

## **Does Intrauterine Insemination in Saudi Female Cause Production of Antisperm Antibodies?**

Hassan S Jamal\*

**Objective:** To determine the possibility of inducing antisperm antibodies in patients undergoing intrauterine insemination with motile sperms from the husband.

**Design:** Clinico-immunological prospective study.

**Setting:** King Abdulaziz University Hospital.

**Subjects:** Fifty patients undergoing Intrauterine Insemination (IUI), were tested for developing antisperm antibodies using agglutination test, complement mediated immobilization test, and immunoglobulin specific indirect immunobead assay.

**Main outcome measures:** Incidence and type of antisperms antibodies.

**Results:** Forty seven out of 50 patient remained negative for antisperm antibodies after 2-6 cycles of IUI. Detection of antisperm antibodies after IUI was evident in 3 patients (6%).

**Conclusion:** Intrauterine insemination does not appear to be a significant or lasting immune response.

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Immunologic effects are important factors at many levels in reproduction including fertilization, implantation and the development of placenta<sup>1</sup>. Possible bad effects on potential development of embryo range from prevention of fertilization<sup>2</sup>, maintenance of the fetoplacental unit<sup>3</sup>, or deleterious fetal and neonatal effects caused by the passage of maternal IgG across the placenta<sup>4</sup>. From the above, we can realize the significance of development of immune factor in infertility couples. Hence, this study was conducted to analyze the risks of IUI and subsequent formation of serum antisperm antibodies.

However, scientific proof that antisperm antibodies cause infertility is still awaited through large prospective studies<sup>5,6</sup>.

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\* Consultant & Associate Professor  
Department of Obstetrics & Gynaecology  
King Abdulaziz University Hospital  
Jeddah  
Saudi Arabia

## **METHODS**

Serum samples were obtained from 50 patients (women) undergoing IUI with husband sperm at KAUH reproductive endocrinology and infertility clinic. The number of IUI ranged between 2-6 inseminations. The blood was taken before the IUI procedure and 6 weeks later.

**The sperm processing was as follows:**

For fresh semen samples, the whole ejaculate was washed in Pure Sperm Media (Nidacon International AB, Sweden) using aseptic technique in a Laminar Flow bench and allowing them to attain ambient temperature.

- Using a sterile Pasteur pipette, we transferred 1-2 ml of the most dense gradient, (90%) into the bottom of a sterile centrifuge tube.
- Using a second sterile pipette, carefully we layered 1-2 ml of the less dense gradient (45%) over the first.
- Using a third sterile pipette, carefully we layered 1-2 ml of the liquefied semen over the two gradients.

Without disturbing the layers, we placed the centrifuge tube and contents into a standard laboratory centrifuge where the tube and contents were centrifuged at 300 XG for 20 minutes. A pellet of sperm formed at the bottom of the centrifuge tube. We isolated the pellet either by removing it to another centrifuge tube with a new Pasteur pipette or by carefully removing all the contents of the original centrifuge until only the pellet remained. We re-suspended the pellet in 2-3 ml of an appropriate sperm washing medium and allowed the tube to stand for a few minutes. We centrifuged again, this time at 200 XG for 10 minutes. We isolated the sperm pellet and repeated the wash / centrifuge process a second time. Then we re-suspended the sperm pellet in a suitable volume of culture medium to attain the required sperm concentration per ml. This sperm pellet would be injected into the uterine cavity by the Nach Lisse Labotect Catheter (Labotect GmbH, Labor-Technik-Gottingen, Germany).

**TESTING FOR ANTISPERM ANTIBODIES**

The presence of serum antisperm antibodies was determined in each patient by using three different tests before and after each insemination. Serum was tested against sperm (antigen) obtained from the same individual to reduce variability of the assays. After swim up in special media for 1 hour, sperm concentration was adjusted to 25-50 x 10<sup>6</sup> sperms/ml for agglutination and immunobead tests, the serum complement was inactivated by heating at 58<sup>0</sup>C for 30 minutes. Agglutination and immobilization assays were carried out as described originally by Franklin and Dukes<sup>7</sup> and Isojima et al<sup>8</sup> respectively.

For the third test ie. indirect immunobead test <sup>9</sup> the serum was first reacted with the sperms, then the serum was removed and the sperms reacted with a suspension of isotype-specific immunobead (IgA, IgG, IgM) from Bio-Rad Richmond, California. The sperms were then scored for surface-bound immunoglobulin. The presence of one or more beads attached to > 20% of motile sperms was considered as positive for antisperm antibodies.

## RESULTS

Fifty Saudi women completed the final study period. The results are summarized in Table 1.

Table 1. **Development of Antisperm Antibodies before and after IUI**

<i>Number of Inseminations</i>	<i>Number of Patients</i>	<i>Positive for Antisperm Antibodies Before IUI</i>	<i>Positive for Antisperm Antibodies After IUI</i>
1	4	None	None
2	11	None	One patient positive for Agglutination (IgG,IgM)
3	16	None	None
4	6	None	None
5	6	None	Two patients positive for Immobilization only
6	7	None	None

The detection of antisperm antibodies after IUI treatment was evident in 3 (6%) patients. Two of these patients showed agglutination - positive and immunobead-positive after 5 IUI cycles. The most interesting finding is that 47 out of 50 patients remained negative for antisperm antibodies after 2-6 IUI cycles.

## DISCUSSION

The major bulk of sexually active women do not have circulating antisperm antibodies, despite their frequent exposure to sperms. A minority of women produce antisperm antibodies but the cause of antibody production is poorly understood. Infection in the genital tract or inflammation may induce the production of antibodies by increasing the local concentration of lymphoid cells and antibody-promoting lymphokines. Microorganisms or their products may also react with the sperm and increase their antigenicity. Many factors on the other hand are believed to inhibit an immune response to sperm in the majority of women. Cervical infiltration of sperm, phagocytosis of sperm-cell in the genital tract, and immuno-suppressive substances present in seminal fluid are just some of the mechanisms described<sup>10</sup>.

Sperms may reach the peritoneal cavity where immunological stimulation may occur. It has been postulated that antisperm antibodies may appear in circulation after intrauterine insemination. In our study we addressed this hypothesis by testing 50 women for the development of serum antisperm antibodies after repeated IUI procedures. Our study revealed that although a large number of washed sperms were placed directly into the uterine cavity facilitating the appearance of sperms in the peritoneum, a very low incidence (6%) of antisperm antibodies occurred after repeated IUI cycles. Furthermore, none of the patients tested positive by all three methods.

## CONCLUSION

**Based on these results we can conclude that exposure of upper female genital tract to washed male gamete (sperm) during repeated IUI with husband's sperms does not significantly stimulate the production of serum antisperm antibodies.**

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