Evaluation of semen quality in a purebred Montbéliard Bull in Burkina Faso

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Abstract

Artificial insemination is a key tool for genetic improvement across Sub-Saharan Africa. However, limited data exists on the quality of semen locally produced from exotic purebred bulls under tropical conditions. This study aimed to assess the quality of the semen and freezability of a six-year-old purebred Montbéliard bull in Burkina Faso. Over an eight-week period, 16 ejaculates were collected twice weekly and assessed for volume, colour, pH, mass and individual motility, morphology, viability, and concentration. Samples meeting quality thresholds were extended to 100×10^6 sperm/mL with AndroMed®, equilibrated at 4 °C, frozen in liquid nitrogen vapor, and thawed at 37°C. Pre-freeze motility increased from 77.5 ± 5.8% (Week2) to 90% (Weeks7–8), while post-thaw motility ranged from 47.5 ± 5% (Week1) to 75 ± 5.8% (Week7), with significant cryoinjury only in Weeks 1, 2, and 6. Semen's volume remained stable (4.50–5.88mL), and its concentration varied (0.65–1.19 billion/mL). Correlation and PCA analyses revealed strong (>0.6) associations between pre- and post-thaw motility and temporal stabilization of semen traits. Multiple linear regression modeling indicated that including collection week as a temporal covariate

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substantially improved prediction of post-thaw motility ($R^2 = 0.632$, $p = 0.013$). These results highlight the importance of repeated collections and temporal factors in enhancing semen cryotolerance for improving local AI programs.

Keywords: cattle, Montbéliard, semen, motility, viability, cryopreservation

Évaluation de la qualité de la semence chez un taureau Montbéliard de race pure au Burkina Faso

Résumé

L'insémination artificielle est un outil majeur pour l'amélioration génétique en Afrique subsaharienne. Cependant, peu d'études évaluent la qualité de la semence produite localement par des taureaux pure race exotiques dans des conditions tropicales. Cette étude a évalué la qualité et la cryoconservation du sperme d'un taureau Montbéliard au Burkina Faso. Sur huit semaines, 16 éjaculats ont été collectés deux fois par semaine et analysés pour le volume, la couleur, le pH, la motilité de masse et individuelle, la morphologie, la viabilité et la concentration. Les échantillons de qualité ont été dilués à 100×10^6 spermatozoïdes/mL avec AndroMed®, équilibrés à 4 °C, congelés dans la vapeur d'azote liquide et décongelés à 37 °C. La motilité avant congélation variait de 77,5 ± 5,8 % à 90 ± 0 %, et la motilité post-décongélation de 47,5 ± 5,0 % à 75 ± 5,8 %, avec des lésions cryogéniques significatives seulement aux Semaines 1, 2 et 6. Le volume est resté stable (4,50–5,88 mL) et la concentration a fluctué (0,65–1,19 milliard/mL). Les analyses multivariées ont montré de fortes corrélations entre la motilité avant et après congélation et que l'inclusion de la semaine de collecte améliorait la prédiction de la motilité post-décongélation (R^2 = 0,632, p = 0,013). Ces résultats mettent en évidence l'importance des collectes répétées et des facteurs temporels pour optimiser la cryotolérance de la semence et les programmes locaux d'insémination artificielle.

Mots clés : bovin, Montbéliard, semence, motilité, viabilité, cryoconservation

Introduction

Undernutrition remains a major global concern. After remaining relatively stable between 2014 and 2019, the global prevalence of undernutrition increased from 8.4% in 2019 to approximately 9.9% in 2020, a significant rise partly attributed to the global health crisis (FAO, 2021). In Africa, nearly 21% of the population is affected by this issue (FAO, 2021). In Burkina Faso, the livestock sector plays a pivotal role in ensuring food security and strengthening the resilience of farming households. It provides not only food products (milk, meat, eggs) but also essential services such as organic manure for crop production and animal traction (MRA, 2011). The diversity of livestock species is a strategic asset for securing food supply and sustaining livelihoods, particularly in developing countries (FAO, 2010; HERRERO *et al.*, 2014; FAO, 2021). In Burkina Faso, livestock plays a central role in the

national economy: more than 80% of the rural population engages in livestock production, either as a primary or supplementary source of income. The sector contributes between 10 and 20% to the Gross Domestic Product (GDP) and accounts for approximately 40% of agricultural value added, second only to cotton (FAO, 2019; MRAH, 2019).

Livestock production in the country is predominantly based on local breeds raised in extensive systems (87–98%), semi-intensive systems (2–11%), or intensive systems (1–2%), depending on the species (FAO, 2019). However, these local breeds generally exhibit low genetic potential (TRAORÉ, 2018, TAPSOBA *et al., 2*024), which limits the sector's overall productivity. To address this limitation, the Ministry in charge of Animal and Fisheries Resources has implemented several genetic improvement strategies, including artificial insemination (AI).

Nevertheless, the high cost of imported bovine semen remains a major obstacle. A brief survey conducted at the "Centre de Promotion de l'Aviculture et de Multiplication des Animaux Performants" (CPAMAP) revealed that the cost of imported semen ranged from 13,24$ to 22,06$ per dose. This represents approximately 18.45% to 31.25% of the total cost of artificial insemination (AI) performed under induced estrus, and between 50% and 83.33% of the AI cost under natural heat conditions. To reduce these costs, Burkina Faso established an Artificial Insemination Center (AIC). Among its core missions is the local production and distribution of bovine semen to promote genetic progress through AI. This production process typically relies on commercial extenders. To reduce production costs, semen is obtained from purebred bulls derived through embryo transfer, then processed, frozen, and stored locally. However, the quality of locally produced semen remains poorly documented, particularly under the climatic and operational conditions specific to West Africa. Regarding this context, the present study aims to assess the quality of locally adapted Montbéliard bull semen produced in Burkina Faso, with the broader objective of contributing to the genetic improvement of the national cattle herd.

I. Materials and Methods

Animal

The bull used for semen collection was a 6-year-old purebred Montbéliard. The collected bull was receiving a daily diet of forage

supplemented with 12 kg of concentrate, with clean water provided ad libitum.

Semen collection and evaluation

Semen collection was carried out between August and October 2024. Semen was collected twice weekly, on Tuesdays and Fridays, in the early morning at 7:00 AM using an artificial vagina maintained at a temperature of 42–44 °C. The copulation duration was measured in seconds using a digital stopwatch, starting from the moment the teaser bull was presented to the collection bull until ejaculation. Immediately after collection, the semen samples were evaluated both macroscopically and microscopically following standard procedures as described by TAPSOBA *et al.* (2023).

Macroscopic evaluation included the assessment of semen volume, color, pH, and motility. Semen collected in the tube was immediately transported to the laboratory and maintained in a water bath at 37 °C until analysis. Ejaculate volume was determined by direct reading of the graduated collection tube. Semen color was assessed visually by direct observation, and pH was measured using pH indicator strips following immersion in the collected sample. Sperm mobility was evaluated under a light microscope following the method described by Shukla (2002).

Microscopic evaluation encompassed mass activity, progressive motility, viability, morphology, and concentration.

- Mass motility was assessed by placing a 10 µL drop of raw semen on a pre-warmed glass slide and at 100× magnification examined under a light microscope. The motility was scored on a scale from 0 to 5. where 0 = none, 1 = very weak, 2 = weak, 3 = intermediate, 4 = strong, and 5 = very strong.
- Individual motility was evaluated by diluting the semen physiological saline solution (1 drop for 4 ratio). The mixture was placed on a glass slide, covered with a coverslip, and examined at 400× magnification. Motility was assessed subjectively based on the proportion of progressively motile spermatozoa.
- Viability and morphology were evaluated using eosin-nigrosin staining. A drop of semen and four drops of the stain were applied to a slide, air-dried, and then observed under a microscope at 400× magnification.

- Sperm concentration was determined by using an IMV ® spectrophotometer.

Semen Dilution, Freezing, and Post-Thaw Evaluation

Raw semen samples were selected for freezing if they met the following criteria: a minimum volume of 1 mL, a mass motility score of at least 3 (on a 0–5 scale), 50% or more individual motility, less than 25% abnormal spermatozoa, and a sperm concentration high enough to provide 25×10^6 spermatozoa per 0.25 ml AI dose. The selected ejaculates were diluted with a commercial extender (AndroMed®) to a final concentration of 100×10^6 sperm/mL. The extended semen was loaded into 0.25 mL straws and equilibrated at 4 °C for 4 hours. Following equilibration, the straws were frozen by exposure to liquid nitrogen vapor at a height of 8 cm for 10 minutes, then plunged directly into liquid nitrogen at −196 °C for storage purpose. After 24 hours of storage in liquid nitrogen, straws were thawed in a water bath at 37 °C for 30 seconds, transferred to 1.5 mL Eppendorf®, and maintained at 37 °C during post-thaw evaluation.

Statistical Analysis

Statistical analyses were conducted using R studio (version 2024.12.1+563). Data were initially cleaned and formatted, with numeric conversion and removal of missing values. Descriptive statistics were computed for all semen quality parameters, and boxplots were used to assess distributions. A paired two-tailed Student's t-test was applied to compare pre-freeze and post-thaw motility, both overall and by collection week. To identify predictors of post-thaw motility, a multiple linear regression model was fitted using ejaculate volume, sperm concentration, mass motility, and copulation duration as independent variables.

$$\text{Post-thaw motility} = \beta 0 + \beta 1(\text{Volume}) + \beta 2(\text{Concentration}) + \beta 3(\text{Mass motility}) + \beta 4(\text{Copulation duration}) + \epsilon$$

Model assumptions normality of residuals, homoscedasticity, and absence of multicollinearity were verified using Q–Q plots, residuals vs. fitted plots, and variance inflation factors (VIF < 5). Standardized beta coefficients were estimated to assess variable importance. A one-way ANOVA was performed to examine weekly variation in post-thaw motility, preceded by tests for homogeneity of variance. Correlations between variables were assessed using Pearson's coefficient, and results were visualized via heatmap. Finally, Principal Component

Analysis (PCA) was conducted to explore the underlying structure and relationships among semen quality traits and to reduce dimensionality for data interpretation. The analysis included standardized quantitative variables: ejaculate volume, sperm concentration, mass motility, progressive motility (pre-freeze and post-thaw), copulation duration, and collection week. PCA was performed using the FactoMineR and factoextra packages in R.

II. Results

General Semen Characteristics

Semen qualitative and quantitative parameters

The collected male exhibited a scrotal circumference of 36 cm, a testicular volume of 220 cm^3, and a body weight of 856 kg. The ejaculates mostly exhibited a whitish coloration, with some variations ranging from milky white to yellowish white (Figure 1).

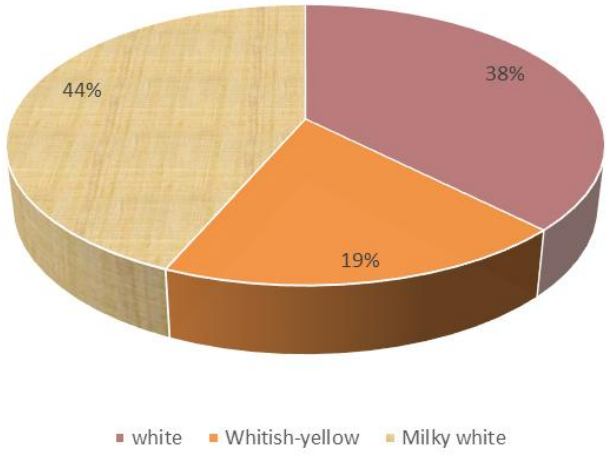

Figure 1 : Ejaculates colors

The table I summarizes the trends in means± standard deviations of the quantitative semen parameters over the eight-week collection period. Semen volume remained relatively stable across the weeks, ranging from 4.50 mL to 5.88 mL. In contrast, sperm concentration in the semen showed more marked fluctuations, varying from 0.65 billion/mL (Week 3) to 1.19 billion/mL (Week 4). Week 3 was characterized by a notably

lower concentration, whereas Weeks 4 and 7 exhibited higher values. Mass motility remained generally stable, with values ranging between 3.0 and 4.25. Nonetheless, some variations were observed, including a slight decline in Week 4 (3.0) and an increase in Week 7 (4.25). Copulation time varied considerably from week to week, reaching an exceptionally high value in Week 5 (437.75 ± 606.15 seconds, with the shortest duration recorded at 17 seconds.

Table I: Means and standard deviations of bovine semen quantitative parameters over time

W_i	Volume (ml)	Concentration (10^9)	Mass motility	pH	Copulation duration (secondes)
W1	*5.75±1.04*	*1.13±0.23*	*3.5±0.58*	6.55±0.07	62.25±31.20
W2	*4.50±0.00*	*1,44 ± 0,40*	*3.5±0.58*	6.90±0.28	50.50±38.79
W3	*5.75±1.50*	*0.65±0.51*	*3.5±0.58*	6.55±0.07	69.25±55.61
W4	*5.75±1.55*	*1.19±0.27*	*3±0*	6.90±0.28	142.00±65.62
W5	*5.38±0.75*	*0.94±0.30*	*3.75±0.50*	6.55±0.07	437.75±606.15
W6	*5.88±1.49*	*0.85±0.43.*	*4±0.82*	6.60±0.14	126.75±235.51
W7	*5.50±1.91*	*1.18±0.28*	*4.25±0.50*	6.65±0.07	17.00±12.08
W8	*5.00±1.08*	*0.97±0.14*	*3.75±0.50*	6.80±0.42	19.75±6.95

W_i : week$_i$

Abnormal sperm percentages ranged from 10% (Weeks 2, 5) to 28% (Weeks 1, 4, 6), with intermediate values of 19% (W3), 16% (W7), and 13% (W8) (figure 2).

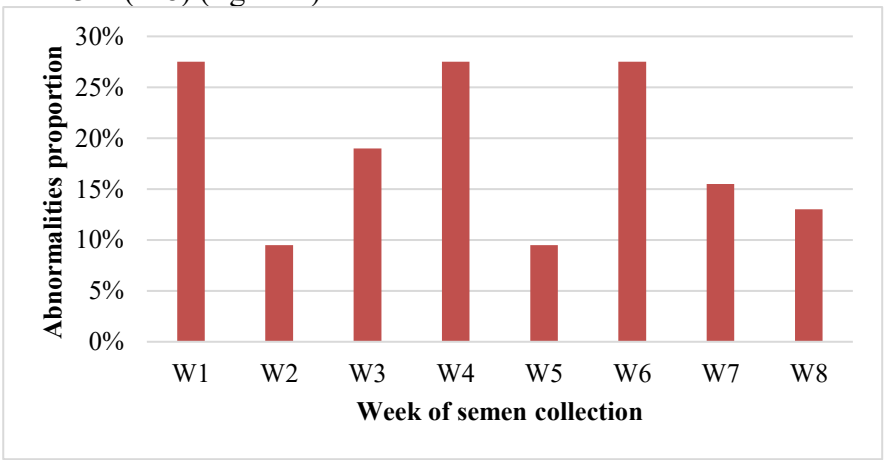

Figure 2: Semen abnormalities across weeks of the trial

Evaluation of individual pre-freeze and post-thaw motility by week

The variation in the means of pre-freezing and post-thaw individual motility over the collection weeks is summarized in Table II below. Pre-freezing motility values range from 75 ± 5.77% (Week 2) to 90 ± 0% (Weeks 7 and 8). Weeks 7 and 8 demonstrate stable and maximal pre-freezing motility. Post-thaw motility values range from 47.5 ± 5% (Week 1) to 75 ± 5.77% (Week 7). Week 1 exhibits the lowest post-thaw motility, whereas Week 7 shows the highest. The data from the eight weeks indicate significant variations in sperm motility before freezing and after thawing. Weeks 1, 2, and 6 showed significant differences between pre-freezing and post-thaw motility. No significant differences were observed in the remaining five weeks of the trial ($p>0.05$).

Table II: Means and standard deviations of pre-freezing and post-thaw motilities by collection time

W_i	Pre-freeze motility	Post-thaw motility	P-value	Significance
W1	77,5 ± 50	47, 5±5	0,0001	***
W2	75 ± 5,77	55 ± 12,9	0,0405	*
W3	80 ± 00	60 ± 11,5	0,1657	NS
W4	77,5 ± 50	67,5 ± 15	0,2522	NS
W5	82,5 ± 50	73,8 ± 4,79	0,1328	NS
W6	87,5 ± 50	65 ± 10	0.0028	**
W7	90 ± 00	75 ± 5,77	0,3248	NS
W8	90 ± 00	65 ± 17,30	0,4823	NS

*** : $p<0,001$; ** : $p<0,01$; * : $p<0,05$ NS : not significant ; W_i = $week_i$

Correlations among sperm parameters

The correlations between the parameters are presented in Figure 3. The heatmap shows that coitus duration is moderately correlated ($r = 0.5$) with post-thaw motility, mass motility, and ejaculate volume. Pre-freeze and post-thaw motilities exhibit a strong positive correlation. In contrast, sperm concentration shows negative relationship with all other parameters.

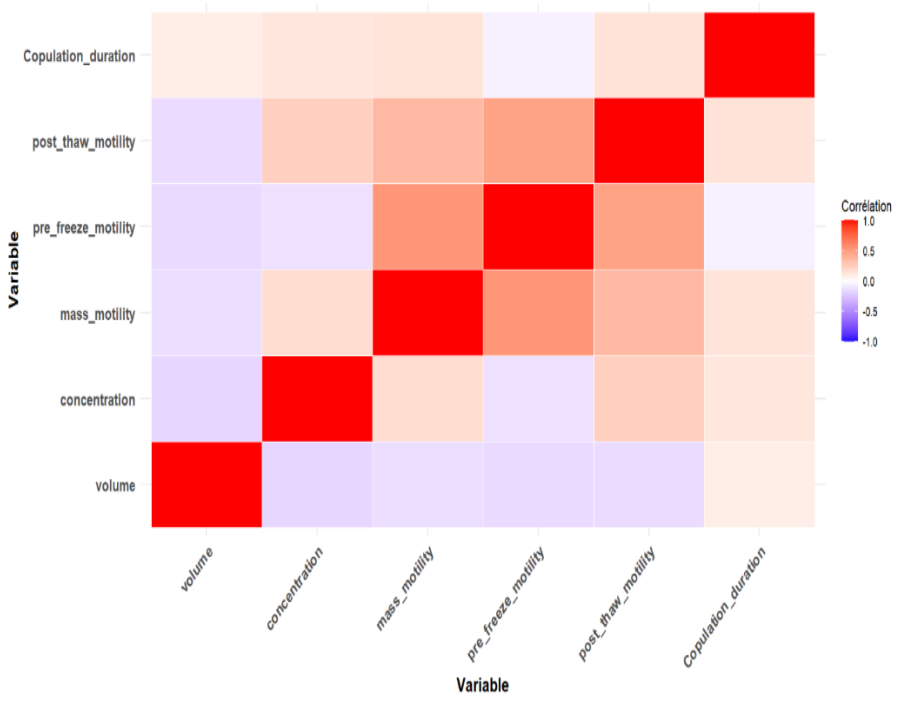

Figure 3: Heatmap depicting correlation among the qualitative parameters of semen

Principal component analysis of temporal variation in sperm motility and concentration

The first two dimensions of the biplot explains 54.3% of the total variance (figure 4). The figure highlights an opposition between sperm motility parameters (pre-freeze, post-thaw, and mass motility) and sperm concentration. Additionally, copulation duration follows a similar trend to concentration. Furthermore, the distribution of points, corresponding to the different semen collections (two per week), reveals a temporal evolution of sperm parameters. Early collections (1–10) are widely dispersed. Intermediate collections (10–20) tend to show stabilization, with data points converging toward the origin of the factorial plane. Later collections (>20) are more clustered and oriented along the axes associated with sperm concentration and post-thaw motility.

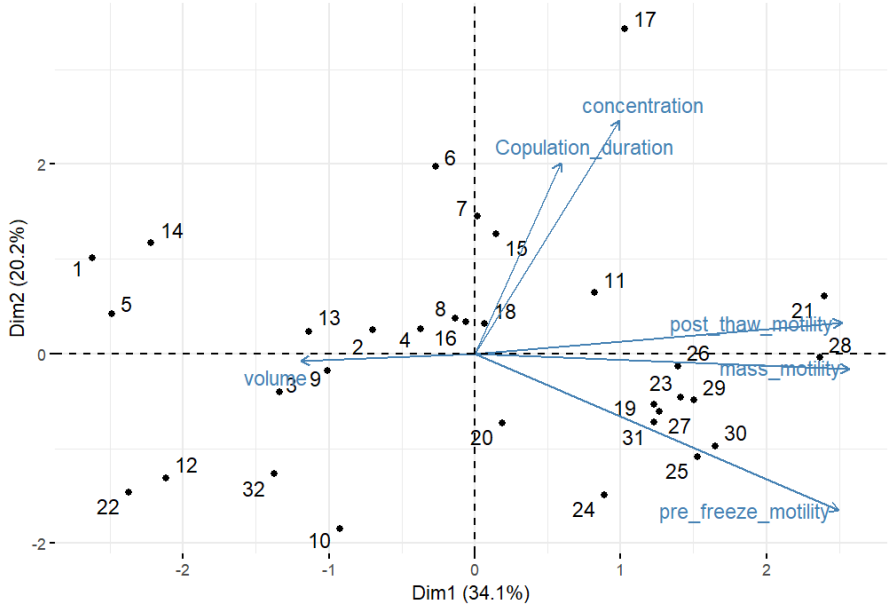

Figure 4: Principal Component Analysis (PCA) plot showing the data distribution across time

Models Assumption Verification

Normality of residuals was assessed using Q-Q plots and Shapiro-Wilk test ($W = 0.949$, $p = 0.133$). Homoscedasticity was verified through residuals vs. fitted plots and Breusch-Pagan test ($BP = 4.437$, $p = 0.350$). Multicollinearity was evaluated using variance inflation factors, with all VIF values < 5 indicating absence of concerning multicollinearity. Autocorrelation was tested via Durbin-Watson statistic ($DW = 1.299$, $p = 0.016$).

The initial model explained 18.8% of variance in post-thaw motility (Adjusted $R^2 = 0.067$, $F(4,27) = 1.56$, $p = 0.213$). Mass motility emerged as the strongest predictor based on standardized beta coefficients ($\beta = 0.305$), but it did not reach statistical significance ($p = 0.099$) (Table III)

Table III: Multiple linear regression coefficients for post-thaw motility prediction

Predictor	Unstandardized β	SE	Standardized β	t-value	p-value
Intercept	36.636	19.609	-	1.868	0.073
Volume	-0.907	1.918	-0.084	-0.473	0.640
Concentration	5.725	6.252	0.164	0.916	0.368
Mass motility	6.708	3.926	0.305	1.709	0.099
Copulation duration	0.018	0.023	0.133	0.760	0.454

The inclusion of the collection week as a temporal covariate to the basic model improved the model performance (R^2 = 0.632; adjusted R^2 = 0.429; p = 0.013. The week-adjusted model demonstrated superior performance across all metrics: highest explanatory power (R^2=0.632), statistical significance (p=0.013), lowest information criteria (AIC=249.03), and improved prediction accuracy (RMSE=9.98). (Table IV) Among single-parameter models, mass motility alone showed a modest but significant association with post-thaw motility (R^2 = 0.132, p = 0.041). Adding sperm concentration slightly improved model fit (R^2 = 0.165) but did not reach statistical significance (p = 0.073).

Table IV: Model comparison metrics for predicting post-thaw sperm motility

Model	R^2	Adj. R^2	AIC	BIC	F-statistic	p-value	RMSE	MAE
Week-adjusted	0.632	0.429	249.03	267.58	3.117	0.013	9.98	7.23
Mass only	0.132	0.103	256.46	260.25	4.563	0.041	12.51	10.14
Mass + Conc	0.165	0.107	257.22	262.61	2.863	0.073	12.48	9.87

AIC: Akaike Information Criterion**, BIC:** Bayesian Information Criterion**,** **RMSE**: Root Mean Square Error, **MAE**: Mean Absolute Error

III. Discussion

Semen Color

During collection, the color of the ejaculate ranged from whitish to yellowish-white, consistent with observations by SOUDRE *et al.* (2005) in Azawak bulls and later confirmed by Coulidiati (2021) in the same breed. HANZEN (2016) suggests that a whitish or milky white coloration typically indicates good semen quality. However, a yellowish hue may reflect natural variation in ejaculate color due to factors such as diet and seasonality. According to PAREZ and DUPLAN (1987), this yellow coloration could be attributed to high sperm concentration or the presence of lipid pigments (lipochromes) derived from the seminal vesicles. Practically, such color remains acceptable for both fresh use and cryopreservation, as bovine semen is considered normal when it ranges from pale white to bright yellow (PAREZ and DUPLAN, 1987).

General Semen Characteristics

The observed ejaculate volumes (4.50 to 5.88 mL) align with the range reported by AMANN and ALMQUIST (1986) for taurine bulls (3–8 mL). The bull used in this study was 36 months old, weighed 821 kg, and had paired testes weighing 307g. These values, however, remain below those documented in zebu breeds such as Azawak (3.85 ± 1.97 mL; SOUDRE*et al.*, 2005) and hybrid types like the Borgou (N'DIAYE *et al.*, 2000). The relative stability in ejaculate volume over the weeks likely reflects the bull's progressive adaptation to collection frequency, a phenomenon previously reported by SÖDERQUIST *et al.* (1991).

Trends in Volume and Concentration

In the early weeks, a correlation was noted between volume and concentration trends; however, this relationship diminished over time. This pattern is similar to findings by CHENOWETH *et al.* (1992), who demonstrated that the relationship between ejaculate volume and concentration may be influenced by collection frequency. Sperm concentration varied between 0.65 and 1.19 billion/mL across the weeks, lower than values reported for Tarentaise bulls (2.72 ± 0.46 billion/mL) and Azawak bulls (2.65 ± 0.76 billion/mL) (COULIDIATI, 2021). This fluctuation is consistent with findings by SALISBURY *et al.* (1978) and FOOTE (2002), who noted that while ejaculate volume

tends to be stable in bulls, concentration can vary depending on collection frequency and environmental conditions Mass motility remained relatively stable throughout the collection period, with values ranging from 3 to 4.25. These results are in line with those of BARTH and OKO (1989) and COULIDIATI (2021), who reported mass motility scores between 3 and 5 in breeding bulls. Coital time exhibited marked variability, with a particularly high peak in week 5. This outlier may be attributed to reduced libido on that specific collection day, during which the bull displayed atypical behavior and walked extensively before ejaculation could be induced. This prolonged activity may have influenced sexual arousal and the collection process.

Pre- and Post-Thaw Motility

Pre-thaw motility results are comparable to those reported by GRAHAM AND MOCE (2005). The gradual improvement observed up to week 7 may reflect the bull's behavioral adaptation to semen collection procedures. Post-thaw motility was lowest in week 1 (47.5%), consistent with prior observations suggesting that initial ejaculates are often of lower quality (COULIDIATI, 2021; KOUDA, 2021).

Correlations Among Sperm Parameters

The negative correlation between sperm concentration and post-thaw motility supports findings by BARBAS and MASCARENHAS (2009), who attributed this relationship to the detrimental effects of excessively high sperm density on cryosurvival. The strong positive correlation between pre- and post-thaw motility highlights the predictive value of initial semen quality for cryopreservation outcomes.

Data Visualization and Principal Component Analysis

Principal Component Analysis (PCA), used to visualize data trends and the distribution of ejaculates over time, explained 54.3% of total variance. The biplot revealed a clear opposition between motility parameters (pre-thaw, post-thaw, and mass motility) and sperm concentration. This finding is consistent with GARCÍA-HERREROS *et al.* (2005), who showed that motility and concentration may be influenced by different physiological mechanisms, excessive concentration, for instance, may reduce seminal plasma fluidity, thereby impairing motility.

Moreover, the progressive stabilization of semen parameters across the collection period likely reflects the bull's adaptation to repeated semen

collection. This adaptation was also noted by SÖDERQUIST *et al.* (1991), who reported that bulls subjected to frequent collections may exhibit stabilized semen quality over time.

Modeling post thaw motility

The basic model explained a limited proportion of variance in post-thaw motility with mass motility showing the highest standardized effect. Incorporating the collection week as a temporal covariate improved model performance, reduced information criteria, and enhanced predictive accuracy, suggesting substantial temporal structure in the data. Comparable improvements in predictive capacity after adding time-dependent variables have been reported in semen quality modeling (Jiang et al., 2017; Yánez-Ortiz et al., 2024), supporting the inclusion of temporal effects in cryopreservation models. Among univariate models, mass motility alone showed a modest but significant association, consistent with its recognized role as a key predictor of sperm cryosurvival (Tu et al., 2022).

Conclusion

This study successfully assessed the semen quality of a locally adapted purebred Montbéliard bull, offering valuable insights into the semen characteristics over an extended collection period. Key parameters such as ejaculate volume, sperm concentration, motility, and cryopreservation outcomes were evaluated. The results indicate that the Montbéliard bull maintains consistent semen quality, with stable pre- and post-thaw motility, adequate sperm concentration, and favorable ejaculate volume. The study also highlighted temporal variations in sperm motility, particularly the improvement in post-thaw motility over time, suggesting that repeated semen collections can enhance cryopreservation outcomes. These findings are crucial for optimizing semen quality assessments in locally adapted purebred bulls and improving breeding strategies in bovine populations.

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Conflict of interest

The authors declare no conflict of interest.

Authors contributions

TASR and KP and DM designed the study, HM, TGF, YB, SSE corrected the manuscript, AT, BB and ZM, supervised the experiment, SY, SS and KG collected the data. TASR did the data analyses and drafted the manuscript.

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