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General Review

Immunologically Mediated Abortion (IMA)

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Roughly 20% of all clinical pregnancies evolve into "spontaneous abortions". The causes of spontaneous abortion have been determined in under 60% of the total and comprise genetic, infectious, hormonal and immunological factors. In some cases the immune tolerance mechanism may be impaired and the foetus immunologically rejected (IMA, immunologically mediated abortion). The immunological mechanism implicated depends on the time in which pregnancy loss takes place. During preimplantantion and up to the end of implantation (13th day) the *cell-mediated immune mechanism* (potential alloimmune etiologies) is responsible for early abortion. This mechanism involves immunocompetent decidual cells (eGL, endometrial granulated lymphocytes) already present during predecidualization (late luteal phase) and their production of soluble factors or cytokines. Once the implantation process is over, after blastocyst penetration of the stroma and the decidual reaction of uterine tissue, IMA could be caused by *cell-mediated* and *humoral mechanism* (antipaternal cytoxic antibodies or autoantibody etiology), by the production of paternal anti major histocompatibility complex antibodies, or even by an autoimmune disorder leading to the production of autoantibodies (antiphospholipid antibodies, antinuclear antibodies or polyclonal B cell activation). The diagnostic work-up adopted to select IMA patients is crucial and includes primary (karyotype of both partners, toxo-test, hysterosalpingography, endometrial biopsy, thyroid function tests, serum hprolactin, luteal phase dating) and secondary (full hemochromocytometric test, search for LE cells, lupus anticoagulant, anticardiolipin, antinuclear antibodies, Rheumatoid factor, blood complement VDRL) investigations. Therapeutical approaches vary. If autoimmune disorders are demonstrated therapies with different combinations of corticosteroids, aspirin and heparin or intravenous immunoglobulin are administered. Otherwise, therapy with paternal or donor peripheral blood mononuclear cells should be instituted.

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INTRODUCTION

Materno–foetal immunologic interaction has yet to be fully defined. However, many studies have demonstrated that the immune system plays a major role in determining the success of implantation and pregnancy outcome through a process called "maternal recognition".

Roughly 70% of all pregnancies fail to go to term, 50–60% of which are lost within the first month of pregnancy (Fig. 1) [1] and hence may go unnoticed [2]. It has also been estimated that 20% of all pregnancies (which includes preimplanted embryos, chemical pregnancies, and clinical pregnancies) evolve into "spontaneous abortions" [3].

Spontaneous abortions therefore constitute a major clinical problem, especially for sub-fertile couples who already have a high failure rate due to infertility. The literature defines recurrent abortion as the occurrence of three or more clinically diagnosable abortions prior to the 20th week of pregnancy [3, 4]. Even though there exists a 10% probability of spontaneous abortion in healthy women (controls) leading to early pregnancy loss [4], it is highly unlikely that this would recur three times in a row. The frequency of recurrent abortion

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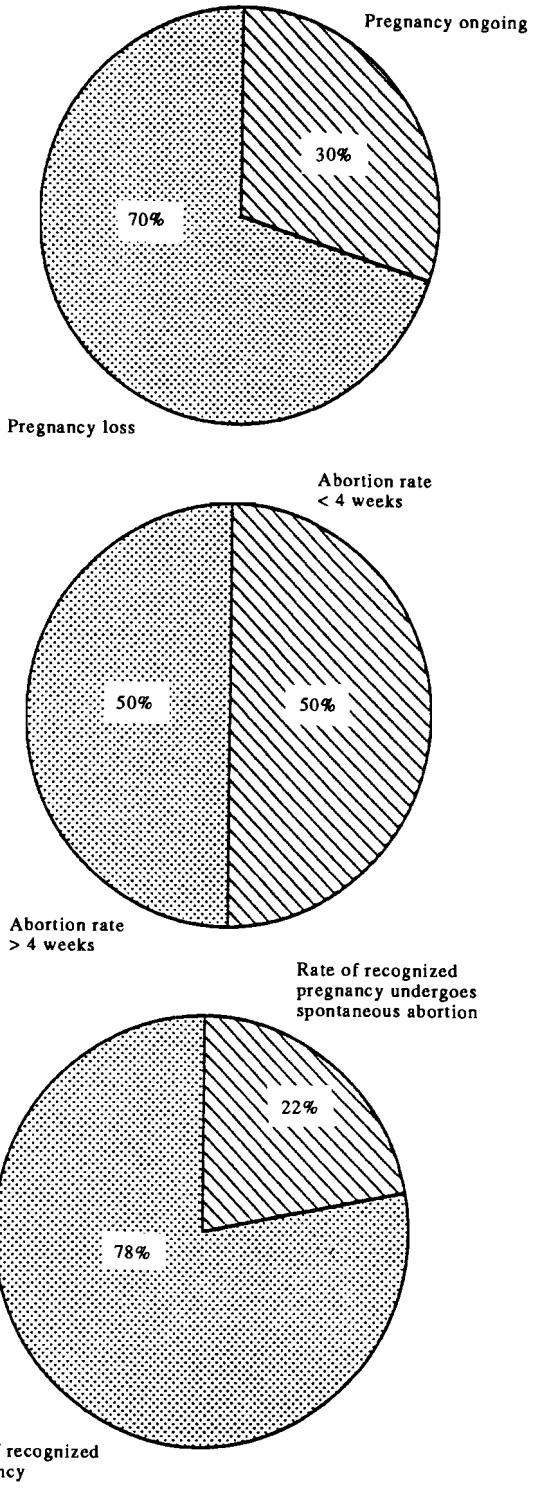


Fig. 1. Incidence of pregnancy loss.

among pregnant women is around 0.4–0.8% [5]. The risk of recurrent abortion increases with the number of past abortions: after one abortion the risk is 24%; after two consecutive abortions it is 26%; after three abortions, 32% (Fig. 2) [1, 4]. The possible causes of recurrent abortion have been determined in under 60% of the total and comprise genetic, infectious, hormonal and immunological factors (Table 1). It has also been

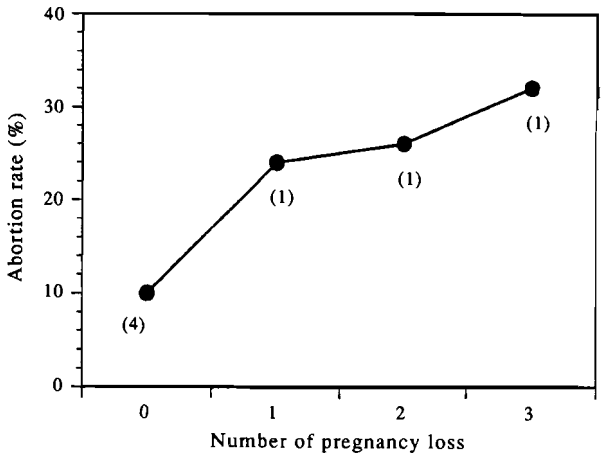


Fig. 2. The risk of pregnancy loss progressively increased with the number of episodes of abortion (the numbers in parentheses are the references). From Bulletti *et al.*: *Fert. Steril.* In press).

claimed that most early stage abortions are due to an error in the implantation process accounting for pregnancy loss in 78% of humans [8]. Other authors gauge the range of nidation from 20 to 43% [9].

Although percentages on the incidence of immunologically mediated abortion (IMA) vary in the literature, there is no doubt that immune abortion exists [4]. This is borne out by the fact that women with immune disorders (e.g. connective tissue disease) are significantly more likely to experience recurrent spontaneous abortion (usually in the second trimester). Another example is women with systemic lupus erythematosus (SLE) [10] in which thrombosis in the spiral arteries of the utero–placental circulation cause ischemia of decidua and placenta. Although there are no controlled studies, pregnancy outcome has been enhanced by combined therapy with immunosuppressive drugs (high-dose corticosteroids) and platelet antiaggregants (low-dose aspirin).

Furthermore, IMA is linked with the physiopathology of "immune tolerance": the maternal immune system tolerates the foetus (50% of the antigens are paternal). In some cases the immune tolerance

Table 1. *Etiology of recurrent abortion (from Dudley [69], modified)*

Etiology	Incidence	Refs
Genetic	2.6–7.7%	[5, 6]
Anatomic	15.4–27%	[5, 6]
Cervical incompetence	9.0–12%	
Uterine septus	up to 16.8%	
Uterine leiomyomata	up to 18%	
Infectious	<1%	[5, 6]
Hormonal	5.1–35%	[5, 6]
Thyroid dysfunction	1.7%	
Luteal phase defect	5.1–35%	
Immunologic	~40%	[5, 6]
(unexplained abortion)	30–50%	[7]

mechanism may be impaired and the foetus immunologically rejected (i.e. IMA). Systemic involvement has recently been implicated when no cause can be found for primary recurrent abortion [4]. Even when recurrent abortions occur after one or more previous pregnancies brought to term (secondary recurrent abortion), they are considered immunologically mediated.

Further, the success rate without treatment in patients selected according to these criteria shows that some women cannot be considered IMA patients and IMA accounts for between 37 [11] and 90% [12] of the total. As a result, the percentage of pregnancies brought to term decreases in proportion to the increase in the percentage of spontaneous abortions [13].

More knowledge of the mechanisms underlying IMA, a more rigid selection of IMA patients and larger case series will clarify what is meant by immunologically mediated abortion.

BASIC ASPECTS (Etiology)

In normal conditions (successful pregnancies) the maternal immune system (IS) does not react against spermatozoa or embryo even when they express antigen exogenous to the maternal system. This maternal immune system "tolerance" probably lies in the fact that immunoregulatory mechanisms protect the foetus [1]. When such mechanisms are impaired spontaneous abortion ensues since paternal immune responses cause the embryo (foetus and trophoblast) to stimulate the maternal IS [14]. Yet a single mechanism cannot be responsible for the success or failure of a pregnancy. The immunological mechanism implicated also depends on the time in which pregnancy loss takes place. During preimplantation and up to the end of implantation (13th day) the cell-mediated immune mechanism is probably responsible for early abortion. This mechanism involves immunocompetent decidual cells (T lymphocytes) already present during predecidualization (late leuteal phase) [15] and which surround the blastocysts within 48 h of contact, positioning themselves in the space following the implantation process [16, 17] and implying that the maternal IS controls embryo implantation and foetal growth.

Some studies have shown that efficient functioning and an adequate number of decidual cells are required to maintain the semiallogenic foetus (50% exogenous antigens) and pregnancy has been likened to a straightforward natural allograft [18, 19]. Other studies have demonstrated that the similarity between regions D/DR and B of the maternal and paternal major histocompatibility complex (MHC) is a crucial factor in the early stages of foetal development [20, 21]. In fact, spontaneous abortion has been prevented in humans by maternal sensitization (a reverse immunization) with MHC antigens from paternal leukocytes [11, 22, 23].

In human, blastocyst implantation occurs on the 6th or 7th day after fertilization [24, 25]. The blastocyst then penetrates the implantation site by means of proteolytic enzymes [24]. In many cases, for reasons which are still unclear, the implantation process is delayed and blastocyst development comes to a halt. The signal(s) leading to nidation are not fully known. Some claim that hormones and other paracrine and/or autocrine factors (e.g. progesterone and prostaglandins) constitute basal signals for nidation, influencing blastocysts and uterine receptivity alike [26]. The success of implantation also depends on adequate chorionic gonadotropin (HCG) levels which may be a basic signal for nidation. In fact, implantation was enhanced by exogenous HCG administration in women with recurrent spontaneous abortion [27]. Successful implantation therefore requires HCG production together with maternal recognition of alloantigens [28].

Once the implantation process is complete, after blastocyst penetration of the stroma and the decidual reaction of uterine tissue, the vascular sinuses develop to ensure a continuous exchange between maternal and foetal tissues. From now on, IMA could be caused not only by a cell-mediated immunological mechanism, but also by a humoral mechanism or by the production of paternal antiMHC antibodies (or antibodies to non-MHC antigens), or even by an autoimmune disorder leading to the production of autoantibodies. Monoclonal antibody studies have shown that antigens able to stimulate the maternal IS are present on the peri-implantation stage blastocyst and antigen expression varies throughout fertilization until the placenta is formed [29, 30].

For pregnancy to proceed, the immunological mechanism underlying implantation and foetal survival, be it cell-mediated or humoral, must ensure that the maternal IS not only reacts to foetal antigen stimulation, but must also prevent and/or block the arrival or activity of cells cytotoxic to foetus and placenta. The trophoblast represents the interface between maternal and foetal tissue and studies on mice have shown that it gives the embryo a sort of "basal immunoprotection". The trophoblast's immunological features are:

- —weak antigenicity to immune damage by lymphocytes or cytotoxic antibodies [31];
- —physical barrier [32];
- —recruits or signals migration into uterine lymphatics and decidua of lymphocytes able to suppress maternal reactivity [33];
- —local production of progesterone and other immunosuppressive hormones [34];
- —promotes the production of blocking factors able to bind several antigenic sites [1].

CELLULAR IMMUNE RESPONSES MECHANISM (Potential alloimmune etiologies)

Classic histological studies have demonstrated that the human endometrium contains lymphoid and myeloid immunocompetent cells and other cells involved in antigen presentation which migrate in the late secretory phase and during the decidual reaction [35, 36]. These cells play a key role in maternal IS recognition. In particular, there are more T-helper lymphocytes (CD4+)(T-h) than T-suppressor lymphocytes (CD8+)(T-s) in normal human endometrial biopsies throughout the menstrual cycle except in the late luteal phase (period of implantation) and menstruation when T-s lymphocytes prevail [35].

An impaired T-h/T-s ratio in favour of T-h lymphocytes has been demonstrated in endometrial biopsies from women with a history of recurrent spontaneous abortion [35], suggesting that a deficit in T-s cell function is responsible for foetal rejection. In the mouse, foetal reabsorption was associated with a diminished number of decidual suppressor cells [37].

Recent target studies have described the immunohistochemical features of the endometrial and decidual lymphocyte population [15]. Leukocytes make up the major cell component of the decidua while T lymphocytes account for 20%. Phenotypic characterization has identified leukocytes present in the decidua and endometrium in the late secretory phase. They are intensely positive for CD56 [natural killer (NK) lineage marker] but do not express other NK lineage markers such as CD16 ("classic" NK marker), 57 and 11b. They are present scattered throughout decidua but aggregate around endometrial glands and arterioles [15]. Macrophages (CD14+) are also present and some are MHC-II positive.

These phenotypically unusual lymphocytes have been classified as eGL (endometrial granulated lymphocytes) since they all have a dense cytoplasm populated by small uniformly distributed granules. Bulmer *et al.*, [15] demonstrated that culture supernatants of decidual cells have an immunosuppressor activity disclosed by specific tests. Culture supernatants of eGL cells alone are less immunosuppressive and may even act as immunostimulants. This implies that there exist other decidual cells capable of suppressing an immune response and that eGL activity must entail the production (or lack of production) of soluble factors or cytokines.

Other data suggest that decidual immunosuppressive activity is closely linked to the presence of the embryo. *In vitro* studies have shown that mouse blastocysts have immunosuppressive properties crucial to the success of implantation [38]. Moreover, 43% of human embryos grown *in vitro* produce immunosuppressive factor(s) [39]. It has therefore been speculated that stimulated decidual cells (eGL) could: (1) "present" foetal antigens to the maternal IS to protect the foetus from rejection [28, 31, 40] or (2) locally produce soluble factors, probably including TGF-β (transforming growth factor-β), to suppress the local maternal immune response whose toxicity is caused by NK cells producing TNF-α (tumour necrosis factor-α) [15, 41–44]. Alternatively, both mechanisms could join in a highly sensitive interplay. The origin, biological effect, mechanism of action and study models of the growth factors implicated in immunologic control and which may be responsible for IMA are listed in Table 2.

Figure 3 summarizes the functional interactions envisaged between different factors and target cells. In Fig. 3.1, TGF-β and hypothesized TGF-α produced by eGL may cause local immunosuppression of TNF-α mediated maternal NK cytotoxicity or they may complete with TNF-α directed on trophoblastic cells [4–7, 11, 12]; in Fig. 3.2, TGF-β or PGE-2 derived from other decidual cells (non-eGL) may determine local immunosuppression of TNF-α mediated maternal NK cytotoxicity [4–7, 11, 12]; in Fig. 3.3, TGF-β from embryo (only hypothesized) may exert local immunosuppression of TNF-α mediated maternal NK cytotoxicity [4–7, 10–13]; in Fig. 3.4, PGE-2 derived from macrophages (CD14+) may block the bioactivity of interleukin-2 (IL-2) and/or may block the IL-2-receptors and/or IL-2-production [4, 14]. Furthermore in Fig. 3.5, GM-CSF and/or IL-3 derived from eGL or other lymphocytes may complete with TNF-α receptor and may exert protective effect for resorption of foetus [6, 8, 9]. Finally in Fig. 3.6, CSF-1 derived from eGL and/or other lymphocytes and/or embryo [5, 6, 9, 16] could determine resorption of foetus at high dosage and protection of foetal rejection when delivered at low dosage. It remains unsettled exactly how these mechanisms involving cell elements and autocrine or paracrine cytokines achieve or influence immunological recognition and tolerance by the maternal IS.

The embryo expresses only class I and not class II MHC antigens. These antigens are present at a very early stage (8 cells) of blastogenesis (day 2) [55]. Women with a history of chronic spontaneous abortion have a greater genetic expressivity of classes I and II and an impaired immune response to paternal MHC antigens [56]. This is thought to be due to a weak maternal protective mechanism and unbalanced maternal immune regulation. However, the view that immunosuppressive factors protecting against IMA (Table 2) block the production or activity of other cytokines has met with more consensus [37]. In both experimental murine and human models recurrent spontaneous abortion was associated with poor local suppressive uterine activity or few specific cells or impaired production or activity of specific factors [57]. Other workers speculate that there is a reduced induction by embryonic decidual factors for the production of adequate immunosuppressive factors of embryonic

Table 2. Origin, biological effect, mechanism of action and study models of the growth factors implicated in immunologic control and which may be responsible for IMA

Factors	Source	Type of study	Biological effect	Mechanism of action	Refs
TGF-β and TGF-β-like substances	Decidual T-cells (CD56 + CD3-CD16-) Decidual lymphocyte no-T no-B Other Decidual cells Embryo (6–8 cells) ?	Murine Decidus. Strongly hypothesized in Human Decidua	Protection for recurrent IMA	Local immunosuppression of TNF-α mediated maternal NK cytotoxicity (1°)	Bulmer *et al.* [15] Mowbray [41] Tabibzadeh [42] Clark *et al.* [43] Tamada *et al.* [44] Altmann *et al.* [45]
TGF-α	Decidual T-cells (?)	Hypothesized Human Decidua	Protection for recurrent IMA	Inhibition bioactivity of IL-2	Mowbray [41]
PGE-2	Macrophages (CD14 +)	Human Decidua	Protection for recurrent IMA	Blockage of receptors and production of IL-2	Bulmer *et al.* [15] Parhar *et al.* [46]
CSF-1 (M-CSF)	Maternal T-cells	Murine Decidua (*2)	In pregnant mouse: a—resorption of foetus (high dosage) b—protection of foetal rejection (low dosage)	Increase of human of cytotrophoblast cells PRs of HCG and HPRL (*3)	Tabibzadeh [42] Pollard *et al.* [47] Wegmann [49] Mowbray [41] Wegmann [49]
GM-CSF and GM-CSF-like substances	Maternal T-cells (?) (*4)	Murine Decidua	Protective effect for resporption of foetus	Unknown mechanism	Wegmann *et al.* [48] Mowbray [41]
IL-3	Murine Decidual cells (?)	Murine Decidua	Protective effect for spontaneous abortion in pregnant mouse	Unknown mechanism	Wegmann [49]
P4	Maternal Serum	Human Model	Ipotizzed protective effect for spontaneous abortion	Ipotizzed suppression of maternal cellular cytotoxicity	Fized *et al.* [50]
Thymic Humoral Factor (THF) Complete Freund Adjuvant (CFA)	Exogenous origin	Murine Model	Reverse of tendency of pregnancy loss in pregnant mouse	Immunoregulation of maternal IS	Toder and Strassiburger [51]
TNF-α	Maternal Lymphocytes (CD56 + (?)) (*5)	Murine Model	Abortigen cytotoxic effect	NK activity effector again foetal antigens (?) Direct cytotoxic effect on trophoblast cells	Mowbray [41] Tabibzadeh [42] Baines and Gendron [52]

TGF-β, transforming growth factor-β; TGF-α, transforming growth factor-α; CSF-1, colony stimulating factor-1; M-CSF, macrophage stimulating factor; GM-CSF, granulocyte macrophage-colony stimulating factor; IL-3, interleukin 3; IL-2, interleukin 2; TNF-α, tumour necrosis factor α. (*1) Other possible mechanisms are: (a) competition with TNF-α directed on trophoblastic cells; (b) competition with TNF-α on target cells having immunosuppressive activity. (*2) Other possible production sites are: (a) term-placenta and amniotic fluid [53]; (b) embryo (6–8 cells) [54]; (c) endometrium [42]. (*3) Another possible mechanism is: cellular binding of CSF-1 on specific receptor on invasive trophoblast [41]. (*4) Another possible production site is: embryo (2–4/6–8 cells) [54]. ?, When the effect is only hypothesized or not well documented.

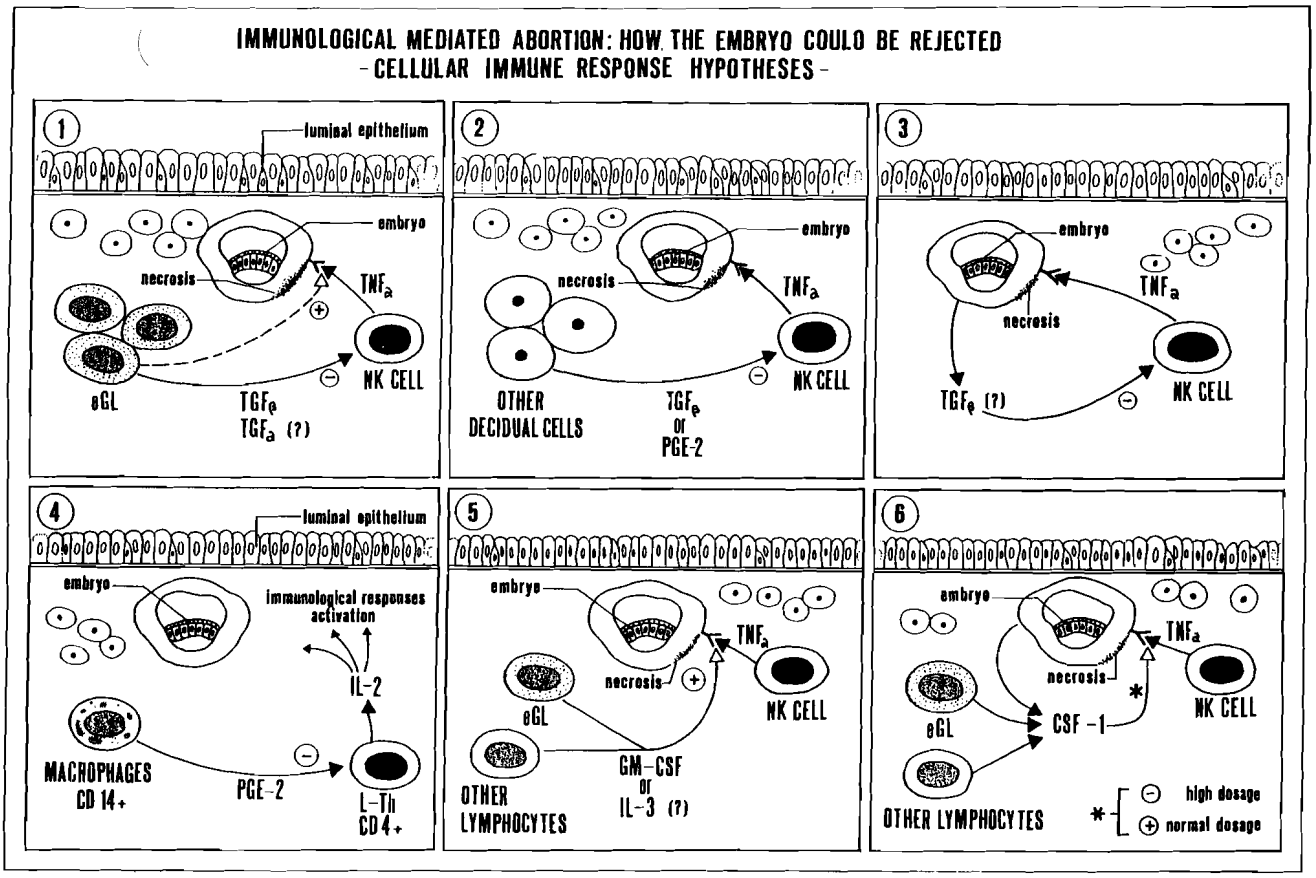


Fig. 3. The growth factors involved in the IMA and possible mechanism of action. TGF-β, transforming growth factor-β; TGF-α, transforming growth factor-α; tumour necrosis factor-α; eGL, endometrial granulated lymphocytes; PGE-2, prostaglandin E-2; L-Th, lymphocyte T helper (CD4+); IL-2, interleukin 2; GM-CSF, granulocyte-macrophage-colony-stimulating-factor; IL-3, interleukin 3; CSF-1, colony stimulating factor-1(M-CSF, macrophage-colony-stimulating-factor).

origin (TGF-β) [1]. These three possibilities are outlined in Fig. 4.

Others claim that the cytokines produced by lymphocytes can constitute growth factors for embryo and placenta (irrespective of the immune problem) [58–60]. In addition, the cytokines produced by macrophages, EGF and PDGF, stimulate embryonic growth [61]. This could mean that besides a suppressor cell deficiency, a deficit in growth factor producing cells could be responsible for recurrent abortion [62]. Another function for decidual immune system cells which produce luteotrophic substances *in vitro* in response to GnRH has been postulated [63] as well as the production of progesterone by mouse granulosa cells [64]. Others hold that LH induces suppressor cell activity [65]. Finally, maternal lymphocytes could produce LH-like substances in response to allogenic trophoblast tissue [66]. Thus successful implantation depends on an adequate ratio between maternal alloantigenic recognition and chorionic gonadotropin levels.

Yet another possibility is that human embryo and trophoblast could simply be attacked by cell-mediated activation of the IS triggered by infection or by initial stimulation by spermatozoon antigens or by the very recognition of trophoblast antigens [1]. Trophoblast antigens could even stimulate a sub-population of decidual leukocytes (in women with IMA) to produce factors toxic to trophoblast proliferation and the embyro. In fact, when treated with trophoblast extracts, cultured leukocytes from women with IMA induced production of embryotoxic factors (hypothesis confirmed on mouse embryos) [67].

Humoural Immune Responses Mechanism (Antipaternal Cytotoxic Antibodies or Autoantibodies Etiology)

Antipaternal cytotoxic antibodies

During normal pregnancy, the mother may develop cytotoxic IgG antibodies against paternal antigens [68]. These IgG antibodies appear in the first trimester; their concentration dwindles towards term to increase again in the post-partum period [69]. Some immunologists believe that these antibodies play a major role in maintaining pregnancy, although how they do so remains unclear [70]. The presence of IgG antibodies is a marker of adequate maternal "immunological recognition" of paternal antigens and some consider

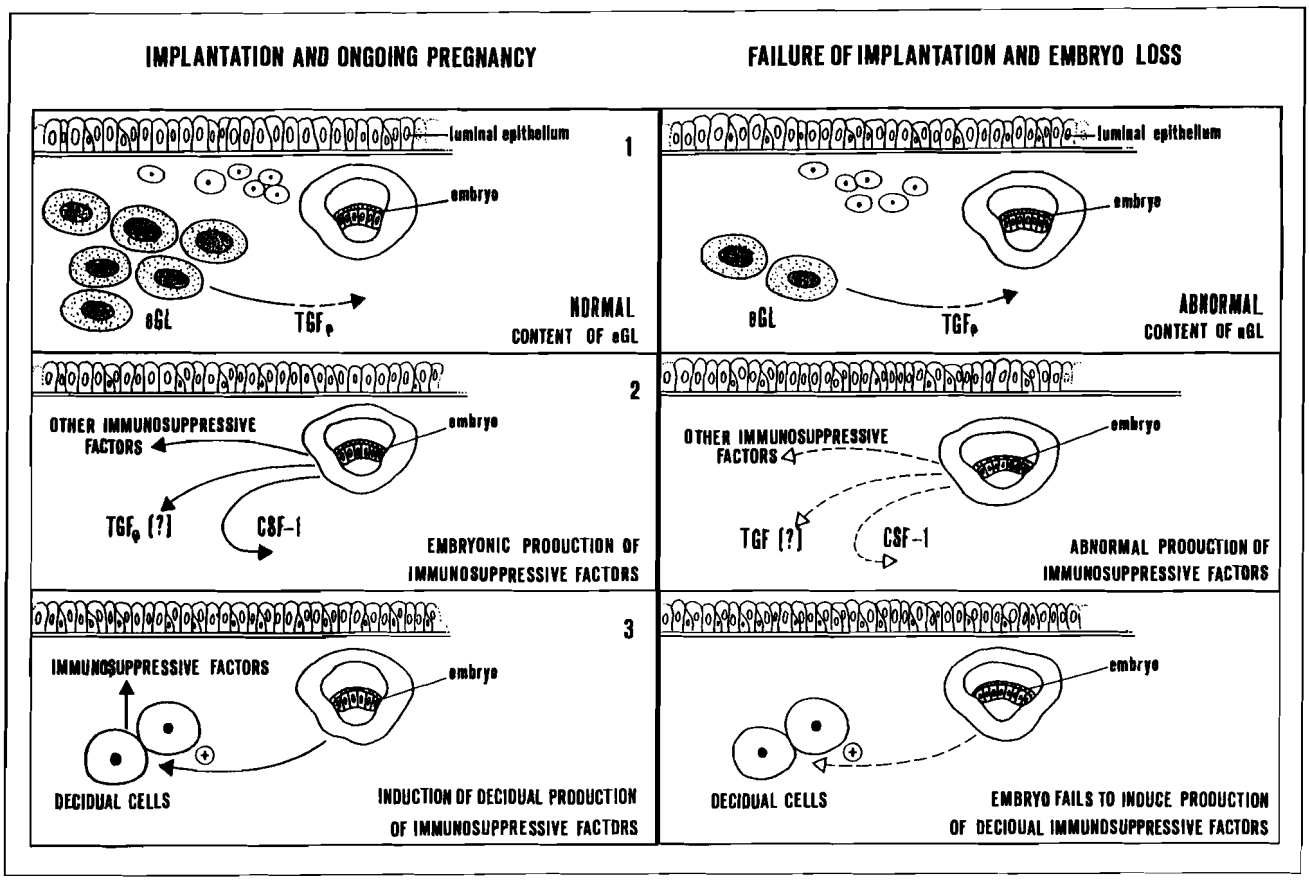


Fig. 4. Growth factors, implantation and embryo loss. IMA could be associated with three conditions that may be responsible for low uterine immunosuppressive activity: (1) reduced number of cells that exert immunosuppressive activity; (2) low production of specific growth factors; and (3) possible low induction of decidual cells from the embryo due to decidual immunosuppressive factors.

it sufficient to rule out recognition failure [70]. However, even though their presence indicates an efficient positive maternal immune response, high antibody titres could cause secondary immune abortions [71]. In addition, the absence of paternal antileukocyte cytotoxic antibodies is still arduous to interpret. Only 20% of women produce these antibodies during their first successful pregnancy and they are present in under 50% of multipara [69].

The successful outcome of pregnancy has long been held to depend on the production of an "immunologic blocking factor" in response to trophoblast development and the factor(s) could be maternal serum IgG [72]. Whether these blocking IgG are really a sub-group of what have already been defined as paternal antileukocyte cytotoxic antibodies, or a class apart remains unclear. In any case, maternal blocking factors have yet to be established and the ability of maternal serum to inhibit the cellular response to paternal antigens may not necessarily depend on serum antibodies alone, but entail the production of specific lymphokines by immunocompetent cells [1].

Blocking factors

The term "blocking factors" has recently been used as a synonym for blocking antibodies (IgG) which are thought to have three main mechanisms [68, 71, 73]:

(1) they bind to maternal lymphocytes and block recognition of paternal antigens;
(2) they bind to paternal (trophoblastic) antigens blocking them and preventing maternal IS stimulation or the antigens present a maternal trophoblast–lymphocyte cross-reaction (TLX antigens);
(3) they bind by acting as antiidiopathic antibodies at the antigenic sites of antibodies present on maternal lymphocytes and prevent binding with paternal antigens (Fig. 5).

The blocking effect can be detected at the end of the first and at the start of the second trimester peaking during the second trimester and persisting even after pregnancy [69]. Blocking antibodies are commonly found in women without IMA, they are absent in nullipara and statistically fewer or absent in women

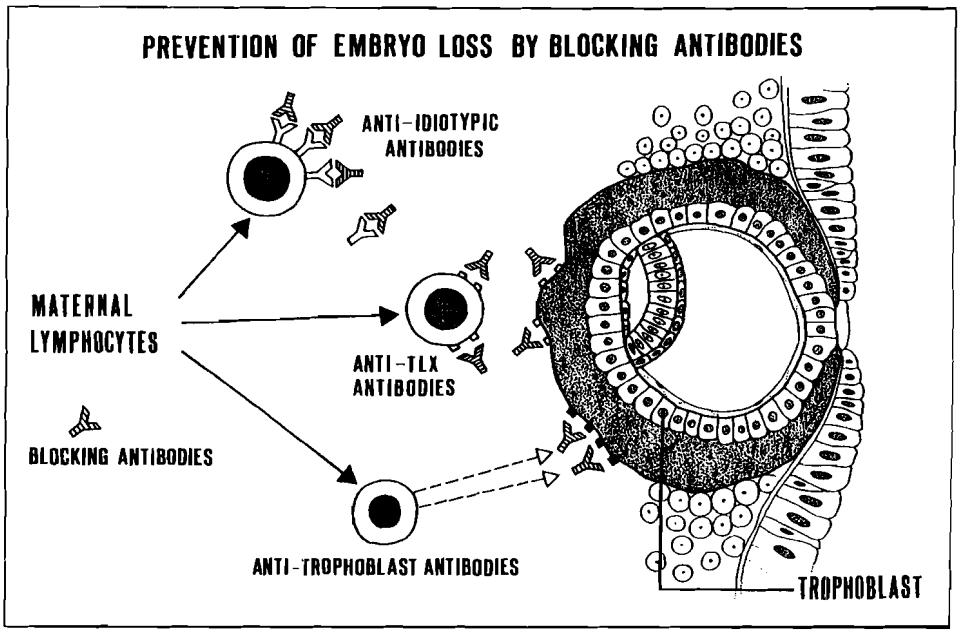


Fig. 5. Possible mechanisms by which blocking antibodies may prevent recurrent abortion.

with IMA [68]. Nowadays the production of blocking antibodies is held to be a prerequisite for successful pregnancy. Immunization (or sensitization) with paternal lymphocytes or lymphocytes from a donor will have a blocking effect. However, gammaglobulinemic women can have normal pregnancies [74] so the blocking effect could merely be the visible part of a much more complicated mechanism.

Autoantibody etiologies

Some autoimmune disorders may increase the risk of spontaneous abortion [70]. Since the reported association between antiphospholipid antibodies (lupus anticoagulant and anticardiolipin) and recurrent spontaneous abortion (e.g. in SLE), other autoantibodies have been implicated in recurrent pregnancy wastage [68] (Table 3).

There are three serotypes of antiphospholipid antibodies:

(a) patients with false positive syphilis tests;
(b) patients with lupus anticoagulant antibodies (LACA); and
(c) patients with anticardiolipin antibodies (ACA).

False positive reactions to syphilis tests may suggest LAC or ACA, but specificity is low and not significantly related to IMA, unlike the presence of LAC and ACA [69]. With treatment, 95% of LAC positive women (10% of the total) will have recurrent spontaneous abortions. Most occur in the first trimester, but 30–40% abort in the second trimester. Only 15% of untreated patients manage to bring a pregnancy to term. The most likely mechanism is placental thrombosis with decidual vascular disease [83] caused by the

Table 3. Percentage of habitual aborters with positive tests

	When a potential autoimmune etiology exists									
	% with ANA		% with LAC		% with ACA		% with PBCA*		No. of patients	
Authors (Refs)	Unexpl.[a]	Expl.[b]	Unexpl.	Expl.	Unexpl.	Expl.	Unexpl.	Expl.	Unexpl.	Expl.
Dudley and Branch [69]	ND[c]	ND[c]	10%	ND	ND	ND	ND	ND	65	0
Harger *et al.* [75]	16.3%	ND	ND	ND	ND	ND	ND	ND	277	
Gleicher *et al.* [77]	ND	ND	ND	ND	ND	ND	70.8%	ND	24	0
Xu *et al.* [76]	40%	ND	ND	ND	ND	ND	ND	ND	30	30
Cowchock *et al.* [80]	29%	5%	ND	ND	ND	ND	ND	ND	14	16
Cowchock *et al.* [81]	30%	14%	3%	0%	13%	0%	ND	ND	61	21
Unander *et al.* [79]	9%	—	ND	ND	42%	—	ND	ND	99	0
Edelman *et al.* [82]	7%	—	10%	—	ND	ND	ND	ND	120	0
Maier and Parke [78]	20%	14%	10%	0%	50%	14%	ND	ND	29	14

ND, not determined; [a]unexplained, patients without an apparent cause for recurrent abortion; [b]explained, patients with an apparent cause for recurrent abortion. [c]polyclonal B Cell activation = PBCA. (Modified from Maier and Parke [78].)

following factors [68]:

—decreased prostacycline production by vascular tissues;
—inhibition of protein C activation;
—inhibition of antithrombin III;
—decreased release of plasminogen activation;
—increased activity of Von Willebrand's factor.

Twenty to 25% of patients with significant LAC levels have thromboembolic diseases [84].

The real nature of ACA antibodies is a matter of debate. All patients with LAC have ACA, but not all patients with ACA have LAC [85], so that LAC could be an ACA sub-group.

Antinuclear Antibodies (ANA)

Indirect immunofluorescence assay for ANA (titre 1:80) was positive in 16.3% of IMA patients [75]. Pregnancy outcome was successful in 52% of these women compared with 65% in ANA-negative women with recurrent pregnancy losses [75]. This non-significant difference and clinical assessment mean that this group of patients cannot be considered at risk for autoimmune disease or significantly more at risk for subsequent spontaneous abortions [75].

Polyclonal B Cell Activation

Abnormal activation of B lymphocytes has been reported in women with spontaneous abortion, infertility and endometriosis [77]. In particular, 70.8% of women with recurrent abortion present autoantibodies (88% of infertile patients). Many women with unexplained infertility and recurrent spontaneous abortion thus present polyclonal B cell activation which may be the cause of these disorders even without clinical signs of autoimmune disease (reproductive autoimmune failure syndrome). Studies in monkeys with histories of habitual abortion [86] found autoantibodies to laminin and other basement membrane proteins like type IV anticollagen antibodies.

In conclusion, all lines of research have demonstrated that immunological and paraimmunological factors are involved in triggering spontaneous abortion. The immunologic events proceed stepwise with a "cascade" mechanism of action. The three major steps are [87]: (I) recognition; (II) start; and (III) amplication. The last two steps have been identified in IMA, but the initial process of recognition remains unclear. A deficient or abnormal presentation of the trophoblast antigen (MHC) by the foetus is probably involved, but it is difficult to pinpoint the exact moment when these antigens change during the early stages of gestation [88].

In vitro studies have shown that the monomorphic antigen (HLA) suppresses progesterone receptors. This has led to speculation that an impaired number of progesterone receptors in the trophoblast could jeopardize pregnancy outcome [87]. Only by dwelling on the "recognition" process will it be possible to identify the mechanism underlying the first step and thus open the way to a specific targeted treatment.

CLINICAL APPROACH

There are two major limitations in IMA management: (1) lack of information on the rationale behind different therapeutic strategies; and (2) lack of evidence on implementing experimental animal results in humans.

Diagnostic Work-up

The diagnostic criteria adopted to select IMA patients are crucial and will determine subsequent clinical management of the patient. The diagnostic work-up includes clinical investigation and laboratory tests which should follow an established pattern in order to identify those patients with recurrent abortion who would benefit from immunotherapy.

Primary screening

There is now general consensus that only women with three or more consecutive spontaneous abortions should be considered. Patients are also defined as primary or secondary aborters.

This initial assessment is designed to exclude women in whom recurrent spontaneous abortion is due to one or other of the possible causes listed in Table 1. The following tests are performed [76, 78, 89, 90]:

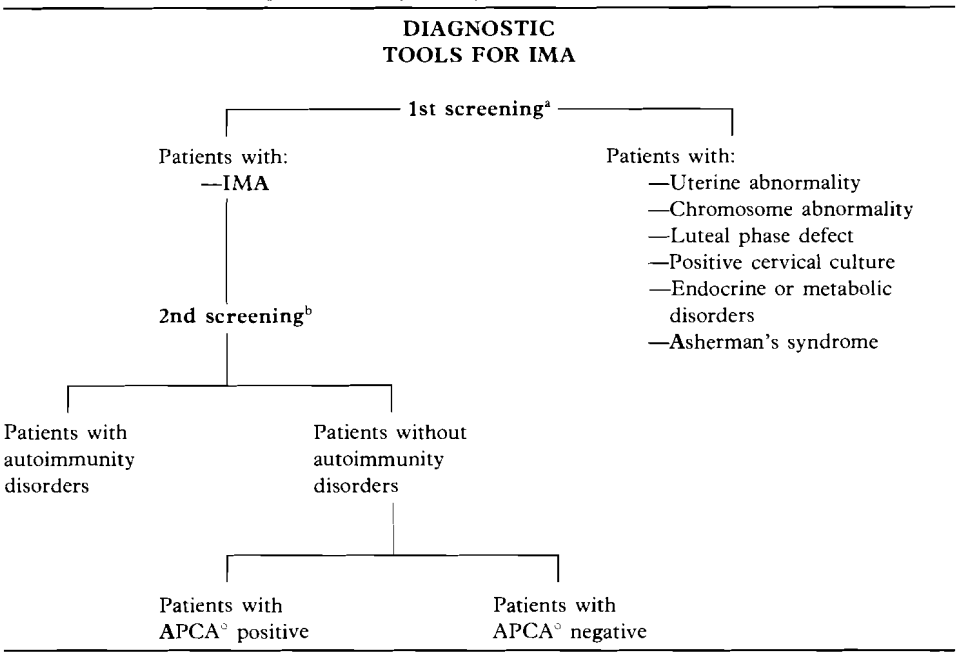
—karyotype of both partners using Q and R band techniques;
—OGTT (oral glucose tolerance test);
—toxo-test (toxoplasmosis serology);
—HSG (hysterosalpingography) or hysteroscopy to rule out anatomic malformations, intrauterine formations and cervical incompetence;
—endometrial biopsy (and/or washing?);
—thyroid function tests (T3, T4, FT3, FT4);
—serum hPRL (prolactin);
—luteal phase dating (luteal phase of at least 12 days and serum progesterone around 8 ng/100 cc).

These tests will rule out patients with uterine abnormalities, chromosome abnormalities, luteal phase defects (LPD), positive cervical culture, endocrine or metabolic disorders and Ascherman's syndrome, selecting women with a probable immunological etiology.

Secondary screening

One of the goals of this series of tests is to confirm or exclude autoimmune disorders. This is a prerequisite for possible treatment and to select the type of treatment [91] (Table 4: IMA diagnostic management).

Table 4. Diagnostic management for patients with or without IMA



[a]1st screening: karyotype of both partners, OGTT, toxo test, hysterosalpingography or hysteroscopy, endometrial biopsy, thyroid evaluation, serum prolactin, luteal phase evaluation.
[b]2nd screening: PT, PTT, serum factors of complement (C3, C4), LE cells, LAC, ACA, ANA, rheumatoid, factor, VDRL.
APXA°, antipaternal cytotoxic antibodies.

Full biochemical screening entails specific tests [78]:

(1) full hemochromocytometric test;
(2) PT, PTT (if altered add blood fibrinogen, AT III and platelet neutralization tests);
(3) search for LE cells;
the association between LAC antibodies and recurrent spontaneous abortion was first noted in a woman with SLE [92].

Studies focussing on spontaneous abortion during the first and second trimesters have demonstrated the association between LAC and elevated ACA levels; 50% of these women had clinically evident SLE [79, 93]. However, other studies have shown that despite the significant association between recurrent abortion and SLE, women with pre- or sub-clinical autoimmunity also have a high incidence of IMA [78, 90]. This finding together with the major incidence of autoimmune disease in women [90] warrants the search for different serum autoantibodies even without clinical signs of disease;
(4) LAC and ACA;
Table 5 lists the main indications for LAC and ACA tests [69, 76]. The sensitivity of the LAC varies widely in the different kits available [94]. ACA antibodies are determined by sensitive and specific enzyme-linked immunosorbent assay (ELISA) [95]. Antiphospholipid antibody syndrome has been defined on the basis of these antibody levels (only permanently elevated IgG levels are considered indicative) and clinical features [96] (Table 6);
(5) rheumatoid factor [78];
(6) blood complement (C3, C4) [78];
(7) VDRL;
in patients positive for VDRL the fluorescent treponema antibody absorption test (FTA-ABS) is recommended [78];
(8) ANA antibodies [69].

Other autoantibodies have also been implicated [78]: native DNA (Farr test), Smith's antigen (Sm), extractable nuclear antigens Ro, La and Ribonucleo-

Table 5. Indication for lupus anticoagulant antibody testing. (modified from Dudley, [69])

(1) Obstetric and gynaecological problems:
Recurrent pregnancy loss
Foetal death
Early onset severe preeclampsia
Pregnancy related thrombosis (venous or arterial)
Intrauterine growth retardation
Pregnancy complicated by clinical autoimmune disease
False-positive serologic test for syphilis
Chorea gravidarum
(2) Other medical problems:
Thromboembolic disease, including stroke
Transient ischemic attacks
Idiopatic seizures in patients with systemic lupus erythematosis
Idiopatic thrombocytopenic purpura
False-positive serologic test for syphilis

Table 6. Suggested criteria for antiphospholipid antibody syndrome

Clinical features	Laboratory features
Venous thrombosis	Ig G anticardiolipin
Aterial thrombosis	antibody (>20 IU)
Recurrent pregnancy loss	Lupus Anticoagulant
Thrombocytopenia	Ig M anticardiolipin antibody (>20 IU) with Lupus Anticoagulant

Patients should have at least one clinical and one laboratory feature during the course of the disease. (Modified from Dudley and Branch [69].

protein (RNP), centromere (ACmA) and spermatozoon (Immunobead test).

This diagnostic work-up leads on to a further step, identifying patients without autoimmune disorders (without evident antiphospholipid syndrome and/or other high titre autoantibodies in over two samples 6–8 weeks apart) [69]. Another step in these women is disclosing the presence of antipaternal cytotoxic antibodies (APCA) [89]. APCA can be assayed by the reactivity between undiluted maternal serum and paternal lymphocytes in peripheral blood (at 22°C) [97]. Rabbit serum acts as complement and results gauge the proportion of cell death in the test sample (positive when serum killer is >20%) [97].

To conclude, laboratory tests will disclose APCA antibodies in 44% of women with recurrent pregnancy losses [78]. However APCA are also found in women with recurrent abortion of non-immunologic origin [78] so that tests for LAC, ACA, VDRL and ANA and determination of factors C3 and C4 are recommended in this patient category.

THERAPEUTIC STRATEGIES

Patients with antiphospholipid syndrome and recurrent abortion

Women with high levels of antiphospholipid antibodies have a significant risk of recurrent abortion and a significantly greater incidence of delayed embryo growth and foetal death [98]. Management is not standardized and different treatments have proved effective [99]. No multicentre randomized treatment trials have been conducted up to now. Some studies report an enhanced pregnancy success rate administering antiphospholipid antibodies to women with recurrent abortion [97]. Different protocols prescribe different combinations of corticosteroids, aspirin and heparin aimed to suppress LAC and normalize coagulation [99]. These combinations include: subcutaneous heparin [100], low-dose aspirin with heparin [101], low-dose aspirin with high-dose prednisolone [102] and low-dose prednisolone with azathioprine [99]. The success rate of pregnancies after treatment is 66.9% [100] compared with 50% in untreated women.

More recently, intravenous immunoglobulin (IVIg) treatment has been proposed in women with recurrent adverse pregnancy outcome caused by high antiphospholipid antibody levels. IVIg therapy appears to inhibit anticoagulant antibodies in pregnant and non-pregnant women by suppressing autoantibody production [98, 103, 104]. IgG are also thought to compete with receptors on the macrophage surface, blocking the Fc receptor [99]. In addition, IVIg administration increases the number of T-s cells [105]. Recently, an idiotype–antiidiotype interaction has been implicated as the mechanism underlying IVIg suppression of anticoagulant activity [104]. Table 7 lists the clinical findings and treatment modalities of the different protocols reported in the literature.

Table 7. Therapeutic approach of recurrent abortion in patients with antiphospholipid antibodies

		Therapeutic regimen				
	No. of patients	Corticosteriods mg/die	Aspirin mg/die	Heparin	Ig mg/kg	Mode of Ig administration
Carreras *et al.* [106]	1	—	—	—	400	At 17, 22 and 27 weeks gestation
Scott *et al.* [107]	1	60	80	—	400	At 8 and 14 weeks gestation
Parke *et al.* [108]	1	—	80	500 U two times a day	600	Monthly infusion from 6 before conception till delivery
Wapner *et al.* [109]	1	—	80	unreported dose	1000	Monthly doses from 9 to 34 weeks gestation
	1	—	80	unreported dose	1000	Monthly doses from 10 to 33 weeks gestation
Katz *et al.* [110]	1	—	80	1000 U three times daily	400	At 24, 28, 29 and 31 weeks gestation
Lubbe *et al.* [111]	6	40	75	—	—	
Brunch *et al.* [102]	20	60	81	—	—	

Modified from Orvieto *et al.* [99].

Patients without autoimmune disease and recurrent abortion

There is no consensus on how these women should be treated and controlled studies are lacking.

Therapy with paternal [11] or donor [56] peripheral blood mononuclear cells has been undertaken for some time. This immunotherapy is based on the idea of TLX cross-reactivity and induction of blocking factors (Fig. 5) although the exact mechanism of action remains a mystery [4, 69]. Some consider this treatment the equivalent of kidney transplant candidates receiving donor blood transfusions which enhance graft success, probably by stimulating the production of antiidiotypic antibodies [112].

Other immunotherapy studies claim that administration of paternal lymphocytes triggers production of P4, a serum inhibitor of maternal cellular cytotoxicity reaction in women with normal pregnancies but not in IMA [113, 114]. Immunization with paternal or donor lymphocytes can lead to a maternal immune reaction which enhances pregnancy outcome by stimulating production of blocking antibodies [69].

The potential side-effects of this therapy (A B O isoimmunization, risk of blood infections, etc.) should only come into play in prolonged therapies, but adequate data are lacking [69]. Administration can be subcutaneous, intradermal or intravenous. Isolated mononuclear cells [89] from paternal or donor blood (approx. 80–85 million cells) are suspended in 4cc of saline solution (0.9%) and subsequently injected. Immunization is repeated 4 weeks later [89].

Success of lymphomonocyte immunotherapy has been gauged in terms of enhanced pregnancy outcome after treatment. The literature reports percentages of successful outcome around 75–80% compared with 32% in untreated (control) women [115–119].

Authors suggest different indications and different groups of women eligible for immunotherapy:

—only women with primary abortion (with no children, all previous pregnancies having resulted in abortion) [120];
—women with primary or secondary abortion (the latter with one or more children before subsequent recurrent abortions) [119];
—all women with IMA with not more than one child and lacking antipaternal cytotoxic antibodies (APCA-negative) [115];
—women with primary and secondary abortion with an impaired *in vitro* response to paternal lymphocyte stimulation [56].

Patients with the highest pregnancy success rate are women with primary IMA (69% pregnancies with healthy children, against 56% with secondary IMA and 32% control women) and APCA-negative prior to therapy, becoming positive after immunization. 75% of women who developed APCA antibodies after lymphomonocyte immunotherapy had successful pregnancies, whereas only 40% of those who remained APCA-negative had a successful pregnancy outcome [89].

Determination of maternal mixed lymphocyte reactivity (MLR) to paternal antigens did not prove a significant selection criterion for patients since only slight differences in pregnancy outcome emerged between MLR-hyporeactive and -normoreactive women [89].

In conclusion, cellular immunotherapy has long-term effects of subsequent pregnancies and is a potentially effective treatment for most women with primary IMA [89].

There are currently no statistically valid data on IVIg immunotherapy in women without antiphospholipid antibodies or other autoimmune disorders. Again, the approach in these cases is based on the mechanism of blocking factors and the administration modalities are the same as those listed for patients with antiphospholipid syndrome [99] (Table 7).

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REFERENCES

1. Hill J. A. and Anderson D. J.: Immunological mechanisms in recurrent spontaneous abortion. *Archsm Immun. Ther. Exp.* 38 (1990) 111–119.
2. Wilcox A. J., Weimberg C. R. and Nisula B. C.: Incidence of early loss of pregnancy. *New Engl. J. Med.* 319 (1991) 189–194.
3. Edmonds D. K., Lindsay K. S., Miller J. F., Williamson E. and Wood P. J.: Early embryonic mortality in women. *Fert. Steril.* 38 (1982) 447–453.
4. Redman C. W. G.: Are there immunologically mediated abortions? If so, which mechanisms? Does immune abortion exist? *30th Forum Immun.* (1991) 169–175.
5. Stray-Pedersen B. and Stray-Pedersen S.: Etiologic factors and subsequent reproductive performance in 195 couples with a prior history of habitual abortion. *Am. J. Obstet. Gynec.* 148 (1984) 140–146.
6. Harger J. H., Archer D. F., Marchese S. G., Murracca-Clemens M. and Garver K. L.: Etiology of recurrent pregnancy losses and outcome of subsequent pregnancies. *Obstet. Gynec.* 62 (1983) 574–581.
7. Tho P. T., Byrd J. R. and McDonough P. G.: Etiologies and subsequent reproductive performance of 100 couples with recurrent abortion. *Fert. Steril.* 32 (1979) 389.
8. Webb P. D. and Glasser S. R.: Implantation. In *Human In Vitro Fertilization and Embryo Transfer* (Edited by D. P. Quigley). Plenum Press, New York, (1989) p. 341.
9. Faulk W. P. and McIntyre J. A.: Trophoblast survival. *Transplantation* 32 (1981) 1–5.
10. Harris E. N., Asherson R. A. and Hughes G. R. V.: Antiphospholid antibodies-autoantibodies with a difference. *A. Rev. Med.* 39 (1988) 261–271.
11. Mowbray J. F., Gibbings C., Liddel H. H., Reginald P. W., Underwood J. L. and Beard R. W.: Controlled trial of treatment of recurrent spontaneous abortion by immunization with paternal cells. *Lancet* I (1985) 941–943.
12. Junco D. J.: Association of autoimmune condition with recurrent intrauterine death. *Clin. Obstet. Gynec.* 29 (1986) 959–968.
13. Parazzini F., Acaia B., Ricciardiello O., Fedele L., Liati P. and Candiani G. B.: Short-term prognosis when no cause can be found for recurrent miscarriage. *Br. J. Obstet. Gynec.* 95 (1988) 654–658.

14. Beer A. E., Quebbeman J. F., Ayers J. W. I. and Haines R. F.: Major histocompatibility complex antigens maternal and paternal immune responses and chronic habitual abortions in humans. *Am. J. Obstet. Gynec.* **141** (1981) 987–999.
15. Bulmer I. N., Longfellow M. and Ritson A.: Leukocytes and resident blood cells in endometrium. In *The Primate Endometrium* (Edited by E. Gurpide and C. Bulletti). *Ann. N.Y. Acad. Sci.* New York (1991) pp. 57–68.
16. Johnson M. H.: Antigens of the peri-implantation trophoblast. In *Immunobiology of Trophoblast* (Edited by R. G. Edwards, C. W. S. Howe and M. H. Johnson). Cambridge University Press, Cambridge (1974) p. 87.
17. Howe C.: Lymphocyte physiology during pregnancy: *In vivo* and in vitro studies. In *Immunobiology of Trophoblast* (Edited by R. G. Edwards, C. W. Howe, and M. H. Johnson). Cambridge University Press, Cambridge (1974) p. 87.
18. Voisin G. A.: Immunologie de la reproduction: relation materno-foetale (implications fondamentales et perspectives d'application). In *Colloques INSERM* (Edited by G. Chaouat) INSERM Pubbl, Paris (1987) p. 3.
19. Robertson M.: Tolerance, restriction and the MIS enigma. *Nature* **332** (1988) 18–19.
20. Gill T. J. III.: Immunogenetics of spontaneous abortions. *Humans Tranpl.* 35 (1983) 1–6.
21. Coulam C. B., Moore S. B. and O'Fallon W. M.: Association between major histocompatibility antigens and reproduction performance. *Am. J. Reprod. Immun. Microbiol.* **14** (1987) 54–58.
22. Taylor C. and Faulk W. P.: Prevention of recurrent abortion with leukocyte transfusions. *Lancet* **11** (1981) 68–70.
23. Mowbray J. F.: Immunology of early pregnancy. *Am. J. Reprod. Immun. Microbiol.* 3 (1988) 79–82.
24. Flamigni C., Bulletti C., Polli V., Ciotti P. M., Prefetto R. A., Galassi A. and Di Cosmo E.: Factors regulating interaction between Trophoblast and human endometrium. In *The Primate Endometrium* (Edited by E. Gurpide and C. Bulletti). *Ann. N.Y. Acad. Sci.* New York (1991) pp. 176–190.
25. Psychoyos A. and Marted D.: Embryo-endometrial interaction at implantation. In *Implantation of the Human Embryo* (Edited by R. G. Edwards, J. M. Purdy, and Steptoe P. C.). Academic Press, London (1985) p. 195.
26. Kennedy T. G.: Embryonic signals and the initiation of blastocyst/implantation. *Aust. J. Biol. Sci.* **36** (1983) 531–543.
27. Casper R. F., Wilson E., Collins J. A., Brown S. E. and Parker J. A.: Enhancement of human implantation by exogenous chorionic gonadotropin. *Lancet* **11** (1983) 1191–1194.
28. Harbour D. V. and Blalock J. E.: Lymphocytes and lymphocytic hormones in pregnancy. *PNEI* 2 (1989) 55–59.
29. Fenderson B. A., Hahnel A. C. and Eddy E. M.: Immunohistochemical localization of two monoclonal antibody-defined carbohydrate antigens during early murine embryogenesis. *Dev. Biol.* **100** (1983) 318–327.
30. Vernon R. B., Linnemeyer P. A. and Hamilton Ms.: A monoclonal antibody, MA 21, recognizes a surface component that is present on F9 teratocarcinoma cells and that appears vectorially on the trophectoderm of peri-implantantion-stage mouse blastocytes. *J. Reprod. Immun.* 15 (1989) 1–20.
31. Elcock J. M. and Searle R. F.: Antigen-presenting capacity of mouse decidual tissue and placenta. *Am. J. Reprod. Immun. Microbiol.* 7 (1985) 99–103.
32. Chaouat G., Wegmann T. G. and Kolb G. P.: The murine placenta as an immunological barrier between the mother and the foetus. In *Immunological Review* (Edited by G. Mullar). Munksgard, Copenhagen (1983) p. 74.
33. Clark D. A. and McDermot M.: Active suppression of host vs graft reaction in pregnant mice III. Developmental kinetics, properties and mechanisms of induction of suppressor cells during first pregnancy. *J. Immun.* 127 (1984) 1276–1280.
34. Stites D. P. and Siiteri P. K.: Steroids as immunosuppressants in pregnancy. *Immunology* 75 117–138.
35. Dallembach-Hellweg G.: The normal histology of the endometrium. In *Histopathology of the Endometrium* (Edited by Dallembach-Helweg). Springer-Verlag. Berlin (1987) pp. 25–92.
36. Sen D. K. and Fox H.: The lymphoid tissue of the endometrium. *Gynecologia* **163** (1967) 371–378.
37. Clark D. A., Chaput A., Walken C. and Rosenthal K. L.: Active suppression of host vs reaction in pregnant mice: VI. Soluble suppressor activity obtained from decidua of allopregnant mice blocks the response to IL-2. *J. Immun.* **134** (1985) 1659.
38. Van-Vlasselaer P. and Vandeputte M.: Immunosuppressive properties of murine trophoblast. *Cell. Immun.* 83 (1984) 422–432.
39. Daya S. and Clark D. A.: Production of immunosuppressive factor(s) by preimplantation human embryos. *Am. J. Reprod. Immun. Microbiol.* 11 (1986) 98–101.
40. Tabibzadeh S. S., Gerber M. A. and Satyaswaroop P. G.: Induction of HLA-DR antigen expression in human endometrial epithelial cells *in vitro* by recombinant γ-interferon. *Am. J. Path.* 125 (1986) 90–96.
41. Mowbray F.: Immunological factors in human abortion. Does immune abortion exist? *30th Forum Immun.* (1991) 207–211.
42. Tabibzadeh S.: Human endometrium: An active site of cytokine production and action. *Endocrine Rev.* 12 (1991) 272–286.
43. Clark D. A., Flanders K. C., Banwatt D., Millarr-Book W., Manuel J., Stedronska-Clark J. and Rowley B.: Murine pregnancy decidua produces a unique immunosuppressive molecule related to transforming growth factor β-2. *J. Immun.* 144 (1990) 3008–3014.
44. Tamada H., McMaster M. T., Flanders K. C., Andrew G. K. and Dey S. K.: Cell type-specific expression of transforming growth factor β-1 in the mouse uterus during preimplantation period. *Molec. Endocr.* 4 (1990) 965–972.
45. Altman D. J., Schnjeder S. L., Thompson D. A., Cheng H. L. and Tomasi T. B.: A Transforming Growth Factor β2 (TGF β2)-like immunosuppressive factor in amniotic fluid and localization of TGF β2 in the pregnant uterus. *J. Exp. Med.* 172 (1990) 1391–1401.
46. Parhar R. S., Kennedy T. G. and Lala P. K.: *Cell Immun.* **116** (1988) 392–410.
47. Pollard J. V. P., Bartocci A., Arceci R. J., Orlofsky A., Lander M. B. and Stanley E. R.: Apparent role of the macrophage factor, CSF-1 in placental development. *Nature* **330** (1987) 484–486.
48. Wegmann T. G., Athanassakis J., Guilbert L., Branch D., Dy M., Menu E. and Chaouvat G.: The role of M-CSF and GH-CSF in foresting placental growth, fetal growth and fetal survival. *Transpl. Proc.* 21 (1989) 566–568.
49. Wegmann T. G.: Does immunoabortion exist? *30th Forum Immun.* (1991) 185–188.
50. Fizet D., Bouzgarrou and Veron G.: Lymphocyte immunization generates immunosuppressive factors in women with recurrent abortions. *Gynec. Obstet. Invest.* 30 (1990) 8–11.
51. Toder V. and Strassiburger.: "Non specific Immunopotentiation and pregnancy loss" Does Immune abortion exist? *30th Forum Immun.* (1991) 181–184.
52. Baines M. G. and Grendon R. L.: Are both endogenous and exogenous factors involved in spontaneous fetal abortion? Does Immune abortion exist? *30th Forum Immun.* (1991) 154–159.
53. Ringler G. E., Contifaris C., Strauss III J. F., Allen J. I. and Geir M.: Accumulation of Colony-Stimulating factor 1 in amniotic fluid during human pregnancy. *Am. J. Obstet. Gynec.* **160** (1989) 655–656.
54. Zolti M., Zion B-R., Meiron R., Shemesh M., Bider D., Mashiach S. and Apte R. N.: Cytokine involvement in oocytes and early embryos. *Fert. Steril.* **56** (1991) 265–272.
55. Warner C. M. and Spannus D. J.: Demonstration of H-2 antigens on preimplantation mouse embryos using conventional antisera and monoclonal antibody. *J. Exp. Zoo.* 230 (1984) 37–52.
56. Beer A. E., Quebbeman J. F., Ayers J. W. I. and Haines R. F.: Major histocompatibility complex antigens maternal and paternal immune responses and chronic habitual abortions in humans. *Am. J. Obstet. Gynec.* 141 (1981) 987–999.
57. Croy B. A., Crepan M., Yamashiro S. and Clark D. A.: Further studies on the transfer of Mus Caroll embryos to immunodeficient Mus Musculus. In *Reproductive Immunology: Materno-Foetal Relationship* (Edited by G. Chaouat). Colloque INSERM PARIS, 154 (1987) p. 101.
58. Billington W. D.: The maternal recognition of the trophoblast. In *Reproductive Immunology* (Edited by D. A. Clarck and A. Croy) Elsever Science, Amsterdam (1986) p. 40.

59. Athanassakis I., Bleackley R. C., Paetkan V., Guilbert L., Barr P. J. and Wegmann T. G.: The immunostimulatory effect of T cells and T cells lymphokines on murine fetally derived placental cells. *J. Immun.* **138** (1987) 37–44.
60. Muller R., Tremblay J. M., Adamson E. D. and Verna I. M.: Tissue and cell type specific expression of two human c-one genes. *Nature* **304** (1983) 454–456.
61. Tsutsumi O. and Oka T.: Epidermal growth factor deficiency during pregnancy causes abortion in mice. *Am. J. Obstet. Gynec.* **156** (1987) 241–244.
62. Clark D. A., Croy B. A., Wegmann T. G. and Chaaouat G.: Immunological and para-immunological mechanisms in spontaneous abortion: recent insights and future directions. *J. Reprod. Immun.* 12 (1987) 1–12.
63. Ebaugh M. S. and Smith E. M.: Characterization of human lymphocyte immunoreactive luteinizing hormone. In *Federation of American Societies of Experimental Biology Proceedings.* New Orleans, LA (1989) Abstr. 1474.
64. Kirsch T. M., Vogel R. L. and Elickinger G. L.: Macrophages: a source of luteotropic cybernins. *Endocrinology* **113,** (1989) 1910–1915.
65. Fucks T., Hammarstrom L., Smith C. I. and Brundin J.: Sex dependent induction of human suppressor T cells by chorionic gonadotropin. *J. Reprod. Immun.* 4, (1982) 185–190.
66. Dickman W. J. and Cauchi M. N.: Lymphocyte induced stimulation of human chorionic gonadotropin production by trophoblastic cells *in vitro. Nature* 271 (1978) 377–378.
67. Hill J. A., Haimovici F., Schiff I. and Anderson D. J.: Trophoblast antigens induce production of toxic factors by leukocytes from a subgroup of women with unexplained recurrent abortion (RA): further evidence for a cellular mechanism in spontaneous abortion. In *Society for Gynecologic Investigation.* Atlanta, GA (1987) Abstr. 30.
68. Scott J. R., Rote N. S. and Brauch D. W.: Immunologic aspects of recurrent abortion and fetal death. *Obstet. Gynec.* 70 (1987) 645–656.
69. Dudley D. J. and Branch D. W.: New approaches to recurrent pregnancy loss. *Clin. Obstet. Gynec.* 32 (1989) 520–532.
70. Mowbray J. F. and Underwood J. L.: Immunology of abortion. *Clin. Exp. Immun.* **60** (1985) 1–7.
71. McIntyre J. A., McConnachie P. R., Taylor C. G. and Faulk W. P.: Clinical, immunologic, and genetic definitions of primary and secondary recurrent spontaneous abortions. *Fert. Steril.* 42 (1984) 849–855.
72. Rocklin R. E., Kitzmiller J. L., Caepenter C. B., Garvoy M. R. and David J. R.: Maternal-fetal relation: absence of an immunologic blocking factor from the serum of women with chronic abortions. *New Engl. J. Med.* 22 (1976) 1209–1213.
73. Suciu-Foca N., Reed F., Rohowsky C., Kung P. and King D. W.: Antiidiotypic antibodies to Anti-HLA receptors induced by pregnancy. *Proc. Natn. Acad. Sci. U.S.A.* **80** (1983) 830–834.
74. Holland N. and Holland P.: Immunological maturation in an infant of a gammaglobulinemic mother. *Lancet* 2 (1966) 1152–1155.
75. Harger J. H., Rabin B. S. and Marchese S. G.: The prognostic value of antinuclear antibodies in women with recurrent pregnancy losses: a prospective controlled study. *Obstet. Gynec.* 73 (1989) 419–424.
76. Xu L., Chang V., Murphy A., Rock J. A., Damewood M., Schlaff W. and Zacur H. A.: Antinuclear antibodies in sera of patients with recurrent pregnancy wastage. *Am. J. Obstet. Gynec.* **163** (1990) 1493–1497.
77. Gleicher N., El Rolly A., Confino E. and Friberg J.: Reproductive failure because of autoantibodies: unexplained infertility and pregnancy wastage. *Am. J. Obstet. Gynec.* **160** (1989) 1375–1385.
78. Maier D. B. and Parke A.: Subclinical autoimmunity in recurrent aborters. *Fert. Steril.* **51** (1989) 280–285.
79. Unander A. M., Norberg R., Hahn L. and Arfors L.: Anticardiolipin antibodies and complement in ninety-nine women with habitual abortion. *Am. J. Obstet. Gynec.* **156** (1987) 114–119.
80. Cowchock S., Dehoratius R. D., Wapner R. J. and Jackson L. G.: Subclinical autoimmune disease and unexplained abortion. *Am. J. Obstet. Gynec.* **150** (1985) 367–375.
81. Cowchock S., Smith J. B. and Gocial B.: Antibodies to phospholipids and nuclear antigens in patients with repeated abortions. *Am. J. Obstet. Gynec.* 155 (1986) 1002–1010.
82. Edelman P. H., Rouquette A. M., Verdy E., Elias A., Cabane J., Cornet D., Barrat J., Chavinie J., Salat-Barous J. and Sureau C. L.: Autoimmunity, fetal losses lupus anticoagulant: beginning of systemic lupus erythematosus or new autoimmune entity with gynaeco-obstetrical expression? *Hum. Reprod.* 5 (1986) 295–297.
83. Hanly J. G., Gladmann D. D., Roseth Laskin C. A. and Urowtz M. B.: Lupus pregnancy: a prospective study of placental changes *Arthritis Rheum.* **31** (1988) 358–366.
84. Glueck H. I., Kant K. S., Weiss M. A., Pollack V. E., Miller M. A. and Coots M.: Thrombosis in systemic lupus erythematosus: relation to the presence of circulating anticoagulants. *Archs Int. Med.* **145** (1985) 1389–1395.
85. Pattison N. S., Mckay E. J., Liggins G. C. and Lubbe W. F.: Anticardiolipin antibodies: their presence as a marker for lupus anticoagulant in pregnancy. *N.Z. Med. J.* **100** (1987) 61–64.
86. Carey S. W. and Klein N. W.: Autoantibodies to laminin and other basement membrane proteins in sera from monkeys with histories of reproductive failure identified by cultures of whole rat embryos. *Fert. Steril.* **51** (1989) 711–718.
87. Szekeres-Bartho-J.: "On the immunology of spontaneous abortion": Letters to the Editor. *Lancet* 1 (1988) 261.
88. Redmann C. W. G.: "Are there Immunologically Mediated Abortions? If so, which mechanisms?" Does Immune Abortion exist? *30th Forum Immun.* (1991) 169–175.
89. Carp H. J. A., Toder V., Gazit E., Orgad S., Mashiach S., Serr D. M. and Nebel L.: Selection of patients with habitual abortion for paternal leukocyte immunization. *Archs Gynec. Obstet.* 248 (1990) 93–101.
90. Gleicher N. and El-Roeiy A.: The reproductive autoimmune failure syndrome. *Am. J. Obstet. Gynec.* **159** (1988) 223–227.
91. Creagh M. D., Malia R. G., Cooper S. M., Smith A. R., Duncan S. L. B. and Graves M.: Screening for lupus anticoagulant and anticardiolipin antibodies in women with fetal loss. *J. Clin. Path.* **44** (1991) 45–47.
92. Nilson I. M., Asted B., Hedner U. and Berezin D.: Intrauterine death and circulating anticoagulant antithromboplastin. *Acta Med. Scand.* **197** (1975) 153–159.
93. Howard M. A., Firkin B. G., Healey D. L. and Choong S. C. G.: Lupus Anticoagulant in women with multiple spontaneous miscarriage. *Am. J. Hemat.* 26 (1987) 175–178.
94. Brandt J. T., Tripplett D. A., Musgrave K. and Orr C.: The sensitivity of different coagulation reagents to the presence of lupus anticoagulants. *Archs Path. Lab. Med.* **11** (1987) 120–130.
95. Loizou S., McCrea J. D., Rudge A. C., Reynolds R., Boyle C. C. and Harris E. N.: Measurement of anticardiolipin antibodies by an enzyme-linked immuno-sorbent assay (ELISA): standardization and quantitation of results. *Clin. Exp. Immun.* **62** (1985) 738–745.
96. Harris E. N.: Syndrome of the black swan. *Br. J. Rheum.* **26** (1987) 324–326.
97. Mittal K. K., Mickey J. F., Singall D. P. and Terasaki P. I.: Serotyping transplantation, XVIII. Refinement of microdroplet cytotoxicity test. *Transplantation* **6** (1968) 913–927.
98. Brown H. L.: Antiphospholipid antibodies and recurrent pregnancy loss. *Clinical Obstet. Gynec.* **34** (1991) 17–26.
99. Orvieto R., Achiron A., Beu-Rafael Z. and Achiron R.: Intravenous immunoglobulin treatment for recurrent abortions caused by antiphospholipid antibodies. *Fert. Steril.* **56** (1991) 1013–1020.
100. Reece, Gabrielli S., Cullen M. T., Zheng X. Z., Hobbins J. C. and Harris E. N.: Recurrent adverse pregnancy outcome and antiphospholipid antibodies. *Am. J. Obstet. Gynec.* **163** (1990) 162–169.
101. Elder M. G., De Swiet M. and Robertson A.: Low-dose aspirin in pregnancy. *Lancet* 1 (1988) 410–414.
102. Branch D. W., Scott J. R., Kochenour N. K. and Hershgold E.: Obstetrics complications associated with lupus anticoagulant. *New Engl. J. Med.* 313 (1985) 1322–1326.
103. Sacher R. A. and King J. C.: Intravenous gamma-globulin in pregnancy: a review. *Obstet. Gynec. Surv.* **44** (1988) 25–29.
104. Carreras L. O., Perez G. N., Martinuzzo M. E., Malan-Borel I., Malbran A. and Said P. B.: Partial neutralization of a Lupus Anticoagulant by Human Immunoglobulin. *Thrombosis Haemostasis* **64** 1(1990) 32–37.

105. Arsura E. L., Bick A., Brumer N. G. and Grob B.: Effects of repeated doses of intravenous immunoglobulin in myasthenia gravis. *Am. J. Med. Sci.* **295** (1988) 438–443.
106. Carreras L. O., Perez G. N., Vega H. R. and Csavilla F.: Lupus anticoagulant and recurrent fetal loss: successful treatment with gammaglobulin. *Lancet* **2** (1988) 393–394.
107. Scott J. R., Branch D. W., Kochenour N. K. and Ward K.: Intravenous immunoglobulin treatment of pregnant patients with recurrent pregnancy loss caused by antiphospholipid antibodies and Rh immunization. *Am. J. Obstet. Gynec.* **159** (1989) 1055–1060.
108. Parke A., Maier D., Wilson D., Andreoli J. and Ballow M.: Intravenous gamma-globulin, antiphospholipid antibodies and pregnancy. *Ann. Int. Med.* **110** (1989) 495–496.
109. Wapner R. J., Cowchock F. S. and Shapiro S. S.: Successful treatment in two women with antiphospholipid antibodies and refractory pregnancy losses with intravenous immunoglobulin infusions. *Am. J. Obstet. Gynec.* **161** (1989) 1271–1274.
110. Katz V. L., Thorp J. M., Watson W. J., Fowler L. and Heine R. P.: Human immunoglobulin therapy for preeclampsia associated with lupus anticoagulant and anticardiolipin antibody. *Obstet. Gynec.* **76** (1990) 986–988.
111. Lubbe W. F., Palmer S. J., Butler W. S. and Liggins G. C.: Fetal survival after prednisone suppression of natural lupus anticoagulant. *Lancet* **1** (1983) 1361–1363.
112. Takcuchi H., Sakagami K. and Seki Y.: Antiidiotypic antibodies and suppressor cells induced by donor-specific transfusion in potential kidney transplant recipients. *Transpl. Proc.* **17** (1985) 1059–1070.
113. Fized D., Bouzgarron R. and Vezon G.: Lymphocyte Immunization generates immunosuppressive factors in women with recurrent abortions. *Gynec. Obstet. Invest.* **30** (1990) 8–11.
114. Fizet D. and Bousquet J.: Characterization of P4, a natural inhibitor of cellular cytotoxicity reaction in pregnant women. *Gynec. Obstet. Invest.* **20** (1985) 179–185.
115. Mowbray J. F., Gibbins C. R., Underwood J., Liddel H. and Beard R.: Controlled trial of treatment of recurrent spontaneous abortions by immunization with paternal cells. *Lancet* **I** (1985) 941–943.
116. McIntyre J. A., Faulk W. P., Nichols-Johnson V. R. and Taylord C. G.: Immunologic testing and immunotherapy in recurrent spontaneous abortion. *Obstet. Gynec.* **67** (1986) 169–175.
117. Mowbray J. F., Underwood J. L., Michel M., Forbes P. B. and Beard R. W.: Immunization with paternal lymphocytes in women with recurrent miscarriage. *Lancet* **II** (1987) 679–680.
118. Takakuwa K., Kanazawak K. and Takeuchi S.: Production of blocking antibodies by vaccination with husband lymphocytes in unexplained recurrent aborters. The role in successful pregnancy. *Am. J. Reprod.* **10** (1986) 1–19.
119. Unander A. M. and Lindholm A.: Transfusions of leukocyte rich erythrocyte "concentrates". A successful treatment in selected cases of habitual abortion. *Am. J. Obstet. Gynec.* **154** (1986) 516–520.
120. McIntyre J. A., McConnachie P. R., Taylor C. G. and Faulk W. P.: Clinical immunological and genetic definitions of primary and secondary recurrent spontaneous abortions. *Fert. Steril.* **62** (1984) 849–855.