Free Radical Generation in Ejaculate Samples from Infertile Patients

I. V. Korotkova, M. A. Nikolaeva, V. A. Bozhedomov, E. L. Golubeva, A. Yu. Krutskikh, G. V. Ter-Avanesov, and G. T. Sukhikh

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Generation of free radicals in ejaculate samples from infertile patients was evaluated using chemiluminescent technique. The presence of antisperm antibodies in samples increased the possibility of damages to spermatozoon plasma membranes due to excessive generation of free radicals.

Key Words: *antisperm antibodies; free radicals*

The role of antisperm antibodies (ASAB) in reproduction attracted much recent attention. However, the effect of ASAB on fertilization remains unclear. Some authors revealed a relationship between the presence of ASAB and low probability of pregnancy [3,4], while others put this relationship in doubt [5,15].

Previous studies showed that ASAB affect spermatozoon viability. Cell viability considerably decreases after incubation with ASAB-positive sera from women and men [3]. Incubation with seminal plasma or cervical mucus from ASAB-positive patients decreased motility and viability of spermatozoa [9].

Free radicals (FR) continuously generated in spermatozoon increase membrane fluidity necessary for normal capacitation and acrosomal reaction. The spermatozoon membrane is enriched with polyunsaturated fatty acids, which determine its high fluidity and high sensitivity to damages associated with excessive FR generation [11]. Excessive FR generation activates lipid peroxidation (LPO) in spermatozoon plasma membranes, which causes cell damages.

Low viability of spermatozoa carrying surface ASAB can also result from cytotoxic effects of FR.

Here we studied FR generation in ASAB-positive and ASAB-negative ejaculate samples.

Laboratory of Clinical Immunology, Research Center of Obstetrics, Gynecology, and Perinatology, Russian Academy of Medical Sciences

MATERIALS AND METHODS

ASAB in ejaculate samples were detected by the standart MAR test (mixed agglutinin reaction) [7]. If more than 10% spermatozoa in the sample carried surface ASAB it was considered as ASAB-positive.

Before chemiluminescence measurement, 100 μl ejaculate was washed 3 times with Dulbecco's phosphate buffered saline (DPBS) and centrifuged at 1500 rpm for 5 min. The volume was brought to 500 μl with DPBS. Chemiluminescence was induced with 1 μl 100 mM luminol (5-amino-2,3-dihydro-1,4-phthalazinedione). Chemiluminescence intensity was measured every 35 sec for 40 min on a LKB-Wallac 1256 chemiluminometer (LKB-Wallac) under constant stirring. Control samples contained no luminol.

The intensity of FR generation was expressed in mV/sec. In the control chemiluminescence intensity did not exceed 0.4 mV/sec, which 2-fold surpassed the maximum baseline spontaneous generation under these experimental conditions. Chemiluminescence was maximum 2-10 min after luminol addition. The intensity of chemiluminescence was calculated per cell.

RESULTS

Maximum FR generation in MAR-positive samples was much higher than in ASAB-negative samples (4.17±0.50 *vs.* 1.24±1.50 mV/sec, $p<0.005$, Fig. 1).

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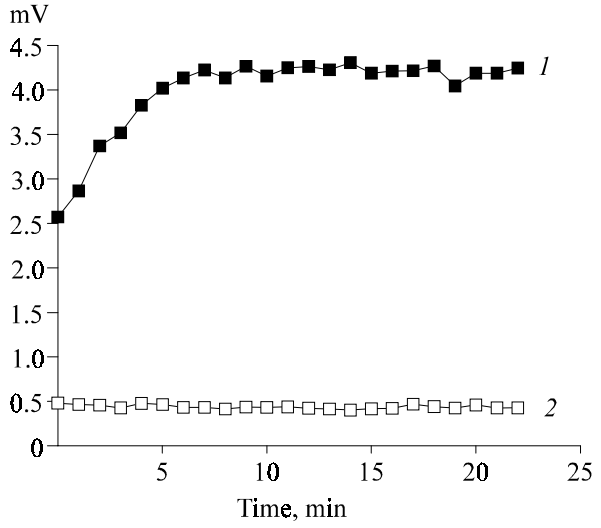

Fig. 1. Chemiluminescence intensity in MAR-positive (*1*) and MAR-negative ejaculate samples (*2*).

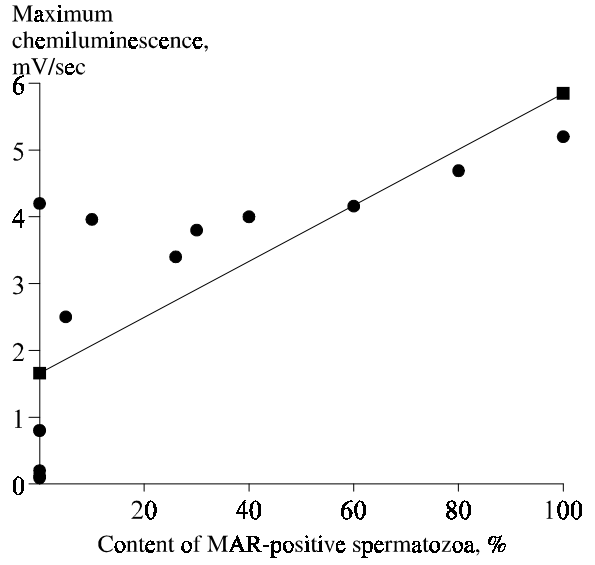

Fig. 2. Correlation between maximum chemiluminescence intensity and percentage of spermatozoa coated with antisperm antibodies.

We revealed a positive correlation (r=0.71, p<0.05) between maximum chemiluminescence in semen samples and the percentage of ASAB-coated spermatozoa (Fig. 2).

The intensity of FR generation depended on the content of MAR-positive spermatozoa in the suspension (Fig. 3, *a*). Increasing the concentration of cells in the test sample by 2 times led to a 4.4-fold increase in the maximum chemiluminescence intensity (p<0.001). When MAR-positive cells were washed 3 times, the maximum chemiluminescence intensity increased by 3.4 times (p<0.005 compared to single washout, Fig. 3, *b*).

The intensive FR generation in MAR-positive semen samples can be attributed to activity of spermatozoa and leukocytes [10] and/or the presence of antioxidants [13].

The regulated process of free-radical oxidation is an important component of spermatozoon metabolism. This process increases fluidity of cell membranes necessary for normal fertilization [1,2,6]. The capacity of the cell antioxidant systems (antioxidant enzymes glutathione peroxidase, catalase, and superoxide dismutase) is limited by low cytoplasmic volume. Intensive FR generation in ASAB-coated spermatozoa is probably associated with peculiarities of their intracellular antioxidant systems. The formation of antigen-ASAB complexes (AG-ASAB) on cell surface can also intensify FR generation.

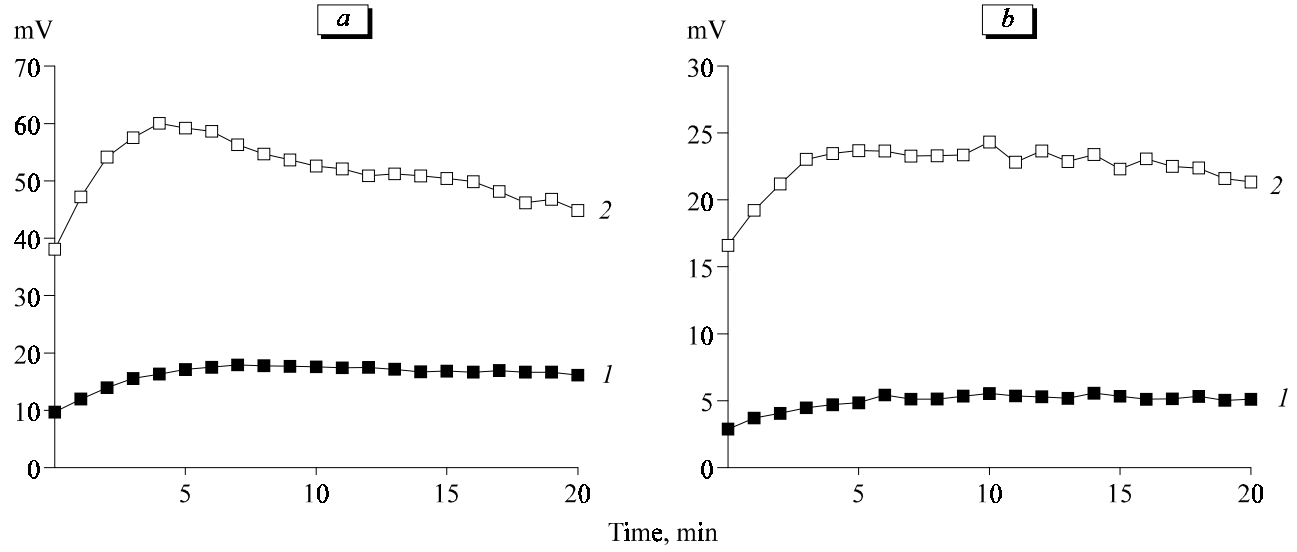

Fig. 3. Intensity of free radical generation as a function of spermatozoon concentration (*a*) and number of washings from seminal plasma (*b*). Ordinate: kinetics of chemiluminescence of MAR-positive ejaculate sample. Spermatozoon concentration (*a*): initial (*1*) and 2-fold increased (*2*). Number of washings (*b*): 1 (*1*) and 3 (*2*).

High concentration of antioxidant enzymes and FR scavengers in the seminal plasma is an important protective factor [13]. Moreover, prostate gland fluid, seminal plasma, and follicular fluid containing antioxidant enzymes protect spermatozoa from reactive oxygen species-induced damages during *in vivo* fertilization [1]. In our and previous experiments with ejaculate samples from infertile patients [1,8] intensification of FR generation after centrifugation and isolation of MRA-positive spermatozoa can be explained by removal of antioxidants.

The effects of spermatozoon concentration in samples on the intensity of FR generation can also be related to exhaustion of the protective antioxidant reserves, which accompanies the increase in cell count.

Our previous studies showed that the increase in cell concentration is accompanied by intensive aggregation of AG-ASAB complexes on the surface of spermatozoa and their shedding (removal) from cell surface [12]. Thus, intensive shedding of AG-ASAB complexes is probably associated with increased FR generation, which intensifies LPO in spermatozoon plasma membranes [14].

Our results indicate that the presence of ASAB in ejaculate samples modulates FR generation in the semen from infertile patients. This probably contributes to decreased viability of spermatozoa in ASAB-containing samples.

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