ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LI, 6 SECTIO DD 1996

DEPARTMENT AND CLINIC OF REPRODUCTION,
FACULTY OF VETERINARY MEDICINE, AGRICULTURAL ACADEMY IN LUBLIN

Zygmunt WRONA, Jan KRZYŻANOWSKI

An Attempt at the Establishment of Breed Specificity of Sperm Antigenes of Bulls in Poland*

Próba ustalenia swoistości rasowej antygenów nasienia buhajów użytkowanych w Polsce

INTRODUCTION

The identification of fertility disturbance of the immunological base does not present diagnostic problems any more. The introduction of modern methods based on immunoenzymatic techniques (7, 15, 29) or immunomagnetic antibody separation (34) should enable making fast and reliable diagnosis in the near future. As a consequence of a proper diagnosis establishment there are some activities undertaken to eliminate a disease cause. A natural way of a spermagglutinin titre increase in female organism is to hinter the antigenes further access to the immunological system. Practically, it is realized by giving up any further natural mating or insemination.

The other methods that facilitate fertilization and fertilized egg cell implantation, despite a high spermagglutinin titre, are: a) addition of immunosuppressive activity compounds to the sperm (28, 33), b) insemination of washing sperm (1, 6, 10), c) intrauterine insemination (1, 6, 10), d) change of a getter (2, 5, 30, 31, 39). This last method does not induce any problem of the law, ethical or moral nature as it does in the case of man and it has been applied for a long time despite a lack of any theoretical grounds. However, its use depends on the determination of a degree to which female organism spermagglutinins formed formerly will react with the antigenes contained in another male's sperm. Therefore, a question is to establish a specificity degree of produced antibodies directed against antigenically operated components of sperm of the same male or males of other breeds.

The objective of the present researches was to determine if the antibodies directed against antigenically operated sperm components are of specific breed or are also directed against the antigenes of sperm of bulls of other breeds.

MATERIAL AND METHODS

The immune sera directed against the sperm of the bulls of 4 breeds (holstein-fresian /hf/, black-white Polish /cb/, red-white Polish /czb/, red Polish /cp/) were obtained by immunizing the rabbits with sperm of three bulls of each breed according to the Hunter and Hafs method (13).

* The paper accomplished within the CPBP 05.06. programme.

Such a selection of bulls breed was induced by prevailing occurrence of females of these breeds in the Polish raising and breeding. As the antigenes there was also used the sperm of bulls of simental and limousin breeds whose sperm is used for commercial crossing with males of above-mentioned breeds. The sperm for immunization was collected in an arificial vagina. Having made an introductory estimation and concentration calculation, the gathered ejaculate served some other purpose provided the spermatozoa mass movement was + + or + + +, individual movement – 80% and spermatoza concentration $\geqslant 0.8 \times 10^9$ spermatozoa/cm^3 of the ejaculate.

THE SPERM ANTIGENES PREPARATION FOR RABBITS IMMUNIZATION

The full sperm collected from three bulls of the same breed was merged together and subjected to homogenization by means of an ultrasonic disintegrator till the complete disintegration of sperm cells was obtained (with the application of microscopic control of homogenization efficacy). Afterwards total protein level of homogenized sperm was determined after the biuret method and then a dilution was made, its value being 250-300 g protein in 0.3 ml. The mixture prepared in this way was preserved with mertiolate and frozen.

THE IMMUNIZATION OF RABBITS

For immunization there were selected rabbits of 2.5-3.0 kg of body weight fed with full-portion LSK and 24 hours access to water. The rabbits were immunized three times with fortnight intervals by a mixture of 0.3 ml homogenized sperm and 0.5 ml of the Freud's complete adjuvant administered intracutaneously in 3-4 spots on the back after hair shaving. After a lapse of two weeks' time since the last immunization, the rabbits were exsanguinated and the obtained serum was poured into 1 ml tubes and stored in the temperature –20°C. The serum meant for the immunodiffusive and immunoelectrophoretic examinations was deprived of complement by the incubation on the water bath in the temperature 56°C for 30 minutes prior to freezing.

In order to establish a breed specificity degree of the obtained immune sera there was applied an immunodiffusion method according to M a n c i n i (17), the method of classical immunophoresis (27) as well as the microagglutination test (8). The frozen sperm was used as antigens to the microagglutination test and homogenized sperm for immunodiffusive and immunoelectrophoretic examinations. The homogenized sperm was prepared analogically as for rabbits immunization.

THE IMMUNODIFFUSION METHOD AFTER MANCINI

To make up 1.5% gel with additive of 25% of undiluted immune serum, agar of Difko firm was employed. The homogenized full sperm was used as antigene. The plates were placed in the humid chamber for 48 hours. Afterwards the plates were taken out and the readings were performed.

THE METHOD OF CLASSICAL IMMUNOELECTROPHORESIS

The examinations after the classical immunophoresis method (27) were performed employing 1% agar gel (Fluk's agarose AG). On the denoted and warmed up to 55°C defatted standard glasses, 1% agarose solution was poured out in the amount so that a gel layer was 2 mm. After the agarose had clotted, a punch for gel curving out was used to obtain two round vessels for the antigenes and a furrow between them to deposit immune serum containing the antibodies. The examined antigenes of 15 µl capacity (about 10 µl of protein) were laid into the vessels and subjected to electrophoresis in a standard apparatus for electrophoresis of 0E-210 type, at 120 V voltage for two hours. In the electrophoresis as well as in the preparation of 1% agarose gel there was employed a medinale--veronal buffer of 8.6 pH and the ionic strength – 0.05. Immediately after the electrophoresis was

completed, the amount of about 200 µl of rabbit immune serum directed against the sperm of studied bull breeds was deposited into the formerly prepared furrow.

THE MICROAGGLUTINATION TEST

In order to establish the breed specificity of the obtained mmune sera, the microagglutination test was performed, which is widely used by many authors (2, 3, 5, 8, 12, 14, 18, 32). A value of an agglutination titre was fixed with regard to antigenes contained in the frozen sperm. The rabbit immune serum directed against the sperm of bulls of 4 breeds was initially diluted with PBS in the ratio 1:5. The final dilution was equal 1:2,560. The thawed sperm was laid upon a serologic plate with the immune serum and then the plate was vibrated in order to mix up the serum and sperm thoroughly. Next the plate was set in a thermistor at 30°C for 15 minutes. After the serologic plates had been removed from the thermistor they were examined under the microscope zoom 125 to determine the agglutination. An agglutination degree was estimated according to the Smith's method (8).

1st degree (+) – single clusters of sperm cells (2 or 3 conglutinated sperm cells) in the field of vision,

2nd degree (++) – numerous clusters of conglutinated sperm cells, a slight number of sperm cells moving around freely,

3rd degree (+++) – great clusters of conglutinated sperm cells, no sperm cells moving around freely.

THE RESULTS

The results of the microagglutination test of the sperm cells and blood serum were presented in Tables 1-4. Table 1 gives the results of a reaction of immune serum directed against the full sperm of cb breed bull and the sperm of all the breeds of bulls used in the experiment. Quite obvious is the reaction "+++" of the serum with the sperm of a bull of cb breed. The weakest agglutination response was recorded for the sperm of bulls of hf and limousin breed. Alike, a low agglutination titre (1:160) was noted for the sperm of red Polish bull breed. It is worth-while noting that in both, immune serum and the sera directed against the sperm of bulls of other breeds, there was observed a partial paralysis of spermatozoa tails manifested by their distinct archwise bend causing a circular movement of sperm cells.

Table 2 shows the test findings of the sperm microagglutination with immune serum directed against the sperm of bulls of hf breed. From them it explicity follows that the sperm cells of cb breed bulls were agglutinated most poorly by the immune serum directed against the antigenes contained in the sperm of bulls of hf breed. The low agglutination titres also appeared with the sperm of cp breed bull. Table 3 presents the results of bull sperm agglutination with the immune serum directed against the full sperm of red-white Polish breed bulls (czb). In the case of this immune serum there occurred agglutination of the sperm cells in all the breeds of bulls, and its intensity was the highest in relation to cp breed bull sperm, obviously apart from the highest titre with regard to the bulls' sperm of the same breed.

Tab. 1. The results of the test on spermatozoa agglutination of bulls of 6 breeds with rabbit immune serum directed against the sperm of cb breed bulls

Antigene kind	Degree of serum dilution									
	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
Sperm of hf breed bull	+	+	+	+	+	–	–	–	–	–
Sperm of cb breed bull	+++	+++	+++	+++	++	++	++	++	+	+
Sperm of cp breed bull	+	+	+	+	+	+	–	–	–	–
Sperm of czb breed bull	++	+	+	+	+	+	+	+	–	–
Sperm of simental breed bull	+	+	+	+	+	+	–	–	–	–
Sperm of limousin breed bull	+	+	+	+	–	–	–	–	–	–

+++ 3rd degree of agglutination; ++ 2nd degree of agglutination; + 1st degree of agglutination; – no agglutination (Table 1–4).

Tab. 2. The results of the test on spermatozoa agglutination of bulls of 6 breeds with rabbit immune serum directed against the sperm of hf breed bulls

Antigene kind	Degree of serum dilution									
	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
Sperm of hf breed bull	+++	+++	+++	+++	++	++	++	++	++	+
Sperm of cb breed bull	+	+	+	–	–	–	–	–	–	–
Sperm of cp breed bull	+	+	+	+	–	–	–	–	–	–
Sperm of czb breed bull	+	+	+	+	+	+	–	–	–	–
Sperm of simental breed bull	+	+	+	+	+	–	–	–	–	–
Sperm of limousin breed bull	+	+	+	+	+	–	–	–	–	–

Tab. 3. The results of the test on spermatozoa agglutination of bulls of 6 breeds with rabbit immune serum directed against the sperm of czb breed bulls

Antigene kind	Degree of serum dilution									
	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
Sperm of hf breed bull	++	++	+	+	+	+	+	+	–	–
Sperm of cb breed bull	++	++	++	++	+	+	+	+	–	–
Sperm of cp breed bull	++	++	++	++	++	++	+	+	+	–
Sperm of czb breed bull	+++	+++	+++	+++	++	++	++	++	++	+
Sperm of simental breed bull	+	+	+	+	+	+	+	+	–	–
Sperm of limousin breed bull	+	+	+	+	+	+	+	+	–	–

Tab. 4. The results of the test on spermatozoa agglutination of bulls of 6 breeds with rabbit immune serum directed against the sperm of cp breed bulls

Antigene kind	Degree of serum dilution									
	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
Sperm of hf breed bull	+	+	+	+	+	+	+	+	+	–
Sperm of cb breed bull	+	+	+	+	+	+	+	+	–	–
Sperm of cp breed bull	+++	+++	+++	+++	+++	++	+	+	+	+
Sperm of czb breed bull	+	+	+	+	+	+	+	+	+	–
Sperm of simental breed bull	+	+	+	+	–	–	–	–	–	–
Sperm of limousin breed bull	+	+	+	+	–	–	–	–	–	–

Tab. 5. The results of the immunodiffusive examinations of rabbit immune serum directed against the sperm of bulls of 4 breeds

Antigene kind	Rabbit immune serum directed against the sperm bull breed			
	hf	cb	cp	czb
Sperm of hf breed bull	a	c	b	c
Sperm of simental breed bull	–	c	c	c
Sperm of cb breed bull	c	a	b	b
Sperm of cp breed bull	a	b	a	b
Sperm of czb breed bull	c	c	b	a
Sperm of limousin breed bull	c	b	c	c

a – above 4 precipitative rings; b – 3–4 precipitative rings; c – up to 2 precipitative rings; – no precipitative rings (Table 5–6).

Tab. 6. The results of the immunoelectrophoretic examinations of rabbit immune serum directed against the sperm of bulls of 4 breeds

Antigene kind	Rabbit immune serum directed against the sperm bull breed			
	hf	cb	cp	czb
Sperm of hf breed bull	a	c	b	c
Sperm of simental breed bull	–	c	c	c
Sperm of cb breed bull	c	a	b	b
Sperm of cp breed bull	a	b	a	b
Sperm of czb breed bull	c	b	b	a
Sperm of limousin breed bull	c	c	–	c

Table 4 gives the results of the agglutination of the sperm cells of the examined bull breeds with the immune serum directed against the sperm of red Polish breed bulls (cp). There was found an occurrence of a spermagglutinins titre up to the values 1:1,280 for the sperm of cb, hf and czb breed bulls. The lowest titre was stated for the sperm of simental and limousin breed bulls (1:40).

The immunodiffusive examinations according to the Mancini's method were presented in Table 5. The data enclosed proved that the poorest antigenic consanguinity occurred between the immune serum directed against cb breed bulls' sperm and the antigenes contained in the homogenized sperm of hf, simental and czb breed bulls. The immune serum directed against hf breed bulls' sperm reacted the strongest with an antigene contained in the sperm of cp breed bull (3-4 precipitative rings). However, no precipitative rings with the antigenes of simental breed bulls' sperm were observed.

The results of the immunoelectrophoresis examinations were demonstrated in Table 6. They proved explicitly that immune serum directed against cb breed bulls' sperm has got the least number of antibodies directed against the antigenes of hf, simental and limousn breed bulls' sperm, while the greatest number against the antigenes was contained in the sperm of cp, nd czb breeds bulls.

DISCUSSION

The problem of sperm antigenic specificity of a particular breed of bulls has had an interest for researchers for a long time (9, 13, 16, 19, 21, 22, 35). It is very difficult to state these relations owing to a great number of breeds occurring within one country region (local breeds) or intentional and casual interbred crossings found there. A nearly unlimited possibility of sperm purchase and its dispatch to any place in the world are propitious to interbred crossings. The breed mixing occurs mainly in the countries of relatively poor reproduction control and without any specified breeding program. A selection of bull's sperm enabling insemination of cows affected by immunologic infertility should be performed each time basing on the examinations' results that allow to establish the height of spermagglutinins' titre in the blood serum for the sperm of these breeds which should be used for insemination with the view to the breeding reasons.

From the many years' investigations it turns out that there is no total specificity of antibodies to the antigenes, occurring in the sperm owing to the common for all the breeds antigenic components such as the antigenes of a blood group, (20, 26), which are also present in the sperm. Under the conditions of natural immunization that occurs in the case of manifold or ineffective mating or insemination, an immunogenic response of particular sperm components that operate antigenically is hardly predictable. The activity of inhibitors of

immunological response (4, 19, 23) in the sperm ought to be taken into account as well as the presence in the female's genital tracts substances "hiding" heterogeneity of components introduced with the sperm (23).

On the grounds of available research works (5, 11, 14, 16, 24, 25) as well as the present author's investigations (36, 37, 38) it can be assumed that a significant agent causing occurrence of a high antispermatozoal antibodies titre is manifold appearance of ineffective insemination in regular intervals of time, in accordance with the oestrous cycle (at least 21-23 days).

In the carried out experiments there were found antigenic components mutual for the bulls of different breeds while on comparing the breeds it appeared that their number was different. The weakest antigenic affinity for the antibodies directed against the sperm of bulls of cb breed was stated for the sperm of bulls of hf and simental breeds, then the sperm of limousin and cp breeds successively. It should be stated that the bulls selected for this experiment were thoroughbred in 100%. Nowadays, in the situation when very many geffer bulls of cb breed have an "admixture" of blood of hf breed bulls, one should take into account the appearance of a higher titre of spermagglutinins in the blood serum of cows with fertility disturbances of the immunological base in relation to the sperm of bulls in 100% of sb breed, although these cows were inseminated with the sperm of cb breed bulls only. A weak antigenic affinity of the sperm of simental breed bulls which was recorded in our experiments is worth noting as it gives a chance to fertilize cows with a high titre of spermagglutinins directed against both the antigenes of the sperm of bulls of cb and hf breeds. However, it is mating that produces off-spring meant only for fattening. Still, it makes possible to regenerate the lactation and provides the chance to decrease the spermagglutinin titre down to a level that enables insemination with the sperm of a bull against whose sperm there was a high titre.

REFERENCES

1. Argarwal A.: Treatment of immunological infertility by sperm washing and intrauterine insemination. Arch. Andrology **29**, 207, 1992.
2. Bhatt G., Vyas K. K., Purohit S. K., Jatker P. R.: Studies on immunoinfertility in repeat breeder cows. Indian Vet. J. **56**, 184, 1979.
3. Boettcher B.: The molecular nature of spermagglutinins and sperm antibodies in human sera. J. Reprod. Fert. Suppl. **21**, 151, 1974.
4. Bouvet J. P., Condere J., Pillot J.: *In vivo* and *in vitro* immunosuppression in mice by a 100-110 Kd fraction from boar seminal plasma. Am. J. Reprod. Immunol. Microb. **14**, 135, 1987.
5. Bratanov K., Dikov V., Rabiev G., Danov D.: Studies on the immunology of reproduction. VII. The role of spermantibodies in the insemination of cows with incomplete uterine involution and cows with latent endometritis. Vet. Sci., Sofia, **2**, 323, 1965.

6. Byrd W., Ackerman C., Carr B. R., Edman C. D.: Treatment of refractory infertility by transcervical intrauterine insemination of washed spermatozoa. Fert. Steril. **48**, 921, 1987.
7. Czuppon A. B.: Clinical evaluation of the new aperm antibody slide-test for detection of antibodies to spermatozoa in cervical mucus and seminal plasma. J. Clin. Chem. Clin. Biochem. **26**, 35, 1988.
8. Deo S., Roy D. J.: Investigations on repeat breeding cows and buffaloes. Immunological agglutination of spermatozoa in the cervical mucus. Indian Vet. J. **48**, 572, 1971.
9. Docton F. L., Ferguson L. C., Lazear E. J., Fordyce E.: The antigenicity of bovine spermatozoa. J. Dairy Sci. **35**, 706, 1952.
10. Galle P. C., McRae M. A., Colliver J. A., Alexander J. S.: Sperm washing and intrauterine insemination for cervical factor, oligospermia, immunologic infertility and unexplained infertility. J. Reprod. Med. **35**, 116, 1990.
11. Griffin J. F. T., Nunn W. R., Hartigan P. J.: An immune response to egg-yolk semen diluent in dairy cows. J. Reprod. Fert. **25**, 193, 1971.
12. Harsh M., Satyavir Y., Uma S., Arun K., Praveen M.: Circulating iso- and auto-antibodies to human spermatozoa in infertility. Indian J. Pathol. Microb. **33**, 161, 1990.
13. Hunter A. G., Hafs H. D.: Antigenicity and cross-reactions of bovine spermatozoa. J. Reprod. Fert. **7**, 357, 1964.
14. Konchev L., Pavlova S., Danev A.: Assessment of circulating agglutinating antisperm antibodies in buffalo cows with unexplained infertility and an attempt to identify buffalo sperm isoantigens. Am. J. Reprod. Immunol. **29**, 62, 1993.
15. Lander M. F., Hansen P. I., Drost M.: Antisperm antibodies in cows after subcutaneous and intrauterine immunization. Vet. Rec. **5**, 461, 1990.
16. Malinowski I. F.: Oplodotvorijaemost korov v zavisimosti od titra spermoantitiel v syvorotkie krovi. Zivotnovoctvo **2**, 77, 1976.
17. Mancini G., Carbonara A. O, Meremans J. F.: Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry **2**, 235, 1968.
18. Max A.: Analiza przyczyn nieskutecznego unasieniania krów na podstawie badań klinicznych, hormonalnych i immunologicznych. Medycyna Wet. **46**, 352, 1990.
19. Max A.: Immunologiczne aspekty niepłodności. Prz. Hod. **1**, 29, 1991.
20. Menge A. C., Stone W. H., Tyler W. J., Casida L. E.: Immunological studies on fertility and sterility. IV. Fertility of cattle and rabbits inseminated with semen treated with antibodies produced against semen, spermatozoa and erytrocytes. J. Reprod. Fert. **3**, 331, 1962.
21. Menge A. C.: Induced infertility in cattle by isoimmunization with semen and testis. J. Reprod. Fert. **13**, 445, 1967.
22. Mundo de Cote J. C., Velazquez E. A., Garza R. J., Valencja M. J.: Aspectos immunologicos dela infertilidad eu bovinos y su repercusion eu la reproduccion. Veterinaria. Mexico **11**, 63, 1980.
23. Naz R. K., Menge A. C.: Antisperm antobodies: origin, regulation and sperm reactivity in human infertility. Fert. Steril. **61**, 1001, 1994.
24. Neuman P., Göbel H., Gottschall A., Meyer U., Tiemann U.: Zur Bedeutung immunologischer Regelmechanismen in der Fortpflanzung. Mh. Vet.-Med. **44**, 335, 1989.
25. Panchal M. T., Dholakia P. M., Derashri H. J., Kodagali S. B.: Investigation on immunoinfertility in repeat-breeding buffaloes. Indian J. Anim. Sci. **60**, 1211, 1990.
26. Parish W. E., Carron-Brown J. A., Richards C. B.: The detection of antibodies to spermatozoa and the blood group antigens in cervical mucus. J. Reprod. Fert. **13**, 469, 1967.
27. Rao S. S., Sadri K. K.: The antigenic composition of buffalo semen. J. Comp. Path. **70**, 1, 1960.

28. Scarselli G., Livi C., Emmi L., Chelc E., Noci I., Pellegrini S.: Analysis of the action of corticosteroid treatment in immunologic infertility: a preliminary report and an alternative hypothesis. Am. J. Reprod. Immunol. Microbiol. **15**, 57, 1987.
29. Shai D., Yoseph D., Yser Y., Naot Y.: A reverse enzyme-linked immunosorbent assy for detection of antisperm antibodies in sera and genital tract secretions. Fert. Steril. **54**, 894, 1990.
30. Sirazdinov R. R., Kuznecov A. K.: Level of natural sperm antibodies in the blood in relation to the effectiveness of insemination in cows. Sbornik Naucz. Trudov. Leningrad. Vet. Institut. **71**, 112, 1982.
31. Strzeżek J., Bućko T., Jastak Z.: Wstępne obserwacje miana spermaglutynin w surowicy krwi krów i jałówek rasy ncb. Medycyna Wet. **29**, 236, 1973.
32. Tornov A.: On the natural isospermoagglutinins in bovine blood serum. Veterinary Sci. (Sophia) **4**, 83, 1967.
33. Ulćová-Gallová Z., Mráz Z., Planićková E., Mackú F., Ulć I.: Local hydrocortisone treatment of sperm – agglutinating antibodies in infertile women. Int. J. Fertil. **33**, 421, 1988.
34. Viqano P., Fusi F. M., Brigante C., Busacca M., Viquali M.: Immunomagnetic separation of antibody-labelled from antibody-free human spermatozoa as a treatment for immunological infertility. A preliminary report. Andrologia **23**, 367, 1991.
35. Wrona Z.: Immunologiczne uwarunkowania zaburzeń płodności. Annales UMCS, Sect. DD **49**, 27, 1994.
36. Wrona Z.: Badania nad niepłodnością immunologiczną u krów. I. Dynamika narastania przeciwciał przeciwplemnikowych po doszyjkowej immunizacji nasieniem buhaja. Annales UMCS, Sect. DD, **50**, 21, 1995.
37. Wrona Z.: Badania nad niepłodnością immunologiczną u krów. II. Czas utrzymywania się przeciwciał przeciwplemnikowych w surowicy krwi i śluzie szyjkowym po doświadczalnej immunizacji nasieniem buhaja. Annales UMCS, Sect. DD, **50**, 45, 1995.
38. Wrona Z.: Badania nad niepłodnością immunologiczną u krów. III. Obserwacje kliniczne. Annales UMCS, Sect. DD, **50**, 61, 1995.
39. Zafracas A. M., Samouilidis S. C., Cricorion A. B., Köhler-Samouilidis G.: Ein einfacher Test zum Nachweis von Spermantikörpern in Genitalschleim. Tierärztl. Umsch. **42**, 973, 1987.

STRESZCZENIE

Celem przeprowadzonych badań była próba ustalenia, czy przeciwciała skierowane przeciwko antygenowo oddziałującym składnikom nasienia są swoiste dla rasy, czy też skierowane są również przeciwko antygenom nasienia innych ras buhajów. Surowice odpornościowe skierowane przeciwko nasieniu czterech ras buhajów (hf, cb, czb, cp) uzyskano immunizując króliki nasieniem tych ras.

W badaniach posługiwano się metodą immunodyfuzji, klasycznej immunoelektroforezy oraz testem mikroaglutynacji. Jako antygenów używano nasienia czterech ras buhajów oraz dodatkowo nasieniem buhajów rasy simental i limousin. W przeprowadzonych badaniach stwierdzono występowanie wspólnych komponent antygenowych dla różnych ras buhajów, przy czym ich ilość była różna przy porównaniu różnych ras. Najsłabsze powinowactwo antygenowe dla przeciwciał skierowanych przeciwko nasieniu buhajów rasy cb stwierdzono ze strony surowicy odpornościowej skierowanej przeciwko nasieniu buhajów rasy hf i simental. Słabe powinowactwo antygenowe w stosunku do pozostałych ras buhajów stwarza możliwość zacielania krów z wysokim mianem spermaglutynin skierowanych zarówno przeciwko antygenom nasienia buhajów rasy cb, jak i hf.