



## **Effect of ambient temperature, season, age and breed on testicular thermoregulation in bulls used for artificial insemination in Tunisia**

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### **Abstract**

The objective of this study was to determine the effect of ambient temperature, season, breed and age on testicular thermoregulation by measuring scrotal surface temperature (SST), in 13 bulls (3 Holstein, 5 Tarentaise and 5 Brune des Alpes) used for artificial insemination in Tunisia. Results show that a gradient of temperature exists between the top to the bottom of the testicle. In addition, the ambient temperature affect significantly ( $P<0.05$ ) SST. However, statistical analysis showed no significant difference in testicular temperatures ( $P<0.05$ ) between breeds and the different classes of ages. In the same way, season did not affect ( $P<0.05$ ) testicular thermoregulation of Holstein and Brune des Alpes bulls. The results indicate that breed, age and season do not affect significantly testicular temperatures in bulls. However, ambient temperature affects significantly scrotal surface temperature.

**Key words:** Bull, testes, thermoregulation, breed, age

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### **Introduction**

Thermoregulation of the testes is essential for sperm production (Waites, 1970; Setchell, 1978). For normal spermatogenesis in bulls, the testes in the scrotum must be maintained at a temperature 2 to 5°C lower than body temperature (38°C). Bovine testicular temperature must not exceed 33–34.5°C (Wildevus and Entwistle, 1983; Barth and Bowman, 1994). Adverse effects of elevated testicular temperature on sperm production (Ross and Entwistle, 1979; Wolfe et al., 1985), semen quality (Wildevus and Entwistle, 1986; Coulter, 1988; Barth and Oke, 1989; Vogler et al., 1993) and male fertility (Waites, 1970; Setchell, 1978) have been documented for many species of domestic animals. Researchers have shown that the surface temperature of the scrotum is highly correlated with deep testicular temperature (Gold et al., 1977; Cena and Clark, 1978; Coulter 1988) and that scrotal surface temperature provides accurate information about testicular thermoregulation (Purohit et al., 1985; Wolfe et al., 1985; Coulter, 1988a).

The frozen semen of Holstein, Tarentaise and Brune des Alpes is extensively used for augmenting animal production among the local cows in our country for genetic up gradation. Hence, the present study was undertaken to assess the effect of ambient temperature, season, age and breed on testicular thermoregulation in bulls used for artificial insemination in Tunisia.

### **Materials and Methods**

#### **Animals**

Three Holstein bulls, five Tarentaise bulls and five Brune des Alpes bulls in the age range of 15 to 42 months, weighing 500 to 900 Kg were selected.

#### **Testicular temperatures**

Ambient temperatures varies from 12.5°C (February) to 38.9°C (August). Testicular temperature was measured by infrared thermography (Oakton Temptestr) prior to semen collection, at 3 locations on the posterior aspect of the scrotum, three cm from the top of the testis (SST<sub>1</sub>), three cm from the bottom

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(SST<sub>b</sub>) of the testis, and at the midpoint of the testis (SST<sub>m</sub>) for both left and right testes. All locations were along a vertical axis approximately 3 cm lateral to the median raphe of the posterior aspect of the scrotum.

**Statistical analyses**

All statistical analyses were performed using the Statistical Analysis System (SAS Institute, Cary, NC). Pearson’s correlation coefficient was used to calculate interrelationship between different factors and temperature. One-way ANOVA with Duncan test was used to determine the effect of breed, age, season ambient temperature on testicular temperatures. The model used for analysis of variance:

$$Y_{ij} = \mu + T_i + E_{ij}$$

Y<sub>ij</sub>: SST<sub>t</sub> : top of the testis (°C) ; SST<sub>m</sub>: the midpoint of the testis (°C) ; SST<sub>b</sub> the bottom of the testis (°C)

μ : mean

T<sub>i</sub> : effet of ambient temperature/effet of breed/ effet of season/ effet of age

E<sub>ij</sub> : error

**Results**

**Effect of ambient temperature on testicular temperatures**

Table 1 shows that all breeds and for both left and right testes, testicular temperature is high at the top of the testis, decrease progressively toward the middle of the testis and reaches the minimal value at the bottom.

The ambient temperature has a positive correlation with the testicular temperatures (top, middle and bottom of testis) for all bulls studied (Table 2).

**Relationships between the different testicular temperatures**

Testicular temperatures at different locations and for all breeds (table 3, 4 and 5) are correlated significantly (P <0.05).

**Table 1: Descriptive statistics of the testicular temperatures of the bulls**

		Holstein			Tarentaise			Brune des Alpes		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Right testis	SST <sub>t</sub>	31.50	37.80	34.62	31.40	39.10	34.69	31	38.40	34.53
	SST <sub>m</sub>	27.10	37.50	33.41	29.20	38.20	33.54	28	37.40	33.25
	SST <sub>b</sub>	27.90	38	32.13	23.30	38.30	30.13	21.30	35.80	30.70
Left Testis	SST <sub>t</sub>	30	38.30	34.74	31.20	42.70	35.05	32	39.60	34.99
	SST <sub>m</sub>	23.50	38.50	33.90	27.10	37.20	33.96	29.90	37.00	33.82
	SST <sub>b</sub>	28.70	36.80	32.23	24.80	35.90	30.76	21.40	35.30	31.09

SST<sub>t</sub> : top of the testis (°C) ; SST<sub>m</sub>: the midpoint of the testis (°C) ; SST<sub>b</sub> the bottom of the testis (°C)

**Table 2: Matrix of correlation of Pearson of testicular temperatures with ambient temperature**

	Right testis			Left testis		
	SST <sub>t</sub>	SST <sub>m</sub>	SST <sub>b</sub>	SST <sub>t</sub>	SST <sub>m</sub>	SST <sub>b</sub>
Holstein	0.64	0.55	0.63	0.40	0.49	0.62
AT Brune des Alpes	0.61	0.54	0.51	0.47	0.44	0.50
Tarentaise	0.69	0.72	0.68	0.59	0.63	0.70

AT: ambient temperature; SST<sub>t</sub>: top of the testis (°C); SST<sub>m</sub>: the midpoint of the testis (°C); SST<sub>b</sub> the bottom of the testis (°C)

**Table 3: Matrix of correlations of Pearson for testicular temperatures (Holstein)**

	Right side			Left side		
	SST <sub>m</sub>	SST <sub>b</sub>	SST <sub>t</sub>	SST <sub>m</sub>	SST <sub>b</sub>	SST <sub>t</sub>
SST <sub>t</sub>	0.80	0.64	0.48	0.70	0.62	
SST <sub>m</sub>		0.74	0.47	0.73	0.74	
SST <sub>b</sub>			0.59	0.72	0.82	
SST <sub>t</sub>				0.72	0.75	
SST <sub>m</sub>					0.77	

SST<sub>t</sub> : top of the testis (°C) ; SST<sub>m</sub>: the midpoint of the testis (°C) ; SST<sub>b</sub> the bottom of the testis (°C)

**Effect of breed on testicular temperatures**

The analysis of the variance (table 6) didn't show any significant difference between the testicular temperatures between different breeds.

**Table 4: Matrix of correlations of Pearson for testicular temperatures (Brune des Alpes)**

	Right side			Left side		
	SST <sub>m</sub>	SST <sub>b</sub>	SST <sub>t</sub>	SST <sub>m</sub>	SST <sub>b</sub>	SST <sub>t</sub>
SST <sub>t</sub>	0.70	0.40	0.55	0.57	0.50	
SST <sub>m</sub>		0.24	0.61	0.49	0.38	
SST <sub>b</sub>			0.22	0.43	0.70	
SST <sub>t</sub>				0.5	0.47	
SST <sub>m</sub>					0.66	

SST<sub>t</sub> : top of the testis (°C) ; SST<sub>m</sub>: the midpoint of the testis (°C) ; SST<sub>b</sub> the bottom of the testis (°C)

**Table 5: Matrix of correlations of Pearson for testicular temperatures (Tarentaise)**

	Right side			Left side		
	SST <sub>m</sub>	SST <sub>b</sub>	SST <sub>t</sub>	SST <sub>m</sub>	SST <sub>b</sub>	SST <sub>t</sub>
SST <sub>t</sub>	0.66	0.58	0.60	0.60	0.62	
SST <sub>m</sub>		0.65	0.54	0.70	0.61	
SST <sub>b</sub>			0.43	0.48	0.83	
SST <sub>t</sub>				0.72	0.44	
SST <sub>m</sub>					0.57	

SST<sub>t</sub> : top of the testis (°C) ; SST<sub>m</sub>: the midpoint of the testis (°C) ; SST<sub>b</sub> the bottom of the testis (°C)

**Effect of season on testicular temperatures**

The comparison by analysis of the variance of the testicular temperatures of the Holstein bulls (Table 7).

**Table 6: Variation of testicular temperatures between breeds**

Breeds	Holstein	Brune des Alpes	Tarentaise
Tssd	34.67 ± 1.83 <sup>a</sup>	34.67 ± 1.49 <sup>a</sup>	34.52 ± 1.58 <sup>a</sup>
Tmsd	33.46 ± 2.28 <sup>a</sup>	33.35 ± 2.05 <sup>a</sup>	33.61 ± 1.80 <sup>a</sup>
Tfsd	32.30 ± 2.92 <sup>a</sup>	30.94 ± 2.62 <sup>a</sup>	29.98 ± 2.80 <sup>a</sup>
Tssg	34.75 ± 2.03 <sup>a</sup>	35.16 ± 1.61 <sup>a</sup>	34.90 ± 1.78 <sup>a</sup>
Tmsg	34.04 ± 1.61 <sup>a</sup>	34.23 ± 2 <sup>a</sup>	33.91 ± 3.47 <sup>a</sup>
Tfsg	32.15 ± 2.36 <sup>a</sup>	31.39 ± 2.82 <sup>a</sup>	30.56 ± 2.54 <sup>a</sup>

The numbers of the same line that don't have the same letters are significantly different ( $P < 0.05$ ); SST<sub>tr</sub>: top of the right testis (°C); SST<sub>mr</sub>: the midpoint of the right testis (°C); SST<sub>br</sub>: the bottom of the right testis (°C); SST<sub>tl</sub>: top of the left testis (°C); SST<sub>ml</sub>: the midpoint of the left testis (°C); SST<sub>bl</sub>: the bottom of the left testis (°C)

and Brune des Alpes (Table 8) didn't reveal any significant difference between the two seasons. But (Table 9) shows that testicular temperatures of the Tarentaise bulls revealed significant differences between the two seasons for SST<sub>m</sub>, SST<sub>b</sub> for the right testis and SST<sub>b</sub> for the left side.

#### Effect of age on testicular temperatures

The comparison of the testicular temperatures (Table 10) of the bulls didn't reveal any significant difference between the two classes of ages.

## Discussion

The scrotum, testes, and testicular vasculature have a role in maintenance of testicular temperature below body temperature, which is essential for normal spermatogenesis in the bull (Setchell, 1978; Coulter and Kastelic, 1994). Infrared thermography has been

utilized as a noninvasive method for assessing scrotal surface temperature (Coulter et al., 1988). The bull scrotum is an ideal subject for infrared thermography as it has little hair cover, is a good radiator of infrared energy, and has a temperature range that is optimal for measurement by this instrument (Coulter, 1988).

Our survey shows that the temperatures of the top (SST<sub>t</sub>), the middle (SST<sub>m</sub>) and of the bottom (SST<sub>b</sub>) of the testes are generally similar to previous reports (Vogler et al., 1993; Kastelic et al., 1996). Indeed, the temperature of the bottom of the testicle was on average, lower than the other testicular temperatures. It is in agreement with other studies (Kastelic et al., 1995; Kastelic et al., 1996; Brito et al., 2004). Infrared thermograms of the scrotum from bulls with apparently normal scrotal/testicular thermoregulation had left-to-right symmetry and a decrease in temperature (typical range, 4 to 6°C) from the top of the testis (ventral to the testicular vascular cone) to the bottom (Coulter et al., 1988; Purohit et al., 1985). Indeed, the testicular temperature is maximal at the top and decreases progressively in the middle of the testis and reaches the minimal value at the bottom, confirming the existence of a temperature gradient. Kastelic et al. (1995) reported that the temperature gradient (temperature difference between the top and bottom) was pronounced on the scrotal surface. In other studies, Coulter and Kastelic (1994) reported that the scrotum has a positive temperature gradient. Kastelic et al. (1996) compared scrotal surface temperature (SST) in bulls at ambient temperatures of 15 and 25°C and was higher at the higher ambient temperature. The greatest increase in SST was at the bottom of the testes, the least increase at the top of the testes, with an intermediate

**Table 7: Effect of seasons on the testicular temperatures of the Holstein bulls**

Season	SST <sub>tr</sub>	SST <sub>mr</sub>	SST <sub>br</sub>	SST <sub>tl</sub>	SST <sub>ml</sub>	SST <sub>bl</sub>
Summer	35.67 ± 1.82 <sup>a</sup>	34.79 ± 1.36 <sup>a</sup>	34.00 ± 2.71 <sup>a</sup>	35.81 ± 1.16 <sup>a</sup>	35.51 ± 1.06 <sup>a</sup>	33.79 ± 1.86 <sup>a</sup>
Winter	34.23 ± 1.70 <sup>a</sup>	32.88 ± 2.39 <sup>a</sup>	31.56 ± 2.76 <sup>a</sup>	34.29 ± 2.18 <sup>a</sup>	33.21 ± 3.94 <sup>a</sup>	31.39 ± 2.21 <sup>a</sup>

The numbers of the same column that don't have the same letters are significantly different ( $P < 0.05$ ); SST<sub>tr</sub>: top of the right testis (°C); SST<sub>mr</sub>: the midpoint of the right testis (°C); SST<sub>br</sub>: the bottom of the right testis (°C); SST<sub>tl</sub>: top of the left testis (°C); SST<sub>ml</sub>: the midpoint of the left testis (°C); SST<sub>bl</sub>: the bottom of the left testis (°C)

**Table 8: Variation between seasons of the testicular temperatures of the Brune des Alpes bulls**

Season	SST <sub>tr</sub>	SST <sub>mr</sub>	SST <sub>br</sub>	SST <sub>tl</sub>	SST <sub>ml</sub>	SST <sub>bl</sub>
Summer	35.18 ± 0.93 <sup>a</sup>	33.94 ± 2.05 <sup>a</sup>	32.04 ± 1.58 <sup>a</sup>	35.47 ± 1.11 <sup>a</sup>	34.31 ± 1.24 <sup>a</sup>	32.43 ± 1.80 <sup>a</sup>
Winter	34.25 ± 1.73 <sup>a</sup>	32.88 ± 1.95 <sup>a</sup>	30.06 ± 2.97 <sup>a</sup>	34.92 ± 1.91 <sup>a</sup>	33.82 ± 1.84 <sup>a</sup>	30.55 ± 3.22 <sup>a</sup>

The numbers of the same column that don't have the same letters are significantly different ( $P < 0.05$ ); SST<sub>tr</sub>: top of the right testis (°C); SST<sub>mr</sub>: the midpoint of the right testis (°C); SST<sub>br</sub>: the bottom of the right testis (°C); SST<sub>tl</sub>: top of the left testis (°C); SST<sub>ml</sub>: the midpoint of the left testis (°C); SST<sub>bl</sub>: the bottom of the left testis (°C)

**Table 9: Variation inter season of the testicular temperatures of the Tarentaise bulls**

	SST <sub>tr</sub>	SST <sub>mr</sub>	SST <sub>br</sub>	SST <sub>tl</sub>	SST <sub>ml</sub>	SST <sub>bl</sub>
Summer	35.25 ± 1.60 <sup>a</sup>	34.85 ± 1.20 <sup>a</sup>	31.58 ± 1.61 <sup>a</sup>	35.81 ± 1.70 <sup>a</sup>	35.30 ± 0.90 <sup>a</sup>	32.12 ± 1.91 <sup>a</sup>
Winter	33.83 ± 1.24 <sup>a</sup>	32.46 ± 1.46 <sup>b</sup>	28.48 ± 2.47 <sup>b</sup>	34.16 ± 1.49 <sup>a</sup>	33.05 ± 2.22 <sup>a</sup>	29.10 ± 2.16 <sup>b</sup>

The numbers of the same column that don't have the same letters are significantly different ( $P < 0.05$ ); SST<sub>tr</sub>: top of the right testis (°C); SST<sub>mr</sub>: the midpoint of the right testis (°C); SST<sub>br</sub>: the bottom of the right testis (°C); SST<sub>tl</sub>: top of the left testis (°C); SST<sub>ml</sub>: the midpoint of the left testis (°C); SST<sub>bl</sub>: the bottom of the left testis (°C)

**Table 10: Effect of age on testicular temperatures**

Classes of age		15-20 months	≥ 21 months
Right testis	SST <sub>t</sub>	34.59 ± 1.54 <sup>a</sup>	34.67 ± 1.83 <sup>a</sup>
	SST <sub>m</sub>	33.49 ± 1.91 <sup>a</sup>	33.46 ± 2.28 <sup>a</sup>
	SST <sub>b</sub>	30.44 ± 2.75 <sup>a</sup>	32.30 ± 2.92 <sup>a</sup>
Left testis	SST <sub>t</sub>	36.08 ± 1.98 <sup>a</sup>	36.40 ± 1.52 <sup>a</sup>
	SST <sub>m</sub>	34.14 ± 1.81 <sup>a</sup>	33.91 ± 3.47 <sup>a</sup>
	SST <sub>b</sub>	32.15 ± 2.36 <sup>a</sup>	30.95 ± 2.70 <sup>a</sup>

The numbers of the same line that don't have the same letters are significantly different ( $P < 0.05$ ); SST<sub>t</sub>: top of the testis (°C); SST<sub>m</sub>: the midpoint of the testis (°C); SST<sub>b</sub>: the bottom of the testis (°C)

increase for average SST. The ambient temperature presents positive correlation with the testicular temperatures in the different locations (top, middle and bottom of testis) for all bulls studied. It confirms the fact that the ambient temperature has a large effect on the temperature of the scrotal surface. Kastelic et al. (1996) confirmed that bottom SST was affected most by ambient temperature, with top SST the least affected. The SST gradient was greatest at 5°C and least at 25°C. The ambient temperature has a greater effect on SST (Kastelic et al., 1996) and SST was more labile at the bottom of the scrotum than at the top.

The analysis of the variance didn't show any significant difference between the testicular temperatures of the different breeds. It is in conformity with the results of Brito et al. (2002) who showed that there were no significant differences among genetic groups for top or bottom SST or for SST gradients. The comparison of the testicular temperatures of the Holstein bulls and the Brune des Alpes bulls did not reveal any significant difference between the two seasons. But for the Tarentaise bulls, some important differences exist between the two seasons for SST<sub>m</sub>, SST<sub>b</sub> for the right testis and SST<sub>b</sub> for the left side.

The statistical analysis of the testicular temperatures of the bulls didn't reveal any significant difference between the two classes of ages. This is in agreement with Lunstra and Coulter (1997), who indicated that SST was not related to age. But, Brito et al. (2002) found an effect of age on SST gradient; the gradient was positive in bulls ≤ 36 months of age and negative in older bulls. However, there was no significant effect of age on top, bottom or average SST.

### Conclusion

Results show that breed, age and season do not affect significantly testicular temperatures in bulls. However, ambient temperature affects significantly scrotal surface temperature. Furthermore, the study of the relationship between testicular temperatures and semen quality and sperm production need further investigations.

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