

Original Research Article

Comparative analysis of sperm quality of greater cane rat (*Thryonomys swinderianus*) collected by masturbation and epididymal puncture, in Ivory Coast

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 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 collected 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

Introduction

The greater cane rat is a typical African rodent whose breeding is rapidly expanding in sub-Saharan Africa (Fantodji and Soro, 2004; Mensah et al., 2007; Annor et al., 2014). Controlling its reproduction in captivity like 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 sperm and those of the gonads and male genital glands of the greater cane rat (Hougangbe-Adote et al., 2004; Adebayo et al., 2014; 2016; 2019). The collection of sperm from the greater cane rat was done by masturbation, electroejaculation, but not by epididymal puncture (Soro et al., 2009; Olukole et al., 2010). This work is a contribution to the characterisation of the spermiology of the greater cane rat. 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.

I. Material and Methods

1.1 Material

1.1.1 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.

1.2 Methods

1.2.1 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).

1.2.2 Semen collection by masturbation

Semen collection from greater can rat was performed according to animal protection and welfare law of the European directive 2010/63/EU, by masturbation using the method of Soro et al., (2009).

1.2.3 Semen collection using the epididymal sperm aspiration technique

A dose of 0.1 ml of ketamine hydrochloride per kg live weight is then administered to the animals for general anaesthesia. The method described by Esteves et al., (2011) 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.

1.2.4 Semen evaluation of the greater can rat

Semen evaluation includes macroscopic and microscopic examination using the modified David's method (WHO, 2010).

1.2.5 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.

II. Results

2.1 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.

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

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

- Volume: The Kruskal-Wallis test showed a highly significant difference at $P < 0.05$ between the semen volumes collected by the two collection methods. 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).

- pH: The mean pH of the semen collected was 6.8 for masturbation and 6.5 for aspiration.

- Sperm concentration: Comparative analysis of semen 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 is 512.06 ± 1.1 106/ml.

- Vitality: Overall, the Kruskal-Wallis's test did not yield a significant difference $P > 0.05$ for the comparative analysis of vitality. Mean vitality values were $59.4 \pm 8\%$ for masturbation and $60.3 \pm 4\%$ for aspiration.

2.1.2 Comparative analysis of the spermocytogram of seminal fluid from greater cane rat collected by masturbation and by aspiration

- Sperm shape: Individual sperm analysis revealed normal and abnormal shapes. In manual semen collection, the average rate of normal sperm shape was $78.4 \pm 3.3\%$ versus $21.6 \pm 3.3\%$ of abnormal shapes. 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.

III. Discussion

The comparative study of the parameters of greater cane rat spermatozoa obtained by masturbation and by epididymal puncture showed similarities and differences. For instance, the semen was whitish in colour as also observed by Olukole et al. (2014) in greater cane rat and Bencheikh (1995) in rabbits. This observation differs from that of Houzangbe-Adote et al. (2004) and Soro et al. (2009), who observed a yellowish-white colouration of semen collected by masturbation from male greater cane rats. In fact, according to Simins and Ross (2021), the colouring of the semen is caused by a protein of prostate origin called spermine, whose oxidation would cause the yellowing of the semen, 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 collected 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 volume of semen obtained by masturbation is 0.3 ml higher than that obtained by Olukole et al. (2014). 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 on 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 cynomolgus rats between 7 and 13 months of age, confirm those obtained by Bencheikh (1995) in rabbits from 6.68 to 7.06. According to Korochkina et al. (2014), 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: $59.4 \pm 8\%$ and masturbation: $60.3 \pm 4\%$). These values are lower than the 95 ± 1.16 obtained by electroejaculation by Olukole et al. Bencheikh (1995) obtained mean values of 48.9 to 84.5% in rabbits. These different values of sperm vitality in the greater rats could be related to the sperm collection techniques used, as noted by Cary et al. (2004). The mean values of sperm motility of $53.4 \pm 5.5\%$ (aspiration)

and $51.26 \pm 3.6\%$ (masturbation) obtained in this study are lower than those obtained by Houzangbe-Adote et al. (2004) which ranged from 59 to 70% in greater cane rats aged 7 to 30 months. Olukole et al. (2014) obtained about 73% motility. This difference in observation could be explained by the method of assessment of semen parameters. The assessment performed in this study is based on the modified David model (WHO, 2010). In fact, this method requires two measurements to be taken at 1 h and 3 h after collection. However, Houzangbe-Adote et al. (2004) performed a single reading 1 h after collection, and Soro et al. (2009) and Olukole et al. (2014) did not specify the reading 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 higher than the maximum concentrations obtained by Houzangbe-Adote et al. (2004) of 143 ± 27 106/ml in the 7-8 month interval and by Soro et al. (2009) of 144 ± 2 106/ml. However, they remain low compared to 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.

Conclusion

This study compared the sperm values of male greater cane rats 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.

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18. 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
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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