Thieme

Active Immunisation with Partner Lymphocytes in Female Patients Who Want to Become Pregnant – Current Status

Aktive Immunisierung mit Partnerlymphozyten bei Kinderwunschpatientinnen – der aktuelle Stand

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ABSTRACT

Around 1–3% of all couples who try to have a child are affected by recurrent miscarriage. According to the WHO, recurrent miscarriage is defined as the occurrence of three or more consecutive miscarriages up to the 20th week of pregnancy. There are various causes of recurrent miscarriage; in many cases, the causes remain unclear, with the result that immunological factors are one of the possible causes discussed. For the mother's immune system, the embryo represents a semi-allogeneic transplant, as half of the embryo's genes are of paternal origin. In place of a conventional immune response, the embryo induces a secondary protection mechanism, which contributes to the successful implantation. When performing immunisation with partner lymphocytes, the patient receives an intradermal injection of her partner's prepared lymphocytes into the volar side of the forearm in order to induce immunomodulation with a consequently increased rate of pregnancy and live birth. A prerequisite for this procedure is that all other possible causes of sterility have been ruled out in advance. Due to the highly heterogeneous nature of the data, a significant benefit as a result of the immunisation cannot yet be clearly proven. However, there are signs that the therapy may be effective when using lymphocytes that have been extracted as short a time beforehand as possible. Overall, the treatment represents a safe, low-risk procedure. Following a detailed informative discussion with the couple regarding the chances of success and following a detailed review of the indication and contraindications, immunisation with partner lymphocytes can be discussed with the couple on a case-by-case basis – provided that all other possible causes of sterility have been ruled out in advance.

ZUSAMMENFASSUNG

Etwa 1–3% aller Kinderwunschpaare sind von einem habituellen Abortgeschehen betroffen. Dies ist laut WHO definiert als das Auftreten von 3 oder mehr aufeinanderfolgenden Aborten bis zur 20. SSW. Die Ursachen hierfür sind vielfältig, bleiben in einer Vielzahl der Fälle sogar unklar, sodass unter anderem immunologische Faktoren diskutiert werden kön-

nen. Der Embryo stellt für das Immunsystem der Mutter ein semiallogenes Transplantat dar, da die Hälfte der Gene des Embryos paternaler Herkunft sind. Anstelle einer üblichen Immunantwort induziert der Embryo einen sekundären Schutzmechanismus, welcher zur erfolgreichen Implantation beiträgt. Bei der Immunisierung mit Partnerlymphozyten werden der Patientin aufbereitete Lymphozyten ihres Partners in die volare Seite des Unterarms intrakutan injiziert, um so eine Immunmodulation mit konsekutiv erhöhter Schwangerschafts- und Lebendgeburtenrate zu induzieren. Voraussetzung für dieses Verfahren ist, dass zuvor alle anderen infrage kommenden Sterilitätsursachen ausgeschlossen wurden. Aufgrund der äußerst heterogenen Datenlage kann ein signifikanter Nutzen durch die Immunisierung immer noch nicht eindeutig belegt werden. Es gibt jedoch Hinweise, dass die Therapie bei Verwendung möglichst frisch entnommener Lymphozyten wirksam sein könnte. Die Behandlung stellt insgesamt ein sicheres und risikoarmes Verfahren dar. Nach ausführlicher Aufklärung des Paares über die Erfolgsaussichten und genauer Überprüfung von Indikation und Kontraindikationen kann individuell mit dem Paar eine Immunisierung mit Partnerlymphozyten diskutiert werden – vorausgesetzt, zuvor wurden alle anderen infrage kommenden Sterilitätsursachen ausgeschlossen.

Introduction

As a sign of low reproductive efficiency, couples who are trying to have a child may suffer from failure to conceive following multiple embryo transfers. On the other hand, rapid and unproblematic spontaneous conception may be followed by recurrent loss of the pregnancy within the context of a miscarriage.

Recurrent miscarriage is defined as the occurrence of three or more consecutive miscarriages up to the 20th week of pregnancy [1], with the American Society for Reproductive Medicine (ASRM) defining recurrent miscarriage as just two consecutive miscarriages [2]. 1 % of couples is affected by recurrent miscarriage. The probability of a repeat miscarriage rises as the number of previous miscarriages increases [1]. There are various causes of recurrent miscarriage, including a combination of several factors. Examples of causes include: chromosomal causes (balanced translocation, inversion, mosaic), infections (toxoplasmosis, chlamydia), endocrine causes (PCO, hyperandrogenaemia, hyperprolactinaemia, hyper/hypothyroidism), coagulation disorders (Factor V Leiden mutation, prothrombin mutation), autoimmune diseases (lupus erythematosus, antiphospholipid syndrome), congenital or acquired uterine anomalies (uterine septum, uterus myomatosus) [3]. However, in around 40 % of cases, the cause remains unclear, with the result that immunological factors are one of the possible causes discussed [4].

From the development of the blastocyst through to implantation, an intensive immunological interaction is required between the embryo and the maternal immune system. During the build-up of the extraembryonic membranes, the trophoblast is incorporated into the decidua, erodes maternal blood vessels and therefore maintains the foetomaternal exchange of blood substances and nutrients. As a result of this process, direct contact is established between maternal blood and foetal cells, the syncytiotrophoblasts. The trophoblast invasion into the maternal decidua is influenced by immunological effector cells, in particular the uterine natural killer cells (uNK) (see below) [11]. With the help of the vascular endothelial growth factor (VEGF) and interferon gamma (INF-gamma), they stimulate the conversion of the spiral arteries and are involved in the regulation of the invasion depth. The pregnant uterus can be described as an immune-privileged site, in that the balance between organ preservation and infection defence is significantly shifted in favour of organ preservation. In order to nevertheless provide effective protection from pathogens for the uterus, there are a large number of immunocompetent cells in the decidua, which belong to the innate, and therefore antigen-independent, immune defence. The dendritic cells (DC) take on a special function in the decidua: On the one hand, they can induce antigen-specific cytotoxic T-cell immune responses and, on the other hand, they ensure immunological tolerance under steady-state conditions [5, 6].

In addition, local immunoactive substances, such as galectins and glycodelin, are secreted by glandular uterine epithelial cells [7]. ▶ **Fig. 1** shows the foetomaternal interface with the cells responsible for a successful implantation.

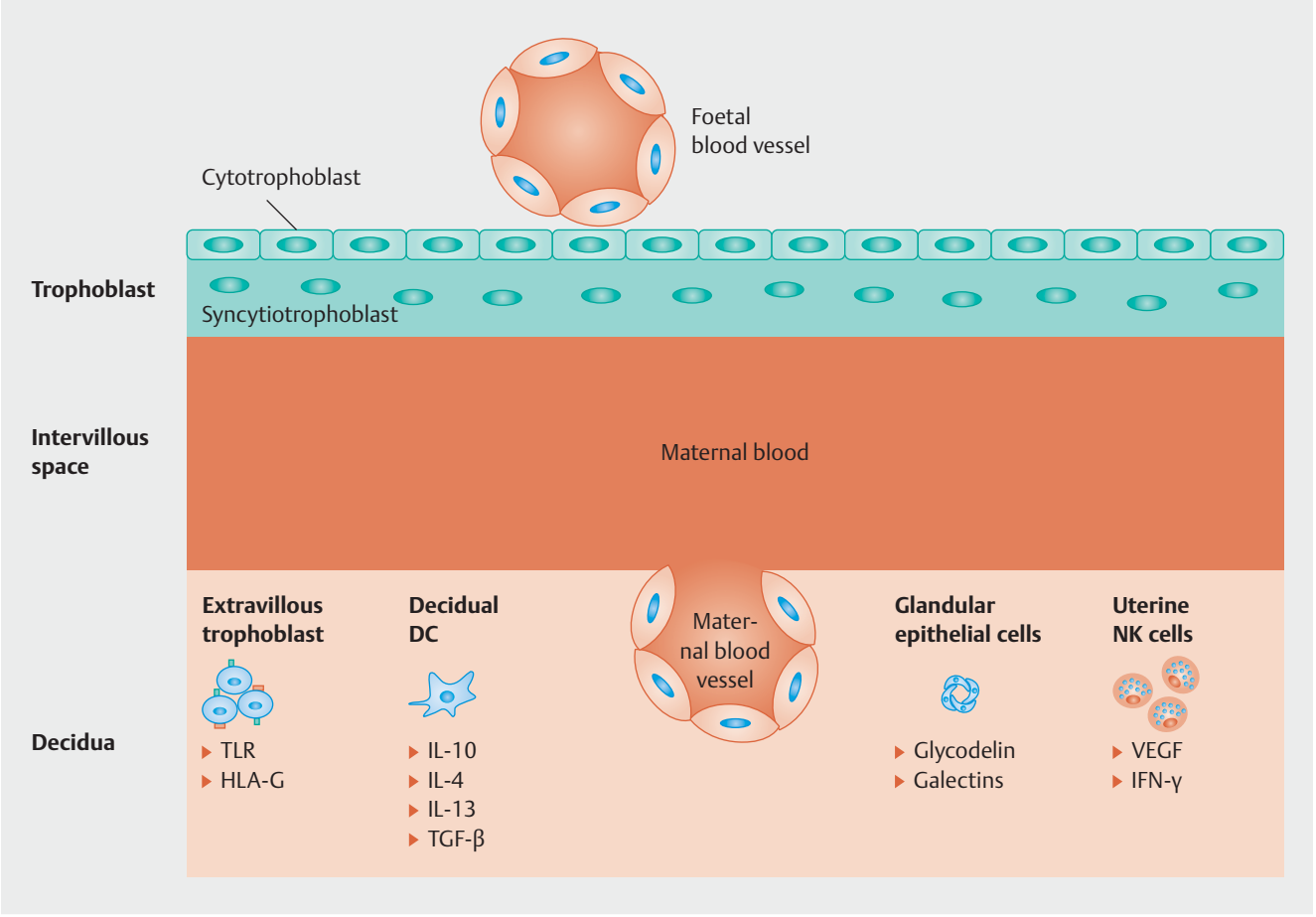
For the mother's immune system, the embryo represents a semi-allogeneic transplant, as half of the embryo's genes are of paternal origin. In place of a conventional immune response, the embryo induces a secondary protection mechanism [8]. The first theories regarding the explanation of this form of "immune tolerance" were described by Medawar in 1953:

1. He assumed a strict anatomical separation between maternal and foetal tissues by means of the placenta.
2. A further hypothesis described the embryo as non-immunogenic, stating that it therefore follows that the embryo cannot generate an immune response.
3. The third theory assumed a maternal immune response that had been weakened by the pregnancy [9, 10].

HLA (Human Leukocyte Antigens)

The last assumption was modified by the concept of the "protective immune response". This was supported by examinations of human leukocyte antigens (HLA), surface proteins of leukocytes and other tissues (▶ **Fig. 2**, Major histocompatibility complex [MHC]). The HL antigens form the individual signature of the cells and play the key role when the immune system is differentiating between endogenous and exogenous structures. Monozygotic twins and 25 % of siblings have an identical HLA pattern.

The extravillous trophoblast invading the decidua does not express the conventional HLA Class I or Class II protein complexes, but rather non-conventional human leukocyte antigens, in particular HLA-G, which are of great importance for the success of the pregnancy. HLA-G inhibits the activity of natural killer cells (NK cells) and type 1 T helper cells (T_H1 cells) and thereby prevents



▶ **Fig. 1** Immunocompetent cells of the foetomaternal interface (according to [17]). In the intervillous space, the trophoblast is in direct contact with the maternal blood. There is a special cellular immunological environment in the decidua. The individual cellular components with their most important molecules are presented here. TLR: toll-like receptor; DC: dendritic cells; TGF-beta: transforming growth factor; uNK cells: uterine natural killer cells; VEGF: vascular endothelial growth factor; IFN-gamma: interferon gamma.

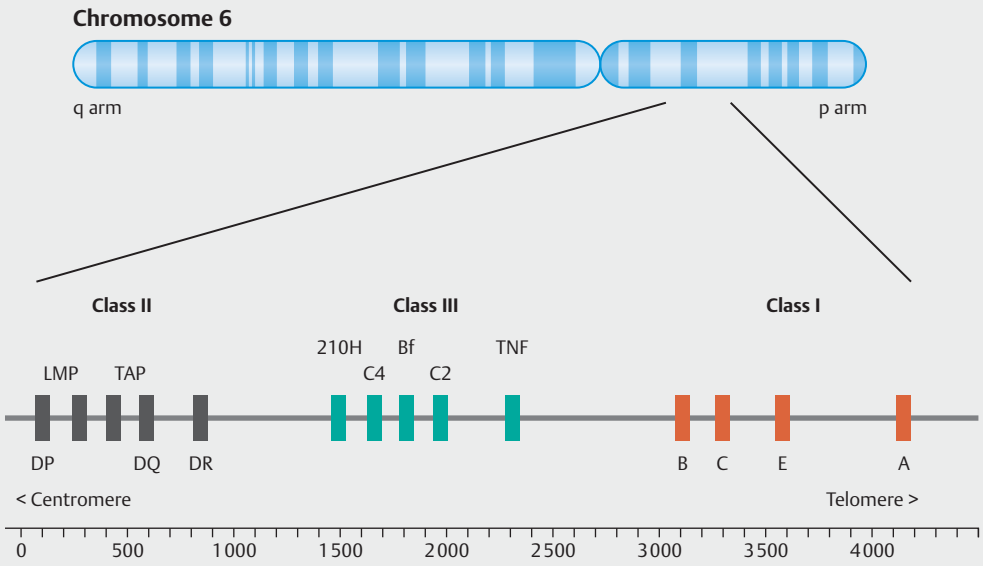
the rejection of the semi-allogeneic embryo. With their regulation and secretion of cytokines and chemokines, the uterine natural killer cells (uNK cells) are responsible for this [11, 13 – 15]. In addition, the trophoblast expresses pathogen recognition receptors on its surface, known as toll-like receptors (TLR), which trigger a tissue and pathogen-specific immune response following activation [12].

Natural Killer Cells (NK)

$CD56^+$ cells, known as natural killer cells (NK cells), represent a primary component of the innate, non-specific immune system. They destroy those somatic cells whose HLA molecules are genetically coded as “foreign” or are modified by infection, such as tumour or virus-modified cells, without previous recognition of a specific antigen. The rapid and non-antigen-dependent elimination of such cells represents an important form of protection against viral diseases and tumour cells, but, at the same time, it poses the risk of autoimmunity. For this reason, the cytotoxic mechanisms of the NK cells are strictly regulated and their maturation requires a specific environment, e.g. one shaped by cytokines and chemokines.

A factor in the changing maternal immune system is the pregnancy-related decrease in natural killer cells (NK) and their production of interferon gamma (IFN-γ). A missing decrease in maternal peripheral killer cells is associated with an increased rate of miscarriage [19, 20].

Uterine natural killer cells (uNK) amount to around 70 % of the immune cells in the foetomaternal interface and they represent a special type of cell shaped by the immunological environment; these cells differ significantly from the peripheral blood NK cells [16]. uNK have significantly more secretory than cytotoxic properties. The uNK cells are inhibited by the HLA-G expressed by the trophoblast [17]. uNK cells have a lytic function within the context of the conversion of the spiral arteries [18]. The secretion of interferon gamma, and of other vasoactive substances, such as vascular endothelial growth factor (VEGF), is important for the development of the placental immune architecture, as well as the vascularisation of the placenta [19, 20].



▶ **Fig. 2** Major histocompatibility complex (MHC). The HLA (human leukocyte antigen) system is located on the short arm of chromosome 6 and is also known as the major histocompatibility complex (MHC). The typical MHC genes are divided into two regions, which code two classes of HLA molecules: HLA Class I (HLA-A, -B, -C), which are found on all nucleated somatic cells and HLA Class II (HLA-DR, -DQ, -DP), which are found only on antigen-presenting cells. The HLA Class III molecules include complement factors, which are involved in the non-specific immune defence. The overall HLA complex comprises around 4000 kilobases (Kb) and is very polymorphic, i.e. there are several genetic variants (alleles) for most gene loci. Source: Zentrum für Humangenetik und Laboratoriumsdiagnostik (MVZ) [Center for Human Genetics and Laboratory Diagnostics (AHC)], Dr. Hirv, Dr. Bangol, Martinsried.

T Lymphocytes

The regulatory T cells (T_{reg}), previously also known as T suppressor cells, are responsible for the self-tolerance of the immune system and prevent the occurrence of autoimmune diseases. They undergo a physiological increase during pregnancy. If this mechanism does not occur, recurrent miscarriages are observed [19].

The T helper cells are a group of T lymphocytes and they have a supporting, "helping" role to play with regard to the immune response. Two subgroups of T helper cells can be described according to the cytokines they secrete: Type 1 T helper cells are involved in the cellular immune response and release interferon gamma (IFN-γ), interleukin-2 (IL-2) and tumour necrosis factor alpha (TNF-α). In this regard, cytotoxic T cells destroy infected cells, for example as a response to a viral infection. In contrast, type 2 T helper cells are involved in the humoral immune response and secrete the cytokines IL-4, IL-5, IL-9, IL-10 and IL-13. These cytokines strengthen antibody production as well as the proliferation and function of eosinophil granulocytes. T_H1 responses suppress T_H2 responses and vice versa.

The cytokines of the T_H1 and T_H2 cells influence implantation as well as foetal development and differentiation.

In 1993, Wegmann et al. described the theory of a balance between the T_H1/T_H2 cytokines and stressed that foetal survival is only possible if the T_H2 cytokines are dominant compared to the T_H1 cytokines (so-called "shift" in favour of the type 2 T helper cells over the type 1 T helper cells) [21]. In the following years, further studies were carried out, which confirmed this hypothesis and concluded that an increased level of T_H1 cytokines (INF-γ, IL-2 and TNF-α) is associated with an increased rate of miscarriage [22, 23]. TNF-α suppresses the growth of the trophoblast by inducing apoptotic processes in its cells [18, 19].

Although it was demonstrated that cytokines are essential during pregnancy, the T_H1/T_H2 paradigm changed to the effect that the $T_H2 > T_H1$ dominance should not be presented in such a dogmatic manner. The cytokine networks are structured in a highly synergistic and redundant manner, with the result that it is difficult to examine and assess individual cytokines in detail. More recent investigations see an epiphenomenon of a modified hormone and cytokine balance as being responsible for the successful outcome of a pregnancy, rather than the presence of a very dominating T_H2 cytokine pattern [24].

The individual immune response stages in the early phase of pregnancy have not yet been clarified in detail and require further research. If dysregulation occurs within the individual meticulous stages of immunomodulation, this results in a miscarriage rate of up to 50 % [25, 26].

For couples trying to have a child who are suffering from recurrent miscarriage, there are many therapy approaches aimed at exerting a modulating effect on the immune system in order to thereby increase the pregnancy rate. In addition to active immunisation with partner lymphocytes, there is also a range of additional immune therapies designed to have a positive effect on implantation rates, such as glucocorticoid administration, intralipid infusions, intravenous immunoglobulin administration and therapy with anti-TNF-α agents [3, 27, 28]. Of these various therapies,

immunisation with partner lymphocytes has been subject to most investigation [29, 30].

In addition to a direct influence on the immune system, immunological therapy approaches could also have an impact on psychological causes of recurrent miscarriage in the form of a placebo effect. "Tender loving care" (TLC) is one of the concepts that emphasises the importance of psychological factors [31, 32]. Within the context of this concept, the pregnant woman is subject to close monitoring from both a clinical and psychosomatic perspective, e.g. regular ultrasounds during the early stages of pregnancy, which go well beyond the designated scope of prenatal care. Stray-Pedersen divided women suffering from recurrent miscarriage for whom anatomical causes had been ruled out into two groups: One group received psychological support as well as close gynaecological monitoring and the other group did not. Significantly higher pregnancy rates were observed in the patients in the TLC group (86 vs. 33 %; $p < 0.001$) [31]. Despite good results, the TLC concept is still missing scientific validation by means of randomised controlled studies in terms of evidence-based medicine. As such, it would appear that further studies are needed in this regard.

In observational studies, a possible immunological effect cannot be distinguished from the placebo effect of the therapy. Only placebo-controlled studies can therefore be used to assess the immunological effect.

Immunomodulation by Means of Active Immunisation with Partner Lymphocytes

Before preparations for the immunisation can start, certain prerequisites for both partners must be reviewed: contraindications for the female recipient include, for example, the presence of an autoimmune disease (lupus erythematosus, antiphospholipid syndrome, Crohn's disease, ulcerative colitis or multiple sclerosis), chronic diseases that may necessitate a transplant at a later date (diabetes mellitus, cystic fibrosis, polycystic kidney disease) or transplants in the medical history. If the partner has an increased risk of transmitting infectious diseases or malignant cells, he will not be approved for a lymphocyte donation.

The aim of the active immunisation with partner lymphocytes is to stimulate the immune system, thereby leading to improved immunorecognition in the subsequent pregnancy. The active immunisation was developed and used for the first time in the 1980s.

As a general rule, whole blood is taken from the partner and the lymphocytes are isolated from the whole blood by means of density gradient centrifugation. Under sterile conditions, the lymphocytes are washed multiple times and then suspended in saline solution. Partner lymphocytes are currently classified as advanced therapy medical products (ATMP) in Germany, as the lymphocytes play a different role in the patient's body than they would in the donor's body. A manufacturing authorisation and processing in a cleanroom are therefore required. The finished product is usually administered to the patient in the form of an intracutaneous injection into the volar side of a forearm. A test for antipaternal HLA antibodies can be carried out 4–6 weeks after immunisation. If the production of antibodies is detected, the couple should aim for a pregnancy within the following 12 months; otherwise, the immunisation can be repeated.

The immunisation should strengthen the maternal immune response, which is aimed at paternal antigens on the trophoblast. The detection of antipaternal antibodies indicates that the immunisation has induced a maternal immune response [33]. In the literature, there are several studies that describe an increased pregnancy rate following immunisation associated with the simultaneous presence of antipaternal antibodies [33–35]. Carp et al. also established a connection between positive detection of antibodies following immunisation and successful pregnancy: 50 % of patients in whom antibodies were detected fell pregnant, but this figure was just 37 % when there were no antibodies detected [33]. However, it is unknown whether the antipaternal HLA antibodies exert a direct effect or whether they are simply a marker for the successful modulation of the maternal immune system.

Possible complications and the side effects profile following immunisation are roughly equivalent to those following intradermal vaccination against viral infectious diseases. Possible side effects include local reactions, such as redness, swelling or burning; rarer side effects include systemic, flu-like symptoms, which occur in 8 % of cases. There is no particular risk of anaphylaxis or autoimmune diseases [36, 37]. Despite previous testing on viral diseases, the transmission of infections cannot be completely ruled out.

The efficacy of the method has been assessed in numerous studies and overview analyses. ▶ **Table 1** shows an overview of the randomised studies, which have been included in the current meta-analyses of Wong et al., 2014 [28], Liu et al., 2016 [38] and Cavalcante et al. 2017 [39] [40–62]. In addition to the study design and the number and age of the patients enrolled, the table also lists the time, dosage and administration route of the immune therapy, the substance for the treatment group and the placebo group, the outcome regarding live birth and/or advanced pregnancy, as well as information regarding success monitoring, storage or other particular characteristics.

The first meta-analysis regarding immunisation with partner lymphocytes was published in 1993 by Fraser et al. [63]. In this case, 4 randomised studies regarding immunotherapy with lymphocytes or infusion of trophoblast membranes were carried out. The studies showed no improvement with regard to the rate of live births [63].

In 1991, during the 11th annual meeting of the American Society for Reproductive Immunology, the Ethics Committee of the Society for Immunotherapy initiated a multicenter study to standardise the treatment protocol and increase the study size. To this end, data was compiled from 15 sites. Nine randomised studies were analysed by two independently operating analysis teams. It was demonstrated that there was an increased rate of live births following immunotherapy in female patients suffering from recurrent miscarriage (odds ratio [OR] 1,16, 95 % confidence interval [95 % CI] 1.04–1.34). A significant increase in the rate of live births was described when antipaternal HLA antibodies were detected in the mother before the pregnancy (RR 1.17, 95 % CI 1.06–1.27) [54].

▶ **Table 1** Current study situation for immunisation with partner lymphocytes: a comparison of three meta-analyses (Wong LF et al., 2014, Liu Z. et al., 2016, Cavalcante MB et al., 2016) with the respective included studies.

	Study	Year	Design	Number of patients	Age, years	Treatment time	Treatment group	Placebo group	Storage	Dosage, administration route	Outcome parameters (LB ± advanced pr.)	Successful outcome		p	Taken into account in the meta-analysis			Comment
												Treatment group	Placebo group		Wong 2014	Liu 2016	Cavalcante 2016	
1	Mowbray JF et al. [40]	1985	RCT, do-blind, paired sequence analysis	49	N.sp.	Before pr.	PLy	MLy from 20 ml blood	N.sp.	400 ml citrated blood, 3 ml i. v.; 1 ml i. d.; 1 ml s. c.	LB + pr. ≥ 28 WoP within 12 months	77 % (17/22)	37 % (10/27)	0.01	x	x		
2	Cauchi MN et al. [41]	1991	RCT, do-blind, paired sequence analysis	46	N.sp.	Before pr.	PLy	NaCl	N.sp.	100–1000 × 10^6 Ly; 1 ml i. v.; 1 ml i. d. +s. c.	LB + pr. ≥ 20 WoP	62 % (13/21)	76 % (19/25)	1.0	x	x	x	
3	Ho HN et al. [42]	1991	RCT, do-blind	99	28.5 ± 2.6	Before pr.	PLy/ DLy	MLy	N.sp.	100–200 × 10^6 Ly; 2 ml i. d.	LB + pr. ≥ 20 WoP	78 % (39/50)	65 % (32/49)	> 0.1	x	x	x	2. Immunisation if neither pregnancy nor antibodies detected within 6 months
4	Gatenby PA et al. [43]	1993	RCT, do-blind, paired sequence analysis	38	33 ± 4.6	Before pr.	PLy	MLy	N.sp.	400 × 10^6 Ly; 3 ml i. v.; 1 ml i. d.; 1 ml s. c.	LB	68 % (13/19)	47 % (9/19)	0.1	x	x	x	
5	Carp HP et al. [44]	1997	RCT, do-blind	42	31 ± 4.26 (24–45)	Before pr.	PLy	N.sp.	N.sp.	Various	LB	45 % (5/11)	19 % (6/31)	NS		x	x	
6	Kilpatrick DC et al. [45]	1994	RCT, do-blind	22	N.sp.	Before pr. + 1 × rep. up to 6th WoP	PLy	MLy	N.sp.	50–200 × 10^6 Ly from 100 ml blood 4 ml, i. d., s. c., i. v.	N.sp.	67 % (8/12)	60 % (6/10)	N.sp.	x	x		Unpublished data, according to Wong LF et al., 2014
7	Clark DA, Daya S [46]	1991	RCT, do-blind	18	N.sp.	Before pr.	PLy	NaCl	N.sp.	40 × 10^6 Ly i. d.	LB	63 % (7/11)	28 % (2/7)	N.sp.		x	x	
8	Pandey MK [47]	2003	RCT, do-blind	19	N.sp.	Before pr.	PLy	MLy/NaCl	N.sp.	5 × 10^6 L, i. d.	N.sp.	86 % (12/14)	20 % (1/5)	N.sp.		x	x	
9	Yanping C et al. [48]	2011	RCT, do-blind	94	N.sp.	Before and 3 × up to 12th WoP	PLy	N.sp.	N.sp.	20–40 × 10^6 Ly; 2 ml i. d. (6–8×)	N.sp.	84 % (41/49)	53 % (24/45)	N.sp.		x	x	Article in Chinese, information according to Liu Z et al., 2016

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▶ **Table 1** Current study situation for immunisation with partner lymphocytes: a comparison of three meta-analyses (Wong LF et al., 2014, Liu Z. et al., 2016, Cavalcante MB et al., 2016) with the respective included studies. *(Continued)*

	Study	Year	Design	Number of patients	Age, years	Treatment time	Treatment group	Placebo group	Storage	Dosage, administration route	Outcome parameters (LB ± advanced pr.)	Successful outcome		p	Taken into account in the meta-analysis			Comment
												Treatment group	Placebo group		Wong 2014	Liu 2016	Cavalcante 2016	
10	Lin S et al. [49]	2012	RCT, do-blind	84	N.sp.	Before and every 2 weeks up to 16th WoP	PLy	N.sp.	N.sp.	10 ml citrated blood s. c.	N.sp.	79% (33/42)	40% (17/42)	N.sp.		x	x	Article in Chinese, information according to Liu Z et al., 2016
11	Aiwu W et al. [50]	2013	RCT, si-blind	78	N.sp.	Every 3 weeks up to 12th WoP	PLy/ DLy	TCM	N.sp.	20–30 × 10^6 Ly; 1 ml s. c.	N.sp.	82% (32/39)	46% (18/39)	N.sp.		x	x	Article in Chinese, information according to Liu Z et al., 2016
12	Bin T et al. [51]	2013	RCT, do-blind	888	N.sp.	Before and 2 × during Pr.	PLy/ DLy	N.sp.	N.sp.	25 ml citrated blood; 0.2 ml 4–6× i. d.	N.sp.	84% (250/ 297)	43% (254/ 591)	N.sp.		x	x	Article in Chinese, information according to Liu Z et al., 2016
13	Hong L et al. [52]	2003	RCT, do-blind	29	N.sp.	Before and during early Pr.	PLy	N.sp.	N.sp.	20–30 × 10^6 Ly; 0.3 ml i. d. (3×)	N.sp.	86% (18/21)	25% (2/8)	N.sp.		x	x	Article in Chinese, information according to Liu Z et al., 2016
14	Stray-Pederson S [53]	1994	RCT, do-blind	64	N.sp.	N.sp.	PLy	N.sp.	N.sp.	N.sp.	N.sp.	73% (24/33)	71% (22/31)	N.sp.	x			Unpublished data, according to Wong LF et al. 2014
15	Coulam CB et al. [54]	London	RCT	67	N.sp.	N.sp.	PLy	PLy, from 40 ml blood, 2/3 i. v., 1/6 i. d., 1/6 s. c.	N.sp.	Ly from 400 ml blood, 2/3 i. v., 1/6 i. d., 1/6 s. c.	LB	68% (25/37)	47% (14/30)	N.sp.			x	
		Taipei	RCT, do-blind	102	N.sp.	Before Pr., boost with Ly from 50 ml blood i. d. after 6 months or during Pr.	PLy	MLy	N.sp.	100–200 × 10^6 Ly, i. d.	LB	77% (41/53)	65% (32/49)	N.sp.			x	

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▶ **Table 1** Current study situation for immunisation with partner lymphocytes: a comparison of three meta-analyses (Wong LF et al., 2014, Liu Z. et al., 2016, Cavalcante MB et al., 2016) with the respective included studies. *(Continued)*

	Study	Year	Design	Number of patients	Age, years	Treatment time	Treatment group	Placebo group	Storage	Dosage, administration route	Outcome parameters (LB ± advanced pr.)	Successful outcome		p	Taken into account in the meta-analysis			Comment
												Treatment group	Placebo group		Wong 2014	Liu 2016	Cavalcante 2016	
		Melbourne	RCT, do-blind	42	N.sp.	Before pr.	PLy	NaCl	N.sp.	Ly from 100–150 ml blood, 1/2 i. v., 1/2 i. d. and s. c.	LB	65 % (13/20)	73 % (16/22)	N.sp.			x	
		Aalborg	RCT, do-blind	76	N.sp.	Before Pr., boost up to 6th WoP	PLy/ DLy	MLy	N.sp.	Ly from 400 ml blood, i. v.	LB	65 % (31/48)	57 % (16/28)	N.sp.			x	
		Sydney	RCT, do-blind	39	N.sp.	Before pr.	PLy	MLy	N.sp.	Ly from 400 ml blood, 2/3 i. v., 1/3 i. d., s. c.	LB	68 % (13/19)	60 % (12/20)	N.sp.			x	
		Paris	RCT, do-blind	52	N.sp.	Before pr.	PLy	MLy	N.sp.	Ly from 400 ml blood, 4/5 i. v., 1/5 i. d.	LB	65 % (17/26)	54 % (14/26)	N.sp.			x	
		Milan	RCT	30	N.sp.	Before pr.	PLy	None	N.sp.	Ly from 400 ml blood, i. v., i. d., s. c.	LB	63 % (10/16)	79 % (11/14)	N.sp.			x	
		Edinburgh	RCT, do-blind	22	N.sp.	Before Pr. + boost up to 6th WoP	PLy	MLy 40–60 ml blood	N.sp.	100 ml blood, $50–200 \times 10^6$ Ly, i. v., i. d., s. c.	LB	67 % (8/12)	60 % (6/10)	N.sp.			x	
		Hamilton	RCT	N.sp.	N.sp.	Before pr.	PLy	NaCl	N.sp.	50×10^6 Ly	LB	N.sp.	N.sp.	N.sp.			x	von Coulam et al. not analysed because a bias was suspected due to the fact that the analysis was carried out in Hamilton itself

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▶ **Table 1** Current study situation for immunisation with partner lymphocytes: a comparison of three meta-analyses (Wong LF et al., 2014, Liu Z. et al., 2016, Cavalcante MB et al., 2016) with the respective included studies. *(Continued)*

	Study	Year	Design	Number of patients	Age, years	Treatment time	Treatment group	Placebo group	Storage	Dosage, administration route	Outcome parameters (LB ± advanced pr.)	Successful outcome		p	Taken into account in the meta-analysis			Comment
												Treatment group	Placebo group		Wong 2014	Liu 2016	Cavalcante 2016	
		Total		430							LB	68% (158/231)	61% (121/199)	< 0.01			x	**No effect:** concentration (>/<300 × 10^6 Ly), time of immunisation, HLA sharing **Effect:** age, number of previous miscarriages (primary miscarriages p = 0.025, secondary miscarriages NS
16	Illeni MT et al. [55]	1994	RCT, do-blind	44	N.sp.	Before pr.	PLy	None	N.sp.	400 ml blood, 200 × 10^6 Ly; 1 ml i. v.; 1 ml i. d.; 1 ml s. c.	LB + Pr.	73% (16/22)	64% (14/22)	NS	x	x	x	3 years (1988–1991), follow-up after 24/25 months as a median
17	Collins J et al. [56]	1994	RCT, 10 sites	456	Up to 45	N.sp.	PLy	N.sp.	N.sp.	N.sp.	LB	62% (153/245)	52% (109/211)	0.026			x	
18	Ober C et al. [57]	1999	RCT, do-blind, 6 sites	171	33 ± 4.3 (23–41)	Before Pr., rep. after 6 months if no Pr. has occurred	PLy	NaCl	Overnight at 1–6 °C	200 × 10^6 Ly 3 ml i. v., 1 ml s. c.; 1 ml i. d.	LB + Pr. ≥ 28th WoP, within 12 months	36% (31/86)	48% (41/85)	0.11	x		x	Excluded in the meta-analysis of Liu et al. because the study was discontinued due to the fact that there were more miscarriages in the treatment group than in the control group

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▶ **Table 1** Current study situation for immunisation with partner lymphocytes: a comparison of three meta-analyses (Wong LF et al., 2014, Liu Z. et al., 2016, Cavalcante MB et al., 2016) with the respective included studies. *(Continued)*

	Study	Year	Design	Number of patients	Age, years	Treatment time	Treatment group	Placebo group	Storage	Dosage, administration route	Outcome parameters (LB ± advanced pr.)	Successful outcome		p	Taken into account in the meta-analysis			Comment
												Treatment group	Placebo group		Wong 2014	Liu 2016	Cavalcante 2016	
19	Daya S et al. [58]	1994															x	
		London	RCT	39	N.sp.	N.sp.	PLy	MLy from 40 ml blood, 2/3 i. v., 1/6 i. d., 1/6 s. c.	N.sp.	Ly from 400 ml blood, 2/3 i. v., 1/6 i. d., 1/6 s. c.	N.sp.	65 % (13/20)	37 % (7/19)	0.08			x	
		Taipei	RCT, do-blind	53	N.sp.	Before Pr., boost with 50 ml blood after 6 months if no Pr.	PLy/ DLy	MLy	N.sp.	100–200 × 10^6 Ly, i. d.	N.sp.	70 % (19/27)	58 % (15/26)	0.34			x	
		Melbourne	RCT	31	N.sp.	Before pr.	PLy	NaCl	N.sp.	Ly from 100–150 ml blood, 1/2 i. v., 1/2 i. d. and s. c.	N.sp.	56 % (9/16)	73 % (11/15)	0.46			x	
		Aalborg	RCT, do-blind	40	N.sp.	Before Pr., boost up to 6th WoP	PLy/ DLy	MLy	N.sp.	Ly from 400 ml blood, i. v.	N.sp.	68 % (17/25)	40 % (6/15)	0.08			x	
		Sydney	RCT	28	N.sp.	Before pr.	PLy	MLy	N.sp.	Ly from 400 ml blood, 2/3 i. v., 1/3 i. d., s. c.	N.sp.	42 % (5/12)	38 % (6/16)	1.0			x	
		Paris	RCT, do-blind	52	N.sp.	Before pr.	PLy	MLy	N.sp.	Ly from 400 ml blood, 4/5 i. v., 1/5 i. d.	N.sp.	63 % (17/27)	40 % (10/25)	0.17			x	
		Edinburgh	RCT, do-blind	31	N.sp.	Before Pr. + boost up to 6th WoP	PLy	MLy 40–60 ml blood	N.sp.	100 ml blood, 50–200 × 10^6 Ly, i. v., i. d., s. c.	N.sp.	50 % (8/16)	27 % (4/15)	0.27			x	

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▶ **Table 1** Current study situation for immunisation with partner lymphocytes: a comparison of three meta-analyses (Wong LF et al., 2014, Liu Z. et al., 2016, Cavalcante MB et al., 2016) with the respective included studies. *(Continued)*

	Study	Year	Design	Number of patients	Age, years	Treatment time	Treatment group	Placebo group	Storage	Dosage, administration route	Outcome parameters (LB ± advanced pr.)	Successful outcome		p	Taken into account in the meta-analysis			Comment
												Treatment group	Placebo group		Wong 2014	Liu 2016	Cavalcante 2016	
		Hamilton	RCT, si-blind	11	N.sp.	Before pr.	PLy	NaCl	N.sp.	50×10^6 Ly, s. c.	N.sp.	43 % (3/7)	25 % (1/4)	1.0			x	
		Meta-analysis of all 8 RCTs		285	N.sp.	Before Pr., partly boost	PLy/ DLy	NaCl/MLy		Various (see above)		61 % (91/ 150)	44 % (60/ 135)				x	**No effect:** concentration (>/<300 $\times 10^6$ Ly), time of immunisation, HLA sharing, age **Effect:** number of previous miscarriages
20	Scott JR et al. [59]	1994	RCT, do-blind	22	N.sp.	Before pr.	PLy or DLy	NaCl	N.sp.	400–900 $\times 10^7$ Ly, i. v.	N.sp.	60 % (6/10)	42 % (5/12)	N.sp.	x	x		
21	Christiansen OB et al. [60]	1994	RCT, do-blind	66	30 (21–44)	Before Pr. 2 × immunisation (rep. after 1 month), then rep. every 5 months until Pr.	2 DLy compatible with AB0 and Rhesus	MLy	N.sp.	150 ml blood, 150–460 $\times 10^6$ Ly i. v.	LB	71 % (31/43);	48 % (11/23)	NS	x	x	x	
22	Reznikoff-E MF [61]	1994	RCT, do-blind	52	N.sp.	Before Pr., partly during Pr.	PLy	MLy	N.sp.	400×10^7 Ly, 5 ml; 4 ml i. v., 1 ml i. d.+s. c.	N.sp.	65 % (17/26)	54 % (14/26)	N.sp.	x	x		Unpublished data, according to Wong LF et al., 2014
23	Pandey MK et al. [62]	2004	RCT, do-blind	124	N.sp.	Up to 6 × every 4 weeks, until MLR-Bf titre ≥ 30	PLy	MLy/DLy/ NaCl	Overnight (37 °C/ 5 % CO_2)	5×10^6 Ly, i. m., i. d., s. c., i. v., each 0.25 ml	LB	78 % (25/32)	17 % (16/92)	< 0.01	x	x	x	No treatment if MLR-Bf already positive

AB: antibody, do-blind: double-blind, i. m.: intramuscular, i. d.: intradermal, i. v.: intravenous, n.sp.: not specified, LCT-XM: cross test with paternal lymphocytes in lymphocyte toxicity test, LB: live birth, Ly: lymphocytes, MLR-Bf: mixed lymphocyte reaction blocking antibody, MLy: maternal lymphocytes, NS: not significant, PLy: paternal lymphocytes, RCT: randomised controlled trial, s. c.: subcutaneous, si-blind: single-blind, DLy: donor lymphocytes, Pr.: pregnancy, WoP: week of pregnancy, TCM: traditional Chinese medicine, rep.: repeat

In 2001, the Cochrane Library published a meta-analysis of immunological treatment options for recurrent miscarriage, including lymphocyte immunisation. The last update of this meta-analysis in 2014 comprised 12 studies relating to immunotherapy with partner lymphocytes with a total of 641 patients, with 316 women in the case group and 325 women in the control/placebo group. No significant effect on the live birth rate following immunisation was demonstrated (OR 1.22, 95% CI 0.89–1.69) [28]. There was also no proof of an increased rate of live births for immunisation with donor lymphocytes (OR 1.39, 95% CI 0.68–2.82) [28].

The results of this Cochrane analysis are criticised by a range of scientists [29, 62, 64]. The main point of criticism was that the results of the study by Ober et al. [57] were included, which published the first and only data to date that showed a negative effect, i.e. even an increase in miscarriages, following immunotherapy.

Ober et al. stored the partner's blood, from which the lymphocytes were to be prepared, at a temperature of 1–6 °C in order to be able to extend the period of time between the blood draw and immunisation. Clark et al. demonstrated that a sufficient number of CD200+ cells is required to achieve an immunomodulatory effect in immunotherapy with lymphocytes. CD200 is expressed on dendritic cells, among others, and can induce immunomodulation in the recipient within the context of immunisation. In this regard, the immunosuppressive component of the immune system is supported by the T_{reg} cells with the help of the transforming growth factor beta (TGF-β) [65]. Storage at low temperatures reduces the CD200+ cell count [65]. Clark et al. argued that recurrent miscarriages following immunotherapy with lymphocytes must be attributed to genetic causes on the part of the embryo, an as yet undetected autoimmune disease in the patient or immunotherapy performed with an insufficient number of CD200+ cells [64].

Furthermore, Ober et al. included patients with autoimmune diseases (positive ANA titre) in the study, which has a negative effect on the results following immunotherapy with lymphocytes [57]. Further points of criticism were the lack of success monitoring (detection of antipaternal HLA antibodies) following immunisation, different methods of administration of the lymphocytes (intradermal, subcutaneous, intravenous) as well as different dosages and lymphocyte concentrations [29, 62, 64].

A repeat analysis of the data from the Cochrane Library, excluding the results of Ober et al. [57], 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) [28].

In 2014, Liu et al. published a new meta-analysis in the American Journal of Reproductive Immunology in order to correct the errors and/or weaknesses of the Cochrane analysis regarding this topic [38]. In this new meta-analysis, 18 randomised clinical studies from the period 1985–2013 were included; with a total of 1738 patients: 739 in the case group with immunisation with partner or donor lymphocytes and 999 patients in the control group. Liu et al. demonstrated a significant effect on the rate of live births following immunisation: 77.8% live births were recorded in the group following immunisation, compared with 46.1% in the control group (OR 4.02, 95% CI 3.23–5.00) [38]. A subgroup analysis regarding different immunisation protocols also revealed a significant increase in the rate of live births when the immunisation was performed before and during the pregnancy (OR 4.67, 95% CI 3.70–5.90 vs. OR 2.00, 95% CI 1.39–2.88) [38]. A further subgroup analysis indicated a better outcome when using no more than 100×10^6 lymphocytes per administration (OR 1.52, 95% CI 1.04–2.22) [38].

Yu et al. investigated the various methods of administration and demonstrated that the best results were achieved with intradermal immunisation [66].

In 2002, the US Food and Drug Administration (FDA) decided that active immunisation with partner lymphocytes would only be performed under study conditions. The reason for this was the aforementioned data of Ober et al. [57] from 1999. The current AWMF (Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften [German Association of the Scientific Medical Societies]) guideline 015/050 of 2013 is also circumspect with regard to this therapy. The reason for this is the lack of evidence of active partner immunisation for the treatment of recurrent spontaneous miscarriage. The only literature source indicated is a Cochrane analysis [67] from 2006, which also includes the work of Ober et al.

A recent review by Cavalcante et al. [39] from 2017 included 6 meta-analyses. Two of these – the above-mentioned works of Fraser et al. and Wong et al. – showed no increase in the rate of live births [28, 63], while the other four demonstrated a significant effect with regard to the rate of live births following immunisation with partner lymphocytes [38, 39, 54, 56, 68].

Conclusion

Immunisation with partner lymphocytes is a treatment option for recurrent implantation failure or recurrent miscarriage if all other possible causes have been ruled out in advance. Due to the highly heterogeneous nature of the data, a significant benefit as a result of the immunisation cannot yet be clearly proven. However, there are signs that the therapy may be effective when using lymphocytes that have been extracted as short a time beforehand as possible. Overall, the treatment represents a safe, low-risk procedure. Following a detailed informative discussion with the couple regarding the chances of success and following a detailed review of the indication and contraindications, immunisation with partner lymphocytes can be discussed with the couple on a case-by-case basis, provided that all other possible causes of sterility have been ruled out in advance.

Conflict of Interest

The authors declare that they have no conflict of interest.

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