Effect of Serum sperm antibodies in women on embryo quality / pregnancy outcome following IVF a controlled study

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abstract

A high titer in the male, specifically in the semen, reduces the potential for fertility. The high titer leads to inhibition of sperm progression in the mucus, as shown by poor post-coital tests. Women of over half of the men with positive ASA achieved pregnancy following IUI. In the female, the importance of the effect of antibodies present in the mucus against the sperm has been stressed. However, whether the presence of circulating ASA in the female interferes with egg fertilization, embryo development, and pregnancy rates is still controversial. The present study was conducted to evaluate the circulating ASA, defined as greater than 20% IgG or IgA, on the fertilization rate, cleavage rate, and pregnancy rate in patients undergoing IVF-ET. These rates were compared with 61 controls who had the procedure on the same day, but had no ASA in their serum. In the ASA group the number of fertilized oocytes per patient was similar. There were no differences in the cleave rate compared to controls. There was a significant difference in the clinical pregnancy rate between the two groups. Specifically, 7 out of 61 pregnancies were achieved in the ASA group versus 16 of 61 in the control group ($p < 0.03$). ASA did not interfere with the fertilization / cleavage rates of embryos *in vitro*. However, ASA may interfere with the effect on pregnancy rate.

Introduction

The presence of sperm antibodies in the female seem to be associated with impaired reproductive function. There is an association between poor post-coital test and the presence of sperm antibodies in the initial entry of the sperm into the mucus. However, it does impair the progressive motility. A typical feature is the presence of the shaking heads phenomenon. The presence of sperm antibodies on the head, midpiece, tail, and tail tip have been identified with varying functional significance. Those antibodies that bind to the head appear to be of the highest significance since they can interfere with the fertilization process. The presence of sperm antibodies in the serum has been previously reported(Clarke et al, 1985). However, the correlation is far better between cervical mucus than serum antibodies. However, though the

mucus is the only readily accessible site for evaluation of the presence and effect of ASA, fertilization and implantation take place elsewhere, specifically in the oviduct or endometrium, where ASA could be present.

Fertilization rate is lower in patients with circulating ASA(Clarke et al, 1986; Kamada et al, 1985; Mandelbaum et al, 1987), however, the specific features associated with embryonal development have not been evaluated. This could be of importance since the ASA present on the granulosa cells, the oocyte, or in the follicular fluid could, theoretically, interfere with embryo development. A compounding factor is the technique by which the sperm antibody measurement is carried out since disagreement remains as to which is the best test to be used for this purpose. The effect of ASA on pregnancy has not been evaluated following IVF when fertilization is detached from implantation.

The present study was conducted to evaluate the effects of ASA in maternal serum on fertilization, cleavage, and pregnancy rates. This was compared to randomly selected patients who underwent IVF-ET on the same day.

Materials / Methods

A total of 122 patients undergoing IVF were studied. Before stimulation protocols were initiated. Each patient was tested for sperm antibodies. In 61 patients, positive(greater than 20%) serum ASA IgG of IgA was detected. These patients were compared with 61 patients chosen on a random basis with negative ASA who had egg retrieval on the same day. Most patients were given Lupron followed by hMG/hCG regimens, estradiol, and ultrasound measurements. When the follicles exceeded 16mm, hCG was administered and, within 36 h, the eggs were retrieved using the vaginal route. Following retrieval, the eggs were placed in culture media containing HTF / BSA, and were inseminated with sperm (50,000 per dish). The eggs were observed with 24 h to determine if fertilization had occurred. Eggs which did not fertilize were reinseminated by adding fresh sperm.

1) Assessment of embryo quality: The embryo quality was assessed by estimating the rate of mature follicle fertilization / cleavage rate using an inverted microscope with Hoffman Optics at 200 and 400 magnification light microscopy.

2) Sperm antibody assay: The method for ASA measurement was previously published (Check / Bollendorf, 1992). It is based on the indirect Immunobead test which utilizes beads consisting of micron-size polyacrylamide spheres covalently linked to rabbit antihuman antibodies. The serum was initially incubated at 56°C for 30 minutes to inactivate the complement. The assay was performed by adding the serum to donor sperm which was free of ASA and incubation for 30 minutes at 37°C. Following incubation, the sample was centrifuged at 3500 rpm for 8 seconds. Subsequently, the sample was washed three times with D-PBS/BSA solution. Five ul of the sperm suspension was then added to an aliquot of IgG and IgA immunobead suspension. The percentage of binding and binding sites were examined on a slide preparation using phase contrast microscopy. Binding of at least 3 beads per sperm was considered positive. the test was considered to be positive at greater than 50%, and weakly

positive. The test was considered to be positive at greater than 50%, and weakly positive at 20 to 49%; 0 to 19% were considered as negative results.

Statistical analysis was carried out using Student's t test and chi square analysis.

Results

Fertilization rate in the two groups was similar (65% vs 68%). The mean number of fertilized eggs in the ASA patients was similar to that of controls (Table 1). There were also no differences in the cleavage rates of the two groups. The number of fragmented embryos was also similar (38% vs 50%).

Table 2 shows the pregnancy rate and outcome in patients with ASA. When compared to controls, there was a two-fold lower rate of pregnancy which is statistically significant if compared to the rate of pregnancies that reached term, specifically 5 out of 61 experimental group pregnancies versus 13 of 61 pregnancies in the control group ($p < 0.03$). The pregnancy outcome and type of ASA reveals no differences between IgA and IgG(Table 3). The distribution of the antibodies was similar for both IgG and IgA. There were no differences in the number of the embryos transferred for the ASA / control groups(4.2 vs 4.4, respectively).

Discussion

The clinical pregnancy rates in patients with ASA is significantly lower than controls. However, this is not caused by poor embryo quality since the number of transferred embryos were similar. The egg quality, ability of the sperm to fertilize, and the developmental rate of normal embryos *in vitro* was also similar to that of controls. This study is of importance since it took into account the use of a matched control group which was randomly selected on the same day that the IVF procedure was performed on the ASA patients. The laboratory quality, techniques, culture media, and the embryologist who performed the procedure were also controlled factors in the present study. It appears that ASA in the follicular fluid, granulosa cells, or attached to the zona pellucida do not interfere with embryo formation *in vitro*. Whether this occurs *in vivo* cannot be excluded. Sperm-specific antibodies may interfere with the ability of the sperm to bind to the zona pellucida(Bronson et al, 1982, 1983). The presence of ASA in maternal serum are associated with the presence of Interferon gamma which causes an autoimmune interaction and may have a toxic effect on the embryo(Witkin et al, 1992).

Once the embryo is replaced into the uterus, it is exposed to ASA present in the uterine secretions, thus interfering in the implantation process and reducing successful pregnancy rate. There is a tendency for pregnancy rate to decline 12.5% versus 18% in controls. The major difference between our previous study and the current investigation is that the controls in that study were not matched for the day of the procedure.

Infertility is not due to the process of failed fertilization, though one might argue that, *in vivo*, the egg and sperm may be exposed to ASA present, perhaps in the oviduct, which might interfere in the process locally. The present study is at variance with a previous report by Witkin et al. (1992) which found a significant reduction in the fertilization rate. However, the study

Table 1 **Fertilization / cleavage of eggs in ASA and control groups(mean ISD)**

	ASA Group (n=61)	Control N=61
No. of fertilized eggs	5.8 ± 0.7	6.3 ± 0.7
No. of cleaved embryos	5.7 ± 0.7	6.3 ± 0.6

Table 2 **Pregnancy outcome in patients with positive ASA and controls**

Parameters	ASA Group (n=61)	Control (n=61)
Delivery	5	16
Spontaneous abortion	1	3
Chemical pregnancy	1	0
Ectopic pregnancy	1	0

Table 3 **Type and percentage of ASA in pregnant patients**

Subject	% IgA	% IgG	Outcome
1	96%	24%	Delivery
2	42%	33%	Delivery
3	33%	15%	Delivery
4	7%	37%	Delivery
5	62%	16%	Delivery
6	8%	38%	SA
7	35%	62%	Ectopic
8	35%	62%	Chemical

SA = Spontaneous abortion

employed maternal serum in the culture media.

The preparation of the oocyte for fertilization and embryo development does not require any specific washing or other procedures to reduce antigenicity. However, this provides only a partial solution for these couples since the pregnancy rates and outcomes are different, despite adequate embryos, in IVF patients who have ASA in their serum. Therefore, newer approaches, perhaps including pre-IVF use of steroids, should be contemplated in these couples to reduce the rate of both implantation and post-implantation failure. Based on this data, all couples should be screened for ASA before attempting IVF.

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