Volume 56 (11) : November, 2003
Irish Veterinary Journal

peer reviewed

peer reviewed

The prevalence of anti-sperm antibodies in cattle in the Eastern Anatolian region of Turkey

Ali Risvanli[1], Muhterem Aydin[1], Erdal Kaygusuzoglu[1], Hakan Bulut[2], A. Mukremin Apaydin[1] and Yusuf Bolat[2]
1 Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, University of Firat, Elazig, Turkey.
2 Department of Virology, Faculty of Veterinary Medicine, University of Firat, Elazig, Turkey.

Sera from 200 cows and heifers of different breeds were subjected to an enzyme immunoassay (EIA) for anti-sperm antibodies. Fifteen (7.5%) of the animals tested were positive for the antibodies. The prevalence of the antibodies was greatest amongst the cows that had had three or more inseminations: six (17.65%) of 34 cows tested positive.

Key words
Cattle,
Anti-sperm antibody,
Turkey.

Irish Veterinary Journal
Volume **56:** 560 - 562, 2003

Introduction

In any species, the role of anti-sperm antibodies (ASA) in infertility is controversial. It could be concluded from some of the data published in the literature that ASA might be influential in inhibiting fertility (Basu *et al.*, 1990; Branson *et al.*, 1985; Lee *et al.*, 1993; Mettler *et al.*, 1980); other authors believe that ASA are irrelevant to infertility (Farahani *et al.*, 1981; Max, 1990). Antibodies in semen could be expected to affect sperm motility, viability, or the ability to fertilize the egg. In females, the glandular epithelium of the cervix is considered capable of producing a local immune response. Antibodies in cervical mucus or oviductal fluid could impede sperm transport or interfere with the acrosome reaction (Alexander and Anderson, 1989; Basu *et al.*, 1990; Cunningham *et al.*, 1991; Lander *et al.*, 1990).

There are several hypotheses to explain why anti-sperm antibodies develop in animals. Immune responses vary from one animal to another and certain animals may have a genetic predisposition for a greater response. It has been suggested that if spermatozoa are present during an infection of the reproductive tract, the infectious agents somehow potentiate the immune response against sprermatozoa by an adjuvant effect (Alexander and Anderson, 1989; Cunningham *et al.*, 1991). Another possibility is that cross-reacting antibodies (i.e., antibodies that are formed against one antigen but can also react with a second antigen) may develop in animals and impair fertility (Alexander and Anderson, 1989; Paolichhi *et al.*, 2000).

In the present study, we investigated the prevalence of anti-sperm antibodies in serum samples from cows and heifers in the Eastern Anatolian Region of Turkey. We also examined the relationships between the prevalence of anti-sperm antibodies and various host factors that might provide some indications as to why the animals produced the antibodies and what effect, if any, they had on fertility.

Correspondence:

Ali Risvanli
Department of Obstetrics and Gynaecology,
Faculty of Veterinary Medicine,
University of Firat,
Elazig,
Turkey.
E-mail: arisvanli@yahoo.com
Tel: +90 424 237 00 00/6169
Fax: + 90 424 238 81 73

Materials and methods

A total of 200 cows and heifers, from one to 11 years old, were included in the study. The breeds of the animals are given in Table 1. The pregnancy status of the animals was determined by rectal and ultrasonographic examinations. Data related to the age, breed, gestation and artificial insemination were provided by the owners. Blood samples (10ml) were drawn and serum was obtained according to standard procedures. Serum samples were stored frozen at –20°C until the assays were performed.

In the enzyme immunoassay, we followed the procedures described earlier with slight modifications (Bolat *et al.*, 1996). Washings and dilutions were made with phosphate-buffered saline (PBS) containing 0.02% Tween-20, pH 7.2. Incubations were carried out at room temperature for one hour. To prepare

peer reviewed

antigen for the assays, bull ejaculate was washed in PBS twice and resuspended, at a concentration of $5x10^6$ spermatozoon / ml, in PBS containing 0.25% gluteraldehyde only. A hundred µl from this suspension was added to the test wells of the 96-well plates coated with poly-L-lysine (100 µg / ml) in 0.01M bicarbonate coating buffer. As negative controls, PBS containing 0.25% gluteraldehyde only was put in some wells. Blockings were done with the addition of 100µl of PBS containing 10% horse serum to the wells. Serum samples were diluted in PBS at 1 to 50 ratio and were tested in duplicate. As secondary antibody, goat anti-bovine IgG conjugated with horse radish peroxidase (Sigma Chemical Co., St. Louis, MO, USA) was used. The chromogen substrate was o-phenylene diamine. The reactions were stopped with addition of 100µl 1M H_2SO_4 to the wells and absorbance was determined at 450nm wave length (Medispec, ESR 200 EIA Plate Reader). To determine the cut off point for positivity, the following method was used (Bolat *et al.*, 1996). Average absorbance obtained from negative wells was multiplied by three and absorbance value obtained was used as a cut off value for positive samples.

The statistical analyses of the results were made by SPSS for Windows (1993).

TABLE 1: The prevalence of the anti-sperm antibodies (ASA) according to the breed of the animals

Breed	ASA (+)		ASA (-)		
	No.	%	No.	%	Totals
Holstein	2	5.00[b]	38	95.0	40
Swiss Brown	8	10.67[b]	67	89.33	75
Eastern Anatolian					
Native	2	11.11[b]	16	88.89	18
Crossbreed	3	5.66[b]	50	94.34	53
Simmental	0	0[a]	14	100.00	14
Totals	15		185		200

[a, b] *The difference between the group percentages is significant (P<0.01).*

TABLE 2: The prevalence of the anti-sperm antibodies (ASA) according to age and reproductive status of the animals

	NON-PREGNANT (n=125)				PREGNANT (n=75)				
	ASA (+)		ASA (-)		ASA (+)		ASA (-)		
	N	%	N	%	N	%	N	%	Total ASA (+)
Heifers (n=37)	-	-	15	40.54	4	10.81	18	48.65	4
Maiden (n=15)	0	0.00	15	100.00	-	-	-	-	0
Pregnant (n=22)	-	-	-	-	4	18.18	18	81.82	4
Cows (n=163)	9	5.52	101	61.96	2	1.23	51	31.29	11
Up to 4 years (n=56)	4	7.14	27	48.21	1	1.79	24	42.86	5
5-8 years (n=72)	3	4.17	46	63.89	1	1.39	22	30.56	4
9-11 years (n=35)	2	5.71	28	80.00	0	0.00	5	14.29	2
TOTALS	9	7.20	116	92.80	6	8.00	69	92.00	15

TABLE 3: The prevalence of the anti-sperm antibodies (ASA) according to the number of inseminations of the animals

The number of inseminations	ASA (+)		ASA (-)		Totals
	No.	%	No.	%	
Non inseminated	2	3.51[b]	55	96.49	57
once	5	6.94[b]	67	93.06	72
twice	2	5.41[b]	35	94.59	37
3 or more times	6	17.65[a]	28	82.35	34
Totals	15		185		200

[a, b] *The difference between the group percentages is significant (0.01).*

Results and discussion

Overall, anti-sperm antibody was detected in the serum of 15 (7.5%) animals. The results have been tabulated according to breed (Table 1), the age and reproductive status (Table 2) and number of inseminations (Table 3). The prevalence of anti-sperm antibodies appeared to increase significantly (to 17.65%) amongst cows that had three or more inseminations (P<0.01, Table 3). It is tempting to speculate that in this group of animals, at least, anti-sperm antibodies might have played a role in the repeat-breeding. However, for circulating antibodies to be a factor in fertility regulation, they must come into contact with spermatozoa within the female tract. We had no evidence that the circulating antibodies had crossed the blood-uterus barrier.

Our study also demonstrated that anti-sperm antibodies were present in six (8%) of 75 pregnant animals. Thus, additional factors such as the titre or other properties of the antibodies would have to come into play if, indeed, these antibodies exert an anti-fertility effect. Similar results have been reported by others (Gokcen *et al*., 1986-1987; Wang and Xie, 1990).

In the literature, there is not sufficient information about the titres of anti-sperm antibodies in different breeds of cattle. In our study, none of the Simmental breed had anti-sperm antibodies. This is a rather striking result. However, the number of the animals in this group was small and it may be misleading to draw any conclusions about the observation.

In conclusion, the presence of anti-sperm antibody in the serum of cows seemed to be affected most by the factors such as the number of artificial inseminations animals experienced and the breed of the animal. The exact role played by these antibodies in relation to fertility requires further study.

References

Alexander, N.J. and Anderson, D.J. (1989). Immunological factors in reproductive fitness. In: *Reproduction in Mammals: Book 4: Reproductive Fitness*. pp176-209. Edited by C.R. Austin and R.V. Short. Cambridge: Cambridge University.

Basu, S., Bandopadhyay, S.K. and Choudhury, R.R. (1990). Comparative efficacy of different serodiagnostic methods for detecting immunologic infertility in repeat breeding cows. *Indian Journal of Animal Reproduction* 11: 124-127.

Bolat, Y., Bulut, H., Ozdarendeli, A. and Doymaz, M.Z. (1996). Development of enzyme-linked immunosorbent assay for detect of antibodies to infectious bovine rhinotracheitis / infectious pustular vulvovaginitis virus in cattle. *Firat University Journal of Health Sciences* 10: 282-288.

Branson, R., Cooper, G., Hjort, T., Ing, R., Jones, W.R., Wang, S.X., Mathur, S., Williamson, H.O., Rust, P.F., Fudenberg, H.H., Mettler, L., Czuppon, A.B. and Sudo, N. (1985). Anti-sperm antibodies, detected by agglutination, immobilization, microcytotoxicity and immunobead-binding assays. *Journal of Reproductive Immunology* 8: 279-299.

Cunningham, D.S., Fulgham, D.L., Rayl, D.L., Hansen, K.A. and Alexander, N.J. (1991). Antisperm antibodies to sperm surface antigens in women genital tract infection. *American Journal of Obstetrics and Gynecology* 164: 791-796.

Farahani, J.K., Tompkins, W. and Wagner, W.C. (1981). Reproductive status of cows and incidence of antisperm antibodies. *Theriogenology* 15: 605-612.

Gokcen, H., Minbay, A., Cekgul, E. and Carlı, T. (1986-1987), Studies on the relationship between presence of anti-sperm agglutinins and some cases of infertility. *Journal of Faculty of Veterinary Medicine University of Uludag* 1-2-3: 141-148

Lander, M.F., Hansen, P.J. and Drost, M. (1990). Antisperm antibodies in cows after subcutaneous and intrauterine immunisation. *Veterinary Record* 126: 461-462.

Lee, C., Nie, G.J., Joo, H.S. and Momont, H. (1993). An enzyme linked immunosorbent assay (ELISA) for the detection of antisperm antibodies in horse serum. *Theriogenology* 40: 1117-1126.

Max, A. (1990). Analysis of causes of ineffective insemination of cows based on clinical, hormonal and immunological examinations. *Medycyna-Weterynaryjna* 46: 352-354.

Mettler, L., Shirwani, D. and Gradl, T. (1980). The occurrence of sperm antibodies in human reproduction. *American Journal of Obstetrics and Gynecology* 136: 106-116.

Paolichhi, F.A., Casaro, P.A., Gimeno, G.J., Kartebani, L.G. and Mozzolli, A.B. (2000). Antisperm response in rams experimentally infected with *Brucella ovis*. *Small Ruminant Research* 36: 7-15.

SPSS (Statistical Package for Social Sciences) for Windows copyright©, spss, inc. 1993.

Wang, G.L. and Xie, C.X. (1990). The relationship between antisperm antibodies and progesterone in the serum of infertile dairy cows. *Acta Veterinaria et Zootecnica-Sinica* 21: 26-30 ■