THE INFLUENCE OF DIFFERENT CONTACT LEVELS WITH MALE ON THE VAGINAL CYTOLOGY IN RABBITS UNDER THE TROPICAL HUMID CONDITION

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ABSTRACT

This study aimed to determine the influence of the contact with the male on vaginal cyclicity in the female rabbit through the evaluation of the cellular content of the vaginal lumen. Twelve rabbit does of mixed parity were divided equally into three treatments: Auditory and Olfactory contacts (AUD-OLF); Auditory, Olfactory and Visual contacts (AU-OL-VI); No contacts (NO-COT) with the male. Animals were housed in pair. In AUD-OLF and AU-OL-VI treatments the male animal was housed in a separate compartment between the females, while visual contact between the male and females was blocked with a screen in AUD-OLF. NO-COT was housed in another pen about 200 m away. Vaginal smears were daily collected from all the females for 29 days and the cells classified into superficial, intermediate and parabasal epithelial cell. From 10 different fields, each cell type was counted and then expressed as percentage of total. The rectal temperature of the animals was measured just prior to each smear collection. Intermediate cells appeared dominant (5.63-72.82%) in the AUD-OLF treatment, whereas parabasal cells predominate (20.24-90.66%) in the NO-COT group. Parabasal and intermediate cells occurred at equal proportions in the AU-OL-VI animals. The type of contact had no clear cut effect on the appearance of the superficial cells with only slight differences among treatments (9.20, 11.05 and 13.52% for AUD-OLF, AU-OL-VI and NO-COT, respectively; $P<0.05$). On the contrary, the intermediate cell numbers were highest in AUD-OLF, followed by AU-OL-VI and NO-COT (59.44, 40.07 and 28.82%; $P<0.05$). The reverse was the case with parabasal cells with values of 31.37, 48.87 and 57.64% ($P<0.05$), respectively. Rectal temperature was significantly ($P<0.05$) higher in NO-COT animals (38.20^{o}C). Rectal temperature had significant ($P<0.01$) positive (0.40) and negative (-0.44) relations with parabasal and intermediate cell count, respectively. Superficial and intermediate cells were significantly ($P<0.01$) and negatively related (-0.30) as also were intermediate and parabasal cells (-0.91). The association between superficial and parabasal cells was also negative (-0.11), but not significant. In conclusion the vaginal smear from normal adult rabbit shows no distinct pattern of occurrence of superficial, intermediate and parabasal epithelial cells as in spontaneous ovulators; female rabbit isolated completely from the influence of male shows more parabasal cells in the vaginal lumen which is characteristics of anoestrous animals and auditory, olfactory and visual contacts with the male appear determinant to prepare the female rabbit for sexual receptivity.

Key words: Rabbit, Vaginal smear, Male influence, Rectal temperature, Humid tropics.

INTRODUCTION

Spontaneous ovulators such as Guinea pig show cyclicity in behavioural oestrus and vaginal epithelial cells, and behavioural oestrus correspond to the preponderance of superficial cells in the vaginal lumen (Nelson, 2000). On the contrary in non-spontaneous/induced ovulators, such as rabbit, mating induces a neuro-endocrinological reflex, which provokes an LH pulse that leads to ovulation (Bedford, 1970). Rabbit do not present a cyclic appearance of the exfoliated vaginal cells and copulation and fertilization occur regardless of which of the vaginal epithelial cell types predominate (Kunde and Proud, 1929; Tsiligianni *et al*., 2004).

Touma *et al*. (2001) showed that in *Galea musteloides*, a wild cavy and an induced ovulator, acoustic-visual-olfactory stimuli and not necessarily copulation, was enough to stimulate oestrus and ovulation. Ypsilantis *et al*. (1996) also reported vaginal cycling in the rabbit using cytological examination of the vaginal smears. They showed that there is an increase followed by decrease in the percentage of superficial cells, lasting 1-2 and 2-3 days, respectively. More recently, Tsiligianni *et al*. (2004) reported that the cellular content of the vaginal lumen can be used to select rabbit does for superovulatory treatment, as more normal zygotes were obtainable from does with predominantly parabasal and intermediate cells, prior to superovulatory treatment. So far the specific role of the rabbit buck in influencing receptivity, ovulation and corpus luteum activity in the female rabbit has not been elucidated.

The objective of the present study was to determine the influence of acoustic, visual and olfactory stimuli from the male on vaginal cyclicity in the female rabbit, through the evaluation of the cellular content of the vaginal lumen.

MATERIALS AND METHODS

Animals and experimental design

Twelve rabbit does of mixed parity were divided equally into three treatments thus: Auditory and Olfactory contacts (AUD-OLF); Auditory, Olfactory and Visual contacts (AU-OL-VI); No contacts (NO-COT) with the male. Animals were housed in pair. In AUD-OLF and AU-OL-VI treatments the male animal was housed in a separate compartment between the females, while visual contact between the male and females was blocked with a screen in AUD-OLF. NO-COT was housed in another pen about 200 m away. Animals were kept in this condition for 2 weeks before the commencement of data collection.

Data collection

Vaginal smears were collected from all the females every other day for a total length of 29 days. The smear collection procedure involved applying gentle pressure on the vulva to evert the lower end of the vagina. A 5 cm cotton tipped swab stick was carefully inserted into the vagina as far as it could go without force and then twisted gently while being withdrawn. The swab was rolled over a clean glass slide to form two parallel tracks of smear materials. The smear was immediately fixed in absolute methanol, air dried and stained with Leishman stain. The vaginal smears were examined at magnification 100× and the cells classified into superficial, intermediate and parabasal epithelial cell (Bowen, 1998). From 10 different fields, each cell type was counted and then expressed as percentage of total. The rectal temperature of the animals was also measured just prior to each smear collection.

Statistical Analysis

The percentage composition of the different types of epithelial cells in each smear was represented with stack bars for individual animals in each treatment group. The mean percentage composition of each cell type was also compared between the treatment groups using one way ANOVA and Tukey test. Pearson correlation analysis was run between the rectal temperature and epithelial cell types.

RESULTS AND DISCUSSION

The percent constitution of superficial, intermediate and parabasal epithelial cells as observed in every smear for all the 12 experimental animals is shown in Figure 1. Animal CH4 was excluded from smear collection on days 9 and 11 to allow it recover from a minor leg injury sustained on day 8. In contrast to Ypsilantis *et al*. (1996) and in agreement with Kunde and Proud (1929) we could not observe a specific pattern for the appearance of the different cell types in the smears.

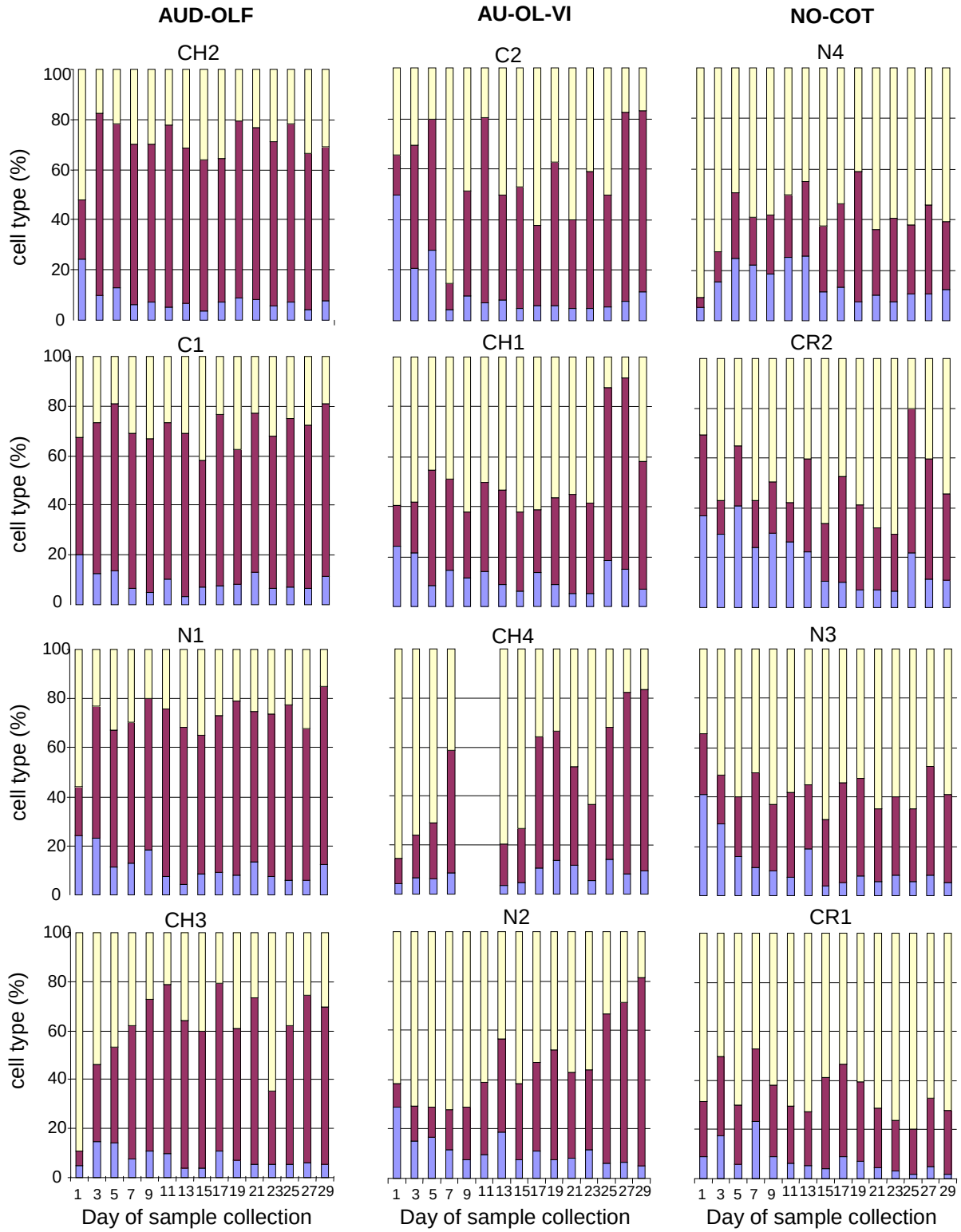

Figure 1: Percent composition of superficial ▭ intermediate ▭ and parabasal ▭ epithelial cells in the vaginal smear of 12 rabbits with different exposure to the male over a 29 day period. Treatments include Auditory and Olfactory contacts (AUD-OLF), Auditory, Olfactory and Visual contacts (AU-OL-VI) and No contacts (NO-COT). Letter on top of each grouped bars represents the animal's tag No

Superficial cells number did not give any pattern of increase and decrease (as described by Ypsilantis *et al*., 1996) and only on 3 out of 178 smears were the values above 40% (on days 1, 3 and 1 in animal C2, CR2 and N3, respectively). Intermediate cells appeared the dominant cell type (5.63–72.82%) in the smears from animals under AUD-OLF treatment whereas parabasal cells predominate (20.24–

90.66%) in the NO-COT treatment group. Parabasal and intermediate cells appeared to occur at equal proportions in the animals under AU-OL-VI. These results showed that sexual receptivity (oestrus) in rabbit doe is not signified by predomination of superficial cells in the vaginal lumen, as is found in guinea pig.

The mean composition of the different epithelial cells under the 3 treatment groups is shown in Table 1. The 3 levels of contact seem to have no clear cut effect on the appearance of the superficial cells and there were only slight difference between groups (range 9.20–13.52%). On the contrary, the mean values for intermediate and parabasal cells were significantly ($P<0.05$) different among the 3 contact levels. The intermediate cell incidence was highest in AUD-OLF (59.44 %) followed by AU-OL-VI (40.07%) and then NO-COT (28.82%). The reverse was the case with parabasal cells with values of 31.37, 48.87 and 57.64%, respectively. In spontaneous ovulators exposed to physical contact with male, intermediate and parabasal cells normally dominates the vaginal smear of pubertal, except at oestrus, while parabasal cells is the paramount cell type in the smear of pre-pubertal (anoestrus) females (Young, 1937; Ola *et al*., 2005). We may use this to explain the preponderance of parabasal cells in the smear of NO-COT (no contact with male) animals as been in a state of anoestrus. Since parabasal cells occurrence was highest in and also dominate the smears from does isolated from male influence (NO-COT), it thus appears that olfactory and visual/auditory contacts with the male rabbit as in AUD-OLF and AU-OL-VI are determinant to prepare the female rabbit ready for sexual receptivity.

Rectal temperature was significantly ($P<0.05$) higher in NO-COT animals (38.20^{o}C, Table 1) and this could be attributed to the housing of the animals which was in pens more exposed to the weather factors. Pearson correlation analysis (Table 2) revealed that rectal temperature of the animals had significant ($P<0.01$) positive (0.40) and negative (-0.44) relations with parabasal and intermediate cell count, respectively. This could also explain higher occurrence of parabasal cells and the reduced appearance of the intermediate cells in the vaginal smears of the NO-COT animals. On a weaker level ($P<0.05$, 2-tailed analysis) rectal temperature was also positively correlated to superficial cell count. Superficial and intermediate cells were significantly ($P<0.01$) and negatively related (-0.30) so also was intermediate and parabasal cells (-0.91). The association between superficial and parabasal cells was also negative (-0.11) but not significant. Unlike in spontaneous cyclers where appearance of superficial cells excludes the parabasal cells and is indicative of behavioural oestrus, rabbit presents both epithelial cell types in the same slide. It may mean that even when the superficial cells dominate the smear they may not indicate oestrus. In rat proestrus stage is accompanied by large numbers of parabasal cells in the smear and mating may occur at the later end of this stage during which the superficial and cornified cells will start to appear in large numbers (Long and Evans, 1922). Rabbit mates successfully even when the vaginal lumen is devoid of identifiable epithelial cells (Kunde and Proud, 1929).

Table 1: Percent constitution of different types of exfoliated epithelial cells in the vaginal lumen of rabbits

	AUD-OLF	AU-OL-VI	NO-COT	P level
Rabbits no. (Sampling no.)	4 (60)	4 (58)	4 (60)	
Superficial cell (%)	9.20 ± 0.63^{b}	11.05 ± 1.02^{ab}	13.52 ± 1.26^{a}	0.05
Intermediate cell (%)	59.44 ± 1.71^{a}	40.07 ± 2.53^{b}	28.82 ± 1.22^{c}	0.05
Parabasal cell (%)	31.37 ± 1.59^{c}	48.87 ± 2.54^{b}	57.64 ± 1.58^{a}	0.05
Rectal temperature (^{o}C)	37.17 ± 0.07^{b}	37.35 ± 0.08^{b}	38.20 ± 0.09^{a}	0.05

Means ± SEM with different superscript letters on the same row differ significantly ($p<0.05$, Tukey test).

Table 2: Pearson correlation between rectal temperature and the epithelial cell types

	Rectal Temperature	Superficial cell	Intermediate cell	Parabasal cell
Rectal temperature	1.00	0.14^{a}	-0.44^{b}	0.40^{b}
Superficial cell		1.00	-0.33^{b}	-0.11
Intermediate cell			1.00	-0.91^{b}
Parabasal cell				1.00

[a]Significant correlation at 0.05; [b]Significant correlation at 0.01

CONCLUSIONS

The vaginal smear from normal adult rabbit shows no distinct pattern of occurrence of superficial, intermediate and parabasal epithelial cells.

Female rabbits isolated completely from the influence of male show more parabasal cells in the vaginal lumen which is characteristics of anoestrous animals.

Auditory, olfactory and visual contacts with the male appeared determinant to prepare the female rabbit for sexual receptivity.

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