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The presence of anti-*zona pellucida* antibodies in blood serum of pregnant and infertile mares and cows

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The report deals with the frequency of appearance of anti-*zona pellicuda* antibodies (anti-ZP antibodies) in mares and cows. Blood samples were withdrawn from 176 mares and 200 cows of different breeds and ages, maintained in the Sanliurfa province, Turkey. The anti-ZP antibodies in the sera were determined with a commercial porcine ELISA test kits. Two mares (1.14%) and four cows (2.00%) appeared anti-ZP positive (anti-ZP+). Of these, three animals were found pregnant (one mare and two cows) and three were infertile.

KEY WORDS: antibodies / cow / mare / *zona pellucida*

Despite advances in understanding the physiology of reproduction, in 30 to 40% of infertile animals the cause of their infertility remains unknown. Several authors have suggested that antibodies against *zona pellucida* (anti-ZP antibodies) may play a role in the pathogenesis of the idiopathic infertility in these animals. Indeed, experimental

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immunizations of mares with anti-ZP antibodies resulted in infertility [Kirkpatrick *et al.* 1992, Asbury and Lyle 1993, Turner *et al.* 2002, Liu *et al.* 2005].

Zona pellucida (ZP) is an extracellular matrix that surrounds the oocyte, and plays the key role during fertilization. The ZP both lets the sperm in and stays intact to protect the cleaving embryo. Within the ZP three sulfated glycoproteins (ZP_1, ZP_2 and ZP_3) are found. Studies to determine the function of ZP proteins have largely concentrated on their sperm receptor activity. Since it was found that ZP had a strong organ-specific but species-cross-reactive antigenicity, then immunization of females with ZP glycoproteins proved to impair fertilization in a variety of animals. Immunization of females with ZP glycoproteins leads to block of fertility in several animal models. Studies on active immunization with anti-ZP antibodies demonstrated that in most animal species immunized, a temporary infertility could be induced along with the elevation of antibody titers, always associated with ovarian failure and destructive changes in ovarian follicles. When native ZP antigens from heterospecies were used for immunization, the immunized animals often produced self-reactive anti-ZP antibodies and this condition resulted in infertility [Skinner *et al.* 1984, Topper *et al.* 1997, Garza *et al.* 1998, Hinsch *et al.* 1998, Kolle *et al.* 1998, Briggs *et al.* 1999].

It is clear that the ZPs from a wide variety of mammals are closely (40-90%) similar. They have been isolated and characterized in a number of animals and there is considerable homology in the mammalian species so far studied. For example, the homologies between the bovine and the porcine ZP proteins reach 84.3% [Wassarman *et al.* 1999, Zhu and Naz 1999].

The reduced fertility has mainly been attributed to antibody interference with sperm-egg binding, causing anovulation and abnormal cycles. However, some ovarian dysfunctions, a decrease in ovarian weight and follicular development, and abnormal follicular differentiation have also been reported. There is also evidence that polyclonal anti-ZP antibodies may prevent the sperm from penetration of the ZP, but have no effect on sperm binding to the ZP [Mahi-Brown *et al.* 1982, 1985, 1988, Hasegawa *et al.* 1992, Barber and Fayrer-Hosken 2000].

In the present study, we have measured the rate of anti-ZP antibodies in mares and cows of different breeds, at different ages and of varying reproductive status (suboestrus, anoestrus, metritis, ovarian cysts, repeat breeding, pregnancy).

Material and methods

Used were 176 mares, 157 of which were purebred Arab and 19 Thoroughbred, at the age ranging from 2 to 29 years. The mares were from a randomly selected province of Sanliurfa located in south-eastern region of Turkey.

Out of a total of 200 cows used, 82 were Brown-Swiss, 32 Holstein, 20 Simmental and 66 crossess, ageing between 3 and 12 years, from eastern region of Turkey. Pregnancy status of mares and cows was established ultrasonographically on day 20-25 after insemination and confirmed on day 30-35 with rectal palpation. Features of the animals, such as age and number of matings (repeats) were obtained from their owners. The gynecological examinations were done with several methods (rectal palpation, vaginal examination, ultrasonography, and hormone assays) in non-pregnant animals. Metritis was determined ultrasonographically, and with histopathological examination *via* uterine biopsy. In 131 mares and 83 cows several infertility problems - suboestrus, anestrus, metritis, ovarian cysts and returns - were observed.

Blood samples (10 ml) were withdrawn and serum obtained, according to standard procedures. Sera were stored at -20^{0}C until assayed. The presence of anti-ZP antibodies in sera of animals was determined by commercial ELISA test kits (BIOSERV DIAGNOSTICA, Zona-Pellucida-Antibody-ELISA, BS-20-20, Germany).

Next, the results were compared and evaluated with the information gathered from the animal owners. Statistical analyses of the results were performed with chi-square test in SPSS [1999] packet programme according to Snedecor and Cochran [1980].

Results and discussion

Anti-ZP antibodies were identified in two mares and four cows (1.14 and 2% of animals examined, respectively). Results are presented in Table 1 and 2 across animals' breeds, age and reproductive status. None of these factors affected the frequency of appearance of anti-ZP in mares in cows significantly.

Table 1. Distribution of results within mares

Animals category	Anti-ZP+		Anti-ZP-	
	n	%	n	%
Purebred Arab (n=157)	2	1.27	155	98.73
Thoroughbred (n=19)	0	0.0	19	100
Total (n=176)	**2**	**1.14**	**174**	**98.86**
Age 2-7 years (n=48)	1	2.08	47	97.92
Age 8-15 years (n=79)	0	0.0	79	100
Age 16-29 years (n=49)	1	2.04	48	97.96
Total (n=176)	**2**	**1.14**	**174**	**98.86**
Pregnant (n=45)	1	2.22	44	97.78
Infertile (n=131)	1	0.76	130	99.24
Total (n=176)	**2**	**1.14**	**174**	**98.86**

No significant differences between categories (P>0.05).

Table 2. Distribution of results within cows

Animals category	Anti-ZP+		Anti-ZP-	
	n	%	n	%
Brown-Swiss (n=82)	1	1.22	81	98.78
Holstein (n=32)	1	3.13	31	96.87
Simmental (n=20)	0	0.0	20	100
Crosses (n=66)	2	3.03	64	96.97
Total (n=200)	**4**	**2.00**	**196**	**98.00**
Age 2-4 years (n=99)	2	2.02	92	97.98
Age 5-7 years (n=67)	2	2.99	65	97.01
Age >7 years (n=34)	0	0.0	34	100
Total (n=200)	**4**	**2.00**	**196**	**98.00**
Pregnant (n=117)	2	1.71	115	98.29
Infertile (n=83)	2	2.41	81	97.59
Total (n=200)	**4**	**2.00**	**196**	**98.00**

No significant differences between categories (P>0.05).

The ZP has strong immunogenic properties, and the raised antisera effectively block fertilization. High level of anti-ZP antibody titers often correlate well with the incidence of infertility. However, in most cases, infertility is associated with loss of ovarian function characterized by suppression of folliculogenesis. To explain ovarian dysfunction some authors indicate that the presence of oophritogenic T cell epitopes within the immunogen can give rise to an inflammatory response that is directed to the developing oocytes by the presence of autoantibody computer [Wood *et al.* 1981, Skinner et al. 1984, Bagavant *et al.* 1994, Gupta *et al*. 1997].

Also according to the pregnancy status, anti-ZP antibodies positive rate may vary. In the present study, no significant difference in per cent of anti-Z plus and anti-Z minus frequency was stated between pregnant and unfertile females of both species. But, we have determined that anti-ZP antibodies may be found in certain titers in pregnant animals too. This situation shows that to exert their effects on fertility the anti-ZP antibodies need to reach high titers in blood and local tissues. If not, the infertility will last over the whole length of the reproductive life of a female. Under such circumstances, in this report, we were not able to determine whether the infertility was reversible. However, passive immunization has demonstrated that when antibody titer declines, fertility returns.

No significant differences were identified between the groups when the results were assessed according to breed, age and reproductive status. But, this report also demonstrates that anti-ZP antibodies were present in one (2.22%) pregnant mare and in two (1.71%) pregnant cows. Thus, additional factors such as the titer or other properties of the antibodies would have to come into play if, indeed, these antibodies exert an anti-fertility effect. The exact role played by these antibodies in relation to fertility requires further study.

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Obecność przeciwciał anty-*zona pellucida* w surowicy krwi ciężarnych i jałowych klaczy i krów

S t r e s z c z e n i e

Badaniami objęto surowice krwi 176 klaczy i 200 krów różnych ras, będących w różnym wieku i stanie fizjologicznym. Posługiwano się testem ELISA. Dodatni wynik testu uzyskano w przypadku dwóch klaczy i czterech krów (odpowiednio 1,14 i 2% wszystkich samic każdego gatunku), spośród których jedna klacz i dwie krowy były w ciąży, a trzy były jałowe.