

STUDIES ON THE LYSOZYME ACTIVITY IN THE MILK OF FEMALE DONKEYS (*EQUUS ASINUS*) IN RELATION TO REPRODUCTIVE PHYSIOLOGY

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ABSTRACT

The lysozyme activity (LZA) in milk samples from 23 Jennies was measured on alternative days during the first 60 days postpartum with modified turbidimetric method. Progesterone profile in milk was determined by a comparative enzyme immunoassay. LZA ranged between 203 278 and 34 340 U/ml with an average of $93\ 023 \pm 28\ 128$ U/ml. Lysozyme activities remained very high until the end of foal heat in all jennies. These values declined abruptly to a 25% lower level at the end of foal heat and followed a linear trend downward until 50th day p.p. and afterward, these values stabilized until the day 59 of the study. Jennies, who conceived in foal heat showed 15000 U/ml LZA significantly higher than those, which had not conceived in foal heat, until the end of foal heat. Lysozyme activity and progesterone content showed a significantly ($P < 0.001$) negative correlation during foal heat and in subsequent heats. It is concluded that considerably higher lysozyme activity in milk can firstly be considered as a protective factor for the newborn and secondly, for the low susceptibility of jenny's udder to infections. Moreover, it might play an important role in an intensive and rapid postpartal regeneration process of the uterus.

Key words: Lysozyme activity, milk, reproduction, progesterone, donkey.

INTRODUCTION

Milk contains a large number of specific and non-specific immunologic factors aimed at the protection of the newborn. They have been found to promote intestinal growth and maturation in neonatal period and to have immunomodulating effects throughout life. Since the immune system of newborn foal is least developed during early postnatal period, the need for possible transfer of immunity during this time may be the greatest. Among the non-specific immunoprotective factors, lysozyme – an extraordinary bacteriolytic protein – is a component of the antibacterial system, which also affects the general immune system. Latvietis et al. (1995) have reported that addition of a lysozyme containing preparation to avian food significantly enhanced the T- and B lymphocytes, circulating immune factors and lysozyme in serum along with daily growth.

It was first realized by Gill et al. (1985) that a correlation between lysozyme activity (LZA) in blood and time of new conception in mares may exist. Later on, we found that mares conceived in foal-heat exhibited markedly higher lysozyme activity in milk until the end of foal-heat compared with those, which had not conceived in the foal heat (Sarwar et al. 1996). These findings were reconfirmed by Hatzipanagiotou et al. (1998), who described a highly significant relationship between lysozyme activity and conception in a mixed herd of mares. To date, there is no literature available on lysozyme activity in jenny's milk. Hence, this study has

been undertaken to elucidate: i) the course of lysozyme activity in jenny's milk during early lactation period, ii) a possible correlation between lysozyme activity in milk and reproductive and hormone status in jennies, and iii) the influences of different environmental factors like parity and age.

MATERIALS AND METHODS

This study was conducted on 23 jennies, aged from 5 to 18 years, living in Germany, Holland and Switzerland. Total contingent was comprised of 5 primiparous and 18 multiparous jennies, while two age-groups consisted of 14 young (<10years) and 9 old (>10years) jennies.

Samples of 10 ml jenny's milk were collected in PVC tubes on alternate days during the first 60 day p.p. The samples were frozen immediately after milking and stored at $-20\ ^\circ\text{C}$ until analysis. Lysozyme activity in the jenny's milk was determined with the help of a modified turbidimetric method using *Micrococcus lysodeiktiticus* as substrate. The averages of coefficients of variance (CV) for inter- and intraassay amounted to 3.65% and 3.59 %, respectively. The progesterone content in milk was determined by competitive enzyme immunoassay (EIA). For analysis the oestrous cycle was divided into follicular phase with milk progesterone up to 1.0 ng/ml inclusive and luteal phases of more than 1.0 ng/ml.

Statistical analysis: The data were expressed in Mean \pm S.D. The differences between mean values of different

groups were worked out with covariance analysis. The interrelationships between lysozyme activity and progesterone content were ascertained in each animal using Spearman's correlation coefficient. All computations were performed with the help of statistical computer software SAS (SAS Institution Inc. Cary, NC, USA).

RESULTS

Lysozyme activities (LZA) in all 690 samples ranged between 34 340 and 203 278 U/ml of jenny's milk with an average of $93\ 023 \pm 28\ 128$ U/ml. On 1st and 3rd day postpartum the colostral LZA were higher than that of mature milk. All jennies exhibited a significant decline at the end of foal heat (latest until 24th day p.p.). After decline, the activities followed a downward trend until

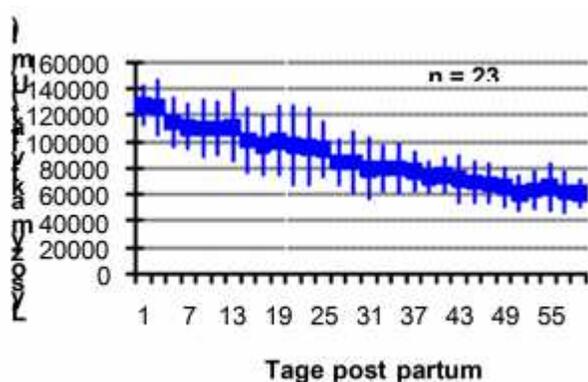


Fig. 1: Course of lysozyme activity (Mean ± SD) in the milk of jennies (*Equus asinus*) during early lactation period

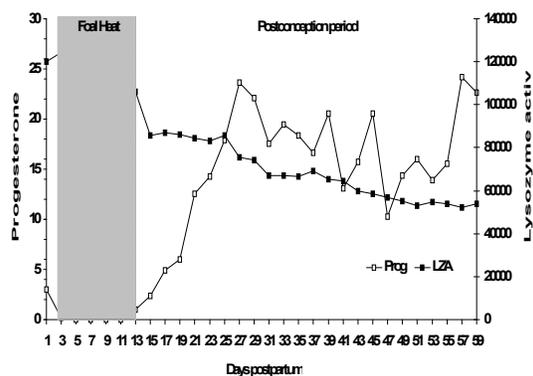


Fig. 3: Comparison between the courses of lysozyme activity and progesterone content in the milk of jennies which had conceived during foal heat (n =16) throughout study period

50th day p.p. and then stabilised until the 59 days p.p. (Fig 1).

Jennies, which had conceived during foal heat showed by 15000 U/ml higher lysozyme activity than those which had not conceived in foal heat, until the end of foal heat (Fig. 2). All jennies showed a strongly negative correlation ($P < 0.001$) between lysozyme activity and progesterone profile up to the end of foal-heat (Fig 3). Each jenny, which had not conceived during foal-heat showed a strong ($P < 0.001$) negative correlation between lysozyme activity and progesterone content during subsequent heats until the end of study (Fig. 4). A typical case of 'Vereni', which had not conceived until 3rd heat postpartum.

Multiparous and old (>10 years) mares exhibited significantly ($P < 0.05$) higher mean value than primiparous and young (5-9 years) animals up to the end of foal-heat.

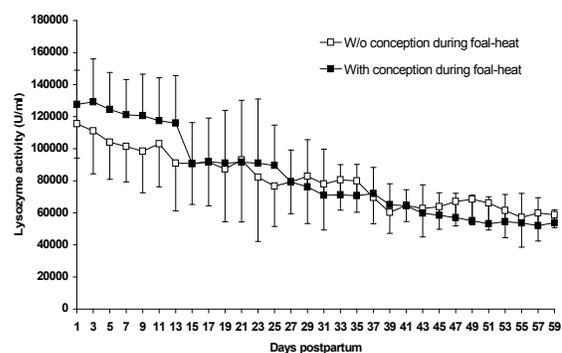


Fig. 2: Comparison between the courses of lysozyme activity in the milk of jennies with conception in foal heat (n= 16) and jennies without conception in foal heat (n= 7) throughout study period

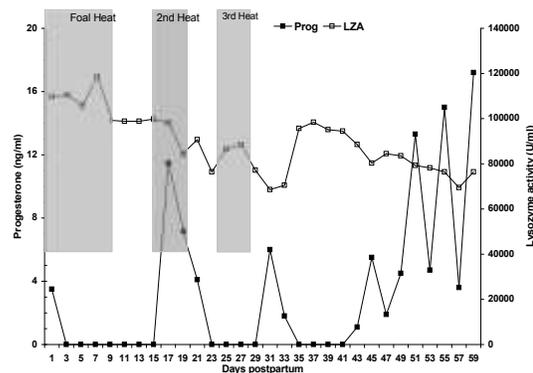


Fig. 4: Comparison between the lysozyme activity (LZA) and progesterone content (Prog) in the milk of a jenny named "Vereni" which had not conceived until third heat postpartum

DISCUSSION

Lysozyme activities averaged $93\ 023 \pm 28\ 128$ U/ml with a range from 34 340 to 203 278 U/ml in milk of 23 jennies during the period from 1st to 60th day postpartum. No literature is available on lysozyme activity in jenny's milk to discuss these results. As compared to human beings and other animals, the average lysozyme activity in jenny's milk was the highest. This average value was almost 50% higher than in mare's milk (Jauregui-Adell 1974; Sarwar 1996; Sonntag 1996; Rieland 1997).

In human beings various authors reported a range between 9 880 to 52 000 IU/ml, with an exception of Sanches-Pozo et al. (1987), who recorded an enormously higher mean value (235 300 U/ml) in human milk.

LZA in cow's milk has been extensively studied both in physiological and pathological conditions. There is a common agreement that cow's milk contains a very low concentration of lysozyme (0-58.5 U/ml). It has been justified by the fact that lysozyme is released by the broken neutrophils in serum and cow's neutrophils contain extremely low concentration of lysozyme.

Radwan and Elmarimi (1987), Farid et al. (1984) and Ismail et al. (1984) reported a substantial rise of LZA milk in mastitis among different bovine species. Persson et al. (1992) suggested that the neutrophils are the most probable source of lysozyme during the inflammation of mammary gland. Bovine neutrophils like those of human beings and other mammalian species play a double role in inflammation: phagocytosis and degradation of microorganisms, and induction of the inflammatory reaction by the induction of chemotaxis and enhancement of phagocytosis (Jain 1986). Thus, high concentrations of lysozyme are released in inflammation due to high turnover rate of neutrophils. In addition, lysozyme does not possess only antimicrobial characteristics but is also important in the cellular immunity. Lysozyme activity of milk has been found hundred times higher than the values reported in serum for mares and foals. This suggests that lysozyme is solely transferred from serum to milk not only by diffusion but also by additional selective mechanisms involved. Rieland (1997) did not find a correlation between lysozyme activity and somatic cell count in mare's milk. It is suggested that most probably a reasonable part of equine milk lysozyme is locally contributed by secretion from the mammary epithelial cells.

The finding of a strong decline on 13th day p.p. on average is in good agreement with previous literature. Sarwar et al. (1996) and Hatzipanagou et al. (1998) described a strong decline (20-25%) on 12th and 9th day p.p. in mare's milk, respectively. Sanches-Pozo et al. (1987) carried out their investigations from 6th day p.p. onward and recorded a decline in lysozyme activity on

the 12th day p.p. Following decline, the values, however, maintained a constant level until the end of the study (30th day p.p.). McClland et al. (1978) and Hennart et al. (1991) studied the daily changes in lysozyme activity in human milk during first week of lactation. They observed a strong fall during first 3 - 4 days of lactation, and thereafter, the values remained at a constant level throughout the week.

Abrupt ejection from the protected uterine milieu to the extra uterine environment exposes newborn to a great infectious challenge. At the time of birth, the newborn has no optimally developed immune system particularly with regard to the secretary immunity. So newborn needs specific and non-specific immune factors by maternal milk. Thus, comparatively high values of lysozyme activity in jenny's milk can, above all, be considered as a protective factor for the newborn. Besides, high concentration of lysozyme in jenny's milk can be considered as a significant factor for the low susceptibility of jenny's udder to infections. In this context, low lysozyme concentration and high incidence of mastitis in cows can especially be mentioned.

In the present study, jennies who did conceive in the foal heat showed significantly higher LZA than the jennies, which did not conceive during foal heat. Since the lysozyme is chiefly produced by polymorphonuclear neutrophilic leucocytes (PMNL), it is assumed that similar high level of lysozyme activity will concurrently be present in blood and uterus, which might enhance the regeneration and cleaning process as well as antimicrobial protection which ultimately contribute to better fertility rate. The lower lysozyme activity in jennies, which did not conceive in foal-heat may be an indicator for reduced defence activity at this time. It is further supported by the short luteal phase in these animals, which indicates an irritation of uterine mucosal membrane with a higher prostaglandin secretion (Pascoe 1986). An early regression of corpus luteum is observed in many cases in mares (Hugheo et al. 1977; Allen 1979). Furthermore, it will be interesting to mention in this connection that Reinhold (1975) found a reduced lysozyme activity in a bacterial infection of mammary glands. He suggested that it might be due to the binding of lysozyme to bacteria. The results of Latvieties et al. (1995) as referred in introduction of this have also proved that lysozyme has an immuno-stimulating effect in addition to local antimicrobial action.

Taken as a whole, the results discussed here suggest a special role of a good functional non-specific defence system – as this parameter LZA in the milk studied here – in a rapid and intensive postpartal regeneration process concurrently in progress in uterus and consequently for better conception chances in the foal heat. The possibility to determine the prognosis for conception chances in a specific jenny during foal heat with the help of lysozyme determination test needs

further investigation. The precision, feasibility and quickness as well as the cost of such a lysozyme test can play an important role.

According to McCue (1993), progesterone inhibits the lactogenesis through its inhibitory effect on epithelial cells of mammary alveolar tissue. These cells are also considered a possible source of the lysozyme in the milk. The present findings that the lysozyme content in the milk after foal heat or with the beginning of the C. L-phase decline support this notion. Since the PMNL are considered as a main producer of lysozyme, these results are also in agreement with the findings of Killingbech (1973) and Vander Plasche et al. (1982) who reported an inhibition of PMNL activity under progesterone dominance. It needs particular attention that lysozyme activity in jennies which did not conceive during foal-heat raised slightly again during subsequent heats. This suggests that there is again, first of all, an inhibitory effect of progesterone and secondly an activating effect of oestrogen to observe. The fact that the second peak of lysozyme activity after parturition in contrast to the foal heat acquired a low level of LZA may be attributed to a lower oestrogen production during second and third postpartal heats (Enbergs et al. 1999).

Elevated LZA during early postpartal period in relation to parity and age can be attributed to augmented training of specific and non-specific immune mechanisms over time. These results extend over to the previous literature (Sarwar et al. 1996). Senft et al. (1974) and Götze et al. (1977) also have reported higher lysozyme activities in multiparous cows than their primiparous counterparts.

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