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Vaginal lumen cytology and peripheral sex hormone concentrations at different vaginal orifice statuses in the female greater Grasscutter

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***Target audience:** Animal physiologists, reproductive physiologist and animal scientists*

Abstract

This study aimed to characterise the oestrous cycle of the female greater grasscutter (Thryonomys swinderianus) through investigating the pattern of exfoliated vaginal epithelia cells and peripheral sex hormone profiles in two different vaginal orifice status (open and close) displayed by the animal. Vaginal smears and blood samples were collected every other day from 12 non pregnantgrasscutterdoes for 7 days regardless of the vaginal orifice status. The cells in the smear were classified into parabasal, intermediate, superficial and metoestrus epithelial cell. Blood plasmawas assayed for estradiol (E_2) and progesterone (P_4) hormones. Irrespective of the vaginal status, intermediate cells were predominant with 54% and 56.7% for open and close vagina orifice, respectively. Superficial cells were15 % in open vagina status as against 9 % in the close vagina status. E_2 concentration was significantly higher ($P < 0.05$) in the open vagina status (6.75 vs 3.91 ng/ml) whereas P_4 concentration was similar in both statuses. In conclusion, the similarity in the occurrence of vaginal lumen cells at open and close status indicated that the grasscutter could be an induced ovulator while the higher E_2 concentration in the open vagina status could mean higher sexual receptivity at this stage.

Keywords: Greater grasscutter; induced ovulator; sex hormones; vaginal cytology

Description of Problem

In most areas of sub-Saharan Africa especially West Africa region, the greater grasscutter(*Thryonomys swinderianus*, Temminck 1827) is a highly priced meat source because of its excellent taste and high nutritional value (1). The inherent potential of the grasscutter to increase per capital animal protein consumption cannot be easily realized due to the little information available on its reproductive biology. Since maintaining an animal's reproductive performance in captivity is the major determinant of its successful domestication (2), research into the grasscutter reproductive biology is inevitable.

Sex hormones bring about cyclic changes in the vaginal epithelium with different pattern of epithelial cells exfoliated during the oestrous cycle (3). The epithelial cells are categorised into parabasal, intermediate, superficial and metoestrus cells (4). The changes in the concentration of the ovarian hormones and vaginal exfoliation pattern has been used to characterize the oestrous cycle of many domesticated animals including dog, cattle, cat, sheep, goat, rabbit and rodents such as guinea pig, rat and chinchilla (3;5; 6; 7; 8; 9; 10). The predominance of superficial cells, indicating oestrus is associated with high oestrogen levels while parabasal and intermediate cells dominance is related to the luteal stage in spontaneous ovulators (3). In rabbit, an induced ovulator, the vaginal exfoliation pattern is generally irregular and

superficial cells predominance does not occur (5; 12).

Information on the exfoliative vaginal cytology of the grasscutter is not available and hormonal assays have not been significantly employed to investigate its reproductive cycle. Therefore, this study provides basal information on the types of vaginal exfoliated cells as well as peripheral levels of estradiol and progesterone in non pregnant female greater grasscutter.

Materials and Method

Twelve female greater grasscutter were housed in the pit type pens at the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife. The animals were sexually matured weighing 1.0 – 1.5 kg and were kept away from mating. Vaginal smear and blood sample were collected from each animal every other day over seven days irrespective of whether the vagina orifice was open, sealed or closed. When sealed or closed it was gently opened up for smear collection. Vaginal smear was collected with a cotton swab onto a clean glass slide. The smears were air-dried and stained with Leishman stain. They were observed under the light microscope at a magnification of ×100 for the presence of epithelial cells and leucocytes. Cell types were counted from 10 different microscope fields and the occurrence of each cell type reported as percentage of the total. Blood ($\leq$ 1 ml) was collected by lateral saphenous vein venipuncture after restraining the animal. To restrain the animal one person held down the animal while its head was covered with a piece of cloth. We got better result this way than the use of anaesthetic agent. Plasma was retrieved from the blood samples for estradiol (E_2) and progesterone (P_4) hormonal assay using the EIA kits (Cat No BC-1113 and 1111, respectively) from BioCheckInc, Foster City, CA 94404, USA. For statistical analysis and interpretation, both open and sealed vaginal orifice statuses were regarded as open vagina. The mean percentage of the epithelia cells were compared within each vagina status by one-way Analysis of Variance. Because of the limitations imposed on this experiment (discussed later in the next section) mean daily concentrations of P_4 and E_2 in the different vaginal status were simply plotted against day of sample collection. In addition, the overall mean P_4 and E_2 concentrations were compared between the two vaginal statuses using student t test procedure. All statistical analyses were tested at 5 % level of significance using SPSS 17.0 software.

Results and Discussions

As shown in Table 1, the least period between opening and closure of the vagina orifice was 1 day (in animal 7) while the longest interval was 6 days (in animal 3). The occurrence of close vagina is higher and significant ($P<0.05$) compared to the occurrence of open vagina. Nonetheless, the opening and closing of the vagina orifice do not show any regular pattern. However, the erratic manefestation of the opening and closing of the vagina orifice corroborate the observations of previous studies (11; 19) where the researchers concluded that the erratic opening and closing of the vagina showed that the grasscutter was an induced ovulator and that the animal mated and concieved regardless of the status of the vagina.

Epithelial cell types found in the vaginal smears of the grasscutter does in this study were parabasal, intermediate, superficial and metoestrus cells similar to those observed in other hystricomorphic rodents such as the chinchilla and the agouti paca (13; 9). These cell types have also been reported in the rabbit (14; 12), which is an induced ovulator. The smears revealed an array of inconsistent cytological pattern. For example, Figures 1a and b, which were the smears, obtained from

doe with open and close vaginal orifice, respectively, shows similar mix of different epithelial cells. In other smears animals with similar vaginal orifice presented different vaginal exfoliative cellular patterns on the same day.

In Table 2, the mean percentage abundance of vaginal epithelial cells is shown against the status of the vagina. Intermediate cell types predominated the smears in both open and close vagina status, similar to what has been reported for the rabbit (14; 12) and the cow (16). However, in the cow, superficial cells were more abundant (>21%) than what is observed in this study and was even more than the intermediate at oestrus (16) perhaps due to the cow being a spontaneous ovulator. It was noticed that the occurrence of the superficial cells tended towards being higher in the open vaginal status. This could indicate some level of better sexual receptivity at this status and is in support of the findings of (11).

However, the result of the vaginal cytology generally suggests that it may not be useful to predict oestrus, just as the vaginal orifice status does not influence receptivity, mating and conception in the female grasscutter (15).

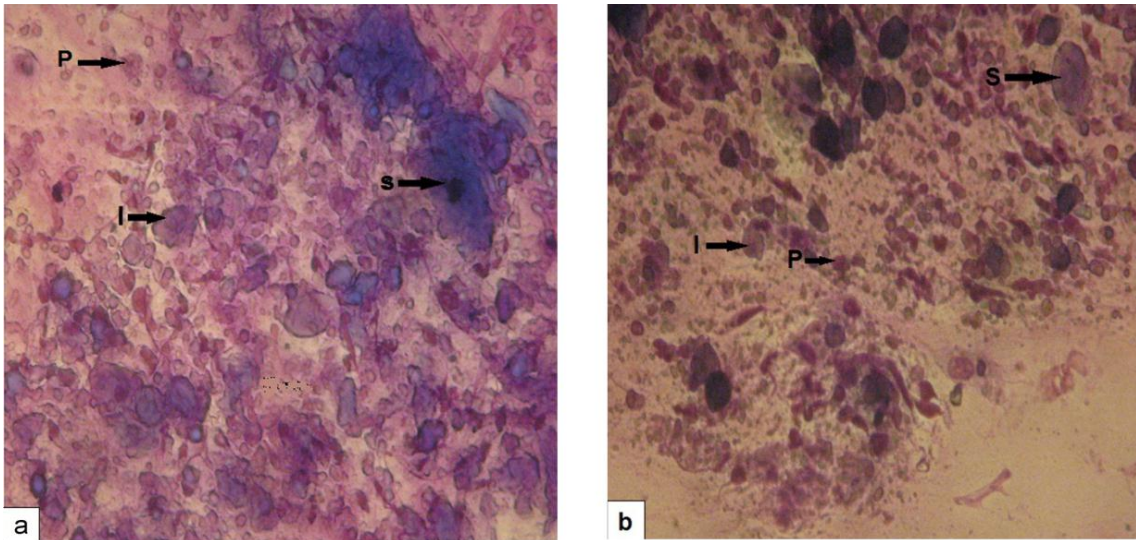


Figure 1: Vaginal smears from non pregnant grasscutter does with an open (a) and a close (b)vaginal orifice showing mixture of parabasal (P), intermediate (I) and superficial (S) epithelial cells (×100).

As shown in Figure 2 the concentration of E_2 was generally higher in open vaginal status being almost twice the level in the close vaginal status. On the contrary, the levels of P_4 were similar in both conditions. Higher E_2 level is generally associated with higher sexual receptivity, in both spontaneous (16) and induced (17) ovulators. Thus, the higher level of E_2 in the grasscutter does with open vaginal orifice could be taken as an indication of higher sexual receptivity. The concentration of P_4 observed in both vaginal status were similarly low (<2.0 ng/ml). These results are in agreement with those obtained in domesticated guinea pig (*Cavia aperea*) which is an hystricomorph rodent like grasscutter. With a spontaneous ovulation, concentration of E_2

were higher in female guinea pigs with open vagina while P_4 also remained low (18).

The graphical plot of the mean E_2 and P_4 concentrations (Figure 2) of the grasscutter does in either open or close vagina status on the three sampling days showed a rise in the E_2 concentration of the close above the open vaginal status in opposition to P_4 concentration. This observation could support the reported sexual receptivity and conception of grasscutter does at both open and close vagina status(15). This is also the situation in rabbit, an induced ovulator, which can conceive even when the sexual receptivity indicators are very poor (18). However, observation of the sex hormone concentrations in the grasscutter over a longer period would be more meaningful especially when it is known that the duration and intervals of the vaginal orifice opening is inconsistent (19).

Regardless of the vaginal status all the vaginal epithelia cells with the exception of parabasal cells correlated positively but insignificantly with the vagina status (Table 3) contrary to the findings of (12). Parabasal cells in contradiction to other vaginal epithelia cells had a negative relationship to the vaginal status (r = -0.411). Nevertheless, like the other epithelia cells the relationship was not significant. It is noteworthy that only metoestrous cells are significantly correlated to the superficial cells while all others are not significantly correlated implying that the abundance of one cell is not related to the other.

Also, P_4 concentrations correlated positively (0.400) and significantly ($p<0.05$) with the vaginal status. However, though E_2 concentrations correlated positively with the vagina status, the association was not significant (0.133). These observations suggest that the abundance of the vaginal epithelial cells and sex hormones' concentrations, especially E_2 in the grasscutter, is not dependant on a specific vaginal status, hence, in the grasscutter doe, vaginal status may not be a dependable marker of sexual receptivity and fertility.

Table 1: Daily vagina status (open or close) of experimental grasscutters.

ANIMAL	DAILY VAGINA STATUS	CLOSE STATUS	OPEN STATUS
1	5C, 1O, 1C	6	1
2	7C	7	0
3	1O, 6C	6	1
4	4C, 2O, 1C	5	2
5	1O, 3C, 3O,	3	4
6	2C, 1O, 4C	6	1
7	2C, 1O, 1C, 1O, 2C	4	3
8	1O, 3C, 1O, 2C	5	2
9	2C, 10, 4C	6	1
10	7C	7	0
11	1O, 2C, 3O, 1C	3	4
12	3O, 1C, 2O, 1C	2	5
Total		60	24
Mean + SE		5.0+ 0.50 [a]	2.0+ 0.48 [b]

[ab]Means with different superscript on the same row differ significantly (P <0.05, Duncan test; SE= standard error)
O = open; C = close

Table 2.Mean percentage abundance of vagina epithelia cells against vagina status in experimental female grasscutters.

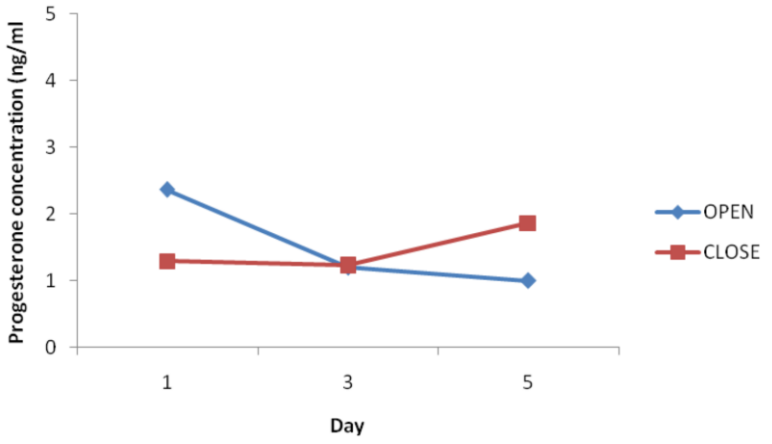
Vagina status	n	Cell type				SEM	Prob
		Parabasal	Intermediate	Superficial	Metoestrus		
Open	24	22.30[b]	54.00 [a]	15.30 [b]	8.33 [b]	7.47	0.02
Close	60	32.70 [b]	56.70 [a]	9.00 [c]	6.67 [c]	3.31	0.03

[ab]Means with different superscript on the same row differ significantly (P <0.05, Duncan test; n= number of occurrence of a particular vagina status)

Table 3: Pearson correlation between vaginal status, epithelial cell types and sex hormone concentrations

	Vaginal status	Parabasal Cell	Intermediate Cell	Superficial Cell	Metoestrus Cell
Vaginal status	1	-0.411	0.087	0.593	0.213
Parabasal Cell		1	-0.733	-0.327	-0.114
Intermediate Cell			1	-0.327	-0.526
Superficial Cell				1	0.812+
Metoestrus Cell					1
Estradiol concentration	0.133				
Progesterone concentration	0.400+				

+ Significant correlation at 0.05(2-tailed analysis)



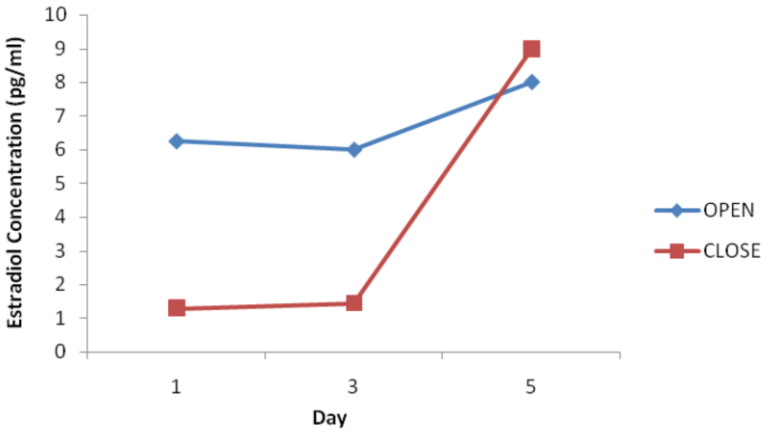


Figure 2: Daily mean concentrations of estradiol (pg/ml) and progesterone (ng/ml) at different vaginal orifice status

There were some challenges in collecting blood and vaginal smear samples of the experimental animals which shortened the duration of the study. This is due to difficulty in restraining the animals for smear and blood collection. In addition, the fragile skin and thin vessels of the animals as well as mortality from handling discouraged frequent blood and smear collection. It was difficult to replace the animals because of no ready source of supply.

Conclusions and Applications

1. Intermediate epithelial cells predominated in the vaginal smears collected from non-pregnant grasscutter does.
2. Smears collected in either open or close vaginal status did not present any specific cytological pattern that could be used to identify any of the states.
3. E_2 concentrations appeared to rise in does with open vaginal status while P_4 remained low in both vaginal states.
4. These findings allude to the suggestion that the grasscutter could be an induced ovulator.

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References

1. Mensah, G. A. and Okeyo, A. M. (2005). Continued harvest of the diverse African Animal Genetic Resources from the wild through domestication as a strategy for sustainable use: A case of the larger Grasscutter (*Thryonomys swinderianus*). ILRI publication.
2. Dukelow, W. R. (1978). Ovulation detection and control relative to optimal time of mating in non-human primates. Symposium of the Zoological Society of London, 43: 195 - 206.
3. Reddy, K. C. S., Raju, K. G. S., Rao, K. S. and Rao, K. B. R. (2011). Vaginal cytology, vaginoscopy and progesterone profile: Breeding tools in bitches. *Iraqi Journal of Veterinary Sciences*, 25 (2): 51–54.
4. Bowen, R. (1998). Classification of vaginal epithelia cells.Retrieved July 10, 2012, from

http:// arbl. cvmbs. colostate. edu/hbooks/pathphys/reprod/vc/cells.html

5. Kunde, M. M. and Proud, T. (1929).The ineffectiveness of vaginal smears in predicting the oestrous cycle in the rabbit. *American Journal of Physiology*, 88: 446-452.
6. Mingoas, J. L. K. and Ngayam, L. L. (2009). Preliminary findings on vaginal epithelial cells and body temperature changes during oestrous cycle in Bororo zebu cow. *International Journal of Biological and Chemical Sciences,* 3 (1): 147-151.
7. Sharma, M. And Sharma, N. (2016). Vaginal cytology: An historical perspective on its diagnostic use. *Advances in Animal and Veterinary Sciences*, 4(6): 283-288.
8. Amilton, C. S. , Diego, C. V. , Bruno, M. B., Gleidson, B. O., Daniela, M. O., Ferdinando, V.F. B., Moacir, F. O. and Antônio, C. A-N. (2015). Characterization of the estrous cycle in *Galea spixii* (Wagler, 1831). *Brazilian Journal of Veterinary Research,* 35(1):89-94.
9. Noguiera, T. M. R., Toniollo, G. H. and Giannoni, M. L. (2005)**.** Estrous cycle colpocytology in captive pacas (*Agouti paca*, Linnaeus, 1766) ARS *Veterinaria Jaboticabal*, SP, Vol. 21, Suplemento: 209-214.
10. Ola, S. I., Sanni, W. A. and Egbunike, G. N. (2006).Exfoliative vaginacytology during the oestrous cycle of West African Dwarf goats. *Reproduction, Nutrition Development,* 46: 87-95.
11. Henry, A.J. (2011). Reproductive performance of grasscutter does at first parity and growth performance of their F1 generation. *Asian Journal of Animal Sciences*, 5(4): 289 - 295.
12. Ola, S. I. and Oyegbade, M. O. (2008). The influence of different contact levels with male on the vaginal cytology in rabbits under the tropical humid condition. Proceedings of 9th World Rabbit Congress, Verona, Italy: 417-422.
13. Tayfur, B., Narin, L. and Gu¨ ner, B. (2002).Diagnosis of sexual cycle by means of vaginal smear method in the chinchilla (*Chinchilla lanigera*). *Laboratory Animals,* 36:51–60.
14. Tsiligianni, Th., Saratsi, A., Besenfelder, U., Anastasiadis, A., Vainas, E., Saratsis, Ph. and Brem, G. (2004).The use of cytological examination of vaginal smears in the selection of rabbits for superovulation. *Theriogenology,* 61: 989–995.
15. Addo, P. G.,Dodoo, A., Adjei, S., Awumbila, B. and Awotwi, E. (2002).Determination of the ovulatory mechanism of the grasscutter (*Thryonomys swinderianus*). *Animal Reproduction Science,* 71: 125–137
16. Tongku, N.S., Juli M., Rohaya, Cut N.T., Dian, M., Sri, W., Juliana, R., Nurhafni, Budianto, P. and Herrialfian. (2015). Determining proportion of exfoliative vaginal cell during various stages of estrus cycle using vaginal cytology techniques in Aceh Cattle. *Veterinary Medicine International,* 2016: 1 - 5
17. Boiti, C., Besenfelder, U., Brecchia, G., Theau-Clement, M. and Zerano, M. (2006). Reproductive physiopathology of the rabbit doe. In: Recent Advances in Rabbit Research (Edited by Maertens L. and Coudert P.). Institute of Agriculture and Fishery Research, Belgium: 3 - 19.
18. Touma, C., Palme, R. and Sachser, N. (2001). Different types of oestrous cycle in two closely related South American rodents (*Cavia aperea* and *Galea musteloides*) with different social and mating systems. *Reproduction,* 121: 791-801.
19. Addo, P. G., Awumbila, B., Awotwi, E. and Ankrah, N-A. (2007)**.** Reproductive characteristics of the female grasscutter (*Thryonomys swinderianus*) and formulation of colony breeding strategies. *Livestock Research for Rural Development*. Volume 19, Article #59.Retrieved October 25, 2012, from http:// www. lrrd. org/ lrrd 19/4/addo19059.htm