

Semen Characteristics of Crossbred Bucks Anglo-Nubian × Sahelian Goats in Mali

IDRISSA SACKO¹, MADOU DAO¹, SOULEYMANE SANOGO^{1,2*}, B. MICHEL OROUNLADJI², KALY DIAKITÉ¹, DIAKARIDIA TRAORE³, MAMADOU D. COULIBALY¹, AMADOU B. CISSE¹

¹Centre Régional de Recherche Agronomique (CRRA) de Kayes, Institut d'Economie Rurale (IER), Mali; ²Centre International de Recherche-Développement sur l'Élevage en Zone Subhumide (CIRDES), Burkina Faso; ³Centre National d'Insémination Artificielle Animale (CNIA), Bamako, Mali.

Abstract | This study's goal was to perform macroscopic and microscopic characterization of Anglo-Nubian × Sahelian crossbred goat's semen in Mali. From January to August 2020, a total of 45 ejaculates have been collected from 03 bucks (5/8 Anglo-Nubian, 3/8 Sahelian Goat) using artificial vagina. Semen volume, mass motility and live and dead sperm counts (spz) were measured by direct reading and electronic microscopy with a direct image transfer system on screen, respectively. Sperm concentration and total sperm count in ejaculate were determined by microscopy using single chamber Neubauer cell. Results show that ejaculate volume was influenced ($p < 0.05$) by individual (buck). Besides, individual and period of collection had no impact ($p > 0.05$) on mass motility. However, the period of collection significantly ($p < 0.05$) affects ejaculate volume, vitality, sperm concentration and mortality rate. The overall averages obtained for the different parameters studied were 0.92 ml for semen volume; 3.91 for mass motility; 73.69% for vitality; $2.11.10^9$ spz/ml for sperm concentration, $2.11.10^9$ spz and 26.54% for total sperm count in ejaculate and spz mortality rate respectively. March-April was found to be more suitable for semen collection. This study emphasized the high semen quality of crossbred bucks, which could be candidates for a goat genetic improvement program.

Keywords | Anglo Nubian, Crossbred bucks, Sahelian goat, Sperm quality

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***Correspondence** | Souleymane Sanogo, Centre International de Recherche-Développement sur l'Élevage en Zone Subhumide (CIRDES), Burkina Faso; **Email:** souley.sanogo@cirdes.org

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INTRODUCTION

Small ruminants are raised with a significant art by rural and urban population in Mali. The current size of the goat population was estimated at 26,486,240 head (DNPIA, 2019). The use of different breeds of small ruminants helps many poor households to overcome their economic and social issues (Wasso et al., 2018; Sacko, 2021). Despite their numerical and socioeconomic importance, local breeds of goat have very low productivity (0.2 to 1 liter/day of milk and adult live body weight of 20 to 35 kg) compared to exotic breeds (3 to 5 liters/day;

90 to 150 kg/adult) and their crossbred products (2 to 3 liters/day; 40 to 80 kg/adult), Dao (2016). The low milk and meat production of local breeds is characteristic to their low genetic potential and dependent on the absence of a genetic improvement program (Boly et al., 2001). Moreover, this low production is linked to the lack of control over the collection and processing methods of semen, as well as the lack of adequate equipment. In this respect, crossing these indigenous breeds with exotic breeds for improvement appears to be a real and rapid opportunity to boost goat production and productivity in Mali (Sanogo et al., 2012). This improvement in productivity cannot be

achieved without a good mastery of semen collection and processing techniques. The availability of suitable breeding equipment to produce enough crossbred to satisfy the growing demand of farmers is also a plus.

The control of reproduction in goat farming, allows the grouping of births and facilitates the management of pregnant or lactating females (Leboeuf et al., 2003). Moreover, reproductive performance is found to be one of the main factors determining the efficiency of the animal production and its failure can lead to a significant economic loss in breeding (Abdelsalam and Mohammed, 2011; Jibril et al., 2013). Experiments have proven the feasibility of the approach, but its cost remains the limiting factor for producers (FAO, 2008).

Both on station and in the field, crossbred Anglo-Nubian goat x Sahelian goat have better milk productivity than local goats (Sanogo et al., 2012). These crossbreeds adapt well to the climatic conditions in Mali and deserve to be disseminated to breeders. Unfortunately, the number of these crossbred animals available on station is still insufficient to satisfy the growing demand of breeders in this country. The use of artificial insemination, especially from fresh semen locally produced, seems in the short term, one of the best alternatives to boost production and solve the inadequacy. Artificial insemination has several advantages. These include: (i) the rapid dissemination of genetic progress, (ii) the introduction of exotic breeds through semen in areas where live animals cannot withstand climates and pathologies, (iii) the control of venereal contact diseases, and (iv) the reduction of expenses related to maintenance of males on farms (Knox, 2016). This technique promotes the rapid dissemination of genetic progress through the “male route”, between several females that can be inseminated from the same ejaculate.

The present study is part of the research dynamics of the mastery of tools for scaling up goat seed technology to make it accessible to breeders. This can allow to improve the genetic potential of their goat herd and their incomes while contributing to ensure the food and nutritional security of the populations.

The results of the study will contribute to improve the access of breeders to a greater number of genetically improved goats through a better knowledge of semen characteristics and the selection of the best bucks for semen quality production for artificial insemination.

MATERIALS AND METHODS

STUDY AREA

The trial was carried out at the Research Station of the Regional Agricultural Research Center (CRR) of Samé/

Kayes in Mali. The climate is of the Sudano-Sahelian type with isohyets ranging from 550 to 750 mm of water per year. The average temperature is 28°C with a maximum of 44°C in the hot dry season. The rainy season extends from June to September with an average of 55 days of rain. Evapotranspiration varies between 2300 and 2500 mm per year.

ANIMAL MATERIAL

The sample was composed of three (3) crossbred bucks (5/8 Anglo-Nubian, 3/8 Sahelian Goat) from which 45 ejaculates were collected. The choice of the buck was made on the basis of the conformity of the criteria such as: absence of defect or malformation of the testicles, their sire's performance, their individual performance, the performance of their collaterals, phenotypic criteria of the breed and good health status. The selected bucks based on those criteria were representative of the crossbred population.

The billy goats were 5.52 years with an average weight of 71.37±7.37 kg. Such adult bucks have been chosen for the experiment, based on the findings of Atara et al. (2018). According to those researchers, adult bucks should be preferred over young bucks for breeding purpose because they perform better than the latter in terms of semen quantitative parameters. The experimental animals were fed on grazing pasture from 8:00 am to 1:00 pm in the morning and from 3:00 pm to 6:00 pm in the afternoon. When they returned from the pasture, they were supplemented with cottonseed cake (300 g per head) and salt lick. Brucellosis test was performed on all the experimental animals. Previously, all animals were internally and externally dewormed and vaccinated against pasteurellosis and Peste des Petits Ruminants.

SEMEN COLLECTION AND EXAMINATION EQUIPMENT

The collection material consisted of three (3) complete artificial vaginas, graduated cone tubes, a cauldron, gloves, knee protectors, non-spermicidal lubricating gel and absorbent paper. The examination was done with an electronic microscope equipped with a system of image transfer to the computer with a heating plate at 37-38°C, a spectrophotometer, a water bath, a single-chamber Neubauer hematometer, a vortex mixer, a centrifuge, micropipettes (10µl, 50µl, and 1000µl), slide and slide boxes, racks for tubes and micropipettes, test and hemolytic tubes. Solutions were also used for sperm analysis including:

- Formolated NaCl solution: 1 ml of formalin supplemented to 100 ml with the 9% NaCl solution.
- Eosin-Nigrosin solution: 1g of eosin + 2g of Nigrosin.

DETECTION OF THE HEAT ON THE FEMALES

Females underwent heat synchronization hormone treatments to facilitate buck mounting and semen

collection. The protocol used was based on the protocol of Maher (2010, 2012). The products used were vaginal sponges impregnated with FGA 30 mg, chronogest PMSG 600 (1.5 ml) and Enzaprost (0.2 ml).

SEMEN COLLECTION

On the eve of each collection session, the bucks were subjected to a general health examination and to the operations of maintenance and washing of the posterior trunks, abdominal regions with soap, bleach and rinse with tap water. Semen collection was done from January to August 2020 using the artificial vagina with a frequency of one or two collection sessions per month depending on the synchronization results. At each collection session, one or two samples (depending on the condition of the sire) were collected at 10 to 20 minute intervals.

In order to allow a good erection, the buck was presented 2 or 3 times to the female goat in heat. The time of preparation of the mating lasted about 10 minutes and includes two or three false mounting depending on the physical condition of the sire. The operator squats next to the immobilized female. After two or three false mounts without intromission, collection is performed with the artificial vagina at the next mount, when the buck approaches vigorously for mating.

MACROSCOPIC ANALYSIS OF SEMEN

The volume of the ejaculate is read directly from the graduations of the conical collection tube and by weighing on a nano-gram scale to minimize the errors made when reading the graduations of the tube. It is done immediately after collection without taking into account the foamy part of the ejaculate. The color of the collected semen was assessed visually after collection at the same time as the volume reading. Viscosity was assessed in two ways after liquefaction (30-60 min) in the laboratory. It is firstly appreciated through visual assessment and, secondly by stretching through the tip of the micropipette. The viscosity is qualified as follows: increased, normal and abnormal viscosity.

MICROSCOPIC ANALYSIS OF SEMEN

Mass motility was assessed through the deposition of two 10- μ l drops of pure sperm (without dilution) on a coverslip and then examined under a hot stage microscope at objective 40 for 20 to 30 seconds at the scale of 0 to 5 (Baril et al., 1993). The concentration was calculated using the Neubauer hemacytometer following the same procedures as Baril et al. (1993), which consisted of diluting 0.01 ml (10 μ l) of pure semen in 4 ml of formalinized saline, a volume of 1/400. The actual concentration of ejaculate was determined by the following formula:

$$\left(\frac{A+B}{2}\right) * \left(\frac{100}{4}\right) * 400 = \left(\frac{A+B}{2}\right) * 10^4 \text{ spz/mm}^3$$

Either, $\left(\frac{A+B}{2}\right) * 10^7 \text{ spz/ml de semence pure}$

Where A and B refer to the sperm (spz) count in the large squares of each of the two corresponding grids. The Neubauer hemacytometer used for counting had only one counting chamber.

DETERMINATION OF THE PERCENTAGE OF DEAD SPERM

Dead sperms count was determined by mixing 10 μ l of pure semen with 10 μ l of 2% eosin dye shaken for approximately 10 seconds and adding 20 μ l of 4% nigrosin while shaking to homogenize the mixture. 10 μ l of this mixture was spread on a slide identified by the number of the male and the date of harvest. The slide was then observed under a microscope at object 100 while putting on a drop of immersion oil. A count of 100 to 200 sperms was performed distinguishing between live and dead sperms.

DATA ANALYSIS

The relative frequencies of the different modalities of the qualitative characteristics (color and viscosity) of the bucks' semen were calculated. Analysis of variance were performed to compare quantitative sperm characteristics (mass motility, ejaculate volume, vitality, concentration, and mortality rate of sperm) as a function of animal and month of semen collection. The Student Newman-Keuls test was used for comparison of means in case of significant difference ($p < 0.05$) through agricolae package (de Mendiburu, 2020). Pearson correlations and coefficient of determination (R^2) were determined in order to establish relationships between sperm parameters. The data were analyzed using R 4.1.2 statistical package (R Core Team, 2021).

RESULTS AND DISCUSSION

COLOR AND VISCOSITY OF GOAT SEMEN

The buck's semen was whitish in color (100%) while 91% of the semen's samples collected showed a normal viscosity (Figure 1).

SPERMATIC CHARACTERISTICS ACCORDING TO THE ANIMAL

For all quantitative sperm parameters considered, only ejaculate volume showed significant difference ($p < 0.05$) among bucks (Table 1). Indeed, the ejaculate volume was more abundant in the buck 999 (1.31 \pm 0.58 ml) compared to the buck 998 (0.77 \pm 0.36 ml) and 1382 (0.72 \pm 0.18 ml). The overall average ejaculate volume was 0.92 ml while the overall mean mass motility score was 3.91 and the average vitality was 73.69%. The sperm concentration was 2.11 $\times 10^9$ /ml. The sperm mortality rate was 26.54%.

Table 1: Sperm characteristics (mean ± standard error) of crossbred bucks.

Variables	Goat 998	Goat 999	Goat 1382	Overall	p-value
Mass motility	4.00±0.50	3.93±0.73	3.85±0.49	3.91	0.802
Ejaculate volume (ml)	0.77±0.36 ^b	1.31±0.58 ^a	0.72±0.18 ^b	0.92	< 0.001 ^{***}
Vitality (%)	80.46±8.79	71.81±12.75	71.96±9.98	73.69	0.118
Concentration (10 ⁹ /ml)	1.70±1.07	1.93±1.15	2.41±1.73	2.11	0.415
Concentration per ejaculate (x10 ⁹ spz)	1.59±1.53	2.75±2.48	1.89±1.67	2.11	0.311
Mortality rate of sperm (%)	19.54±8.79	28.19±12.75	28.53±11.44	26.54	0.131

a, b: values assigned by different letters on the same line are significantly different (p < 0.05); spz: spermatozoa; ^{***}: highly significant difference and p < 0.001

Table 2: Sperm characteristics (mean ± standard error) of crossbred bucks by month of collection.

Variables	January - February	March-April	May-June	July-August	Overall	p-value
Mass motility	4.23±0.60	3.80±0.42	3.86±0.38	3.69±0.63	3.91	0.080
Ejaculate volume (ml)	0.89±0.38 ^b	1.32±0.44 ^a	0.88±0.61 ^b	0.67±0.27 ^b	0.92	0.007 ^{**}
Vitality	67.45±9.43 ^b	70.86±13.89 ^{ab}	80.32±5.66 ^a	78.53±8.89 ^a	73.69	0.015 [*]
Concentration per ml (x10 ⁹)	2.58±2.14 ^{ab}	2.77±0.98 ^a	1.70±0.47 ^b	1.35±0.71 ^b	2.11	0.041 [*]
Concentration per ejaculate (x10 ⁹)	2.30±1.96 ^{ab}	3.81±2.44 ^a	1.48±1.00 ^b	0.95±0.70 ^b	2.11	0.002 ^{**}
Mortality rate of sperm	33.32±11.44 ^a	29.13±13.89 ^{ab}	19.67±5.66 ^b	21.47±8.89 ^b	26.54	0.016 [*]

a, b: values assigned by different letters on the same line are significantly different (p < 0.05); spz: spermatozoa; ^{*}: significantly different and p < 0.05; ^{**}: highly significantly different and p < 0.01

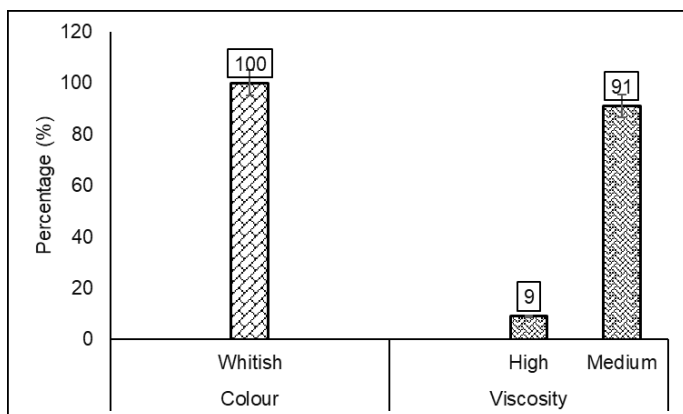


Figure 1: Frequency of qualitative characteristics of buck's semen.

SPERM CHARACTERISTICS ACCORDING TO THE MONTH OF SEMEN COLLECTION

The months of semen collection had significant effect (p < 0.05) on ejaculate volume, sperm vitality, concentrations per ml and per ejaculate (Table 2). March and April had the highest values of ejaculate volume (1.32 ± 0.44 ml). Sperm vitalities in May-June (80.32 ± 5.66 %) and July-August (78.53 ± 8.89 %) were higher (p < 0.05) than in January-February (67.45 ± 9.43 %). Semen sperm concentration was higher in March-April (2.77 ± 0.98 x 10⁹ /ml) than in May-June (1.70 ± 0.47 x 10⁹ /ml) and July-August (1.35 ± 0.71 x 10⁹ /ml). Per ejaculate, the sperm concentration was also higher (3.81 ± 2.44x10⁹) in March-April than in the following months. The mortality rate of sperm was higher in January-February (33.32 ± 11.44%) than in May-June (19.67 ± 5.66 %) and July-August (21.47 ± 8.89%).

CORRELATION AND LINEAR MODELS BETWEEN SPERM PARAMETERS OF BUCKS

Pearson correlations performed between sperm parameters yielded strong and positive coefficients (r = 0.69; p < 0.001) between sperm concentration per ejaculate and ejaculate volume (Table 3). Concentration per ejaculate and per ml showed strong, positive correlations (r = 0.83; p < 0.001). The correlation coefficient (r = - 0.99; p < 0.001) between sperm vitality and sperm mortality rate was negative and high (Table 3).

Table 3: Correlation matrix between sperm parameters of crossbred bucks.

Variables	Motil	Vol_ml	Vit_%	Conc	Tx_spz_m	Conc_ejac
Motil						
Vol_ml	0.02					
Vit_%	0.12	-0.31 [*]				
Conc	-0.07	0.29 [*]	-0.26			
Tx_spz_m	-0.14	0.30 [*]	-0.99 ^{***}	0.23		
Conc_ejac	-0.11	0.69 ^{***}	-0.39 ^{**}	0.83 ^{***}	0.36 ^{**}	

Motil: Motility; Vol_ml: Volume in ml; Vit_%: Vitality in %; Conc: Concentration; Tx_spz_m: mortality rate of sperm; Conc_ejac: Ejaculate concentration; ^{***}: strong correlation and p < 0.001.

The coefficients of determination between the concentration per ejaculate and its volume (R²= 0.48), between the concentration per ejaculate and per ml (R²= 0.68) and that between sperm vitality and its mortality rate

($R^2 = 0.98$) were relatively high (Table 4).

Table 4: Predictive models for ejaculate concentration and sperm vitality.

Relationship between variables	Models	R^2	r	p-value
CE - VE	CE = -0.62 + 2.89 VE	0.48	0.69	< 0.001
CE - Cml	EC = -0.35 + 1.14 Cml	0.68	0.83	< 0.001
Vit - Tspz	Vit = 98.92 - 0.95 Tspz	0.98	-0.99	< 0.001

EC: ejaculate concentration; EV: ejaculate volume; Cml: concentration per milliliter; Vit: sperm vitality; Tspz: sperm mortality rate; R^2 : coefficient of determination; r: correlation coefficient.

Throughout this study, which focused on the characterization of semen parameters of crossbred bucks Anglo-Nubians × Sahelian goats, no abnormalities in semen color were observed in the buck. Indeed, the semen collected color ranging from white to whitish. Similar results were reported by Haro et al. (2019) who had obtained a majority percentage of 69.2% for the milky white color exhibited by the semen of Sahelian goats in Burkina Faso. The high viscosity of the semen coupled with the whitish color is evidence of the normal appearance of the semen (Haro et al., 2019). The individual (buck) selected for semen collection influences the ejaculate volume. This result can be explained by age and environmental factors that act differently on individuals that can increase or decrease their seminal production. Semen parameters are influenced by age of the bucks (Mia et al., 2013; Atara et al., 2018). The time (month) of semen collection during the year has influenced on ejaculate volume, sperm vitality, sperm concentration in the semen and sperm mortality rate.

The overall average volume (0.92 ml) of ejaculate observed is less than 1.28 ml and 1.54 ml reported by Haro et al. (2019) on the Sahelian buck in Burkina Faso and Singh et al. (2019) on Beetal goats in India, respectively, as well as the range of 1 to 1.5 ml obtained in Alpine goats (Baril et al., 1993). This volume is greater than 0.51 ml reported by Souley (2013) on the Sahel goat in Niger. The quantity and quality of animal semen is influenced by genetic type, diet, collection frequency, and season (Dotche et al., 2019). The difference in the volume of semen might reflect their different genetic potentiality and genetically superior bucks could produce higher volume of semen as found also by Swarna et al. (2022). The result also agrees with the findings of the authors (Islam et al., 2008; Sultana et al., 2013) who reported a significant individual variation on semen volume of Black Bengal bucks.

The mass motility (3.91) obtained in this study is higher than those obtained (2.90 and 3.17) in India (Singh

et al., 2019) and Burkina Faso (Haro et al., 2019), respectively. This difference can be explained not only by the agroclimatic conditions that are different depending on the regions but also by the genotypic factors that are not similar. Delgadillo et al. (1999), found that sperm motility is low between January and April (≈ 3.02) and rises between May and November (≈ 3.55). In this study, mass motility is similar during all collection months, but higher than those obtained by these authors (Delgadillo et al., 1999). However, the results of the mass motility are in agreement with the results of Swarna et al. (2022). A score of 3 for mass motility is usually discarded. This is not the case in this study. Sperm motility is the first and foremost criteria of a semen sample whether it would be selected or discarded (Swarna et al., 2022). Similarly, fertility will be good as concluded by Issa et al. (2001) for whom, ejaculates with a mass motility score lower than 4 have a lower fertility (60.9%).

The average vitality (73.69%) obtained for the bucks during this study is better from May to August. It is however, lower than that reported by Souley (2013) on the Sahel buck in Niger and 94.5% reported by Haro et al. (2019) in the Sahel billy goat in Burkina Faso. The sperm motility found in the present study was also lower than 80.83% reported by Siddiqua et al. (2016). The results of Souley (2013) were similar to those of Haro et al. (2019) on the same breed in Niger and Burkina Faso. Vitality provides information on the rate of live sperm and dead sperm from an ejaculate. According to Kabera (2008), for the use of semen in artificial insemination, the standard requires that the percentage of live sperm in the semen should be greater than 60%. Therefore, the semen from the bucks in the study is suitable for use in artificial insemination.

The variations in concentration between the months of collection in the study are contrary to those reported in the work of Delgadillo et al. (1999). Their results showed that the minimum sperm count per ejaculation occurs between February and April ($1.4 \cdot 10^9$ spz/ejaculation), while the maximum number is observed between May and September ($2.8 \cdot 10^9$ spz/ejaculation). These differences may be related to variations in climatic factors and animal feeding.

The strong and positive correlations between sperm concentration and ejaculate allows the development of predictive models for estimating or quantifying sperm concentration in crossbred bucks. These results provide alternatives for reducing the time and cost of ejaculate concentration determination. It is also easier to estimate sperm vitality, which changes in the opposite direction of the mortality rate.

CONCLUSIONS AND

The results of this study show conclusive qualitative and quantitative characteristics of the semen of the crossbred bucks (5/8 Anglo-Nubian and 3/8 Sahelian Goat). The quantitative and qualitative parameters show that the semen of the crossbred billy goats is in the range of semen that can be cryo-conserved for a dissemination of this genotype in order to increase the productivity of goat resources in Mali through artificial insemination. Linear regressions were used to estimate sperm vitality as a function of mortality rate with an accuracy of 98%. Similarly, the concentration of ejaculate can be estimated with 68% accuracy based on the concentration per milliliter.

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NOVELTY STATEMENT

This is the first to report reproductive potential of Crossbred Bucks Anglo-Nubian × Sahelian Goats, especially on the characteristics of the sperms in West Africa.

AUTHOR’S CONTRIBUTION

Conceptualization, SI, DM, SS, TD, CMD; methodology, SI, DM, SS, OBM, TD, CMD, CAB, DK; data analysis, OBM, SI; writing-original draft preparation, SI, SS, OBM, DM; writing-review and editing, SI, SS, OBM, CMD, CAB. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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