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RESEARCH ARTICLE

The Effectiveness of Paternal Lymphocyte Immunotherapy for Recurrent Miscarriage in Couples with Human Leukocyte Antigen Sharing: A Novel Approach

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ABSTRACT

Background: The aetiology of recurrent pregnancy loss (RPL) is varied and ranges from genetic abnormalities, autoimmune, uterine structural abnormalities, thrombophilic disorders, endocrinologic dysfunction, infective to idiopathic factors. Reproductive immunology may provide an area of opportunity in treatment of idiopathic cases. Research has indicated that any amount of HLA compatibility among spouses leads to immunological perturbations leading to higher RPL rates. These disturbances in alloimmune parameters are found to be significantly reduced after a successful immunotherapy with paternal lymphocytes immunotherapy (LIT) among couples who share HLA.

Aim: To analyze the role of alloimmune factors in couples who are considered unexplained RPL by testing HLA sharing between the partners and to determine the effect of lymphocyte immunotherapy (LIT) on live birth rate in couples with HLA sharing.

Methods: This retrospective observational study was conducted in a single tertiary center in Bangalore for a duration of three years. Couples who satisfied the inclusion and exclusion criteria were selected and HLA sharing between the partners was tested. Couples with HLA sharing received LIT before and during pregnancy. The pregnancy and live birth rates were calculated and compared with couples with HLA sharing who did not receive LIT.

Results: Out of the 199 couples who were screened for HLA sharing among partners, 146 couples had different degrees of HLA sharing. 81 couples received LIT and 32 did not opt for LIT and were taken as control group. The pregnancy and live birth rates were significantly higher in the LIT group compared to control group (77.7% vs 40.6%, p-0.0001, OR 5.1, 95% CI 2.10-11.4 and 56.7% vs 21.8%, p-0.0002, OR 4.6, 95% CI 2.13-13.8 respectively). Miscarriage rates were similar between the two groups.

Conclusion: Partner lymphocyte immunotherapy is a novel treatment option in improving the pregnancy outcomes among women with unexplained RPL and HLA sharing among partners.

Institutional ethical committee registration number- EC/22/000115
Ethical committee approval number for study-EC/OA/46/2023

INTRODUCTION:

A miscarriage is the spontaneous demise of a pregnancy in utero prior to the period of viability (23 weeks 6 days). Recurrent pregnancy loss (RPL) is a condition seen in about 0.5% to 2.3% of couples.[1] There are different definitions for RPL given by the various gynaecological societies. Recent ESHRE 2017 defines RPL as two or more pregnancy loss, consecutive or non-consecutive up to 24 weeks of gestation excluding biochemical, ectopic and molar pregnancies.[2]

The aetiology of RPL ranges from genetic abnormalities, antiphospholipid antibody syndrome, uterine anatomic abnormalities, thrombophilic disorders, endocrinological dysfunction, infective to idiopathic factors. According to ESHRE[2], more than half the cases of RPL are due to idiopathic causes. Reproductive immunology may provide an area of opportunity in treatment of such idiopathic cases.

There is a plethora of immune reactions at play at the maternal fetal interface during any ongoing pregnancy. A well balanced and modulated immune system is vital for continuation of a healthy pregnancy. Any disruption in the immune cross talk between maternal and fetal tissues along with failure of maternal immune tolerance mechanisms can lead to unexplained miscarriages.

The embryo presents as a semi allogenic graft to the maternal immune system since half its genes are paternal in origin. Despite the embryo being akin to a foreign body, the conventional maternal immune responses are not mounted against the embryo. Instead, certain protective immune mechanisms are activated during pregnancy.[3] Sir Peter Brian Medawar (father of reproductive immunology) was among the first to identify the immunological paradox occurring in pregnant women in response to the foetus (semi-allograft). He proposed a theory where the foetus escapes the notice of the maternal immune system due to production of maternal "blocking antibodies".

Role of immunological causes for unexplained RPL is still debatable and various immune therapies proposed are controversial. Some of the proposed immunological mechanisms for unexplained RPL are lack of blocking antibody formation, excessive natural killer cell activity, dysregulation of T- helper 1 (Th 1) and T-helper 2 (Th 2) cell responses and T regulatory cell hypoactivity.

Paternal lymphocyte immunotherapy (LIT) is one among the various immunotherapies which are at experimental stage. The role of paternal LIT in RPL couples has been studied since 1980. It was seen that the alloimmune parameters are found to be significantly suppressed in successful immunotherapy among couples receiving LIT. The solution injected in LIT is a lymphocyte concentrate derived from the processing of the peripheral blood of the male partner or a third party donor to promote maternal blocking antibody production, thus creating a favourable immunological milieu for embryonic implantation. Till the 2000s the beneficial role of this therapy was questioned, but recent studies have shown improved gestational outcomes in couples with unexplained RPL.[1,4] In this retrospective study, we intended to evaluate the effectiveness of LIT on pregnancy outcomes among couples with recurrent miscarriage and human leukocyte antigen (HLA) sharing.

AIMS and OBJECTIVES

1. To analyse the role of alloimmune factors for RPL in couples who otherwise are considered as unexplained RPL by testing HLA sharing between the partners.
2. To determine the effect of lymphocyte immunotherapy (LIT) on live birth rate in couples with HLA sharing.

SUBJECT AND METHODS:

This retrospective observational study was conducted in a single tertiary center (Gunasheela Surgical and Maternity Hospital) in Bangalore for a duration of three years between August 2019 to July 2022. Approval of scientific review committee and ethics committee of Gunasheela Surgical and Maternity Hospital, Basavanagudi, was obtained (Institutional ethical committee registration number-EC/22/000115 and ethical committee approval number for study-EC/OA/46/2023).

The sample size was calculated based on a previous publication by M Sarno et al,[1]with 95% CI and 80% power the minimum sample size calculated to be 56 (i.e., 28 in each group - LIT and No LIT group). Inclusion criteria were women aged < 40 years with BMI $< 30 kg/m^2$ and ≥ 2 pregnancy losses and who also had HLA sharing between the partners. Some of these women had thyroid abnormalities and diabetes mellitus which were well controlled, hence were included. Exclusion criteria consisted of couples with chromosomal abnormalities, presence of autoimmune factors like antiphospholipid antibodies, antinuclear antibodies, women with thrombophilias, uterine abnormalities, pelvic inflammatory disease and genital tuberculosis, severe male factor infertility and no HLA sharing between the partners.

Subjects who satisfied the inclusion and exclusion criteria were analyzed in this study. Data was collected retrospectively.

HLA typing library preparation: Full length amplification of HLA – A, B, C, DRB1, DQB1 and DPB1 loci using NGSgo – MX6 – 1 kit

(GenDXNGSgo) using the long-range PCR method and the concentration of the resulting amplicons obtained using Fluorescent based method (Qubit 3.0).

Human leucocyte antigen (HLA) typing was done in each of the partner to look for sharing in the HLA genes at six loci - A, B, C, DRB1, DQB1, DPB1. Two alleles for each locus were tested. Partners with HLA antigen sharing were assigned a score ranging from 1–12. In couples with score of >1/12, paternal lymphocyte immunotherapy was offered.

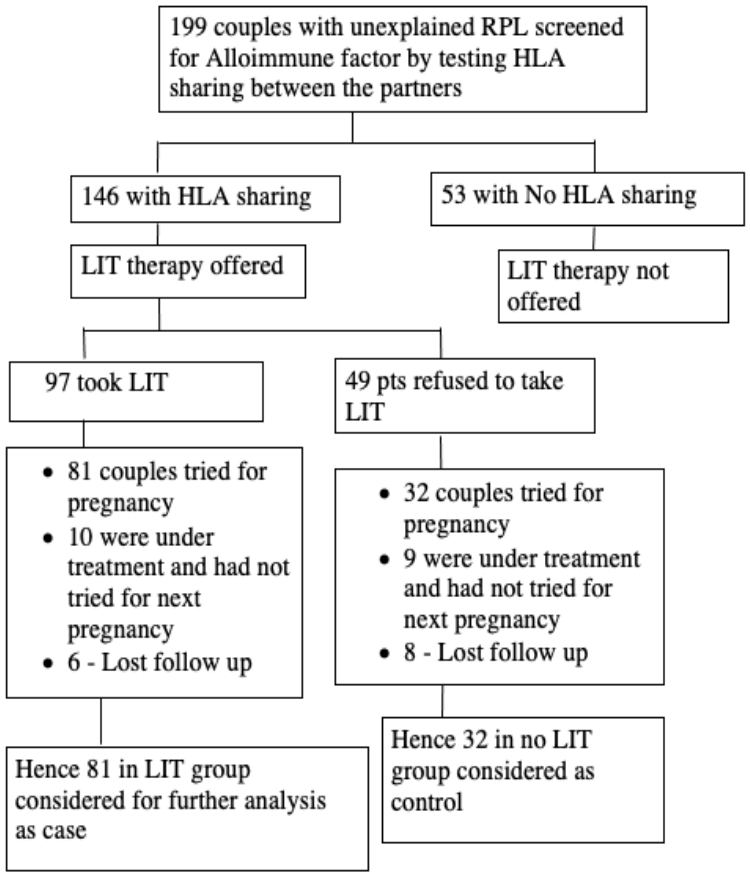
A total of 199 couples with unexplained RPL were screened for alloimmune factors by testing HLA sharing between the partners. Out of 199 couples, 146 had HLA sharing and LIT was offered. The remaining 53 couples did not have HLA sharing and hence were not offered LIT. Among 146 couples with HLA sharing, 97 couples took LIT, out of which 81 tried for pregnancy. The remaining 49 out of 146 couples who shared HLA antigens refused to take LIT. 32 couples among these who tried for another pregnancy were taken as control subjects for the study (Flow chart 1). Hence, we were able to include 81 patients in LIT group and 32 patients as control (no LIT group) in the HLA antigen sharing cohort for final analysis.

The lymphocyte immunization therapy protocol followed in the study was as follows:

80ml of fresh blood from male partner was collected and centrifuged repeatedly at 3500rpm for 10 minutes under laminar air flow. WBCs were separated and washed in saline and resuspended in 1ml of saline solution. Repeat centrifugations were done. Around 2-3ml of WBC concentrate was administered to the female partners intramuscularly. Only Rhesus D compatible patients were included for LIT.

Lymphocyte immunization therapy was given before (2-3 doses) and during pregnancy (1-6 doses) at intervals of 4-6 weeks. Based on the degree of HLA sharing, if the score was </= 3/12, women received 3 doses of LIT during pregnancy. If the HLA sharing score was > 3/12, women received LIT till 28 weeks of pregnancy accordingly. Pregnancy positive patients were followed up and analyzed. During pregnancy, all patients were given progesterone support in the form of vaginal progesterone. Successful pregnancy was considered when pregnancy crossed 24 weeks of gestation and these women were followed up till delivery.

Flowchart 1:



Statistical Analysis:

The baseline characteristics of the study population are described as mean and standard deviation for continuous variables. Categorical variables are described as numbers and percentages. We compared groups by using the Student's t-test or Kruskal–Wallis test for numerical variables and Fisher's exact-test for categorical variables. The collected data were transferred to an Excel 2010 worksheet (Microsoft Corp., Redmond, WA), and SPSS 20.0 software (SPSS Inc., Chicago, IL) was used for statistical analysis. We considered $p < 0.05$ statistically significant.

RESULTS:

Table 1 describes the baseline characteristics of the study population between the two groups (LIT group and no LIT group). No significant differences were noted with respect to age, body mass index (BMI), married life, anti mullerian hormone(AMH) and previous pregnancy losses.

Table 1 – Baseline characteristics of study population

Variables	LIT group (n=97)	No LIT group (n=49)	P value
Age	32.7 ± 4.6	33.39 ± 3.93	0.345
BMI	27.07 ±5.32	26.51 ± 3.02	0.491
Married life (years)	7.32 ±3.31	7.45 ± 3.75	0.829
AMH	3.92 ±3.18	3.59 ± 2.83	0.536
Pregnancy Loss	3.05 ± 1.45	2.71 ± 1.24	0.158

Table 2 describes the degree of HLA sharing among both the LIT and the no LIT control groups. The degree of sharing ranges from 1/12 to 12/12 alleles. The table also includes the pregnancy rates and live birth rates (LBR) for each degree of HLA sharing among both the groups. Most of the women in the study had one to four HLA allele sharing with their partners. There were a few women having HLA sharing up to 9 alleles out of twelve. As we compared the pregnancy and live birth rates among the LIT and no LIT group, we saw better pregnancy and live birth rates among the LIT group compared to the no LIT group.

Table 2 – Comparison of HLA sharing score between the two groups of LIT and pregnancy outcome

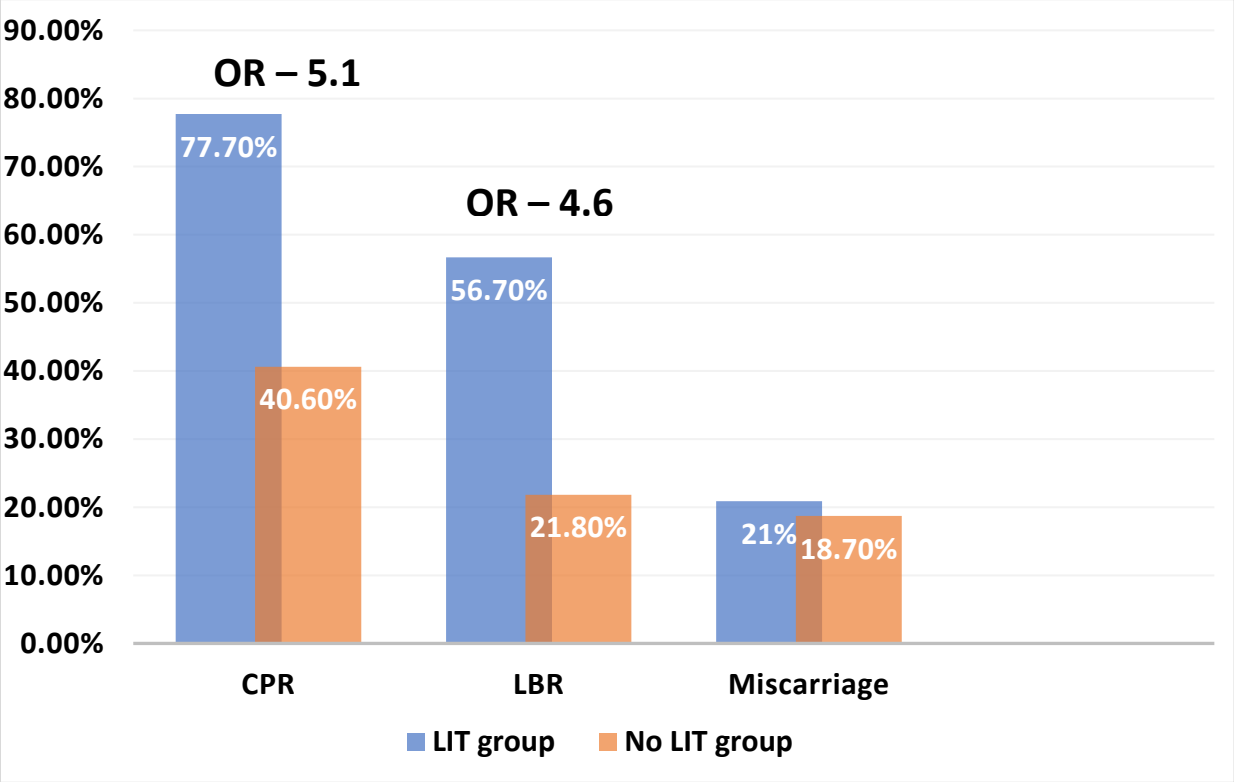
HLA sharing score	LIT taken			LIT not taken (49)		
	Number (n = 97) (81 tried for pregnancy)	Pregnancy (n=63)	Live Birth Rate(n=46)	Number (n = 49) (32 tried for pregnancy)	Pregnancy (n=13)	Live birth rate(n=7)
1	30	15	12(40%)	20	8	4(20%)
2	26	18	8(33.3%)	17	4	3(17%)
3	16	13	10(62.5%)	6	1	0
4	13	9	8	2	0	0
5	9	5	5	1	0	0
6	0	0	0	2	0	0
7	0	0	0	1	0	0
8	1	1	1	0	0	0
9	1	1	1	0	0	0
10	1	1	1	0	0	0
11	0	0	0	0	0	0
12	0	0	0	0	0	0

Only couples who tried for another pregnancy in both, the LIT group (81) and no LIT group (32) were included in final analysis. Overall, clinical pregnancy rate (CPR) and LBR was significantly higher in LIT group compared to the No LIT group (77.7% vs 40.6%, p-0.0001, OR 5.1, 95% CI 2.10-11.4 and 56.7% vs 21.8%, p-0.0002, OR 4.6, 95% CI 2.13-13.8 respectively). Miscarriage rates were similar between the two groups (20.9 vs 18.7%, p-0.64). (Table 3) (Figure 1)

Table 3 – Comparison between LIT and No LIT group and pregnancy outcomes

Variables	LIT group (81)	No LIT group (32)	OR	95% CI	P value
Clinical pregnancy rate	**63/81 (77.7%)**	**13/32 (40.6%)**	**5.1**	**2.10 – 11.4**	**0.0001**
Miscarriage rate	17/81 (20.9%)	6/32 (18.7%)	0.79	0.3 – 2.09	0.64
Live Birth Rate	**46/81 (56.7%)**	**7/32 (21.8%)**	**4.6**	**2.13 – 13.8**	**0.0002**

Figure 1- Bar diagram depicting odds ratio of clinical pregnancy rate, live birth rate and miscarriage rates among LIT and No LIT groups.

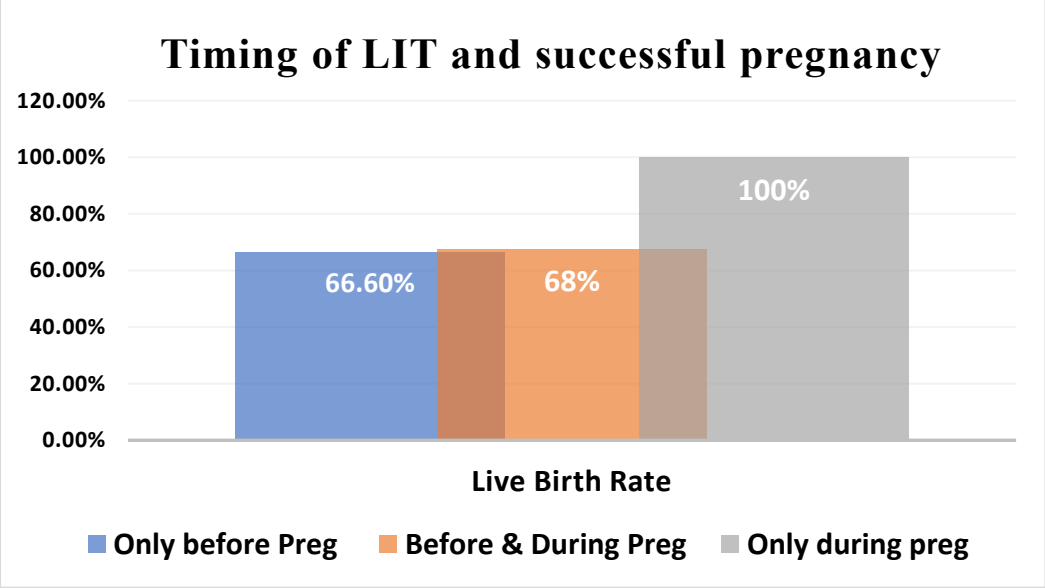


In the LIT group, 63 women had a clinical pregnancy. Pregnancy outcome with respect to timing of LIT was analyzed. Some women did not take LIT prior to pregnancy (Women with an unplanned pregnancy) and some did not take during pregnancy for various reasons. There was no significant difference in the live birth rates between the groups who took LIT only before pregnancy and couples who took LIT both before and during pregnancy. Surprisingly all eleven women who took LIT only during pregnancy, continued successfully and had a live birth. (Table 4) (Figure 2)

Table 4 – Comparison between timing of lymphocyte immunotherapy and live birth rate

Timing of LIT (63)	**Live Birth (46) N(%)**
Only before pregnancy (18)	12 (66.6%)
Before and during pregnancy (34)	23 (67.6%)
Only during pregnancy (11)	**11 (100%)**

Figure 2- Bar diagram comparing timing of lymphocyte immunotherapy and live birth rate



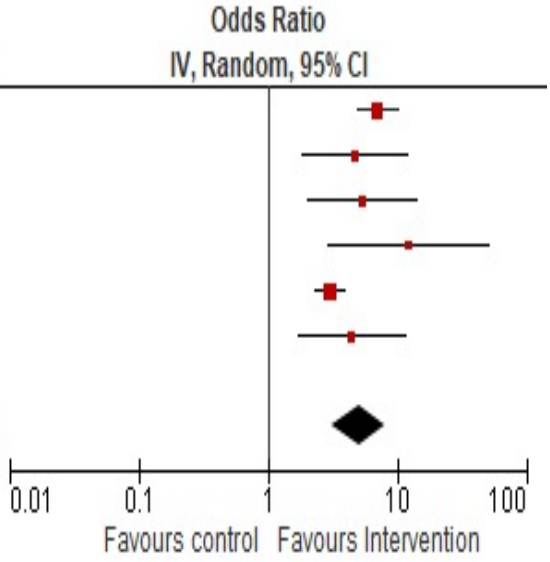
This forest plot (Table 5) compared the benefit of LIT in RPL women in our study and six similar studies. There was a clear advantage favoring LIT seen consistently in all the studies. (Odds ratio=5.04, 95% CI: [3.16 to 8.05]; P<00001).

Table 5-Forest plot describing the role of LIT in couples with HLA sharing and recurrent pregnancy loss

Study or Subgroup	log[Odds Ratio]	SE	Weight	Odds Ratio IV, Random, 95% CI
Aiwu et al.	1.953	0.1794	25.4%	7.05 [4.96, 10.02]
Gunasheela et al.	1.5454	0.483	13.4%	4.69 [1.82, 12.09]
Lin et al.	1.6845	0.4907	13.1%	5.39 [2.06, 14.10]
Pandey et al	2.5055	0.7334	7.8%	12.25 [2.91, 51.57]
Sarno et al.	1.1086	0.1362	27.1%	3.03 [2.32, 3.96]
Yanping et al.	1.4996	0.4884	13.2%	4.48 [1.72, 11.67]
Total (95% CI)			100.0%	5.04 [3.16, 8.05]

Heterogeneity: Tau² = 0.19; Chi² = 16.49, df = 5 (P = 0.006); I² = 70%

Test for overall effect: Z = 6.79 (P < 0.00001)



DISCUSSION:

The HLA system is a complex set of genes encoding the Major Histocompatibility Complex (MHC) proteins, or HLA molecules, in humans. These encode cell-surface proteins which are responsible for the regulation of the immune system in the human body. Even though the mother's immune cells are exposed to foetal HLA antigens which are semi allografts, the maternal immune system diverges the immune response in protective manner to have a successful pregnancy. During pregnancy, the villous (syncytiotrophoblast) trophoblast cells in the placenta operate as an immune barrier to shield the foetus from probable maternal rejection because they don't possess HLA antigens on their cell surface. HLA-C, HLA-E, and HLA-G alleles, on the other hand, are found on extravillous trophoblast cells. When there is a dysregulation of maternal and foetal immune cross talk, the protective and tolerant immune response fails to protect the pregnancy and results in a miscarriage. For an uneventful progress of pregnancy, the mother's immune system activates an alloimmune response, which is mediated by HLA molecules.

Role of Human leucocyte antigens (HLA) in placentation, implantation and pregnancy loss:

Human leucocyte antigen-C(HLA-C) is the main classical HLA antigen expressed on the trophoblastic cells and functions by supporting placentation .[5]HLA C antigen interacts with uterine NK cells and identifies foetal HLA antigens and accounts in initiating protective mechanism for trophoblast invasion.[6] They are expressed on extra villous trophoblasts and binds to killer immunoglobulin receptors (KIR) on NK cells and promotes trophoblast invasion. HLA C also promotes maternal tolerance by altering the activity of CD8+ cells and T regulatory (Treg) cells.[7] It activates maternal Treg cells and inhibits immune response to paternal antigens, thus protecting the pregnancy.

Human leucocyte antigen-G (HLA G) is a non-classical HLA antigen present on placenta which promotes placentation and implantation. HLA G mRNA levels are also correlated with embryo cleavage and successful pregnancy acceptance by mother's immune system.[8] HLA G on interaction with the maternal immune cells establishes foetal tolerance by opposing the cytotoxic activity of NK cells and CD8+ T cells, suppressing the CD4+ T cell proliferation and controlling T reg cells activity.[8] It interacts with certain receptors like Leukocyte Immunoglobulin Like Receptor B1 (LILRB1-inhibitory receptors on APC), CD160 (receptor on T lymphocytes, NK and endothelial cells)
and KIR2DL4 (receptor on NK cells).[8,9]

Human leucocyte antigen-E (HLA E) generates maternal immune tolerance by interacting with NK cell receptors CD94/NKG2A inhibitory receptors and the CD94/NKG2C activating receptors. Also, HLA-E presents signal peptides of HLA-A, B, C and G to inhibitory receptor NKG2 on NK cells. Hence, it's the degree of functioning of inhibitory and activating receptors of NK cells which depicts the maternal immune tolerance to fetus.[10,11]

Class II HLA antigens HLA DR, DQ, DP are mainly involved in allograft rejection by presenting peptides to CD4+ T cells present on macrophages, dendritic cells and B cells. Generally, it is absent in feto-maternal interface, thus helps in continuation of pregnancy without recognising fetus as a foreign body. Class II HLA genes have established association with pregnancy complications like preeclampsia[12] intrauterine growth restriction[13] and pregnancy loss. [14,15,16] HLA-DQB1 allele 03:03:02 has been implicated with a greater risk of miscarriages in South Indian women.[17] Moreover, increased expression of HLA-DQ2/DQ8 has been noted in RPL women.[18]

In our study we have evaluated HLA genes sharing between the partners at HLA 6 loci A, B, C, DR B1, DQ B1, DP B1. We could not evaluate the other HLA genes like HLA G, E due to financial constraints and high-cost burden and non-availability of testing kits during the study period.

Role of HLA antigens sharing in RPL:

Disturbances in the HLA system and its consequences can be grouped under alloimmune related aetiology of recurrent miscarriages. It is proposed that any amount of sharing of human leukocyte antigens between the two partners (Sharing of HLA antigens at the A, B, D/DR and G loci) could prevent the formation of the protective blocking antibodies, thus leading to a miscarriage. Research also has indicated that any amount of HLA compatibility among spouses leads to higher RPL rates as is seen among the inbred populations who have a higher incidence of RPL.[16] HLA antigen sharing between the partners causes maternal hypo responsiveness to paternal antigens causing pregnancy failure.[19] Increased HLA compatibility among couples results in an inadequate foetal tolerance inducing response. This has been connected to the mother's inability to recognise the semiallogenic nature of the foetal tissues. Therefore, ***HLA incompatibility*** confers a definite reproductive benefit rather than ***histocompatibility*** unlike in the other organs where transplant is considered. Both primary and secondary RPL patients have been found to exhibit sharing of HLA alleles, with the former sharing the HLA-A and HLA-DQ antigens, and the latter sharing HLA-A, HLA-B, HLA-DR, and HLA-DQ antigens.

The various immunological treatment options available for treatment in RPL patients include intralipid infusions, corticosteroid therapy, intravenous immunoglobulin infusions, granulocyte-colony-stimulating factor (G-CSF) and tumor necrosis factor (TNF)-alpha-blocker. Immunization with partner or third-party lymphocytes is an effective therapeutic option for couples with HLA allele sharing.[20]

Role of LIT in unexplained RPL:

The mechanism of LIT is to stimulate the production of anti-paternal cytotoxic antibodies, anti-idiotypic antibodies (Ab2) and mixed lymphocyte reaction blocking antibodies (MLR-Bf). It also reduces the natural killer cell activity, improves Th-1/Th-2 balance leaning towards a Th-2 predominance and improves regulatory T (Treg) cell profile, thus creating a favourable environment in utero favouring implantation of the embryo.[4]

Taylor and Faulk[21] were the first to bring in the concept of paternal lymphocyte immunotherapy as a therapeutic measure for women with idiopathic RPL in the 1980's. They based their study from observations on renal allograft studies showing a

delay in graft rejections secondary to third party blood transfusions. The same idea was applied in couples with RPL having HLA sharing. Many studies have been performed since then to evaluate its safety and efficacy among RPL women. Beer et al.[22] also based his study on benefit of paternal lymphocyte immunotherapy in inducing maternal "blocking antibodies" and resulting in a healthy pregnancy. Literature has shown that paternal lymphocyte immunization therapy also induces the production of multiple other antibodies such as Anti-Paternal Cell Antibodies (APCA)[23,24] anti T- Cell Receptor (TCR) idiotypic antibodies[25] and Mixed Lymphocyte Reaction Blocking factor (MLR-Bf)[26] among women with RPL which help in achieving successful pregnancies. In addition to the above, LIT brings about other beneficial immunological changes during pregnancy such as non-specific T cell suppression[27], reduction in maternal IL-2 receptor levels, a shift towards Th2 type immune response [28] and reduced natural killer (NK) cell activity. Although previous studies remain inconclusive in this regard, there is some evidence that pregnancy failure does occur when the above protective immune changes do not occur in a pregnancy[2,29,30].

The Cochrane Library published a meta-analysis in 2001 elaborating the various immunological treatment options for RPL, one among which was for was lymphocyte immunisation. It included 12 studies and a total of 641 patients receiving partner LIT. The case group consisted of 316 women and control/placebo group had 325 women. They concluded that there was no significant improvement of live birth rates following LIT (OR 1.22, 95% CI 0.89–1.69).[31] The results of the above meta-analysis was criticised by many researchers[32,33]. The criticism was centred around the results of the meta-analysis which mainly based their conclusion on the results of the study by Ober et al which was the first and only study to show a negative effect of LIT on pregnancy rates. [29]Two points of their study were criticised. Firstly, they used paternal lymphocytes that was stored overnight rather than fresh lymphocytes in their study. Such cells lose their immunogenic effect on storage. Secondly, they did not exclude patients with autoimmune conditions. Patients with autoimmune conditions involving antiphospholipid and antinuclear antibodies may not respond well with LIT. Thus, a repeat analysis of the Cochrane analysis data on the above topic was undertaken in 2014 excluding the results of Ober et al.[29] The results of the revised analysis in 2014 observed a significant increase in the rate of live births following immunisation with partner lymphocytes (OR 1.63, 95% CI 1.13–2.35; p = 0.009).[31]

Another meta-analysis by Liu et al. [34]was published in 2014 in an attempt to overcome the mistakes and/or weaknesses of the Cochrane analysis regarding this topic. This new meta-analysis included 18 randomised clinical studies involving 1738 patients with patients segregated into case (received LIT) and control groups (no LIT). The results demonstrated a significant improvement in live births (77.8%) in the LIT group compared to 46.1% in the control group (OR 4.02, 95% CI 3.23–5.00). Another review article by Cavalcante et al. [35] from 2017 included 6 meta-analyses. Four out of the six studies showed a clear benefit of immunisation with partner lymphocytes with significant increase in live birth rates.

In our study, the overall clinical pregnancy rates (CPR) and live birth rates were significantly higher in LIT group compared to the group who declined LIT. (Table 3). The live birth rate in our study after LIT is comparable with studies by Sarno et al.[1], and Gunther et al.[36], (60% and 53% respectively). (Table 6)

Table 6. Summary of selected studies on live birth rates after LIT in couples with recurrent miscarriage in comparison to our study.

Study	Year	LIT group n (%)	No LIT group n (%)	Comment
Pandey et al[26]	2004	21/25 (84)	6/20 (30)	Treatment: LIT using partner's blood, prior to pregnancy Control: LIT from the patient's blood Route: IV + ID + SC + IM
Yanping et al[37]	2011	41/49 (84)	24/45 (53)	Intradermal LIT from partner's blood, prior to and during pregnancy Control: No LIT
Lin et al[38]	2012	33/42 (79)	17/42 (41)	Subcutaneous LIT from partner's blood, prior to and during pregnancy. Control: No LIT

Aiwu et al[39]	2013	250/297 (84)	254/591 (43)	Subcutaneous LIT from the third party or partner's blood, prior to and during pregnancy. Control: No LIT
Sarno et al[1]	2019	452/752 (60)	114/344 (33)	Intradermal LIT from partner's blood, before and/or during pregnancy. Control: No LIT
Günther et al[36]	2021	17/32 (53)	None	Intradermal LIT from partner's blood, before pregnancy.
Present study (Gunasheela et al)	**2023**	**46/81 (56.7%)**	**7/32 (21.8%)**	**Intramuscular LIT from partner's blood before and during pregnancy**

Majority of the studies used intradermal and subcutaneous routes to give LIT. Study by Pandey et al used intradermal, intravenous, subcutaneous and intramuscular routes, while in our study we used only the intramuscular route. We did not notice any major adverse reactions with LIT, with only a few patients complaining of minimal pain and swelling at injection site.

A study by Yu et al. compared the various routes of administration and demonstrated that the best results were achieved with intradermal immunisation.[40]

Regarding the timing of immunotherapy and pregnancy, Liu et al., performed a subgroup analysis of the various immunisation protocols and observed a significant increase in the rate of live births when the immunotherapy was given before and during the pregnancy (OR 4.67, 95% CI 3.70–5.90 vs. OR 2.00, 95% CI 1.39–2.88).[34] Our study revealed no difference in the live birth rates with regards to the timing at which the LIT was given (only before, during or both before and during pregnancy). Although all the women who took LIT only during pregnancy had a live birth in our study, no definite conclusion can be drawn on this group because of the small number of patients. The take home message here is that the patients who did not utilise the immunomodulation by LIT prior to pregnancy can still get the benefit of immunomodulation during pregnancy to achieve a successful outcome.

Conclusion:

Ours is a unique study because it's one of the few studies that have looked at HLA antigen sharing between the partners and used a novel scoring system in deciding LIT for unexplained RPL patients. LIT significantly improves the chances of live birth in unexplained RPL and it has shown that LIT had a positive effect on pregnancy whether it was taken only before pregnancy, during pregnancy or both. Retrospective nature and small sample size are the obvious drawbacks of this study. Proper randomization of subjects is not possible as pregnancy loss is a sensitive issue. It may have been a better study if blocking antibodies were tested before and after LIT. This we may consider as an extension of this study at a later date.

Conflicts of interest statement:

The authors have no conflicts of interest to declare.

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HLA TYPING REPORT

Patient Details

First Name :---
Last Name : ---
Hospital : Gunasheela IVF Centre
Physician : Dr. Devika Gunasheela
Diagnosis : Secondary Infertility
Specimen Type : Blood

Date Of Birth : ---
PRN : ----
Tattva ID : ----
Received On : --------
Reported Date : -- ----

A*	B*	C*	DRB1*	DQB1*	DPB1*
01:01:01:01	40:06:01:02	03:02:02:01	03:01:01:01	02:01:01:01	04:01:01:01
33:03:01:01	58:01:01:01	15:02:01:01	15:01:01:01	06:02:01:01	04:01:01:01

Typing Status: Complete

Donor Details

First Name : ----
Last Name : ----
Hospital : Gunasheela IVF Centre
Physician : Dr. Devika Gunasheela
Diagnosis : NA
Specimen Type : Blood

Date Of Birth : -----
PRN : -----
Tattva ID : -----
Received On : -----
Reported Date : ------

A*	B*	C*	DRB1*	DQB1*	DPB1*
24:02:01:04	40:06:01:02	01:02:01:01	03:01:01:01	02:01:01:01	02:02:01:01
33:03:01:01	58:01:01:01	03:02:02:01	15:01:01:01	06:01:01:01	13:01:01:02

Typing Status: Complete

Matching Ratio with Patient : 7/12 **Matched Locus: A, B,C, DRB1 & DQB1**

Reported By
Sachin Shetty
Scientist

Verified By
Dr. Swathi Shetty
Molecular Geneticist

End of Report