

Inhibitory effects of human seminal plasma on an ELISA used to detect anti-sperm antibodies: Implications for the determination of sperm quality

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Abstract

The in vitro inhibitory effect of human seminal plasma on an ELISA used to detect anti-sperm antibodies have been observed. The mean inhibition rate of seminal plasma samples from 75 men was $61.5 \pm 23.1\%$. The inhibition rate of 29 samples from normal sperm group was $71.14 \pm 18.25\%$, while that of 46 samples from the abnormal sperm group was $55.43 \pm 23.98\%$. The results show that human seminal plasma from semen with high quality sperms possesses a high inhibitory rate to anti-sperm antibody reactions, suggesting its efficiency for immunosuppression of humoral immune reactions. Its possible implications are discussed. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Human seminal plasma; Inhibition; Anti-sperm antibody; ELISA; Sperm quality

1. Introduction

Semen of mammalian species contains a built-in system to prevent immunologic sensitization of females against antigens of sperm and seminal plasma in spite of repeated coitus. This prevention of sensitization is likely due to the presence of a potent immune response inhibitor originating from

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some of the sex accessory glands of the male. Failure of this built-in system in the male results in an immune response to seminal antigens in the female. This seminal immunity, when of the right kind and magnitude, results in clinical immune-fertility (Prakash, 1981). Over the years, significant progress has been made in identifying the immunosuppressive factors in seminal plasma and their possible action in vitro. The seminal plasma completely suppressed both primary and secondary humoral immune responses in mice. It also inhibited complement-mediated hemolysis in a standard immune hemolytic assay (Anderson and Tarter, 1982; Tarter and Alexander, 1984). Human seminal plasma suppressed delayed-type hypersensitivity responses to intravaginally deposited sheep red blood cells and sperm (Lee and Ha, 1989). Seminal plasma could cause inhibition of T-lymphocyte activation (Imade et al., 1997). Several authors have provided further evidence about suppression of the immune response by seminal plasma (Quayle et al., 1987). Observations that human seminal plasma contains factors that inhibit anti-sperm antibody reactions in vitro are reported here. Its implications for sperm quality are discussed.

2. Materials and methods

2.1. Subjects and samples collection

Semen samples from 75 men at ages of 25–46 were obtained after abstinence for 3–5 days.

2.2. Preparation of human sperm membrane antigens

Pooled semen samples obtained from 20 males with normal fertility history were liquefied and washed 5-times with PBS. Precipitated sperm preparations were suspended at 4°C for 1 h in Tris–HCl buffer containing 0.05% NP-40 and centrifuged at $17\,000 \times g$ for 30 min. The supernatants were collected for protein determination (Huang, 1993).

2.3. Preparation of human seminal plasma

Semen samples were liquefied and centrifuged at $1000 \times g$ for 10 min. The supernatants were collected for subsequent tests (Huang, 1993).

2.4. Routine examination of semen

According to the WHO laboratory manual for the examination of human

semen and semen–cervical mucus interaction (2nd edition, London, Cambridge, 1987), the samples were examined respectively for semen density, survival rate, viability and morphology, and then classified into normal and abnormal groups. The seminal plasmas were prepared by centrifugation at 3000 RPM for 10 min and stored at -20°C for subsequent tests.

2.5. Determination of the inhibitory effects on an ELISA to detect anti-sperm antibodies (AsAb)

Flat bottom polystyrene plates were incubated with sperm membrane antigens overnight at 4°C followed by washing in phosphate buffered saline (PBS) solutions containing 0.05% Tween-20 (PBS-T). A total of 100 μl of seminal plasma samples and 10 μl of AsAb positive sera were added to the plate and incubated for 1 h at 45°C , washed 3 times in PBS-T before the addition of 50 μl of a 1:500 dilution of horseradish peroxidase conjugated goat anti-human IgG. After incubation for 1 h at 45°C and three washes with PBS-T, 50 μl of OPD- H_2O_2 solution in substrate buffer were added and color was developed for 10 min at 37°C . The positive and the blank controls were carried out by addition of 100 μl of PBS-T and 10 μl of AsAb positive sera, or 110 μl of PBS-T, respectively. All ELISA assays were read in an automatic ELISA reader at 492 nm. Calculation of the inhibition rate of seminal plasma to AsAb reaction was as follows:

$$\text{ISR} = \frac{A_{\text{positive cell}} - A_{\text{seminal plasma cell}}}{A_{\text{positive cell}} - A_{\text{blank cell}}} \times 100\%$$

3. Results

3.1. Determination of the inhibitory effects on an ELISA to detect AsAb

A total of 75 samples of seminal plasma showed the inhibitory effects on AsAb reactions. The mean inhibition rate was $61.5 \pm 23.1\%$. The inhibition rate of 29 samples of the normal group was $71.14 \pm 18.25\%$ and that of 49 samples of the abnormal group was $55.43 \pm 23.98\%$, suggesting that the inhibition rate of the abnormal group is lower than that of normal group.

3.2. The relationship between the inhibition rate and the quality of sperm

The inhibition rate of seminal plasma to AsAb reaction was closely associated with the percentage of sperm survival rate, viability, density and

morphology. Seminal plasma samples with a survival rate more than 70% and less than 50% showed obvious differences in their inhibition rate to the AsAb reaction ($P < 0.01$), (Table 1). Seminal plasma samples with viability grades A, C and D also showed significant differences in their inhibition rate to AsAb reaction ($P < 0.05$, $P < 0.01$), (Table 2). Seminal plasma samples with normal morphology more than 70% and less than 50% had significant differences in their inhibition rate to AsAb reaction ($P < 0.05$), (Table 3). Seminal plasma samples of sperm density more than

Table 1

Relationship between the sperm survival rate and the inhibition to anti-sperm antibodies (AsAb) reaction by corresponding seminal plasma

Sperm survival rate (%)	<i>n</i>	ISR (%) $\bar{x} \pm$ S.D.	<i>P</i>
≥ 70	39	69.62 ± 16.68	
≥ 50	19	58.74 ± 23.76	< 0.05
< 50	17	43.72 ± 25.24	< 0.01

Table 2

Relationship between the sperm viability and the inhibition to anti-sperm antibodies (AsAb) reaction by corresponding seminal plasma

Sperm viability	<i>N</i>	ISR (%) $\bar{x} \pm$ S.D.	<i>P</i>
A	44	67.33 ± 19.74	
B	18	58.48 ± 23.29	> 0.05
C	8	48.76 ± 29.84	< 0.05
D	5	41.48 ± 24.65	< 0.01

Table 3

Relationship between the normal morphologic sperm and the inhibition to anti-sperm antibodies (AsAb) reaction by corresponding seminal plasma

Normal morphologic sperm (%)	<i>n</i>	ISR(%) $\bar{x} \pm$ S.D.	<i>P</i>
≥ 70	46	64.74 ± 21.12	
≥ 50	17	60.43 ± 26.60	> 0.05
< 50	12	50.59 ± 23.93	< 0.05

Table 4

Relationship between the sperm concentration and the inhibition to AsAb reaction by corresponding seminal plasma

Sperm concentration ($\times 10^6/\text{ml}$)	<i>n</i>	ISR (%) $\bar{x} \pm \text{S.D.}$	<i>P</i>
≥ 60	34	67.06 ± 21.51	
≥ 20	20	57.97 ± 23.24	>0.05
< 20	21	55.88 ± 24.60	>0.05

$60 \times 10^6/\text{ml}$ showed a higher inhibition rate than those with sperm density less than $60 \times 10^6/\text{ml}$, but was not significant ($P > 0.05$), (Table 4).

4. Discussion

Human seminal plasma can interfere, either directly or indirectly, with the function of most cells of the immune system including T cells, B cells, NK cells and macrophages. The immunosuppressive effects of human seminal plasma are mediated by several factors (Kelly, 1995). The prostaglandins of the E series predominate and raise intracellular AMP in leukocytes. By this mechanism they suppress lymphocyte proliferation, and natural killer cell activity, and are likely to modify cytokine release from antigen presenting cells. Therefore, acquired and innate responses (including immune surveillance) in the reproductive tract will be curtailed, at least temporarily, after intercourse. Semen contains several inhibitors of complement and a unique reservoir of CD59, a major complement inhibitor, is found on the prostasomes (sub-micron organelles with lipid membranes). The prostasomes also inhibit lymphocyte proliferation and the activity of phagocytic cells. Other suppressive agents are present in semen and may exert specific effects, e.g. transforming growth factor-beta which may inhibit primed responses to antigen, and receptors for the Fc fraction of gamma-globulin which might bind inflammatory agents. Among others, the presence of a 17-kDa CD4-masking factor in human seminal plasma may be relevant to the modulation of maternal immunity at insemination and to the control of sexual transmission of HIV-1 (Autiero et al., 1991).

Human seminal plasma can also impair the activity of antibody and complement molecules. Witkin et al. (1983) reported a IgG-Fc binding protein in seminal fluid. The seminal fluid fraction appeared to specifically react with the Fc portion of IgG. The seminal fluid Fc-binding protein was isolated by affinity chromatography on Fc coupled to CNBr-activated Sepharose 4B.

We suggest that the effects of the addition of seminal plasma on an ELISA used to detect antisperm antibodies include:

1. Human seminal plasma may adhere to sperm membrane antigens coated in the plate well, therefore, interferes with the binding of antisperm antibodies to coated antigens.
2. Human seminal plasma Fc-binding protein binds to the Fc portion of antisperm antibody, and therefore, blocks the binding site for subsequent enzyme-labeled secondary antibodies.
3. Human seminal plasmas adhered to the Fc of antisperm antibodies or sperm membrane antigens may transfer to bind the enzyme conjugated secondary antibodies' Fc portion, therefore, interfere with the enzyme activity in the ELISA used in the experiment (Fig. 1).

The observation might be related to events that occur *in vivo*. Seminal plasma can impair the activity of antibody at different stages and therefore inhibit the humoral immune response against sperms to ensure the passage of sperm through the female reproductive tract for successful fertilization. Evolutionarily, the good quality sperm might enable the seminal plasma to inhibit the anti-sperm antibody reaction, as it does in this experiment. Maybe the poor quality sperm lack some of immunosuppressive factors, and as a result, will somewhat trigger an immune response in the male or female against sperm. It is reasonable that the good quality sperm are given full the entire of immunosuppressive factors by the seminal plasma to make them 'be better and do better', even when anti-sperm antibodies exist.

The results revealed that the immunoinhibitory effect of human seminal plasma on anti-sperm antibody reactions was associated with the quality of sperms. The high inhibitory rate of the seminal plasma corresponds to the high quality of semen. Therefore, the determination of the inhibitory rate of human seminal plasma to anti-sperm antibody reactions may become one indication reflecting fertility in clinical reproductive immunology.

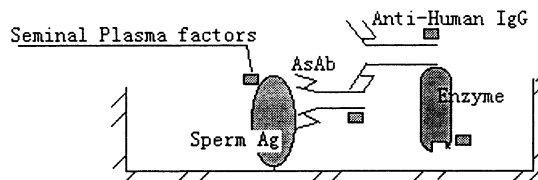


Fig. 1. Scheme of the effects of the addition of human seminal plasma on an ELISA used to detect antisperm antibodies. Seminal plasma is expected to interfere the detect system at several stages: (1) adhere to sperm membrane antigens; (2) bind to Fc portion of human antisperm antibody; (3) bind to Fc portion of anti-human IgG to which an HRP was conjugated as an enzyme label, thus interfering the activity of HRP; and a less possibility that they inhibit the HRP with activity in the final stage of enzyme catalyzed color development, due to the dissociation of seminal plasmas from their binding site into the solution for color development.

Furthermore, the observation might be consistent with the finding that human seminal plasma contains a component, which binds immunoglobulins (Ig) (Witkin et al., 1983; Liang et al., 1993). This seminal plasma may possess immunosuppressive activity and may modulate the activities of the immunosurveillance system of the reproductive tract. A major concern is that following infection of the cells of the cervix with virus, repeated exposure to human seminal plasma may accelerate the progression of disease and damage the local immune system. This also raises the question that if anti-HIV vaccine can effectively prevent individuals from the transmission of HIV through the human reproductive tract due to the inhibitory effects of human seminal plasma on antibody activity (Lu et al., 1999). Therefore, a better understanding of those complicated phenomena is needed for future therapy development or contraceptive development.

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