



Effects Of Breed, Body Weight And Scrotal Circumference On Epididymal Sperm Abnormalities Of Imported Beef Bulls In Algeria

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Abstract: The present study was designed to investigate the influence of breed, body weight, scrotal circumference and its correlation with epididymal sperm abnormalities of 69 beef bulls imported and raised in Algeria. Semen samples were collected after slaughter from the cauda epididymis and stained using the Eosin-Nigrosin stain. Percentage of head abnormalities, detached heads, mid-piece, tail abnormalities, proximal and distal cytoplasmic droplets were recorded. The main types of sperm abnormalities observed were tail defects (33.46% ± 8.61), and distal cytoplasmic droplets (23.78% ± 12.76). Body weight and scrotal circumference have a significant effect ($p < 0,05$) on tail abnormalities, similarly, the body weight has significant effect ($p < 0,05$) on acrosome abnormalities. Body weight and scrotal circumference were negatively correlated with detached head ($p < 0,01$) and Proximal droplets ($p < 0,05$). Detached heads were more prevalent ($p < 0,05$) in Aubrac and Blonde d'aquitaine bulls compared with Charolais bulls. In brief the bulls of >650Kg body weight and scrotal circumference >35cm, show a slight improvement in performance with regard to the majority of epididymal sperm abnormalities. These finding are important for the selection and conservation of epididymal sperm from beef bulls, prior to slaughter, for use in reproductive biotechnology programs.

Key works: abnormality; beef bull; breed; scrotal circumference; epididymis; semen morphology.

1. Introduction

From an economic point of view, the fertility of the herd is of major importance for the beef producer, since all his income comes from the calves born. The use of epididymal spermatozoa in reproductive biotechnology has been widely reported in a variety of species [10,16,17,19,20,25,29], its represent an alternative for the storage and utilization of gametes recovered from individuals with acquired fertility problems or who die suddenly. Sperm collected from the epididymis tail are also fertile and morphologically similar to those collected from the ejaculate, indicating that absence of seminal plasma does not affect the morphology of epididymal

sperm[11]. Typically, scrotal circumference measurement as well as semen quality analysis are considered an initial option for evaluating fertility potential and sexual maturity (1,9)

In this study impacts of breed, body weight, and scrotal circumference on epididymal sperm abnormalities of beef bulls imported by Algeria (for meat production originate) from Europe have been evaluated.

2. Materials and methods

2.1 Animals and clinical examination

Material from 69 beef bulls originate from Europe and imported in Algeria for meat production, with an average age of 17 months, at from three different breeds raised in Algeria. Bull distribution by breed was Charolais (n = 28), Aubrac (n=32) and Blonde d'aquitaine (n = 9). Bulls were classified into 3 groups according to their breed, body weight, and scrotal circumference (Table 1).

Testis and epididymides were collected at slaughter for Annaba abattoir located in Eastern Algeria (36° 54'15''N, 7°45'5'' E) were immediately put in a container with crushed ice to be transferred to the laboratory. The body weights, scrotal circumference of animals were taken prior to slaughter and a general clinical examination was performed, together with a special clinical examination of the external genital tract by inspection and thorough palpation of testes and epididymides and gonads with an abnormal appearance (abscess, adhesions, etc.) were excluded from this study. The time to arrival at laboratory was 2 h.

Table 1: Bulls classified according to the parameters used

Parameters	Groups		
	I	II	III
Breed	Charolais / Aubrac/ Blonde d'aquitaine		
Body weight (kg)	<650 kg	650 to 750kg	> 750kg
Scrotal circumference (cm)	<35cm	35 to 37cm	> 37cm

2.2 Testicular biometry, semen collection and laboratory analyses

Prior to collecting the testes, the Body weight (BW) of each animal was recorded before slaughter using a mechanical balance. Then, the scrotal circumference (SC) was obtained with a flexible metric tape. It was measured as the largest diameter of the testes and scrotum after pushing the testes firmly into the scrotum [4,9].

Semen samples were collected from the cauda epididymal by retrograde flushing method. Cauda epididymidis and ductus deferens were isolated from the rest of the epididymis by making a cut with a scalpel near the junction of the corpus and the proximal cauda. After that, the lumen of the ductus deferens was cannulated with a blunted 22G needle. Sperm cells were then flushed in a retrograde direction from the ductus deferens through the cauda epididymidis [30].

Morphology of sperm were assessed in smears by eosin-nigrosin staining. Sperm morphology was also evaluated using a phase contrast microscope in 1000× magnification under immersion oil. A total of 100 cells were counted as described by Barth and Oko [3], the results are expressed as percentages of different abnormalities sperm[6].

Pathologies included the presence of proximal and distal cytoplasmic droplets, abnormal acrosomes, abnormal head (defects in size and shape, nuclear vacuoles, and others), detached heads, abnormalities of the midpiece (distal midpiece reflex, bowed midpiece and others) and tail (bent tail, coiled tail, and others). Cytoplasmic droplets were classified as proximal or distal cytoplasmic droplets depending on their attachment position on the cell [12].

2.3 Statistical analysis

Statistical analyses were performed using the IBM SPSS statistical 25 (Inc). The data were analysed by using descriptive statistics, simple correlation and simple regression. The relationship between the various parameters studied was verified by the significance test of the Pearson correlation coefficients (SPSS Inc). The p-value of < 0.05 were considered significant. A 1 factor analysis of variance (ANOVA) (SPSS) was used.

3. Results

The presence of proximal and distal cytoplasmic droplets, Head abnormalities, acrosome abnormalities, detached heads, mid-piece and tail abnormalities owing to breed, body weight and scrotal circumference of the bulls under study have been presented in Figure 1 and Figure 2, respectively.

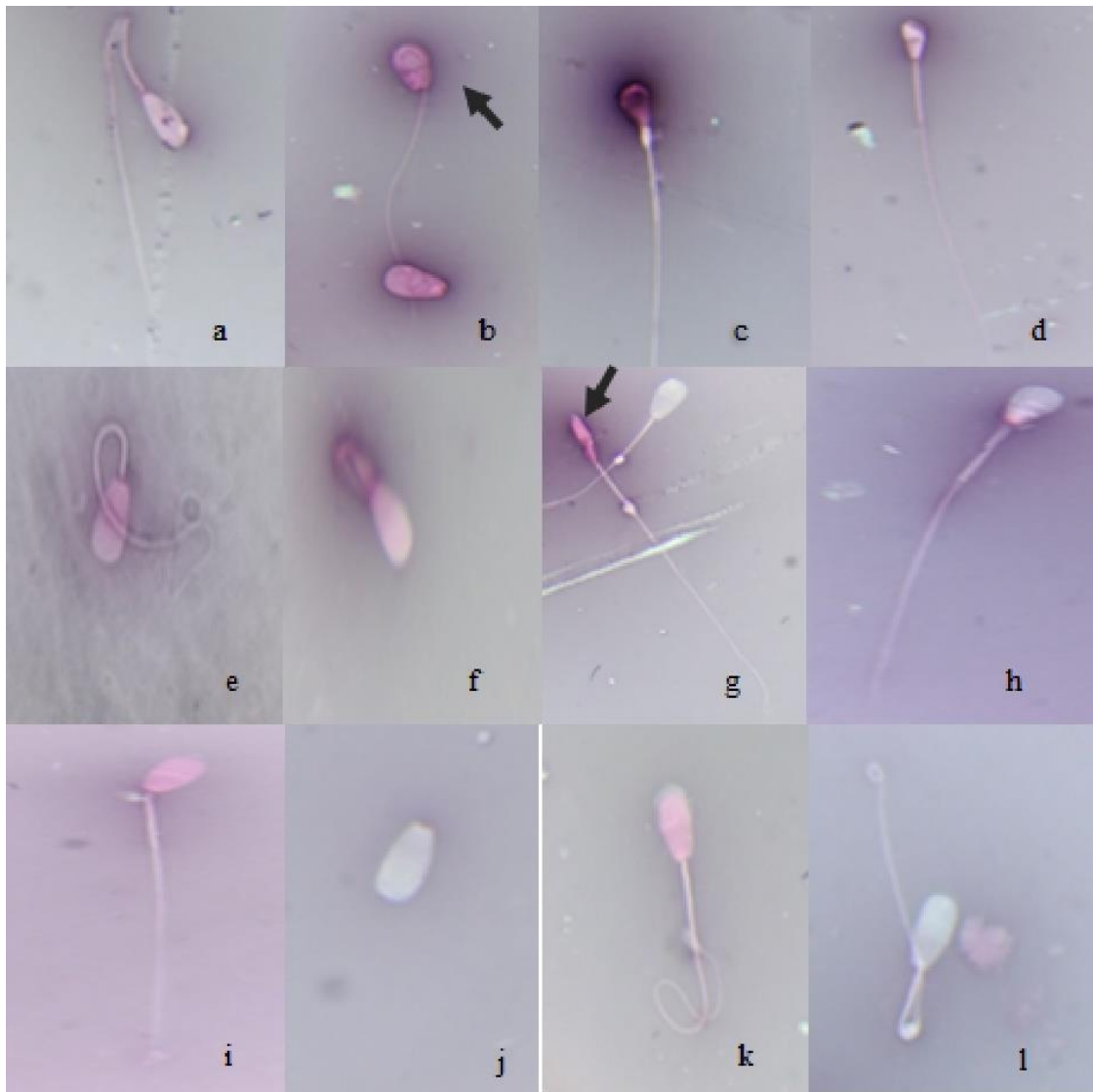


Figure 1. Different morphological abnormalities in bull sperm cells (simple anomaly) (Phase contrast optics, 1000x). (a, i) Bent tail, (b) round head, (c) pyriform head, (d) small abnormal head, (e, f, k) coiled tail, (g) narrow head, (h) pyriform head, (j) detached head, (l) folded tail. Eosin–nigrosin-stained smears.

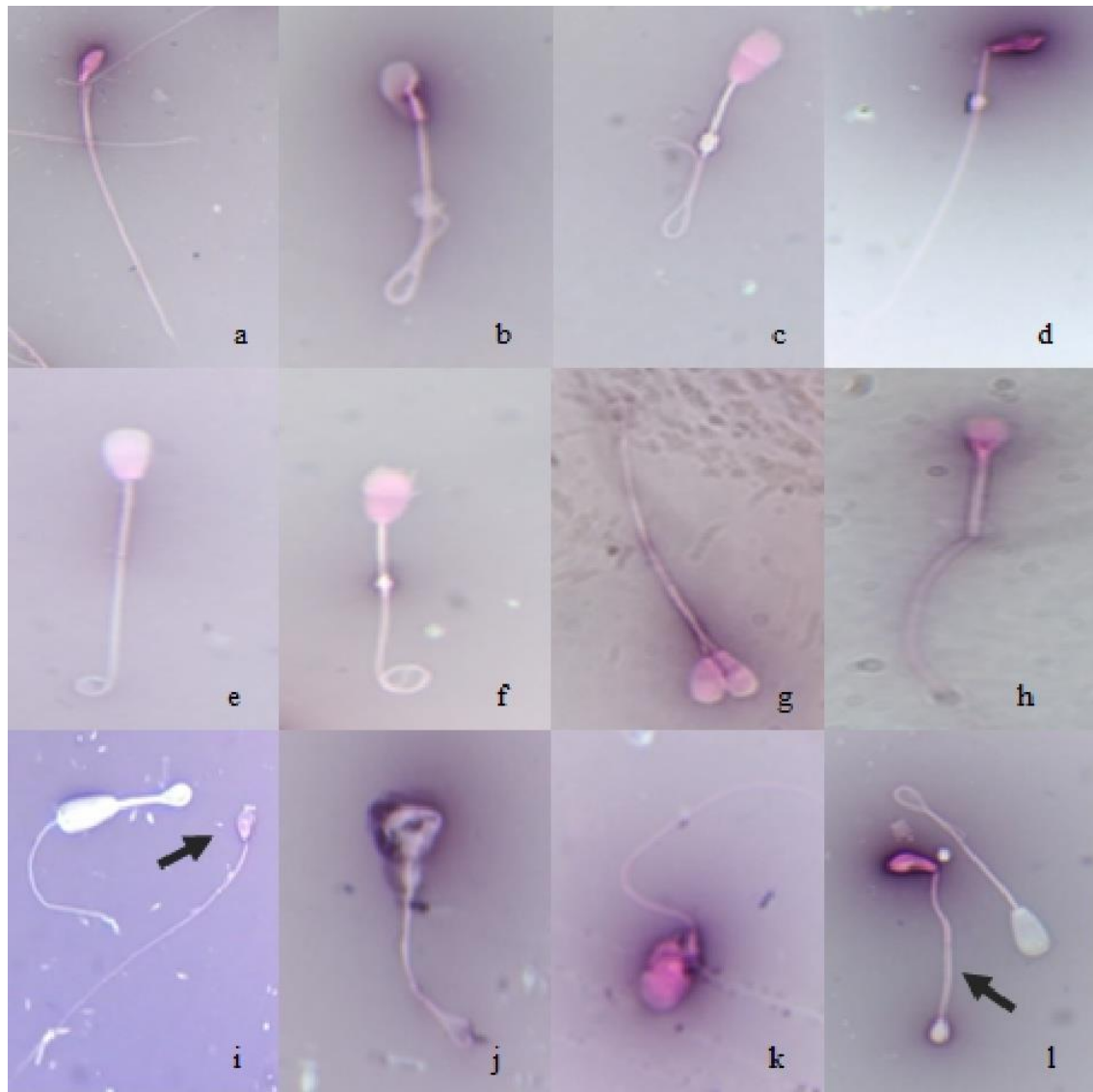


Figure 2. Different morphological abnormalities in bull sperm cells (associated anomalies) (Phase contrast optics, 1000x). (a) Double tail and pyriform head, (b) acrosome defect and folded tail, (c) distal droplet and anticoiled tail, (d) bent with distal droplet and coiled tail, (e) Round head and terminally coiled, (f) distal cytoplasmic droplet and terminally coiled, (g) Double head and tails, (h) thick midpiece and pyriform head, (i) underdeveloped head, (j) giant head with proximal droplet and terminally coiled, (k) giant head, (l) pinhead and terminally coiled. Eosin–nigrosin-stained smears.

3.1 Breed:

Data corresponding to values obtained for sperm abnormalities according to the breed of Beef Bulls are presented in Table 2. Overall, the percentage of tail (33.46% \pm 8.61%) and distal cytoplasmic droplet defects (23,78% \pm 12.76%) recorded represent the highest abnormalities in the three breeds bulls.

Even though the percentage of distal and proximal cytoplasmic droplets abnormalities and abnormal acrosome were slightly higher in charolais bulls compared with other breeds, there was no significant difference between breed and sperm abnormalities. Except for, the mean percent of detached heads were higher ($P < 0.01$) in Aubrac and blonde d'aquitaine bulls compared with charolais bulls.

Table 02: Mean \pm SD of sperm abnormalities according to the breed of Beef Bulls

Parameter	Breed			Overall
	Charolais (n = 28)	Aubrac (n = 32)	Blonde d'aquitaine (n = 9)	
Head abnormalities (%)	2,71 \pm 1,69	3,23 \pm 4,14	3,90 \pm 4,98	3,11 \pm 3,46
Tail abnormalities (%)	34,99 \pm 10,52	32,40 \pm 6,70	32,48 \pm 8,35	33,46 \pm 8,61
Midpiece abnormalities (%)	00,00	0,08 \pm 0,39	0,09 \pm 0,28	0,05 \pm 0,28
Proximal droplets (%)	3,19 \pm 3,12	2,51 \pm 1,12	2,55 \pm 1,11	2,7 \pm 2,1
Distal droplets (%)	21,68 \pm 11,77	25,69 \pm 13,55	23,55 \pm 13,22	23,78 \pm 12,76
Detached heads (%)	0,92 \pm 1,47 ^a	2,96 \pm 3,79 ^b	3,62 \pm 5,44 ^b	2,22 \pm 3,48
Abnormal Acrosome (%)	0,12 \pm 0,31	0,08 \pm 0,32	0,00 \pm 0,00	0,09 \pm 0,29

3.2 Body weight:

The average results of the sperm abnormalities according to the body weight of Beef Bulls were shown in table 3. The body weight was found to have a significant effect on the acrosome and tail sperm abnormalities ($P < 0,05$). The highest proportion of total sperm abnormalities (72,18%) was recorded in bulls of <650 kg body weight, and the lowest in >750 kg weight groups (61,91%).

Table 03: Mean \pm SD of sperm abnormalities according to the body weight of Beef Bulls

	BW	Overall
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Parameter	<650 kg (n = 20)	650 to 750kg (n = 11)	> 750kg (n = 38)	
Head abnormalities (%)	3,25 ± 1,53	2,65±0,77	3,16 ± 4,54	3,11± 3,46
Tail abnormalities (%)	37,71 ± 10,16 ^a	33,48 ± 5,64 ^b	31,22 ± 7,74 ^b	33,46± 8,61
Midpiece abnormalities (%)	00,00	0,00	0,09 ± 0,38	0,05± 0,28
Proximal droplets (%)	3,26 ± 3,26	3,35 ± 2,21	2,39 ± 1,21	2,79± 2,16
Distal droplets (%)	26,01 ± 16,60	25,03 ± 5,76	22,25 ± 11,97	23,78± 12,76
Detached heads (%)	1,74 ± 1,48	1,27 ± 1,50	2,75 ± 4,45	2,22± 3,48
Abnormal Acrosome (%)	0,21 ± 0,46 ^a	0,00 ± 0,00 ^b	0,05 ± 0,19 ^b	0,09± 0,29

^{a, b} *p*<0.05 Means with different superscript within classes differ significantly

3.3 Scrotal circumference:

The results on the sperm abnormalities due to scrotal circumference of the experimental bulls are presented in table 4. A significant effect of SC on tail abnormalities was recorded (*p*<0,05). The highest percentage was observed in <35cm SC, and the lowest in >37cm scrotal circumference groups. The variations of the other sperm abnormalities studied were not significant between the groups (*p*>0,05).

Table 04: Mean ± SD of sperm abnormalities according to the scrotal circumference of Beef Bulls

Parameter	CS			Overall
	<35cm (n = 18)	35 to 37cm (n = 23)	> 37cm (n = 28)	
Head abnormalities (%)	3,16 ± 1,61	2,44±1,18	3,62 ± 5,18	3,11± 3,46
Tail abnormalities (%)	37,69 ± 10,99 ^b	32,84 ± 5,26 ^a	31,25 ± 8,43 ^a	33,46± 8,61
Midpiece abnormalities (%)	00,00	0,09 ± 0,44	0,05 ± 0,20	0,05± 0,28
Proximal droplets (%)	3,40 ± 3,44	3,01 ± 1,78	2,23 ± 1,08	2,79± 2,16
Distal droplets (%)	23,04 ± 15,86	25,35 ± 12,19	22,97 ± 11,28	23,78± 12,76
Detached heads (%)	1,37 ± 1,24	2,66 ± 3,72	2,40 ± 4,19	2,22± 3,48
Abnormal Acrosome (%)	0,14 ± 0,34	0,13 ± 0,39	0,01 ± 0,09	0,09± 0,29

^{a, b} *p*<0.05 Means with different superscript within classes differ significantly

3.4 Correlation between all parameters

Correlation between breed, body weight, scrotal circumference and sperm abnormalities are listed in table 5.

Table 5: Pearson correlation between breed, body weight, scrotal circumference and epididymal sperm abnormalities in beef bulls

	Normal morphology	Head abnormalitie (%)	Detached head	Abnormal Acrosome	Proximal droplet	Distal droplet	Midpiece abnormalitie	Tail abnormalitie
BW	0,30*	-0,08	-0,37**	0,09	-0,28*	-,122	0,17	-0,23
SC	0,22	-0,01	-0,33**	0,05	-0,27*	-,069	0,21	-0,16
Breed	0,07	0,11	0,30*	-0,12	-0,13	0,09	0,14	-0,12

** Correlation is significant at the 0.01 level.

* Correlation is significant at the 0.05 level.

SC : Scrotal Circumference ; BW : body weight

4. Discussion

The effects of breed, scrotal circumference and body weight on the incidence of sperm abnormalities are detailed in the following paragraphs.

In the present study, the predominant anomaly recorded on all samples was that of the tail ($33.46\% \pm 8.61$), followed by the presence of distal cytoplasmic droplets with a frequency of $23.78 \pm 12.76\%$. It has been reported that the high percentage of tail abnormality found may be an artifact, as a result of thermal shocks when transporting testes in ice packs from the slaughterhouse to the laboratory [3,22]. There are other underlying factors that may contribute to the tail abnormality such as poor testicular thermoregulation, testicular degeneration, hypo-osmotic conditions or epididymal transit failures [18]. The present study indicated that body weight and scrotal circumference of bulls had significant effect on the percentage of tail abnormalities ($p < 0,05$), where the highest value was recorded from bulls with body weight >650 kg ($37,71 \pm 10,16\%$) and scrotal circumference <35 cm ($37,69 \pm 10,99\%$), the lowest value was recorded in bulls with body weight >750 kg ($31,22 \pm 7,74\%$) and scrotal circumference >37 cm ($31,25 \pm 8,43$). Body weight were positively correlated ($p < 0,05$) to percent of the normal morphology.

The second main anomaly is the presence of distal cytoplasmic droplets with a frequency of $23.78 \pm 12.76\%$ which is in agreement with the results of Martin et al [16] and lower than that reported by chaveiro et al; Goovaerts et al and Bertol et al [5,7,13,28]. Distal cytoplasmic droplets are simply remnants of spermatid cytoplasm on spermatozoa occurring during the process of spermiogenesis, reflecting one of the most prominent

changes during the maturation process [24]. Their presence is considered normal for spermatozoa recovered directly from the cauda of the epididymis of bulls [5], since their appearance on spermatozoa by an unnatural way is a physiological process, their existence simply reflects a maturation phase before ejaculation [30]. However, epididymal spermatozoa even with distal cytoplasmic droplets have the capacity of fertilization as it was shown by Martin et al [17]. Sperm with distal cytoplasmic droplets can be used in artificial insemination and in particular in assisted reproductive techniques such as in vitro fertilization and intracytoplasmic injection in studies performed in cattle [15,17,19,21]. No significant effect was noted on the appearance of distal cytoplasmic droplets between groups ($p > 0,05$).

Abnormalities of the head, proximal cytoplasmic droplet, and acrosome are classified as major defects, associated with reduced fertility [8]. The prevalences of spermatozoa with abnormal heads, proximal cytoplasmic droplet and abnormal acrosomes recorded in our study are relatively low ($3.11 \pm 3.46\%$), ($2.79 \pm 2.16\%$) and ($0.09 \pm 0.29\%$) respectively and are lower than those reported in other studies [5,7,13,22]. The body weight was found to have a significant effect on the acrosome abnormalities ($p < 0.05$). The highest percentage of acrosome abnormalities was observed in $<650\text{kg}$ ($0,21 \pm 0,46\%$) and the lowest in $650 < 750\text{kg}$ ($00,00\%$) weight groups. Similar impact of body weight on semen characteristics in bulls was also recorded earlier by others [14,26,27,31]. Body weight and scrotal circumference were negatively correlated ($p < 0,01$, $p < 0,05$) with Proximal droplets.

The percentages of sperm recorded with detached heads were $2.22 \pm 3.48\%$, similar to the results ($5.4 \pm 5.6\%$) of Persson et al [22], The bulls in this study were never used for breeding purposes and thus the increase in this abnormality could be due to accumulation of sperm in the epididymis, which leads to degradation of the head-tail junction according to Barth [2]. In our study, there was significant variation in the percentage of detached head abnormalities ($p < 0,05$) between the breed bulls under study, where the lowest percentage was recorded in charolais bulls, followed by Aubrac and Blonde d'aquitaine bulls. Body weight and scrotal circumference were negatively correlated ($p < 0,01$, $p < 0,05$) with detached head.

5. Conclusion

In brief scrotal circumference body weight and breed influenced the prevalence of some epididymal sperm abnormalities. This study suggest that the bulls of $>650\text{Kg}$ body weight and scrotal circumference $>35\text{cm}$, show a slight improvement in performance with regard to the majority of epididymal sperm abnormalities. These finding are important for the selection and conservation of epididymal sperm from beef bulls, prior to slaughter, for use in reproductive biotechnology programs.

REFERENCE

- 1 Alapati R., Stout M., Saenz J., Gentry Jr G., Godke R. & Devireddy R. 2009. Comparison of the permeability properties and post-thaw motility of ejaculated and epididymal bovine spermatozoa. *Cryobiology*. 59(2): 164–170.
- 2 Barth A.D. 2013. Bull Breeding Soundness Evaluation Manual | The Western Canadian Association of Bovine Practitioners. Available at: <<https://www.wcabp.com/products/bull-breeding-soundness-evaluation-manual>>. Accessed 03/2022.
- 3 Barth A.D. & Oko R. 1989. Abnormal morphology of bovine spermatozoa.
- 4 Barth A.D. & Ominski K.H. 2000. The relationship between scrotal circumference at weaning and at one year of age in beef bulls. *The Canadian Veterinary Journal*. 41(7): 541.
- 5 Bertol M.A.F., Weiss R.R., Thomaz-Soccol V., Kozicki L.E., Fujita A.S., Abreu R.A. de & Green K.T. 2013. Viability of bull spermatozoa collected from the epididymis stored at 18-20 C. *Brazilian Archives of Biology and Technology*. 56(5): 777–783.
- 6 Brito L., Barth A., Wilde R. & Kastelic J. 2012. Effect of growth rate from 6 to 16 months of age on sexual development and reproductive function in beef bulls. *Theriogenology*. 77(7): 1398–1405.
- 7 Chaveiro A., Cerqueira C., Silva J., Franco J. & da Silva F.M. 2015. Evaluation of frozen thawed cauda epididymal sperms and in vitro fertilizing potential of bovine sperm collected from the cauda epididymal. *Iranian Journal of Veterinary Research*. 16(2): 188.
- 8 Chenoweth P.J. 2005. Genetic sperm defects. *Theriogenology*. 64(3): 457–468.
- 9 Claus L.A.M., Barca Junior F.A., Junior C.K., Pereira G.R., Favaro P. da C., Ferreira F.P., Galdioli V.H.G., Seneda M.M. & Ribeiro E.L. de A. 2021. Testicular shape, scrotal skin thickness and testicular artery blood flow changes in bulls of different ages. *Reproduction in Domestic Animals*. 56(7): 1034–1039.
- 10 Cunha A., Carvalho J., Kussano N., Martins C., Mourão G.B. & Dode M. 2016. Bovine epididymal spermatozoa: Resistance to cryopreservation and binding ability to oviductal cells. *Cryobiology*. 73(3): 348–355.
- 11 Cunha A.T.M., Silva L.P., Carvalho J.O. & Dode M.A.N. 2020. Shape and size of epididymal sperm from Gir bulls using atomic force microscopy: A nanoscale characterization of epididymal sperm. *Reproductive Biology*. 20(1): 37–41.
- 12 García-Vázquez F.A., Hernández-Caravaca I., Matás C., Soriano-Úbeda C., Abril-Sánchez S. & Izquierdo-Rico M.J. 2015. Morphological study of boar sperm during their passage through the female genital tract. *Journal of Reproduction and Development*. 2014–170.
- 13 Goovaerts I., Hoflack G., Van Soom A., Dewulf J., Nichi M., de Kruif A. & Bols P. 2006. Evaluation of epididymal semen quality using the Hamilton–Thorne analyser indicates variation between the two caudae epididymides of the same bull. *Theriogenology*. 66(2): 323–330.
- 14 Gopinathan A., Sivaselvam S., Karthickeyan S. & Kulasekar K. 2018. Effect of body weight and scrotal circumference on semen production traits in crossbred Holstein Friesian bulls. *The Indian Journal of Animal Reproduction*. 39(1): 24–27.
- 15 Howes E., Hurst S. & Jones R. 2001. Actin and actin-binding proteins in bovine spermatozoa: potential role in membrane remodeling and intracellular signaling during epididymal maturation and the acrosome reaction. *Journal of Andrology*. 22(1): 62–72.
- 16 Martins C., Driessen K., Costa P.M., Carvalho-Neto J., De Sousa R., Rumpf R. & Dode M. 2009. Recovery, cryopreservation and fertilization potential of bovine spermatozoa obtained from epididymides stored at 5 C by different periods of time. *Animal Reproduction Science*. 116(1–2): 50–57.
- 17 Martins C., Rumpf R., Pereira D. & Dode M. 2007. Cryopreservation of epididymal bovine spermatozoa from dead animals and its uses in vitro embryo production. *Animal Reproduction Science*. 101(3–4): 326–331.
- 18 Mwaanga E.S., Kataba A., Parés Casanova P.-M., Phiri E. & Lundu T. 2014. Caudal epididymal sperm morphology and body measurements relationships of the Gwembe dwarf bucks. *International Journal of Agricultural Policy and Research*, 2014, Vol. 2, Núm. 10, p. 346-351.
- 19 Nazari H., Ahmadi E., Hosseini Fahraji H., Afzali A. & Davoodian N. 2021. Cryopreservation and its effects on motility and gene expression patterns and fertilizing potential of bovine epididymal sperm. *Veterinary Medicine and Science*. 7(1): 127–135.

- 20 Papa F.O., Melo C., Fioratti E., Dell'Aqua Jr J., Zahn F. & Alvarenga M.A. 2008. Freezing of stallion epididymal sperm. *Animal Reproduction Science*. 107(3–4): 293–301.
- 21 Perry V. 2021. The role of sperm morphology standards in the laboratory assessment of bull fertility in Australia. *Frontiers in Veterinary Science*. 8: 672058.
- 22 Persson Y., McGowan M. & Söderquist L. 2006. Comparison between the sperm morphology in semen samples obtained from yearling beef bulls by transrectal massage of the ampullae and cauda epididymal dissection. *Reproduction in Domestic Animals*. 41(3): 233–237.
- 23 Persson Y. & Söderquist L. 2005. The proportion of beef bulls in Sweden with mature spermograms at 11–13 months of age. *Reproduction in Domestic Animals*. 40(2): 131–135.
- 24 Robaire B. & Hermo L. 1988. Efferent ducts, epididymis, and vas deferens: structure, functions, and their regulation. *The Physiology of Reproduction*. 1: 999–1080.
- 25 Santiago-Moreno J., Toledano-Díaz A., Pulido-Pastor A., Gómez-Brunet A. & López-Sebastián A. 2006. Birth of live Spanish ibex (*Capra pyrenaica hispanica*) derived from artificial insemination with epididymal spermatozoa retrieved after death. *Theriogenology*. 66(2): 283–291.
- 26 Sarder M., Joarder O. & Ali M. 2001. Studies on phenotypic and genotypic variation in the semen traits of seven AI bulls. *Bangladesh J. Genet. Biotechnol.* 2: 35–42.
- 27 Siddiqui M., Bhattacharjee J., Das Z., Islam M., Islam M., Haque M., Parrish J. & Shamsuddin M. 2008. Crossbred bull selection for bigger scrotum and shorter age at puberty with potentials for better quality semen. *Reproduction in Domestic Animals*. 43(1): 74–79.
- 28 Silva A.E.D.F., Dias A.L., Unanian M.M., Freitas A.R. de & Bloch Junior C. 2003. Peptides content and morphophysiological evaluation of epididymis and ejaculated sperm in bovine. *Revista Brasileira de Zootecnia*. 32(6): 1890–1900.
- 29 Toyonaga M., Sato Y., Sasaki A., Kaihara A. & Tsutsui T. 2011. Artificial insemination with cryopreserved sperm from feline epididymides stored at 4 C. *Theriogenology*. 76(3): 532–537.
- 30 Turri F., Madeddu M., Gliozzi T., Gandini G. & Pizzi F. 2012. Influence of recovery methods and extenders on bull epididymal spermatozoa quality. *Reproduction in Domestic Animals*. 47(5): 712–717.
- 31 Waite R., Dwyer C., Beggs D., Mansell P., Stevenson M. & Pyman M. 2019. Scrotal circumference, bodyweight and semen characteristics in growing dairy-breed natural-service bulls in Tasmania, Australia. *New Zealand Veterinary Journal*. 67(3): 109–116.