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Research Article

EXFOLIATIVE VAGINAL CYTOLOGY AND VAGINAL ELECTRICAL RESISTANCE: IMPORTANT TOOLS FOR ESTRUS DETECTION IN EWES

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ABSTRACT

Present study was aimed to examine the efficiency of Exfoliate vaginal cytology (EVC) and Vaginal electrical resistance (VER) for estrus detection in ewes. Eight adult healthy multiparous Gaddi ewes were synchronized with double shot of $PGF_{2\alpha}$ @ 187.5 µg, at 11 days apart. Vaginal cytology and electrical resistance were recorded successively for next five days after the 2nd injection of $PGF_{2\alpha}$ to determine the standing estrus in these animals. Decrease in percentage of parabasal and intermediate cells, while increase in percentage of superficial and anuclear cells was observed in the present study. Lower proportion of parabasal, intermediate cells (7.25±1.03, 22.63±2.53), followed by higher proportion of superficial and anuclear cells (41.63±1.52, 28.50±1.91), respectively at Day 4 could be regarded as the time of standing estrus in Gaddi sheep. Vaginal electrical resistance revealed decreasing trend at first two days of observation (358.7±38.98 vs 343.0±36.54 Ω) followed by significant increase during Day 3 (457.0±42.04 Ω; $p<0.05$) with peak value at Day 5 (478.0±26.01 Ω) of estrus. Correlation among EVC and VER revealed significant positive correlation with superficial ($p<0.001$) and anuclear cells ($p<0.01$), while negative significant ($p<0.001$) correlation with intermediate cells.

In conclusion, EVC in conjunction with VER can be employed for prediction of day of standing estrus/mating in ewes with absence of endocrine investigations. Furthermore, the day when a decreasing trend in the percentages of parabasal and intermediate cells followed with increasing trend in the percentage of anuclear and vaginal electrical resistance accompanied by peak level of superficial cell can be regarded as the exact day for mating in ewes.

Keywords: Exfoliate Vaginal Cytology (EVC), Vaginal Electrical Resistance (VER), Gaddi ewe

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Sheep husbandry plays pivotal role in rural economy by providing employment and income to marginal and landless households (Arora *et al.*, 2002). Sheep is a seasonally polyestrous animal with optimum reproduction during ideal time of the year which is usually spring (Abecia *et al.*, 2011). Estrus signs in the ewe are less pronounced than in the doe and are mostly expressed in the presence of ram (Altincekic and Koyuncu, 2012). Estrus detection in ewes can be accomplished on basis of observing behavioural modulations of ewe as well as ram. Zaid (2011) recorded discrete changes in the ratio of cornification of epithelial and basal cell at the time of ovulation in sheep. Vaginal cytology can be used to depict typical information regarding various hormonal alterations that occur throughout the estrous cycle in sheep (Zohara *et al.*, 2014; Jamwal, 2021) and goats (Ribeiro *et al.*, 2019). Comparative percentage of various vaginal epithelial cells owing to hormonal changes during the cycle may aid in predicting estrus phase (Sharma and Sharma, 2016). Vaginal electrical resistance, is the tissue's capacity to resist the flow of externally applied low electric current (Yamauchi *et al.*, 2009), alterations to the reproductive tract's histology and biochemistry which are brought about by ovarian endocrine activity (Aboul-Ela *et al.*, 1983). Vaginal electrical resistance reading varies during oestrus cycle may be correlated to its stages, providing crucial information for productive and efficient reproduction. Hence, the present study was designed to evaluate the usefulness of EVC and VER for predicting the day of standing estrus/mating in ewes.

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MATERIALS AND METHODS

A total number of eight adult healthy multiparous Gaddi ewes aged 2.16±0.16 yrs. (range 1.5-3 years), weighing 29.62±1.19 kg (range 25-35 kg) and devoid of any genital tract pathology were selected. The animals were housed in semi-intensive rearing system at Livestock Farm Complex, CSK Himachal Pradesh Krishi Vishvavidalaya, Palampur where they were allowed to graze in pasture for duration of six hours a day. The farm was located at 32.6 N, 76.3 E, altitude 1290.8 m and receives an annual rainfall of 13.50 mm. The mean annual minimum and maximum temperatures of the location was 16 °C and 31 °C, respectively in the breeding season (November to March, Department of Agronomy, College of Agriculture, CSKHPKV, Palampur). Additionally, the ewes were fed 20 gm of mineral mixture (HimChelate®, Himalaya, India) daily during the study period. The ewes also had the access of ad libitum drinking water round the clock. Estrus synchronisation was achieved by administration of intramuscular injections of $PGF_{2\alpha}$ @ total dose 187.5 µg

Table 1. Average characteristics (Mean±SEM) of vaginal epithelial cells (%) and vaginal electrical resistance (VER, Ω), in Gaddi sheep

Days Following 2nd PG Injection	Vaginal Epithelial Cells (%)				Vaginal Electrical Resistance (Ω)
	Parabasal	Intermediate	Superficial	Anuclear	
Day 1	44.00±2.28^{c}	36.71±2.80^{bc}	11.86±1.67^{a}	7.43±2.37^{a}	358.7±38.98^{ab}
Day 2	22.14±4.08^{b}	40.57±3.03^{c}	25.14±4.54^{b}	12.14±3.38^{ab}	343.0±36.54^{a}
Day 3	11.86±2.63^{a}	26.86±6.23^{ab}	43.57±5.27^{c}	17.71±2.15^{b}	457.0±42.04^{bc}
Day 4	7.25±1.03^{a}	22.63±2.53^{a}	41.63±1.52^{c}	28.50±1.91^{c}	467.0±26.10^{c}
Day 5	11.50±1.34^{a}	24.17±2.15^{a}	26.00±1.79^{b}	38.33±1.56^{d}	478.0±26.01^{c}

Values of different superscript (a-d) in the same column differs ($p<0.05$)

(Pragma®, Intas Animal Health, India) 11 days apart (Naderipour *et al.*, 2012; Jamwal, 2021). Thereafter, the ewes were monitored for behavioural estrus by teasing with adult healthy ram (morning-evening, 30 minutes each) after the second injection of $PGF_{2\alpha}$ successively for next 5 days (Day of 2nd $PGF_{2\alpha}$ injection designated as Day 0). Ewes exhibiting standing estrus during the period were allowed to be served naturally.

Vaginal Cytology: To record vaginal epithelial cells sequential exfoliative vaginal cytology was conducted during the estrus period. Sterile vaginal swab (Himedia®) was gently inserted into anterior vagina in properly restrained ewe. The swab was gently rolled against the vaginal mucosa two to three times before being removed. Swab was then rolled over clean, grease free glass slide and allowed to air dry. The smear was then fixed in 70 % of methanol for about 15 minutes, followed by 45 minutes of Giemsa staining at 1:8 dilutions. Smears were then dried off and subjected to examination under 40X magnification of microscope. The cells were categorized as parabasal, intermediate, superficial and anuclear (Jamwal, 2021).

Vaginal electrical resistance (VER): Vaginal electrical resistance was recorded using DRAMINSKI estrus detector®. Firstly, the probe was thoroughly cleaned with antiseptic solution and then inserted intravaginally. The data was recorded after three circular spins. Recording were taken twice daily and the mean value was used to denote the daily reading.

Statistical analyses were carried out using SPSS Statistics Version 25. The data was analysed through one-way ANOVA to find out statistical significance ($p<0.05$). Relationships among the EVC and VER were tested using Pearson correlation test.

RESULTS AND DISCUSSIONS

Perusal of Table 1, Fig. 2 revealed significantly higher (44.0±2.28%, $p<0.05$) population of parabasal cells at Day 1 of estrus detection followed by decreasing trends

Table 2. Correlation of EVC and VER in Gaddi ewes

Parameters	IM	SF	AN	VER	PB
Intermediate (IM)	1	-	-	-	-
Superficial (SF)	-.631**	1	-	-	-
Anuclear (AN)	-.612**	.277	1	-	-
Vaginal Electrical Resistance (VER)	-.654**	.503**	.383*	1	-
Parabasal (PB)	.354*	-.732**	-.637**	-.290	1

**correlation is significant at $p<0.001$; *correlation is significant at $p<0.01$

(22.14±4.08% to 11.50±1.34%) upto Day 5. Intermediate cells follow the identical pattern, with a slight increase up to Day 2 (36.71±2.80% v/s 40.57±3.03%) and then a significant decrease (26.86±6.23% v/s 22.63±2.53%, $p<0.05$) at Day 3 and 4, respectively. Contrarily, significantly higher ($p<0.05$) concentration of superficial (43.57±5.27%) were observed on Day 3 of estrus detection (Table 1), whereas anuclear cells attained maximum level on Day 5 (38.33±1.56%) in present study. Lower proportion of parabasal, intermediate cells (7.25±1.03, 22.63±2.53), followed by higher proportion of superficial and anuclear cells (41.63±1.52, 28.50±1.91), respectively at Day 4 could be regarded as the time of standing estrus in Gaddi sheep. Zohara *et al.* (2014) and Sitaresmi *et al.* (2018) characteristically observed a comparatively lower proportion of parabasal cells (0.0) on the day of estrus in indigenous ewes and Ettawa-Sannen does. Similarly, lower proportion of intermediate cells 4.3 (indigenous ewes; Zohara *et al.*, 2014) and 8.23±6.1 (Ettawa-Sannen does; Sitaresmi *et al.*, 2018) were also observed during estrus. In contrast, Rasad and Setiawan (2017) observed higher proportion of parabasal (22.1) and intermediate cells (23.5) during estrus.Anggriawan *et al.* (2017) concluded that characteristic large proportion of intermediate cell predominates during proestrus followed by superficial cells during estrus and parabasal cell during diestrus in fat tailed ewes. Similar trends of lower parabasal and intermediate cells (4.3±0.8, 16.5±2.1) along with higher

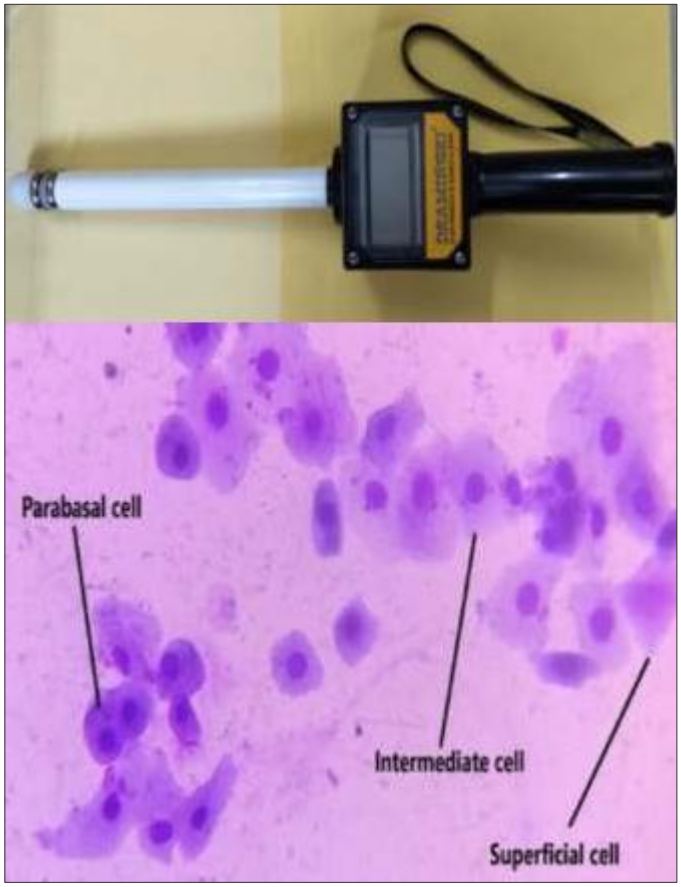

Fig. 1(a, b). DRAMINSKI estrus detector® and Vaginal cytology (X400)

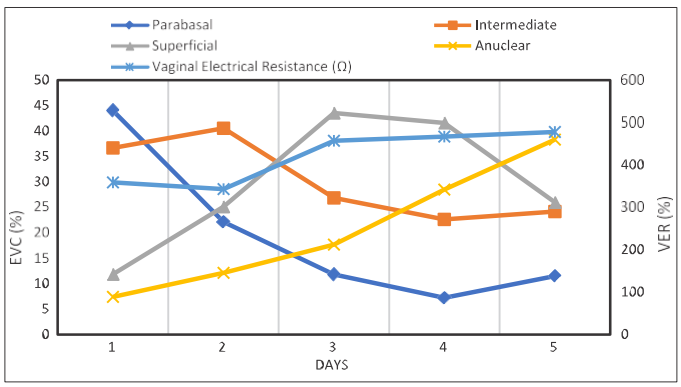

Fig. 2. Average characteristics (Mean±SEM) of vaginal epithelial cells (%) and vaginal electrical resistance (VER, Ù), in Gaddi sheep

proportion of superficial and anuclear cells (41.0±5.9 and 25.2±1.6) at Day 4 were observed earlier in Gaddi ewes (Jamwal, 2021).

Vaginal electrical resistance (VER) recorded during the present study (Table 1) revealed decreasing trend at first two days of observation (358.7±38.98 vs 343.0±36.54 Ω) followed by significant increase during Day 3 (457.0±42.04 Ω; $p<0.05$) with peak value at Day 5 (478.0±26.01 Ω) of estrus (Table 1, Fig. 1). Present finding was consistent with the earlier findings (Talukder *et al.*, 2018), where the lowest VER during estrus onset

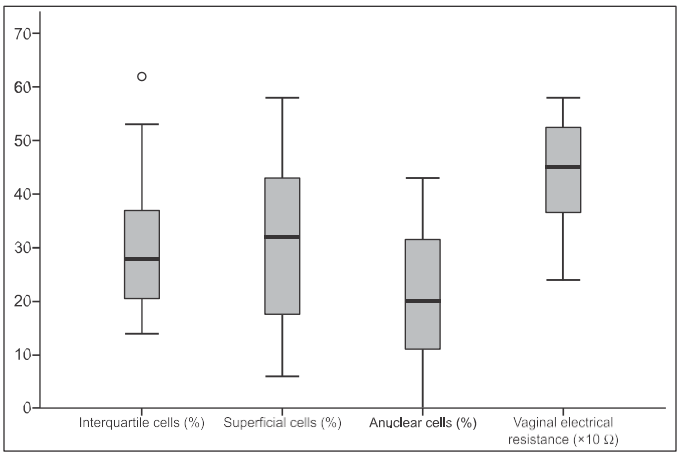

Fig. 3. Box-and-Whisker plots of the different vaginal -exfoliative cytology cells and –electrical resistance. The thick line is the median; the lower and upper lines of the 'box' are the first and third quartile. The box includes 50% of the data. The 'whiskers' extend to the farthest data point closer than 1.5 interquartile ranges from the 'box'. The circles can be considered 'outliers'.

(370.0±82.0 Ω) was observed which increases with duration. Similar, lowest value of VER at Day 2 (332.5 ± 55.4 Ω) with peak attained on Day 4 (545.0±66.4 Ω) was observed earlier in Gaddi sheep (Jamwal, 2021). Nain *et al.* (2018) reported significantly lower values of VER during proestrus and estrus phase, than other reproductive phases in goats, Contrarily, Prathibha *et al.* (2018) reported higher VER values in teaser ewes during estrus than teaser ewes outside estrus (570.0±24.6 vs 442.9±14.6 Ω). Changes in the VER during the different reproductive stages of estrous cycle may be due to alteration in estrogen and progesterone ratio (Rezac *et al.*, 2001). Positive correlations among VER and progesterone concentration were also observed in Chios ewes during breeding and in Kymi ewes during NB season synchronized with progesterone and eCG preparations (Theodosiadou and Tsiligianni, 2015).

Correlation among EVC and VER (Table 3) reveals significant positive correlation with superficial ($p<0.001$) and anuclear cells ($p<0.01$), while negative significant ($p<0.001$) correlation with intermediate cells.

CONCLUSION

In conclusion, VEC in conjunction with VER can be employed for prediction of day of standing estrus/mating in ewes with absence of endocrine investigations. Additionally, the day when a decreasing trend in the percentages of parabasal and intermediate cells followed with increasing trend in the percentage of anuclear and vaginal electrical resistance accompanied by peak level of superficial cell can be regarded as the exact day for mating in ewes. However, more studies are required in the similar tract to unfold the mystery of estrus detection in Gaddi ewes.

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