

# PASSIVE IMMUNIZATION IN THE CLINICAL MANAGEMENT OF SECONDARY SPONTANEOUS RECURRENT MISCARRIAGE

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## ABSTRACT

**Objective:** To investigate whether intravenous immunoglobulin (IVIG) can improve the chance of livebirth in women with a history of recurrent spontaneous miscarriage (RM).

**Study design:** Randomized, double-blind, placebo- controlled trial.

**Patients and interventions:** Fifty four women with a history of secondary (RM) were randomly assigned to receive doses of (IVIG) or placebo (Saline) starting as soon as pregnancy test was positive.

**Results :** The live birth rate (LBR) was significantly higher in the IVIG group when compared with placebo-infused group even after exclusion of cases of ectopic pregnancy and cases with low serum  $\beta$ hCG and progesterone levels.

## INTRODUCTION

Of all clinically detected pregnancies approximately 10% end in miscarriage (1). Recurrent spontaneous miscarriage (RM) is defined as a series of at least three pregnancy losses, a condition that affects 1% of women who desire a child (2). RM is classified as either primary (never achieved a live birth) or secondary (normal pregnancy or pregnancy that progressed to at least 26 weeks followed by recurrent abortion) (3). The frequency of fetal aneuploidy decreases with the number of previous losses (4). On the contrary, several studies have reported that the prevalence of immunological disturbances (5) and of particular class I and class II HLA alleles (6,7) increases with the number of previous pregnancy losses.

A series of autoantibodies (8,9) and a T-helper type I cytokine bias (10) can be found with increased prevalence in these patients as well. However, there is so far no definitive diagnostic test that can identify women who have such alloimmune dysfunction.

In patients negative for autoantibodies, active immunization with allogenic lymphocytes (ALT) from the partner (PLT) or third party donors (DLT) have been used.

ALT increases significantly the chance of livebirth among patients with primary RM with no auto-or allo-antibodies whereas no effect could be detected in patients with secondary RM (11), (12).

On the other hand, intravenous immunoglobulin (IVIG) has been used for several decades as treatment for a variety of immune-related diseases including; immune thrombocytopenic purpura,

autoimmune neuropathies, systemic lupus erythematosus, Guillain-Barré syndrome, Kawasaki syndrome and others (13).

IVIg action may be mediated partially through induction of apoptosis in activated peripheral blood lymphocytes (14). Elevated levels of natural killer cells (NK) have been found in blood of women having miscarriages of karyotypically normal pregnancies (15).

Results of the seven placebo-controlled published studies using IVIg were conflicting with only two trials showing significant or almost significant treatment effect (16,17). The remaining five trials, however showed no effect. (18-22).

The Cochrane meta-analysis reported no increase in livebirth after IVIg (23), but the updated meta-analysis of the trials showed that treatment may be efficient in patients with secondary RM(24) unlike those with primary RM.

This placebo-controlled study was carried out to determine the clinical outcome of pregnancy in patients with secondary RM with IVIg treatment during their current pregnancy.

## **MATERIALS & METHODS**

### *Criteria of eligibility in this study :*

- \* History of  $\geq 4$  confirmed miscarriages- with the present male partner- before the end of the twenty sixth gestational week, of which the last three had been consecutive.
- \* Normal uterine cavity as proved by hysterosalpingography or hysteroscopy.
- \* Normal parental chromosomes by G-band technique.
- \* Regular menstruation (cycle length 21-35 days).
- \* Positive pregnancy test.
- \* Absent endocrinological abnormality.
- \* Written informed consent.

No age restrictions were imposed allowing women up to 45 years of age to participate in this study. Pregnancies obtained by IVF or controlled ovarian hyperstimulation were excluded. None of the patients had immunoglobulin A (IgA) deficiency.

The presence of an autoimmune rheumatic disease was not a cause of exclusion in this study.

### *Treatment protocol :*

Fifty four women meeting the above mentioned eligibility criteria were recruited for this study. Patients were instructed to conduct a pregnancy test if menstruation was three days overdue. Once pregnancy was confirmed, the first infusion was given.

After randomization, IVIg and equivalent amounts of saline (placebo) were administered. They could not be distinguished and the codes were blind for both the patients and the investigators. The typical infusion schedule was comprised of weekly infusions of 25g. IVIg from gestational week 5-9, and afterwards infusions were given fortnightly until gestational week 14 in women with only first trimester miscarriages and to gestational week 24 in women with second trimester losses, when no further infusions were given. The IVIg given in this study was GAMMARAAS<sup>®</sup> immunoglobulin IV 5% (human) manufactured by Shanghai RAAS and packed by VACSERA; Egypt. The product is double virus inactivated by virus filtration and low pH

### *Measures of effect :*

The primary outcome measures were; (a) livebirth rate (LBR) after exclusion of patients with serum progesterone  $< 35$  nmol/L and  $\beta$ hCG  $< 100$

U/L at the time of the first infusion, (b) LBR after exclusion of patients with ectopic pregnancies, (c) LBR after exclusion of a,b.

Secondary outcome measures were; gestational age at delivery, birth weight and Apgar scores. To have meaningful results 27 patients were assigned for each group. For each patient excluded or lost to follow up, one patient was added to maintain statistical power.

**Monitoring and laboratory tests:**

Transvaginal ultrasound scans were performed every second week from the 6th till the 12<sup>th</sup> gestational weeks to confirm the presence of heart beats and gestational age. Quantitative  $\beta$  subunit of hCG was measured every week till week 10. If the increase of  $\beta$ hCG was insufficient or vaginal bleeding occurred, weekly ultrasound examinations were undertaken. Survey by transabdominal ultrasound for congenital anomalies was done in gestational weeks 18 and 26. After the 26<sup>th</sup> week the routine pregnancy follow up was done.

Losses were considered fetal, if they occurred after the 10<sup>th</sup> week; embryonic before the 10<sup>th</sup> week but after documentation of fetal heart rate.

Pregnancies lost before depiction of fetal heart rate were pre-embryonic.

Checking for hepatitis B antigens and antibodies against hepatitis C and HIV 1 and 2 was done on fresh samples obtained at the time of inclusion, in week 24 and 3 months after birth, or last infusion in case of miscarriage. Hepatitis C and HIV antibodies and Hbs Ag virus were monitored using an enzyme immunoassay (EIA). Anticardiolipin antibodies (ACA) were measured using an enzyme linked immunosorbent assay (ELISA).

**Statistical analysis:**

Data were expressed as means  $\pm$  SD. Paired-t-test and chi-square tests were used to compare results between IVIG and placebo groups.

**RESULTS**

From July 2000 to January 2004, 54 women with a history of secondary recurrent miscarriages, who were confirmed to be pregnant before conclusion of the trial were randomized and included in this study.

**Table I : Characteristics of patients at time of inclusion .**

	IVIG (n = 27)	Placebo (n = 27)	P
Age (years)	30.7 $\pm$ 2.4	31.8 $\pm$ 1.1	NS
No with 4 previous losses	10 (37)	13 (48)	NS
No with 5 previous losses	9 (33)	7 (26)	NS
No with $\geq$ 6 previous losses	8 (30)	7 (26)	NS
Anti cardiolipin positive	9/27 (33%)	8/27 (30%)	NS
Hormones at randomization			
* serum $\beta$ hCG (u/L)	623.2 $\pm$ 102	549.5 $\pm$ 133	NS
* serum progesterone (nmol/L)	82.4 $\pm$ 14	76.3 $\pm$ 12	NS
Body weight (Kg)	72.7 $\pm$ 16	68.9 $\pm$ 14	NS

NS = non significant

Data are presented as means  $\pm$  SD.

All patients completed the infusion schedule until pregnancy was concluded by birth, or miscarriage.

Therapies used in management of RM e.g; progesterone, hCG, aspirin, heparin, prednisone, or allogenic lymphocyte immunization were not received by any patient during the course of this study. There was no statistical difference in the levels of  $\beta$ -hCG, and serum progesterone before the first infusion between IVIG and placebo groups (Table I).

Patients receiving IVIG achieved a LBR of

(21/27) 78% compared to (9/27) 34% in placebo infused patients,  $P < 0.01$  (Table II).

All pregnancy losses in IVIG group occurred in the first trimester (one fetal, one pre-embryonic, and two embryonic), whereas four miscarriages in the placebo group, occurred in the second trimester.

Seventy eight percent (7/9) of ACA positive patients assigned to IVIG group achieved a live birth. None of the ACA positive placebo patients had a live birth. Neonatal data did not differ between IVIG and placebo groups; (Table III).

**Table II : Pregnancy outcomes in participant groups .**

LBR	IVIG (n = 27)	Placebo (n = 27)	P
Total	21 / 27 (78%)	9 / 27 (34%)	< 0.01
After Exclusions *	21 / 25 (84%)	9 / 27 (34%)	< 0.01
Appropriate hormones °	21 / 24 (88%)	9 / 23 (39%)	< 0.01

\* Exclusions of the 2 ectopic pregnancies in the IVIG group.

° Serum  $\beta$ HCG > 100 iu/L and serum progesterone > 35 nmol/L at first infusion.

**Table III : Neonatal Data .**

	IVIG (n = 27)	Placebo (n = 27)	P
Birthweight (g)	3122.5 $\pm$ 241	3097.4 $\pm$ 213	NS
Gestational age (wks)	37.2 $\pm$ 2.4	38.1 $\pm$ 1.3	NS
Apgar score $\leq$ 7 at 5 min	0	0	NS
Cesarean section	12	11	NS
Neonatal disorders	3	1	NS
Sex of liveborn infants (male / female / unknown)	6/5/0	3/6/0	P < 0.01

Data are presented as means  $\pm$  SD.

For neonatal abnormalities, in IVIG group two infants were born with alloimmune thrombocytopenia, which was corrected postpartum by giving IVIG to these infants and they were healthy thereafter.

Another infant in IVIG group had cleft palate. In the placebo group only one infant had talipes calcaneocavus.

Tests for antibodies against hepatitis C and HIV remained negative in all patients through and after pregnancy.

Few mild side effects viz; arthralgia, low grade fever, malaise, myalgias and local skin rash were noted in 13 patients receiving IVIG but they were mild in all patients.

Unexpectedly, there was a higher overall live-born female/male gender ratio which was significantly different from the 1:1 ratio.

## DISCUSSION

The mechanisms that normally prevent a mother from rejecting her semi-allogenic conceptus are unclear.

The majority of recent research has focussed on immunologic causes but appropriate testing and treatment for RM due to immunologic causes is yet to be determined.

In 1988, Carreras et al.<sup>(25)</sup> suggested that IVIG could be an alternative to leukocyte infusions in patients with RM.

IVIG is a highly purified human IgG prepared from pooled plasma with diverse antibody profile because thousands of donors contribute to the pool<sup>(26)</sup>.

The use of IVIG to treat primary and secondary deficiencies in antibodies began 25 years ago.

Today, passive immunotherapy is attracting renewed interest as a new means to treat neurodegenerative, infectious, autoimmune and neoplastic diseases<sup>(27-29)</sup>.

Besides IVIG has been found to induce tolerance and improve clinical outcome after organ transplantation, and may work in the prevention of recurrent pregnancy loss<sup>(30)</sup>.

The results of uncontrolled pilot studies using IVIG were promising with successful pregnancy rate of 82% in women with RM<sup>(31-33)</sup>.

However, The results of the following five randomized controlled trials conducted to evaluate IVIG in RM were conflicting.

At a first glance, these diverging results provided no compelling reason to continue using IVIG in RM.

Nevertheless, the trials of immune therapy are too heterogenous in their inclusion of patients and treatment protocols to reach firm conclusions.

For instance, in this trial 58% (31 / 54) of patients had more than four previous miscarriages.

In the two placebo-controlled trials of the Danish group; 70-100% of patients had > 4 previous RM compared with only 19%<sup>(18)</sup> to 53%<sup>(20)</sup> of patients in other placebo controlled trials.

Based on the revised Cochrane meta-analysis showing improvement of livebirth only in patients with secondary RM; we selected only those patients in our study. A current literature search of RCTs using IVIG revealed that two trials were limited to women with primary RM (no live births)<sup>(18,19)</sup>, one involved only women with secondary RM,<sup>(16)</sup> and two trials included both primary and secondary RM<sup>(17-20)</sup>.

There are many indications that women with at

least four to five miscarriages and women with secondary RM have more immunological background for their problem (hence more likely to get IVIG therapeutic benefit) than those with fewer miscarriages and no previous live birth<sup>(34,35)</sup>.

Patients with IgA deficiency were excluded from this study. Similarly they were excluded from 4 RCTs<sup>(16,19-20)</sup>.

The prevalence of IgA deficiency is 1 per 1000 and severe side effects are encountered, albeit rarely, in these patients when IVIG infusion is given to them; e.g.: nephrotoxicity, alopecia, aseptic meningitis and retinal necrosis<sup>(26)</sup>.

Regarding treatment protocols, IVIG infusion was started before conception in three trials<sup>(17)</sup>,<sup>(36)</sup>,<sup>(20)</sup>, and during the first trimester in the present study and in three RCTs<sup>(18)</sup>,<sup>(16)</sup>,<sup>(19)</sup>.

To date there was no consensus regarding the dose employed as well. The doses given in RCTs varied widely, for example, Coulam et al.<sup>(17)</sup> gave 500 mg/kg bw in follicular phase and 500 mg / kg bw / 4 weeks until 30 weeks of gestation, whereas, Christiansen et al.<sup>(16)</sup> gave 30-40 g / person in gestational weeks 5 and 6, 20-30 g / person in gestational weeks 7, 8 and every 2 weeks till the 4<sup>th</sup> gestational week.

Shadowing experience with autoimmune thrombocytopenia Yamada and his coworkers<sup>(37)</sup> used a massive dose of 20 g/d for 5 consecutive days, a total of 100 g during gestational weeks 4-7 usually between 4-5 wks gestation. The regimen of IVIG infusion adopted in this study was identical to that given by the Danish co-workers after the year 2000<sup>(24)</sup>.

Karyotypic analysis of abortuses was not done in this study since the more the number of previous losses the less the frequency of fetal aneuploidy<sup>(4)</sup>.

On the other hand, the live birth rate was significantly higher in IVIG vs placebo infused patients in this study unlike the insignificant difference in aggregate LBR of the 5 RCTs between the two groups.

Despite years of use, the mechanism of IVIG immunomodulation is still unclear.

It was recently shown<sup>(38)</sup> that the protective effect of IVIG is due to up regulation of the inhibitory Fc receptor (Fc  $\gamma$  RIIB) on macrophages in the reticuloendothelial system (RES).

In addition IVIG induces RES blockade<sup>(20)</sup>. Moreover, IVIG use in immuno inflammatory conditions is associated with reduction in the serum concentrations of inflammatory markers such as IL-6 and TNF  $\alpha$  which are produced by macrophages and T lymphocytes CD4<sup>(22)</sup> and high serum TNF  $\alpha$  levels were found in pregnant women with a history of RM<sup>(39)</sup>.

In this study, we measured serum creatinine in weeks 5 and 24 and at 3 months after birth or last infusion, to detect any renal troubles.

Acute renal failure is a rare IVIG complication with approximately 150 cases reported in literature.<sup>(20)</sup> It has been associated with diabetes, advanced age, preexisting renal disease and administration of intravenous immunoglobulin in sucrose-based solutions and rarely with maltose stabilized preparations<sup>(40)</sup>. Sucrose, maltose, sorbitol, glycine, glucose or albumin are used as stabilizing agents to prevent immunoglobulin aggregation.

In this study we used GAMMARAAS<sup>®</sup> IVIG 5% (human) manufactured by Shanghai RAAS packed by VACERA Egypt. This product is stabilized by sorbitol to avoid the osmotic insult induced by sucrose on proximal renal tubules<sup>(41)</sup>.

Finally, intravenous immunoglobulins are tools

of an emerging technology in current medicine: immunoregulation.

The immune system has abandoned the status of mere champion of the body against infectious microbes and has assumed a position in the organic integration system with complex mechanisms essentially present in all diseases.

So far this is the only Egyptian RCT using IVIG in secondary RM.

Standardized IVIG regimens and more larger scaled placebo-controlled trials should be done to establish its use in RM.

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