

Profile of Endometrial Secretory Proteins in Repeat Breeder Jersey Crossbred Cows

Bhavna^{1*}, Pankaj Sood² and Upma Mrall³

¹Division of Veterinary Gynaecology and Obstetrics, FVSc & AH, SKUAST, Jammu, India

²Department of Teaching Veterinary Clinical Complex, COVAS, CSK-HPKV, Palampur, India

³Department of School Education, Jammu, J&K, India

*Corresponding author: drbhavna260@gmail.com

ABSTRACT

The present study was conducted to evaluate, both quantitatively and qualitatively, the endometrial secretory proteins in uterine flushing collected on day 12 of estrous cycle in 10 normal and 10 repeat breeder Jersey crossbred cows. In the estrous cycle preceding the one in which uterine flushing were to be collected, all the repeat breeder cows were evaluated by phenolsulphonphthalene dye test to exclude the possibility of ovarobursal adhesions and fallopian tube blockade, both of which are established reasons of repeat breeding. The total protein content in the uterine flushing was higher in normal than repeat breeder cows (9.9 ± 1.6 mg/ml vs. 7.6 ± 0.5 mg/ml). On SDS-PAGE analysis, six protein bands of molecular weight 41, 53, 63, 69, 85 and 91 kDa were recorded in all the normal, but only in 8 of the 10 repeat breeder cows. The remaining 2 repeat breeder cows revealed an absence of three proteins of 63, 69 and 91 kDa. In conclusion, a lower total protein content and/or absence of specific proteins could be a potential reason of repeat breeding.

Keywords: Crossbred cows, endometrial secretory proteins, SDS-PAGE, estrous cycle

Repeat breeding, evinced in 21.9% of the infertile cattle (Kaikini *et al.*, 1983), has an intriguing etiology in cases without any palpable genital lesions, purulent discharge and estrous cycle abnormalities. Judicious use of fertile semen from certified bulls through AI eliminates the role of male in regulating fertility. Hence, the onus is on a better understanding of the diverse dam related factors leading to pregnancy failure. The uterine endometrium secretes an array of proteins that mediate a dialogue between the fetus and the dam so that the former progresses normally *in utero*. Limited studies have compared endometrial secretory proteins (ESP) between normal and repeat breeder - exotic (Guisse and Gwazdauskas, 1987) and Indian (Minhas and Saxena, 2008) cows. The information in a particular breed cannot be extrapolated to others due to a likelihood of breed variation

(Malayer and Hansen, 1990). Hence, the intent of present study was to investigate the ESP in repeat breeder (ovarobursal adhesions and fallopian tube blockade tested) compared to normal Jersey crossbred cows.

MATERIALS AND METHODS

Animals and management

The Jersey × Red Sindhi crossbred cows (exotic inheritance of 50.0 to 75.0%) included in the study were raised under standard managerial practices, including feeding (NRC, 2001), in the Agricultural University Livestock Farm, Palampur. The cows were normal cyclic and were either normal (N; n=10) or repeat breeders (RB; n=10). The RB cows had a history of 5 to 8 inseminations prior to inclusion in the study.

Evaluation of ovarobursal adhesions and fallopian tubes

The normalcy of reproductive tract and genital discharge at estrus was confirmed in the N and the RB cows. To exclude ovarobursal adhesions and fallopian tube blockade as a cause of repeat breeding, all the RB cows were subjected to phenolsulphonphthanalene dye test (Sood *et al.*, 2004). The test was performed at a weekly interval during mid-diestrus phase of estrous cycle preceding to the one in which uterine flushing were collected for evaluation of ESP.

Collection of uterine secretions

At day 12 (day 0 = day of estrus), the uterine horn ipsilateral to the ovary bearing CL in the N and the RB cows was catheterized with a sterile Rusch catheter. Thereafter, 40 ml of sterile phosphate buffer saline [PBS; pH 7.4] was slowly infused through the catheter. The PBS solution was left in the uterus for five minutes whereby the uterus was gently massaged transrectally. Subsequently, transrectal manipulation allowed the flush to flow out through the catheter and was collected in a sterile vial. The flush was immediately transported on ice to the laboratory where it was centrifuged at 10,000 rpm for 15 minutes at 4°C. The supernatant was aspirated and stored at -20°C till further analysis.

Quantification and characterization of ESP

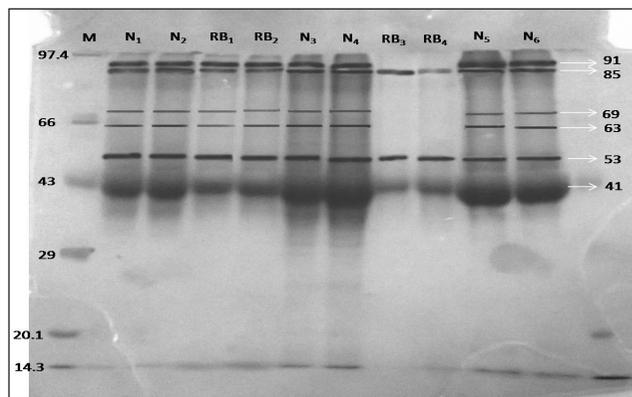
The ESP was quantified and characterized using one dimensional SDS-PAGE technique (Laemmli, 1970). The resultant bands obtained were evaluated after staining by Coomassie brilliant blue. The molecular weight of different proteins was compared with standard molecular weight markers used alongwith the sample.

RESULTS AND DISCUSSION

The total protein content in the uterine flushing at day 12 in the N and the RB cows was 9.9 ± 1.6 mg/ml and 7.6 ± 0.5 mg/ml, respectively.

The present findings simulate to a trend of higher protein content of 11.5 ± 2.3 mg to 16.2 ± 4.1 mg in normal (highest value at estrus) than 6.7 ± 5.4 mg to 11.4 ± 4.1 mg in the repeat breeder cows (highest value at day 15) (Guise and Gwazdauskas, 1987). Similarly, the uterine flushing from abattoir samples in the repeat breeders consistently revealed lower total protein (Ayalon *et al.*, 1978). Contrarily, a more recent study has reported significantly higher total protein in the uterine secretions of repeat breeders than normal cows (9.6 ± 5.4 mg/ml versus 1.3 ± 0.2 mg/ml) and was attributed to probably higher secretory proteins, cellular debris and tissue damage in repeat breeders with apparently clear genital discharge (Minhas and Saxena, 2008). Total protein concentrations of 4.79 mg/ml in the repeat breeder cows (Reinhold, 1953) and 1.07 ± 0.03 mg/ml in the normal cows (Rao and Sheshagiri, 1998) are also on record.

As is evident from Fig. 1 showing SDS-PAGE analysis, a total of six different protein bands of molecular weight 41, 53, 63, 69, 85 and 91 kDa, respectively, were observed in the entire N and eight repeat breeder (RB) cows.



N – Normal cyclic; RB – Repeat breeder; M – Marker

Fig. 1: SDS-PAGE analysis showing different proteins (kDa) in uterine flushing on day 12 of estrous cycle in normal (N) and repeat breeder (RB) cows

However, in the remaining two repeat breeder cows, depicted as RB₃ and RB₄, three proteins

of 63, 69 and 91 kDa were absent. Stage specific secretion of proteins by bovine uterine endometrium has been reported (Bartol *et al.*, 1981). A most complex array of 25 different proteins, both qualitatively and quantitatively, has been recorded at estrus (Williams *et al.*, 1992) and has been attributed to stimulatory action of high estradiol (Bartol *et al.*, 1981). Progesterone at high concentration, on the other hand, reduced the variety of endometrial proteins to at least eight (Guise and Gwazdauskas, 1987). The latter could be a plausible reason of detecting only six different proteins during day 12 of estrous cycle in the present study.

The proteins of 41, 53 and 91 kDa were the major proteins in the N and the RB cows. In endometrial explants, the major secretory proteins at day 14 has been 21.4 kDa, whereas on day 0, 9 and 18 the endometrial proteins of 12.7, 19.1, 32 and 66.5 kDa have been the major detected proteins (Williams *et al.*, 1992). A more recent study in Indian Holstein crossbreds revealed six major protein bands of >100, 68, 52, 33, 24 and 20 kDa in normal cows and >100, 68, 52, 33, 20 and <10 kDa in the repeat breeder cows. The latter findings also suggested the proteins of 24 kDa in normal and <10 kDa in repeat breeders to be the molecular markers (Minhas and Saxena, 2008). Compared to the latter study, the absence of ESP of molecular weight <41 kDa in the current investigation could be due to variation in estimation method or the denaturing conditions used to cleave the polypeptides in SDS-PAGE. However, stage of sample collection, breed of cow, side of uterine horn ipsilateral to the ovary bearing corpus luteum and environment (Williams *et al.*, 1992) could be the other reasons affecting the isolation of ESP.

The absence of 63, 69 and 91 kDa proteins in two RB-2 cows could be due to subnormal progesterone concentration, as low progesterone makes the endometrium deficient in proteins and other nutrients necessary for embryo (Guise and Gwazdauskas, 1987). Moreover, difference in progesterone concentration could have been

the reason for low total protein content in the uterine flushing of RB than the N cows in present study.

CONCLUSION

It is concluded that a low protein content or absence of specific protein from uterine endometrium may be the plausible reason of repeat breeding in the cows under investigation, which is further supported by absence of ovaro-bursal or fallopian tube abnormality, known to cause 5.5% of infertility/sterility in cattle (Abalti *et al.*, 2006). Endometrial proteins besides ensuring histotropical nutrition, also act as transport and immunoregulatory molecules, protease inhibitor and lysosomal enzymes (Pentacost and Teng, 1987).

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