Journal of Andrology, Vol. 18, No. 3, May/June 1997

Seminal Plasma Lactoferrin Concentrations in Normal and Abnormal Semen Samples

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ABSTRACT: Although the iron-chelating protein lactoferrin is secreted by the seminal vesicles, the precise role of lactoferrin in semen is unclear. This study aimed to determine whether there is any association between seminal lactoferrin concentrations and normal and abnormal semen samples with and without leucocytospermia. Lactoferrin concentrations were measured by radial immunodiffusion of semen samples from 368 men attending a regional andrology referral center. Routine seminal analysis, including the presence of leucocytospermia, was also performed. Results showed increased seminal lactoferrin in samples showing oligospermia (13.3 mg/100 ml) and oligoasthenospermia (13.4 mg/100 ml) compared to normospermic samples (11.2 mg/100 ml). There were no significant differences in seminal lactoferrin between normospermic samples and azoospermic samples or asthenospermic samples with normal sperm density. Although there was a trend toward increased lactoferrin concentration with leucocytospermia, this was not significant. Possible causes for raised lactoferrin in association with oligospermia are discussed.

Key words: Leucocytospermia, oligospermia, asthenospermia, infertility.

J Androl 1997;18:302–304

Lactoferrin is a medium-molecular-weight protein (84 kDa) with iron-binding properties. It was first isolated in bovine milk and subsequently in human breast milk (Schäfer, 1951). The protein is present in many human secretions and is also found in the epithelial lining of the stomach, colon, lung, and genital tract, and in neutrophils (Groves, 1971). It was first identified in human seminal plasma by Masson et al (1966) and is secreted by the seminal vesicles (Hekman and Rümke, 1968; Tauber et al, 1975).

By virtue of its iron-chelating effect, lactoferrin is bacteriostatic (Masson and Heremans, 1966) and may therefore have a role in the defense against microorganisms (Masson, 1970). In breast milk, lactoferrin has been shown to act as an antimicrobial protein suppressing coliform bacteria in the neonate (Bullen et al, 1972). However, its role in semen remains unclear.

Lactoferrin levels have been reported to be raised in association with asthenospermia and oligospermia when compared to normospermia, although the numbers analyzed were small (Auterio et al, 1991). Wolff et al (1990) showed a significant relationship between leucocytospermia and a reduction in total numbers of sperm and sperm motility. Leucocytospermia is suggestive of genital-tract infection (WHO, 1992), and lactoferrin concentrations may alter in such circumstances.

The purpose of this study was to determine any association between seminal plasma lactoferrin concentration and normal and abnormal semen samples with and without leucocytospermia.

Materials and Methods

Semen samples were obtained from 368 men attending a regional andrology infertility clinic. All semen samples were obtained by masturbation into a sterile nonspermicidal container after 4 days of sexual abstinence. The samples were allowed to liquefy for 30 minutes at room temperature. Routine seminal analysis, according to standard WHO criteria (1992), was then performed. In cases of complete azoospermia, serum follicle-stimulating hormone (FSH) was also determined. Leucocytospermia was defined as $>1 \times 10^6$ white cells/ml by light microscopy.

Seminal lactoferrin concentrations were then determined on the supernatant after centrifugation at $1,000 \times g$ for 10 minutes. Single-radial-immunodiffusion assay was used (Mancini et al, 1965). Goat antisera to lactoferrin (Nordic Immunology Laboratories, Tilberich, Netherlands) was added to 1% agarose (Hyland Pharmaceuticals, Feltham, UK) and made into 1.5 mm plates that were then subdivided in two. Standardized human colostrum, lactoferrin concentration 10 mg/100 ml, was used as a standard for lactoferrin. Seminal plasma from each sample and standard colostrum was then added to three different plates. The diffusion through the agarose was measured (usually 20–30 mm)

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Received for publication November 1, 1996; accepted for publication January 24, 1997.

Table 1. *Number of samples displaying the different semen parameters and leucocytospermia*

	Number of samples	Samples with leucocytospermia
Normospermia (>20 million/ml, >40% motility)	73	15 (20.5%)
Asthenospermia (>20 million/ml, <40% motility)	107	19 (17.4%)
Oligoasthenospermia (<20 million/ml, <40% motility)	115	17 (14.8%)
Oligospermia (<20 million/ml, >40% motility)	53	11 (20.8%)
Azoospermia (serum FSH > 9 IU/L)	10	1 (10.0%)
Azoospermia (serum FSH < 9 IU/L)	10	1 (10.0%)
Totals	368	64 (17.4%)

FSH, follicle-stimulating hormone.

after 48 hours' incubation, then the area was calculated and compared with the control in all plates, and the lactoferrin concentration calculated. The standard error of the lactoferrin concentration was earlier evaluated as 1.08%. Samples from all groups were run concurrently, and the technician performing the lactoferrin assay was blind to the results of the seminal analysis.

Data were assessed for normality using the Shapiro-Wilk test and described using the median and interquartile ranges. The Mann-Whitney *U*-test was used to compare groups.

Results

Of the 368 samples, 73 had normal semen parameters, 107 had asthenospermia with normal sperm density, 53 had oligospermia with normal sperm motility, 115 had oligoasthenospermia, and 20 had azoospermia (Table 1). A diagnosis of testicular germ cell hypofunction was made on the basis of raised serum FSH levels >9 IU/L in 10 of the azoospermia group, and the remaining 10 were presumed to have obstructive azoospermia.

Leucocytospermia was present in 64 samples (17.4%). The number of samples with leucocytospermia was 15 (20.5%) in the normospermic group, 19 (17.8%) in the asthenospermic group, 11 (20.8%) in the oligospermic group, 17 (14.9%) in the oligoasthenospermia group, and two (10%) in the azoospermic group. Leucocytospermia was not significantly raised in any of the groups.

There was no significant difference in median seminal lactoferrin concentrations between the normal group (11.2 mg/100 ml), the asthenospermia with normal sperm density group (12.6 mg/100 ml), and both azoospermia groups (11.2 mg/100 ml and 10.2 mg/100 ml). However, the oligospermia group (13.4 mg/100 ml) and the oligoasthenospermia group (13.3 mg/100 ml) both showed a significantly increased median seminal lactoferrin concentration (Table 2).

Table 2. *Median seminal lactoferrin (and interquartile ranges) in the different sperm groups*

	Seminal lactoferrin (mg/100 ml)
Normospermia (n = 73)	11.2 (6.3–15.7)
Asthenospermia (n = 107)	12.6 (7.3–18.6)
Oligoasthenospermia (n = 115)	13.3* (8.9–21.7)
Oligospermia (n = 53)	13.4* (9.2–21.7)
Azoospermia (n = 20)	11.1 (8.8–20.4)

* Normal vs. oligoasthenospermia, $P = 0.016$; normal vs. oligospermia, $P = 0.025$.

The median lactoferrin concentration in the leucocytospermia group (n = 64) was raised at 12.4 mg/100 ml compared to 11.4 mg/100 ml in the nonleucocytospermia group (n = 304), but this was not statistically significant.

Discussion

This large study analyzing 368 samples shows that seminal lactoferrin concentrations are raised in samples showing oligospermia and oligoasthenospermia. There was no significant difference in lactoferrin concentrations between complete azoospermia, whether presumed obstructive or associated with raised serum FSH levels, and normospermia samples. Auterio et al (1991) suggested that lactoferrin is raised in asthenospermia as well as oligospermia. Numbers in their study were small, and it is unclear whether the asthenospermic group had normal sperm density. Our study showed there was no significant difference between asthenospermia with normal sperm density and normospermic samples.

The reason for raised lactoferrin in individual semen samples remains unclear. Leucocytes are present in most ejaculates (Wolff and Anderson, 1988; Barratt et al, 1990); however, excessive presence of these cells (leucocytospermia) is traditionally associated with clinical and subclinical genital-tract infection (WHO, 1992). In this study, although median lactoferrin concentration was higher, there was no statistically significant difference in the between-samples exhibiting leucocytospermia and those that did not. This may be because the method for identifying leucocytes from

other seminal cells and the various white-cell subsets is unreliable (Barratt et al, 1988). Alternatively, seminal leucocytes or increased lactoferrin concentrations may present in semen later than the period of acute infection. In this study, there was no history or clinical evidence of either vesiculitis or prostatitis.

Although lactoferrin concentrations were raised in oligospermia, there was no increase in lactoferrin concentration in cases of azoospermia. Lactoferrin is not present in semen from men with congenital absence of the seminal vesicles (Hekman and Rümke, 1968). A decrease in the seminal vesicle secretion caused by high-dose testosterone injection leads to lower lactoferrin concentrations in seminal plasma (Carpino et al, 1994). Therefore, seminal lactoferrin may reflect seminal vesicle function and the lack of a rise in cases of azoospermia may reflect concomitant abnormalities in the seminal vesicles (Lizana et al, 1987; Aumuller and Riva, 1992). Seminal fructose, which is also secreted from the seminal vesicles, has been used to assess seminal vesicle function (Gonzalez et al, 1989); however, only where there are significant abnormalities of the seminal vesicles is there a reduction of fructose concentrations.

Animal studies on the mouse epididymis show lactoferrin mRNA is stimulated by 17β-estradiol (Yu and Chen, 1993), and in the human female reproductive tract, lactoferrin concentration in vaginal mucus peaks just after the menses (Cohen et al, 1987), which also implies lactoferrin secretion is stimulated by estradiol. In men, serum and testicular estradiol have been shown raised in association with oligospermia (Krause, 1988; Levalle et al, 1994). Therefore, increased circulation of seminal estradiol could cause increased seminal lactoferrin secretion. Whether the rise in serum and testicular estradiol is directly involved in causing the oligospermia is at present uncertain.

Further studies need to measure serum and seminal estradiol as well as seminal lactoferrin in men with oligospermia to determine whether raised estradiol is the causative factor of raised lactoferrin. Also, better identification of white cells, in particular activated neutrophils and other leucocyte subgroups, is needed in order to determine whether lactoferrin is secreted as part of an acute or chronic infective condition.

Acknowledgments

We thank D. B. McClelland, J. M. McGrath, and R. R. Samson for kindly providing the standardized human colostrum.

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