

**Incidence of antisperm antibodies and
its association to subfertility in
couples undergoing
assisted reproductive technologies,
at a selected centre.**

By

Varuni Tennakoon



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association to subfertility in couples undergoing
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**Thesis submitted to the University of Sri Jayawardenepura for the
award of the Degree of Master of Philosophy in Immunology on
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I certify that the candidate has incorporated all corrections, amendments and additions recommended by the examiners.



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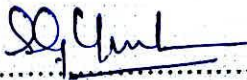

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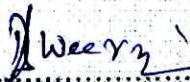
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Declaration by the Supervisors

We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University of Sri Jayawardenepura for the purpose of evaluation.



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Dr. James W Catt

**Dedicated to my parents,
My husband Chamil and my two sons
Dinu and Mithu**

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Abbreviations

ART	- Assisted reproductive technologies
ASA	- Antisperm antibodies
Chi-sq	- Chisquare
cm	- Centimetres
CR	- Cleavage rate
ELISA	- Enzyme linked immunosorbent assay
ESHRE	- European society for human reproduction and embryology
ET	- Embryo transfer
Fc	- Fragment crystalizable
FCM	- Flow cytometry
FR	- Fertilization rate
FSH	- Follicular stimulating hormone
GnRH	- Gonadotrophin releasing hormone
hCG	- Human choriogonadotrophin
IBT	- Immunobead test
ICSI	- Intracytoplasmic sperm injection
Ig	- Immunoglobulin
i-IBT	- Indirect immunobead test
IU	- International units
IUI	- Intra uterine insemination
IVF	- In vitro fertilization
MAR	- Mixed antiglobulin reaction

mil	- Millions
ml	- Millilitres
mm	- Millimetres
n	- number
PID	- Pelvic inflammatory disease
PR	- Pregnancy rate
rpm	- rounds per minute
SD	- Standard deviation
SIT	- Sperm immobilization test
TAT	- Tray agglutination test
WHO	- World health organization
ZP	- Zona pellucida

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ABSTRACT

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 subfertility are 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 subfertility will enable to streamline the treatment. The present study was performed to investigate the incidence of ASA in subfertile couples, and their effects on fertilization processes and pregnancy outcome following ART procedures (intra uterine insemination-IUI / in vitro fertilization-IVF).

Two hundred and thirty subfertile 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). Spermatozoa, seminal plasma and serum samples in males and cervical mucus, serum and follicular fluid in females were analyzed for ASA. The test was considered positive if 30% or more of the motile

sperm were attached to the latex particles. The isotype (i.e. IgA, IgG) and location of ASA (i.e. head, midpiece, tail of the sperm) on the spermatozoa were observed. In couples who underwent IVF, fertilization rate and day 03 cleavage rate of embryos were assessed. The pregnancy and miscarriage rates following each ART procedure were noted.

The incidence of ASA was 20.87% among the subfertile couples. It was 12.61% in males and 8.26% in females. No significant correlation observed with presence of ASA and age, duration of marriage/subfertility, type of subfertility 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 who have had previous IUIs (11.7%) compared to the women who did not have IUIs (5.88%).

The total fertilization rate was significantly higher (P -value=0.001) and the total cleavage rate was significantly lower (P -value=0.037) in ASA positives than that of the ASA negatives. No significant difference was observed in fertilization and cleavage rates among the Ig isotypes. However, IgA isotype of ASA demonstrated the highest fertilization rate and the lowest cleavage rate. Head or midpiece+tail bound ASA on spermatozoa exhibited 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%). 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.