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Reproductive Immunology in Mares

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ABSTRACT

In mare breeding, infertility arising from various causes, leads to important economical losses. In these animals, infertility can occur due to several factors such as infections, genetics, hormones and management problems and other etiologies that cannot be clearly demonstrated. With regard to this aspect, idiopathic infertility remains an important subject in mare breeding. Recently, immunological infertility - both humoral and cellular - has been frequently discussed. The causes of the infertility arising from humoral immunity, which is considered within the context of antigen-antibody reactions, include anti-sperm antibodies (ASA), anti-zona pellucida antibodies (anti-ZP antibodies) and anti-ovarian stroma antibodies. These antibodies affect the fertility in a broad spectrum, ranging from subfertility to sterility. Cellular immunity play an important role, especially in the occurrence of bacterial endometritis. On the other hand, there are many researches emphasizing the use of leukocytes in the treatment of endometritis in mares.

Key words: Reproductive, immunology, mare

INTRODUCTION

Fertility of mares is economically important. Factors causing infertility or subfertility include infectious diseases, functional abnormalities, genetic structural disorders and management problems. However, even in ideal laboratory and clinical conditions, the cause of only 60-70% of infertility and subfertility can be determined; this rate decreases to under 50% in field conditions. Therefore, identification of the causes of idiopathic infertility is an important subject. In recent times, it has been suggested that immunological factors play an important role as one of causes of idiopathic infertility (Alexander and Anderson, 1989; Risvanli and Kaygusuzoglu, 2004).

In the female body, immunological infertility can develop against the zona pellucida, spermatozoa, seminal plasma and the cauda epididiymis extract. Other than during of gamete specific structures, infertility can develop due to immunity against GnRH, hCG and eCG (Alexander and Anderson, 1989; Naz, 1999; Dalin *et al*., 2002).

There are various suggestions on the development of immune response in the animal body against the afore-mentioned structures. It has not been demonstrated clearly whether this immune response arises from an antigen-antibody reaction or a cellular reaction in which lymphocytes or leukocytes are involved.

The objective of the present compilation is to demonstrate the importance immunological factors on infertility in mares.

THE RELATION BETWEEN CELLULAR IMMUNITY AND INFERTILITY

Being one of the most important causes of infertility, the relationship between endometritis and leukocytes, is the most frequently emphasized subject in mares. Ferreira-Dias *et al*. (2005) have

suggested that in mare, phagocytosis, oxidative burst and lymphocyte types in the circulation do not show variation according to anestrus or other phases of the reproductive cycle of the mare, this being a display of the fact that the immune status is not affected by seasonal ovarian activity. Uterine factors may contribute to the injured function of uterine polymorph nuclear cells in susceptible mares. The phagocytic activity of neutrophils in the uterus has been reported to be lower in mares, which are more susceptible to uterine infections. The cause of this fact has been suggested to be the negative effects of uterus secretions on phagocytosis (Troedsson *et al*., 1993). The primary cause of uterine dysfunction has been incompetent opsonization of bacteria in uterine secretions as opposed to a primary dysfunction of neutrophils. The phagocytic activity in susceptible and resistant mares has been similar; the migration of blood and uterine neutrophils from susceptible mares has lower (Watson *et al*., 1987; Cheung *et al*., 1985; Liu *et al*., 1985).

Fresh homologous leukocytes could be used in the treatment of bacterial endometritis (Castilho, 1994). Intrauterine infusion of homologous leukocytes with plasma gives more rapid result, compared to other bacterial endometritis treatment methods such as uterine irrigation and the use of ecbolic drugs (Mattos *et al*., 1999a, b). Furthermore, intrauterine infusion of homologous leukocytes with plasma following the insemination increases the pregnancy rates (Mattos *et al*., 1997, 1999a). In recent studies, intrauterine recombinant human interleukin 8 infusions activate the neutrophil migration, which is attributed to the strong chemoattractive activity of recombinant human interleukin 8 (Zerbe *et al*., 2003b). In this context, it is being suggested that frozen, lysed leukocytes can be used in the treatment of bacterial endometritis (Neves *et al*., 2007). Such applications should be repeated daily until disappearance of the clinical symptoms. Phagocytic activity of neutrophils, antibacterial proteins secreted from granulocytes, opsonizing factors in the plasma (these factors are lower in number in frozen leukocytes) and the chemotactic characteristics of leukocytes have been demonstrated as the reasons for success in the treatment of endometritis by fresh leukocytes (Castilho, 1994; Zerbe *et al*., 2003a; Faurschou and Borregaard, 2003; Asbury, 1984; Engelke *et al*., 1999). Frozen leukocytes have also been suggested to act similarly. The infusion of blood plasma as a thawing agent for frozen and lysed leukocytes could provide a more efficient opsonization, allowing frozen or lysed neutrophils to promote a more rapid bacterial elimination (Neves *et al*., 2007).

The use of some immunomodulators is also one of the preferred methods in the immunological treatment of bacterial endometritis. As some immunomodulators such as Mycobacterium phlei cell wall extract are able to restore the local inflammatory mechanisms in mares, they can be used in the prevention of postmating endometritis becoming chronic (Fumusoa *et al*., 2003).

The relation between cellular immunity and fertility in mares is not limited to endometritis, phagocytosis and neutrophils. There are also some other subjects that may be included under this title. For example, PGE_2 interferes with lymphocyte blastogenesis by T-lymphocyte growth factors dependent mechanisms. It is suggested that the PGE_2 present in the uterus of the early pregnant mare may be one of the factors involved in immunosuppression at the time of maternal recognition of pregnancy (Watson and Zanecosky, 1991). In mares, lymphocyte suppression in the pre-ovulatory follicle liquid shows an increase, especially before ovulation, this situation being related to an immonosupression occurring in response to lymphokine stimulation (Watson and Zanecosky, 1990). Related with this issue, it may be also declared that the compatibility of lymphocyte antigen gene frequency of the stallion and the mare during mating is not related to reproductive insufficiency in the mare (Park *et al*., 1989).

When the relationship between immunology and infertility is investigated, cytokines are one of the subjects on which emphasis should be placed. Cytokines are intercellular signal proteins secreted from immune and non-immune cells. Interleukin-1β, interleukin-6 and tumor necrosis factor alpha are known proinflammatory cytokines showing systemic and local effects and playing roles in the development of acute phase reactions. These cytokines are also related to various reproductive activities such as ovulation, implantation and dilatation of the cervix. Pro-inflammatory cytokines are produced by the endometrial cells of the uterus and leukocytes (Baranao, 1997).

Interleukin-1 system components (Interleukin-1β, Interleukin-1α, Interleukin-1 receptor antagonists and Interleukin-1 receptors) have been demonstrated to be synthesized in various regions of the ovary. These factors have been localized in the various ovarian cell types, such as the oocyte, granulosa and theca cells, in several mammalian species. It has been determined in various mammalian species that these factors are localized in theca and granulosa cells on the oocytes. It is known that interleukin-1 system plays an active role in various reproductive activities, such as ovulation and oocyte maturation and steroidogenesis of ovary (Gerard *et al*., 2004;. Martoriati *et al*., 2002).

It has been suggested that an increase or disorders in cytokine expression may be effective in the development of endometrial fibrosis in the equine uterus and subsequent embryo loss (Cadario *et al*., 2002).

THE RELATION BETWEEN HUMORAL IMMUNITY AND INFERTILITY

Many antigenic substances enter the female body during mating and foaling, such as bacteria, viruses and sperms, which may contribute to both local and generalized antibody formation consequent to various reactions. These antibodies are related to reproduction at different levels.

Immunoglobulin M concentrations in the mare blood sera show no variation according to stages of the cycle. On the other hand, the concentrations of IgA and IgG in the uterus are higher at estrogenic stage compared to progestatitve stage; however, the opposite is true for IgM (Widders *et al*., 1985).

Sexually active females (only estrus phase in mares) encounter millions of spermatozoa regularly. At each mating or breeding, the mares receives millions of immunologically factors and since spematozoa and associated seminal plasma are highly antigenic, immune responses that could impair fertilization might be expected to develop. However, there are many opinions on why this immune response does not lead to infertility, even to sterility in females. The most important of these suggestions is that these ASA developing against spermatozoa, must reach a specific titer in the female body (Kanchev *et al*., 1993). The titer of these antibodies has been suggested to be related with the concentration of spermatozoa within the semen and the number encountering the cell (Harlow and David, 1988). Furthermore, a different opinion suggests that spermatozoa, which are mucosa antigens, need to enter the female blood circulation and that ASA are more frequent in animals with genital diseases such as metritis and vaginitis, or during mating than in females with ruptures in genital organs and that ASA are lower in females with intact genital organs (Alexander and Anderson, 1989; Cunningham *et al*., 1991). In addition, it has been suggested that the route of administration of spermatozoa also plays an important role and that intravenous, intramuscular or intraperitoneal injection during mating than in females with Menge *et al*. (1982) and Lander *et al*. (1990).

There are many proteins on a spermatozoon; therefore, it is not well known which one of these proteins causes an immune response within the female body. There are many antigenic structures such as FA1 and lactic dehydrogenase C4 on the spermatozoa that can facilitate the formation of ASA in the female body (Naz, 1999; Bradley *et al*., 1997; Holland *et al*., 1997).

Porcine zona pellucida has been reported to have a contraceptive effect in the mare. It has been suggested that the antibodies against zona pellucida reduce fertility by blocking oocyte-sperm interaction; and that a 50-60% decrease in the blood zona pellucida antibody titer terminates the contraceptive action (Liu *et al*., 1989; Kirkpatrick *et al*., 1991; Willis *et al*., 1994). It has been observed that administration of these vaccines at least twice produces contraceptive action for 8 months. There are some studies on the use of zona pellucida as an immunocontraceptive, especially in the control of reproduction of equines in wild life, such as horses, donkeys and zebras (Kirkpatrick *et al*., 1992; Kitchener *et al*., 2002; Kirkpatrick and Turner, 2002). Such an immonocontraception method offers the following advantages for mares: it is reversible, its long term use produces no negative effects on the genital organs and it can be applied at a distant from the animal. Furthermore, GnRH vaccines are used in mares as an immunocontraceptive. It has been reported that as a result of such applications, a reduction was observed in ovarian activities and hormonal levels, but despite the reduction in the ovarian activities, estrus symptoms continued with various intervals and durations (Dalin *et al*., 2002).

In a study designed to determine the prevalence of anti-ZP antibodies in mares with different fertility, two of the tested mares (14%) were found positive. However, mares positive for anti-ZP antibodies were detected in both pregnant and infertile animals. Detection of anti-ZP antibodies also in pregnant animals has been reported to be an indication for requirement of higher blood and tissue concentrations of these antibodies for development of their effects on fertility. Furthermore, new studies are necessary to investigate whether infertility resulting from these antibodies is reversible or not (Risvanli *et al*., 2006).

Anti-sperm antibodies developed in the female genital tract can cause sterility, infertility or subfertility, affecting reproductive activities in various points, such as semen motility, oocyte-sperm fecundation, capacitation, penetration and zygote development. These antibodies have been determined by various methods in various regions of spermatozoa (head, tail, surface) in seminal plasma, blood sera, in some tissues such as the uterus and in the oviduct (Alexander and Anderson, 1989; Turner and Kirkpatrick, 2002).

In a study (Nie *et al*., 1993), in mares immunized against sperm cells or seminal plasma, the sera ASA rate was found to be higher in the group immunized against sperm cells. Furthermore, the same study reports that the rate of anti-sperm IgA in the uterine medium was higher in the sperm group. However, it has been determined that the results obtained hardly display the clinical findings. Lee *et al*. (1993) determined ASA in blood sera of maiden mares, following their subcutaneous immunization with sperm cells; the antibody levels persisted through week 5 and then slowly declined until week 15.

In another study, it was reported that 10.23% of mares were ASA positive in serum, these antibodies were lower in the animals that had mated ≥3 times and the ASA prevalence showed no variance according to breed, age, pregnancy status of animals and the presence or absence of infection in the uterus (Risvanli *et al*., 2005).

Despite all these discussed subjects, there are studies suggesting that ASA cause infertility at very low levels and to a higher frequency, they cause subfertility and that such an infertility is temporary (Naz, 1999; Kanchev *et al*., 1993). In addition, the development of such type of

immunological infertility in the female body requires development of some stress conditions and use of some anti-inflammatory medicines, including cortisone, or a genetic disposition (Alexander and Anderson, 1989). In another study, seminal plasma including spermatozoa has been suggested to carry some factors blocking the development of immunological response in the female body (Risvanli and Kaygusuzoglu, 2004; Kelly, 1995).

The use of GnRH vaccines for the control of sexual habits in the stallion, especially at 4 years of age or younger, is possible and because of the obligation in providing the desired anti-GnRH antibody titer levels, it is impossible in older stallions. One of the important side effects of GnRH vaccines in the control of sexual behaviors is the suppression of sperm production. However, this suppression terminates at the end of 4 months in stallions under the age of 4 (Turkstra *et al*., 2005; Van der Meer *et al*., 2001). In recent years, in Australia a GnRH vaccine, named Equity™ has been developed for estrus control and suppression of undesired estrus behaviors in mares; however, this vaccine has not been registered yet for use in stallions (Stout, 2005).

CONCLUSION

It should be considered that immunological factors also have an effect on fertility in mares and in this context, within idiopathic infertility, situations resulting from these factors should also be taken into account and this matter should be evaluated on a larger scale with further studies. With respect to comprehensiveness of fields within reproductive immunology, as in human medicine, centers including laboratories employing specialized personnel should be established in veterinary medicine, especially in equine medicine and immunological tests should be included in the routine tests for the mare infertility. In this context, further rapid reproductive immunology tests should be developed and generalized for use in field conditions.

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