Original Paper

A correlation study between steroid hormone levels and anti-sperm antibodies in serum and seminal plasma of men with or without reduced sperm motility

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Summary

Male infertility is a multi-factorial disorder. The functional ability of spermatozoa is primarily determined by their motility. Many factors are responsible for reduction in sperm motility but immunological and / or hormonal factors are vital. At present, the impact of immunological factor along with hormonal imbalance in reducing sperm motility is not known. In the present study, steroid hormones (testosterone, estradiol, progesterone and cortisol) and anti-sperm antibodies (ASAB) were measured in serum and seminal plasma of males with normal or reduced sperm motility. The ASAB was not detected in serum samples of volunteers with normal sperm motility but detected in 22.7% of serum samples of volunteers with low sperm motility. On the other hand ASAB was detected in seminal plasma of volunteers with normal sperm motility (31.8%) and low sperm motility (36.3%). Significantly low levels of testosterone and cortisol ($p<0.001$ and $p< 0.05$, respectively), and significantly high levels of estradiol and progesterone ($p< 0.005$ and $p< 0.001$, respectively) were observed in the seminal plasma of samples with low sperm motility when compared with samples with normal sperm motility. A correlation was observed between presence of ASAB and altered steroid levels such as low cortisol, and high progesterone and estradiol in seminal plasma of volunteers with low sperm motility. There was no difference in serum steroid levels. These results suggest a relationship between testicular steroid hormone levels with autoimmunity to sperm antibodies.

Key words: anti-sperm antibodies, male infertility, sex steroids, sperm motility.

Introduction

Infertility affects 13-18% of couples (Iammarrone et al., 2003). The pathogenesis of male infertility can be reflected in defective spermatogenesis, defective sperm transport, abnormal sperm morphology or defective sperm functional parameters (Somnath Roy, 1999). Motility is the prime functional parameter that determines the fertilizing ability of spermatozoa. The cause underlying loss of sperm motility may be either hormonal, biochemical, immunological or infection (Armstrong et al., 1999; Diemer et al., 2000; Luboshitzsky et al., 2002; Lambardo et al., 2004)

It has been observed that anti-sperm antibodies (ASAB) are present either systemically in blood or locally in seminal plasma of approximately 10% of infertile male patients (Eshire Capri Workshop, 1998). These ASAB impair the fertilizing ability of spermatozoa by acting negatively on sperm motility and result in poor cervical mucus penetration and in-vitro gamete interaction (Lambardo et al., 2004). The other cause of loss of sperm motility has been experimentally shown to be hormonal imbalance either in blood or in semen (Bujan et al., 1993; Luboshitzsky et al., 2002). There are other factors such as oxidative stress which are related with abnormal sperm morphology such as presence of cytoplasmic droplet on the immature spermatozoa making them non-functional with reduced sperm motility (Ollero et al., 2001). The biochemical composition of semen such as reduced fructose levels also cause reduction in sperm motility in asthenozoospermic cases but this concept is not widely supported (Gonzales and Villena, 2001). Hormonal imbalance and autoimmunity may influence each other to reduce sperm motility, since these two systems work in close association (Kiess and Belohradsky, 1986). There are only few studies showing either no correlation between autoimmunity and hormonal factor (Bozhedomov and

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Teodorovich, 2005), or influence of sex hormones on immune system (Giglio et al., 1994). Therefore, an attempt has been made to study the possible correlation between hormone levels and autoimmunity in reducing sperm motility.

Methodology

Subjects

The male partners of forty four infertile couples (Andrological Outpatients Clinic at NIHFW, New Delhi), twenty two with normal seminal parameters (group I) and an equal number with severely reduced sperm motility but normal sperm counts (asthenozoospermic semen samples, group II) [as per WHO guideline (World Health Organization, 1999)] in the age group of 25-42 years, were selected for the study. Blood was drawn and semen samples were collected after 2-3 days of abstinence. Serum and seminal plasma were separated by centrifugation and the complement factor was inactivated by incubation at 56°C for 30 min.

Detection of anti-sperm antibodies

The presence of immobilizing ASAB in serum and seminal plasma samples was detected according to Isojima et al. (1968). Positive and negative control samples were run along with each experiment. Normal spermatozoa were incubated with test serum or seminal plasma sample in the presence of exogenous complement factor. Sperm immobilization values (SIV) were calculated using the formula,

$$SIV = \frac{\text{\% motility of sperm in control negative}}{\text{\% motility of sperm in test sample}}$$

The sperm immobilization value ³ 2.0 was suggestive of presence of ASAB.

Hormone estimation

Serum as well as seminal plasma levels of steroid hormones such as cortisol, testosterone, progesterone were estimated, cortisol and progesterone adopting ELISA technique developed by Basu et al. (2006) and testosterone by radioimmunoassay method of Abraham (1974).

Statistical analysis

Data were expressed as mean ± SEM and subjected to analysis adopting Student's t-test. Differences were considered significant if the probability of their occurrence was <0.05.

Results

Detection of ASAB

In men with normal sperm motility, ASAB was not detected in serum samples whereas 31.8% of seminal plasma samples contained ASAB. In men with reduced sperm motility, 22.7% of serum samples and 36.3% of seminal plasma samples had ASAB (Table 1).

Table 1 : Presence of ASAB in the serum and seminal plasma samples of males with or without reduced sperm motility

Presence of ASAB	Serum (%)	Seminal plasma (%)
Low sperm motility samples (n=22)	22.7%	36.3%
Normal sperm motivity samples (n=22)	ND	31.8%

Steroid hormone levels

Estradiol

The mean level of estradiol in seminal plasma was significantly higher in males with reduced sperm motility than those with normal sperm motility [94±11.5 *versus* 56±4.6 pg/mL, ($p < 0.005$)] (Table 2).

Table 2: Hormones present in the serum and seminal plasma of male partners of infertile couple with or without reduced sperm motility (Mean ± SEM)

Hormones	Serum - Low sperm	Serum- Normal sperm motility	Seminal low motility	Plasma sperm	Seminal plasma Normal sperm motility
Cortisol (ng/ml)	242±18 (n=16)	265±17 (n=17)	75±5* (n=19)		95±5 (n=18)
Testos terone (ng/ml)	4.6±0.2 (n=16)	4.8±0.4 (n=20)	0.46±0.07** (n=18)		0.99±0.07 (n=18)
Estradiol (pg/mL)	----	----	94±11.5*** (n=17)		56±4.6 (n=18)
Proges terone (ng/mL)	2.63±0.3 (n=17)		3.02±0.28 (n=17)		2 ± 0.2 (n=20)

* $p < 0.05$; ** $p < 0.001$; *** $p < 0.005$

Testosterone

No significant difference was found between the mean serum testosterone levels of men with normal

sperm motility (4.8 ± 0.4 ng/mL) and reduced sperm motility (4.6 ± 0.2 ng/mL), whereas mean seminal testosterone levels were found to be significantly lower in men with reduced sperm motility than in men with normal sperm motility (0.46±0.07 *versus* 0.99±0.07 ng/mL, p< 0.001) (Table 2).

Cortisol

The mean serum cortisol levels of men with reduced sperm motility (242±18 ng/mL) and normal sperm motility (265±17 ng/mL) did not show significant difference, whereas significantly reduced seminal cortisol levels were found in males with reduced sperm motility when compared to those with normal sperm motility (75±5 *versus* 95±5 ng/mL, p<0.05) (Table 2).

Progesterone

There was no significant difference in the mean serum progesterone level of men with low sperm motility (2.63 ± 0.29 ng/mL) and normal sperm motility (3.02±0.28 ng/mL). However, the mean seminal progesterone level was significantly higher in men with low sperm motility compared to those with normal sperm motility (3.7±0.35 *versus* 2±0.2 ng/mL, p< 0.001) (Table 2).

Discussion

In this study, we have shown that presence of ASAB along with low sperm motility has correlation with the altered steroid hormone levels in the seminal plasma. Moreover, antibodies were also found in seminal plasma samples from cases with normal sperm motility but the levels of hormones in serum as well as seminal plasma were unaltered. The development of anti-sperm antibodies in the adult males is correlated with spontaneous or idiopathic factors arising from local or systemic immune response in cases such as homosexuality, testicular trauma (improper biopsy), varicocele, mumps orchitis, spinal cord injury, congenital absence of vas and vasectomy (Hjort et al., 1974; Wolff and Schill, 1985; Golomb et al., 1986; Shulman et al., 1972, 1992; Patrizio et al., 1992a, b; Siosteen et al., 1993). These causes are mostly mechanical or infection-oriented. Many immunosuppressive factors, such as prostaglandins, zinc, transglutaminase, Fc receptor binding protein, prostasomes, etc., are present in the seminal plasma to suppress immune response to sperm auto-antigens (Hargreave et al., 1993). Steroid hormones are also present in the semen but their immunosuppressive properties are not documented. However, they are known to affect the sperm functions. E.g., increased estradiol levels or decreased testosterone levels either in the blood or in the seminal plasma were found to hamper the spermatozoal motility (Purvis et al., 1975; Bujan et al., 1993; Luboshitzsky et al., 2002). Earlier studies have shown significant changes in the levels of hormones such as testosterone, dihydrotestosterone, estradiol, estrone, LH and FSH in vasectomized men compared to age-matched controls (Adamopoulos et al., 1976; Alexander et al., 1980). In the present study we found low seminal testosterone levels in males with low sperm motility.

Cortisol levels in seminal plasma were first determined by Brotherton, which ranged from 31 to 94 ng/mL (Brotherton, 1990). Since corticosteroids are administered to suppress the autoimmunity, it was logical to estimate cortisol levels in the serum and seminal plasma. Low seminal cortisol levels in ASAB-positive samples were suggestive of immunosuppressive role of this hormone. Smith et al. (2002) also observed low cortisol levels in follicles of females with endometriosis. A direct correlation was observed between low cortisol levels and presence of ASAB in the seminal plasma.

Estrogen levels were found to be very high in ASAB-positive seminal plasma samples with low sperm motility. Similarly, higher progesterone levels were observed in ASAB-positive samples with low sperm motility. Earlier, it was shown that exogenous administration of estradiol in the rats significantly increased the IgA and IgG type of antibodies in the uterus, suggesting estradiol-dependent development of specific antibodies in the genital tract (Wira and Stern 1991). Steroids, such as estrogen and progesterone, are known to regulate lymphocyte proliferation through cytokine action (Grekova et al., 2002) and participate in humoral immune responses by modulating antibody synthesis (Canellada et al., 2002). Thus, it could be said that higher estrogen and progesterone levels may be responsible for higher levels of antibodies in the genital tract, which might ultimately hamper the motility of spermatozoa.

Immune-regulation is a complex process, which involves both the central nervous system and endocrine system (Kiess and Belohradsky, 1986). The reason for abnormal steroidogenesis is not known. The possible explanation is that altered hormone synthesis leads to either stimulation or inhibition of certain protein molecules such as cytokines which stimulate the production of antibodies against spermatozoa (Wira and Stern, 1991). The factor that triggers this immuno-endocrinopathy is not clearly known but it could be stress, as observed by Arck et al. (2002) during female pregnancies. Further studies are required to understand the underlying mechanism.

Thus, this study shows that i) cortisol has immunosuppressive activity, ii) estrogen and progesterone stimulate the humoral immune response, and iii) testosterone is responsible for reduction in sperm motility. It is concluded from this study that development of ASAB may be correlated with altered seminal steroid levels in reducing sperm motility.

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