ANTISPERM ANTIBODIES IN SERUM AND CERVICAL MUCUS OF NORMAL AND REPEAT BREEDING COWS.

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ABSTRACT

Indirect ELISA was performed to determine the antisperm-antibodies (asp-abs) in serum and cervical mucus from 14 normal and 53 repeat breeding crossbred cows .85.71% sera and 78.57% cervical mucus from normal breeding cows showed asp-abs titre of 1:160 and 1:40, respectively .On the other hand 100% cows repeating 3-5 times or more showed the presence of asp-abs in serum and mucus with the titre of 1:3120 and 1:1280, respectively. The significant observations made under this study were that although a good proportion of normal cyclic cows were positive for asp-abs but with lower level of titer in serum and mucus. The cows having more unsuccessful inseminations showed higher asp -abs with high titre in serum and mucus.

Key words: Cross bred cows, Repeat breeding, Anti sperm antibodies, Serum and Cervical mucus.

Repeat breeding is a commonly encountered problem in cross bred cows leading to considerable economic losses to the dairy farms. Among the various causes of infertility circulating asp-abs have been reported to be an important factor for unexplained infertility in woman (Franklin and Dukes,1964, Bratanov,1969) and also in cows (Casida,1961 Menge ,1967 and Shanker, *et al.* ,1985). Like the other systems or organs of the body reproductive organs can also produce humoral antibodies against different antigens including sperm and seminal plasma (Yadav and Agarwal, 2002). The sperm agglutinating asp- abs may cause infertility by disturbing the physiological mechanisms and process of fertilization like sperm migration, penetration, capacitation, acrosomal reaction etc. and early embryonic death (Krishna and Rao, 1999). However, in India, the branch of bovine immuno infertility is not been studied elaborately .The present study was therefore, undertaken to investigate the asp-abs in serum and cervical mucus from normal and repeat breeding cows and to elucidate the relationship of asp-abs titre with the repeat breeding condition.

Total of 67 crossbred cows owned by private dairy farms, maintained under similar conditions were included under the study. Of which 14 were normal breeding and 53 were repeat breeding cows. The repeaters were grouped as per the times they repeated. The cows were diagnosed to have repeat breeding problem following the criteria given by Roberts (1971).

Collection of Samples: Serum samples from all the 67 cows were collected during estrus and preserved at -20°C for the study. The cervical mucus from all the cows were also collected aseptically following the procedure of Zemjanis (1970) and were stored at -20°C for further study. The mucus samples were mucolysed with magnetic stirrer just before preservation.

Indirect ELISA for detecting antibodies against bovine spermatozoa: Indirect ELISA was standardized to detect asp-abs in the sera and mucus of the cows. The ELISA test was selected for detecting the asp-abs

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as the test has been considered to be a sensitive test for detecting asp-abs in different species of animals(wang,1989, Lander *et al.*, 1990, and Lee *et al* .,1993) .

Preparation of spermatozoal antigen: Freshly collected bull semen was centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded and the spermatozoal pellet was washed thrice with Phosphate Buffer Solution (PBS, P_H 7.4). Approximately 1 gm of washed spermatozoa was dissolved in 5 ml of PBS. Lysis of sperm was made by repeated freezing and thawing and centrifuged at 5000 rpm for 15 minutes .The supernatant was collected and stored at -20° C and used as antigen .The antigen was titrated and a titer of 1:10 was found to be appropriate for the ELISA.

Test procedure: Microtitre ELISA plate was used to perform the ELISA. The 96 wells ELISA plates were coated with the spermatozoal antigen dissolved in carbonate bi-carbonate buffer (P_H 9.6) with the appropriate dilution and adding 50 µl per well of the plates. Coating was done by keeping the plates overnight at 4° C. Coated plates were washed at least three times with PBS + $tween_{20}$. The test samples were added to the coated plates by making serial two fold dilution from 1:10 dilution in the blocking buffer (PBS $Tween_{20}$ with 5 % horse serum). Test serum samples were also added to the plates containing no antigens. Plates were incubated at 37° C for 1 hour, washed and antibovine horse radish peroxidase (HRPO) conjugate (SIGMA) was added using 1:1000 dilution in the blocking buffer and further incubated at 37°C for 1 hour. Plates were again washed and the substrate chromogen (H_2O_2 + OPD) mixture was added and allowed to react for 15 minutes. The reaction was stopped by adding $1NH_2So_4$ and the optical density (OD) value was recorded in ELISA reader (DYNATECH) using 490 nm filter. A test sample was considered positive when the OD value of a sample was 0.1 or above after subtracting the OD value of the sample in the plate without antigen. The highest dilution of the sample which showed positive OD value was considered as the titre of the sample.

Out of the 14 sera samples from normal breeding cows 85.71% were positive for to asp-abs as the titre ranges from 1:20 to 1:160.On the other hand 78.57% cervical mucus from the same group of cows were positive reactors with 1:10 to 1:40 level of titre. One normal cyclic cow showing 1:20 titre in serum was negative reactor in cervical mucus. This lower level of asp-abs titre in normal cyclic cows might not interfere with the normal process of fertility as the seminal plasma contains immunosuppressive agent (MIM-Male Inhibitory Materials) which might counteract the asp-abs (Hess and Marcus, 1980). Besides, the uterine secretory protein also have got some degree of immunosuppressive effect which might help in fertility in cows with low asp-abs titre (Segersen, *et al.*, 1984). Both the serum and cervical mucus from repeat breeding cows exhibited higher asp-abs titre than that of the normal breeding cows and the titre found to increase along with the increasing numbers of inseminations .The serum and mucus of cows repeated 3-5 times showed the asp-abs titre from 1:160 to 1: 640 and 1:80 to 1:320, respectively. Interestingly, the cows repeated 12-15 and 15-20 times showed average asp-abs titre from 1:1280 to 1:3120, respectively in serum. Similarly, in mucus the average titre from both these two groups of cows showed the asp-abs titre from 1:320 to 1:1280. It was evident that though 100.00% sera and mucus from each of the repeat breeding groups were positive for asp-abs but the cervical mucus showed lower level of asp-abs titre than that of the serum of that respective group of cows. In the present study the higher asp-abs titre in serum and cervical mucus of repeat breeding cows might be due to the boostering effect on asp-abs production from every unsuccessful service .Krishna and Rao ,(1999) reported that the asp-abs titre level as high as 1:2560 may cause infertility in cows and buffaloes. The lower level of the asp-abs titre in mucus might be due to the dilution of antibodies in copious mucous at estrus when it was collected. Another probable reason for lower asp-abs titre in mucus is the gradient phenomena where the antibody titer in serum with higher concentration diffused into the uterine lumen to give lower level of titre. Besides, Symons and Herbert,(1971) reported that the epithelium of the female genital tract at all level prevented the diffusion of Ig-G from relatively higher level in tissue to the lower level in luminal fluid . The present results were in concurrence with the findings of Bratanoff and Dikoff (1960), Pavlichenko *et al.*, (1970), Sirazdinov, (1973), Gokcen *et al.*, (1990), Gondotra and Sharma, (1991) and Jain and Gupta, (1991). However,

Bhatta, *et al*., (1979),Seshagiri, *et al*.,(1987) reported much lower positive reactor repeat breeding cows with Double Gel Diffusion test and Slide agglutination test, respectively. The higher level of asp-abs titer in serum and mucus in the present study might be because of the use of ELISA, which was regarded as the most sensitive and effective test for detection of asp-abs (wang, 1989, Lander *et al*., 1990, and Lee *et al*.,1993).

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