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Assessment of Sperm Morphology in Zebu Bulls, under Field Conditions in the Tropics

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Contents

Sperm morphology was studied in 302 extensively managed Zebu bulls (aged 1.5–9 years), classified as sound (n = 166) or unsound (n = 136) for breeding, under field conditions in the dry tropics of Costa Rica. Single semen samples were collected by electro-ejaculation and fixed in formol-saline solution immediately after collection. Sperm morphology was determined in the field on wet smears using a microscope equipped with phase-contrast optics, and further determined in the laboratory on air-dried smears stained with carbol-fuchsin. The frequencies of sperm abnormalities (such as abnormal acrosome, head, neck, mid-piece, tail, and presence of cytoplasmic droplets) were recorded as a percentage of the total number of counted spermatozoa (400 cells). Zebu bulls considered unsound for breeding showed a higher mean prevalence ($p < 0.05$) of knobbed acrosomes (4.0 versus 0.9%), head defects [specifically, nuclear invaginations and heads with abnormal shapes and sizes (27.6 versus 4.0%)], abnormal tails (11.2 versus 4.7%), and proximal droplets (8.4 versus 1.6%), compared with bulls considered sound for breeding. In these latter bulls, the abnormality most commonly seen was the presence of single bent tails with an entrapped cytoplasmic droplet (3.0 ± 3.7%). Young Zebu bulls (i.e. bulls under 2 years of age) showed a higher percentage of missing acrosomes, and proximal cytoplasmic droplets, than older sires (12.1 versus 2.4%, and 23.9 versus 3.6%, respectively; $p < 0.05$), interpreted as an indication of low ejaculation frequency and sexual immaturity, respectively. Bulls with a long scrotum and soft testicular consistency (TC) at palpation showed higher percentages of abnormal sperm heads in the ejaculate than bulls with a normal scrotal length (SL) and a normal TC (32.7 versus 12.8% and 30.7 versus 10.3%, respectively; $p < 0.05$). In addition, Zebu bulls with a scrotal circumference (SC) ≤ 30 cm showed a higher prevalence of proximal cytoplasmic droplets than bulls whose SC was > 30 cm (9.8 versus 2.6%, $p < 0.05$). A higher mean percentage of abnormally sized and shaped heads, especially undeveloped and narrow at the base, was more frequently found in stained smears than in unstained samples (26.0 versus 9.9%, $p < 0.05$), which clearly underlines the importance of using both stained and wet smears when assessing sperm head morphology. However, for a quick assessment of sperm morphology under field, tropical conditions, phase-contrast microscopy provides useful information for the spermiogramme evaluation.

Introduction

Evidence of a relationship between the fertility of a breeding bull and its semen quality was reported as early as in the beginning of the twentieth century (Williams and Savage 1925; Lagerlöf 1934). A significant correlation has also been demonstrated between the fertility of frozen bull semen and the frequencies of some sperm abnormalities, particularly those related to sperm head forms, defective acrosomes, and the presence of proximal cytoplasmic droplets (Söderquist et al. 1991).

The assessment of the spermiogramme is of utmost importance during the breeding soundness examination (BSE) of Zebu bulls extensively reared in the tropics. Under these conditions, evaluating a bull's fertility by the conception rate of the herd may be misleading, as breeding is usually based on natural mating, with several bulls in the breeding herd. Furthermore, most farms do not keep reproductive records.

Although sperm morphology alone is not the only variable in the spermiogramme related with the potential fertility of a semen sample, the assessment of other parameters, such as sperm concentration and motility may, however, provide erroneous information. In bulls under extensive rearing, electro-ejaculation is the method most currently used to obtain an ejaculate from *Bos indicus* bulls. Nevertheless, this technique induces an ejaculate with a larger volume of semen, decreasing the reliability in the assessment of sperm concentration in the semen sample (Carroll et al. 1963; Ball et al. 1983; Madrid et al. 1988; León et al. 1991; Chacón et al. 1999a). In addition, the presence of other factors in the farms, such as breeding the herd in multiple-sire systems, bull's dominance, and using of continuous or seasonal mating may affect ejaculation frequency in Zebu bulls, thus reducing the significance of assessment of sperm motility under field conditions. Therefore, sperm morphology seems to be the most reliable component of the spermiogramme, which together with an exhaustive clinical and genital evaluation of the animal provides detailed information for estimating the potential fertility of those bulls under field conditions in the tropics, disclosed as breeding soundness examination.

Various techniques have been used to assess sperm morphology in the bull, including light microscopy of stained and unstained preparations, and transmission or scanning electron microscopy (for further reading, see Barth and Oko 1989). Under field conditions, however, use of phase-contrast microscopy appears to be the most practical method for evaluating sperm morphology (Lindford et al. 1976) although the results should be confirmed subsequently using stained smears and, if needed, by using other techniques.

Few studies have been carried out to describe the frequencies of different sperm abnormalities found in ejaculates of extensively reared *Bos indicus* bulls and, furthermore, to demonstrate why evaluation needs to be done with both phase-contrast on wet smears and light microscopy on dried stained semen smears.

The present investigation was therefore undertaken to study the frequencies of sperm abnormalities using

light microscopy with phase contrast optics on fixed wet smears, followed by a examination of dried, stained smears, of the same single semen samples collected by electro-ejaculation from Zebu bulls extensively managed under tropical conditions. The rationale behind double-checking the sperm head morphology with wet and dried stained smears was to assess the value of phase-contrast microscopy as a single method for evaluating sperm morphology under field conditions in the tropics.

Materials and Methods

Animals

Three hundred and two healthy Zebu (*Bos indicus*) breeding bulls from the dry Pacific region of Costa Rica (9°43′–11°13′N, 84°46′–85°57′W) were examined under field conditions for breeding soundness purposes. The bulls belonged to Brahman (n = 138), Indobrasil (n = 70), Nellore × Brahman (n = 39), Nellore (n = 33), Gyr (n = 12), and Indobrasil × Brahman (n = 10) breeds. The BSE included a clinical genital examination and the collection of a semen sample by electro-ejaculation (Chacón et al. 1999a). The mean age of the bulls was 4 years, ranging from 18 months to 9 years of age. All bulls were under the same nutritional management, which was based on pastures and supplementation *ad libitum* with salt blocks. During the dry season, molasses were additionally given. At the time of the BSE, the bulls were kept mating either continuously or temporarily, with a single or multiple bull breeding system, depending on the farm.

Clinical evaluation

The author performed a clinical examination of the bulls at the farm. The specific clinical examination in the 302 healthy bulls included the scrotal circumference (SC), testicular consistency (TC), and scrotal length (SL). The SC was measured using a standard scrotal metal-tape (Nasco®, Wisconsin, USA), and TC was determined subjectively by palpation and classified as 'normal', 'soft' or 'hard'. The SL was classified as 'normal' or 'long', as described by Chacón et al. (1999a), according to the distance between the distal end of the scrotum and the hock joint.

Semen collection and evaluation

Following the clinical examination, semen samples were obtained using a three-electrode electro-ejaculator (Standard Precision Electronics Inc., Colorado, USA), after at least 3 min of manual rectal stimulation of the accessory glands and pelvic urethra. A semen sample was thereafter fixed in buffered formol-saline solution (Hancock 1952) in a ratio of 1 : 10 (v/v), for evaluation of sperm morphological abnormalities under phase-contrast microscopy (×1000). The morphology of acrosome and size and shape of the sperm head (subjectively assessed), as well as the occurrence of nuclear invaginations, abnormal tails, and the presence and location of cytoplasmic droplets was assessed on 400 spermatozoa/sample. At the laboratory, a semen smear was further prepared using a small drop of the fixed semen sample, air-dried and stained with carbol-fuchsin (Williams and Utica 1920; Lagerlöf 1936) to determine head morphology (size and shape). A total of 400 spermatozoa were counted under light microscopy (Zeiss®, Berlin, Germany) at 1000×. The frequencies of abnormal spermatozoa were recorded and, according to the system described by Lagerlöf (1934) and Bane and Nicander (1965), divided into the following categories: Acrosome – missing (includes also swollen and irregular outline) or knobbed; Head – nuclear pouches, crater and crest defect, narrow at the base (included pear-shaped), rounded, tapered, giant, undeveloped and detached normal; Neck – broken or abaxial; Mid-piece – double, stump, accessory; Tail – coiled around the head, tightly coiled under the head, double-folded, coiled principal pieces, right-angled with cytoplasmic droplet, bent with or without entrapped cytoplasmic droplet; and Droplets – proximal or distal.

Classification after breeding soundness evaluation

Given that, during the BSE, those bulls found to have clinical inflammatory or congenital defects of the reproductive organs were excluded from the study, the healthy 302 males were classified as sound or unsound for breeding, based on sperm morphology findings, as described by Chacón et al. (1999a). Bulls classified as breeding-sound were sires having ≤ 15% of knobbed acrosomes, abnormal sperm heads, proximal droplets and mid-pieces and/or a maximum of 30% total sperm abnormalities, whereas those classified as breeding-unsound did not meet the criteria for breeding soundness.

Statistical analysis

Mean percentages (± SD) were calculated for every sperm abnormality. Means were summarized separating the bulls into categories, based upon the following variables: age – < 2 years, ≥ 2 < 7, or ≥ 7 years old; TC – soft, normal or hard; SL – normal or long; SC (only for bulls older than 2 years) – ≤ 30 cm or > 30 cm; and classification after BSE – sound or unsound for breeding. An analysis of variance (ANOVA) was performed between categories, using the Bonferroni test for comparisons of means (SAS® 1988). Additionally, percentages (± SD) of sperm defects with a suspected genetic origin (Blom 1966; Koefoed-Johnsen et al. 1980; Kojima 1988a,b) were calculated for the 302 bulls studied. The mean percentage of abnormal heads (size and shape) counted in either carbol-fuchsin stained smears or formol-saline wet smears were compared using ANOVA for each abnormality.

Results

At the time of the BSE, the 302 bulls were healthy, in good condition, and free of any clinical congenital or inflammatory diseases in their reproductive organs. The mean percentages (± SD) of sperm abnormalities,

according to classification after BSE, are given in Table 1. Table 2 summarizes the mean percentages of sperm abnormalities (excluding specific sperm defects) by sperm region, according to age, TC, SL, and SC. Typical examples of sperm abnormalities as classified in this study, are depicted in Figs 1 and 2. In general, there was a low prevalence of abnormalities located in the sperm neck (0.2 ± 0.6%) and mid-piece (0.6 ± 4.8%) in the population studied. However, an exceptional case was found of a bull with a specific sperm abnormality, the tail stump defect, present in all of its spermatozoa (Chacón and Rodríguez-Martínez 1988). There were no statistical differences for neck/mid-piece abnormalities among the different categories of classification (BSE, age, TC, SL and SC).

Table 1. Mean percentages (± SD) of sperm abnormalities according to classification after breeding soundness evaluation in Zebu bulls

Sperm abnormality	Unsound (n = 136)	Sound (n = 166)
Acrosome		
Missing	3.2 ± 8.5	2.8 ± 10.7
Knobbed[a]	4.0 ± 7.3	0.9 ± 1.7
Head		
Double	0.1 ± 1.3	0.1 ± 0.1
Nuclear pouches[a]	3.2 ± 10.2	0.3 ± 1.0
Crater defect[a]	4.7 ± 10.6	0.6 ± 1.3
Nuclear crest[a]	0.4 ± 2.0	0.1 ± 0.1
Narrow at the base[a]	11.5 ± 16.2	1.8 ± 2.2
Rounded	0.1 ± 0.3	0.1 ± 0.2
Tapered[a]	1.7 ± 4.2	0.3 ± 0.6
Giant	0.2 ± 0.5	0.1 ± 0.2
Undeveloped[a]	10.7 ± 17.5	1.3 ± 2.2
Detached normal	4.0 ± 5.7	2.8 ± 11.9
Total abnormal heads[a]	27.6 ± 28.2	4.0 ± 4.1
Neck[b]	0.3 ± 0.5	0.2 ± 0.6
Mid-piece[b]	1.0 ± 7.5	0.3 ± 0.8
Tail		
Coiled around the head[a]	0.5 ± 1.4	0.1 ± 0.3
Tightly coiled under the head[a]	4.0 ± 8.0	1.9 ± 2.6
Double-folded[a]	1.8 ± 3.7	0.9 ± 2.4
Coiled principal piece	0.4 ± 0.9	0.4 ± 1.5
Right-angled tail with droplet	0.4 ± 1.5	0.3 ± 0.8
Bent tail without droplet	1.5 ± 3.7	1.8 ± 4.5
Bent tail with droplet[a]	8.3 ± 13.3	3.0 ± 3.7
Total abnormal tails[a]	11.2 ± 14.4	4.7 ± 6.7
Cytoplasmic droplets		
Proximal[a]	8.4 ± 13.3	1.6 ± 2.4
Distal	1.8 ± 4.9	0.9 ± 2.1

[a] Indicates significant statistical difference between groups ($p < 0.05$).
[b] Because of the low prevalence of sperm neck and mid-piece abnormalities, they are given as totals, and not listed separately.

Sperm morphology and classification after breeding soundness evaluation

There were no statistically significant differences in the mean percentages of spermatozoa with a missing acrosome between the bulls classified as sound or unsound for breeding.

Morphologically abnormal heads constituted the most prevalent deviation found in the spermiogramme of Zebu bulls graded unsound for breeding. This category of bulls showed higher ($p < 0.05$) frequencies of sperm heads with pear-shaped and narrow-at-the-base forms (Fig. 1n–o), undeveloped shapes (Fig. 1 l), nuclear abnormalities and tapered heads (Fig. 1i–k and 1p, respectively), compared with the bulls classified as sound for breeding (Table 1). No statistically significant differences were found for other head abnormalities, according to the grading of the bulls after BSE (Table 1). In bulls considered sound for breeding, detached normal head (Fig. 1h) was the most common head abnormality found (Table 1).

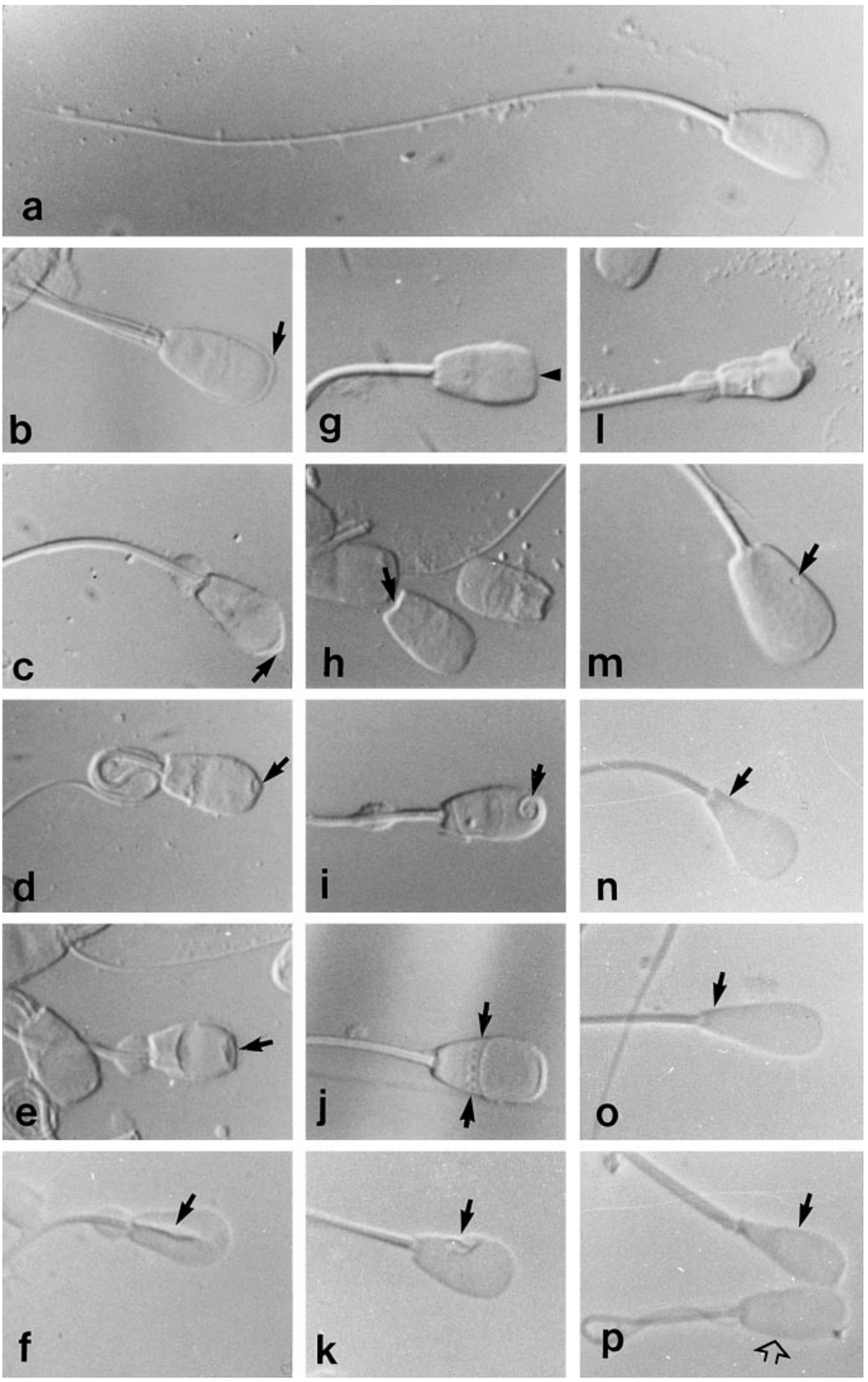
Concerning other abnormalities, bent tails with an entrapped cytoplasmic droplet (Fig. 2 l) was the most common tail abnormality found in both breeding soundness evaluation grading, followed by spermatozoa with tails coiled under the head and double-folded tails (Table 1) (Fig. 2h–i and 2j, respectively).

The Zebu bulls considered unsound for breeding had also a higher prevalence of proximal cytoplasmic droplets (Fig. 2a) than bulls ranked sound for breeding (Table 1). In contrast, the mean percentage of distal droplets (Fig. 2b) was low and did not differ statistically between the groups.

Table 2. Mean percentages (± SD) of sperm abnormalities summarized per region and according to age, testicular consistency, scrotal length and scrotal circumference in Zebu bulls

Variable	Missing acrosomes	Head	Tail	Proximal droplet	Distal droplet
Age (years)					
< 2 (n = 11)	12.1 ± 21.0[a]	16.3 ± 14.9[a]	4.2 ± 1.9[a]	23.9 ± 23.5[a]	0.8 ± 0.8[a]
≥ 2 < 7 (n = 252)	2.4 ± 7.6[b]	14.8 ± 22.4[a]	8.0 ± 11.2[a]	3.6 ± 7.6[b]	1.2 ± 3.6[a]
≥ 7 (n = 39)	4.5 ± 15.9[b]	13.5 ± 24.7[a]	8.0 ± 9.2[a]	3.1 ± 5.3[b]	2.1 ± 3.6[a]
Testicular consistency					
Soft (n = 67)	5.4 ± 11.5[a]	30.7 ± 29.5[a]	7.7 ± 9.0[a]	4.0 ± 8.8[a]	1.0 ± 3.0[a]
Normal (n = 228)	2.3 ± 9.4[a]	10.3 ± 17.7[b]	7.8 ± 11.3[a]	4.4 ± 9.4[a]	1.3 ± 3.8[a]
Hard (n = 7)	2.0 ± 2.7[a]	2.1 ± 2.8[b]	13.4 ± 10.2[a]	4.5 ± 5.7[a]	3.5 ± 3.9[a]
Scrotal length					
Normal (n = 273)	2.5 ± 9.5[a]	12.8 ± 19.3[a]	7.6 ± 10.9[a]	4.6 ± 9.6[a]	1.4 ± 3.8[a]
Long (n = 29)	7.6 ± 12.5[a]	32.7 ± 32.2[b]	9.5 ± 9.9[a]	2.0 ± 3.2[a]	0.9 ± 2.1[a]
Scrotal circumference (cm)[A]					
≤ 30 (n = 38)	0.4 ± 1.6[a]	11.6 ± 21.4[a]	7.4 ± 11.4[a]	9.8 ± 14.3[a]	1.1 ± 2.1[a]
> 30 (n = 253)	3.0 ± 9.7[a]	15.0 ± 22.9[a]	8.1 ± 10.9[a]	2.6 ± 5.0[b]	1.3 ± 3.8[a]

Means in the same category and column with distinct letter are statistically different ($p < 0.05$).
[A] Includes only bulls older than 2 years.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Sperm morphology and age

Presence of the proximal cytoplasmic droplet was the most prevalent abnormality found in Zebu bulls younger than 2 years ($p < 0.05$) (Table 2). In addition, the mean percentage of missing acrosomes was significantly higher in this group than in older bulls ($p < 0.05$) (Fig. 1b; Table 2). No statistically significant differences were found for other abnormalities between the age groups.

Relationship between sperm morphology, TC and SL

The sperm morphology in Zebu bulls with a soft TC and a long scrotum was characterized by a higher prevalence of head abnormalities, compared with that of bulls with a normal TC and SL (Table 2, $p < 0.05$). Bulls with a hard TC showed a lower mean percentage of abnormal heads in the ejaculate than bulls with a soft TC (Table 2, $p < 0.05$). Neither TC nor SL affected the mean percentages of abnormal acrosomes or tails, or the presence of cytoplasmic droplets.

Sperm morphology and SC

A higher prevalence of proximal cytoplasmic droplets was the only difference found between Zebu bulls older than 2 years with SC of ≤ 30 cm ($n = 38$), and bulls whose SC > 30 cm (Table 2, $p < 0.05$).

Specific sperm abnormalities

In general, all the specific sperm defects reported in the literature on *Bos taurus* bulls were found in the Zebu sires studied herein. Although the overall mean percentage for these abnormalities was less than 5%, some bulls showed high frequencies, especially, spermatozoa with nuclear crater and tail defects. The tightly coiled tail under the head (Fig. 2h–i) was the most commonly seen, appearing in 27 bulls in a frequency of more than 10% and one bull having 65% of its spermatozoa affected. A significant, albeit low, correlation was found between the occurrence of this defect and the double-folded tails in the same semen samples of the studied Zebu bulls ($r = 0.25$, $p < 0.0001$). The crater defect (Fig. 1k) appeared in 21 bulls in a frequency of more than 10% and in one bull affecting 64% of the spermatozoa. The knobbed acrosome appeared in 15 bulls in more than 10% of the spermatozoa and in one bull with 34% of its spermatozoa affected. The different variants reported for this defect in *Bos taurus* bulls (Barth 1986) were observed in the Zebu samples. These pictures were characterized by the protuberant form (the most commonly found) (Fig. 1c,d), and the flatted acrosome in the apical region (Fig. 1e,g).

Carbol-fuchsin versus phase-contrast microscopy for evaluation of sperm head morphology

Evaluation of sperm head morphology (i.e. size and shape) in smears stained with carbol-fuchsin allowed the detection of a higher percentage of narrow-at-the-base and undeveloped heads, compared with the complementary wet smears examined under phase-contrast microscopy ($12.0 \pm 1.1\%$ and $11.7 \pm 1.3\%$ versus $3.2 \pm 1.1\%$ and $5.3 \pm 1.3\%$, respectively; $p < 0.001$), in samples from the same ejaculate. No statistically significant differences ($p > 0.05$) were found between methods regarding rounded, tapered, giant or double heads. The use of carbol-fuchsin for determination of head size and shape therefore made it possible to diagnose 16 more bulls as unsound than did the use of phase-contrast microscopy of formol-saline-fixed samples (136 versus 120), which gives a sensitivity of 88% for the latter method.

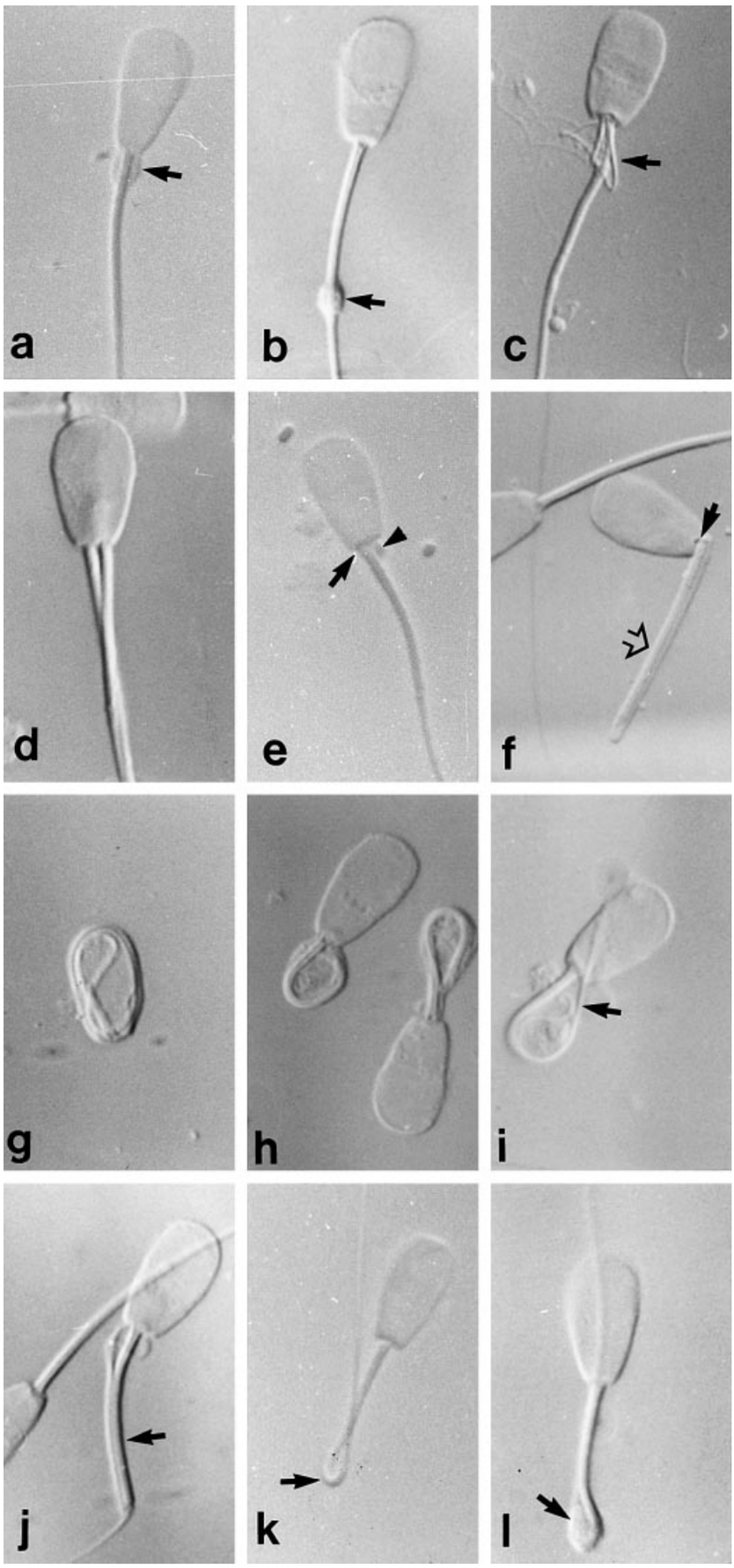
Discussion

Breeding soundness evaluation of Zebu bulls in the tropics is almost only carried out in the field. Due to management conditions and also to the hostile behaviour of the breeding bulls, the collection of semen as a part of the breeding examination is done via electroejaculation. Assessment of sperm morphology, an essential component of the examination, is carried out, with obvious advantages, on site, since the breeding bulls can then be classified as sound or unsound for breeding, and either culled or brought back to the breeding herd.

Under these working conditions, the morphological evaluation of the semen is routinely carried out on formol-saline-fixed samples, using phase-contrast microscopy, or otherwise, practitioners use quick staining methods such as eosin-nigrosin (Chenoweth et al. 1984). The phase-contrast method has some disadvantages, as it does not provide the best resolution to assess the morphology of the sperm head (size and shape), for which air-dried stained smears are usually applied (Rodríguez-Martínez et al. 1997), but, on the other hand, staining methods may mask nuclear and acrosome defects in the spermatozoa (Bane and Nicander 1965; Chenoweth et al. 1984), or may produce shedding on the spermatozoa of the cytoplasmic droplet (Sekoni et al. 1981).

Discrepancies in the percentages of abnormal spermatozoa determined by different methods within the same semen sample have previously been reported

Fig. 1 (a–p): Nomarsky differential interference contrast microscopy of formol-saline fixed spermatozoa (wet smears) from Zebu bulls, depicting the different morphological categories observed at the sperm head level (1800×). a; normal morphology. b; lifted acrosome (arrow), note the absent apical ridge. c–e and g; different forms for the knobbed acrosome defect showing the protuberant shape (arrow in c and d), and the flat form (arrow in e and g). Note in e also the undeveloped (small size) head. f; nuclear crest (arrow). h; detached normal heads, note the normal shape of the implantation fossa. Figures i–m depict abnormalities in the sperm nucleus: i; a single nuclear pouch posterior to the apical margin of the acrosome (arrow), j; diadem defect (nuclear pouches), showing the typical appearance at the post acrosome sperm domain (between arrows), k; lateral crater defect (the arrow shows the irregular contour of the defect at the lateral side of the head which is the most common variant found in Zebus). Figures l–m show variations in sperm head size: l; undeveloped (small), m; giant head with a nuclear pouch (arrow). Figures n–p depict frequently seen variations in the sperm head shape of Zebu bulls: n–o; pear-shaped and narrow at the base heads [note the narrowing at the base level (arrow) and the normal width in the anterior region], p; tapered head (upper spermatozoa), note the narrowing of the whole head in contrast with the normal head shape of the neighbouring spermatozoon (open arrow)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(Salisbury et al. 1942; Mercier and Salisbury 1947; Sprecher and Coe 1996). The higher percentages of abnormal-sized and -shaped sperm heads detected hereby in carbol-fuchsin stained smears allowed the diagnosis of a higher number of unsound bulls complementing the preliminary assessment of fixed, wet smears. This emphasizes the importance of using both methods when assessing sperm morphology.

This higher sensitivity of the carbol-fuchsin staining is based on the enhanced contrast obtained in the contour of the stained cell (Williams and Utica 1920). Despite the usefulness of combining the methods to evaluate sperm morphology, there is the necessity to evaluate the breeding soundness of the Zebu bulls on site and give the farmer an immediate prognosis of the bull's performance. As it is difficult to perform microscopy using carbol-fuchsin stained smears under field conditions, a convenient procedure is to perform a preliminary evaluation using wet smears under phase-contrast microscopy on site and then, at the laboratory, do a follow-up of the stained smears of those samples which, according to the judgement of the evaluator, need to be more closely studied.

The low prevalence of sperm abnormalities from the neck and mid-piece regions on spermatozoa from the Zebu bulls studied is in agreement with reported findings on *Bos taurus* bulls (Barth and Oko 1989). However, some exceptional cases of bulls with a high percentage of these defects in their ejaculates have been reported in both *Bos taurus* (Arriola et al. 1985; Foote et al. 1992) and *Bos indicus* sires (Chacón and Rodríguez-Martínez 1998).

The fact that the bent tail with an entrapped cytoplasmic droplet was the most common abnormality found in Zebu bulls classified as sound after BSE is in accordance with previous reports by Igboeli and Rakha (1971), Fayemi and Adegbite (1982) and Wildeus and Entwistle (1984) on *Bos indicus* bulls managed extensively. However, special attention should be paid to the interpretation of the spermiogramme of bulls with this abnormality in view of its fluctuating prevalence obtained in consecutive ejaculations (Chacón et al. 1999b). In addition, more research is needed to disclose the reasons behind this fluctuation and the potential effects of the abnormality on fertility under natural mating in tropical extensive breeding conditions.

Spermatozoa with tails tightly coiled under the head and double-folded were found in Zebu bulls, abnormalities previously described by Blom (1966), Koefoed-Johnsen and Andersen (1971) and Wenkoff (1978) in *Bos taurus*. The double-folded tails found in the Zebu samples showed the same disarray in the tail ultrastructure as reported in the tightly coiled tails, which is characterized by the presence of a common cell membrane enclosing cytoplasmic-like material together with the abnormal axonemal complex (Chacón and Rodríguez-Martínez, unpublished results). Both tail abnormalities should therefore be grouped as one and the same defect.

When interpreting the sperm morphology component of the spermiogramme of Zebu bulls, the relationship between the age of the animal and some sperm abnormalities should also be considered. Reports regarding sperm abnormalities commonly found in pre- and post-pubertal bulls are scarce in Zebu sires. The higher prevalence of missing acrosomes in bulls younger than 2 years may be a consequence of sperm senescence in the epididymides as a result of sexual rest or low mating frequency (Saacke and Marshall 1968; Wells et al. 1971), as in the tropics, bulls are normally placed with cows after they turn 2 years of age. The higher percentage of proximal cytoplasmic droplets found in these bulls compared with older animals might indicate sexual immaturity. Similar results have been published for young *Bos taurus* bulls (Madrid et al. 1988; Barth and Oko 1989).

The higher prevalence of proximal cytoplasmic droplets in bulls older than 2 years and a SC of ≤ 30 cm may be the result of a delayed sexual maturity, which is commonly seen in Zebu bulls (Chenoweth et al. 1996; Vale Filho et al. 1996). Alternatively, it may reflect a disruption in the sperm maturation process, as it has been shown in bulls with low fertility (Rao et al. 1980). A follow-up of these bulls is needed to rule out the first-mentioned possibility and at the same time, to determine the minimal required thresholds for SC, so that Zebu bulls can be selected as breeders once they have reached a certain SC-value.

The higher prevalence of abnormal heads in bulls having a soft TC and a long scrotum compared with bulls of normal TC and normal SL fits with previous reports in Zebu bulls extensively reared (Chacón et al. 1999a). In regard to Zebu bulls with a hard TC, the similar prevalence found for the abnormalities studied in this group and in bulls with normal consistency, suggests that fibrotic and/or calcification changes in the tubules, associated with low sperm output, are not necessarily reflected in sperm morphology deviations. However, this result should be carefully interpreted in view of the low number of Zebu bulls included in this group.

Several systems for classification of sperm abnormalities have been proposed (Lagerlöf 1934; Blom 1972; Ball et al. 1983; Chenoweth 1997). Some of these classify sperm abnormalities according to their origin as being either 'primary' or 'secondary' (Ball et al. 1983; Wenkoff 1988), or 'major' and 'minor' (Blom 1972). Lately, the (US-based) Society for Theriogenology proposed a system of using a single minimal threshold (70%) of normal spermatozoa in the ejaculate (Chenoweth et al. 1992).

Fig. 2 a–l: Nomarsky differential interference contrast microscopy of formol-saline fixed sperm (wet smears) from Zebu bulls, depicting the different morphological categories observed at the sperm neck, mid-piece and tail levels (1800×). In a; proximal and, in b; distal cytoplasmic droplets (arrows). c; disorganized mid-piece, d; double mid-piece in a spermatozoon with a giant head, e; abaxial tail implantation (arrow) with accessory mid-piece (arrow head), f; broken neck (angled-tail implantation, arrow) with double-folded tail (open arrow). Figures g–l represent tail abnormalities often found in Zebu sires: g; coiled around the head, h–i; tightly coiled under the head, note in h the upper spermatozoon with nuclear pouches and in i part of the principal piece out of the coiled complex (arrow), j; double-folded (arrow), k; single bent tail without droplet entrapped showing the level of the reflex at the distal portion of the mid-piece (arrow), l; single bent tail with an entrapped cytoplasmic droplet (arrow)

The procedure applied in this study of summarizing the abnormalities as totals by sperm domain, as well as counting the specific defects separately, not only allows assumptions to be made about the aetiology of the abnormality, but also allows a better prognosis to be made of the potential recovery of the breeding capacity of the bull, since abnormalities with a genetic origin may not change significantly in repeated semen collections, in contrast to acquired abnormalities (Kojima 1988b).

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