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## **Cytology Approach In the Determination of the Sexual Cycle of Captive Greater Cane Rat (*Thryonomys swinderianus*, Temminck 1827)**

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### **Abstract**

We undertook this study to identify oestrus of greater cane rat. Sexual behavior of 14 females was observed for 6 months. Vaginal samples were performed and stained refer to Papanicolaou (1942). Our results showed that there are four very irregular stages of sexual cycle with cane rat. At the anoestrus stage, vulva is sealed. Females refuse projections. Vaginal smears are basophils. The index estrogen is about 10%. Vaginal mucus is viscous and yellowish at all stages of the cycle except at the oestrus where it becomes fluid. At oestrus, the vulva is open and oozing. Smears are acidophilic and inflammatory. The estrogenic index is 90%. The female is docile and easily accepts the projection. While determine the physiological state of the females before mating, we have been able to achieve without couplings aggressive males.

**Keywords:** cane rat, sexual cycle, cytology.

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## **1. Introduction**

Grasscutters rearing seems to be a focus for the rural population and for some traders in Saharan Africa. According to [1]; this initiative aims to diversify agricultural activities on the one hand and to contribute to raising the level of income of the rural population on the other. Despite the significant progress made in recent decades, grass cutter rearing is far from being completely controlled [2]. According to Tamegnon [3] some data are still inaccessible. The transience of heat that remains imperceptible led to organize permanent or temporary polygamous mating. Females subjected to the coupling modes undergo significant trauma [4,5]. A good productivity of the species breeding also depends on the control of reproduction. Pap smears are a simple cytological technique that can be used to determine the fertile period of females. Given the importance of this parameter in controlling the reproduction rate of farrowing for improving farming cane rats, it seemed interesting and even necessary to know the precise time of fertilizing projection.

This study was undertaken to determine the periods of heat of cane rats through cytological examines. We hope to determine the moments of receptivity to achieve couplings without the trauma of the females.

## **2. Materials and methods**

### **2.1 *Animal material***

Twenty (20) grasscutters [14 females] (aged 4-36 months) and [6 males] (aged 7-36 months) were used. The females were divided in two groups: 7 nulliparous (aged 4 - 10 months) and 7 multiparous (aged 21-36 months). We considered five (5) full male and a neutered male.

### **2.2 *Technical equipment***

It consists of an optical microscope and a counter. A digital camera type canon 14.1 mega pixels is used for photography. A sterile cotton swab disposable is used for vaginal swabs. The fixation was made with hair lacquer. Reagents cytology (Harris haematoxylin, Orange G, polychrome, absolute ethanol, hydrochloric acid, toluene, Eukitt, distilled water) were used.

### **2.3 *Methods***

#### **2.3.1 *Conduct of the trial and data collection***

Cane rats are housed in individual pens before mating. They are fed with green fodder (*Panicum* sp) supplemented by grass cutter pellets. All selected females are empty before the start of the experiment. A health prophylaxis consisting in pens daily cleaning, daily renewal of litter and disinfecting by spreading superphosphate on the floor was performed. Females are brought to male for mating following the evolution of their sexual cycle through observation of cytological aspects of the vagina. A sterile cotton swab is moistened disposable. It is inserted into the vagina until 1/3 of the swab. In contact with the mucosa, or the caudal part of the vagina, the swab is rotated. It is gently extracted from the genital tract and spread on the slide for analysis.

At the time of the mating, they are transferred to the pens of males. Monogamous and polygamous couples are well made. We coupled multiparous as nulliparous seemed unreceptive to males. They thus served as a control. The observations are made once a day early in the morning and between 4h30mn to 5h30mn during six months.

### **2.3.2 Method for the determination of vulva index**

The periodic variations in the appearance of the vulva have been appreciated by a vulva index. Numerical coding system as proposed by Adjanohoun [4] and modified for the occasion was used to express the observed at the vulva as following changes:

- Index vulva (IV0), the vulva is closed by a continuous vaginal membrane;
- Index vulva (IV1), vulva is closed by a crust throughout;
- Index vulva (IV2), the vulva is open and lips congested;
- Index vulva (IV3), the vulva is closed by a crust that partially covers.

### **2.3.3 Method of examination of vaginal mucus and determination of O.I**

Vaginal mucus was characterized by its viscosity and color. For cytological study of cell vulvar wall, on average two smears were made per week for 6 months. The smears were stained by the Papanicolaou staining technique [6]. The estrogen index (O.I) was determined by reading the optical microscope to smear magnification (x 10), (x 40) and (x100). Selected to perform the cell counts are those areas where the cells are sufficiently insulated from each other. At 40 x magnification, there are about 200 cells. Counting is done by using a counter. The index is expressed as a percentage (%).

### **2.3.4 Statistic Analysis**

The results were expressed as means with standard deviations. The comparison of means was done using Tukey test HSD  $p < 0.05$ .

## **3. Results**

### **3.1 Changes of the vulva, mucus, vaginal cells and sexual behavior of nulliparous**

#### **3.1.1 Anoestrus**

The closure of the vulva vaginal membrane (Fig. 1) characterizes the physiological stage. On average, it takes 11 days but may persist up to 32 days. Vaginal mucus is highly viscous and yellowish (Table 1). The estrogenic index is about 8% (Table 2). The females are very agitated and refused any contact with males. They remain indifferent to the male neutered. Pap smears are basophils and inflammatory (Fig. 2).

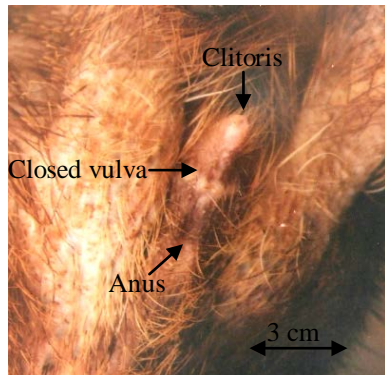


Fig.1. Anoestrus

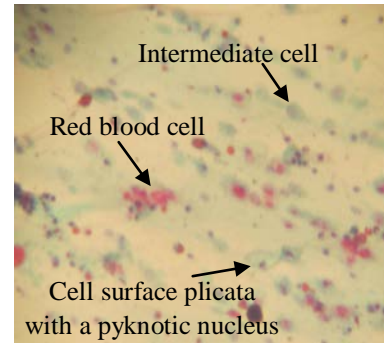


Fig.2. Basophil Pap smear, × 50

Table 1. Sexual behavior and changes in the appearance of mucus during the cycle

Phase of the cycle	Appearance and color of mucus			Sexual Behavior	
	yellowish viscous	Whitish Viscous	Fluid translucent	Restless	docile
Sexual rest	+++++	-	-	+++++	-
Sexual activity	± à +++	+++++	+++++	+++	+++++

Note: The sign (-) indicates an absence of the element, (±) indicates little or no and (+++++) Strongly observed.

Table 2. The mean changes in the IO during the sexual cycle aulacodine

Stage of cycle	Nulliparous female	Multiparous female
Anœstrus	8%	8%
Pro-œstrus	30%	33%
œstrus	45%	50%
Post-œstrus	17%	9%

### 3.1.2 Pro-oestrus

Vaginal vulva is encumbered by a yellowish crust whose peculiarity is that it closes the vulva vestibule (Fig. 3). It lasts 25 days. Vaginal mucus is viscous yellowish. The estrogenic index is about 30%. The females are very rough. Smears are acid-basophils and not inflammatory (Fig. 4).

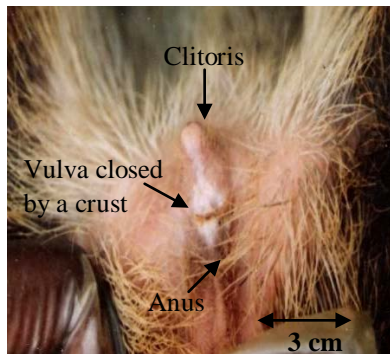


Fig.3 Pro oestrus stage

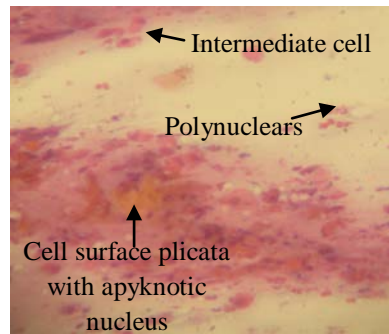


Fig. 4 Baso-acidophil, Pap smear × 50

### 3.1.3 Oestrus

The vulva is opened with a translucent fluid moistening lip (Fig. 5). This phenomenon lasts 2 days. Vaginal mucus is fluid and clear. The estrogenic index is about 45%. Females are relatively rough. The cytology showed inflammatory acidophilus vaginal smear (Fig. 6).

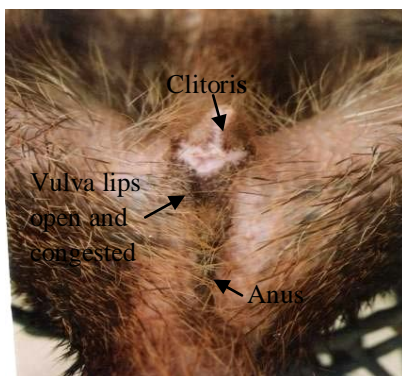


Fig.5 Oestrus stage

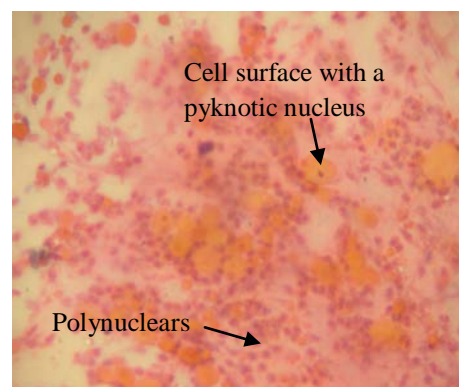


Fig.6 Acidophil Pap smear, × 50

### 3.1.4 Post oestrus

The post-estrus or IV3 stage is characterized by a crust partially obstructing the lips of the vulva. The size of the crust to gradually regress and centered as shown in Fig. 7. Duration is on average  $24 \pm 3$  days. Smears are acid-basophils (Fig.8).

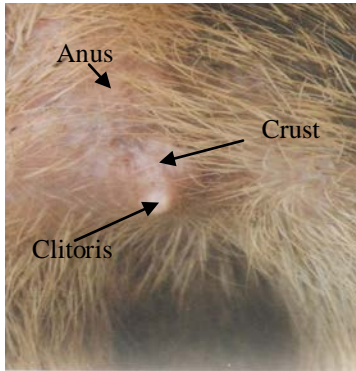


Fig.7 Post oestrus

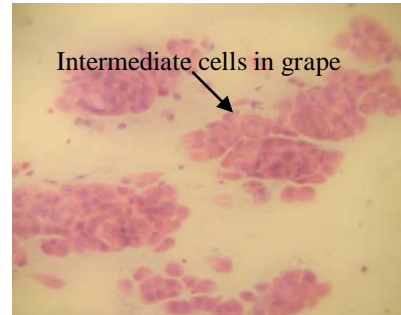


Fig. 8 Acid-basophil smear

## 3.2 Changes of the vulva, mucus, vaginal cells and sexual behavior of multiparous females

### 3.2.1 Anoestrus

It lasts 5 days. The vulva vestibule is sealed by a vaginal membrane. Vaginal mucus is highly viscous and yellowish outside couplings. It can sometimes be a viscous bleeding after positive pregnancy test. The estrogenic index is about 14%. Pap smears are basophils and little inflammatory. The females are very panicky and do not accept projection.

### 3.2.2 Pro-oestrus

The presence of a blocking crust along the lips characterizes this stage. It takes about 15 days. Females are panicky but after diligent during the male, they can be bred. The mucus has varying shades, but still viscous. Apart couplings, viscous mucus is yellowish. Shortly after mating, it is viscous whitish. Similarly, several days after mating it can sometimes be sticky and bleeding. The estrogenic index is about 33%. Smears are acid-basophils, inflammatory and sometimes bleeding.

### 3.2.3 Oestrus

The vulva opens and oozing. This phenomenon lasts 10 days. The estrogenic index is about 63%. Females are receptive to males. We have seen attempts to overlap the male neutered, they teased a lot in this state. Vaginal mucus is very fluid and clear. Smears are inflammatory and acidophilus.

### 3.2.4 Post-oestrus

It is characterized by the presence of a crust unlike the pro-oestrus is off in the middle of the vulva. The mucus is usually yellowish viscous. The color also varies depending on whether the female is pregnant or not. It is outside the couplings yellowish, but becomes bleeding several days after intercourse about a month. It is also possible to whitish shades. At this stage, the estrogenic index is about 25%. The females are indifferent to males in general. The post-estrus lasts 24 days. Smears are acid-basophils.

### **3.2.5 Test of Tukey**

The Tukey HSD test showed significant differences ( $F = 16.0832$   $p = 6.38 \text{ E-}29$ ,  $p < 0.05$ ) changes in the estrogenic index between on the one hand the different stages of the oestrous cycle and secondly between nulliparous and multiparous females.

## **4. Discussion**

We observed that the organization of the sexual cycle was greatly influenced by the degree of parturition of cane rat. However in multiparous, the phases of cane rat sexual activity were very different with very different frequencies (38 to 51 days). Thus the pro-oestrus (IV1) lasted on average 14-25 days. The oestrus (IV2) between 4-10 days post oestrus (IV3) lasted 24 days on average.

This periodicity of the cycle is beyond that described by Gayrard [7] in mammals in general. According to this author proestrus lasted mammals usually between 2-3 days, except a few hours estrus in the mare made 3-10 days post-estrus of 12-15 days. We are tempted to describe as atypical the cane rat sexual cycle as is the case with the rabbit.

At oestrus (IV2), the smear is acidophilus and inflammatory with more than 60% of keratin superficial cells as also found Adjanohoun [4]. At this stage, some females have a particular multiparous feature a demonstration heat sexual behavior. They sniff the vulva of their partners try to ride the castrated male and are obedient to the touch. These behaviors were also observed in cattle and ewes [8,9]. All these signs are associated with a manifestation of heat from the female of the species studied. It is therefore evident that cane rat experiences periods of heat, even if they are discrete in many cases, as noted by [10] and Adjanohoun [4].

Outside oestrus smears are acid-inflammatory and basophils with bottoms rich in mucus. The distinction between stages of pro-oestrus, post-oestrus or anoestrus as meant Adjanohoun [4] is possible at these stages by determining the OI. However, observations of mucus were able to differentiate the phases of the cycle. Thus, oestrus is characterized by fluid and clear mucus. After intercourse, it is often hemorrhagic. In post oestrus, it is viscous and white. It thus characterizes the formation of coagulum post coital as shown Adjanohoun [4]. Pro-oestrus and anoestrus, it takes a viscous yellowish appearance. Bleeding is sometimes observed during embryonic regression around the 30th day of gestation.

At pro-oestrus the OI is about 30% regardless of the degree of parturition and the female is in a state of aggression. The lowest rates were recorded in IO anoestrus (8% -14%). Females at this stage are very rough. TUKEY HSD test showed significant differences at  $p < 0.05$  between changes OI one stage of the cycle to

another and between groups of nulliparous and multiparous females on the other. This effectively showed an irregular sexual cycle with the greater cane rat.

Some females can be blocked at a stage of the cycle for several weeks as found also Adjanohoun [4]. We came to observe OI peaks of over 80%. That is the conclusion for females mated to stage pro oestrus (IV1). This would result in a discharge of estrogen as meant Maillet et al. [11]. This discharge may be the result of a vaginal stimulus [12]. It was also noted the calmness with which the couplings were held at IV2 stage would be a presumption of estrus. While females mated anoestrus has been victims of aggressiveness on the part of the male.

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