FINAL REPORT NSF

Section 1

Information regarding Project/Project Personnel:

i) Research Grant Contract Number : RG/2007/HS/02

ii) Title of the Project
Prevalence of antisperm antibodies (ASA) and its association to subfertility in couples undergoing assisted reproductive technologies (ART) at a selected centre

iii) Principal Investigator
Professor Surangi Gayaneetha Yasawardene

iv) Co-Investigators
Dr Varuni Tennakoon

v) Institute(s) where research was being carried out
1. Research Laboratory, Dept. of Anatomy, Faculty of Medical Sciences, University of Sri Jayewardenepura.
2. IUI Laboratory, 'Prarthana', Centre for ART, No 1175, Cotta Road, Rajagiriya

vi) Date of award
19/03/2007

vii) Date of completion of Project
03/04/2009

viii) Total allocation of funds (Rs)
Nine hundred and forty five thousand, seven hundred and six rupees.
Rs 945,706.00 for a period of two years

ix) Total spent (Rs)
Three hundred and thirty seven thousand, two hundred rupees.
Rs 337,200.00

x) Number of Research Students employed
None

xi) Post graduate degree completed with dates
MPhil Degree – Registered at University of Sri Jayewardenepura and at final stages of thesis writing

xii) Number of Technical Assistants and/or labourers employed and period of service
None

xiii) Publications/Communications arising from the project during the reporting period

Full Papers

1. Varuni Tennakoon, Surangi G.Yasawardene and Deepal S. Weerasekera. Antisperm antibodies: Incidence, isotypes and location on spermatozoa, their implications on fertilization and on pregnancy rate at a selected centre in Sri Lanka.

Sri Lanka Journal of Obstetrics and Gynaecology 2010; 32: 8-16.

2. Varuni Tennakoon, Deepal S. Weerasekera and Surangi G.Yasawardene. Successful pregnancy outcomes following in-vitro fertilization in infertile couples with high levels of antisperm antibodies.

Sri Lanka Journal of Obstetrics and Gynaecology 2010; 32: 47-48.

Published Abstracts

1. Tennakoon V, Weerasekera DS, Yasawardene SG, Ralapanawe MSB. Successful pregnancy following intra cytoplasmic sperm injection (ICSI) and embryo transfer in a female with high antisperm antibody (ASA) levels.
40th Annual Scientific Sessions, Sri Lanka College of Obstetricians & Gynaecologists 3-4 Nov 2007.

2. Tennakoon V, Weerasekera DS, Yasawardene SG. Effect of antisperm antibodies on fertilization and cleavage rates in subfertile couples undergoing in-vitro fertilization.
40th Annual Scientific Sessions, Sri Lanka College of Obstetricians & Gynaecologists 3-4 Nov 2007.

3. Tennakoon V, Yasawardene SG. Incidence of Antisperm Antibodies (ASA) in male and female subjects undergoing subfertility treatment at a selected centre.
63rd Annual Session of Sri Lanka Association for the Advancement of Science. 5-8 Dec. 2007.

4. Tennakoon V, Weerasekera DS and Yasawardenen SG. Detection of Antisperm antibodies, pattern of distribution on spermatozoa and its implications on assisted reproductive technologies.
41st Annual Scientific Sessions, Sri Lanka College of Obstetricians & Gynaecologists In association with Asia - Oceania Federation of Obstetrics & Gynaecology and supported by Indian College of Obstetrics and Gynaecologists 27-29 June 2008.

5. Tennakoon V, Weerasekera DS and Yasawardenen SG. Descriptive analyses of Antisperm antibodies (ASA) in a subfertile population.
41st Annual Scientific Sessions, Sri Lanka College of Obstetricians & Gynaecologists In association with Asia - Oceania Federation of Obstetrics & Gynaecology and supported by Indian College of Obstetrics and Gynaecologists 27-29 June 2008.

6. Tennakoon V, Yasawardenen SG and Weerasekera DS. The influence of Antisperm antibodies (ASA) on embryo cleavage and pregnancy rate in subfertile couples undergoing Assisted Reproductive Technologies (ART).
Sri Lanka Medical Association 122th Anniversary academic Sessions, March, 2009.

7. Tennakoon V, Yasawardenen SG and Weerasekera DS. Effects of antisperm antibodies on fertilization, cleavage and pregnancy rate in infertile couples undergoing In-vitro Fertilization at a selected centre in Sri Lanka.
The Third World Congress on Mild Approaches in Assisted Reproduction, Yokohama, Japan, 30-31 July 2010.

8. Tennakoon V, Yasawardenen SG and Weerasekera DS. Effects of immunoglobulin isotype and sperm surface location of antisperm antibodies on fertilization, cleavage and pregnancy rate in human – A Sri Lankan study.
The Third World Congress on Mild Approaches in Assisted Reproduction, Yokohama, Japan, 30-31 July 2010.

Section 2

Executive Summary of the project

The presence of antisperm antibodies (ASA) can reduce fecundity in both males and females. The immuno-regulatory mechanisms of generation of ASA, their effects on gametes and gamete interactions have been studied extensively, however, some of its clinical implications on infertility is disputed so far. The literature in the field is quite scarce in Sri Lanka. With the availability of assisted reproductive technologies (ART), detection of the possible causes of infertility will enable to streamline the treatment. The present study was performed to investigate the incidence of ASA in infertile couples, to detect effects of these antibodies on fertilization processes and pregnancy outcome following ART procedures (intra uterine insemination-IUI / in vitro fertilization-IVF) and compare the results in such couples and couples without ASA.

Two-hundred and thirty infertile couples were studied from January 2006 to January 2009. Relevant clinical data were obtained by self administered questionnaire and clinical examination. Presence of ASA was elicited using mixed antiglobulin reaction latex bead test (SpermMAR, Fertipro NV, Belgium). In males spermatozoa, seminal plasma and serum samples were analyzed for ASA and in females cervical mucus, serum and follicular fluid were taken for analysis. The test was considered positive if 30% or more of the motile sperm were attached to the latex particles. 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) were observed. In couples underwent IVF, fertilization rate and day 03 cleavage rate of embryos were assessed. The pregnancy/miscarriage rates following each ART procedure were noted.

The incidence of ASA was 20.87% among the infertile couples with males having a higher incidence (12.61%) than the females (8.26%). There was no significant correlation observed with presence of ASA and age, duration of marriage, duration of infertility, type of infertility and occupation of both males and females. A statistically significant association (P-value=0.036) between presence of ASA and a history of genital surgery was observed in males. The incidence of ASA was proportionately higher among women those who had previous IUIs (11.7%) compared to the women who did not have IUIs (5.88%). Also a significant association of ASA was observed in females with pelvic inflammatory disease.

The total fertilization rate was significantly higher (P-value=0.001) in ASA positives than that of the ASA negatives. The total cleavage rate was significantly lower (P-value=0.037) in ASA positives than that of ASA negatives. There was no significant difference observed in fertilization rates and cleavage rates among the Ig isotypes. However, IgA isotype of ASA was observed as having the highest fertilization rate and the lowest cleavage rate. Head or midpiece+tail bound ASA on spermatozoa were observed to have more negative effects on cleavage rate. In ASA positives there was a marked increment in pregnancy rate when they underwent IVF (19.23%) than IUI (13.64%). The effects of isotype and location of ASA on clinical pregnancy rate and miscarriage rate could not be evaluated statistically due to small number of subjects in some categories. However, it was noted that best samples for screening for ASA for male would be IgA ASA on spermatozoa and for female IgA and IgG ASA in serum.

Section 3
Report in detail

(i) Background

Historical perspective

The definition of infertility is that the inability of a couple to conceive irrespective of regular unprotected intercourse for a period of one year. Studies show that one in five reproductive age couples have infertility. 15% of them have unexplained infertility. There is now mounting evidence to support the immunomodulation of fertility in many of these couples. One major aspect of this immunomodulation may be the presence or absence of antisperm antibodies (ASA) (Mazumdar et al. 1998).

Landsteiner and Metchnikoff first demonstrated that spermatozoa were immunogenic more than a century ago (Bronson 2000). The role of naturally occurring antisperm-antibodies (ASA) as a cause of male infertility was recognized since Rumke and Hellingar reported the presence of serum sperm-agglutinating antibodies in infertile men in 1959. Later, experiments by Edwards (1964), McLaren (1964), Menge (1971) and others showed that immunity to sperm of the same or heterologous species impaired their reproductive performance. At the same time that the experimental induction of immunity to spermatozoa was shown to cause induced infertility in animals (Bronson RA 2000).

With the development and the encouraged use of accurate and easy assays for the screening of ejaculates for sperm-bound antibodies, the development of sperm function assays, and finally the progress in assisted reproductive medicine, the role of ASA in infertility as well as the potential for their treatment is becoming better defined (Francavilla et al. 2007).

Antisperm antibodies

Antisperm antibodies are naturally occurring antibodies against sperm antigens. These antibodies present within the reproductive tract may be both transudates from the blood or secreted locally by submucosal plasma cells (Bronson RA 2000).

Different classes and their subclasses of ASA have been identified including IgA, IgG and IgM. The precise mechanism of generation of these antibodies is not clear. However, antibodies against intrinsic sperm antigens and those that are acquired during the process of sperm maturation have been detected. ASA have been detected in body fluids such as male and female serum, seminal plasma, on spermatozoal surface, ovarian follicular fluid, tubular fluid, cervical and vaginal secretions.

Sperm antibodies bound directly to sperm cell membrane epitopes could be situated on the head, midpiece and tail of the sperm (Mazumdar et al. 1998, Bronson RA 2000). Those which are present on the tail are thought to prevent sperm transport through the female reproductive tract, a problem which can be circumvented by in vitro fertilization (IVF), while head-directed antibodies appear to impair the interaction between the spermatozoon and the oocyte (De Almeida et al. 1989). The impairment of fertilizing capacity depends on the percentage of spermatozoa carrying antibodies in the ejaculate and on the type(s) of antibody present (De Almeida et al. 1989, Bronson RA 2000).

With the advent of in vitro fertilization techniques it was possible to establish stronger correlations between antisperm antibodies and failure of fertilization. In vitro fertilization rates were reduced in the infertile couples with antisperm antibodies in the sera or in the husband's seminal plasma (Fernando DMS 2001).

Development of ASA in males

Studies using monoclonal antibodies in subhuman primates have shown that new antigens are expressed on developing spermatocytes and spermatids after the initiation of spermatogenesis (Isahakia et al., 1988). These antigens, to which the immune system is not tolerant, could play a role in the genesis of auto-immunity to sperm. It has been postulated that the development of auto-immunity to spermatozoa may be prevented by sequestration of auto-antigens on germ cells by the presence of the blood–testis barrier. (Setchell et al. 1988, Bronson RA 2000).

Blood testis barrier

Developmental abnormalities of the formation of the blood– testis barrier, its traumatic disruption, or unilateral focal cryptic intra-testicular obstruction at the level of the seminiferous tubules could therefore lead to antisperm antibody formation (Bronson RA 2000, Fu et al., 2006).

Infection of the genital tract

Infections also can lead to partial or complete blockade of the duct system of the testis, occlusion of vas and prostatic ducts. Organisms can act as initiators of ASA formation through the inflammatory process. This response may lead to the formation of antibodies against the bacterial membrane carbohydrates that could cross react with sperm surface carbohydrates (Witkin and Toth, 1983).

Reduced immunosuppressive activity

Ineffective or reduced immunosuppressive activity is another potential mechanism for ASA formation. Studies have compared lymphocyte subpopulations in the blood of men with and without ASA. Liu et al., 1993 and Mazumdar and Levine, 1998 have found that men with ASA had an increased percentage of B cells and a decline in suppressor T cell function.

Development of ASA in females

Production of ASA in women may occur in a variety of ways. Deficiencies in the mucosal immune components of the female genital tract or inadequate immunosuppression, characters of sperm and seminal plasma are believed to be responsible for ASA in the female. However, the reason most women do not develop an immune response after repeated sperm exposure is not clear.

Damage to mucosal layer of the genital tract

Mechanical or chemical disruption of the mucosal layer of the female genital tract may permit exposure to foreign sperm antigens and, ultimately, ASA formation. Kuttech et al., 1990 suggested that due to repeated exposure of sperm to intraepithelial lymphocytes, a state

of immune tolerance and unresponsiveness occurs. However, when mucosal barrier is damaged, sperm antigens may provoke a local immune reaction result in the production of IgA. More significant exposure of sperm antigens would result in systemic response producing antibodies in serum mainly IgG.

Immunological factors

Immunological factors present in the semen can also cause formation of ASA in female. Presence of atypical antigens on the sperm or defective immunosuppressive properties of the sperm can cause development of ASA in female as a consequence of the inability of the sperm to suppress the lymphocytes (D Cruz and Haas, 1993).

Deposition of semen at sites other than vagina

Marshburn and Ketteh, 1994 demonstrated that deposition of semen at sites other than the vagina can induce an immunological response. It is stated that bypassing the cervical filtration and the protective mechanisms in the female genital tract is responsible for the production of ASA in females. Women undergoing repeated intra uterine insemination with washed spermatozoa, are also thought to be prone to develop ASA (Livi et al., 1990).

Effects of Antisperm antibodies

The precise mechanism for ASA-mediated fertility impairment is still unclear. Most studies have shown deleterious effects of ASA in almost all of the steps of fertilization, cleavage and implantation. However, there are some other studies which have given controversial results.

On sperm motility and sperm cervical mucus interaction

Antisperm antibodies may disrupt normal sperm function by damaging sperm motility. This may occur after exposure to ASA in the presence of heterologous complement (Mazumdar et al., 1998). And also reduced sperm survival caused by antisperm antibodies, can affect fertilization (Marshburn and Kutteh, 1994, Bohring C 2005).

The effect of sperm motility seems to vary with the class of antibody, the site of binding to the sperm and the load of antibody attached to each sperm (Bronson et al., 1984).

The role of ASA in inhibiting sperm penetration into the cervical mucus has been illustrated by many investigators. Kremer and Jager 1988, Eggert-Kruse et al., 1991, Check et al., 1994, francavilla et al., 2007 etc have shown that antibodies impede the migration of spermatozoa through cervical mucus in their studies.

On sperm zona pellucida binding

Binding of sperm to the zona is a species specific reaction with specific receptors on the sperm as well as on the zona (Wassarman, 1990).

Liu et al., 1991, Francavilla et al., 1997 and Bates 1997 showed that failure of fertilization of human oocytes in IVF programs due to presence of ASA interfering sperm binding to zona pellucida. It is well confirmed by subsequent studies (Marin-Briggiler et al., 2003).

On acrosome reaction

It is believed that ASA could interfere with this process by masking the sited of induction or by reducing membrane mobility and impeding its occurrence (Bandoh et al., 1992, Bates 1997, Marin-Briggiler et al., 2003). However, there are variable results observed in different studies.

Post fertilization effects

Research is limited on the influence of ASA on post fertilization effects such as pronuclear formation, embryo cleavage, on implantation and early phase of growth of the foetus. Some of the early research studies show that sera of women contained ASA attached to sperm head, the fertilization rate was significantly low but in women with ASA attached to the tail tip there was no difference in the fertilization rate. A similar correlation was found when ASA binding to the sperm head influenced the acrosome reaction and the binding of ASA on tail and/or midpiece was not associated with a significant alteration of viability and motility (Bohring et al., 2004).

Prevalence of ASA and immune infertility

It is difficult to estimate the actual prevalence of ASA given the vast array of diagnostic testing modalities available and their subsequent interpretations. Lot of studies have been carried out in men and women of different populations to determine the ASA prevalence and the incidence of immune infertility. ASA prevalence has been determined using different screening assays such as radiolabeled agglutinin assays, ELISA and IBD.

A study conducted in Germany in 1993 by Eggert-Kruse revealed a low incidence of less than 2% incidence of ASA in cervical mucus of women in an unselected infertile population (Eggert-Kruse, 1993). In contrast, Ekwere in 1995 reported a high incidence of circulating antibodies of 44% in a study among Nigerian men where the prevalence of genital tract infections was high (Ekwere, 1995). Therefore the prevalence of ASA among infertile population ranges a wide array depending on the detection assay and reporting centre.

Clinical significance

The clinical significance of ASA is one of the main areas of controversy. Helmerhorst et al., in a review has stated three reasons for clinicians not testing for ASA routinely. The reasons are lack of standardized and universally accepted assay to detect ASA, lack of consensus on clinical consequences of ASA and absence of evidence for a mechanistic explanation on how ASA impair conception. (Helmerhorst et al., 1999)

Numerous studies have revealed that antisperm antibodies may interfere with fertility. Not all ASA cause infertility. Current tests cannot differentiate the infertility-related ASA from those that do not interfere with infertility, because the antigenic specificities of these ASA are not known (Chiu et al., 2004). Clinical evidence has also accumulated that the ASA-related impairment of sperm transport suggested by the prior studies can be circumvented in the majority of cases by laboratory assisted reproduction.

Treatment options

Although the understanding of the aetiology of ASA has widened, the therapeutic measures have not made the same strides. Several methods are available at present to overcome the potentially deleterious effects of ASA-mediated infertility.

Immunosuppressive therapies

Some studies have shown a slight increase in pregnancy rates with ASA positive males treated with steroids. But the potential adverse effects and lack of well designed studies in many cases have decreased the enthusiasm for use of steroids for treatment of immunologic infertility (Bronson R, 1999).

Assisted reproductive technologies (ART)

Recently ART have been used to treat immunological infertility. Several studies have examined the use of Intrauterine insemination (IUI), In Vitro Fertilization (IVF), and intracytoplasmic sperm injection (ICSI) procedures.

(ii) Scientific scope of the project (overall and specific objectives)

The aim of this study was to determine the prevalence of Antisperm Antibodies on spermatozoal surface, in seminal plasma, cervical mucus, serum and follicular fluid (in IVF) among infertile couples (who were seeking treatment at a selected fertility centre in Sri Lanka) and to determine the pregnancy outcome after IUI and ART.

In Sri Lanka, very few studies have been carried out to determine the prevalence of ASA among infertile couples. The detection for the presence of ASA in infertile couples is not done as a routine test at present even in the presence of possible risk factors.

With the recent advances in the field of infertility, more sophisticated methods for diagnosis and management of infertilty are becoming available in Sri Lanka. Treatment procedures such as IUI, IVF and ICSI are accessible to the general population.

A logical approach for screening and investigating infertile couples will enable effective treatment. The findings of this study show the incidence of the presence of ASA in infertile couples and its effect on pregnancy rate with different ART procedures. The data provide the importance of screening for ASA in unexplained infertility and useful insights for the management of ASA positive infertile couples. Therefore the findings of this study helps to save time and direct infertile couples to suitable treatment modalities, alleviating great portion of physical, emotional and financial strains they go through.

Since the data relating to the prevalence and effects of ASA for Sri-Lankan infertile population is scarce, this study also provides basic figures for any future references.

Objectives

General Objective

To determine the prevalence of Antisperm Antibodies in semen (sperm and seminal fluid) and serum of the male partner and in serum, cervical mucus and follicular fluid (in IVF) of the female partner among infertile couples and compare the pregnancy outcome after IUI and ART, in such couples and couples without ASA

Specific Objectives

1. To determine associated factors(from the questionnaire) for the presence of ASA in ASA-positive males and females
2. To determine the relationship between the site of binding of ASA to spermatozoa and the fertilization/pregnancy rates
3. To determine the fertilization and cleavage rates and the quality of the embryos in IVF/ ICSI in ASA positive and negative couples
4. To determine the pregnancy rates and percentage of spontaneous pregnancy loses in IUI / IVF / ICSI in ASA positive and negative couples

(iii) Materials and Methods

Study Design

This prospective analytical study was conducted at 'Prarthana', Centre for ART, 1175, Cotta Road, Rajagiriya. The study commenced in January 2006 and subjects were recruited up to January 2009. Infertile couples who seek treatment at 'Prarthana', Centre for ART were taken in to this study. The total study population was 230 infertile couples; 460 individuals.

Following a brief explanation about the nature of the study, study sample and duration, the investigations that would be performed and why they were performed, and the justification for carrying out this study, those who volunteered to participate were interviewed after obtaining written consent. The infertile couples were also explained that refusal to participate would not affect their treatment / care in anyway and they could withdraw at anytime and it would not affect them adversely in any manner.

A questionnaire was filled for each subject with regard to take a proper history and to determine the probable cause; if any, for the presence of ASA during the interview. Thereafter both husband and wife were subjected to a general examination.

In the male partner semen and serum was examined for ASA-IgA and IgG. In the female partner, those who underwent IUI, cervical mucus and serum was examined for ASA-IgA and ASA-IgG . And those who underwent IVF / ICSI, cervical mucus, serum and follicular fluid was examined for ASA- IgA and ASA-IgG . The ASA was detected using sperm MAR test kits.

Following the assessment of each couple, one of the Assisted Reproductive Technology (ART) treatment modality was recommended; without considering the status of ASA results. That is IUI, IVF/ICSI. Therefore all the infertile couples underwent either IUI or IVF/ICSI procedure. These procedures are discussed in detail later in this chapter.

Confirmation of Pregnancy

Biological pregnancy was confirmed at 8 weeks of period of gestation by ultrasound scan with a live foetus with a heart beat.

All pregnant patients were followed up at least first three months of period of gestation to see any spontaneous pregnancy losses.

Sample size

The sample size of 230 couples was statistically adequate for this study. The power of test results range from 0.6-0.8 in this study and this indicates the adequacy of sample size.

Sample selection

The volunteered subfertile couples who seek treatment at "Prarthana", Center for ART, No.1175, Cotta Road, Rajagiriya were recruited for the study.

Inclusion criteria

1. Primary / secondary infertility
2. Age of female partner between 21 to 40 years

Exclusion criteria

1. Any ovulatory dysfunction or obvious physical barrier to conception.
2. Females having endometriomas, cysts or uterine fibroids > 3 cm on transvaginal scans at the time of the ART procedure
3. Couples who used donor gametes
4. Males having a sperm count of < 1 million/ml and motility of < 10% were excluded from the study, as it could affect the results of sperm MAR test.

Data collection methods / Procedures

An informed written consent was taken from all the subjects. An investigator administered questionnaire was filled and following information were obtained from both husband and wife.

Information from questionnaire

Age of the husband and wife, duration of marriage, type of infertility and duration of infertility were noted. The medical and surgical histories from both partners were obtained. More concern was paid to obstetrics and gynaecological histories in the female partner and history of mumps, genito-urinary infections and surgeries, trauma to genitalia in the male partner. The current illnesses or any medication that the couple was on, was also asked. A special emphasis was paid to illnesses and diseases of autoimmune origin including Hypothyroidism, Hyperthyroidism, Rheumatoid arthritis etc. Details of the occupational and social history were also taken at the interview. Social habits such as consumption of alcohol and smoking were questioned. In relevant subjects, the quantity and frequency of substance usage were noted.

Examination

A detailed general examination was performed on both husband and wife. In the female partner, a speculum examination of vagina and a transvaginal scan was performed to assess uterus and adnexae. The basic transvaginal scan was done to exclude any fibroids, cysts, endometriomas which could affect the success of treatment procedures and pregnancy.

Investigations

Cervical mucus, serum and follicular fluid in females who underwent IVF were tested for antisperm antibodies with indirect sperm MAR test. Details of this test will be discussed later in this chapter.

In the male, semen and serum of the male partner were tested for antisperm antibodies with direct and indirect sperm MAR tests which will be discussed later in this chapter.

All tests, procedures and safety measures were conducted according to the guidelines of the WHO laboratory manual.

Sample collection

Peripheral blood was collected by venepuncture and cervical mucus was taken by a micropipette on the same day from the female partner for detection of ASA. Isolated serum was stored at -20^0 C until assayed. Follicular fluid was taken after oocyte isolation and centrifuged. Supernatant was stored at -20^0 C until assayed. Detection of ASA was performed on cervical mucus at the time of collection.

Semen was collected into the sterile container by masturbation method following 3 days of abstinence and detection of ASA was performed soon after.

Mixed Agglutination Reaction (MAR) of sperms

Sperm mixed agglutination reaction using sperm MAR test kits (Fertipro NV, Belgium) was performed to detect ASA in test samples. The principle of detecting ASA using sperm MAR test was discussed under methods for testing ASA. In male partners direct sperm MAR test was used to detect ASA on spermatozoa and indirect sperm MAR test was used for seminal plasma and serum. In female partners indirect sperm MAR test used to detect ASA in cervical mucus, serum and follicular fluid. Follicular fluid test samples could be obtained only from the females who underwent IVF/ICSI treatment. Each test sample was checked for both IgA and IgG types of antisperm antibodies. In each test, the site of binding (i.e. head, mid piece, tail) of latex beads to the spermatozoa was noted.

The test was carried out according to the instructions given by the manufacturer. Each test kit was pre-tested with a control sample before examining the test samples.

Sperm MAR IgA test

Kit Contents

- 1 vial SpermMar IgA latex particles coated with specific monoclonal anti-IgA

Direct SpermMar IgA test

10 microlitres of fresh semen, 10 microlitres of Sperm Mar latex particles were place on a microslide. Sample and the latex particles were mixed with the edge of a cover glass. Then the cover glass was put on the mixture and the mixture was observed under a light microscope using a 400x magnification. The result was read after 10 minutes. 100 motile sperms were counted and the number of sperm with beads attached to them was expressed as a percentage. The site of binding of latex beads to spermatozoa was observed.

Indirect SpermMar IgA test

Donor spermatozoa with no ASA (previously checked and confirmed) were washed by letting them swim up in the sperm rinse medium(pH = 7.4-7.5). The serum/ follicular fluid/seminal plasma specimen was serially diluted with sperm rinse medium(pH = 7.4-7.5). 50 microlitres of the suspension of motile donor sperm was incubated with 50 microlitres of serum/ seminal plasma/ cervical mucus/ follicular fluid for 1 hour at 37^{0}C. Then 2 ml of sperm rinse was added and centrifuged for 10 minutes at 400g. The supernatant was aspirated and re-

suspended the pellet with 50 microlitres of sperm rinse medium. 10 microlitres of fresh sperm suspension, 10 microlitres of SpermMar latex particles were place on a microslide. Then the sample and the latex particles were mixed with the edge of a cover glass and followed the same steps as mentioned in Direct SpermMar IgA test.

Sperm MAR IgG test

Kit Contents

- 1 vial SpermMar Latex Particles 0.7 ml – suspension of polystyrene latex particles of approximately 2-4 micron in diameter coated with human IgG
- 1 vial SpermMar Antiserum 0.7 ml – monospecific antiserum directed towards the Fc fragment of human IgG

Direct SpermMar IgG test

10 microlitres of fresh untreated semen, 10 microlitres of SpermMar latex particles, 10 microlitres of SpermMar antiserum were placed on a microslide. The sample and the Latex reagent were mixed well with the edge of a cover glass. The antiserum also was mixed with the latex reagent and sample mixture. Then the cover glass was put on the mixture and the mixture was observed under a light microscope using a 400x magnification. The result was read after 10 minutes. 100 motile sperms are counted to determine the reactive percentage. The site of binding of latex beads to the spermatozoa was noted.

Indirect SpermMar IgG test

Donor spermatozoa (free of ASA – previously checked and confirmed) were washed by letting them swim up in the sperm rinse medium(pH = 7.4-7.5). The serum/ follicular fluid/seminal plasma/cervical mucus specimen was serially diluted with sperm rinse medium (pH = 7.4-7.5). 50 microlitres of diluted serum/follicular fluid/seminal plasma was mixed with 50 microlitres of washed motile donor sperm and was incubated for 60 minutes at 37^0C. 10 microlitres of sperm-serum/follicular fluid/ seminal plasma/cervical mucus mixture, 10 microlitres of SpermMar latex particles, 10 microlitres of SpermMar antiserum were placed on a microslide. Then the sample, latex particles and antiserum were mixed with the edge of a cover glass and followed the same steps as mentioned in Direct SpermMar IgG test.

Analysis of ASA

100 moving sperms were observed and the number of sperms bound to latex beads was calculated.

$$\% \text{ total binding} = \frac{\text{sperm no. with bound beads}}{\text{total counted sperm no.}} \times 100\%$$

At least 30% of sperms show binding taken as positive for ASA (WHO guidelines)

The binding of the beads could be directed towards head, mid-piece or tail of the sperm. The binding site/sites were noted.

The analysis of the samples were performed at IUI laboratory, 'Prarthana', Centre for ART and Research laboratory, Department of Anatomy, Faculty of Medical Sciences, University of Sri-Jayawardanapura.

Fertilization rate and Cleavage rate in IVF / ICSI

Following oocyte aspiration, the eggs were selected and separated from follicular fluid. The selected oocytes were placed on a centre well dish which contained embryo culture medium 1 (G-IVF^{TM} PLUS - Vitrolife, Sweden); a medium for preparation and handling of gametes and for in-vitro fertilization. Following that, the oocytes were washed with the same medium and transferred to a four well dish, one well having 1-4 oocytes. The four well dish contained the same culture medium (G-IVF^{TM} PLUS).

Method of fertilization

Standard IVF

Semen sample was prepared by density gradient method as mentioned in section 2.4.4. The prepared sperm sample was pre incubated for a period of 2 hours at 37^0 C in 6% CO_2 and in 94% air before insemination. A drop of sperms which contained $1x10^6 - 2\ x10^6$ number of sperms was placed adjacent to the oocytes in one well of four well dish. Then the gametes were kept in an incubator at 37^0 C in 6% CO_2 and in 94% air for 18 - 20 hours. After 20 hours, the cumulus cells surrounding the oocytes were denuded using denuding pipette (G26711, COOK, Ireland). Fertilization of oocytes were assessed at this point and the day 01 embryos were placed on a new four well dish which contained an embryo culture medium-2 (G-1^{TM} v5 PLUS - Vitrolife, Sweden); a bicarbonate buffered medium containing human serum albumin, hyaluronan and gentamicin as an antibacterial agent. It is a medium for culture of embryos from the pronucleate stage to day 2/3. The dishes were kept in an incubator at 37^0 C in 6% CO_2 and in 94% air for further growth. Embryos were cultured for 03 days and day 03 cleavage was observed. Embryos were assessed according to the Veeks criteria. Best quality embryos were selected, 02 fresh embryos were transferred to the woman and rest cryopreserved.

ICSI

Following oocyte separation from follicular fluid, they were washed and medium and transferred to a four well dish as described early in this page. The cumulus cells surrounding the oocytes were then denuded using denuding pipette (G26711, COOK, Ireland) in embryo culture medium-1 (G-IVF^{TM} PLUS). Denuded oocytes were transferred to an ICSI dish. ICSI was performed using the Micromanipulator (RI-UK) which has an inverted microscope (Nikon, edipse TE2000-S). The oocyte was fixed by the holding pipette (COOK, Ireland) and a motile sperm from the prepared sperm sample was taken into the injection pipette (COOK, Ireland). The sperm was then injected to the cytoplasm of the oocyte by the injection pipette. Then the gametes were transferred to a new four well dish which contained Embryo culture medium-2 (G-1^{TM} v5 PLUS - Vitrolife, Sweden) and kept in an incubator at 37^0 C in 6% CO_2 and in 94% air for 24 hours for further growth. Further steps from this stage, were as same as mentioned in standard IVF.

Fertilization rate

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.

$$\text{Fertilization rate} = \frac{\text{No. of total fertilized oocytes}}{\text{No. of total inseminated oocytes}} \times 100$$

Cleavage rate

Day 03 cleavage of the embryo was observed under the inverted microscope. Regular appearance (fragmentation), cell orientation, speed of cleavage and cytoplasmic granularity was considered when assessing the quality of embryo. Embryos with 6-8 evenly placed cells with no or mild to moderate fragmentation and cytoplasmic granularity were considered as well cleaved embryos.

$$\text{Cleavage rate} = \frac{\text{No. of total good quality embryos at Day 03}}{\text{No. of total fertilized embryos at Day 01}} \times 100$$

(iv) Results

1 Prevalence of antisperm antibodies

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

The most prevalent type of antisperm antibody in both males (n=25) and females (n=10) was IgA (72.92%). The gross data are shown in Figure 1.

2 Demographic details of the couple

2.1 Age

In the total study population, the age range for the females was 21 to 40 years and for the males the age range was 27 to 49 years. The mean age ± SD of the females was 33.38 ± 4.17years and the mean age + SD for the males was 36.86 + 4.97 years.

The prevalence of antisperm antibodies with the age distribution was analysed by 'Chisquare test'. There was no significant association between the presence of ASA and the age factor in both females (Chi-sq=0.039, P-value=0.998) and males (Chi-sq=3.532, P-value=0.473).

2.2 Duration of marriage and duration of infertility

The duration of marriage ranged from six months to more than ten years. There were 11 couples who were married for six months to one year and 15 couples who were married for more than ten years at the time of recruiting for the study. The mean duration of marriage was 5.8 years. The prevalence of antisperm antibodies with the duration of marriage of infertile couples was analysed by 'Chisquare test'. There was no significant association between the presence of ASA and duration of marriage (Chi-sq=8.45, P-value=0.207). Interestingly, there were no ASA positives among the couples who were married for six months to one year period.

The duration of infertility ranged from six months to 19 years. There were 27 couples having infertility for less than one year and 15 couples having infertility for more than ten years. The mean duration of infertility was 5.3 years. The prevalence of antisperm antibodies with duration of infertility was analysed and there was no significant association found between the presence of ASA and duration of infertility (Chi-sq=7.750, P-value=0.257).

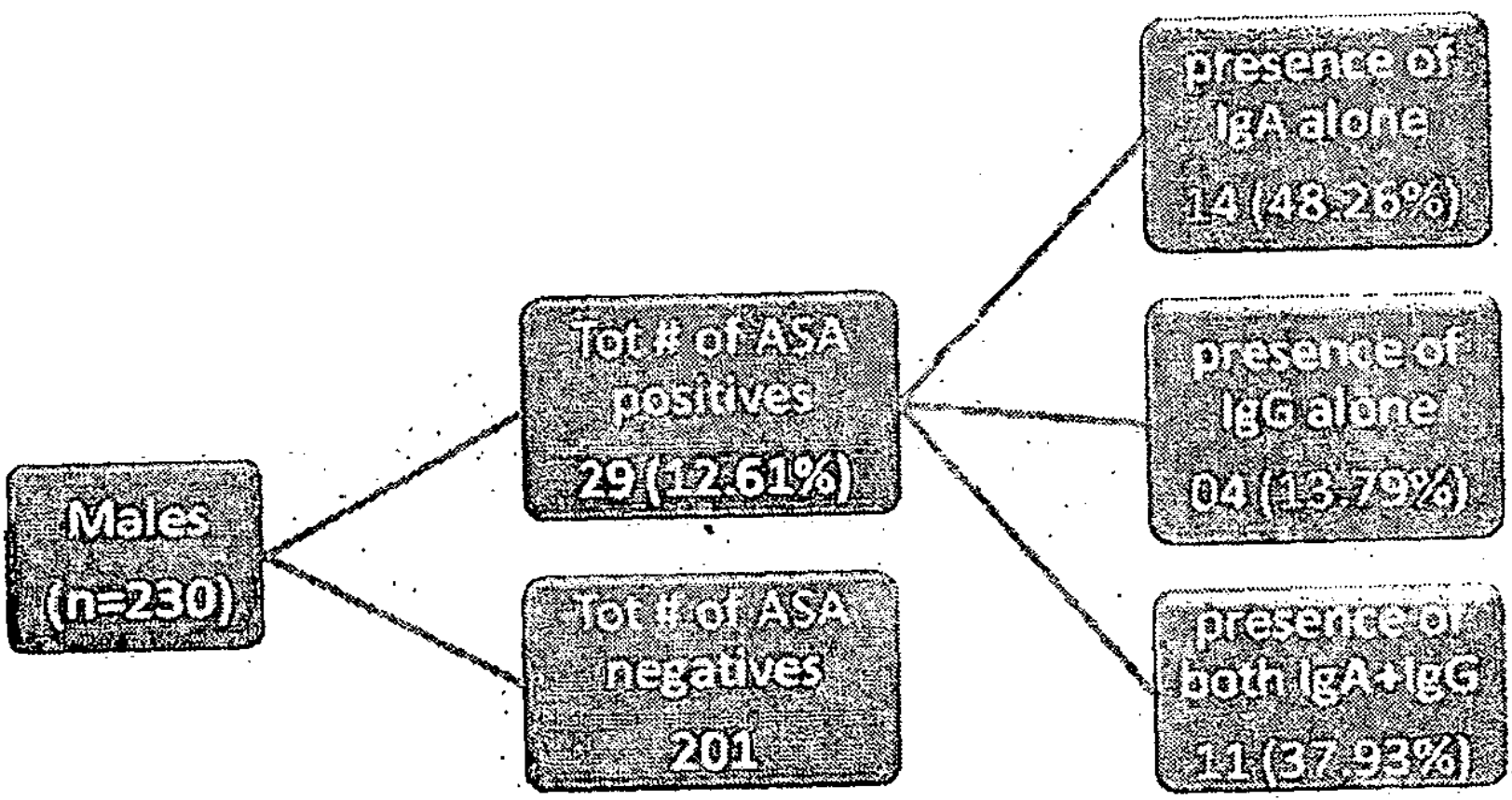
2.3 Occupation

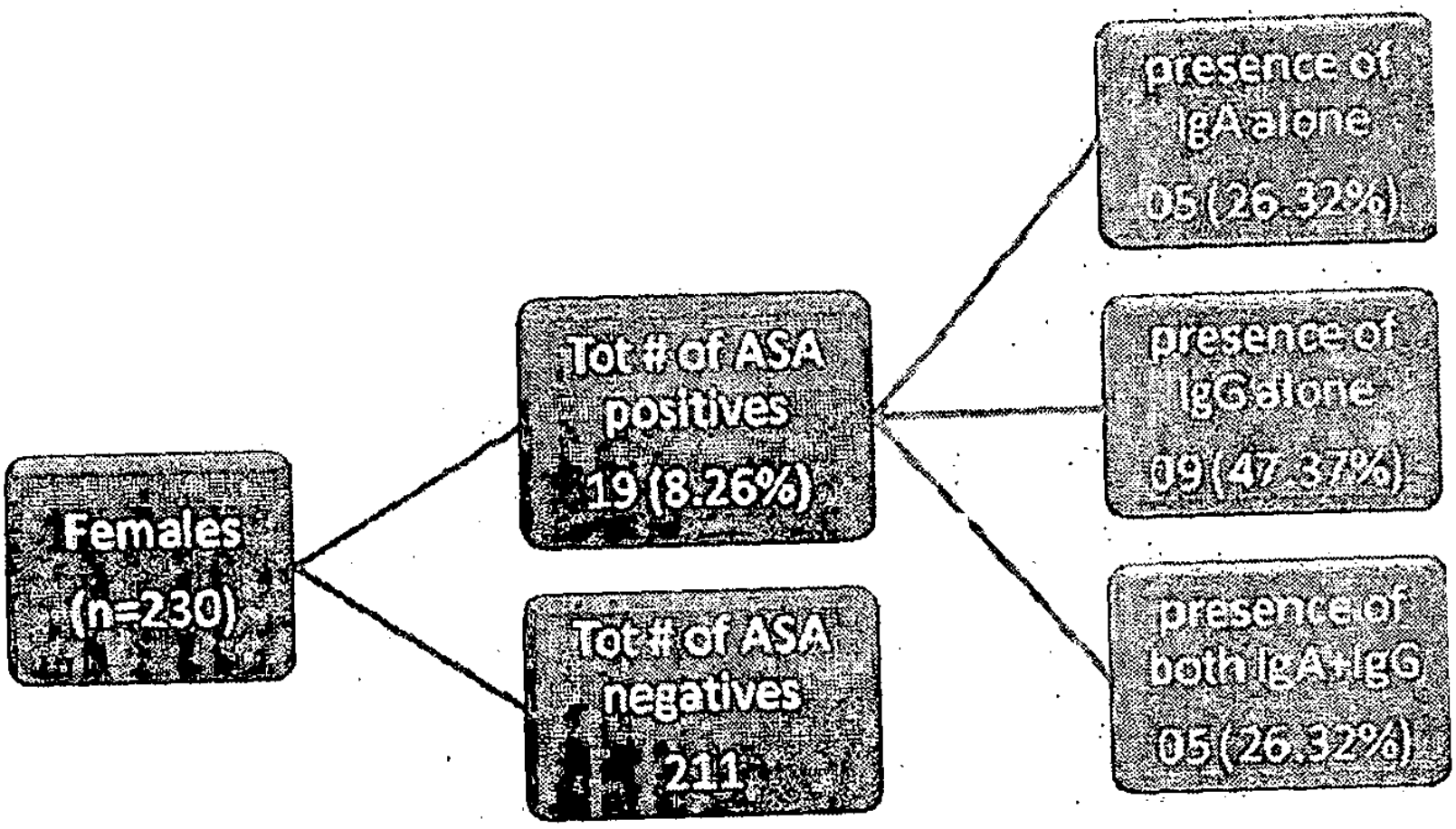
The occupation of males and females were grouped into seven categories for the statistical analysis (Table 2.3).

The percentage of males exposed to heat, vibration or radiation was 4.78 (n=11). It was observed that none of these subjects were positive for antisperm antibodies. The prevalence of antisperm antibodies in different occupational categories was analysed and no significant

Figure1

Prevalence of ASA and its isotypes among infertile couples




Females
(n=230)
Tot # of ASA positives
19 (8.26%)
Tot # of ASA negatives
211
presence of IgA alone
05 (26.32%)
presence of IgG alone
09 (47.37%)
presence of both IgA+IgG
05 (26.32%)

association noted in both males (chi-sq=6.064, P-value=0.300) and females. The pattern of distribution of ASA in different occupational categories is shown for males and females are shown in table 2.3.

Table 2.3. Pattern of distribution of ASA in different occupational categories in males and females

Category	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Tot # of males	18 (7.83%)	115 (50%)	12 (5.22%)	07 (3.04%)	64 (27.83%)	14 (6.09%)	0
Tot# of ASA (+) males	01 (5.56%)	14 (12.16%)	0	01 (14.29%)	12 (18.75%)	01 (7.14%)	0
Tot # of females	18 (7.83%)	84 (36.52%)	01 (0.4%)	01 (0.4%)	11 (4.78%)	04 (1.74%)	111 (48.26%
Tot # of ASA (+) females	01 (5.56%)	08 (9.52%)	0	0	0	0	10 (9.01%)

Category 1 – professionals , 2 – managers and clerical, 3 – skilled workers, 4- service Personnel, 5- businessmen, 6- other, 7- non employed

3 Type of infertility

Of the study population, 76% (n=174) couples had primary infertility while 24% (n=56) couples had secondary infertility. The prevalence of antisperm antibodies with the type of infertility was analysed and showed no significant association (Chi-sq=0.765, P-value=0.382). However, the prevalence of ASA among secondary infertile couples is proportionately higher (25%) than the primary infertile couples (19.54%) in the study sample

4 Causes for formation of antisperm antibodies

4.1 Male causes

4.1.1 Associated genito-urinary conditions

The conditions and surgeries that would breach the blood testis barrier and facilitate formation of antisperm antibodies were taken into consideration. Of the study sample 3.04% (n=7) males had undergone genital surgeries as varicocoelectomy and herniorrhaphy. Out of

these males two (n=2) were positive for ASA. It was observed as a statistically significant (Chi-sq=4.407, P-value=0.036) association between the presence of ASA and a history of genital surgery.

4.1.2 Associated autoimmune conditions and illnesses

It was observed that 27.83% (n=64) of the males had Mumps in childhood, none had mumps orchitis however. Of the males who had mumps during childhood, 17.19% (n=11) were positive for ASA. Prevalence of ASA with childhood mumps was analysed and no significant association (Chi-sq=1.687, P-value=0.194) was found.

None of the males gave a history of rheumatoid arthritis, autoimmune thyroid disease or any other systemic autoimmune disease.

Other co-morbid illnesses as hypertension, hypercholesterolemia, diabetes mellitus - type II etc. were observed in 10.43% (n=24) of males. Prevalence of ASA with these illnesses was analysed and no significant association was found.

4.1.3 Use of addictables

Of the total sample 67.8% (n=156) of the males have never or very occasionally smoked or consumed alcohol. Although 31.3% (n=72) of the males consumed alcohol no one admitted that they were addicted. It was observed that 13% (n=30) of the males were smokers. There was no one who was addicted to other addictables such as Heroin. The prevalence of antisperm antibodies in the groups consuming addictables and not consuming addictables was analysed and no significant association (Chi-sq=0.982, P-value=0.322) was found.

4.2 Female causes

The possible contributory factors in the female for the formation of antisperm antibodies were derived from the initial interview. 40.87% (n=94) of females had experienced intra-uterine insemination (IUI) with prepared sperms before recruitment for the study. Out of them 11.7% (n=11) were positive for ASA. Though there was no statistically significant relationship found, the prevalence of ASA was proportionately higher among women those who had previous IUIs (11.7%) compared to the women who did not have IUIs (5.88%).

It was observed that 24.3% (n=56) of females had a history of termination of pregnancy (spontaneous or induced) and among them 7.14% (n=04) were positive for ASA. There was no significant association found between the presence of ASA and a history of termination of pregnancy.

It was also observed that 9.1% (n=21) had autoimmune diseases as autoimmune thyroid diseases, asthma, rheumatoid arthritis and psoriasis. However, non of these females were positive for ASA. Another 3.9% (n=9) had co-morbid illnesses as hypertension; hypercholesteroleamia, diabetes mellitus – type II and hyperprolactenemia. Out of these females 22.22% (n=02) were positive for ASA. A proportionately higher prevalence of ASA is noted among females who have co-morbid illnesses.

None of the females of the study sample were smokers or consuming alcohol or any other addictables

5 Distribution of ASA in males and females

Gross data of the distribution of ASA in the total study sample is shown in table 5

Table 5 Gross data of the distribution of different antisperm antibody types in different body fluids in the total study sample

Body fluid	Total # of males		
	IgA	IgG	IgA + IgG
Spermatozoa	03		06
Seminal plasma	02	-	01
Spermatozoa + Seminal plasma	09		02
Serum	-	04	01
Spermatozoa + Seminal plasma Serum			01
	Total # of females		
	IgA	IgG	IgA + IgG
Cervical mucus	05	-	01
Serum		01	
Follicular fluid	-	03	01
Serum + Follicular fluid		05	02
Cervical mucus + Serum + Follicular fluid	-	-	01

5.1 Distribution of different antisperm antibody types in males and females

Tabulated data for different antisperm antibody types in males and females are shown in table 5.1a and table 5.1b

The most prevalent antisperm antibody type in males was IgA. Out of the total ASA positive males (n=29), 14 demonstrated IgA isotype alone and 11 demonstrated IgA isotype with IgG. Hence, the IgA positivity in males was 86.21% (25/29). In contrast IgG positivity among them was 51.72% (15/29). Interestingly no one demonstrated IgA alone in their serum. And no one demonstrated IgG alone in their semen. Hence, it is observed that semen always contained IgA isotype alone or together with IgG. Serum always contained IgG alone or together with IgA. Also this data shows that presence of ASA is higher in semen (n=24) than serum (n=6) in males. In semen, 21 (72.41%, 21/29) men showed ASA on spermatozoa.

Fifteen men (51.72%, 15/29) showed ASA in seminal plasma and from that only 03 men (10.34%, 03/29) showed ASA only in seminal plasma. Hence it is evident that screening for ASA on spermatozoa covers higher number of individuals who are positive for ASA than it covers when screening seminal plasma for ASA.

The IgA positivity among females was 52.63% (10/19). The IgG positivity among them was 73.68% (14/19). In females, again, no one demonstrated IgA alone in serum and follicular fluid as well. And no one demonstrated IgG alone in cervical mucus. Therefore the previous observation also applies to females as well; that cervical mucus always contained IgA isotype alone or together with IgG, serum and follicular fluid always contained IgG alone or together with IgA. Most females had ASA in serum and follicular fluid (n=14) than in cervical mucus (n=7).

This data postulates the fact that males possess more locally secreted ASA, having demonstrated higher percentage of antibody positivity in semen and females possess more systemically transudated ASA, having demonstrated higher percentage of antibody positivity in serum and follicular fluid.

5.2 Pattern of localization of ASA in males and females

It is observed that anisperm antibodies are frequently present as tail bound form in males (48.23%) and females (47.37%). It is noted that presence of ASA bound to whole surface of the spermatozoa is infrequent in both males (3.45%) and females (10.53%).

Table 5.2 shows the pattern of localization of ASA in the study sample and with regard to type of antisperm antibody. There was no particular type of ASA was observed significantly bound to a particular location on spermatozoa. It is observed that both IgA and IgG isotypes of ASA could bound to any location on spermatozoa.

Table 5.1a Distribution of different antisperm antibody types in different body fluids in males and females

ASA type	Tot # of males		Tot # of females		
	Semen	Serum	Serum	Cervical mucus	Follicular fluid
IgA alone	14 (48.28%)			05 (26.32%)	
IgG alone		04 (13.79%)	06 (31.58%)		08 (42.1%)
Both IgA + IgG	10 (34.48%)	02 (06.9%)	03 (15.79%)	02 (10.53%)	04 (21.05%)

Table 5.1b Distribution of different antisperm antibody types in different components of semen

ASA type	Tot # of males		
	On spermatozoa	In seminal plasma	In both spermatozoa and seminal plasma
IgA alone	03 (10.34%)	02 (06.9%)	09 (31.03%)
IgG alone			
Both IgA + IgG	06 (20.69%)	01 (03.45%)	03 (10.34%)

Table 5.2 Pattern of localization of antisperm anibodies on spermatozoa in different types of ASA in males and females

Location of antibodies	# of males			
	IgA	IgG	IgA + IgG	Total
Head bound	04 (13.79%)	-	02 (06.9%)	06 (20.69%)
Tail bound	07 (24.14%)	03 (10.34%)	04 (13.79%)	14 (48.23%)
Mid piece + tail bound	03 (10.34%)	01 (03.45%)	04 (13.79%)	08 (27.59%)
Whole sperm bound			01 (03.45%)	01 (03.45%)

	# of females			
	IgA	IgG	IgA + IgG	Total
Head bound		02 (10.53%)	02 (10.53%)	04 (21.05%)
Tail bound	02 (10.53%)	05 (26.32%)	02 (10.53%)	09 (47.37%)
Mid piece + tail bound	01 (05.26%)	02 (10.53%)	01 (05.26%)	04 (21.05%)
Whole sperm bound	02 (10.53%)	-	-	02 (10.53%)

6 Effects of antisperm antibodies

Figure 6 shows the gross data of total number of couples that underwent different treatment procedures, presence of ASA and pregnancy outcome.

6.1 Effects of ASA on fertilization and cleavage of embryos

6.1.1 Effects of ASA (irrespective of type and location) on fertilization and cleavage of embryos

Effects of ASA on fertilization of oocytes and cleavage of embryos were observed in couples who underwent in vitro fertilization. Table 6.1.1a and Table 6.1.1b show the fertilization rates and cleavage rates of embryos among ASA negatives and ASA positives. It was observed that in ASA positives the total fertilization rate (69.38% - both insemination methods; 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%). The same observation was made in standard IVF and ICSI, where the fertilization rates were higher and cleavage rates were lower in ASA positives than in ASA negatives. This supports the fact that ASA have negative effects on post fertilization events.

Another observation made as irrespective of the type of ASA or the location of binding of ASA on spermatozoa, the total ASA positives did not show a significant difference in fertilization rate or cleavage rate when undergoing standard IVF and ICSI as the method of insemination, with ASA negatives.

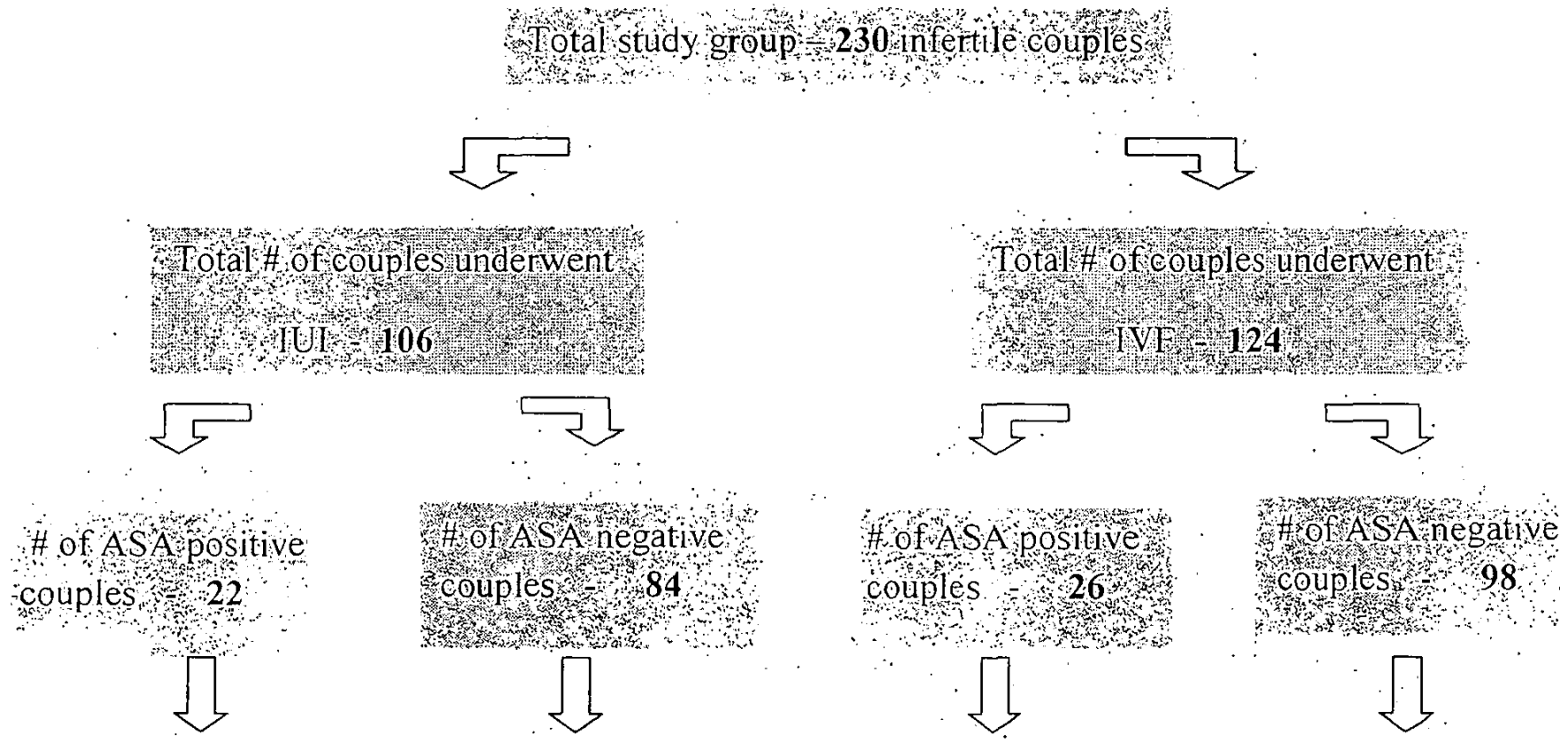
6.1.2 Effects of type of ASA on fertilization and cleavage of embryos

Table 6.1.2a and Table 6.1.2b show the fertilization and cleavage rates corresponding to the immunoglobulin (Ig) isotype of ASA. Among the Ig isotypes it was observed that there was no significant difference in fertilization rates and cleavage rates when oocytes undergo standard IVF or ICSI as the method of insemination. 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 of ASA was observed as having the highest fertilization rate and the lowest cleavage rate.

6.1.3 Effects of location of ASA on spermatozoa, on fertilization and cleavage of embryos

Table 6.1.3a and Table 6.1.3b show the fertilization and cleavage rates in standard IVF and ICSI corresponding to the location of ASA among couples that underwent IVF. It is observed that 50% (n=24) of the ASA positives had ASA bound to the tail of the spermatozoa. Among

Figure 6 Flow chart of total number of couples that underwent different treatment procedures, presence of ASA and pregnancy outcome



Number of inseminations/embryo transfers	22	84	26	98
Number of clinical pregnancies	03	17	05	22
Number of spontaneous pregnancy losses	0	01	02	06

Table 6.1.1a Insemination, fertilization and cleavage of oocytes in standard IVF, ICSI and as a total among ASA positives and ASA negatives

	# of inseminated oocytes			# of fertilized oocytes			# of cleaved embryos		
	Total	**Standard IVF**	**ICSI**	**Total**	**Standard IVF**	**ICSI**	**Total**	**Standard IVF**	**ICSI**
ASA positives	258	75	183	179	55	124	96	31	65
ASA negatives	808	347	461	473	213	260	292	137	155

Table 6.1.1b Fertilization rates and cleavage rates of embryos among ASA negatives and ASA positives

	Total fertilization rate	**Total cleavage rate**	**Standard IVF**		**ICSI**	
			Fertilization rate	**Cleavage rate**	**Fertilization rate**	**Cleavage rate**
ASA positives	69.38%	53.63%	73.33%	56.36%	67.76%	52.42%
ASA negatives	58.54%	61.73%	61.38%	64.32%	56.4%	59.62%

Table 6.1.2a Insemination, fertilization and cleavage of oocytes corresponding to the type of ASA in standard IVF, ICSI and as a total among ASA positives

	# of inseminated oocytes			# of fertilized oocytes			# of cleaved embryos		
	Total	**Standard IVF**	**ICSI**	**Total**	**Standard IVF**	**ICSI**	**Total**	**Standard IVF**	**ICSI**
IgA	29	08	21	24	06	18	11	03	08
IgG	106	36	70	75	28	47	37	13	24
IgA +IgG	123	31	92	80	21	59	48	15	33

Table 6.1.2b Immunoglobulin (Ig) isotype of ASA and corresponding fertilization rates and cleavage rates of embryos

	Total fertilization rate	**Total cleavage rate**	**Standard IVF**		**ICSI**	
			Fertilization rate	**Cleavage rate**	**Fertilization rate**	**Cleavage rate**
IgA	82.76%	45.83%	75%	50%	85.71%	44.4%
IgG	70.75%	49.33%	77.77%	46.43%	67.14%	51.06%
IgA + IgG	65.04%	60%	67.74%	71.43%	64.13%	55.93%

Table 6.1.3a Insemination, fertilization and cleavage of oocytes corresponding to the location of ASA in standard IVF, ICSI and as a total among ASA positives

	# of inseminated oocytes			# of fertilized oocytes			# of cleaved embryos		
	Total	**Standard IVF**	**ICSI**	**Total**	**Standard IVF**	**ICSI**	**Total**	**Standard IVF**	**ICSI**
Head	111	27	84	76	19	57	39	10	29
Tail	102	29	73	67	20	47	41	15	26
Midpiece+tail	39	19	20	33	16	17	13	06	07
Whole sperm	06	0	06	03	0	03	03	0	03

Table 6.1.3b Location of ASA on spermatozoa and corresponding fertilization rate and cleavage rate of embryos

	Total fertilization rate	Total cleavage rate	Standard IVF		ICSI	
			Fertilization rate	**Cleavage rate**	**Fertilization rate**	**Cleavage rate**
Head	68.45%	51.34%	70.37%	52.63%	67.86%	50.88%
Tail	65.69%	61.19%	68.97%	75%	64.38%	55.32%
Midpiece+tail	84.62%	39.39%	84.21%	37.5%	85%	41.18%
Whole sperm	50%	100%	-	-	50%	100%

the rest of the ASA positives 20.83% (n=10), 22.92% (n=11) and 06.25% (n=3) had head, midpiece+tail and whole sperm bound ASA respectively.

It was observed that in standard IVF the cleavage rates (52.63% and 37.5% respectively) were markedly lower when ASA was located around the head and significantly lower (P-value=0.03) when located on midpiece+tail of the spermatozoa compared to the cleavage rate (64.34%) in ASA negatives. When ASA was located around the tail of spermatozoa, there was no significant difference observed in cleavage rate compared to that of ASA negatives. It was also observed that head bound ASA oocytes had a markedly lower (52.63%) and midpiece+tail bound ASA oocytes (37.5%) had a significantly lower (P-value=0.026) cleavage rate than that of the tail bound ASA oocytes (75%).

When oocytes were inseminated by ICSI, there was no statistically significant difference observed in cleavage rates of head (50.88%), tail (55.32%) and midpiece+tail (41.18%) bound ASA than that of ASA negatives (59.62%), except the whole sperm bound ASA. The whole sperm bound ASA had few number of oocytes which made statistical analysis impossible for that category. The differences of cleavage rates between binding sites were more or less alleviated by ICSI over Standard IVF as the method of insemination.

Therefore this data demonstrates that head or midpiece+tail bound ASA on spermatozoa exhibit more negative effects on cleavage rate in standard IVF. However, when ICSI was performed on oocytes, the negative effects of head and midpiece+tail bound ASA on cleavage of embryos were suppressed.

6.2 Effects of ASA on clinical pregnancy rate

6.2.1 Effects of ASA (irrespective of type and location) on clinical pregnancy rate

As shown in Figure 6, 08 pregnancies in ASA positives and 39 pregnancies in ASA negatives were observed following the ART procedure. Among the couples who underwent IUI, the pregnancy rate was proportionately higher in ASA negatives (20.24%) than in ASA positives (13.64%). This observation elicits the fact that ASA may have negative effects not only on sperm-mucus interaction but also on other stages of fertilization. Among the couples who underwent IVF, the pregnancy rate was marginally higher in ASA negatives (22.45%) than in ASA positives (19.23%). Moreover, in ASA negatives, the pregnancy rate is marginally higher when they underwent IVF (22.45%) than IUI (20.24%). In ASA positives there was a marked increment in pregnancy rate when they underwent IVF (19.23%) than IUI (13.64%). Hence, it is evident that ASA positives may have a better chance in achieving pregnancy when they undergo in-vitro fertilization. This observation could not be evaluated statistically because the number of subjects in some categories was less than 5.

6.2.2 Effects of type of ASA on clinical pregnancy rate

A total of 08 clinical pregnancies were observed among the ASA positive (3-IUI, 5-IVF) couples, following the ART procedure. Table 6.2.2 shows the Ig isotypes of ASA with the number of pregnancies. It was observed that couples with IgG isotype of ASA achieved the highest pregnancy rate (23.08%) while couples with IgA isotype of ASA had the lowest pregnancy rate (10.53%).

Table 6.2.2 Occurrence of clinical pregnancies with the type of ASA and location of ASA on spermatozoa

Type of ASA	# of transfers/ASA positive couples	# of clinical pregnancies	Pregnancy rate
IgA	19	02	10.53%
IgG	13	03	23.08%
IgA + IgG	16	03	18.75%
Location of ASA			
Head	10	0	
Tail	24	05	20.83%
Midpiece + tail	11	02	18.18%
Whole sperm	03	01	33.33%

6.2.3 Effects of location of ASA on spermatozoa, on clinical pregnancy rate

Table 6.2.2 shows the location of ASA with the number of pregnancies. Most number of pregnancies was observed when ASA was bound to the tail of the spermatozoa. Importantly, there were no pregnancies achieved when ASA was bound to the head of the spermatozoa. However, one pregnancy was achieved with ASA bound to whole surface of spermatozoa by performing ICSI as the method of fertilization. These observation could not be evaluated statistically because the number of subjects in some categories was less than 5.

6.3 Effects of ASA on spontaneous pregnancy losses

As shown in Figure 6, there were no spontaneous pregnancy losses observed in ASA positives compared to one miscarriage observed in ASA negatives (5.88%; 1/16), when they underwent IUI. However, in couples who underwent IVF, there were 02 (40%; 2/5) spontaneous pregnancy losses observed in ASA positives compared to 06 (27.27%; 6/22) miscarriages in ASA negatives. There were obviously a higher number of miscarriages observed in ASA positive couples who underwent IVF. This observation supports the fact that ASA may have deleterious effects on post implantation events of conception. However, it could not be evaluated statistically because the number of subjects in some categories was less than 5.

(v) Discussion

Sperms have been known to be antigenic for more than a century. Although there is a strong body of evidence that in humans and in other species at least some antibodies that bind to sperm antigens can cause infertility, it is still a debated matter. This is mainly due to the fact that a significant percentage of fertile couples have detectable ASA, and clearly showing that these antibodies have not all disrupted fertility.

Investigations of ASA and improvements in their methods of detection have advanced rapidly during the 1970s, 1980s and early 1990s but not so much during the last few years. This is in large part due to the observation that ICSI could be used as an effective treatment for circumventing immunoinfertility (Bohring C 2002). Most existing techniques used to detect ASA, fall short of being able to distinguish reliably between ASA that contribute to infertility and those that do not (Shibahara H 2003). However, the rational use of current ASA-tests can be effective in screening and quantifying sperm autoimmunization relevant to infertility (Shibahara H 2003). The mixed agglutination reaction latex bead test (spermMAR) provides a rapid assay time, good specificity, isotype and the location of the ASA and the ability to use viable sperms (Mazumdar and Levine 1998).

Epidemiological studies on immunoinfertility in Sri Lanka have been performed only infrequently. The largest of these, concerning antisperm antibodies, has been conducted at the Department of Obstetrics and Gynaecology, Faculty of Medicine, Colombo during 1997 to 2000 (Fernando DMS 2001). The reported incidence of ASA among the sample was 9.5%; among males it was 8.25% while that of females was 1.75% when detected by spermMAR test in semen of males and serum of both males and females. The incidence of ASA has been estimated to range from 9%-36% in infertile couples in world literature depending on the test format, detecting assays and the reporting centre (Lee R et al. 2009). The present study shows an incidence of 20.87% ASA among infertile couples with 12.61% in males and 8.26% in females. This corresponds with world data. However, the incidences are higher than the previous study conducted in Sri Lanka, provided the fact that more test samples were preformed on each subject to screen for ASA in this study. Sinisi et al.; 2008 demonstrated 13% prevalence of IgG type of ASA on spermatozoa surface in 750 infertile men by spermMAR test. A study by Collins JA et al., showed that among 471 couples in whom both partners were evaluated, 42 (8.9%) tested positive for anti-sperm antibodies by immunobead testing, tray agglutination testing or gelatin agglutination test, including 38 (8.1%) male partners and 6 (1.3%) female partners. In the same study they have shown a minor percentage of antibody positivity in fertile couples. It is reported that 2.4% to 10% of fertile couples may possess ASA (Francavilla et al. 2007), implying the fact that not all ASA disrupt fertility. Many studies have showed a higher incidence of ASA in males than the females (Collins JA et al. 1993, Fernando DMS 2001). The present study showed similar results although the difference was not statistically significant.

A trend towards late marriages at present contributes for higher mean age of males and females in infertile population than in the past. The mean age of the females was 33.38 and that of males was 36.86 years in the present study. It corresponds with most of published literature (Esteves SC et al. 2007). Different studies have shown the existence of an age dependence in the

incidence of natural sperm antibodies in normal animals (Flickinger CJ et al. 1997) and that such variation exists in human as well (Mathur S et al. 1981). However, these studies have employed predominantly healthy individuals and demonstrated conflicting results. A large scale study by Kalaydjiev et al., again on healthy individuals has investigated different age groups, starting with newborn and their mothers up to subjects older than 88 years, using four sperm antibody assays. They have showed low incidence of ASA in pre pubertal ages, a rise during reproductive age with a gradual decline with aging (Kalaydjiev SK et al., 2002). The present study did not show a significant difference in incidence among the age groups that were analyzed in both males and females. It can be explained by the fact that the whole study population fell into the reproductive age group and was a selective population of infertile couples. The presented results therefore demonstrated that there is no age dependent tendency in incidence of ASA during the reproductive age among infertile couples.

There was no statistically significant difference observed between the incidence of ASA and duration of marriage in the present study. The same result was observed with duration of infertility as well. Theoretically this observation is expected, since the causes for formation of ASA are well defined in males and females. Interestingly, there were no ASA positives observed among the couples (n=11) who were married for six months to one year period. Mechanical or chemical disruption of the mucosal layer of female genital tract may permit exposure to foreign sperm antigens and, ultimately ASA formation (Mazumdar and Levine 1998). This may explain the absence of ASA in females in early part of marriage. However, the reason most women do not develop an immune response after repeated sperm exposure is not yet established. The other possible explanation for this observation would be the small number of couples that were investigated in that duration of marriage would have demonstrated that result by chance. The lack of local or foreign data in relation to the incidence of ASA with duration of marriage or infertility, makes it impossible to compare the data of the present study.

Occupation of the male partner has been widely studied in relation to the state of infertility. However, the impact on immuneinfertility by the type of occupation is not clear. Occupational exposure to lead or mercury was found to make protein better antigens. The production of autoantibodies to nervous system protein is one example of such effect. Shamy MY et al., have assessed serum ASA levels in healthy male workers exposed to lead (n = 50) or to mercury (n = 39) using ELISA technique. Antisperm antibodies were detected in 90% of workers exposed to lead with the predominance of the IgG type and 84.6% of workers exposed to mercury with the predominance of the IgM type (Shamy et al. 1998). No subjects were exposed to heavy metals in the present study and the incidence of antisperm antibodies in different occupational categories was not significant. Factors such as heat, vibration, radiation and stress have been suggested as risk factors for infertility although its impact on immunoinfertility is questionable.

Antisperm antibodies exhibit deleterious effects on various stages of fertility from sperm-cervical mucus interaction to implantation and early embryo development (Mazumdar and Levine 1998, Cline and Kutteh 2009). Also, it has been shown a tenuous association between the prevalence of ASA in women's sera and recurrent miscarriages. Secondary infertility in men can be found in men who develop varicocoele (Djaladat H et al. 2006) or who undergo genito-urinary surgeries (Vivas et al. 2007); both conditions that are well known for ASA formation. In

the present study, there was no significant difference of incidence of ASA between primary infertility and secondary infertility. ASA may prevail in both types of infertility in both males and females as it could develop with time influenced by other conditions.

Sperm have foreign antigens because they are not present until after puberty and most likely develop in an immune privileged site. There are several hypotheses for ASA formation in men. Theoretically, the blood testis barrier may be breached by a variety of mechanisms resulting in exposure of immunogenic sperm antigens to the immune system, initiating an immune response resulting in an inflammatory reaction and ASA formation (Mazumdar and Levine 1998). It is shown that after vasectomy, approximately 50% of men produce ASA (Chamley and Clarke 2007). Fu GB et al. 2006 have demonstrated that testicular injury can induce and elevation of ASA, which could last a very long time. Likewise, a disproportionately higher incidence of ASA has been shown in spinal cord injured men (Hirsch IH et al., 1992) and men with varicocoeles/varicocoelectomies (Djaladat H et al., 2006). It was observed as a statistically significant association between the presence of ASA and a history of genital surgery in the present study. Majority of them had undergone varicocoelectomy. Theoretically, presence of varicocoeles also can induce formation of ASA as impaired venous drainage of the testis may result in damage to the seminiferous tubules and lead to ASA production and the surgery may alleviate that, though causing disruption of blood testis barrier. Testicular trauma, orchidectomy, vasectomy or spinal cord injuries were not observed in males in the study sample.

Inflammation may lead potentially to genital tract disruption and ASA formation. History of mumps has been shown to cause abnormal semen parameters in a Sri Lankan population (Fernando et al. 1998). However, formation of ASA is basically caused by mumps orchitis. Absence of a history of genital tract infections was a significant observation in the present study. Anyhow, no semen samples were found to be positive for culture in men who had increased pus cells and microorganisms. In the present study it was observed that 27.83% of the males had mumps in childhood, and none had mumps orchitis. Incidence of ASA with childhood mumps was not significant.

In recent studies some authors have suggested a possible cross reactivity of antibodies to spermatozoa in individuals who are having a systemic autoimmune disease. Shirashi Y et al. 2009 have investigated antisperm antibodies in the sera of 70 males with systemic autoimmune diseases and 80 healthy controls, by using the indirect-immunobead test. Among 70 males with systemic autoimmune diseases, the incidence of ASA was 7.1%, and no positives existed in 80 healthy males. The incidence of ASA in males with systemic autoimmune diseases was significantly higher than in the healthy controls. They have concluded that systemic autoimmune diseases may be one of the risk factors for developing ASA in men. However, in the present study, neither the males gave a history of rheumatoid arthritis, autoimmune thyroid disease or any other systemic autoimmune disease, nor the females who gave a history of any systemic autoimmune illness were positive for ASA. Hence, a statistical analysis could not be made and further studies are encouraged in this regard.

Effect of some antihypertensives, anticonvulsants and antipsychotics are known to cause impairment of fertility by affecting the spermatogenesis or by sexual dysfunction (Pandiyan 2000). However, the co-morbid illnesses like hypertension, epilepsy, hyperprolactinaemia and

diabetes mellitus type II have not been studied to determine the association with the incidence of ASA in large scale studies. In the present study there was no statistically significant association found with the presence of co-morbid illnesses in males and females. A definite conclusion of the effect of a co-morbid illness or its drugs cannot be made considering the small number in the present study.

By reviewing the literature on impact of smoking, alcohol and other addictables on immuno infertility, it is evident that the effects remain inconclusive. The studies on smoking and sperm abnormalities show a limited effect of smoking on conventional sperm parameters. It has been observed that smoking had an adverse effect on the progressive sperm motility, irrespective of total amount of cigarettes smoked per day (Hassa H et al., 2006). Supportive evidence is found in the studies of other investigators as well (Fernando DMS 2001, Soares and Melo 2008). Studies on cigarette smoking and incidence of ASA are limited. A study by Ludwikowski et al., 2004 has shown an increased number of antisperm antibodies in smoking patients, though statistically not significant. In the present study there was no significant correlation found with smokers and non-smokers in the incidence of ASA. The data on alcohol are sparser, and show an apparent protective effect of moderate alcohol drinking on sperm parameters, probably due to the antioxidant effect of some alcoholic beverages (Marinelli D et al., 2004). Close CE et al., have studied the relationship of current use of cigarettes, marijuana and alcohol to the parameters of seminal fluid analysis, sperm penetration assay and sperm autoimmunity in 164 men from infertile couples. In that study users of cigarettes, marijuana or alcohol showed no decrease in sperm count, motility or percentage of oval sperm, and no difference in prevalence of antisperm antibodies compared to nonusers. In the present study too, there was no significant correlation found with alcohol consumption and incidence of ASA.

In females another possible contributory factor for the formation of antisperm antibodies is repeated intrauterine inseminations with washed spermatozoa. Intrauterine insemination is widely used for the treatment of infertility as a result of cervical or male factors or empirically before in vitro fertilization. Different studies have shown that in male immunological infertility, well-timed IUI is an effective treatment method (Lahteenmaki A et al. 1995) and that intrauterine insemination does not induce significant ASA production in women (Goldberg JM et al. 1990). In the present study, though there was no statistically significant relationship found, the incidence of ASA was proportionately higher among women those who had previous IUIs (11.7%) compared to the women who did not have IUIs (5.88%). There is a theoretical concern that IUI may induce antisperm antibodies in women as in IUI, semen bypasses the physiological immune barrier of female and washing removes immno-suppressive properties of seminal plasma. Livi et al., have demonstrated the risk of subsequent risk of immunity to sperms in women following intra-peritoneal inseminations..

Antisperm antibodies are immunoglobulins, which are secreted from specially activated B lymphocytes, the plasma cells. The plasma cells may be systemically active and topical activity is also possible. ASA present in the human biological fluids are predominantly of the IgG and IgA class (Krause and Naz 2009). It is reported that sperm bound IgG was present in 8.2% and IgA in 7.1% of men as detected by mixed antiglobulin reaction (Meinerts and Hjort, 1986). Shibahara et al., have reported an incidence of 2.5% IgG and 1.8% of IgA bound to sperm surface in 275 semen samples by using direct immunobead test (Shibahara H et al. 2002). In the

present study the incidence of the type of ASA is little higher to that reported above. This can be explained by the facts that the present study was conducted on a group of infertile couples and that more test samples were screened for ASA in both males and females. It is also observed that the most prevalent antisperm antibody type in males was IgA. It was 86.21% among ASA positive males. In contrast IgG positivity among them was 51.72%. Interestingly no one demonstrated IgA alone in their serum. And no one demonstrated IgG alone in their semen. Also the data showed that presence of ASA was higher in semen (n=24) than in serum (n=6) in males. In semen, ASA was demonstrated on spermatozoa in 72.41% of men. ASA were described in the blood serum of male and female patients. It has become evident that ASA in serum mainly are not a consequence of the contact to sperm antigens, but they are independently existing isoantibodies (Krause and Naz 2009). The IgG antibodies in semen are derived from the serum IgG, but ASA of the IgA fraction originate from a local production. And usually men expressing ASA in semen is higher than in serum (Krause and Naz 2009).

In the present study, the IgA positivity among ASA positive females was 52.63%. The IgG positivity among them was 73.68%. In ASA positive females, again, no one demonstrated IgA alone in serum and follicular fluid as well. And no one demonstrated IgG alone in cervical mucus. Most females had ASA in serum and follicular fluid (n=14) than in cervical mucus (n=7) The occurrence of ASA in cervical mucus is generally quoted to be rare and their concentrations are not correlated to those in blood serum (Krause and Naz 2009).

Hence, it is suggested that semen and cervical mucus always contained IgA isotype alone or together with IgG. Serum always contained IgG alone or together with IgA in both males and females. That observation was valid for the follicular fluid as well. This data postulates the fact that males possess more locally secreted ASA, having demonstrated higher percentage of antibody positivity in semen and females possess more systemically transudated ASA, having demonstrated higher percentage of antibody positivity in serum and follicular fluid. This observation has a clinical significance as it guides the clinicians to opt the best samples for screening males and females for ASA. As it is evident the best samples for screening male would be IgA ASA on spermatozoa and for female IgA and IgG ASA in serum.

There is a diversity of ASA bound to the sperm surface; each of the types of ASA can be bound to the whole sperm surface or selectively to the head, midpiece or tail of the sperm (Yeh et al. 1995, Shibahara et al., 2002). It was observed that anisperm antibodies were frequently present as tail bound form in males (48.23%) and females (47.37%) in the present study. It was noted that presence of ASA bound to whole surface of the spermatozoa was infrequent in both males (3.45%) and females (10.53%). There was no particular type of ASA was observed significantly bound to a particular location on spermatozoa. This finding could not be compared as no data on this regard were available in other published literature. Both IgA and IgG isotypes of ASA could be bound to any location on spermatozoa, though it was found bound predominantly to the tail of the spermatozoa, in accordance to the data of present study.

Numerous studies have shown a negative association between ASA positives with fertilization and cleavage rates. In order for sperm to fertilize an oocyte, several molecular interactions must take place. The sperm binding to the zona pellucida and binding to the oolemma are thought to be mediated by specific protein or protein–carbohydrate interactions, which ASA could

potentially disrupt (Brewis IA et al. 2005). Definitive studies in various animal models have shown an association between sperm antibodies and post fertilization embryonic degeneration (Chamley and Clarke 2007). Firstly, the sperm membrane is integrated into the zygote membrane during the process of fertilization so that sperm antigens are incorporated, into the developing embryo. Secondly, embryonic gene expression commencing from the four to eight cell stage results in the synthesis of various developmental antigens, which can cross-react with sperm antigens (Menge and Naz 1988).

Several studies have demonstrated low fertilization and cleavage rates of embryos in ASA positives. Vazquez-Levin in 1997 demonstrated that fertilization and early embryonic cleavage found to be reduced significantly in ASA positives (Vazquez-Levin et al. 1997). Most of the recent studies also show the negative effects of ASA on fertilization and cleavage rates (Bohring C 2002, Shibaha H et al. 2003, Cline and Kutteh 2009). In contrast, few other authors found that ASA did not affect sperm–oocyte binding (Liu et al. 1991). In the present study, fertilization was not affected due to the presence of ASA. Infact ASA positives showed a significantly higher fertilization rate (P-value=0.001) than ASA negatives. However, the cleavage rate of embryos in ASA positives was significantly lower (P-value=0.037) than that of ASA negatives. Therefore this data indicate that the antibodies to sperm surface antigens may inhibit early cleavage of oocytes although it does not affect fertilization. These findings may suggest that these antigens may constitute an extranuclear cleavage signal for early division of fertilized zygotes (Naz RK 1992). Extensive studies involving many IVF centres are in need to investigate the magnitude of this effect.

A number of studies have been published showing the relationship of isotype and titre of ASA with fertilization (Witkin SS et al. 1992, Bohring C et al. 2004). When evaluating the relationship between Ig isotype of ASA and fertilization, Witkin et al., found that sperm bound antibodies, mainly IgA may directly lower the fertilization rate. Yeh et al., claimed that high titres of IgA and IgM isotypes in semen affect fertilization remarkably; but IgG does not modify the fertilization rate (Yeh WR et al., 1995). Other studies have shown the influence of isotype of ASA not only on fertilization and cleavage but on pregnancy rate as well. Chang et al., concluded that ASA can influence the results of IVF, dependent upon the isotypes of ASA, and IgA isotype in female sera has also being associated with a decrease in pregnancy rate (Chang TH et al., 1993). Vazquez-Levin et al., reported a significant reduction both in the cleavage rate and pregnancy rate (Vazquez-Levin et al. 1997). In the study by Witkin and David, it was found that 44% of women who miscarried were positive for sperm antibodies in their serum compared with only 12% of women who had successful ongoing pregnancies (Witkin and David, 1988). In those studies, examination of the Ig classes of the antibodies revealed that IgA was significantly more common in women who miscarried. In contrast to these studies other authors have not found a statistically significant association (Clarke and Baker 1993). In the present study, the fertilization rates and cleavage rates of Ig isotypes were not significantly affected by the method of fertilization. When comparing with ASA negatives as the standard value, IgA and IgG isotypes had higher total fertilization rates (82.76%, 70.75%) and markedly lower total cleavage rates (45.82%, 49.33%) than that of ASA negatives (58.54%, 61.73%). IgA isotype was observed as having the highest fertilization rate and the lowest cleavage rate. It also shows that IgA isotype has the lowest pregnancy rate (10.53%). This suggests that IgA isotype may have the most deleterious clinical effects on cleaving embryos.

Concerning the relationship between the Ig binding sites and fertilization, Bronson et al., observed that sera containing ASA directed primarily against the sperm head diminished the percentage of oocytes penetrated by sperm and the number of sperm penetrated per oocyte (Bronson RA, 1982). Studies by Witkin et al., Yeh WR et al. and Bohring et al., have published that antibodies reacting with sperm tail and head could decrease drastically the sperm fertilizing potential. The present study showed a marked reduction on cleavage rate when ASA was bound to head and significant reduction (P-value=0.03) when bound to midpiece+tail of the sperm surface with that of ASA negatives in Standard IVF. Head status of spermatozoa long has been assumed to play a key role in fertilization because of sperm's plasma membrabe receptors and its internal nuclear content. The midpiece+tail on the other hand may have high density of ASA on spermatozoa surface which affect cleavage rate. Our study also shows that when ICSI was performed on oocytes, there was no significant difference in cleavage rates among different locations compared to ASA negatives. This is supported by numerous other studies which have shown that ICSI as an effective method of treatment in immunoinfertility (Lambardo F et al. 2004, Shibahara H et al. 2005). This technique bypasses most, if not all, of the steps in conception that are adversely affected by antisperm antibodies in human infertility (Chamley and Clarke 2007).

Concerning the pregnancy rate with the site of binding of ASA, present study demonstrated that most pregnancies (n=5) were observed with tail bound ASA. Interestingly, no pregnancies were observed with head bound ASA. A single pregnancy was achieved in ASA bound to the whole surface of the sperm with ICSI as the method of fertilization. Though the numbers of pregnancies were small to statistically define, it is suggested that head bound ASA may have deleterious post fertilization effects on developing pre implantation embryo. The negative effects of ASA on implantation failure have been reported in several other studies (Cline and Kutteh 2009). Moreover, these studies even show impaired pregnancy rates and increased miscarriage rates among ASA positives (Vazquez-Levin et al. 1997, Cline and Kutteh 2009). Nevertheless, more studies with large number and control groups need to be conducted in this regard to achieve conclusive data.

Summarizing all the observations and conclusions made from the present study, it is evident that some ASA cause infertility or contribute significantly to infertility in humans. It is observed that infertility due to presence of ASA is relatively rare, but not so uncommon. The question that needs to be answered is should one wait for failed fertilization or repeated unsuccessful treatment procedures to perform tests for ASA retrospectively? Present study supports that always it is advisable to detect the presence of ASA at an early stage in infertile workup, for a small individual cost, relative to the total cost for repeated unsuccessful procedures and also to save on time. It is proper time to consider whether detection of ASA should be included in routine IVF test protocols and at early stage in general infertile workup as well. A significant step forward in this field would be the identification of specific antigens that when bound by ASA would specifically affect fertility. This distinction could then lead to changes in treatment of ASA positives and a more individualized approach to therapy saving time, money and emotional stress of infertile couples.

(vi) Conclusions

- There was no significant correlation observed with the incidence of ASA in different age groups of both males and females.

- There was no significant correlation observed with the incidence of ASA with the duration of marriage and duration of infertility.

- There were no relation observed with the presence of ASA and the type of occupation of the subjects of the study sample.

- There was no significant correlation observed with the incidence of ASA with the primary infertile couples and the secondary infertile couples.

- The incidence of ASA was proportionately higher in females who have undergone previous IUI, compared to females who have not undergone previous IUI. However, as the p-value was 0.115, there was no significant tendency of having ASA in women who have undergone previous IUI.

- This data did not show an association with a history of miscarriage induced/spontaneous with the incidence of ASA in females.

- This data did not show an association with the presence of an autoimmune diseas and the incidence of ASA in females. Likewise there was no significant correlation between the incidence of ASA and presence of a co-morbid illness in females.

- The incidence of ASA was proportionately higher in males who gave a history of childhood mumps compared to males who did not have childhood mumps. However, as the p-value is 0.194, there was no significant tendency of having ASA in males who had childhood mumps.

- There was a directly proportionate statistically significant correlation observed in males who have undergone previous genito-urinary surgeries and the incidence of ASA, compared to males who had not undergone such surgeries.

- This data did not show an association with the presence of co-morbid illnesses and the incidence of ASA in males. Likewise there was no significant correlation between the incidence of ASA and the use/non use of addictables in males.

- The incidence of ASA in males in this study group was 12.61%, and in females it was 8.26%. Hence, the incidence of ASA in infertile couples in this study group was 20.87%. This percentage corresponds with local and international values for prevalence of ASA in infertile couples.

- This data showed that in males, precence of ASA is proportionately high in semen (mainly locally secreted) than serum (mainly transudates). In females no difference observed with locally secreted (cervical mucus) sites and transudated (serum and follicular fluid) sites.

- In this study group, among ASA positives, IgA alone was only present in local ASA secretory sites, i.e. semen and cervical mucus. IgG alone was not present in semen and cervical mucus.

- The data demonstrated that most ASA were present in tail bound form in males and females. Few ASA were observed with head bound form and insignificant number of ASA were observed with whole sperm bound form.

- The pregnancy rates were almost similar in ASA positives and negatives following the ART procedure.

- However, there was proportionately higher first trimester miscarriage rate observed in ASA positives than in ASA negatives in females underwent IVF.

- The oocyte fertilization rate was statistically significant in ASA positives than in ASA negatives in standard IVF. Standard IVF eliminates the negative effects of ASA in cervical, uterine and follicular secretions.

- ASA positives had a higher fertilization rate than ASA negatives when oocyte fertilization done by ICSI. This was statistically highly significant. ICSI eliminates the negative effects of ASA in cervical, uterine and follicular secretions and also of ASA bound to spermatozoa.

- The data showed again a statistically high significance in oocyte fertilization in ASA positives than in ASA negatives irrespective of the fertilization method (IVF/ICSI).

- ASA positives had a proportionately lower rate of embryo cleavage than the ASA negatives when fertilized with IVF or ICSI or irrespective of the method of fertilization. This supports the fact that ASA has a negative effect on post fertilization events.

- There were no pregnancies observed in couples who had head bound ASA. Presence of ASA more towards tail piece of the sperm may not have a significant effect on fertilization.

- ICSI was the treatment of choice for the female who had ASA in cervical mucus, serum and follicular fluid.

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(viii) Problems if any, encountered during the implementation of the project

No major problems were encountered during the implementation of the project. As procurement of SpermMar test kits were handled by National Science Foundation the delays in transfer of finances to the University of Sri Jayewardenepura was avoided.

(xi) Major findings and follow up activities

- When oocyte fertilization done by ICSI, ASA positives had a higher fertilization rate than ASA negatives
- Irrespective of the fertilization method (IVF or ICSI), the oocyte fertilization inASA positives were higher than in ASA negatives. This implies that early intervention with ART will be needed in ASA positives.
- ASA has a negative effect on post fertilization events

Section 4

Impact of Research results

(i) Relevance of results achieved to scientific advancement

The present study supports that it is beneficial to detect the presence of ASA at an early stage in infertile workup and thereby to plan the assisted reproductive technology to be used. Detection of ASA should be included in routine IVF test protocols and even at early stage in general infertile workup as well.

(ii) Relevance of results achieved to national/ socio-economic development

By including the detection of ASA in routine IVF test protocols the total cost of certain repeated unsuccessful assisted reproductive technology procedures can be cut down and also can save on time. Thereby the cost of IVF could be reduced to some extent.

(iii) Dissemination / application of research outpt

The detection of the presence of ASA in subfertile couples in Sri Lanka is not done as a routine test at the moment. The detection ASA should be included as a routine test when investigating and treating subfertility. Dissemination of the research results could be done through communicating these results to the relevant professional bodies such as the College of Obstetricians and Gynaecologists and the Sri Lanka Medical Association and the relevant ministry- Ministry of Health so that when guidelines and protocols for the management of subfertility / infertility is made these results could be taken in to consideration.

Section 5

Miscellaneous

(i) List of major equipment acquired during the project period

No major equipment was acquired during the project period.

(ii) List of Publications / communications arising from the project

Full Papers

1. Varuni Tennakoon, Surangi G.Yasawardene and Deepal S. Weerasekera. Antisperm antibodies: Incidence, isotypes and location on spermatozoa, their implications on fertilization and on pregnancy rate at a selected centre in Sri Lanka.

Sri Lanka Journal of Obstetrics and Gynaecology 2010; 32: 8-16.

2. Varuni Tennakoon, Deepal S. Weerasekera and Surangi G.Yasawardene. Successful pregnancy outcomes following in-vitro fertilization in infertile couples with high levels of antisperm antibodies.

Sri Lanka Journal of Obstetrics and Gynaecology 2010; 32: 47-48.

Published Abstracts

1. Tennakoon V, Weerasekera DS, Yasawardene SG, Ralapanawe MSB. Successful pregnancy following intra cytoplasmic sperm injection (ICSI) and embryo transfer in a female with high antisperm antibody (ASA) levels.
40th Annual Scientific Sessions, Sri Lanka College of Obstetricians & Gynaecologists 3-4 Nov 2007.

2. Tennakoon V, Weerasekera DS, Yasawardene.SG. Effect of antisperm antibodies on fertilization and cleavage rates in subfertile couples undergoing in-vitro fertilization.
40th Annual Scientific Sessions, Sri Lanka College of Obstetricians & Gynaecologists 3-4 Nov 2007.

3. Tennakoon V, Yasawardene SG. Incidence of Antisperm Antibodies (ASA) in male and female subjects undergoing subfertility treatment at a selected centre.
63rd Annual Session of Sri Lanka Association for the Advancement of Science. 5-8 Dec. 2007.

4. Tennakoon V, Weerasekera DS and Yasawardenen SG. Detection of Antisperm antibodies, pattern of distribution on spermatozoa and its implications on assisted reproductive technologies.
41st Annual Scientific Sessions, Sri Lanka College of Obstetricians & Gynaecologists In association with Asia - Oceania Federation of Obstetrics & Gynaecology and supported by Indian College of Obstetrics and Gynaecologists 27-29 June 2008.

5. Tennakoon V, Weerasekera DS and Yasawardenen SG. Descriptive analyses of Antisperm antibodies (ASA) in a subfertile population.
41st Annual Scientific Sessions, Sri Lanka College of Obstetricians & Gynaecologists In association with Asia - Oceania Federation of Obstetrics & Gynaecology and supported by Indian College of Obstetrics and Gynaecologists 27-29 June 2008.

6. Tennakoon V, Yasawardenen SG and Weerasekera DS. The influence of Antisperm antibodies (ASA) on embryo cleavage and pregnancy rate in subfertile couples undergoing Assisted Reproductive Technologies (ART).
Sri Lanka Medical Association 122th Anniversary academic Sessions, March, 2009.

7. Tennakoon V, Yasawardenen SG and Weerasekera DS. Effects of antisperm antibodies on fertilization, cleavage and pregnancy rate in infertile couples undergoing In-vitro Fertilization at a selected centre in Sri Lanka.
The Third World Congress on Mild Approaches in Assisted Reproduction, Yokohama, Japan, 30-31 July 2010.

8. Tennakoon V, Yasawardenen SG and Weerasekera DS. Effects of immunoglobulin isotype and sperm surface location of antisperm antibodies on fertilization, cleavage and pregnancy rate in human – A Sri Lankan study.
The Third World Congress on Mild Approaches in Assisted Reproduction, Yokohama, Japan, 30-31 July 2010.

Section 6

Summary statement of Expenditure

The grant was handled entirely by the National Science Foundation. No money was transferred to the University of Sri Jayewardenepura. Sperm Mar test kits were purchased through NSF under consumables.

I am not attaching a statement of expenditure as finances were handled by NSF.

Section 7

(i) Grantees signature

Prof. Surangi G. Yasawardene

(ii) Comments of the Head of the Department

Grantee is the Head of the Department of Anatomy where research was carried out.

Comments of the Dean, Faculty of Medical Sciences

Recommended

29/11/11

Prof Jaya...a Jayawardan
...an
Fa... ...y of ...al Science
University of Sri Jayewardene
Nugegoda.

(iii) Head of the Institution's signature

2011/11/29

Dr. N. L. A. Karunaratne
Vice-Chancellor
University of Sri Jayewardenepura
Nugegoda, Sri Lanka.

Vice-chancellor, University of Sri Jayewardenepura

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