

Detection Of A Uterine Secretory Protein Unique To Uninfected Repeat Breeder (Infertile) Cows By SDS – PAGE

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Citation

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Abstract

We report here the detection of a protein p<10 by SDS-PAGE, exclusively present in the uterine secretions of uninfected repeat breeder (infertile) cows but absent in healthy non-pregnant and pregnant cows. In contrast, proteins p27 (Native PAGE) and p24 (SDS-PAGE) were found to be conspicuously absent in uterine secretions of repeat breeder as well as pregnant cows but present in healthy non-pregnant cows. This suggests that p<10 could be a possible marker of repeat breeding while p27 and p24 could be markers of healthy non – pregnant status in cows, respectively. Proteins p22 and p20 on Native PAGE, and p>100, p68, p52, p33 and p20 on SDS-PAGE, were found to be common between uterine secretions of healthy non-pregnant and repeat breeder cows but absent in case of pregnant cows. It appears that these proteins could possibly be downregulated during pregnancy and may be associated with maintaining a non-pregnant status in cows. The characteristic presence or absence of certain proteins in uterine secretions of repeat breeder cows observed in this study could help in understanding the etiopathology and immunochemical basis of non-infectious infertility in cows.

INTRODUCTION

One of the important factors in successful completion of pregnancy is the presence of certain proteins in the uterine secretions during various stages of pregnancy. If the presence and proper functioning of these proteins contribute to successful reproduction and survival of the fetus to full term, then their absence and improper functioning could also possibly contribute to repeat breeding, sterility, abortion and fetal destruction by maternal immune system.

Since successful reproduction in farm animals is directly related to farm economy, it is vital to study the possible roles of secreted uterine proteins in normal animals and their immunopathology in failure of reproduction (e.g. repeat breeding). The present studies were, therefore, undertaken to determine the protein concentrations and differential protein profile of uterine secretions in repeat breeder cows and to compare them with those of normal healthy non – pregnant cows.

MATERIALS AND METHODS

Uterine secretions were collected from seven healthy non-pregnant cows and seven uninfected repeat breeder cows (which had been artificially inseminated more than three times without success and were devoid of any bacterial infection as apparent from microbial culture tests). The uteri

were flushed with Phosphate Buffered Saline (PBS) and the flushings were collected with the help of Rusch's catheter. Uterine secretions were analyzed for the levels of total proteins and were also fractionated by Poly Acrylamide Gel Electrophoresis (PAGE) to obtain differential protein profiles.

ESTIMATION OF TOTAL PROTEINS

The method for protein estimation reported by Reinhold (1953) was followed with minor modifications. The sample (0.1 ml) was mixed with 4.9 ml of 0.75N NaOH. A reagent blank tube containing 5 ml of 0.75N NaOH was also included. One ml of Biuret reagent was added immediately to each tube, mixed thoroughly and was allowed to stand at room temperature for 20 minutes for development of color. The Optical Density (O. D.) of unknown sample against the reagent blank was read at 545 nm.

POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE)

The uterine secretions were analyzed electrophoretically to study the differential protein profiles in case of pregnant, non-pregnant and repeat breeding cows. Mini Slab Gel System (Genei, Bangalore) was used for this purpose.

Native PAGE: Discontinuous polyacrylamide gels consisting

of resolving gels (7%) and stacking gels (2.5%) were prepared.

SDS-PAGE: The SDS-PAGE gels were prepared using the method described by Laemmli (1970) with minor modifications. Discontinuous polyacrylamide gels consisting of resolving gels (10%) and stacking gels (4%) were prepared. The samples were diluted in the sample buffer in the ratio of 1:4 and heated in a boiling water bath for 1 min.

After loading, the samples were subjected to electrophoresis for 15 min at 120 V and for 1 hour at 200 V. Along with the samples, standard molecular weight markers were also run in each gel. The gels were stained for 1 hour in staining solution (0.5% Coomassie Blue in 10% Acetic Acid and 40% Methanol) and then destained in the destaining solution (70 ml Acetic Acid dissolved in distilled water to make the volume to 1 L) till the background was clear.

RESULTS

TOTAL PROTEIN CONCENTRATIONS

The mean value (\pm S. D.) of total protein concentration in the uterine secretions was 133.8 ± 25.00 mg/100ml (1.338 ± 0.25 mg/ml) in case of non-pregnant cows and 962.1 ± 541.0 mg/100ml (9.621 ± 5.41 mg/ml) in case of repeat breeder cows (Table 1), respectively. The difference was very significant ($p < 0.01$).

Figure 1

Table 1: Total protein concentrations in uterine secretions of repeat breeders and healthy non-pregnant cows

S. no.	Protein concentrations (mg/ml)	
	Non pregnant cows	Repeat Breeders
1.	1.292	15.83
2.	1.454	4.84
3.	1.292	10.18
4.	1.131	16.64
5.	0.969	3.87
6.	1.777	11.63
7.	1.454	4.36
Mean	1.338 ± 0.25	9.621 ± 5.41
\pm S. D.		

NATIVE PAGE PROTEIN PROFILES OF UTERINE SECRETIONS

Three main protein bands of molecular weights 27 kDa, 22 kDa and 20 kDa, respectively, were detected in case of healthy non-pregnant cows while two main protein bands of 22 kDa and 20 kDa, respectively, were detected in case of repeat breeder cows in the Native PAGE protein profiles of uterine secretions. Besides these, 2 – 3 faint bands were also observed in the molecular weight range of 29 kDa to 43 kDa (Table 2, Fig. 1).

Figure 2

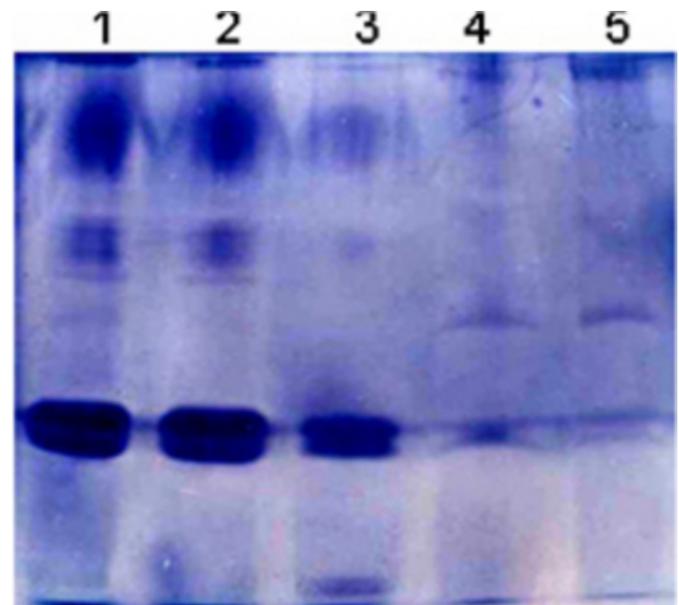
Table 2: Proteins detected in the PAGE protein profiles of uterine secretions of repeat breeders, healthy non-pregnant and pregnant cows

S. n.	Protein Band (kDa)	Presence of protein band in protein profile					
		Native PAGE			SDS PAGE		
		RB	NP	P	RB	NP	P
1	>100	-	-	-	+	+	+
2	80	-	-	+	-	-	-
3	72	-	-	-	-	-	+
4	68	-	-	-	+	+	+
5	52	-	-	-	+	+	+
6	50	-	-	+	-	-	-
7	43-45	-	-	+	-	-	+
8	33	-	-	-	+	+	-
9	29	-	-	-	-	-	+
10	27	-	+	-	-	-	-
11	24	-	-	-	-	+	-
12	22	+	+	-	-	-	-
13	20	+	+	-	+	+	+
14	14	-	-	-	-	-	+
15	<10	-	-	-	+	-	-

RB = Repeat Breeder; NP = Non-Pregnant; P = Pregnant

Figure 3

Figure 1: Native PAGE protein profiles of uterine secretions of repeat breeder and healthy non-pregnant cows. Left to Right Lanes 1 to 3- Uterine secretions of healthy non-pregnant cows and Lanes 4 & 5- Uterine secretions of repeat breeder cows.



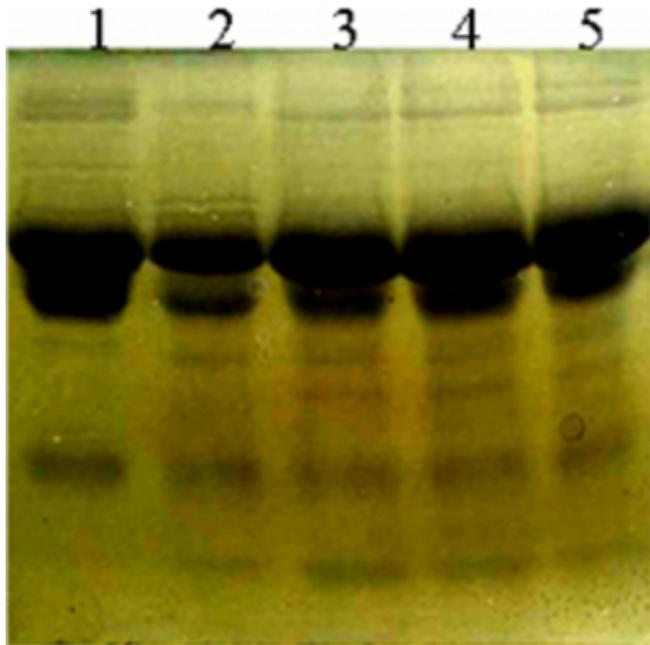
SDS-PAGE PROTEIN PROFILES OF UTERINE SECRETIONS

Six main protein bands of molecular weights >100 kDa, 68 kDa, 52 kDa, 33 kDa, 24 kDa and 20 kDa, respectively, were detected in the uterine secretions of non-pregnant cows. In addition, 4 – 5 bands in the molecular weight range of >100 kDa and 68 kDa were also found to be present.

Six main protein bands of molecular weights >100 kDa, 68 kDa, 52 kDa, 33 kDa, 20 kDa and <10 kDa, respectively, were discernible in the uterine secretions of repeat breeder cows. In addition, 4 – 5 bands in the molecular weight range of >100 kDa and 68 kDa were also found to be present (Fig. 2, Table 2).

Figure 4

Figure 2: SDS-PAGE protein profiles of uterine secretions of repeat breeder and healthy non-pregnant cows. L to R Lanes 1 & 2- Uterine secretions of healthy non-pregnant cows and Lanes 3 to 5 - Uterine secretions of repeat breeder cows.



The unique protein band found in repeat breeder secretion was absent in uterine secretions of pregnant cows and was undetectable by native or SDS PAGE (Figs. 3 & 4)

Figure 5

Figure 3: Native PAGE protein profiles of uterine secretions of pregnant cows. Lane 1- Molecular weight markers, Lanes 2 and 3- Uterine secretions of pregnant cows.

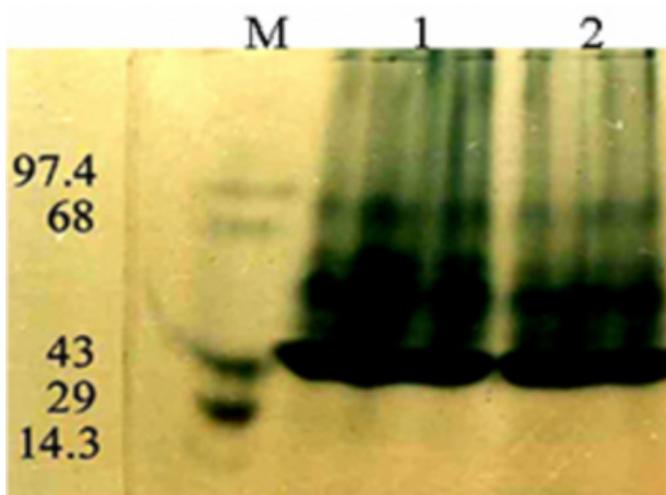
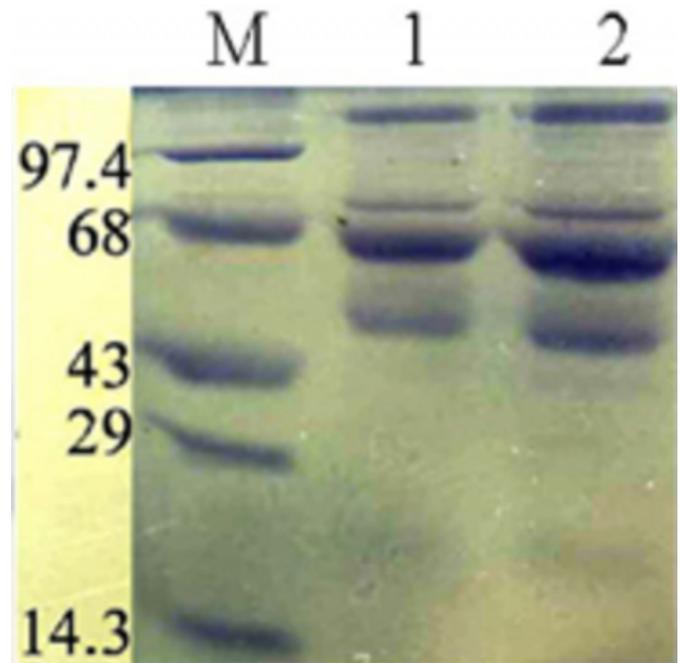


Figure 6

Figure 4: SDS-PAGE protein profiles of uterine secretions of pregnant cows. Lane 1- Molecular weight markers, Lanes 2 and 3- Uterine secretions of pregnant cows.



DISCUSSION

Since uterine secretory proteins play important roles in the maintenance of pregnancy, comparative studies on the total protein concentration and differential profile of proteins in uterine secretions of normal non-pregnant as well as repeat breeding animals could be very useful.

The present study revealed the concentrations of total proteins in the uterine secretions of repeat breeder cows to be very significantly ($p < 0.01$) higher than those of normal healthy non – pregnant cows. The levels of proteins in uterine flushings of healthy cows have been reported to be 107.25 ± 3.62 mg/100 ml [3] while protein levels of uterine flushings of repeat breeder cows have been reported to be 479 mg/100 ml [4]. The higher concentrations of proteins in uterine flushings of repeat breeders were suggested to be due to the increased levels of secretory proteins, cellular debris and tissue damage. The total protein concentration in uterine flushings was reported to decrease significantly after treatment.

In the present study, the PAGE analysis of proteins of the uterine secretions revealed some interesting patterns. On Native – PAGE, the protein p27 was found to be exclusively present in the uterine secretions of healthy non – pregnant cows only whereas, the proteins p22 and p20 were present in

the uterine secretions of both, the healthy non – pregnant and the repeat breeder cows.

On SDS – PAGE, the protein p<10 was found to be exclusively present in the uterine secretions of repeat breeder cows only, whereas, p33 was present in both, the healthy non-pregnant as well as the repeat breeder cows. It was interesting to note that p22 and p20 (Native PAGE) and p33 (SDS-PAGE), common to healthy non-pregnant and repeat breeder cows, were conspicuously absent in uterine secretions from pregnant cows. We have not come across any report on such studies on comparison of protein profiles of uterine secretions of repeat breeder cows with healthy non-pregnant and pregnant cows in the available literature. The exact nature and function of proteins unique to the repeat breeders or non – pregnant cows observed in our study remain to be elucidated. The complete understanding of their roles holds the key to immunological manipulation of sterility and fertility in reproduction and treatment of repeat breeding of non-infectious origin in domestic animals.

CONCLUSIONS

The protein profiles and total protein concentration of uterine secretions of uninfected repeat breeder cows were compared with those of healthy non-pregnant animals. The mean value of total proteins in uterine secretions of repeat breeders was found to be very significantly ($p<0.01$) higher than that of normal healthy non – pregnant cows. On SDS-PAGE, the protein p<10 was found to be present exclusively in the uterine secretions of repeat breeder cows but not in healthy non – pregnant cows. On Native PAGE, proteins p22 and p20, and on SDS-PAGE, proteins p>100, p68, p52, p33

and p20 were found to be common between healthy non – pregnant cows and repeat breeder cows. The proteins p27 (Native PAGE) and p24 (SDS-PAGE) were present in the uterine secretions of healthy non-pregnant cows but absent in case of repeat breeder cows. The exact physiological role and significance of characteristic presence or absence of certain proteins in uterine secretions of repeat breeder cows could not be ascertained.

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