords: scrotal/testicular thermoregulation, semen quality, fertility, cattle, bovine

Implications

The testicular vascular cone (TVC) is comprised of the highly coiled testicular artery surrounded by the venous pampiniform plexus. The TVC, located at the top of the testis, operates as a counter-current heat exchanger, cooling arterial blood before it enters the testis. This role is critical, as blood flow is the primary source of heat within the testis and increased testicular temperature reduces the percentage of morphologically normal, motile and fertile sperm. Bulls with a TVC that facilitated heat transfer had better quality sperm and were more resistant to increased ambient temperatures.

Introduction

Bull testes must be maintained 2°C to 6°C lower than body temperature for production of fertile sperm (Waites, 1970). Several mechanisms contribute to testicular cooling, with

[†] E-mail: jpkastel@ucalgary.ca

https://doi.org/10.1017/S1751731118001167 Published online by Cambridge University Press

substantial contributions by a specialized vascular structure, the TVC (Cook *et al*., 1994). This structure, located above the testis, consists of a highly coiled testicular artery surrounded by a network of small veins (pampiniform plexus) (Hees *et al*., 1984). The TVC functions as a counter-current heat exchanger to transfer heat from the testicular artery to the testicular vein, thereby cooling the blood before it enters the testis (Sealfon and Zorgniotti, 1991). The counter-current heat transfer system in the TVC is similar to that present in the extremities, for example the hands, which reduces heat loss in cold environments (Mitchel and Myers, 1968). To prevent heat loss in extremities, heat is transferred from arteries to veins, thereby preserving core-body temperature. By analogy, in the TVC, heat is transferred from the testicular artery to the testicular venous system, keeping the testes and scrotum cooler than the abdomen (Setchell, 1978). However, if the function of the TVC is impaired, the counter-current heat exchange system is disturbed and spermatogenesis is impaired (Gunn and Gould, 1975).

Anatomy of the testicular vascular cone

The TVC has a unique structure. It begins in the neck of the scrotum, ventral to the superficial inguinal ring and ends at the dorsal pole of the testis, just before the testicular artery enters the testis (Kirby, 1953; Hees *et al*., 1990). The TVC is cone-shaped, 10 to 15 cm long, with a maximum diameter of 5 to 6 cm. Veins anastomose to form a complex pampiniform plexus network around the highly coiled testicular artery, with the coils becoming more elaborate as the artery gets closer to the testis. However, loops usually remain in the same plane and do not form large retrograde coils.

The testicular artery has several structural features, including permanent constrictions of the lumen and sharp bends that may serve to reduce both blood flow velocity and pulse (Free, 1977). The total length of the testicular artery is ~1.5 to 4.5 m (Hees *et al*., 1984). The diameter of the lumen of the testicular artery was approximately consistent throughout the TVC (1.6 to 2.2 mm). However, the thickness of the arterial wall decreased from 300 to 225 to 110 μm at the dorsal, middle and ventral aspects, respectively, of the TVC. Furthermore, at these locations, there were ~45, 35 and 25 layers of smooth muscle cells in the wall of the testicular artery (Hees *et al*., 1984). Decreasing thickness of the arterial wall may have an elastic effect and reduce pulsatility. Space among coils of the testicular artery is filled with a sponge-like mass (Harrison, 1949), with the artery surrounded by the pampiniform plexus, except at the periphery of the TVC. The thickness of the perivascular connective tissue space between veins was ~120 μm in the proximal TVC, but decreased to only 50 μm in the distal portion (Hees *et al*., 1984). Presumably, decreases in arterial wall thickness and arterial–venous distance from the top to the bottom of the TVC increase efficiency of heat transfer from the testicular artery to the venous system, thereby resulting in more profound cooling of blood before it enters the testis.

The TVC has three interconnected systems, each with a distinct distribution and luminal diameter (Hees *et al*., 1984). The first has large veins (wall thickness, 20 to 30 μm) that are oriented parallel to one another and surround the testicular artery. The second system is an intermediate network (luminal diameter, 40 to 70 μm) of veins that is well developed and in close apposition with the testicular artery (Hees *et al*., 1984). The third system, with the smallest vessels, is comprised of venules and venous capillaries that drain into either intermediate or large veins. In this third system, most vessels run parallel to each other and surround the testicular artery in transverse, diagonal or longitudinal directions. The testicular artery is generally not completely surrounded by the venous system and the latter does not have a uniform distribution. Although the large veins of the first system and the intermediate venules of the second system have constrictions and pouches, none of the three venous systems comprising the pampiniform plexus have valves, so blood flow is relatively constant, with minimal delays (Hees *et al*., 1984).

In the bull, there are only a few small branches from the testicular artery that serve the capillary network of the spermatic cord and the epididymis (Hees *et al*., 1984). Occasionally, there are arteries that bypass the epididymis and enter the body of the testis, although their importance is unknown (Kirby, 1953). In the testis and adnexia, total arterial volume was similar to total venous volume (Chubb and Desjardins, 1982). The existence and importance of arteriovenous anastomoses in the vascular supply of the testis in bulls are not clear. These junctions were prominent in histological sections (Hees *et al*., 1984), but not apparent when imaging was done with scanning electron microscopy (Hees *et al*., 1984). In a more recent study of the TVC in *Bos taurus* bulls (Polguj *et al*., 2011), there were several vascular anastomoses amongst venous vessels. In vascular casts, the testicular artery had a mean ± SD diameter of 2.01 ± 0.35 mm, whereas venous-venous anastomoses were 0.07 ± 0.05 mm in diameter. There were also several indirect connections between testicular artery and veins, with some connections towards the arterial lumen and some to the vein in the pampiniform plexus. However, no direct connections between a testicular artery and veins from the plexus were observed. The authors concluded that in theory, the anastomosed regions do not support blood return to the testes. Furthermore, they stated that the major function of the anastomoses was related to effects on blood supply and reduction of arterial pressure before reaching the testes. Similar observations have been made in other mammals, indicating structures with parallel evolution and function.

Development of the testicular vascular cone

In a study characterizing the development of the TVC, beef bulls ($n = 70$) were examined from 10 to 70 weeks of age, whereas the third group of bulls ($n = 44$) was examined only at 74 weeks of age (Brito *et al*., 2012). Testicular vascular cone diameter increased until ~13.5 months of age, which was 1 to 8 weeks before they achieved maximum scrotal circumference. Vascular cone fat thickness also increased with age (from ~2 to 6 mm) and followed a pattern of increase similar to that for backfat

https://doi.org/10.1017/S1751731118001167 Published online by Cambridge University Press

(from ~2.5 to 5 mm). Testicular artery wall thickness and distance from arterial to venous blood in the vascular cone decreased with proximity to the testis (from ~320 to 290 μm and from 775 to 660 μm, respectively). Vascular cone diameter was negatively correlated with scrotal surface temperatures ($r = -0.53$) and with the percentage of sperm head defects ($r = -0.49$) and detached sperm heads ($r = -0.40$), but was positively correlated with the percentage of normal sperm ($r = 0.45$). The arterial–venous blood distance was negatively correlated with the percentage of normal sperm ($r = -0.40$) and positively correlated with the percentage of sperm head defects ($r = 0.38$) and proximal droplets ($r = 0.42$). In that study, TVC diameter increased with age following testicular development, whereas vascular cone fat thickness increased with a pattern similar to the accumulation of backfat. In addition, bulls with increased TVC diameter or decreased distance between arterial and venous blood, both of which were expected to increase the efficiency of testicular cooling, were associated with a greater percentage of morphologically normal sperm and corresponding decreases in percentages of defective sperm.

Role of the vasculature and the scrotum and testes in thermoregulation

The role of blood vessels and their association with temperatures of the scrotum and testes have been reported. Temperature gradients (dorsal minus ventral temperature) were 1.6°C, 0.4°C and −0.2°C on the scrotal surface, scrotal subcutaneous tissues and within the testes, respectively (Kastelic *et al.*, 1995). Therefore, on the scrotal surface, the temperature was highest at the top, whereas within the testes, the temperature was slightly warmest at the bottom. It was concluded that these temperature gradients were related to blood vessels. In that regard, presumably the arterial blood supply to the scrotum is from top to bottom, whereas the testis is the opposite, as the testicular artery passes under the body of the epididymis, starts at the bottom of the testes, and then forms several branches that extend dorsally before entering the testis (Kastelic *et al.*, 1996a and 1997b). Therefore, vascularization of the testes is from the bottom to the top, opposite to that of the scrotum. Furthermore, blood in the testicular artery was presumably cooled going through the TVC, was similar at the bottom of the cone (top of testis) as it was on the ventral pole of the testis, but then it was cooler where the artery entered the testis (temperatures of 34.3 ± 0.8°C, 33.4 ± 0.6°C, 31.7 ± 0.6°C, respectively). Therefore, arterial blood was cooled in the TVC and then remained approximately the same temperature between the bottom of the TVC (i.e. top of testis) and bottom of the testis, but subsequently cooled before its entry into the testicular parenchyma. Therefore, both the scrotum and the testis were warmest at the origin of their blood supply (namely, top of scrotum and bottom of the testis, respectively), but they get cooler distal to that point (Kastelic *et al.*, 1996a and 1997b). Remarkably, *in situ,* these opposing temperature gradients collectively result in a nearly uniform testicular temperature (top to bottom), which is a few degrees cooler than body temperature.

Another study (Kastelic *et al.*, 1997a) was performed to elucidate contributions of the scrotum, testes and testicular artery to scrotal/testicular thermoregulation, at two ambient temperatures (15°C and 25°C), with the higher temperature created by heating the room. Full-length vertical incisions were made on both sides of the scrotum, the testes were drawn out of the scrotum, and one testis was randomly chosen to be placed back in the scrotum, and retained there by clamping the scrotal skin closed (effectively eliminating the scrotal incision). At 15°C and 25°C, temperature gradients (top temperature minus bottom temperatures, in °C) were: scrotal surface with a testis (1.5 and 1.3); scrotal surface without a testis (2.1 and 1.6); surface of exposed testis (−0.6 and 0.0); subtunic of exposed testis (−2.2 and −0.6); core of covered testis (0.0 and 0.4); and core of exposed testis (−1.3 and 0.4). Furthermore, intraepididymal temperatures were always warmer in the caput and corpus compared with the cauda in the covered testes at 15°C (34.8 ± 0.5°C, 35.1 ± 0.4°C and 32.1 ± 0.6°C, respectively, $P < 0.01$) and at 25°C (36.4 ± 0.4°C, 36.2 ± 0.4°C and 34.2 ± 0.5°C, $P < 0.05$). The same pattern was observed for the exposed testes at 15°C (29.8 ± 0.7°C, 29.9 ± 0.7°C and 26.4 ± 0.6°C) and 25°C (32.2 ± 0.6°C, 31.9 ± 0.7°C and 29.6 ± 0.6°C). These findings supported the assertion that the scrotum and testes were warmest at the origin of their blood supply and cooler distally.

Sources of testicular heat

Testicular blood flow and oxygen uptake of were determined in eight Angus bulls (Barros *et al.*, 1999). On average, 12.4 ml of blood per minute passed through the testicular artery. However, this may have been a low estimate, as the magnetic flow meter used in this study necessitated some compression of the artery. Blood in the testicular artery was warmer than that in the testicular vein (39.2°C *v.* 36.9°C, $P < 0.001$) and a greater proportion of hemoglobin saturated with oxygen (95.3% *v.* 42.0% $P < 0.001$). Using flow data and the arterial–venous differential in hemogblobin saturation, we estimated testicular oxygen uptake (1.2 ml/min) and heat production by metabolism (5.8 calories of heat/min). In contrast, based on blood flow and the arterial–venous temperature differential, it was estimated that flow was responsible for 28.3 calories/min. Therefore, we concluded that blood flow, and not metabolism, was the primary source of heat in testes (Barros *et al.*, 1999).

Additional contributions to testicular cooling

Several features of the scrotum affect thermoregulation. A pendulous scrotum allows greater exposure of the scrotal neck to the environment, improving a bull's ability to regulate scrotal and testicular temperatures (Coulter, 1980). The scrotum is able to change its size and surface texture, thereby enhancing its temperature-regulating ability (Setchell, 1978). Several factors may promote heat loss from the scrotum, including scrotal skin which is thin, nearly hairless and has a high density of sweat glands (Waites, 1970). Furthermore, the scrotal skin usually has only a thin layer of

https://doi.org/10.1017/S1751731118001167 Published online by Cambridge University Press

subcutaneous fat and blood vessels are close to the skin surface; both of these features promote radiation of heat from the blood to the environment (Dahl and Herrick, 1959). The dartos muscle is closely adherent to the deep surface of the scrotal skin and responds to changes in ambient temperature by maintaining a scrotal position that preserves the abdomino-testicular temperature gradient.

Scrotal/testicular thermoregulation in *Bos taurus*, *Bos indicus* and crossbred bulls

Mechanisms of testicular thermoregulation, the relationship of scrotal, TVC, and testicular morphology with thermoregulatory capability, and their effects on semen quality and sperm production were studied in 20 *Bos indicus*, 28 crossbred and 26 *Bos taurus* bulls (Brito *et al*., 2004). The ratio of testicular artery length and volume to testicular volume were larger ($P < 0.05$) in *B. indicus* and crossbred bulls than in *B. taurus* bulls (1.03 and 0.94 cm/cm^3 *v.* 0.48 cm/cm^3; 0.034 and 0.047 ml/cm^3 *v.* 0.017 ml/cm^3, respectively). Testicular artery wall thickness (average 192.5, 229.0 and 290.0 μm, respectively) and arterial–venous blood distance in the TVC (average 330.5, 373.7 and 609.4 μm, respectively) were smallest in *B. indicus*, intermediate in crossbred, and greatest in *B. taurus* bulls ($P < 0.05$). Furthermore, the proximity between arterial and venous blood was consistent with decreases in arterial blood temperature after passage through the TVC (5.9°C, 5.0°C and 2.9°C, in *B. indicus*, crossbred, and *B. taurus* bulls, respectively). In crossbred and *B. taurus* bulls, there was a positive top-to-bottom scrotal temperature gradient and a negative testicular subtunic temperature gradient. However, in *B. indicus* bulls, both scrotal and testicular subtunic temperatures gradients were positive. Differences in vascular arrangements, characteristics of the artery (e.g. wall thickness) or thickness of the tunica albuginea may have affected testicular arterial blood and subtunic temperatures in *B. indicus* bulls. The better testicular thermoregulatory capability was associated with increases in pendulosity, testicular artery length and volume, and top-to-bottom gradient of the distance between the artery wall and the veins in the TVC. Increased semen quality was associated with increased testicular volume and scrotal subcutaneous temperature gradient, and with decreased scrotal surface and testicular temperatures. Increased sperm production was associated with increased testicular artery volume, testicular volume, and scrotal subcutaneous temperature gradient, and with decreased testicular artery wall thickness, scrotal circumference, and scrotal surface, testicular subtunic and epididymal temperatures. The authors concluded that morphology of the TVC may contribute to the greater resistance of *B. indicus* bulls to high ambient temperatures by conferring a better testicular blood supply and by facilitating heat transfer between the testicular artery and veins. Testicular thermoregulation was associated with opposing scrotal and testicular subtunic temperatures gradients only in crossbred and *B. taurus* bulls. Scrotal, TVC, and testicular morphology influenced testicular thermoregulatory capability and were associated with differences in semen quality and sperm production.

Pathogenesis of heat-induced changes in sperm morphology

Increased testicular temperature increases metabolism, with a concurrent need for more oxygen; however, blood flow apparently does not significantly increase. As the testis operates on the brink of hypoxia (inadequate oxygenation) when testicular temperature is in the normal range, there is a long-standing assertion that increased testicular temperature increased oxygen demand but blood flow does not increase and consequently, hypoxia is the primary mechanism of disrupted spermatogenesis following increased testicular temperature (Waites and Setchell, 1964). However, this had apparently never been rigorously tested until our group conducted two studies (both were 2×3 factorials) to test the following two hypotheses: (1) hypoxia disrupts sperm quality and production; and (2) hyperoxia prevents hyperthermia-induced reductions in sperm quality and production.

It was reported that mice breathing 12.5%, 15.0%, 21.0% and 100% oxygen had testicular oxygen concentrations of 16, 24, 36 and 102 μmol/l (Baker and Lindop, 1970). Rats breathing 100% oxygen had doubled testicular oxygen saturation compared with those breathing 21% oxygen (Kram *et al*., 1989), whereas 16.0% and 10.8% oxygen constituted mild and frank hypoxia, respectively (Chen *et al*., 2007). In one study (Kastelic *et al*., 2017), we exposed 18 Canadian Arcott rams (nine had an insulated scrotum), to air containing 14%, 21% or 85% oxygen for ~30 h. In that study, scrotal insulation (to increase testicular temperature) substantially reduced sperm motility (from 58 ± 7% to a minimum of 30 ± 10%; mean ± SD) and proportion of morphologically normal sperm (from 87 ± 1% to a minimum 30 ± 4%), but effects due to oxygen were minimal. In the second study (Kastelic *et al*., 2008), 96 male CD-1 mice were maintained at 20°C *v.* 36°C, exposed to 13%, 21% or 95% oxygen twice for 12-h intervals (separated by 12 h at room temperature and 21% oxygen) and euthanized 14 or 20 days after exposure.

There were primarily main effects of temperature; mice exposed to 36°C had significant reductions in several end points compared with mice exposed to 20°C (Table 1).

In both studies, our hypotheses were not supported; sperm quality and production were not consistently disrupted by

Table 1 *Differences in various end points between mice exposed to ambient temperatures of 20°C* v. *36°C (Kastelic* et al., *2008)*

	Ambient temperatures		
End points	20°C	36°C	Probability
Testis weight (mg)	110.4 ± 14.3	101.3 ± 17.6	<0.06
Daily sperm production (sperm/g, $\times 10^6$)	19.6 ± 3.7	16.5 ± 6.1	<0.03
Progressively motile sperm (%)	26.5 ± 12.1	13.8 ± 9.8	<0.001
Normal sperm (%)	77.6 ± 7.9	61.3 ± 5.8	<0.0001
Defective heads (%)	5.2 ± 3.2	17.1 ± 11.8	<0.0001
Total altered spermatids	56.3 ± 38.2	106.7 ± 95.3	<0.05
Total altered germ cells	78.4 ± 49.2	125.7 ± 103.4	<0.05

https://doi.org/10.1017/S1751731118001167 Published online by Cambridge University Press

hypoxia, and hyperoxia did not protect against hyperthermia in mice. Therefore, in two species, our preliminary data challenged the long-standing dogma that hypoxia is the underlying cause of disrupted spermatogenesis after increased testicular temperature.

In the two studies described above, oxygen delivery to testes was inferred (based on rodent data), but not measured. As a follow-up, we recently conducted two studies in rams to determine the effects of hypoxia and testicular hyperthermia, on testicular blood flow and oxygen delivery to the testis and its uptake (unpublished). The objective of the first study was to determine how variations in inspired oxygen concentration affected testicular blood flow, oxygen delivery and extraction, testicular temperature and evidence of hypoxia. Eight rams were maintained under general anesthesia and exposed to successive decreases in oxygen concentration in inspired air (100%, 21% and 13%; once each concentration was achieved, after allowing 20 min for acclimation, it was sustained for 45 min, with assessment after 30 min). As oxygen concentration decreased (100% to 13%), testicular blood flow increased (9.6 ± 1.7 *v.* 12.9 ± 1.9 ml/min/100 g of testis, $P < 0.05$; mean ± SEM). Conductance (normalized flow) increased from 0.46 ± 0.07 to 1.28 ± 0.19 ml/min/mm Hg/100 g testis ($P < 0.05$). Increased testicular blood flow maintained oxygen delivery and increased testicular temperature by ~1°C; this increase in temperature was correlated with increased testicular blood flow ($r = 0.35$, $P < 0.0001$). Furthermore, oxygen utilization increased concomitantly and there were no significant differences among groups in blood pH, HCO_3- or base excess, and no effects of venous-arterial differences in lactate production. We concluded that, under acute hypoxic conditions, the testis maintained oxygen delivery and uptake by increasing blood flow and oxygen extraction, with no indication of anaerobic metabolism.

The objective of the second experiment was to determine effects of increasing testicular hyperthermia on testicular blood flow, oxygen delivery and uptake, and evidence of hypoxia. Under general anesthesia, testicular temperatures of nine crossbred rams were sequentially maintained at 32°C to 34°C, 37°C and 40°C (45 min at each temperature). As testicular temperature increased from 32°C to 34°C to 40°C, there were increases in mean ± SEM testicular blood flow (9.8 ± 2.1 *v.* 12.2 ± 2.2 ml/min per 100 g of testes; $P < 0.05$), oxygen extraction ($31.2 \pm 5.0\%$ *v.* $47.3 \pm 3.1\%$; $P < 0.0001$) and oxygen use (0.35 ± 0.04 *v.* 0.64 ± 0.06 ml/min per 100 g of testes; $P < 0.0001$). However, there was no evidence of anaerobic metabolism, based on a lack of change ($P > 0.05$) in lactate, pH, HCO_3-, and base excess.

The second experiment, as described above, was apparently the first to detect increased testicular blood flow in rams with an intact scrotum. Although in a classic study (Waites and Setchell, 1964) there was no increase in blood flow when testis temperature was increased, it was noteworthy that limited numbers of rams were used and the methodology to measure blood flow was probably not as reliable as the ultrasonic flow probes used in our study. In another study in rams (Mieusset *et al*., 1992), there was increased blood flow in response to increasing testis temperature. However, in that study, the testis was outside of the scrotum (exposed), and as a consequence, it was concluded that, in the absence of the scrotum, the findings did not refute the long-standing notion that testicular blood flow failed to increase in response to increasing testicular temperatures. Interestingly, in one of our previous studies in bulls (unpublished), as the ambient temperature increased from 5°C to 35°C, testicular blood flow almost doubled (2.45 *v.* 4.22 ml/100 g testis per min, $P < 0.05$).

In our recent ram study, increasing blood flow was a primary factor underlying the response to increasing testicular temperature, with a 48% increase in conductance (blood flow normalized by arterial pressure). In addition, there was a 52% increase in oxygen extraction at 40°C *v.* 32°C to 34°C. However, there were no apparent effects on aerobic metabolism, based on no significant changes in lactate, pH, HCO_3-, and base excess. Therefore, these data challenged the classical paradigm regarding scrotal/testicular thermoregulation, as acute testicular hyperthermia caused increases in blood flow and delivery and uptake of O_2, with no indications of hypoxia. Furthermore, these data were entirely consistent with our previous studies in rams (Kastelic *et al*., 2017) and mice (Kastelic *et al*., 2008) that the effects of increased testicular temperature were due to testicular temperature *per se* and not due to secondary hypoxia.

Effects of increased temperature on testicular cells

Increased temperature affects all cell types in the testes, although germ cells are more sensitive than either Sertoli or Leydig cells (Waites and Setchell, 1990). Damage is proportional to the extent of testicular warming and the duration (Waites and Setchell, 1990). Increased temperatures can cause the death of spermatocytes in meiotic prophase; however, sperm that has a greater degree of differentiation may have altered structure or function (Setchell *et al*., 1971). Increased testicular temperature usually decreases progressive motility and live sperm, with more morphologically abnormal sperm, especially head defects (Barth and Oko, 1989). Remarkably, specific defects appear in a predictable sequence, although the percentage of affected sperm are variable (Barth and Bowman, 1994). Following increased testicular temperature induced by scrotal insulation, after 11 to 18 days, there were peaks in distal midpiece reflexes, proximal and distal droplets and knobbed acrosomes. A few days later (21 to 23 days), there were microcephalic and teratoid sperm, nuclear vacuoles and pyriform heads, as well as coiled tails (Barth and Bowman, 1994). Finally, abnormal DNA peaked at ~30 days after insulation (Barth and Bowman, 1994). In the absence of an effect on spermatogonia, the interval from the restoration of normal testicular temperature to appear to normal ejaculated sperm in the ejaculate corresponds to the interval from the beginning of differentiation to ejaculation (Waites and Setchell, 1990). Furthermore, although morphologically sperm may be

https://doi.org/10.1017/S1751731118001167 Published online by Cambridge University Press

present in the ejaculate, they may have reduced fertility and higher rates of embryonic death (Burfening and Ulberg, 1968).

Summary of increased testicular temperature

When the testicular temperature is increased, sperm morphology usually remains normal for a few days (as sperm in the epididymis is minimally affected), followed by the appearance of morphologically abnormal sperm (Barth and Oko, 1989). In contrast, epididymal sperm was affected by increased testicular temperatures in some studies (Kastelic *et al.*, 1996b), whereas in another study, sperm appeared morphologically normal, but frozen-thawed sperm was abnormal. Following increased testicular temperature, it usually takes ~6 weeks for sperm morphology to be restored (Vogler *et al.*, 1991). If the testes become very warm and/or remain warm for an extended interval, restoration of morphologically normal sperm may be delayed. Overall, the greater the extent of testicular warming and the longer the duration, the greater the percentage of abnormal sperm and the longer the interval before recovery.

Consequences of increased testicular temperature

Anything which increases testicular temperature, including testicular inflammation, a fever (e.g. due to pneumonia), a lame bull spending a lot of time lying down, or increased environmental temperatures, will reduce percentages of motile and of morphologically normal sperm, and with severe heating, the number of sperm produced (Kastelic *et al.*, 1996b; Brito *et al.*, 2004). If the increased temperature is of short duration (e.g. 2 or 3 days), there is a consistent interval from the insult to the appearance of specific defects in the ejaculate (Barth and Bowman, 1994). Although the timing is predictable, the extent to which a particular bull will have a specific defect is highly variable (wide range in the percentage of defective sperm). In general, the extent to which sperm are affected is directly related to the degree and duration of testicular heating. Therefore, a mild increase in testicular temperature for 2 days could cause a mild reduction in semen quality, with full recovery in 3 to 4 weeks, whereas a prolonged and severe increase in testicular temperature could result in poor quality sperm for several months (Kastelic et al., 1997b). Occasionally, bulls are permanently affected, with the production of few or no normal, viable sperm.

Increased ambient temperature

It is well established that increased ambient temperature decreases semen quality. For example, only 12 h of 40°C air temperatures and 35% to 45% relative humidity decreased semen quality (Skinner and Louw, 1966). In addition, *Bos indicus* bulls are more tolerant of heat *Bos taurus* bulls (Skinner and Louw, 1966), due to physiological differences. Reductions in semen quality were less dramatic and delayed, with more rapid returns to normal, in crossbred bulls (*B. indicus* × *B. taurus*) compared with *B. taurus* bulls exposed to hot conditions (Johnston *et al.*, 1963). Another study compared *B. indicus* and *B. taurus* bulls over a 2-year interval and noted decreases in sperm motility for *B. indicus* in winter and for *B. taurus* in summer (Godfrey *et al.*, 1990).

In one study (Rhynes and Ewing, 1973), plasma testosterone concentrations decreased after exposure to high ambient temperatures (35°C), perhaps due to increased temperatures increasing release of cortisol, which suppresses LH and testosterone. Furthermore, although plasma testosterone concentrations returned to baseline concentrations within ~2 weeks, sperm quality remained impaired (Rhynes and Ewing, 1973), presumably due to the residual effects of testicular hyperthermia. Effects of environmental temperatures on sperm quality and sperm production were determined over a 1-year interval (Parkinson, 1987). In that study, the percentage of morphologically normal sperm peaked at the lowest temperature (~14.5°C), whereas maximal sperm production occurred at an ambient temperature of 17°C (Parkinson, 1987).

Scrotal insulation

A comparative study was conducted to determine effects of scrotal insulation on sperm production, semen quality and testicular echotexture in *B. indicus* and *B. indicus* × *B. taurus* crossbred bulls (Brito *et al.*, 2004). In Experiment 1, *B. indicus* bulls ($n = 12$) were allocated to control and whole-scrotum insulation groups, whereas in Experiment 2, crossbred bulls ($n = 21$) were allocated into control, whole-scrotum and scrotal-neck insulation groups. Insulation was applied for 4 days (start of insulation = Day 0) and semen collection and testicular ultrasonographic examinations were performed twice-weekly until Day 35. Sperm concentration and total sperm output during the post-insulation period were greater in control groups (overall sperm concentration averages of ~500 *v.* 300×10^6 sperm/ml and ~300 *v.* 190×10^6 sperm/ml; total sperm output averages of 4.6 *v.* 2.9×10^9 sperm/ejaculate and 2.5 *v.* 1.6×10^9 sperm/ejaculate, for Experiments 1 and 2, respectively), with significant differences only in *B. indicus* bulls. Overall, sperm motility in scrotal-insulated *B. indicus* bulls was lower ($P < 0.05$) than in the control group (55% *v.* 69%). After whole-scrotum insulation in crossbred bulls, sperm motility was lower ($P < 0.05$) than pre-insulation levels (~70%) between Days 21 and 31 (~30%), and lower than control levels on Day 24 (~55% *v.* 20%). The proportion of normal sperm after whole-scrotum insulation was lower than pre-insulation and control values from Day 11 to the end of the experiment in *B. indicus* bulls ($P < 0.05$ from Days 14 to 21 and on Day 27, overall means of 90% and 90% *v.* 40% normal sperm in pre-insulation, control and insulated bulls, respectively), and from Days 14 to 25 in crossbred bulls ($P < 0.05$ on Days 14 and 18, overall means of 80% and 75% *v.* 60% normal sperm in pre-insulation, control and whole-scrotum insulation). Insulation of the scrotal neck in crossbred bulls did not significantly affect semen quality. There was considerable variation among bulls in the

https://doi.org/10.1017/S1751731118001167 Published online by Cambridge University Press

incidence of specific sperm defects. Timing of appearance of sperm defects after insulation provided insights into pathogenesis of specific abnormalities. Neither whole-scrotum nor scrotal-neck insulation affected testicular echotexture in either experiment. In conclusion, whole-scrotum insulation decreased both sperm production and semen quality in *B. indicus* and *B. indicus* × *B. taurus* bulls. However, those changes were not associated with changes in testicular echotexture (based on pixel analysis of ultrasonograms).

Insulation of the scrotal neck

As a model to mimic fat deposition in the neck of the scrotum that is common in overconditioned bulls, scrotal necks of five bulls were insulated for 7 days (Days 1 to 8) and semen collected by electroejaculation approximately every 3 days from Days 3 to 35 (Kastelic *et al.*, 1996b). Sperm within the epididymis or at the acrosome phase during insulation appeared to be most affected. Insulated bulls had twice as many sperm with midpiece defects and four times as many with droplets on Day 5, fewer normal sperm and three times as many with midpiece defects and droplets on Day 8, fewer normal sperm on Days 15 and 18, and more sperm with head defects on Days 18 and 21. Semen quality approached that in control bulls by Day 35.

In a second experiment, the scrotal neck was insulated for 48 h. At 24 h after insulation, scrotal surface temperature (SST) had decreased at the top of the testis but had increased at the bottom of the testis, resulting in a significant reduction in SST gradient. However, by 48 h, SST was not significantly different from that before insulation. In contrast, scrotal subcutaneous temperature increased 2.0°C, 1.5°C and 0.5°C at the top, middle, and bottom of the testis, respectively, and intratesticular temperature was increased 0.9°C at the corresponding three locations within the testis 48 h after insulation (compared with pre-insulation). Therefore, compensatory mechanisms were apparently able to restore SST, but not scrotal subcutaneous or intratesticular temperatures.

Infrared thermography

Infrared (IR) thermography is a non-invasive technology to detect IR radiation (Kastelic et al., 1997b). For scrotal IR thermography, the goal is to have the scrotum account for the majority of the image. Therefore, depending on the field of view of the lens, the IR thermography camera is held ~1 m behind a bull (Kastelic *et al.*, 2017). There are some environmental considerations for conducting these examinations (Kastelic *et al.*, 1996c), including the absence of direct sunlight and working in a moderate range of environmental temperatures (ideally, ~10°C to 15°C). In a comprehensive study (Kastelic *et al.*, 1996c), it was reported that although time of day did not affect scrotal surface temperature, bulls should not be examined within several hours after feeding and within 1 h after getting up. Furthermore, the scrotum should be clean and dry, as debris can obscure the image and moisture can cause artificial cooling.

Infrared thermography is a technique that can be used to measure scrotal surface temperatures and thereby make inferences regarding testicular temperature. Infrared thermography cameras typically produce a color image, with specific colors associated with a range in temperature. Therefore, it is possible to immediately interpret the scrotal surface temperature pattern. The area immediately above the top of the testis is typically the warmest area of the scrotum, as considerable heat is dissipated from the underlying, highly coiled testicular artery. Bulls with an apparently normal scrotal/testicular thermoregulation had left-to-right symmetry and temperatures that were 4°C to 6°C cooler at the bottom compared with the top (Kastelic *et al.*, 2012). In contrast, less consistent temperature patterns, including horizontal variation and large areas with high scrotal surface temperature, were regarded as indications of aberrant thermoregulation involving either testes and/or epididymides.

Although most bulls with an abnormal thermogram will have poor semen quality (Kastelic *et al.*, 2012), there are some bulls with bad semen quality that have an apparently normal thermogram. Therefore, whereas IR thermography can be used as an ancillary tool to assess bull breeding soundness, it does not overcome the need to collect and evaluate semen. In a study involving 1-y-old beef bulls used for natural service (45-day breeding season with ~ 18 heifers per bull), pregnancy rates (80 days after bull removal) were similar (83% and 85%) for bulls with a normal or questionable, scrotal surface temperature pattern, respectively. However, bulls with an abnormal scrotal surface temperature had lower fertility (68%, $P < 0.01$; Lunstra and Coulter, 1997). Despite these differences in fertility, all bulls were deemed breeding sound on the basis of a standard evaluation. These data highlighted the potential value of incorporating IR thermography into bull breeding soundness examinations. Historically, IR thermography cameras were expensive and at least some required liquid nitrogen. However, advancements in electronics has resulted in suitable cameras that are portable, do not need liquid nitrogen and are relatively modestly priced (~US$3000), making this a cost-effective tool to provide unique and helpful information.

Ultrasonography

Diagnostic ultrasonography, typically using the same scanners as those used for transectal imaging of the reproductive tract of cows, has been used for examining the reproductive tract of bulls (Coulter *et al.*, 1988). The transducer generates high-frequency sound waves (typically 5 to 7.5 MHz), and then receives the returning backscatter and echoes, which are processed to produce a real-time image. Testicular ultrasonography had no effect on semen quality or sperm production (Coulter *et al.*, 1988). Ultrasonographic anatomy of the bull testis has been described (Pechman and Eilts, 1987). In addition, ultrasonography has also been used to determine the diameter and fat cover thickness of the TVC in bulls (Cook *et al.* 1994; Brito *et al.* 2002), changes in the TVC in association with increasing age in beef bulls (Cook *et al.*, 1994;

https://doi.org/10.1017/S1751731118001167 Published online by Cambridge University Press

Brito *et al*., 2012), and to compare characteristics of the TVC in *B. taurus*, *B. indicus* and crossbred bulls (Brito *et al*., 2004), all of which have been described above.

Regarding ultrasonographic examination of bull testes, in the authors' experience, areas of testicular fibrosis, visible ultrasonographically as irregular, intensely white areas in the testis, are common, particularly in young bulls. In a comprehensive study of these lesions (Barth *et al*., 2008), they appeared as early as 5 to 6 months, and in some bulls increased up to 12 to 14 months. The severity of lesions increased over time in some bulls. The cause of these lesions is unknown, although they were associated with a respiratory virus (BRSV) outbreak in bulls in Argentina. Spermatogenesis was apparently not affected; bulls with ~50% of their testes affected still produced normal sperm. Ultrasonography has been used to examine bull testes, and computerized image analysis has been done to assess the image, with some associations between the ultrasonographic image and semen quality. However, the findings have never been used on another set of bulls, to determine the real value of this approach. Therefore with current approaches, ultrasonography as an adjunct to a standard breeding soundness evaluation, has limited utility. However, it could be useful for detecting specific abnormalities of the testes (e.g. local or generalized swelling of the testis). Perhaps future advances in computerized image analysis, or perhaps utilization of Doppler-based ultrasonography (to assess blood flow), will expand the use of ultrasongraphy for breeding soundness evaluation in bulls.

Effects of diets on scrotal and testicular temperatures

In a series of studies (Brito, 2006), bull calves were fed diets that supplied ~70%, 100% and 130% of their requirements for both energy and protein (all bulls had ample vitamins and minerals). In bulls fed these low-, medium- or high-nutrition diets from 26 to 70 weeks of age, there were no significant differences among diets for top or bottom SST. However, as expected, top SST was higher than bottom SST (overall means ~34°C and 32°C, respectively, $P < 0.0001$). When similar diets were fed to bulls from 0 to 70 weeks of age, bulls fed a low diet had lower SST temperature than those fed a high diet (~31°C *v*. 32.5°C for top SST and ~27°C *v*. 29°C for bottom SST). Importantly, observed overall temperature at the top was higher than at the bottom for all groups (overall mean ~ 32°C *v*. 28°C). In another experiment, bulls were fed either a low- or medium-diet from 10 to 26 weeks and then fed either a medium- or high-diet from 27 to 70 weeks. For the two feeding periods, bulls were fed low/medium, low/high, or medium/medium diets. Bulls fed a low/high diet had a lower SST than the low/high group at the top (~32°C *v*. 34°C) and the bottom (~28.5°C *v*. 30°C). Furthermore, in all groups, the temperature at the top was higher than at the bottom (overall mean ~33°C *v*. 39°C).

Many studies have been conducted on the effects of post-weaning nutrition in beef bulls. In one experiment, replicated over 2 years, 72 Angus, Angus × Simmental, or Hereford × Simmental bulls were fed either a moderate-energy (100% forage) or high-energy (80% grain, 20% forage) diet for 168 days after weaning. Bulls fed the high-energy diet were heavier ($P < 0.0001$; diet by time interaction), had thicker backfat ($P < 0.05$; diet by-line by time interaction), and had a larger scrotal circumference ($P < 0.05$; Coulter *et al*., 1997). There was no significant effect of diet on top, bottom, or average SST. However, bulls fed the moderate-energy diet had a larger (P<0.02) SST gradient (3.9°C *v*. 3.4°C).

Acknowledgments

Appreciation is expressed to numerous colleagues, students and support staff that participated in generating much of the data cited in this review.

Declaration of interest

There are no conflicts of interests to disclose.

Ethics Statement

Unpublished work cited herein was reviewed and approved by institutional animal care committees.

Software and data repository resources

No data or models are deposited in an official repository.

References

Baker DJ and Lindop PJ 1970. Oxygen cathode measurements in the mouse testis. Physiology and Medical Biology 15, 263–270.

Barros CMQ, Oba E, Brito LFC, Cook RB, Coulter GH, Groves G, Olson M and Kastelic JP 1999. Avaliação do fluxo sanguineo e do oxigenio testicular em touros Aberdeen Angus [Testicular blood flow and oxygen evaluation in Aberdeen Angus bulls]. Revista Brasileira de Reprodução Animal 23, 218–220.

Barth AD, Alisio L, Avilés M, Arteaga AA, Campbell JR and Hendrick SH 2008. Fibrotic lesions in the testis of bulls and relationship to semen quality. Animal Reproduction Science 106, 274–288.

Barth AD and Bowman PA 1994. The sequential appearance of sperm abnormalities after scrotal insulation or dexamethasone treatment in bulls. The Canadian Veterinary Journal 35, 93–102.

Barth AD and Oko RJ 1989. Abnormal morphology of bovine spermatozoa. In Defects of the sperm head (ed. AD Barth and RJ Oko), pp. 130–192. Iowa State University Press, Iowa City, IA, USA.

Brito LF, Barth AD, Wilde RE and Kastelic JP 2012. Effect of growth rate from 6 to 16 months of age on sexual development and reproductive function in beef bulls. Theriogenology 77, 1398–1405.

Brito LF, Silva AE, Barbosa RT and Kastelic JP 2004. Testicular thermoregulation in *Bos indicus*, crossbred and *Bos taurus bulls*: relationship with scrotal, testicular vascular cone and testicular morphology, and effects on semen quality and sperm production. Theriogenology 61, 511–528.

Brito LF, Silva AE, Rodrigues LH, Vieira FV, Deragon LA and Kastelic JP 2002. Effects of environmental factors, age and genotype on sperm production and semen quality in *Bos indicus* and *Bos taurus* AI bulls in Brazil. Animal Reproduction Science 70, 181–190.

Brito LFC 2006. Nutrition, metabolic hormones, and sexual development in bulls. PhD thesis, University of Saskatchewan, Saskatchewan, SK, Canada.

Burfening PJ and Ulberg LC 1968. Embryonic survival subsequent to culture of rabbit spermatozoa at 38 degrees and 40 degress C. Journal of Reproduction and Fertilility 15, 87–92.

Chen XQ, Dong J, Niu CY, Fan JM and Du JZ 2007. Effects of hypoxia on glucose, insulin, glucagon, and modulation by corticotropin-releasing factor receptor type 1 in the rat. Endocrinology 148, 3271–3278.

https://doi.org/10.1017/S1751731118001167 Published online by Cambridge University Press

Chubb C and Desjardins C 1982. Vasculature of the mouse, rat, and rabbit testis-epididymis. American Journal of Anatomy 165, 357–372.

Cook RB, Coulter GH and Kastelic JP 1994. The testicular vascular cone, scrotal thermoregulation, and their relationship to sperm production and seminal quality in beef bulls. Theriogenology 41, 653–671.

Coulter GH 1980. Testicular development: Its management and significance in young beef bulls. In Proceedings of the 8th Technical Conference on Artificial Insemination and Reproduction, National Association of Animal Breeding, 15 October 1980, Columbia, SC, USA, p. 1.

Coulter GH and Bailey DR 1988. Effects of ultrasonography on the bovine testis and semen quality. Theriogenology 30, 743–749.

Coulter GH, Cook RB and Kastelic JP 1997. Effects of dietary energy on scrotal surface temperature, seminal quality, and sperm production in young beef bulls. Journal of Animal Science 75, 1048–1052.

Dahl EV and Herrick JF 1959. A vascular mechanism for maintaining testicular temperature by counter-current exchange. Surgery, Gynecology and Obstetetrics 108, 697–705.

Free MJ 1977. Blood supply to the testis and its role in local exchange and transport of hormones. In The testis volume 4 (eds. AD Johnson and WR Gomes), pp. 39–85. Academic Press, New York, NY, USA.

Godfrey RW, Lunstra DD, Jenkins TG, Berardinelli JG, Guthrie MJ, Neuendorff DA, Long CR and Randel RD 1990. Effect of season and location on semen quality and serum concentrations of luteinizing hormone and testosterone in Brahman and Hereford bulls. Journal of Animal Science 68, 734–749.

Gun SA and Gould TC 1975. Vasculature of the testes and adnexia. In: Handbook of physiology section 7 – endocrinology (ed. RO Greep and EB Astwood), pp. 5–117. American Physiology Society, Washington, DC, USA.

Harrison RG 1949. The comparative anatomy of the blood-supply of the mammalian testes. Journal of Zoology 119 (2), 325–344.

Hees H, Leiser R, Kohler T and Wrobel K 1984. Vascular morphology of the bovine spermatic cord and testis. Light- and scanning electron-microscopic studies on the testicular artery and pampiniform plexus. Cell and Tissue Research 237, 31–38.

Hees H, Kohler T, Leiser R, Hees I and Lips T 1990. Vascular morphology of the bovine testis. light- and scanningelectron microscopic studies. Anatomischer Anzeiger 170, 119–132.

Johnston JE, Naelapaa H and Frye JB 1963. Physiological responses of Holstein, Brown Swiss and Red Sindhi crossbred bulls exposed to high temperatures and humidities. Journal of Animal Science 22, 432–436.

Kastelic JP and Brito LF 2012. Ultrasonography for monitoring reproductive function in the bull. Reproduction in Domestic Animals 47, 45–51.

Kastelic JP, Cook RB and Coulter GH 1996a. Contribution of the scrotum and testes to scrotal and testicular thermoregulation in bulls and rams. Journal of Reproduction and Fertility 108, 81–85.

Kastelic JP, Cook RB, Coulter GH and Saacke RG 1996b. Insulating the scrotal neck affects semen quality and scrotal/testicular temperatures in the bull. Theriogenology 45, 935–942.

Kastelic JP, Cook RB, Coulter GH, Wallins GL and Entz T 1996c. Environmental factors affecting measurement of bovine scrotal surface temperature with infrared thermography. Animal Reproduction Science 41, 153–159.

Kastelic JP, Cook RB and Coulter GH 1997a. Contribution of the scrotum, testes, and testicular artery to scrotal/testicular thermoregulation in bulls at two ambient temperatures. Animal Reproduction Science 45, 255–261.

Kastelic JP, Cook RB and Coulter GH 1997b. Scrotal/testicular thermoregulation and the effects of increased testicular temperature in the bull. Veterinary Clinics of North America: Food and Animal Practice 13, 271–282.

Kastelic JP, Coulter GH and Cook RB 1995. Scrotal surface, subcutaneous, intratesticular, and intraepididymal temperatures in bulls. Theriogenology 44, 147–152.

Kastelic JP, Wilde R, Bielli A, Genovese P, Bilodeau-Goeseels S and Thundathil JC 2008. Hyperthermia is more important than hypoxia as a cause of disrupted spermatogenesis. Reproduction in Domestic Animals 43, 166.

Kastelic JP, Wilde RE, Rizzoto G and Thundathil JC 2017. Hyperthermia and not hypoxia may reduce sperm motility and morphology following testicular hyperthermia. Veterinarni Medicina 62, 437–442.

Kirby A 1953. Observation on the blood supply of the bull testis. The British Veterinary Journal 109, 464.

Kram HB, Miyamoto E, Rajfer J, Appel PL and Shoemaker WC 1989. Testicular oximetry: a new method for the assessment of tissue perfusion and viability following torsion and detorsion. Journal of Pediatric Surgery 24, 1297–1302.

Lunstra DD and Coulter GH 1997. Relationship between scrotal infrared temperature patterns and natural-mating fertility in beef bulls. Journal of Animal Science 75, 767–774.

Mieusset R, Sowerbutts SF, Zupp JL and Setchell BP 1992. Increased flow of testicular blood plasma during local heating of the testes of rams. Journal of Reproduction and Fertility 94, 345–352.

Mitchell JW and Myers GE 1968. An analytical model of the counter-current heat exchange phenomena. Biophysical Journal 8, 897–911.

Parkinson TJ 1987. Seasonal variations in semen quality of bulls: correlations with environmental temperature. Veterinary Record 120, 479–482.

Pechman RD and Eilts BE 1987. B-mode ultrasonography of the bull testicle. Theriogenology 27, 431–441.

Polguj M, Jedrzejewski KS and Topol M 2011. Angioarchitecture of the bovine spermatic cord. Journal of Morphology 272, 497–502.

Rhynes WE and Ewing LL 1973. Testicular endocrine function in Hereford bulls exposed to high ambient temperature. Endocrinology 92, 509–515.

Sealfon AI and Zorgniotti AW 1991. A theoretical model for testis thermoregulation. Advanced Experimantal Medical Biology 286, 123–135.

Setchell BP 1978. Naturally occurring and induced dysfunctions of the testis. In: The Mammalian testis (ed. BP Setchell), pp. 359–422. Paul Elek, London, England.

Setchell BP, Voglmayr JK and Hinks NT 1971. The effect of local heating on the flow and composition of rete testis fluid in the conscious ram. Journal of Reproduction and Fertility 24, 81–89.

Skinner JD and Louw GN 1966. Heat stress and spermatogenesis in *Bos indicus* and *Bos taurus* cattle. Journal of Applied Physiology 21, 1784–1790.

Vogler CJ, Saacke RG, Bame JH, Dejarnette JM and McGilliard ML 1991. Effects of scrotal insulation on viability characteristics of cryopreserved bovine semen. Journal Dairy Science 74, 3827–3835.

Waites GM and Setchell BP 1964. Effect of local heating on blood flow and metabolism in the testis of the conscious ram. Journal of Reproduction and Fertility 8, 339–349.

Waites GMH 1970. Temperature regulation and the testis. In: The testis (ed. AD Johnson, WR Gomes and NL Vandemark), pp. 241–279. Academic Press, New York, NY, USA.

Waites GMH and Setchell BP 1990. Physiology of the mammalian testis. In Marshall's physiology of reproduction, Volume 2 (ed. GE Lamming), pp. 1–105. Springer-Verlag, London, England.

https://doi.org/10.1017/S1751731118001167 Published online by Cambridge University Press