



Analysis of Sperm Characteristics in Bulls of the Local Algerian Atlas Brown Breed



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Abstract

THE Atlas brown breed, although dominant within the national herd and well adapted to local climatic conditions, remains underutilized in terms of reproductive and productive performance. This study aimed to characterize the sperm quality of six bulls of this breed, aged 18 to 24 months, based on the analysis of 103 ejaculates. The evaluated parameters included volume, motility, pH, sperm concentration, enzymatic activity (methylene blue test), fructose concentration, as well as sperm vitality and abnormalities. Analyses, conducted using classical methods (microscopy, spectrophotometry, vital staining), showed generally satisfactory results: an average volume of 3.7 mL, progressive motility of 77.7%, a concentration of 0.65×10^9 spermatozoa/mL, and a pH of 6.7. Storage at +4 °C revealed significant correlations ($P < 0.01$) with most evaluated parameters: positive correlations with motility, pH, fructose concentration, and redox potential, and negative correlations with mortality and sperm abnormalities. These findings highlight the reproductive potential of the Atlas brown breed and underscore the importance of systematic andrological evaluation in genetic improvement programs and the conservation of local breeds.

Keywords: Atlas brown breed, bulls, sperm parameters, semen preservation, Algeria.

Introduction

In 2017, the Algerian cattle (*Bos taurus*) herd numbered 2,171,633 heads [1]. The local population represented approximately 78% of the total herd, with a remarkable concentration of two-thirds in the northeastern Region of the Country. This population is characterized by low milk productivity, with an average daily yield of 3 to 4 liters over a 6-month period, or about 595 kg per lactation [2].

The Algerian local cattle population consists of a single breed, the Atlas brown, which represents 48% of the national herd but accounts for only 20% of total production. This local breed is well adapted to the climate, topography, and feed resources of the different regions of the country. However, this adaptation has resulted in variations in size and coat color. Some studies have described the size (1.05 to 1.2 m) and weight (210 to 400 kg) of adult males and females of the Atlas Brown breed [3, 4].

The reproductive capacity of Atlas brown bulls, as well as the morphometric factors that may influence it, has been little described. Yet, this preliminary assessment is essential for the rational use of these bulls, as reproductive capacities depend on age, weight, height, and scrotal circumference [5, 6, 7].

Few data are available on the reproductive performance of Algerian local breed bulls, particularly the Atlas brown. Existing studies report scrotal circumference (SC) and the evolution of serum testosterone levels with age. For instance, an average SC of 13.7 cm at 3 months of age with a testosterone level of 0.7 ng/mL has been observed, whereas bulls aged 38 to 48 months present an average SC of 33.5 cm with a mean testosterone level of 7.2 ng/mL [8].

Several studies have emphasized the importance of clinical examination of the animal and semen

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evaluation to determine fertility [8, 9]. Bull qualification for reproduction is based on three essential elements: good semen quality, good physical health, and strong libido [9]. In this context, Birkner *et al.* [10] showed that bulls with high libido can achieve higher conception rates than those with low libido (51.5 vs. 30.6%). Similarly, Perry *et al.* [11] and Chenoweth *et al.* [12] reported that libido is higher in older bulls than in younger ones, although Hafez [13] noted that older bulls have longer reaction times.

According to the American Society of Andrology [5], bull qualification is based on a general clinical examination (with particular attention to body condition and the locomotor system), a thorough examination of the internal and external genitalia, evaluation of sexual behavior (libido, mounting ability, and intromission), measurement of scrotal circumference according to age [14, 15], and semen analysis.

Scrotal circumference measurement is a key indicator for evaluating breeding bulls and is considered a reliable predictor of daily semen production, especially in young bulls [14, 15]. According to Hopkins and Spitzer [16], the American Society of Theriogenology recommends that all breeding bulls have a minimum scrotal circumference of 30 cm. However, Kasari *et al.* [17] suggested that higher values (32–33 cm) should be used for large breeds such as Simmental, Angus, and Maine-Anjou. It is also noted that no value below 30 cm should be accepted, even for small breeds [16].

Knowledge regarding the sperm quality of this breed remains limited, particularly in young bulls, especially for the evaluation of their fertility. Therefore, this study aimed to analyze the sperm characteristics of six bulls of this breed, aged 18 to 24 months, based on the analysis of 103 ejaculates.

Material and Methods

Animals and source of semen

A total of 103 ejaculates were collected from six bulls. The males were selected based on the morphological characteristics of the local breed (Atlas Brown), age, and libido. The animals were between 18 and 24 months old at the time of semen collection (average: 22 months) (All methods used in this study were conducted in accordance with national regulations and international guidelines on animal welfare, The study protocol was approved by the Scientific Council of the Veterinary Sciences Institute of Blida University, Algeria).

They were fed a forage-based diet roughage: barley (*Hordeum vulgare*) and wheat straw (*Triticum aestivum*) supplemented with concentrates soybean meal (*Glycine max*) and corn (*Zea mays*). General examination revealed that all animals were in good health.

Before semen collection, a training and exercise phase was carried out to habituate and prepare the bulls for mounting; this step is considered essential in the reproductive management of males intended for semen collection and production. The training was performed on sandy ground for about 30 minutes (min) per day (d).

For the second phase (collection), semen was collected using an artificial vagina previously washed and sterilized by moist heat (pressurized steam) in an oven before each session. Immediately after collection, semen was analyzed using classical methods in two steps:

(1) Preliminary analysis based on the evaluation of volume and color (milky-white, indicative of concentration) by direct visual observation.

(2) Objective analysis, including the assessment of motility (percentage of forward-moving spermatozoa observed between slide and coverslip under phase contrast, $\times 400$), pH, BMT test (methylene blue), concentration using a spectrophotometer (optical density determined using a 3.5% sodium citrate solution; cell concentration determined via standard curve; total sperm count per ejaculate calculated by multiplying concentration $\times$ volume, expressed in 1×10^9), fructose concentration and vital staining of spermatozoa [18].

Analyzed parameters

1) Motility

Sperm motility was assessed under a microscope at $\times 400$ magnification on a heated stage (37–38 °C). A drop of semen was placed between a slide and coverslip to observe sperm movement within the microscopic field.

2) pH test

pH was measured to determine hydrogen ion concentration using a chemical indicator (0.2% bromothymol blue).

3) Methylene blue test (BMT)

The BMT test was performed by mixing equal parts of 0.1 cm³ of semen and methylene blue solution. The faster the discoloration, the better the semen quality. This technique evaluates the activity of dehydrogenase enzymes.

4) Fructose concentration analysis

Fructose concentration was determined according to Mann's method [18]. The method is based on the chemical reaction between fructose and resorcinol (2,3-dihydroxybenzoic acid) in an acidic medium and involves two stages:

- Step 1: Deproteinization – 0.1 mL of fresh semen was added to 1.9 mL of distilled water and 2 mL of 10% trichloroacetic acid. The mixture was heated (90 °C) and centrifuged (4000 rpm for 15 min).

• Step 2: Determination – the supernatant was aspirated and mixed with 0.2% resorcinol alcohol in a tube. Optical density was measured at $\lambda = 490$ m μ . The red or pink coloration observed during incubation is directly proportional to fructose concentration.

5) Analysis of live, dead, and abnormal spermatozoa

This method is based on counting spermatozoa under a microscope after simple staining using Ziehl-Enrich's technique [19]. A semen smear was prepared on a slide, cleaned with alcohol-ether, dried, and rinsed with distilled water. After preparation, the slide was examined microscopically to quantify non-stained spermatozoa (live: > 60%), stained spermatozoa (dead; expected < 25%), and stained abnormal spermatozoa.

Dilution and storage of Semen at +4 °C

This technique includes three steps:

1. Preparation of the diluent, consisting of 150 mL of distilled water mixed with egg yolk and placed in a water bath (37 °C);
2. Dilution procedure; this step involves reducing the initial sperm concentration to achieve the desired concentration, while adding the protective diluent
3. Storage of diluted semen, which requires strict precautions to avoid thermal shock. Temperature reduction must be gradual and completed over 3 hours (h); a rapid decrease may cause cell damage.

Statistical analysis

After data collection and organization, a series of statistical analyses was performed using XLSTAT software.

First, a descriptive study of sperm parameters was carried out. The results were expressed as mean (X), standard deviation (SD; δ), and coefficient of variation (CV %), in order to assess the degree of dispersion or variability of the data relative to the mean.

Secondly, a relational analysis was conducted to highlight the correlation between semen storage at +4 °C and the different sperm parameters. This analysis was performed using Fisher's test and Student's t-test.

Results and Discussion

A total of 103 ejaculates collected over a four-month period from local-breed bulls were subjected to statistical analysis regarding semen quality. The mean ejaculate volume was 3.7 ± 0.5 mL, and the mean sperm concentration was 0.65×10^9 /mL, with coefficients of variation of 13.5% and 15.4%, respectively (Table 01). Mass motility averaged $77.9\% \pm 6.01$, and the total sperm count per ejaculate did not exceed 2.2×10^9 . The mean pH and

methylene blue test (BMT) values were 6.7 ± 0.17 and $12 \text{ min} \pm 2.3$, respectively.

A total of 103 ejaculates from six local-population bulls (Atlas Brown breed) were analyzed to study variations in semen quality after preservation at +4 °C. Correlation coefficients between semen parameters showed strong linear relationships among volume, concentration, motility, and preservation at +4 °C across the different bulls (Table 02).

Preservation at +4 °C had a significant effect ($P < 0.01$) (Table 02), and was positively correlated with motility ($r = 0.981$), pH ($r = 0.980$), redox potential ($r = 0.999$), and fructose concentration ($r = 0.998$). Conversely, preservation at +4 °C showed a significant ($P < 0.01$) negative linear correlation with the percentage of dead cells and with live cells ($r = -0.981$).

The analysis of the results obtained in the present study, based on 103 ejaculates from 6 bulls of the Atlas Brown bulls, reveals an overall satisfactory semen quality, considering the rusticity and the modest performance traditionally attributed to this local breed. The mean ejaculate volume ($3.7 \text{ mL} \pm 0.5$) is comparable to the values reported for other local breeds [20, 21, 22], although lower than those of standard breeds such as Holstein or Montbeliard. The mean sperm concentration (0.65×10^9 /mL) reflects moderate cellular production, likely influenced by genetic, nutritional, and environmental factors [23, 5].

Progressive motility ($77.7\% \pm 5.01$) exceeds the commonly accepted threshold of 60% required to consider an ejaculate fertile [20]. This parameter is strongly influenced by the quality of spermatogenesis and the physiological condition of the bull. The stability of pH (6.7 ± 0.17) within a slightly acidic range is consistent with optimal values for sperm survival.

The methylene blue reduction test, an indicator of enzymatic activity, shows an average value of $12 \text{ min} \pm 2.3$, consistent with active cellular metabolism. The fructose concentration ($600.8 \text{ mg}/100 \text{ mL}$) reflects proper functional activity of the seminal vesicles and provides an essential energy source for sperm motility [18, 24].

The correlations established between sperm parameters and storage at +4 °C reveal a highly significant relationship ($P < 0.01$). Motility, pH, redox potential, and fructose concentration show very high correlation coefficients ($r > 0.98$), highlighting the influence of storage on sperm viability. These findings align with those reported by Barth [8] and Chenoweth *et al.* [9], who emphasize the importance of thermal conservation in maintaining fertility.

Conversely, a negative correlation was observed between storage duration and the percentage of live spermatozoa ($r = -0.996$), indicating a progressive decline in cellular viability over time, likely due to increased oxidative stress and decreased energy reserves. Additionally, the increase in dead spermatozoa ($r = 0.913$) and abnormal sperm ($r = 0.939$) supports this degenerative trend, as described by others authors [6, 25].

Finally, the low variability of the measured parameters ($CV < 20\%$, except for the total number of spermatozoa per ejaculate) indicates good homogeneity of the analyzed material, thereby reinforcing the reliability of the obtained data.

Conclusion

The results of this study showed that Atlas Brown bulls exhibit overall satisfactory sperm quality, both at semen collection and during short-term storage. The evaluated parameters suggest that this breed has a good aptitude for the production of semen suitable for artificial insemination. Despite the generally modest zootechnical performances reported under certain breeding conditions, these findings indicate that this local cattle population possesses an interesting reproductive capacity.

This potential could be exploited in genetic improvement programs aimed at optimizing reproductive performance, as well as in strategies for the conservation of local genetic resources. Indeed, preserving this genetic heritage is essential in a context of increasing intensification of livestock systems and growing reliance on imported breeds. Thus, the Atlas Brown breed represents a promising and exploitable biological resource that requires further investigations to better understand its

reproductive performance and its adaptation to local environmental conditions.

Sperm parameters—especially motility, concentration, and viability—were strongly correlated with storage at $+4\text{ }^{\circ}\text{C}$, highlighting the sensitivity of semen to post-ejaculation handling. The integration of systematic andrological evaluation of local breeding bulls, including measurements such as scrotal circumference, could significantly improve reproductive performance in extensive systems.

This work also opens perspectives for further studies incorporating detailed morphological analysis of spermatozoa, biochemical fertility markers, and in vivo fertility assessment, in order to better exploit the Algerian bovine genetic heritage.

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Conflict of Interest

The study was conducted without any conflict of interest.

TABLE 1. Fresh semen quality results (N = 103 ejaculates).

Sperm parameters	Mean (X)	SD (δ)	CV %
Volume (mL)	3.7	± 0.5	13.5
Motility (%)	77.7	± 5.01	6.4
pH	6.7	± 0.17	2.5
Redox potential (BMT)	12	± 2.3	19.2
Sperm concentration ($10^6/\text{mL}$)	0.65	± 0.10	15.4
Total sperm/ejaculate (10^9)	2.17	± 0.84	38.7
Fructose concentration (mg/100 mL)	600.8	± 5.2	0.9

X: Arithmetic mean; δ : Standard deviation (SD); CV: coefficient of variation

TABLE 2. Correlation between semen storage at $+4\text{ }^{\circ}\text{C}$ and sperm parameters using Student's t-test and Fisher's test.

Studied parameters	Cr	Tst 0.01	Tst 0.05	Sr	Tr	P	Regression line (Dr)
Motility (%)	-0.981	3.50	5.88	0.06	16.23	< 0.01	$Y = 0.38x + 26.56$
pH	0.980	3.50	5.88	0.07	13.71	< 0.01	$Y = 0.008x + 5.77$
Redox potential (min)	0.999	3.50	5.88	0.02	49.90	< 0.01	$Y = 0.019x + 20.21$
Fructose concentration (mg/100 mL semen)	0.998	3.50	5.88	0.02	49.80	< 0.01	$Y = 0.474x + 32.27$
Live sperm (%)	-0.996	3.50	5.88	0.02	33.06	< 0.01	$Y = 0.376x + 29.88$
Dead sperm (%)	0.913	3.50	5.88	0.15	5.88	< 0.01	$Y = 0.202x + 22.74$
Sperm with major abnormalities (%)	0.939	3.50	5.88	0.13	6.78	< 0.01	$Y = 0.126x + 0.70$

Cr: correlation coefficient (r); Tst 0.01: Student's test at 0.01 level; Tst 0.05: Student's test at 0.05 level; Sr: Standard error of r; P: probability; Dr: regression line or regression equation.

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تحليل خصائص السائل المنوي لدى ثيران سلالة الأطلس البني المحلية الجزائرية

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الملخص

تُعد سلالة الأبقار البنية الأطلسية من السلالات السائدة ضمن القطيع الوطني، كما أنها متكيفة بشكل جيد مع الظروف المناخية المحلية، إلا أنها لا تزال غير مستغلة بالشكل الكافي من حيث الأداء التناسلي والإنتاجي. هدفت هذه الدراسة إلى توصيف جودة السائل المنوي لدى ستة ثيران من هذه السلالة، تتراوح أعمارها بين 18 و24 شهرًا، وذلك بالاعتماد على تحليل 103 قذفات منوية.

شملت المعايير المُقيّمة حجم القذفة، الحركة، الرقم الهيدروجيني (pH)، تركيز الحيوانات المنوية، النشاط الإنزيمي (باستخدام اختبار أزرق الميثيلين)، تركيز الفركتوز، بالإضافة إلى حيوية وتشوهات الحيوانات المنوية. وقد أظهرت التحاليل، التي أجريت باستخدام الطرق التقليدية (المجهر، القياس الطيفي، التلوين الحيوي)، نتائج مرضية بشكل عام، حيث بلغ متوسط الحجم 3.7 مل، والحركة التقدمية 77.7%، والتركيز 0.65×10^9 حيوان منوي/مل، ودرجة الحموضة 6.7.

كما أظهرت عملية الحفظ عند درجة +4 مئوية وجود ارتباطات معنوية عالية ($P < 0.01$) مع معظم المعايير المدروسة، حيث سُجلت ارتباطات إيجابية مع الحركة ودرجة الحموضة وتركيز الفركتوز والجهد الأكسدي-الاختزالي، وارتباطات سلبية مع نسبة النفوق والتشوهات المنوية.

تُبرز هذه النتائج الإمكانيات التناسلية لسلالة الأبقار البنية الأطلسية، وتؤكد على أهمية التقييم الأندروجيني المنتظم ضمن برامج التحسين الوراثي والحفاظ على السلالات المحلية.

الكلمات المفتاحية: السلالة البنية الأطلسية، الثيران، معايير السائل المنوي، حفظ السائل المنوي؛ الجزائر.