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# Comparative Analysis of Sperm Quality of the Greater Cane Rat (*Thryonomys swinderianus*) Collected by Masturbation and Epididymal Puncture, in Côte d'Ivoire

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Research concept and design have executed by authors AJLO, ZPD, BYEBG, YVG and MIJMD. Author AJLO has performed the collection and the assembly of data, data analysis and interpretation. The article has been written by authors AJLO, ZPD and BYEBG. Authors YVG and MIJMD have performed the critical revision of the article and final approval of article. MGF aided in interpreting the statistical analysis. All authors read and approved the final manuscript.*

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## ABSTRACT

The aim of this study was to compare the semen parameters of manually collected male greater cane rats with those obtained by epididymal aspiration. 15 apparently healthy male greater cane rats, aged 7-13 months, reared in close captivity were used in this study. Thirty (30) semen samples were collected, fifteen (15) by masturbation during the first week and the other half by epididymal aspiration during the second week from the same subjects. The experiment was conducted in accordance with EU Directive 2010/63/EU on the protection of animals in experiments. Semen evaluation included macroscopic and microscopic examination using the modified David's method. The mean volume of semen obtained by masturbation (0.6 ± 29.3 ml) was significantly higher than that obtained by aspiration (0.035 ± 0.4 ml). The Kruskal-Wallis test showed a highly significant difference at $P < 0.05$ between the semen volumes obtained by the two collection methods. Comparative analysis of semen concentration values showed a significant difference $P < 0.05$ using the Kruskal-Wallis test. Collection by masturbation resulted in a mean sperm concentration of 154.33 ± 11.44 106/ml. This value is lower than that obtained by aspiration, which is 512.06 ± 1.1 106/ml. From the comparative analysis it can be concluded that the aspiration method provides good vitality and a higher sperm concentration.

*Keywords: Sperm; epididymal puncture; masturbation; greater cane rat.*

## 1. INTRODUCTION

The Greater Cane Rat is a typical African rodent whose breeding is expanding rapidly in sub-Saharan Africa [1-3]. Managing its reproduction in captivity, as well as that of other livestock (cattle, goats, pigs, rabbits, etc.), requires knowledge of the spermiology of this rodent. Previous studies have determined the functional and structural characteristics of the sperm and those of the gonads and male genital glands of the greater rats [4-7]. Sperm collection from the greater cane rat has been performed by masturbation, electroejaculation, but not yet by epididymal puncture [8,9]. This work contributes to the characterisation of the spermiology of the greater cane rats. The aim of this study is to compare the quantitative and qualitative values of manually collected spermatozoa of the greater cane rat with those obtained by epididymal aspiration.

## 2. MATERIALS AND METHODS

### 2.1 Materials

#### 2.1.1 Biological material

Thirty (30) semen samples were collected from 15 apparently healthy male greater cane rats, aged 7-13 months, reared in close captivity on a private farm in the Abidjan district (RCI). Fifteen (15) were collected by masturbation during the first week and the other half by epididymal aspiration during the second week from the same subjects.

### 2.2 Methods

#### 2.2.1 Selection criteria and breeding methods

Apparently healthy greater cane rats were selected according to the age criteria. Thus, a total of fifteen (15) greater cane rats were considered for the study, divided into three (3) batches of five (5) animals, respectively aged Batch 1 (7-8 months), Batch 2 (9-10 months), and Batch 3 (11-13 months) (Table 1).

#### 2.2.2 Semen collection by masturbation

Semen collection from the Greater Cane Rat was performed in accordance with the Animal Welfare Act, European Directive 2010/63/EU, by masturbation using the Soro et al. [8] method.

#### 2.2.3 Semen collection using the epididymal sperm aspiration technique

The animals were then given a dose of 0.1 ml ketamine hydrochloride per kg live weight to induce general anaesthesia. The method described by Esteves et al. [10] was used for semen collection. After skin depilation and muco-cutaneous disinfection. A longitudinal skin incision of 3 cm was made, opening the cremaster muscle and the vagina to expose the testis and its afferents. The semen contained in the tail of the epididymis was aspirated using an insulin syringe (1 ml). Each puncture produced a volume of semen that was analysed. After collection, the wound was disinfected and sutured.

#### 2.2.4 Semen evaluation of the greater can rat

The evaluation of semen includes a macroscopic and microscopic examination using the modified David's method [11].

### 2.3 Data Analysis

SPSS software version 26.0.0.0 was used to analyse the data. The non-parametric Kruskal-Wallis test was used to compare samples.

## 3. RESULTS

### 3.1 Comparative Analysis of the Spermogram-Spermocytogram of Seminal Fluid from Greater Cane Rat Collected by Masturbation and by Aspiration

The semen was generally whitish in colour and moderately viscous. The other results of the analyses are presented in Tables 2 and 3.

#### 3.1.1 Comparative analysis of the spermogram of seminal fluid collected by masturbation and epididymal puncture in greater cane rat

The semen characteristics were mainly related to volume, pH, viscosity, vitality, motility, concentration and morphology of the spermatozoa.

**- Volume**

In general, the mean volume of semen obtained by masturbation (0.6 ± 29.3 ml) was much higher than that obtained by aspiration (0.035 ± 0.4 ml). The Kruskal-Wallis test showed a highly significant difference at $P < 0.05$ between the semen volumes obtained by the two collection methods. In general, semen volume varied positively with animal age.

**- pH**

In general, we obtained semen with a slightly acidic pH close to neutral, regardless of the collection method. The average pH of the collected semen was 6.8 for masturbation and 6.5 for aspiration.

**- Sperm concentration**

Comparative analysis of sperm concentration values showed a significant difference $P < 0.05$ using the Kruskal-Wallis test. The mean sperm concentration obtained by masturbation was 154.33 ± 11.44 106/ml. This value is lower than that obtained by aspiration, which was 512.06 ± 1.1 106/ml. The analysis of these values within each type of collection also showed a significant difference $P < 0.05$. The semen concentration values were correlated with the age of the animals. For the age interval of 7-8 months, the values obtained were 143.2 ± 2.1 106/ml for masturbation and 492.2 ± 0.7 106/ml for aspiration. These values were higher for animals aged 11-13 months and were 170 ± 10.4 106/ml (masturbation) and 532 ± 1.3 106/ml (aspiration), respectively.

**- Vitality**

Overall, the Kruskal-Wallis test showed no significant difference $P > 0.05$ for the comparative analysis of vitality. The mean vitality values were 59.4 ± 8% for masturbation and 60.3 ± 4% for aspiration. Individual analysis of the variation in vitality within the two groups according to sampling method showed a significant difference $P < 0.05$. Maximum vitality was obtained in animals aged 7-8 months with a value of 79±1.4% and was obtained by MESA for all observations.

**- Mobility**

In general, the Kruskal-Wallis test showed no significant difference $P > 0.05$ for either group or individual analysis. The mean motility values obtained by masturbation were 53.4 ± 5.5% and 51.26 ± 3.6% for aspiration. The mean total sperm motility after 3 hours was 54%.

#### 3.1.2 Comparative analysis of the spermocytogram of the seminal fluid of the greater cane rat collected by masturbation and aspiration

**- Sperm shapes**

Individual sperm analysis revealed normal and abnormal shapes. In manual semen collection, the mean percentage of spermatozoa with normal shape was 78.4 ± 3.3% compared to 21.6 ± 3.3% of spermatozoa with abnormal shape. The abnormalities of the intermediate pieces were analysed according to the head, the intermediate piece and the flagellum. Thus, we successively obtained 14.4 ± 1.4 % for head anomalies, 4.4 ± 0.5 % for midpiece anomalies and 2.7 ± 2 for the flagellum. Similarly, on aspiration, the average rate of normal forms was 77.3 ± 1.5% compared to 22.7 ± 1.5% of

abnormal forms. The average abnormality of the sperm head was 7.24 ± 3.6%, that of the midpiece 12.9 ± 3.2% and that of the flagellum 2.57 ± 4.3%. The Kruskal-Wallis test showed no significant differences P>0.05 for the comparative analysis of the percentages of spermatozoa shapes obtained by the different types of sampling.

## 4. DISCUSSION

The comparative study of the parameters of greater cane rat spermatozoa obtained by masturbation and epididymal puncture revealed similarities and differences. For example, the spermatozoa were whitish in colour, as also observed by Olukole et al. [12] in greater cane rat and Bencheikh [13] in rabbits. This observation differs from that of Houzangbe-Adote et al. [4] and Soro et al. [8], who observed a yellowish-white colouration of semen collected by masturbation from male greater cane rats. In fact, according to Simins and Ross [14], the colouring of the semen is caused by a protein of prostate origin called spermine, whose oxidation would cause the semen to yellow, very often due to a long period of sexual abstinence. The mean volume of semen aspirated from the epididymal tail (0.035 ± 0.4 ml) was much lower than the mean volume of semen obtained by masturbation (0.6 ± 29.3 ml). This result supports the observations of Boersma et al. (2015), who collected small volumes of semen in mice by percutaneous aspiration of epididymal fluid. Similarly, the mean semen volume obtained by masturbation was 0.3 ml higher than that obtained by Olukole et al. [12]. In fact, the semen volume of an animal depends on several genetic, nutritional, physiological, pathological and environmental factors (Neylanne et al. 2015). The pH values (6.5-6.8) measured in semen samples collected successively by aspiration and masturbation are approximately the same as those obtained by Houzangbe-Adote et al. These pH values, obtained in greater cane rats between 7 and 13 months of age, confirm those obtained by Bencheikh [13] in rabbits, ranging from 6.68 to 7.06. According to Korochkina et al. [15], the acidity of semen is due to prostatic secretions. The mean sperm vitality recorded in this study was approximately equal between the two groups (aspiration: 59.4 ± 8% and masturbation: 60.3 ± 4%). These values are lower than the 95 ± 1.16 obtained by electroejaculation by Olukole et al. [12] Bencheikh [13] obtained mean values of 48.9 to 84.5% in rabbits. These different values of sperm vitality in the greater cane rats could be related to the sperm collection techniques used, as noted by Cary et al. [16]. The mean sperm vitality recorded in this study was approximately equal between the two groups (aspiration: 59.4 ± 8% and masturbation: 60.3 ± 4%). These values are lower than the 95 ± 1.16 obtained by electroejaculation by Olukole et al. [12] Bencheikh [13] obtained mean values of 48.9 to 84.5% in rabbits. These different values of sperm vitality in the greater cane rats could be related to the sperm collection techniques used, as noted by Cary et al. [16]. The mean values of sperm motility of 53.4 ± 5.5% (aspiration) and 51.26 ± 3.6% (masturbation) obtained in this study are lower than those obtained by Houzangbe-Adote et al. [4], which ranged from 59 to 70% in greater cane rats aged 7 to 30 months. Olukole et al. [12] obtained approximately 73% motility. This difference in observation could be explained by the method of assessing semen parameters. The assessment performed in this study is based on the modified David model [11]. In fact, this method requires two measurements at 1 h and 3 h after collection. However, Houzangbe-Adote et al. [4] performed a single measurement 1 h after collection, and Soro et al. [8] and Olukole et al. [12] did not specify the measurement mode for determining sperm motility. The mean sperm concentrations obtained by masturbation and aspiration were 154.33 ± 11 106/ml and 512.06 ± 1.1 106/ml, respectively. These mean values are

**Table 1. Distribution of greater cane rat by age category (n=15)**

| Lot | Value | Age (months) | Average weight (g) ± standard deviation |
|---|---|---|---|
| 1 | 5 | 7 - 8 | 2410 ± 18,2 |
| 2 | 5 | 9 - 10 | 3508 ± 12,9 |
| 3 | 5 | 11- 13 | 3624 ± 11,1 |

**Table 2. Data from seminal fluid analysis of greater cane rat obtained by masturbation (n=15)**

| Lot | Volume (ml) | pH | Vitality (%) | Mobility (%) | Concentration ($10^6$/ml) | Normal sperm forms (%) | Abnormal sperm forms (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Head | Intermediate piece | Flagellum |
| 1 [7-8 mois] | 0,6 ± 25,8 | 6,8 | 59,2 ± 6,8 | 54 ± 6,2 | 143,2 ± 2,1 | 79 ± 2,4 | 14,6 ± 1,2 | 3,8 ± 0,4 | 2,6 ± 0,4 |
| 2 [9-10 mois] | 0,6 ± 20,5 | 6,8 | 60 ± 7,1 | 52,2 ± 2,4 | 150 ± 5 | 78,2 ± 2,8 | 14,8 ± 1,6 | 3,5 ± 0,4 | 3,5 ± 0,7 |
| 3 [11-13 mois] | 0,8 ± 40,8 | 6,8 | 59,2± 10,4 | 54 ± 7,9 | 170 ± 10,4 | 78 ± 4,9 | 13,8±1,5 | 6 ± 0,8 | 2,2 ± 0,6 |

**Table 3. Data from seminal fluid analysis of greater cane rat obtained by aspiration (n=15)**

| Lot | Volume (ml) | pH | Vitality (%) | Mobility (%) | Concentration ($10^6$/ml) | Normal sperm forms (%) | Abnormales sperm Forms (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Head | Intermediate piece | Flagellum |
| 1 [7-8 mois] | 0,031 ± 0,7 | 6,5 | 79 ± 1,4 | 54 ± 7,6 | 492,2 ± 0,7 | 78,6 ± 1 | 7,13 ± 1,2 | 12,13 ± 0,9 | 2,13 ± 0,5 |
| 2 [9-10mois] | 0,033 ± 0,4 | 6,5 | 52 ± 2,8 | 51,2 ± 2,2 | 512 ± 1,3 | 77,2 ± 1,7 | 6,6 ± 6,8 | 13,6 ± 8,3 | 2,6 ± 8,5 |
| 3[11-13 mois] | 0,04 ± 0,3 | 6,5 | 50 ± 7,8 | 48,6 ± 1,2 | 532 ± 1,3 | 76 ± 1,9 | 8 ± 2,8 | 13 ± 0,4 | 3 ± 4,1 |

higher than the maximum concentrations obtained by Houzangbe-Adote et al. [4] of 143 ± 27 106/ml in the 7-8 month interval and by Soro et al. [8] of 144 ± 2 106/ml. However, they remain low in comparison with the values obtained by electroejaculation (136.10 ± 9.15 109/ml) and after testicular biopsy (319.3 109/ml). These different values demonstrate the need to establish reference values for spermiology in the greater cane rat.

## 5. CONCLUSION

This study compared the sperm values of male greater cane rat whose semen was collected by two methods, masturbation and epididymal aspiration after microdissection, to determine the possible effects on semen quality. From the comparative analysis it was concluded that the aspiration method provides good vitality and higher sperm concentration. However, a more thorough study involving a large number of animals will allow reference values to be established for greater cane rat semen in order to characterise the species [17].

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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