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Sperm morphological attributes in indigenous male goats raised under extensive husbandry in Ethiopia

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Abstract

This study assessed the morphological attributes of epididymal spermatozoa from indigenous goat breeds managed under conditions of extensive husbandry in Ethiopia. Seventy-four healthy male goats from 5 breeds (Arsi-Bale [AB], Afar, Central Highlands [CH], Boran, and Woito-Guji [WG]) and 3 age groups (< 14 mo [younger]; 14.0 – 19.5 mo [intermediate]; 19.6 – 24 mo [older]) were randomly selected, and their epididymal spermatozoa were evaluated for morphology. While in caput and corpus, the proportion of detached heads, proximal droplets, abnormal mid-pieces, and simple bent tails were greatest ($P < 0.05$). Simple bent tails were lowest in the cauda epididymides whereas inversely the proportion of distal droplets and coiled tail defects was greatest ($P < 0.05$) in the cauda. In the cauda epididymides, defective acrosomes were greatest ($P < 0.05$) for CH goats and the least for Boran and WG. The proportion of loose sperm heads was greatest ($P < 0.05$) for AB. The occurrence of narrow and variable-sized spermatozoa was greater ($P < 0.05$) for lowland (Afar, Boran, and WG) than highland (AB, CH) goat breeds. Afar and CH goats had more ($P < 0.05$) coiled tails than the rest of the goat breeds. Indigenous goats at a younger age had an increased ($P < 0.05$) proportion of loose heads while those in the older age group had a greater ($P < 0.05$) proportion of acrosome defects. Older goats also had an increased ($P < 0.05$) proportion of distal droplets and tail defects compared to goats at a younger age. In conclusion, while sperm-head morphological abnormalities were not severely influenced by breed and age group, these variables affected proportions of loose sperm heads and acrosome defects.

Keywords: sperm morphology; tropical male goats; age; breed; epididymis.

Introduction

We previously focused on the characterization of body size and testicular traits of five indigenous goat breeds under extensive husbandry in Ethiopia (Mekasha *et al.*, 2007). These goat breeds are the dominant slaughter flock for export of goat meat by modern Ethiopian abattoirs.

Collection of ejaculates from domestic animals under extensive husbandry in the tropics is not a simple matter owing to the presence of a multiple-sire herd system, male dominance, and the continuous mating system that makes ejaculation frequency uncontrollable (Chacon, 2001). Moreover, the situation is most often complicated by the lack of adequate infrastructure, which hinders access to the various males of interest. Instead, epididymal spermatozoa have been widely used to accurately characterize sperm morphological attributes in domestic animals, as they are considered representative of testicular function as well as epididymal function and are as fertile as sperm collected from an ejaculate (Igboeli and Foote, 1968; Einarsson *et al.*, 1979). Moreover, evaluation of spermatozoa from the epididymis also enables understanding of sperm maturation events in the different segments of the duct.

The morphological characteristics of spermatozoa are influenced by several factors including the genetic make-up and physiological stage of the animal, nutrition, season, climatic factors, and disease (Barth and Oko, 1989; Dowsett and Knott, 1996; Dana *et al.*, 2000). This implies that evaluation of sperm morphology should be performed along with clinical evaluations for different breeds at different ages and in different environments before using them for breeding. In Ethiopia, such information is non-existent for indigenous male goats under extensive husbandry, despite the fact that the country has a diverse agro-climate and a variety of goat production systems that manage a number of different goat breeds. The fact that the country is situated close to the equator makes it clear that a seasonal effect may be negligible as tropical goats have the ability to breed all year round (Delgadillo *et al.*, 1997). Besides, depending upon nutritional status and breed, most tropical male goats may attain puberty at about 8 mo of age (Madani and Rahal, 1988; Delgadillo *et al.*, 1997).

Previous studies have shown that sperm morphology has a close association with fertility (Williams and Savage, 1925; Lagerlöf, 1934; Soderquist *et al.*, 1991). Poor sperm morphology is an indicator of decreased fertility in many species, including goats (Chandler *et al.*, 1988). An accurate morphological

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examination of spermatozoa thus enables the elimination of males with potentially low fertility prior to the preservation of their semen (Rodriguez-Martinez and Barth, 2007). The present study was conducted to investigate the morphological attributes of epididymal spermatozoa in indigenous male goats under extensive husbandry in Ethiopia.

Materials and Methods

Study population

The study population comprised male goats from 5 breeds indigenous to Ethiopia: Arsi-Bale (AB), Afar, Central Highlands (CH), Boran (also known as "Long-eared Somali"), and Woito-Guji (WG). These are the dominant breeds employed by the chosen abattoirs, as they are marketed within a close radius. However, due to diverse agro-climates and production systems, the distribution of goat breeds differs throughout the country. Thus, the AB and CH breeds dominate the highland agro-climate with its mixed crop-livestock production system while Afar, WG, and Boran dominate the arid and semi-arid agro-climates where pastoral and agro-pastoral forms of production are found. These breeds are described in detail elsewhere (Farm-Africa, 1996; Mekasha *et al*., 2007).

Study site

The field study was conducted at Debre-Zeit (HELMEX) and Mojo (MODERN) abattoirs, which are located about 45 and 75 km, respectively, east of the Ethiopian capital, Addis Ababa. Microscopic evaluation was performed at the sperm laboratory of the Division of Reproduction, Department of Clinical Sciences, Swedish University of Agricultural Sciences (SLU), Sweden.

Sampling

After regular clinical examination, apparently healthy, male goats were screened from each breed as a slaughter flock. From this slaughter flock, representative male goats of each breed were selected using a stratified random sampling technique (AB =15 Afar = 15, CH = 10, Boran = 16, and WG = 18). Following sampling, each goat was given an identification number marked on a plastic tag, which remained with the animal's carcass throughout the slaughter process until the testes and epididymides were carefully removed from each carcass and placed into a pre-labeled plastic bag. Each animal's age was determined from dentition based on the number of permanent incisors (full teeth = < 14 mo [n = 28]; one erupted pair of incisors = 14 – 19.5 mo [n = 32]; two erupted pairs of incisors = 19.6 – 24 mo [n = 14]) as previously reported for African indigenous goats (Wilson, 1989).

Data collection procedure

Sample collection and staining

Immediately after removal, the paired testes and epididymides were transported to the nearby laboratory (International Livestock Research Institute) in a polystyrene box with icebox clamps. Upon arrival at the laboratory, the epididymides were carefully separated from the testes and split into the 3 anatomical segments: caput (head), corpus (body), and cauda (tail). After recording the weight of the segments and testis of paired testicles for a different study, each segment of the right epididymis was incised with a scalpel blade and spermatozoa were aspirated with a pipette into a labeled vial containing buffered formalin solution (Hancock, 1957) for fixation. Two smears were prepared from each sample. The first smear was dried overnight, fixed with a flame, and stained with Williams (carbol-fuchsin-eosin) solution for the evaluation of sperm head morphology as described by Williams (1920) and modified by Lagerlöf (1934). The second smear was made discontinuously to form dense ridges, dried and fixed similarly to the first smear, and stained following a method described by Papanicolaou (1942) for the determination of cells present other than spermatozoa.

Evaluation of sperm morphology

Formalin-fixed sperm samples collected from the different segments of the epididymides were evaluated for sperm morphology both in wet smears and in stained samples. Two-hundred spermatozoa in the wet smears were counted using a phase-contrast microscope at 1,000x magnification with an oil-immersion objective lens. Spermatozoa that displayed morphological abnormalities (proximal cytoplasmic droplet, distal cytoplasmic droplet, loose heads, acrosome defects, acrosome abnormality, nuclear pouches, abnormal mid-piece, simple bent tails, tail coiled under the head, or double-coiled tails) were identified. For evaluation of sperm head morphology, 500 spermatozoa were examined in Williams-stained slides using a light microscope at 1,000x magnification, and the numbers of sperm head abnormalities (pear shape, narrowness at the base, abnormal contour, undeveloped head, narrowness, abaxial implantation, or abnormal loose head) were counted. The relative proportion of morphologically-normal spermatozoa was estimated from both the wet smear- and Williams-stained samples and presented as a range (e.g., the proportion of spermatozoa without defects neither in wet smears nor in Williams-stained samples). Distinctions were made when accounting for cytoplasmic droplets as sperm defects, considering their temporal (proximal) or permanent (distal) location while sperm matured throughout the epididymides. The smears with ridges, stained by the method of

Papanicolaou, were examined using a light microscope at a magnification of 100x, 250x, or 400x for the presence and relative quantity of cells other than spermatozoa (foreign cells) such as cells of the seminiferous epithelium, the epididymides, and inflammatory cells (such as leukocytes). The relative presence of each foreign cell type was classed as: 0 = absent, 1 = scarce, 2 = moderate, or 3 = rich to very rich.

Statistical analyses

Data of sperm morphological attributes were square-root transformed prior to analysis. The General Linear Model (GLM) procedure of SAS was employed for the analysis of the transformed data (SAS, 2004). Breed, age, and segment of the epididymides were considered class variables but analyzed separately. While the effect of breed and age were analyzed based on data from caudae epididymis, sperm maturation was assessed based on data from the 3 segments of the epididymis (caput, corpus, and cauda). Mean differences were examined with Tukey's adjustments and presented as least-squares means (LSM) along with the pooled SEM. Differences due to breed, age, or segment of the epididymides were considered significant at $P < 0.05$.

Results

Effects on sperm maturation

There were no major differences in the number of most sperm head abnormalities along the different segments of the epididymides (caput, corpus, and cauda; Table 1). However, spermatozoa collected from the corpus epididymides had greater proportions of acrosome defects ($P < 0.05$). Similarly, numerically greater proportions of spermatozoa with narrow bases and loose abnormal heads were recorded in the caput epididymides. Nevertheless, even though acrosome abnormality, pear-shaped heads, and total number of pathological heads were numerically less in the cauda epididymis, it was not significant. While a greater ($P < 0.05$) proportion of detached sperm heads were seen in caput and corpus epididymides, it was significantly lower in the cauda. Furthermore, the numerical differences in abaxial implantation and abnormal contour did not reach significance.

Table 1. Sperm morphology (least-squares mean percentage) in indigenous Ethiopian goat spermatozoa collected from different regions of the epididymis (caput, corpus, or cauda).

Variable	Region of the epididymis			SEM
	Caput	Corpus	Cauda	
Sperm head shapes				
Pear shape	4.0	4.3	2.6	0.25
Narrow at the base	0.89	0.66	0.19	0.08
Abnormal contour	0.24	0.24	0.11	0.03
Undeveloped	0.68	0.47	0.68	0.13
Loose abnormal head	0.94	0.69	0.72	0.11
Narrow	0.26	0.34	0.55	0.04
Variable size	3.7	4.6	5.0	0.46
Abaxial implantation	0.11	0.17	0.10	0.01
Total abnormal head shapes	10.8	11.5	9.7	0.74
Loose heads	13.3^{a}	15.5^{a}	4.7^{b}	0.50
Acrosome defects	3.5^{b}	6.6^{a}	4.2^{b}	0.41
Acrosome abnormality	0.38	0.65	0.39	0.08
Nuclear pouches	0.10^{b}	0.20^{b}	0.53^{a}	0.06
Cytoplasmic droplets				
Proximal	31.5^{a}	5.6^{b}	2.8^{c}	0.66
Distal	3.1^{c}	14.0^{b}	31.2^{a}	0.87
Abnormal mid-piece	2.1^{a}	2.7^{a}	1.2^{b}	0.18
Tail defects				
Simple bent tail	7.2^{a}	7.2^{a}	0.6^{b}	0.33
Coiled tail under head	8.4^{a}	6.6^{b}	3.0^{c}	0.34
Coiled tail double folded	4.7^{a}	4.2^{b}	2.2^{c}	0.23
Total	20.6^{a}	18.0^{b}	5.9^{c}	0.68
Normal spermatozoa* (mean range)	(53 - 65)	(46 - 58)	(71 - 81)	(0.8 - 1.3)

[a, b] Means with different superscripts in the same column are significantly ($P < 0.05$) different.

* Included proximal droplets in caput and corpus and distal droplets across the epididymal segments.

The relative percentages of spermatozoa with cytoplasmic droplets, abnormal mid-pieces, and tail defects differed significantly ($P < 0.05$) between the different segments of the epididymides (Table 1). As expected, the greatest proportion of proximal cytoplasmic droplets ($P < 0.05$) was found in the caput while the lowest proportion appeared in the cauda. The proportions of distal cytoplasmic droplets indicated ($P < 0.05$) that morphological sperm maturation was normal. Spermatozoa collected from the caput epididymides exhibited the greatest ($P < 0.05$) proportion of under-the-head coiled tails, double-folded tails, and total tail defects ($P < 0.05$) while the corpus epididymides had moderate levels of all three. The proportions of sperm with abnormal mid-pieces and simple bent tails were similar between the caput and corpus epididymides. Spermatozoa aspirated from the caudae generally had lesser ($P < 0.05$) proportions of abnormal mid-pieces, simple bent tails, under-the-head coiled tails, double-folded tails, and total tail defects than spermatozoa from other segments. Thus, the greatest proportion of morphologically-normal spermatozoa was found in the cauda epididymides. Foreign cells were present in samples from the epididymis, namely boat cells (epididymal epithelium lining), erythrocytes, and leukocytes.

Effect of breed

The effect of breed on sperm head morphology from spermatozoa collected from the cauda epididymides is presented in Table 2. There was no major disparity in most sperm head abnormalities among the goat breeds studied; however, Boran exhibited a numerically greater percentage of total abnormal head morphologies while AB had less and Afar, CH, and WG were intermediate. Similarly, CH had the greatest proportion ($P < 0.05$) of acrosome defects, acrosome abnormalities, and undeveloped spermatozoa. Arsi-Bale goats accounted for the greatest proportion of loose heads ($P < 0.05$) and loose, abnormal sperm heads. There was no strong disparity in spermatozoa with cytoplasmic droplets and abnormal mid-pieces between the breeds; however, CH goats accounted for a numerically greater percentage of distal cytoplasmic droplets. Afar and CH accounted for a greater proportion of spermatozoa with double-folded, coiled tails ($P < 0.05$), under-the-head coiled tails, and total tail defects. The relative percentage of morphologically-normal spermatozoa was greater for WG and Boran and less for CH, with AB and Afar breeds being intermediate.

Table 2. Sperm morphology (least-squares mean percentage) in indigenous Ethiopian goat spermatozoa collected from the cauda epididymis with variables grouped by breed.

Variable	Breeds					
	Arsi-Bale	Afar	Central Highlands	Boran	Woito-Guji	SEM
Sperm head shapes						
Pear shape	2.0	4.3	3.3	4.4	2.0	0.57
Narrow at the base	0.11	0.23	0.38	0.25	0.18	0.05
Abnormal contour	0.03	0.12	0.08	0.22	0.11	0.03
Undeveloped	1.18	0.67	4.23	0.24	0.38	0.59
Loose abnormal head	1.61	0.50	0.29	0.73	0.57	0.09
Narrow	0.42	1.34	0.24	1.04	0.68	0.20
Variable size	2.61	5.84	4.35	7.81	6.84	0.84
Abaxial implantation	0.11	0.08	0.10	0.10	0.07	0.02
Total abnormal head shapes	8.18	13.1	13.01	14.86	10.9	1.77
Loose heads	10.7^{a}	4.8^{b}	4.4b	3.0^{b}	3.94^{b}	0.70
Acrosome defects	3.55^{bc}	4.94^{b}	11.30^{a}	2.03^{c}	2.08^{c}	0.64
Acrosome abnormality	0.18	0.27	0.65	0.56	0.41	0.11
Nuclear pouches	0.21	0.61	0.60	0.70	0.66	0.14
Cytoplasmic droplets						
Proximal	3.32	3.73	2.30	2.03	2.50	0.41
Distal	26.44	28.44	37.55	33.96	32.55	2.34
Abnormal mid-piece	2.56	0.50	0.95	1.23	0.94	0.33
Tail defects						
Simple bent tail	0.65	0.69	0.55	0.80	0.36	0.11
Coiled tail under head	3.43	3.69	3.75	2.53	2.19	0.36
Coiled tail double folded	1.80^{b}	3.41^{a}	3.40^{a}	1.23^{b}	1.83^{b}	0.27
Total	5.90	7.80	7.70	4.56	4.38	0.58
Normal spermatozoa* (mean range)	(69 - 76)	(69 - 80)	(66 - 73)	(74 - 87)	(75 - 86)	(1.3 - 2.7)

[a, b, c] Means with different superscripts in the same column are significantly ($P<0.05$) different.

* Included proximal droplets in caput and corpus and distal droplets across the epididymal segments.

Effect of age

As with breed, there were no major differences across the age groups regarding sperm head abnormalities in spermatozoa collected from the cauda epididymides (Table 3). However, the proportion of total pathological sperm-head morphologies was numerically greater for younger male goats (< 14 mo) and lower for the intermediate age group while older goats were intermediate. While the youngest goats had a greater proportion of loose sperm heads ($P < 0.05$), goats at the intermediate age had the least. On the other hand, older goats had a greater proportion of acrosome defects ($P < 0.05$) while younger goats had less. The proportion of distal cytoplasmic droplets was greater ($P < 0.05$) for goats at an older age compared to goats at a younger age. Age group did not influence the proportions of abnormal mid-pieces. However, older goats had greater ($P < 0.05$) percentages of coiled, double-folded tails, and total tail defects while younger goats had less. Similarly, older goats had a greater ($P < 0.05$) proportion of simple bent tails compared to goats in both the younger and intermediate age groups. The relative proportion of morphologically normal spermatozoa was greater ($P < 0.05$) for goats at the intermediate age compared to younger and older ages.

Table 3. Sperm morphology (least-squares mean percentage) in indigenous Ethiopian goat spermatozoa collected from the cauda epididymis with variables distributed by age group.

Variable	Age group			SEM
	<14 mo	14 – 19.5 mo	19.6 – 24 mo	
Sperm head shapes				
Pear shape	3.0	2.5	2.1	0.47
Narrow at the base	0.18	0.21	0.17	0.04
Abnormal contour	0.15	0.10	0.07	0.03
Undeveloped	0.88	0.21	1.28	0.30
Loose abnormal head	1.22	0.49	0.25	0.27
Narrow	0.55	0.49	0.67	0.09
Variable size	6.20	3.92	5.20	0.74
Abaxial implantation	0.10	0.14	0.02	0.02
Total abnormal head shapes	12.4	7.1	9.8	1.23
Loose heads	5.6^{a}	4.2^{b}	4.0^{ab}	0.57
Acrosome defects	3.6^{b}	4.1^{ab}	7.9^{a}	0.72
Acrosome abnormality	0.46	0.18	0.78	0.11
Nuclear pouches	0.50	0.65	0.32	0.14
Cytoplasmic droplets				
Proximal	2.82	2.85	2.75	0.37
Distal	30.0^{b}	32.2^{a}	33.7^{ab}	2.35
Abnormal mid-piece	1.77	0.64	1.40	0.33
Tail defects				
Simple bent tail	0.63^{ab}	0.43^{b}	0.92^{a}	0.11
Coiled tail under head	2.8	3.2	3.0	0.37
Coiled tail double folded	1.82^{c}	2.26^{b}	3.20^{a}	0.28
Total	5.3^{b}	5.9^{b}	7.2^{a}	0.59
Normal spermatozoa* (mean range)	(68 - 80)	(77 - 84)	(70 - 78)	(1.5 - 2.4)

[a, b] Means with different superscripts in the same column are significantly ($P < 0.05$) different.
* Included proximal droplets in caput and corpus and distal droplets across the epididymal segments.

Discussion

In the present work, we studied sperm morphological attributes among 5 indigenous goat breeds in 3 age groups under extensive husbandry in Ethiopia. We have also compared morphological features of goat spermatozoa along the 3 major epididymal segments (caput, corpus, and cauda). Prior to our study, information on sperm morphological attributes in these indigenous male goats under extensive husbandry was scarce, and reports comparing tropical breeds were lacking.

Following spermiation, sperm maturation through the epididymis is an important step in the formation of physiologically-normal and competent spermatozoa, that is, those capable of fertilization. Various functional and morphological changes are sequentially carried out in the epididymis in relation to the acquisition of normal fertilizing capacity, which is obtained by the time the spermatozoa reach the epididymal body (Turner, 1995; Axner, 2000). Spermatozoa in the cauda are thus considered mature, both morphologically and functionally, and are stored there until ejaculation. In the present study, numerically

greater sperm head abnormalities occurred in the caput and corpus epididymides, and a declined trend was noted in the cauda. A similar decrease in the proportion of abnormal head defects along the epididymal duct has previously been observed in bulls (Rao, 1971) and cats (Axner *et al.*, 1999). The caput and corpus epididymides of the present male goats seemed to be efficient in removing loose sperm heads, as indicated by a significantly greater reduction from these segments towards the cauda. Similarly, it has been demonstrated that abnormal spermatozoa might be phagocytosed in the epididymis, mainly in the caput and corpus and to a lesser extent in the cauda (Sutovsky *et al.*, 2001).

As expected, the proportion of proximal cytoplasmic droplets decreased significantly between the caput and cauda while the proportion of distal cytoplasmic droplets increased significantly between the same areas, implying that sperm maturation took place successfully. A similar trend has been reported in normal bulls (Rao *et al.*, 1980) and normal domestic cat spermatozoa (Axner *et al.*, 1999). Such migration of the cytoplasmic droplets from the proximal to distal mid-piece is a morphological change which, together with certain physiological changes, is part of the normal maturation process that gives the spermatozoa their capacity for motility and fertility (Rao *et al.*, 1980).

In this study, we found that the proportion of tail defects was greater in the caput and corpus epididymides and a significant reduction took place in the cauda. In contrast, other studies have reported a significant increase in the proportion of tail defects as the spermatozoa advance along the epididymal duct (Briz *et al.*, 1996; Axner *et al.*, 1999). On the other hand, Rao *et al.* (1980) found no such differences in sperm tail abnormalities (simple bent and coiled tails) between the caput and cauda epididymides. In our case, the scrotal contents were transported from the abattoir to the laboratory with an ice pack, and we also used refrigerated formol-saline for fixation, which might have induced cold shock in the spermatozoa and thus influenced the sperm tails resulting in their bending and coiling. Earlier studies have also stressed that cold stress and hypo-osmotic conditions could increase sperm tail defects (Barth and Oko, 1989; Persson *et al.*, 2006; Rodriguez-Martinez and Barth, 2007).

Eventually, as expected, the sperm maturation process was reflected in the greater proportion of morphologically normal spermatozoa in the cauda epididymis than the other segments. While spermatozoa in the cauda epididymis were abundant and easy to collect, collection from corpus and caput necessitated a slight finger press, which ultimately increased the presence of epithelial cells in these samples, as reflected in our analysis. The greater proportion of boat cells in the cauda epididymides was due to the fact that these cells originate mainly from the epididymis. The presence of erythrocytes and leukocytes indicates contamination during separation of the epididymides from the testes or during the sperm collection process from the duct, in general.

We have demonstrated that there was no strong discrepancy in total sperm head abnormalities among the five goat breeds studied. Apart from the fact that information is scarce to compare our findings with other tropical goat breeds, the available reports on other species are also inconsistent. Brito *et al.* (2002) found no significant difference in semen quality between bull breeds. In contrast, other studies found a significant influence of breed on the presence of abnormal sperm heads in stallions (Dowsett and Knott, 1996) and bulls (Soderquist *et al.*, 1991). However, in the present study, a comparison based on the prevalent agro-climate and production system revealed that male goats from the lowland agro-climate (Boran, Afar, and WG) accounted for 64.7% of the total sperm head abnormalities. This was due to the fact that the greatest number of sperm head malformations were attributed to narrow sized (82.3%; $P < 0.05$) and variable sized (74.6%; $P < 0.05$) spermatozoa in these breeds. This shows that the higher ambient temperature prevalent in the lowlands might have slightly disturbed the normal spermatogenesis in the testes. Our findings are corroborated by earlier work which highlights the negative effect of high ambient temperature on sperm morphology in bulls (Coulter *et al.*, 1997) and boars (Suriyasomboon *et al.*, 2005).

The greater proportion of acrosome defects in CH, loose heads in AB, and undeveloped spermatozoa in both breeds might be associated with environmental factors such as nutrition, as inadequate nutrient supply has been reported to be the major constraint in the highland mixed crop-livestock production system to which these breeds are exposed (Farm-Africa, 1996). Earlier studies have also confirmed that nutritional factors, mainly those related to underfeeding, affect semen quality (Roberts, 1971), sperm maturity (Dana *et al.*, 2000), and abnormal acrosome development (Barth and Oko, 1989). The greater proportion of loose heads in AB might also be related to accumulation of aged spermatozoa (senescence) in the cauda epididymides (Barth and Oko, 1989; Persson *et al.*, 2006), or to breed, as previously demonstrated in stallions (Dowsett and Knott, 1996).

The lack of differences among the breeds and the lower proportion of proximal droplets found in the cauda epididymis in the present study are indicative of a better sperm maturation process in male goats. In contrast, a breed difference in proximal cytoplasmic droplets has been detected in ejaculated spermatozoa in bulls (Soderquist *et al.*, 1991) and stallions (Dowsett and Knott, 1996). Similarly, no difference was noted in distal cytoplasmic droplets, except that CH breeds accounted for a numerically greater proportion than the other breeds. Normally, distal droplets disappear from the spermatozoa after ejaculation, and their presence in cauda epididymides indicates a normal sperm maturation process. The lack of a difference in total

abnormal sperm tails between breeds is in agreement with earlier work in bulls (Soderquist *et al.*, 1991). The proportion of total tail defects was also lower than the 14% reported for cauda-epididymal spermatozoa in beef bulls (Persson *et al.*, 2006). As the overall proportion of tail defects in the present study was less than 10%, it is considered to be within expected limits of normality. It has been reported that virtually every bull sound for breeding produces at least a small percentage of spermatozoa with tail abnormalities (Barth and Oko, 1989).

Age of the animal is an important physiological factor that modifies the sperm morphological characteristics of domestic animals. This has been demonstrated by earlier studies, which found abnormal sperm head shape to be more prevalent at younger age, to gradually decrease after sexual maturity, and to become greater again at older age, provided that the animal is healthy (Barth and Oko, 1989; Soderquist *et al.*, 1991; Amann *et al.*, 2000). However, other studies found no significant effect of age on major sperm defects (Chandler *et al.*, 1985; Brito *et al.*, 2002). Hallap *et al.* (2006) also reported that acrosome and total head abnormalities did not differ significantly with age in frozen-thawed bull spermatozoa, suggesting that the large within-bull variation might have masked the true picture. Similarly, differences in total and specific sperm head abnormalities in the present study did not reach significance across the different age categories considered, possibly due to variation in age grouping. In spite of this, the present study still demonstrated the normal trend of sperm head shape abnormality along the different physiological stages with the youngest male goats demonstrating 74% greater abnormalities ($P = 0.09$) and the oldest at 38% greater abnormalities compared to those at the intermediate age.

As the proportion of proximal droplets in this study remained smaller across all the age categories studied, it lacked biological importance. The lack of difference and occurrence of a greater proportion of distal droplets across the different age groups was indicative of a better sperm maturation process as the present sperm population was non-ejaculated and collected from the cauda epididymides.

The slightly greater proportion of simple, bent-tail defects in the older than the younger age group could be related to cold shock due to the testicles being transported in an icebox. Besides, as its occurrence in this study was small, it would not interfere with fertility as suggested earlier (Rodriguez-Martinez and Barth, 2007). Similarly, the greater proportion of coiled-tail defects in the older compared to the younger age group might be due to iatrogenic effects or mild testicular or epididymal disturbance. Under normal conditions, however, sperm tail defects decrease against the increasing age gradient, which indicates sexual maturity of domestic animals. The fact that the proportions of coiled and total tail defects were both less than 10% in the oldest goats in the present study indicates that these abnormalities were too small to impede sperm motility. However, the lack of a great disparity in the present goats' age group and the fact that these animals were not at an extreme age might have contributed to the present lack of differences in most sperm morphological attributes. Thus, we conclude that while sperm head abnormalities were not seriously influenced, loose sperm heads and acrosome defects were significantly affected by breed and age in indigenous Ethiopian male goats under extensive husbandry.

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