Abstract

**Objectives:** 1. To investigate the incidence, isotypes and location of antisperm antibodies (ASA) on the sperm surface in an infertile population. 2. To study the influence of isotypes, location of ASA on the sperm surface on fertilization, cleavage rates of embryos and pregnancy rate.

**Methods:** A prospective analytical study was carried out on infertile couples who underwent Assisted Reproductive Technology (ART) procedures at ‘Prarthana’ Centre for in vitro fertilization (IVF) during three year period from 01.01.2006 – 01.01.2009. Presence of ASA was elicited using mixed agglutination reaction latex bead test (Sperm MAR, Fertipro NV, Belgium) in male and female subjects. The isotype and location of ASA, detected by the type (i.e. IgA, IgG) and the site of binding of latex beads to ASA on the spermatozoa (i.e. head, midpiece, tail of the sperm) was analyzed. In females who underwent In-vitro fertilization, fertilization rate and day 03 cleavage rate of embryos were assessed. The pregnancy rates following each ART procedure were noted. A total of 230 infertile couples were investigated.

**Results:** Out of 230 couples, 48 (20.86%) were positive for ASA. Among ASA positives, 19 (39.58%), 13 (27.08%), 16 (33.34%) had IgA, IgG, both IgA, IgG respectively and 10 (20.83%), 24 (50%), 11 (22.92%), 03 (6.25%) had head, tail, midpiece+tail, whole sperm bound ASA respectively. The total fertilization rate and cleavage rate among ASA positives were 69.38% (179/258) and 53.63% (96/179) respectively; and among ASA negatives the rates were 58.54% (473/808) and 61.73% (292/473) respectively. Couples with IgA, IgG and IgA+IgG achieved a pregnancy rate of 10.53%, 23.08% and 18.75% respectively. Out of 08 pregnancies 05, 02, 01 had ASA on tail, midpiece+tail and whole sperm respectively.

**Conclusions:** It was found that presence of ASA has negative effects on fertilization and post fertilization events. IgA isotype of ASA demonstrated the lowest pregnancy rate. When majority of sperms are coated with ASA on the heads or mid piece+tail, cleavage rates were often impaired. Intra-cytoplasmic sperm injection is recommended for such couples to achieve a successful pregnancy.

**Key words:** Antisperm antibodies, incidence, isotypes, binding site, in vitro fertilization, intracytoplasmic injection, pregnancy rate.

Introduction

Research in antisperm antibodies (ASA) began in 1899 when Landsteiner initially reported that sperm could be antigenic if injected into a foreign species. Since then ASA in infertility has been largely investigated and has led to the possibility that naturally occurring male and female ASA could serve as one mechanism for human infertility. The incidence of ASA has been estimated to range from 9%–36% in infertile couples depending on the test format, detecting assays and the reporting centre. ASA have been theorized to negatively impact fertility by affecting sperm motility, cervical mucus penetration, gamete fusion and potentially even the first steps of embryo development. These antibodies present within the reproductive tract may be both transudates from the blood or secreted locally by submucosal plasmacells. Developmental abnormalities of the formation of the blood-testis barrier and its traumatic disruption could
lead to ASA formation in males. In females, mechanical or chemical disruption of the mucosal layer of the female genital tract may permit exposure to foreign sperm antigens and ultimately formation of ASA. Women undergoing repeated intrauterine insemination (IUI) with washed spermatozoa, are also thought to be prone to develop ASA. Removal of immunosuppressive properties of seminal plasma when preparing the semen for IUI could be an added factor. There are three major structural types of antisperm immunoglobulins: IgA, IgG and IgM; each of the three types can be bound to the whole sperm surface or selectively to the head, midpiece or tail of the spermatozoa when assessed by the latex bead technique. IgM is a larger molecule and rarely present in semen or cervical mucus. Also it is rarely detected alone or combined with IgA/IgG. Therefore it is generally considered as having less clinical importance.

The aims of this study were,

1. To find out the incidence of ASA among infertile couples in a Sri Lankan set up.
2. To study the effect of ASA on fertilization, cleavage rate of embryos and clinical pregnancy rate comparing ASA positive and negative couples.
3. To study the influence of isotype and topographical location of ASA on fertilization, cleavage and clinical pregnancy rate of ASA positive couples.
4. To demonstrate whether intracytoplasmic sperm injection (ICSI) is preferable to standard IVF (in vitro fertilization) as the method of oocyte insemination in certain isotypes and locations of ASA in ASA positive couples.

This study also compares the findings with those already published in the literature and provides valuable insights for the investigation and management of infertile couples in Sri Lanka.

Materials and methods

This prospective analytical study was conducted on infertile couples undergoing ART (assisted reproductive technology) procedures at ‘Prarthana’, Centre for IVF, 1175, Cotta Road, Rajagiriya, from 01.01.2006 to 01.01.2009. All infertile couples who consented (informed written consent was taken) and who was seeking treatment at ‘Prarthana’, Centre for IVF were recruited for the study. The total study population was 230 infertile couples; 460 individuals.

The ethical clearance for the study was obtained from the Ethical Review Committee of Faculty of Medical Sciences, University of Sri Jayawardenepura, Gangodawila, Nugegoda, Sri Lanka.

Each couple underwent a single cycle of ART procedure i.e. either IUI or IVF-ET (embryo transfer) as the method of treatment for infertility. The method of treatment for each couple was decided depending on other factors such as age, duration of infertility, previous failed treatment procedures etc. other than presence of ASA. In the male partner spermatozoa, seminal plasma and serum were examined for ASA isotypes of IgA and IgG. In the female partner, cervical mucus and serum were examined for both ASA isotypes. In females who underwent IVF, follicular fluid was also examined for ASA-IgA and IgG.

The ASA were detected using mixed agglutination reaction latex bead test (SpermMAR, Fertipro NV, Belgium). In mixed agglutination reaction (MAR) assay, blood group O Rh positive erythrocytes are coated with human IgG or IgA and are subsequently mixed with washed or unwashed, viable sperms. The sperm erythrocyte agglutination in the presence of ASA is observed by light microscopy. Instead of erythrocytes coated with immunoglobulin, the ‘SpermMAR’ test, which is a commercially available test, have IgA and IgG coated latex particles to detect ASA. This test provides a rapid assay time, good specificity, isotype and the location of the ASA and the ability to use viable sperms.

The direct ‘SpermMAR’ test was performed on spermatozoa and indirect test was performed on seminal plasma, cervical mucus, serum and follicular fluid to elicit ASA. The percentage of motile sperm exhibiting latex bead binding was calculated. A test with >30% of the sperms with bound beads was considered ‘positive for ASA’ (WHO laboratory guidelines). The isotype of the ASA was observed. Furthermore, the site of attachment was also considered: binding to the head, midpiece, tail or to the whole sperm as well as to more than one region was indicated.

Washed sperm samples were used for both IUI and IVF. In couples that underwent IVF, the preovulatory oocytes in the metaphase II and late metaphase I stages of development were considered in determining the fertilization rate. Oocytes were inseminated either by standard IVF or intracytoplasmic sperm injection (ICSI). In standard IVF a drop of sperms containing 1×106-2×106 mil/ml sperms was placed aside of the oocyte in-vitro. In ICSI a single sperm was manually injected into the cytoplasm of the oocyte.
Oocytes were observed 18-24 hours following insemination by standard IVF or ICSI under the inverted microscope. Fertilization of the oocyte by the sperm was confirmed, if the cell had 02 pronuclei and 02 polar bodies. Fertilization rate was assessed as,

Fertilization rate = 
\[
\frac{\text{total no of fertilized oocytes} \times 100}{\text{total number of inseminated oocytes}}.
\]

Day 03 cleavage of the embryo was observed under the inverted microscope. Embryos with 6-8 evenly placed cells with no or mild to moderate fragmentation and cytoplasmic granularity were considered as well cleaved embryos. Cleavage rate was assessed as,

Cleavage rate = 
\[
\frac{\text{no of good quality D3 embryos} \times 100}{\text{total number of fertilized oocytes at day 01}}.
\]

Following each ART procedure the clinical pregnancies were noted. The clinical pregnancy was confirmed at 08 weeks of gestation by an ultra sound scan revealing a foetus with heart beat. Clinical pregnancy rate was assessed as,

Clinical pregnancy rate = 
\[
\frac{\text{total number of clinical pregnancies} \times 100}{\text{Total number of embryo transfers}}.
\]

Two sample z test and Fisher’s exact test were used to analyze statistical significance either with two categories or with ASA negative category. ANOVA was used to analyze data in more than two categories in this study. The fertilization and cleavage rates of ASA negative category were taken as the standard to compare with the ASA positive category.

Results

Incidence of antisperm antibodies

The incidence of antisperm antibodies among the infertile couples of the study sample was 20.87% (n=48, 48/230). Antisperm antibody incidence among the males in the sample was 12.61% (n=29, 29/230) while that for the females was 8.26% (n=19, 19/230).

Effect of ASA on fertilization rate, cleavage rate and clinical pregnancy rate in ASA positives and ASA negatives

Figure 1 shows the number of couples underwent each procedure, number of clinical pregnancies achieved in each procedure and total number of inseminated oocytes, number of fertilized oocytes and number of cleaved embryos in those who underwent IVF. Table 1 shows the fertilization and cleavage rate of embryos among ASA positives and negatives in IVF. It was observed that in ASA positives the total fertilization rate (69.38% both standard IVF and ICSI inclusive) was significantly higher (P-value=0.001) than that of the ASA negatives (58.54%). When comparing the total cleavage rates, it was observed that ASA positives (53.63%) had a significantly lower (P-value=0.037) cleavage rate than that of ASA negatives (61.73%). Therefore, this data shows that although higher fertilization rates are seen in ASA positives, the cleavage rates are significantly lower than the ASA negatives, supporting the fact that ASA have negative effects on post fertilization events.

Among ASA positives, it was observed a proportionately higher clinical pregnancy rate in those who underwent IVF (19.23%) than those who underwent IUI (13.64%). Among ASA negatives the clinical pregnancy rates were 22.54% in IVF and 20.24% in IUI. Statistical significance was not calculated here due to small sample size.

The occurrence of immunoglobulin (Ig) isotypes of ASA

Among ASA positives, 19 (39.58%), 13 (27.08%) and 16 (33.34%) demonstrated IgA, IgG and both IgA+IgG respectively.

Impact of Ig isotypes of ASA on fertilization rate and cleavage rate

Table 2 shows the fertilization and cleavage rates corresponding to the Ig isotypes of ASA. Among Ig isotypes it was observed that there was no significant difference in fertilization rates and cleavage rates when oocytes undergo standard IVF or ICSI. Hence it shows that Ig isotypes do not have an impact on method of insemination of oocytes. Irrespective of the method of insemination, the total fertilization rate of oocytes were higher with IgA (82.76%) and IgG (70.75%) compared to that of ASA negatives (58.54%). The cleavage rates were markedly low in IgA (45.82%) and IgG (49.33%) compared to that of ASA negatives (61.73%), though there is no statistically significant difference observed. In IgA+IgG, the total fertilization rate (65.04%) and cleavage rate (60%) differ marginally with those of ASA negatives. IgA isotype was observed as having the highest fertilization rate and the lowest cleavage rate.