PREVALENCE OF ANTI-SPERM ANTIBODIES IN MARES IN THE SOUTH-EASTERN ANATOLIAN OF TURKEY

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Abstract

This study aimed to determine the prevalence of anti-sperm antibodies (ASAs) in mares in the south-eastern Anatolian of Turkey and the effect of age, mating number, pregnancy status and metritis on the antibody prevalence. One hundred and fifty-seven purebred Arabians and 19 Thoroughbreds were included in the study. The presence of ASAs in blood sera of the animals was determined by enzyme immunoassay. The percentage of animals with ASAs in their serum was 10.23. The prevalence of ASAs was the lowest in mares that had been mated three or more times. There were no differences between the groups when the results were assessed according to age, pregnancy and uterine mucosa status.

Key words: mare, anti-sperm antibodies.

Despite advances in understanding the physiology of reproduction, 30 to 40% of infertile animals have no identifiable cause of their infertility. Several investigators have suggested that antibodies against sperm may play a role in the pathogenesis of the idiopathic infertility in these animals. However, in recent years, studies directed to immunological reasons, especially to ASAs which developed in female body against spermatozoa, have increased. For example, in studies which are related to immunological infertility in mares, immunizations with experimental aim have been done with zona pellucida and as a result of this, infertility has developed in the animals (2, 14, 26).

Lee et al. (16) have reported that titers of ASAs increase in the serum of mares 5 week after they were immunized with sperm or seminal plasma. In studies performed by various investigators, infertility due to ASAs in stallions has been found (4, 22, 28, 30).

It has been reported that ASAs cause infertility by blocking sperm penetration to cervical mucus, causing early embryonic death, hindering sperm-ovum union, causing function disorders of spermatozoa or by leading to the death of spermatozoa (13, 18, 19). The antibodies have been found in blood serum, seminal plasma, cervical mucus and in uterine tissue (1, 5).

It is difficult to estimate the percentages of females that are infertile because of ASAs. There are several reasons which may explain ASAs development in some females. Among these reasons there are factors such as immunological state of the female, sperm concentration in the ejaculate, number of matings, as well as different stress conditions developed in female or corticosteroid use. In addition, it has been reported that spermatozoa have to come in contact with blood to develop ASAs in the animal body; therefore inflammatory states, such as metritis and vaginitis, or trauma and bleeding occurring during mating, have an important role in the development of these antibodies (1, 20, 21, 23).

The aim of this study was to determine the prevalence of ASAs in mares in the Southeastern Anatolian of Turkey and to establish the effect of some factors such as age, mating number, pregnancy, uterine mucosa status on ASA prevalence.

Material and Methods

The study material consisted of 176 mares, 157 of them were purebred Arabian, 19 Thoroughbreds, ranging in age from 2 to 29 years. The mares were randomly selected from a province of Sanliurfa located in south-eastern region of Turkey. Their pregnancy status was established through transrectal palpation at 30 to 35 d after insemination and by ultrasonography at 20 d after insemination. Metritis was determined by ultrasonography and histopathological examination via uterine biopsy. Features of the mares, such as age and number of matings were obtained from their owners. Blood samples (10 mL) were collected and serum was
obtained according to standard procedures. Sera were stored at -20°C until assayed.

The presence of sperm antibodies was determined by EIA (3, 16, 23, 24). In the enzyme immunoassay we followed the procedures described earlier with slight modifications (16). 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 1 h. Antigen was prepared by washing stallion ejaculate twice in PBS and then resuspending the sperm, at a concentration of $5 \times 10^6$ spermatozoon/mL, in PBS containing 0.25% gluteraldehyde. One hundred microlitres of this suspension was added to each of the test wells of 96-well plate coated with poly-L-lysine (100 µg/mL) in 0.01 M bicarbonate coating buffer. As a negative control, PBS containing only 0.25% gluteraldehyde was put in some wells. Blockings were done with the addition of 100 mL of PBS containing 1% bovine serum albumin (Sigma Chemical Co., St. Louis, MO.) to the wells. Serum samples were diluted 1 to 50 in PBS and were tested in duplicate. As a secondary antibody, goat anti-equine IgG conjugated with horseradish peroxidase (Sigma) was used. The chromogen substrate was o-phenylene diamine. The reactions were stopped with addition of 100 mL of 1 M H$_2$SO$_4$ to the wells and absorbance was determined at 450 nm wave length (Medispec, ESR 200 EIA Plate Reader). As a negative control, sera from 5 maiden mares were used. The mean absorbance value of negative sera plus 3 standard deviations were considered as cut off value point for a positive response.

The data obtained were compared with parameters such as age, pregnancy, mating number (times of mating this year) and metritis. Statistical analyses of the results were done with chi-square test in SPSS packet programme (27).

**Results**

In the EIA, the mean absorbance value for negative sera was detected as 0.120 OD. According to EIA results, values greater than 0.230 OD were considered indicative of a positive reaction. The percentage of animals that were found to have ASAs in their blood sera were recorded as 10.23.

When the results were assessed according to the number of matings, the percentage of ASA positive animals were found as the lowest to mares mated 3 or more times ($P<0.05$) (Table 2).

There was no difference between the groups when the results were assessed according to age, pregnancy and uterine mucosa status (Tables 1, 3, 4).

| Table 1 |
| Distribution of the results according to age |
| Age | ASA (+) | ASA (-) |
| n | % | n | % |
| 2-7 (n=48) | 5 | 10.42 | 43 | 89.58 |
| 8-15 (n=79) | 10 | 12.66 | 69 | 87.34 |
| 16-29 (n=49) | 3 | 6.12 | 46 | 93.88 |
| Total (n=176) | 18 | 10.23 | 158 | 89.77 |

The differences between the group percentages are not significant

| Table 2 |
| Distribution of the results according to mating number |
| Mating number | ASA (+) | ASA (-) |
| n | % | n | % |
| Once (n=34) | 5 | 14.71$^a$ | 29 | 85.29$^a$ |
| 2 times (n=84) | 8 | 9.52$^{ab}$ | 76 | 90.48$^{ab}$ |
| 3+ (n=28) | 1 | 3.57$^b$ | 27 | 96.43$^b$ |
| Total (n=146) | 14 | 9.59 | 132 | 90.41 |

$*$ $P<0.05$

$^a, b$ The difference between values shown with different letters in the same column is important
Table 3
Distribution of the results according to pregnancy status

<table>
<thead>
<tr>
<th>Pregnancy</th>
<th>ASA (+)</th>
<th></th>
<th>ASA (-)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant (n=45)</td>
<td>6</td>
<td>13.33</td>
<td>39</td>
<td>86.67</td>
</tr>
<tr>
<td>Non-pregnant (n=131)</td>
<td>12</td>
<td>9.16</td>
<td>119</td>
<td>90.84</td>
</tr>
<tr>
<td>Total (n=176)</td>
<td>18</td>
<td>10.23</td>
<td>158</td>
<td>89.77</td>
</tr>
</tbody>
</table>

The differences between the group percentages are not significant

Table 4
Distribution of the results according to uterine mucosa status

<table>
<thead>
<tr>
<th>ASA</th>
<th>Metritis (+)</th>
<th></th>
<th>Metritis (-)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (n=18)</td>
<td>3</td>
<td>16.67</td>
<td>15</td>
<td>83.33</td>
</tr>
<tr>
<td>Negative (n=158)</td>
<td>27</td>
<td>17.08</td>
<td>131</td>
<td>82.92</td>
</tr>
<tr>
<td>Total (n=176)</td>
<td>30</td>
<td>14.60</td>
<td>146</td>
<td>85.40</td>
</tr>
</tbody>
</table>

The differences between the group percentages are not significant

Discussion

It has been suggested that ASAs are the most common reasons of idiopathic infertility in females (6, 10). Several studies have been performed related to encountering and causing infertility due to ASAs in animals. Kanchev et al. (12) and Max (17) have reported the infertility rate due to ASAs in cows as 3.3% and 4.5%, respectively. In the study by Farahani et al. (8) performed on blood sera of repeat breeder, fertile cows and heifers, the rate of ASAs was found as 26, 32, and 0%, respectively. Also, in the same study, it was suggested that there is a relationship between the fertility of animals and the rate of encountering ASAs. Wang and Xie (29) found that ASAs were present in 36% of 119 infertile cows in contrast to the rate of 3.8% in pregnant cows. Seshagiri et al. (25) have found the presence of ASAs in 59.4% of repeat breeder cows and in 14.5% of regular breeders. In the present study, ASAs were found in 18 of 176 (10.23%) mares.

It has been reported that the presence of ASAs is influenced by several factors such as age, lactation number, mating number, pregnancy status, administration pattern of sperm, genital infections, and immune and hormonal status (9, 15, 21, 29). It has been reported that ASA positive rate varies according to the age and lactation number and this rate increases with the age increase. However, Day (7) has reported that there was no difference between non-mated and multiparous mares in the percentage of animals with ASAs. In the present study, there was no difference between the age groups.

It has been suggested that ASA positive rate varies according to the mating number and reproductive status. In a study performed by Risvanli et al. (24), it was reported that the rate was higher in cows mated three or more times. In the present study, this rate was found to be the lowest in mares mated three or more times and in maiden animals and the highest in non-mated mares after parturition. It seems that the development mechanism and effect pattern of ASAs in mares differ from that in other female animals. From the obtained data, it was concluded that the development of these antibodies in mares takes more time than in the other females and therefore only the antibodies resulting from matings in previous breeding season appear in the period after parturition.

Also according to the pregnancy status, ASA positive rates may vary. It has been reported that ASAs may be found in certain titers in pregnant cows too (10). In a study by Wang and Xie (8) ASA incidence was reported in 3.8% of pregnant cows. In the presented study, there was no difference according to pregnancy status. This situation reflects that ASAs are required to reach high titers in blood and local tissues in order to exert their effects on fertility.

In studies on the development mechanisms of ASAs in the female organism, it has been found that sperm are required to come into contact with blood and inflammatory processes such as metritis and trauma in the genital organs have an important role in this point (1, 11). In this view, it may be expected to encounter more antibodies in animals with metritis. However, in the present study, the results did not vary according to the presence or absence of metritis.

In conclusion, it was found that the prevalence of ASAs was the lowest in mares mated three or more times. There were no differences according to age, pregnancy or uterine mucosa status. In addition, it was concluded that it is required to perform further studies with more details related to the development of ASAs and their effect mechanism on fertility in mares.

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References


27. SPSS (Statistical Package for Social Sciences) for Windows Copyright™, SPSS, inc. 1993.

