Antisperm Antibodies in Blood Sera of Bulls and Correlations With Age, Breed and Ejaculate Quality

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Abstract

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Blood sera from 612 bulls of different age and breed randomly selected in artificial insemination stations and bull rearing stations were examined by ELISA for the presence of antisperm antibodies. At serum dilution 1:40 and higher, the incidence of positive bulls was 35.5%. The first incidence of antibodies was recorded at the age of 5 to 6 months (13.8%), with a significant increase (58.1%; $p < 0.01$) at 9 to 10 months of age. In the group of bulls aged 5 months and older, positive antibody titres were detected in 42.7% of animals. The finding of antisperm antibodies was significantly higher in active donors of semen compared with the candidate breeders that have not been used as regular donors (56.4% vs. 39.2%; $p < 0.01$). Higher frequency of antibodies was detected in bulls of the Black Pied Holstein cattle and their crossbreeds compared with the Czech Red Pied cattle and their crossbreeds (48.4% vs. 35.2%; $p < 0.01$). In positive bulls with a regular semen collection, a lower ejaculate volume was found ($p < 0.01$) as well as lower activity of endogenous reductases in sperms ($p < 0.01$) and decreased sperm velocity after 120 min of heat test ($p < 0.05$). Fertility in those bulls was insignificantly lower. Prognostic and clinical importance of antisperm antibodies for quality assessment of breeding animals is being discussed.

Cattle, testis, sperm, spermatoanalysis, autoantibodies, enzyme like immunoassay, fertility

The development and functions of the reproductive system are controlled by complex neuroendocrine mechanisms. The immune system plays a key role under physiological conditions and in reproductive disorders. The processes leading to sperm formation have some immunologic "privileges". Sperms and their precursors exhibit quite strong antigenic traits. A wide spectrum of antigenic structures is being expressed in the testis during spermatogenesis towards which, ontogenetically, autotolerance mechanisms are inactive. Protection against autoimmunity is provided by the hemotesticular barrier composed predominantly of Sertoli cells isolating the tubular content from the vasculature, and limited lymphatic drainage of the testis. Several other immunoregulatory mechanisms also play a role, e.g. immunosuppressive factors of seminal plasma and both systemic nonspecific and specific factors (immunoregulation cells, cytokines etc.). However, the "isolation" of the sperm antigens is not complete. In the testicular interstitium the cells of the immune system are present, especially macrophages and T lymphocytes. These provide local protection against infection and some other functions, including the supply of growth factors to Leydig cells and precursors of germ cells.

The formation of antisperm antibodies (ASA) and their relation to reproduction belong to so far unresolved questions in the field of reproduction. Generally, ASA formation can be induced primarily during infectious and noninfectious inflammations, or by obstruction of testicular efferent duct. The incidence of ASA was also induced by an accident (Zhang et al. 1990), very low temperature (Fayemi et al. 1992), cryptorchism (Pinart et al.

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1999), vasectomy (Jessop and Ladds 1995), and by excessive male exploitation (Wicher et al. 1987).

The occurrence of ASA is being connected with infertility in both humans and laboratory and farm animals (Menge et al. 1962; Awadi et al. 1984; De Almeida et al. 1986; Zhang et al. 1990; Nouza et al. 1992). Negative effect of ASA on sperm function during *in vitro* fertilization was demonstrated by Tasdemir et al. (1995), Kim et al. (1999), Lombardo et al. (2001), and others.

The first study on the prevalence of antisperm antibodies in blood sera of boars and sows was published by Fayemi et al. (1992); in bulls and boars by Zralý et al. (1997). Most of the unexplained questions concern the etiology and pathogenic mechanisms of functional changes in sperms induced by antibodies, and the subsequent subfertility (Gandini et al. 1995). The objective of the study was to determine the level of antisperm antibodies in the blood sera of bulls of different age and breed in relation to sexual activity, and to assess the effect of the antibodies on semen quality and fertility.

Materials and Methods

Blood sera were obtained from 612 randomly selected bulls in four artificial insemination stations and three rearing stations. The set of animals included 395 bulls of the Red Pied cattle (Czech Spotted cattle and their crossbreeds), and 217 bulls of the Black Pied cattle (Black Pied Holstein cattle and their crossbreeds). The animals were divided into ten age groups to analysis the effect of age on ASA occurrence. To assess the effect of sexual activity on the presence of antibodies, 237 breeding bulls were selected from the set, and 94 of them provided ejaculate twice a week (active semen donors). The remaining set of 143 bulls were candidate breeders that have not so far been used for a regular semen collection. Blood samples were collected by jugular venipuncture, and sera were stored at –18 °C.

Detection of ASA in blood sera was carried out by an enzyme immunoanalytical method (ELISA) as described previously (Zralý et al. 1997). Bull spermatozoa were obtained by ejaculate centrifugation, washing, ultrasound treatment and diluting with a binding buffer. The antigen obtained was incubated in the wells of a microtitre plate P for ELISA tests (Gama, Czech Republic) at 37 °C for 2 h. The plates were washed with a phosphate buffer containing 0.05 % Tween 20 prior to analysis. 200 µl of a thinning solution were pipetted into the first well of row A, and 100 µl into the other wells. Then 5 µl of the examined serum were pipetted into the row A of the plate in the following sequence: into the column 1 only thinning solution for the detection of nonspecific reactions, into the column 2 negative control sera, into the column 3 positive control sera followed by unknown samples. From this basic dilution 1:40 gradual dilutions were obtained by transfer of 100 µl into another row. Incubation of the diluted samples was carried out at the laboratory temperature for 90 min. After incubation and washing the plate was reincubated with 100 µl/well of swine immunoglobulins against bovine immunoglobulines labelled with peroxidase (SwAB x PX, dilution 1:1200, Sevak, Czech Republic) at the laboratory temperature for 60 min. Into the washed well, 100 µl /well of the substrate – 38 mg of 5-aminosalicylic acid (Fluka)/10 ml of distilled water with 1 ml 0.07 % hydrogen peroxide were pipetted. Incubation was carried out at the laboratory temperature for 45 min. Colour intensity was measured using a photometer iEMS Reader MF (Labsystems, Finland) at wave length 492 nm, and evaluation was done by a computer program Genesis. As positive reactions were considered the samples of the examined sera in which the absorbance values were more than two-fold higher compared with the negative control sera. Dilution of blood sera 1:40 and higher was considered as a positive finding.

Spermatoanalysis of ejaculates has been performed in 66 breeding bulls. Analysis of sperms have been performed by comparison of the initial value with the values obtained after 120 min in the form of dynamic functional assays of resistance (Věžník and Švecová 1992). Fertility of bulls was assessed as a percentage of pregnant breeding cows after the first insemination.

Significance of differences among the characteristics was assessed by χ^2 test, the Student's *t*-test, and by the test of the difference of two relative values using the program STAT Plus v.1.10 (Matoušková et al. 1993).

Results

Of 612 bull sera examined, which were grouped according to the age of bulls, 217 (35.5%) were positive for ASA. Until the age of four months, no occurrence of ASA was detected. The first positive findings were recorded in 4 of 29 bulls (13.8%) in the age category 5 to 6 months. The incidence of antibodies was higher in older animals and culminated in the age category 9 to 10 months (58.1%; $p < 0.01$) and was maintained on this level during the whole period under investigation. A mild decrease in the incidence of

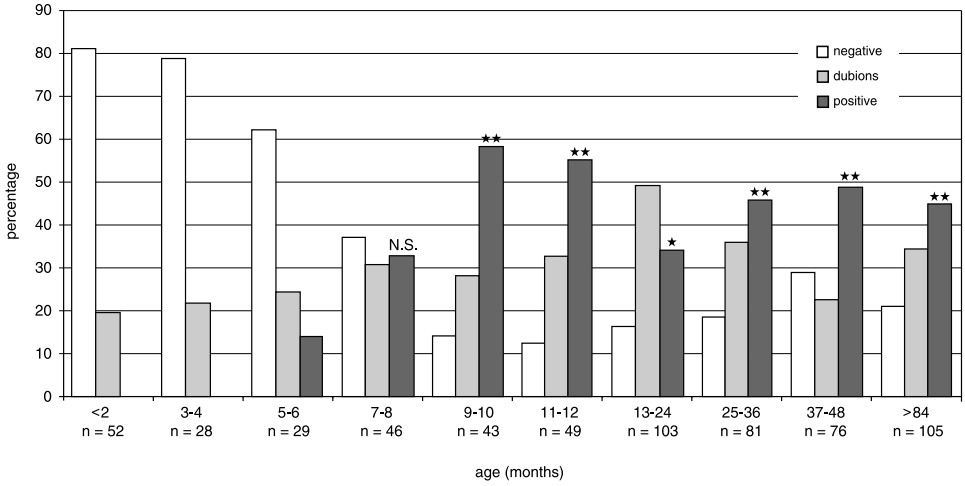

Fig 1. Occurrence of ASA in blood sera of bulls of different age Significance of differences in comprison with the group of 5-6-month-old bulls $*p < 0.05$, $**p < 0.01$

positive animals was detected at the age of 13 to 24 months only (34.0%; $p < 0.05$) as shown in Fig. 1. The frequency of titres was in the whole set as follows: 1:40 – 24.7%; 1:80 – 6.2%; 1:160 – 2.7%; 1:320 – 1.9% of positive findings.

Table 1
Occurrence of ASA in blood sera of bulls of different breeds

Breed	Number of animals	Negative		Dubious		Positive	
		No.	%	No.	%	No.	%
Czech-spotted	395	117	29.6	139	35.2	139	35.2
Black-spotted Holstein	217	32	14.7	80	36.9	105	48.4
χ^2-test		p < 0.01		N.S.		p < 0.01	

Table 2
Relationship between antisperm antibodies in blood sera of bulls and selected spermatologic parameters

Titer of ASA	No.	1	2	3	4	5	6	7	8	9	10	11	12
0 - 40	41	10.44** ± 5.30	1142.05 ± 509.85	69.88 ± 8.91	53.29 ± 16.83	71.78 ± 7.85	62.24 ± 11.12	85.29 ± 22.98	71.96* ± 28.18	187.88** ± 34.61	133.46** ± 31.80	30.02 ± 9.41	39.80 ± 10.29
80	20	6.80** ± 3.38“	966.50 ± 482.41	69.75 ± 9.39	47.50 ± 16.10	72.90 ± 9.97	62.00 ± 17.41	82.84 ± 28.49	55.78* ± 23.77	145.15** ± 30.12	90.90** ± 27.50	26.11 ± 8.11	38.13 ± 14.21

Mean ± SD
* $p < 0.05$
** $p < 0.01$

1 volume of ejaculates (ml)
2 sperm count ($\times 10^3/mm^3$)
3 motile spermatozoa (%) - initial value
4 motile spermatozoa (%) - after 120 min
5 viable spermatozoa (%) - initial value
6 viable spermatozoa (%) - after 120 min
7 sperm velocity (μm/s) - initial value
8 sperm velocity (μm/s) - after 120 min
9 index of endogenous reductases - initial value
10 index of endogenous reductases - after 120 min
11 pathological spermatozoa (%) - initial value
12 pathological spermatozoa (%) - after 120 min

Of 237 breeding bulls evaluated with regard to their sexual activity, positive titres of ASA in 94 active semen donors were determined in 53 cases (56.4%), and of 143 candidate breeders examined, 56 were positive (39.2%; $p < 0.01$).

Analysis of ASA incidence based on bull's breed (Table 1) showed significantly higher proportion ($p < 0.01$) of positive findings in the Black Pied cattle compared with Red Pied cattle (48.4% vs. 35. 2%). The largest differences between both groups were apparent at the age of 9 to 12 months, and 36 to 48 months.

In bulls with ASA titre 1:80 and more a significantly ($p < 0.01$) decreased volume of ejaculate was determined as well as a decreased index of endogenous reductases in the initial values and after 120 min of the heat test ($p < 0.01$), and decreased sperms velocity after 120 min of the heat test ($p < 0.05$), as shown in Table 2. The average fertility of bulls with antibody titres 1:80 and more was 48.1%, and in those with lower ASA titres was 53.1%. The differences were not statistically significant.

Discussion

The first occurrence of ASA was recorded in bulls aged 5 to 6 months, which represents a prepubertal stage in the development of reproductive functions, characterized by the onset of spermatogenesis (Amann and Walker 1983). These findings are in accordance with the results in boars published by Fayemi et al. (1992). The prepubertal stage is a period in which several changes occur in the developing reproductive system. It includes the testosterone induced differentiation of supportive cells to Sertoli cells and a simultaneous differentiation of gonocytes to prespermatogonia and spermatogonia A, so that cells with different antigenic characteristics are formed. Another important increase of antibodies up the age of one year is connected with an intensive cell differentiation of the developed testis in puberty, a fourty times increase of Sertoli cells and onset of spermiohistogenesis (Curtis and Amann 1981). A relatively lower incidence of ASA was recorded in the period between 13 and 24 months of age when all developmental morphological and functional processes are already terminated.

The increasing incidence of ASA in prepuberty and puberty can be connected with not yet fully functional hemotesticular barrier created by firm connections in Sertoli cells (Johnson 1973) which divide the seminiferous tubules into basal and adluminal parts thus preventing the contact of sperms with the cells of the immune system. In older bulls (more than 25 months), no significant changes in the incidence of antibodies were observed unlike of Fayemi et al. (1992) who recorded a continuous increase of antibodies in boars. Similarly, Flickinger et al. (1997) observed in rats ASA formation since the day 56, and a significant increase between 91 and 128 days of age.

Comparison of ASA incidence in mature breeding bulls with regard to their sexual activity revealed significantly higher incidence ($p < 0.01$) in active semen donors than in the candidate breeders. At this point clarifying of ASA formation appears to be necessary. Wicher et al. (1987) observed increased incidence of antibodies in rabbits due to excessive and long-term exploitation with a relatively higher possibility of protein absorption from ejaculate in the epididymis.

Higher incidence of antisperm antibodies in the Black Pied Holstein cattle is in accordance with results of studies on the autoimmune character of aspermia formation in domestic animals. Losos et al. (1968) induced in bulls degeneration of the germinal epithelium by immunization with homologous spermatozoa and testicular homogenates, and found out a different susceptibility of individual breeds.

Antisperm immunity and its relation to infertility is one of the so far unresolved questions in the field of reproduction. The relation of ASA to the fertilizing function of spermatozoa has been recently studied in experiments carried out in both human and veterinary medicine.

The results obtained had already been implemented in diagnostics and treatment of human infertility (Ulčová-Gallová and Mardešic 1996; Lombardo et al. 2001 and others). Binding of ASA to surface antigens of sperms can result in sperm agglutination, inhibition of metabolic processes (lower activity of endogenous reductases), reduction of motility and decrease of sperm velocity. There is also a negative effect on the course of capacitation, acrosome reaction (lower penetration ability) and fertility (Kim et al. 1999). Our results demonstrate a possible effect of several factors on immune functions of testes, ejaculate quality and fertility of breeder bulls. The key role is apparently played by functional stability of the hemotesticular barrier, represented by connections of Sertoli cells projections, as well as the recently demonstrated secretion of immunosuppressive agents (De Cesaris et al. 1992) that specifically inhibit the proliferation of B and T lymphocytes with the ensuing drastic decrease in the secretion of interleukin-2. The above mentioned facts show an evident prognostic importance of ASA for the assessment of breeders. As concerns the clinical importance of ASA in sera, it would be desirable to compare these data with those on the incidence of ASA bound directly to sperm surface.

Výskyt protilátek proti spermiím v krevním séru býků a jejich vztah k věku, plemeni a kvalitě ejakulátu

Na přítomnost protilátek proti spermiím enzymoimunoanalytickou metodou bylo vyšetřeno 612 krevních sér býků různého stáří a plemene, pocházejících z inseminačních stanic a odchovných zařízení býků. Při ředění séra 1:40 a vyšším bylo zjištěno 35.5 % pozitivních zvířat. Prvý výskyt protilátek byl zaznamenán ve stáří 5 až 6 měsíců (13.8 %) s významně vyšším vzestupem (58.1 %; $p < 0.01$) ve věkové kategorii 9 až 10 měsíců. U skupiny býků ve věku 5 měsíců a starších byly pozitivní titry protilátek zjištěny u 42.7 % jedinců. U aktivních donorů semene byl nález protilátek proti spermiím významně vyšší než u býků nevyužívaných k pravidelnému odběru semene (56.4 vs. 39.2 %; $p < 0.01$). Vyšší frekvence protilátek byla zjištěna u býků černostrakatého holštýnského plemene a jejich kříženců ve srovnání s českými červenostrakatými býky a jejich kříženci (48.4 vs. 35.2 %; $p < 0.01$). U pozitivních býků s pravidelným odběrem ejakulátu byl stanoven nižší objem ejakulátu ($p < 0.01$), snížená aktivita endogenních reduktáz spermií ($p < 0.01$) a snížená rychlost pohybu spermií za 120 minut termálního testu ($p < 0.05$). Fertilita u těchto býků byla nesignifikantně nižší. Je diskutován prognostický a klinický význam protilátek proti spermiím pro hodnocení kvality plemenných zvířat.

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References

AMANN, RP, WALKER, OJ 1983: Changes in the pituitary-gonadal axis associated with puberty in Holstein bulls. J Anim Sci **57**: 433-442

AWADI, HH, EL-GHANNAN, FH, EL-SAWAT, SS, EIDROOS, A 1984: Immunological studies on the cervical mucus of the normal and repeat breeder cows and buffaloes and its relation to the problem of infertility. In: Proc Int Congr Anim Reprod Artif Insem, Urbana-Champaign, IL, Vol.III: 443-445

CURTIS, SK, AMANN, RP 1981: Testicular development and establishment of spermatogenesis in Holstein bulls. J Anim Sci **53**: 1645-1657

De ALMEIDA, M, SOUMAH, A, JOHANNET, P 1986: Incidence of sperm associated immunoglobulins in infertile men with suspected autoimmunity to sperm. Int J Androl **9**: 321-330

De CESARIS, P, FILIPPINI, A, CERVELLI, C, RICCIOLI, A, MUCI, S, STARACE, G, STEFANINI, M, ZIPARO, E 1992: Immunosuppressive molecules produced by Sertoli cells cultured in vitro: biological effects on lymphocytes. Biochem Bioph Res Comm **186**: 1639-1646

FAYEMI, OE, MORRISON, RB, JOO, HS 1992: Seroprevalence of sperm antibody in selected Minnesota swine breeding herds. Anim Reprod Sci **27**: 341-345

FLICKINGER, CJ, HOWARDS, SS, BARAN, ML, PESSOA, N, HERR, JC 1997: Appearance of "natural" antisperm autoantibodies ofter sexual maturation of normal Lewis rats. J Reprod Immunol **33**: 127-145

GANDINI, L, LENZI, A, CULASSO, F, LOMBARDO, F, PAOLI, D, DONDERO, F 1995: Study of antisperm antibodies bound to the sperm cell surface and their relationship to circulating ASA. Am J Reprod Immunol **34**: 375-380

JOHNSON, MH 1973: Physiological mechanism for the immunological isolation of spermatozoa. Adv Reprod Physiol **6**: 279-324

JESSOP, TS, LADDS, PW 1995: The immunopathology of unilateral vasectomy in the ram. Vet Immunol Immunopathol **47**: 123-133

KIM, CA, PARISH, JJ, MOMONT, HW, LUN, DP 1999: Effect of experimentally generated bull antisperm antibodies on *in vitro* fertilization. Biol Reprod **60**: 1285-1291

LOMBARDO, F, GANDINI, L, DONDERO, F, LENZI, A 2001: Immunology and immunopathology of the male genital tract – antisperm immunity in natural and assisted reproduction. Hum Reprod Update **7**: 450-456

LOSOS, GJ, WINTER, AJ, McENTEE, K 1968: Induction of testicular degeneration in bulls by isoimmunization. Am J Vet Res **29**: 2295-2306

MATOUŠKOVÁ, O, CHALUPA, J, CÍGLER, M, HRUŠKA, K 1993: Statistický a grafický systém STAT Plus, verze 1.10. Výzkumný ústav veterinárního lékařství, Brno

MENGE, AC, STONE, WH, TYLER, WJ, CASIDA, LE 1962: Immunologic studies on fertility. IV. Fertility of cattle and rabbits inseminated with semen treated with antibodies produced against semen, spermatozoa and erythrocytes. J Reprod Fertil **3**: 331-341

NOUZA, K, KINSKÝ, R, DIMITROV, D 1992: Immunology and immunopathology of reproduction. Folia Biol (Praha) **38**: 170-194

PINART, E, SANCHO, S, BRIZ, MD, BONET, S, GARCIA, N 1999: Characterization of the semen quality of postpubertal boars with spontaneous unilateral abdominal cryptorchidism on the right side. Anim Reprod Sci **55**: 269-278

TASDEMIR, I, TASDEMIR, M, FUKUDA, J, KODAMA, H, MATSUI, T, TONAKA, T 1995: Effect on sperm-immunomobilizing antibodies on the spontaneous and calcium-ionophore(A23187)-induced acrosome reaction. Int J Fertil Menopausal Stud **40**: 192-195

ULČOVÁ-GALLOVÁ, Z, MARDEŠIC, T 1996: Does *in vitro* fertilization (IVF) influence the levels of sperm and zona pellucida (ZP) antibodies in infertile women? Am J Reprod Immunol **36**: 216-219

VĚŽNÍK, Z, ŠVECOVÁ, D 1992: Soubor metod k hodnocení pohlavních funkcí býků. Ústav veterinární osvěty, Pardubice, 184 p.

WICHER, V, WICHER, K,GRUHN, RF 1987: Increased production of antibodies to spermatozoa and seminal fluid in rabbits used as semen donors. Int Arch Allergy Appl Immunol **83**: 104-108

ZHANG, Z, RICKETTS, SW, TANNER, SJ 1990: Antisperm antibodies in the semen of a stallion following testicular trauma. Equine Vet J **22**: 138-141

ZRALÝ, Z, BENDOVÁ, J, ŠIŠÁK, M, DIBLÍKOVÁ, I, ŠVECOVÁ, D, ZAJÍCOVÁ, A, VĚŽNÍK, Z 1997: Occurrence of antibodies to the sperms in blood sera of bulls and boars. Vet Med - Czech **43**: 137-144