Is intravenous immunoglobulins (IVIG) efficacious in early pregnancy failure? A critical review and meta-analysis for patients who fail in vitro fertilization and embryo transfer (IVF)

David A. Clark,^{1,4} Carolyn B. Coulam,² and Raphael B. Stricker³

Submitted June 2, 2005; accepted October 24, 2005; published online January 19, 2006

Problem: Intravenous Immunoglobulins (IVIG) are widely used off label in the treatment of early reproductive failure. As IVIG is expensive, and may have side-effects, evidence of efficacy is needed. Previous results have suggested that the pre-conception treatment of primary recurrent abortion patients might be effective, but the data set has been too small for adequate statistical power. More recently it has been suggested that IVIG may improve the success rate of in vitro fertilization and embryo transfer (IVF) in patients with prior IVF failures, but clinical trials have given conflicting results that need explanation. Systematic reviews generating inconclusive results have focused on methodological rigor to the exclusion of biological plausibility.

Methods: Review of current basic science of design, measurement, and evaluation of clinical trials and basic science mechanisms providing a rationale for treatment. Meta-analysis of published randomized controlled and cohort-controlled trials (updated with two unpublished data sets) evaluating IVIG treatment in IVF failure patients. Live birth rate was used as the most relevant endpoint. The ability of different sources of IVIG to suppress natural killer (NK) cell activity was determined using a standard ⁵¹Cr-release assay in vitro.

Results and conclusions: Meta-analysis of three published randomized controlled trials (RCTs) of IVIG in IVF failure patients shows a significant increase in the live birth rate per woman ($p = 0.012$; Number Needed to Treat for 1 additional live birth, NNT = 6.0 women). Using live birth rate per embryo transferred, and adding data from two cohort-controlled trials to the meta-analysis further supports this conclusion (overall $p = 0.000015$, NNT = 3.7 women). Relevant variables appear to include properties and scheduling of the IVIG, and selection of patients with abnormal immune test results. Different IVIG preparations vary significantly in their ability to suppress NK activity in vitro. A rationale for use of IVIG is provided by a review of mechanisms of IVIG action and mechanisms underlying failure of chromosomally normal embryos.

KEY WORDS: Evidence-based medicine; infertility; IVIG; meta-analysis

INTRODUCTION

Parenteral use of human immunoglobulin was first reported as a treatment for measles in 1935, and intramuscular injection of immunoglobulin in immunodeficiency disorders such as agammaglobulinemia was reported in 1952 (1). Intravenous immunoglobulin administration became feasible

¹ Department of Medicine, McMaster University, 1200 Main St. West Rm 3V39, Hamilton, Ontario, Canada L8N 3Z5.

² Millenova Laboratories, 233 East Erie St., Chicago, Illinois 60611.

³ California Pacific Medical Center, 450 Sutter Street, Suite 1504, San Francisco, California 94108.

⁴ To whom correspondence should be addressed; e-mail: clarkd@mcmaster.ca.

with improved purification methods that removed side-effect-producing aggregates, and IVIG was first licenced for sale in the US in 1981 (Bayer) (1). The benefits of IVIG in replacement therapy and in passive immunotherapy of infectious conditions is not difficult to understand. Fortuitously, IVIG was also found to ameliorate ITP (autoimmune thrombocytopenic purpura), and to be beneficial in a number of autoimmune and inflammatory conditions, including relapsing inflammatory polyneuropathy, Guillian-Barre syndrome and Kawasaki disease where coronary artery inflammation can lead to aneurysms and vessel rupture (1).

Recurrent miscarriage and peri-implantation embryo failure in patients undergoing in vitro fertilization and embryo transfer (IVF) have been proposed to an excess of proinflammatory Th1-type cytokines relative to Th2/3 cytokines, and IVIG has been proposed as a treatment (2). A number of randomized controlled trials (RCTs) and cohort-controlled trials have been done in patients with recurrent miscarriage; the RCT data has been subject to meta-analysis and this has been reported elsewhere (3). The use of IVIG in patients undergoing IVF for infertility and/or early pregnancy failure has been tested in several RCTs. The most recent trial by Stephenson and Fluker, which was reported as negative, has created significant controversy (4-7). Stephenson and Fluker noted previous positive reports by Kleinstein *et al.* in an abstract, and de Placido *et al.* in a formal paper (8,9). We were also aware of an RCT done by Sher *et al.* and two unpublished cohort-controlled trials the details of which had not been published (10-12). In this paper, we focus on the question whether there is controlled trial data to support a hypothesis that IVIG can improve the likelihood of a live birth in patients undergoing IVF?

MATERIALS AND METHODS

Randomized trials were known from previous publications and a Medline search disclosed no additional trials. Therefore, we were not undertaking a systematic review. Two cohort-controlled trials were known, one from an abstract and presentation, and a second, from a review article that pooled IVIG in IVF failure patients with a group of previously reported recurrent miscarriage patients (11,12); in neither had the actual data been published and the unpublished details were provided by the authors for this analysis. In the Coulam trial, IVIG was standard

of care and IRB approval was not required; in the Stricker trial, IRB approval had been obtained for the study of IVIG in pregnancy failure patients.

Meta-analysis was done using a standard program for the Peto method as previously described (3,13). An estimate of heterogeneity by χ^2 was calculated to determine if different studies could be combined, and a pooled estimate of the odds ratio, 95% confidence intervals, and two-tailed *p*-value was generated.

The IVIG preparations Gammagard (Baxter, USA) or Gamimune (Bayer, Canada) were tested for suppression of lysis of ^{51}Cr -labeled K562 cells by a 50:1 effector:target ratio of lymphocyte-purified human PBL in vitro as previously described (14). The mean percent ^{51}Cr release and 1 SEM was determined using sets of six replicate test wells with different concentrations of IVIG, and cytolytic activity was converted to lytic units 20% (LU20) using the standard formula. Percent suppression by IVIG was calculated. The significance of differences was determined using Student's *t*-test.

RESULTS

Table 1 summarizes the results from three RCTs of IVIG in IVF failure/early pregnancy loss patients. The trial by de Placido *et al.* achieved a one-tailed *p* < 0.05 in the original publication as did the trial by Sher *et al.* rejecting the null hypothesis that IVIG did not increase the rate of pregnancy success (9,10). The Stephenson and Fluker trial did not, although one can see that the live birth rate was slightly higher in the IVIG-treated patients. To take all of the evidence into consideration, a meta-analysis was performed to assess the ability of IVIG to increase the probability of a woman taking home one or more babies (live birth rate). Figure 1(A) shows that the pooled odds ratio and 95% confidence interval favored IVIG, and the two-tailed *p*-value (2*P*) was 0.012. There was no significant heterogeneity among the studies, hence pooling was permissible. The success rate for live birth in the control group was 19.3%, the success rate in the IVIG group was 36.0%, and the number needed to treat (NNT) for one additional live birth in the RCTs was 6.0 women. The abortion rate for those achieving clinical pregnancies (Table 1) was also reduced significantly (from 38.5 to 15.8%), but so also the likelihood of achieving a clinical pregnancy increased (from 31.3 to 42.6%). The important statistic was not the pregnancy rate or the abortion

Table I. Randomized Controlled Trials of IVIG in IVF Failure and Occult Loss Patients

Author	Group	No. of patients	IVIG given		Abnormal immunity	No. of embryos transferred	Clinical pregnancy	Number of live births [abortions]
			Pre ^a	Post				
De Placido	IVIG	18	Yes	Yes	nd	45	6	5 ^b [1]
	Placebo	21			nd	61	4(5) ^c	2 ^b [2]
Sher	IVIG + HA	45	Yes	Yes	45 ^d	203	27 ^e	23 [4]
	HA	37			37	144	14 ^e	10 [4]
Stephenson	IVIG	26	Yes	No	14 ^f	77	5	4 [1]
	Placebo	25			15	68	7	3 [4]

^aPre- or post-ovulation.^bCalculated by subtracting number of spontaneous abortions [] from number of clinical pregnancies.^cOne patient had twins.^d100% had anti-thyroid antibodies.^eCalculated by adding number of abortions to number of live births.^fPositive ANA or APL (by ACA or lupus anticoagulant tests).

rate, but live birth rate, which can be improved by a combination of improving implantation, preventing occult losses, and preventing clinical abortions.

Two cohort-controlled trials (Tables II and III) were also evaluated. Here the control group repre-

sented women who did not want to have IVIG, due to the fear of unknown side-effects of a blood product such as IVIG or due to the inability to afford the cost of the IVIG. In the Coulam trial (Table II), the patients were of similar age, the IVIG patients had had

Fig. 1. Meta-analysis of controlled clinical trials of IVIG in patients undergoing IVF and embryo transfer for infertility. (A) Odds ratio (Peto method) and 95% confidence interval for three randomized controlled trials for live birth rate per patient (number of women with 1 or more live births). χ^2 for heterogeneity 0.5905, 2 df, not significant. (B) Odds ratio and 95% confidence limits when data in (A) expressed as live birth rate per IVF embryo transferred and combined with the 2 cohort-controlled trials in Tables II and III. χ^2 for heterogeneity 3.836, 4 df, not significant.

Table II. Effect of Pre-Conceptional IVIG in IVF Failure Patients Positive for Elevated NK Levels $\pm$ Autoantibodies

	IVIG ($N = 95$) ^a	Control ($N = 22$) ^b
Average age	35.1	36.8
Number of IVF failures	8	2
Number of embryos transferred	3.2	2.8
Number of clinical pregnancies	43 (47%)	1 (4%)
Number of multiple pregnancies	20	0
Number ongoing pregnancies	36 (37%)	1 (4%)
One or more live births/number of patients	36/95 (37%)	1/22 (4%)
Number of "take-home babies"/number of embryos transferred	54/304 (18%)	1/62 (2%)
Take-home babies per patient	54/95 (57%)	1/62 (2%)

^aPatients with one or more elevated peripheral blood NK levels $\pm$ abnormal autoantibody test received 0.4 g/kg Gammagard IV prior to oocyte retrieval, and every 4 weeks until term, if pregnant.

^bPatients declined IVIG due to safety or cost concerns.

more prior IVF failures, and the take-home baby rate (LBR) per patient and per IVF embryo transferred was increased. All of the patients had elevated levels of NK cells and some had positive autoantibodies (anti-phospholipid, anti-thyroid, ANA). A similar result from Stricker who selected patients with abnormal immune tests is shown in Table III. Again, the control group patients refused IVIG due to fear or cost considerations. The probability of a live birth per patient was extremely high in this study, most likely due to the increased number of IVF embryos transferred per recipient, and it was not possible to include Table III data with that of Tables I and II in a meta-analysis of number of women with one or more live births. However, it was possible to calculate the probability of a take-home baby per embryo for both the RCTs and two cohort-controlled trials and this corrected for the problem of heterogeneity and allowed us to pool all five studies in a meta-analysis.

Figure 1(B) shows the result. The pooled odds ratio was again statistically significant with respect to a benefit of IVIG. The success rate in the control group was 15.0%, the success rate in the IVIG group was 41.9%, and the NNT for one additional live birth in this set of pooled data was 3.7 women for one additional live birth. A similar analysis of the live birth rate per embryo in the RCTs in Fig. 1(A) also allowed rejection of the null hypothesis that IVIG did not increase the take-home baby rate ($p < 0.05$).

In Fig. 1(B), although the results from the various trials were similar and confidence intervals usually included the means of the other trials, the Stephenson and Fluker trial was the least positive. There were a number of possible explanations for this result apart from the fact that the outcome of trials varies due to mere chance. These will be discussed later. One issue that has not been addressed in the literature concerned properties of the IVIG itself. To

Table III. Effect of Pre-Conceptional IVIG in IVF Failure Patients Positive for Autoantibodies and/or Elevated NK Cell Activity

	IVIG ($N = 44$) ^a	Control ($N = 17$) ^b
Average age	37.5	36.5
Number of IVF failures	4.01	3.18
Number of embryos transferred	5.1	5.2
Number of clinical pregnancies	31 (70.5%)	11 (64.7%)
Number of multiple pregnancies	3	1
Number ongoing pregnancies	27 (87.1%)	1 (9.1%)
One or more live births/number of patients	27/31 (87.1%)	1/11 (9.1%)
Number of "take-home babies"/number of embryos transferred	27/157 (18%)	1/57 (2%)
Take-home babies per patient	30/31 (96.7%)	1/11 (9.1%)

^aPatients received 0.2 g/kg Venoglobulin-S or Gamimune-N IVIG prior to oocyte retrieval and every 4 weeks until the third trimester.

^bPatients who refused treatment.

Fig. 2. Suppression of NK cytolytic activity in vitro. The ^{51}Cr release assay as described in Clark and Chaouat was used. GIM: Gamimune, GGD: Gammagard. Data represent mean and 1 SEM for LU20%. Two different in vitro concentrations are shown. *Significantly different at $p < 0.05$ by Student's t -test.

explore this question, we tested the Bayer Gamimune product (used by Stephenson and Fluker) for the ability to suppress NK cytolytic activity in vitro, and compared the activity to the Baxter Gammagard product used by Coulam. It can be seen in Fig. 2 that both IVIG products suppressed NK activity. However, Gamimune was less potent. Based on the titration curve, it appeared one would require eight times more Gamimune to achieve the suppression achieved by Gammagard.

DISCUSSION

The meta-analyses in this paper indicate that IVIG significantly increased the probability of taking home one or more babies by patients undergoing IVF for infertility and/or early pregnancy loss. In evaluating any meta-analysis, it is necessary to carefully consider trials excluded and those included in the analysis. The only excluded trial was that of Kleinstein *et al.* that was published in abstract form only (8); it was not possible to obtain any details of the patients, randomization, or treatment, and the information required for Table I was unavailable. Three other trials had been published. The RCT by de Placido included patients with three or more IVF failure attempts with patients with two or more very early losses (< 8 weeks) or biochemical pregnancies (occult losses). This study provided the fewest details concerning the patients (and their selection/testing),

and the details concerning randomization and blinding was not described. The RCT by Sher *et al.* targeted IVF failure patients who had anti-thyroid antibodies. IVIG was added to heparin + aspirin (HA) in the experimental group. IVIG was not tested alone. The patients in each group were similar. The RCT reported by Stephenson and Fluker documented the most methodological rigor in terms of randomization and blinding. The patients were also described in considerable detail. From the publication, patients with autoantibodies (APA or ANA) did not show a preferential benefit when given IVIG. However, only 3/26 patients in the IVIG group and 12/25 patients in the saline control group had “unexplained” infertility. Problems such as male factor infertility and tubal factor with hydrosalpinges that were included in the trial would not be expected to respond to IVIG treatment. In the Stephenson and Fluker RCT, the overall pregnancy rates in both groups were low compared to nationally published data, suggesting an underlying problem affecting all patients as a confounder might exist, such as inclusion of patients with hydrosalpinges etc. (15). Based on achieved sample size and assuming all patients could benefit from IVIG, the probability of detecting an improvement of 16.7% with IVIG (as shown in Fig. 1 and the calculation earlier) was only 0.47 (probability of missing such a significant effect was 53%). Therefore, the risk of a type-II statistical error was quite high, and therefore, the

study can be said to have been underpowered in its ultimate execution (13). Of course, the achieved sample size in the other RCTs was also not large, but in a series of small trials, it is expected that some will be statistically significant and some not. In the three RCTs, IVIG was given before and after conception; in the Stephenson *et al.* trial, only a single post-conception dose was given only if the patient had achieved pregnancy. The problem of underpowered studies can be remedied by meta-analysis that pools a series of small data sets to achieve a much more powerful sample. The De Placido and Stephenson trials compared IVIG to placebo, and achieved an average combined sample size of 45 per group with a 9.6% improved success rate with IVIG. The probability of a type-II error (i.e., failing to achieve a one-tailed $p < 0.05$) for a 9.6% improvement would be 64%, and using a more rigorous two-tailed $p < 0.05$, the risk of missing a significant effect of 9.6% would have been 78%. Meta-analysis seeks to avoid risks of such unacceptable magnitude by generating a larger sample size that has more power. In the Sher *et al.* trial, all patients received HA, and the control success rate was higher than in the other two RCTs that used placebo. However, in the Sher *et al.* trial, the question was whether IVIG could further improve the take-home baby rate. IVIG and HA are thought to have different mechanisms of action, and the greater improvement in success rate with IVIG compared to the other two RCTs could have reflected a synergistic effect, or selection of patients with autoimmunity (thought more likely to benefit from immunomodulation), or random variation; with correction for number of embryos transferred, the success rates in the cohort-controlled trials were not significantly different from that the Sher *et al.* RCT in Table IA. In meta-analysis of trials of adjuvant chemotherapy in early breast cancer published in The Lancet, Peto *et al.* have shown it quite acceptable, and indeed, necessary, to combine trials where treatments were slightly different (16). Indeed, a significant result when trials that are heterogeneous with respect to treatments are combined, one obtains a more robust and reliable result. Patient heterogeneity is also allowed as an initial step to detect a benefit of treatment, and subsequent trials usually address the question, is there a subset of patients more likely to benefit from treatment? In the pooled result in Fig. 1(A), the magnitude of statistical heterogeneity was acceptable for pooling, and the probability of missing the observed improvement with this size sample was only 26% for a two-tailed

$p < 0.05$ and 15% for a one-tailed $p < 0.05$, which is very close to the value of 20% (power = 80%) used when estimating sample size for a proposed trial.

Two cohort-controlled studies were also considered in our analysis. In theory, RCTs should be more trustworthy, especially when blinded, since randomization is believed to balance unidentifiable variables in the control and treatment groups. A previous prospective international observational study and meta-analysis of allogeneic leukocyte immunotherapy in recurrent miscarriage patients done by the Ethics Committee of the American Society for Reproductive Immunology also considered both RCTs and cohort-controlled trials (17). It was found that randomization did *not* necessarily balance the control and treatment groups for known prognostic factors, and the double-blind RCTs surprisingly showed a *greater* effect of treatment than did unblinded and cohort-controlled studies (18). Recent studies show that cohort-controlled studies yield results that are similar to RCTs as do randomized versus non-randomized studies (19–21). Since it is important to consider all of the available data when assessing a therapy, we chose to look at cohort-controlled studies as well as RCTs. This is acceptable provided one considers the RCTs separately and not solely as a pooled data set. It can be seen from Fig. 1(B), where we corrected for number of transferred IVF embryos that the outcome of cohort-controlled studies showed a greater benefit in the IVIG group, although not statistically different from most of the RCTs. The results in Tables II and III showed a more striking effect of IVIG. Could this reflect bias due to the controls being treatment rejectors? The control patients could have been under greater stress, and stress has been linked to infertility (and recurrent miscarriages) in genetically susceptible individuals. On the other hand, all of the patients in the cohort-controlled trials had been selected for the presence of immunological abnormalities including increased levels of blood NK activity in vitro. Could such patients have been at a higher risk of failure after embryo transfer? It has been controversial whether patients with anti-phospholipid antibodies such as anticardiolipin (ACL) have a worse prognosis with IVF (possibly due to differences in anticardiolipin antibody assays in different centers), but presence of anti-phospholipid antibodies does seem to identify a subgroup more likely to benefit from treatments such as HA and IVIG (21–24). The success rate in the IVIG-treated patients was also higher in the RCT of Sher *et al.* where the patients had been selected

on the basis of anti-thyroid antibodies. In the cohort-controlled studies, elevated NK activity was also used to select patients for treatment. One effect of IVIG is to suppress elevated NK activity. This has been documented in vivo as well as in vitro. The IVIG used in the Stephenson and Fluker trial was provided by Bayer Canada Inc. (4). Figure 2 shows that IVIG preparations cannot be considered equal, and the Bayer Canada preparation was significantly less active than Gammagard that was used for the study in Table II. In the Stephenson and Fluker trial, IVIG was only administered once following establishment of a pregnancy, so a less potent IVIG preparation used in that study may have been inadequate without subsequent administration during pregnancy, thus contributing to the negative result of the study. Unfortunately, one cannot retrieve samples of the different IVIG preparations used in the studies in Fig. 1 and compare them, but the data in Fig. 2 documents a hitherto unappreciated problem that will need to be considered in the design of any future trials. Stricker *et al.* have suggested that patients who receive continuing treatment during only the first trimester may have a higher risk of subsequent loss than those who continue through to the end of the second trimester, but the data are limited (12). Anti-phospholipid antibodies are associated with second trimester losses, and autoantibodies could potentially cause problems up to the time of parturition. Further studies to determine which patients should be treated beyond the first or second trimester need to be done.

It is important to consider whether immunological tests to select patients for IVIG has any value. As noted above, in the Stephenson and Fluker trial, patients with a positive ANA and/or ACL did not show a striking benefit with IVIG (4). De Placido *et al.* did not describe immune testing, whereas Sher *et al.* selected patients with anti-thyroid antibodies (9,10). Kutteh *et al.* reported as much improvement in implantation rates in APA-negative IVF failure patients with heparin + ASA treatment as in the APA-positive patients when anticardiolipin was the only test used to detect anti-phospholipid antibodies (25). However, in ACL-negative patients there can be other abnormalities such as anti-thyroid antibodies and increased levels of circulating natural killer (NK) cells, which appear to be relevant in selecting appropriate patients for treatment. The two cohort-controlled trials employed extensive testing beyond ANA and ACL to select patients (e.g., α CL and/or α PI and/or α PG and/or α PS and/or α PG, NK cell activity, and anti-thyroid antibodies).

There has been some recent controversy about the value of blood NK cell activity testing. Elevated levels of circulating NK cells, and production of Th1 cytokines that activate NK cells, have been linked to increased rates of spontaneous abortions and to IVF failure (26–30). Recently Moffet *et al.* have challenged the validity of peripheral blood NK assays arguing that uterine NK cells differ significantly from peripheral blood NK cells, so measuring the latter should be meaningless for events at the fetomaternal interface (31). The fact that novel CD56^{bright}CD16[–] uterine NK-lineage cells are particularly prominent in endometrial and decidual tissue does not logically lead to a conclusion that they must be the most important with respect to the causation of recurrent miscarriages and infertility; the fallacy of equating numerosity with importance in immunological inflammation has been documented elsewhere (32). Aoki *et al.* (28) showed that high peripheral blood NK activity was associated with an increased risk of miscarriage when the woman subsequently married and tried to reproduce, and this result was reproduced by Yamada *et al.* (29). Further, Lachapelle *et al.* showed an increased number of blood-type NK cells in the endometrium of women with recurrent spontaneous abortions (33). Importantly, 50% of the women had increased NK cell levels, which is strikingly similar to the proportion of recurrent miscarriages that are of normal embryonic karyotype (34, 35). Chronic stress (post-traumatic stress) can increase NK activity in certain individuals, and predispose to pregnancy loss (36). NK lineage cells also include a subset with $\gamma\delta$ T cell receptor (TcR); NK $\gamma\delta$ T cells in abortion prone CBAXDBA/2 matings are an important source of abortion-promoting cytokines (37), and NK $\gamma\delta$ T cells may be present in pregnancy decidua and produce cytokines important to pregnancy success (38–40). Szekeres-Bartho *et al.* have shown distinct subsets of TcR $\gamma\delta^+$ cells in peripheral blood produce cytokines that promote or antagonize abortions, in agreement with data obtained in the CBAXDBA/2 model (41). The peripheral blood lymphocytes of 50% of recurrent miscarriage patients produce embryotoxic cytokines in response to trophoblast (42), and also make an aberrant Th1 response to HLA-G-transfected cells (43). Taken together, these data suggest that increased numbers and/or activity of circulating blood-type NK-related cells can translate into an unfavorable environment at the fetomaternal interface.

Moffet *et al.* (31) also argued that assays of the cytotoxic activity of blood NK cells in vitro had no

Table IV. Classification of Outcome of Controlled Trials of IVIG in Reproductive Failure

Outcome	Pre-ovulatory IVIG start		IVIG started post-pregnancy	
	RSA trials	IVF failure trials	RSA trials	IVF failure trials
Positive ^a	Coulam [95] (48) Kiprov [35] (49) Stricker [47] (50)	Sher [82] (10) de Placido [39] (9) Stricker [61] Table III Coulam [107] Table II	None	None
Negative	Stephenson [39] (51)	Stephenson [51] (4)	German Grp [64] (52) Christiansen [34] (53) Christiansen [58] (54) Perino [46] (55) Jablonowska [41] (56)	None

meaning as the function of uterine NK cells in vivo was mediated by the cytokines they produce and not by cytotoxicity. However, there are many types of in vitro assays that do not measure functionally relevant activity in vivo. To determine if a laboratory test result is useful clinically, one must calculate its positive and negative predictive value, such as was done by Aoki *et al.* (28). Given that the in vitro NK cell assay measures the activation status of circulating NK lineage cells (and not merely their number), the presence of high levels of circulating NK activity could be significant in a woman with recurrent pregnancy failure where HLA-G at the feto-maternal interface is unable to exert a down-regulating effect. Although the positive predictive value of high blood NK activity levels for a positive benefit of IVIG treatment has yet to be tested in controlled clinical trials (Fig. 1 and Tables II and III notwithstanding), IVIG does down-regulate NK activity in vivo as well as in vitro, and also can cause a $\downarrow$ Th1/ $\uparrow$ Th2 shift in cytokine production including increased production of GM-CSF that has been linked to improved implantation. (44–49). Moffet *et al.* (31) expressed concern about reproducibility of NK assays, but it may be intermittent peaks of NK activity rather than the steady-state average level that is important. NK levels in blood can vary in a given patient (31), but so can blood pressure, and in the latter situation, peaks above normal can be harmful. What is important is positive and negative predictive value for an effect of IVIG.

Regan *et al.* (47) have also recently published an opinion paper suggesting that immunological testing of reproductive failure patients has no value, but not all of the relevant data was considered. Stricker *et al.* reviewed clinical trials of IVIG in RSA and IVF failure patients, and suggested some significant differences between trials that gave a positive result and those that gave a negative result (12). In this

analysis, some of the trials did not have concomitant controls, and RSA and IVF failure studies were combined. Table IV provides revised and updated data comparing positive and negative randomized or cohort-controlled trials with respect to whether IVIG was given pre-conception or was delayed until after a pregnancy was detected. Studies that used historical controls or the patients as their own control were removed from the original analysis, and the results from the two cohort-controlled trials in this current manuscript have been included. The reason patients with pregnancy failure cannot be used as their own control arises from the fact that the probability of success in the next attempt at pregnancy is not zero. The probability of success in the first three IVF cycles remains constant, like the probability of obtaining heads in repeat tosses of a coin; even if one has three tails (failures) in a row, there is still a 50% chance of a head in the next toss. Therefore, a statistically significant rate of success >0 is expected by chance alone. In Table IV, the sample size for each trial is shown in square brackets. If one considers RSA trials only, four trials gave IVIG before conception three of the four were positive, whereas five trials delayed treatment until pregnancy was established and of these none were successful ($p = 0.0476$, Fisher's exact test). Combining RSA + IVF trials, 7/9 pre-conception IVIG trials were successful, and 0/5 post-conception ($p = 0.042$). Among the positive trials, 4/7 used immune tests to select patients for IVIG treatment, and among negative trials, 0/7 selected patients for treatment using immune testing ($p = 0.035$). (This should not be confused with doing a few autoantibody tests on patients and conducting a *post hoc* subset analysis.) Although the categorical analysis in Table IV is *post hoc*, in order to convince sceptics, a further prospective large multi-center randomized double-blind clinical trial with a complete

panel of immune testing is required; Table IV is validated by two *a priori* hypotheses. First, a treatment to prevent a condition is more likely to work if it is given before that condition occurs, and second, the treatment is more likely to work if the recipients actually have the condition that the treatment corrects. However, administrators of “managed care” can be unreasonably rigid given their underlying mission is to avoid spending money rather than ensuring patients receive potentially beneficial treatments. Regulatory authorities can also become strict if they perceive that a treatment is being advertised and sold for the purpose of treating a condition “off label.” Therefore, for the benefit of patients, and anticipating obstructionism, one may need to be proactive and see that the necessary clinic trial gets done.

A relevant example of controversy created by conflicting (but underpowered) randomized controlled trials of IVIG usage concerns patients with recurrent spontaneous abortions. Anti-phospholipid antibodies, autoimmunity, and activated T and natural killer cells have also been found at an increased frequency in these patients, and from Table I, it is apparent that some of the improvement in live birth rate in IVIG-treated patients was related to a 4.9% reduction in the spontaneous abortion rate; spontaneous abortion occurs at an increased frequency in IVF patients achieving pregnancies (26,27,60,61). It is therefore appropriate to examine relevant literature on IVIG in recurrent spontaneous abortions to determine if such an effect might be restricted to the IVF group or rather, occurred in non-IVF patients with recurrent miscarriages. A meta-analysis of IVIG trials in recurrent miscarriages using the primary patient data from these trials was published in 1999 (3). This analysis suggested that pre-implantation IVIG in primary recurrent miscarriage showed a trend towards IVIG. The data set was insufficiently powerful to generate a $p < 0.05$ for either primary or secondary recurrent miscarriage patients. Since that analysis, the only new data has come from a second trial of IVIG in secondary recurrent miscarriage done by Christiansen (57). Christiansen combined his two data sets and obtained a $p < 0.05$ for a benefit of IVIG (the two trials were counted separately in Table IV). When his new data was added to the previous data where IVIG was started with a positive pregnancy test (post-conception), the two-tailed p -value remained >0.05 . A one-tailed $p < 0.05$ was achieved, so one could reject the hypothesis that IVIG did not improve the success rate, but this was a *post hoc* analysis of data and there was no *a priori*

reason why IVIG should be more effective if started after conception in patients with secondary recurrent miscarriages as distinct from primary recurrent miscarriage patients. It is argued that pre-conception treatment is better in both primary recurrent miscarriage patients and in IVF failure patients. When pre- and post-conception IVIG data in secondary recurrent miscarriage patients were combined, the one-tailed p -value was >0.05 (not significant). If the same difference were present in a larger data set, a p -value < 0.05 could be obtained, and the lack of significance of what data is currently available may reflect lack of power (so concluding there is no difference may represent a type-II statistical error). In other words, while one cannot reject the null hypothesis that any benefit from pre-conceptional IVIG could be due to chance, one cannot accept it either. It has also been argued that a larger treatment effect of IVIG would be obtained if patients were selected who had immunological abnormalities (53). The analysis provided by Table IV suggests that idea may be valid. Firm conclusions would require validation by a further randomized controlled trial that would have to be done on US soil to satisfy regulators.

What might be the ideal for further clinical trials? Obviously, one would stratify patients with 0, 1, 2, 3, ... abnormal test results, and the analysis of each subgroup would be justified by the *a priori* hypothesis, and exclude patients with conditions unlikely to benefit as discussed above. To be ethical, there would have to be equipoise, meaning that none of the investigators or IRB members have any reason to believe IVIG has any protective activity. In light of the data and analysis in this paper, equipoise would be challenged. On the other hand, if a regulatory agency insisted on such a trial, and one believed IVIG could be beneficial, the only way to help the patients would be to do the trial and it would become unethical not to do so. A clinical trial must also be feasible. A trial with a placebo arm is not acceptable to most women, and recruitment to a trial of IVIG in secondary recurrent miscarriage patients in Canada proved difficult; indeed, at least one patient who aborted and found out she had been receiving placebo, and one who did not achieve pregnancy after 6 months and was taken off trial were devastated. It is our thesis that a reasonable comparator would be heparin + aspirin, which represents potentially effective treatment and is inexpensive (10). A viable RCT for IVF failure and/or recurrent miscarriage patients would most likely include the combination as one of its arms and have IVIG + HA, and IVIG alone in the two other arms.

Such a trial would only be credible if all clinical miscarriages were karyotyped, full immunological testing was done, and patients receiving treatment had monitoring of their NK cells and NK cell subsets to determine if the IVIG actually worked in vivo in each patient. In the interim, IVIG is still likely to be used in selected cases where other treatments have failed. At least 1/2 of IVIG use is for unlicensed off label indications where level 1 evidence from double-blind randomized controlled trials is unavailable (due to rarity of the condition or unwillingness of subjects to take placebo) or, where evidence is just emerging in the form of meta-analyses of numerous small and underpowered trials (60). "Off label" use of drugs is common, and both legally and ethically acceptable provided the patient is informed and consents to the treatment.

We did not set out to do a systematic review of IVIG use in IVF failure. Systematic reviews that pool acceptable RCTs in a meta-analysis or logistic regression analysis usually set acceptability criteria based on the reported methodological rigor of each trial. For example, method of randomization, concealment, and blinding, completeness of follow-up, and analysis by intention to treat are deemed important (although we have seen violations of these principles even in purportedly *good* studies) (3,13). Unfortunately, systematic reviews assume that if rigor was not documented in a paper, there was bias; we prefer analysis of the primary patient data (17) with cross-examination of the data submitters before and during a consensus conference. However, perfection is elusive and clinical decision-making must proceed lest one have clinical paralysis for want of RCTs, so in this paper, we have critically analyzed the best available evidence. Systematic reviews also neglect the issue of biological rigor. Did the patients included in the trial actually have a/the condition that IVIG was expected to correct (13)? Was the IVIG given in a manner that would reasonably be expected to correct the underlying pathophysiology (13)? Was there a secondary endpoint that would indicate the IVIG was altering the patient's underlying physiology as expected (13)? Did all of the patients actually have the lab testing done that was reported in a published report of the trial? In an antibiotic trial, for example, one would need proof that the patient was infected with an organism sensitive to the antibiotic, achieved adequate blood levels, and had no infected artificial implants where antibiotic susceptibility is reduced. In a sufficiently large randomized trial, the noise will be diluted out and an effect will be seen, albeit less

than in appropriately selected patients; the problem is achieving the necessary sample size, which may exceed 20,000.

Is there any mechanistic rationale for the use of IVIG in early pregnancy failure? The first consideration concerns what causes failures, and the second concerns how IVIG may act. Pregnancies that are lost prior to implantation present clinically as infertility and those that are lost during the stages of implantation are manifest as chemical pregnancy or occult loss. Just as in the case of abortion or post-implantation pregnancy loss, pre- and peri-implantation losses can be the result of problems within the embryo itself or within the environment in which the embryo implants. Unlike abortions, tissue is not available for analysis in pre- and peri-implantation losses so it is more difficult to determine which pregnancies are lost because of abnormal embryos. Data obtained from studies using pre-implantation genetic diagnosis indicates that over half of fertilized eggs are chromosomally abnormal (63,64). It is known that the rate of chemical pregnancy (occult loss) is two to four times that of clinical spontaneous abortion (61). In mice, the mechanisms underlying occult loss differ from those of spontaneous abortion (resorption). The latter, for example, has been linked to unopposed action of up-regulated levels of the fgl2 prothrombinase molecule (63). Human recurrent abortion has been similarly linked to increased fgl2 (63). Occult loss in mice does not require fgl2, and is actually increased in frequency in fgl2 knock-out mice (65). Occult loss has also been attributed to alloantigen-specific maternal T cell rejection of embryos occurring when indoleamine 2,3-dioxygenase (IDO) is blocked (66). Bacterial lipopolysaccharide (LPS) and stress can also cause occult losses (65,67). In the case of LPS, unlike spontaneous abortions, loss does not require the type 1 receptor for TNF- α (68). Stress-induced loss is mouse strain specific, and a similar genetically determined susceptibility most likely applies to humans as stress does not normally lead to loss of unwanted pregnancies (69). However, a common theme in patients with IVF failure/infertility and/or recurrent miscarriages has been increased NK activity, increased Th1/Th2 cytokine ratios, and presence of autoantibodies, and patients undergoing IVF have an increased miscarriage rate which, from Table 1, appears to be countered by IVIG (12,26,27,29,30,44–47,60,61,70). Freshly isolated allogeneic (paternal) leukocytes that can prevent spontaneous abortions in appropriately selected patients do not appear

effective in preventing losses at the occult stage (before 6 weeks gestation) (13), and in a mouse model of stress-triggered loss, paternal leukocytes have proven ineffective (71,72). A significant problem in interpretation of studies of therapy of very early losses has been to distinguish between implantation failure and failure as a chemical pregnancy immediately after implantation. As the mechanisms may be different, the syndrome is heterogenous (61,69), and presence of a group of patients unlikely to respond to treatment makes detection of a significant treatment effect more difficult.

With respect to mechanisms of action of IVIG, Sewell and Jolles (1) have set out four separate mechanistic components of IVIG: (a) Actions mediated by the antigen-recognizing part of IgG; (b) Actions of the opposite end of the molecule on Fc receptors; (c) Actions due to binding of complement by the Fc component of IgG; (d) Immunomodulatory substances other than antibody in the IVIG preparations. Some of the beneficial effects of IVIG in disease states may involve more than one mechanism (73). Recently, the CD200 tolerance signal has been shown to be important for suppression of NK activity (as shown in Fig. 2) by IVIG (14); interestingly, the CD56^{bright} subset of NK cells and NKT cells, which have been viewed as of particular importance at the feto-maternal interface express receptors for CD200 (74). CD200 may be particularly important as the CD200 molecule binds to CD200R2/3 on antigen-presenting cells, which then activate CD4⁺25⁺ Treg cells (75); these Treg cells prevent autoimmune disease, and also prevent spontaneous abortion and infertility in mice (76,77). These data suggest that additional measurements of CD200-dependent IVIG effects should be made in patients in RCTs to determine if the treatment is working. Current controversy would not likely survive the impact of such data.

REFERENCES

1. Sewell WAC, Jolles S: Immunomodulatory action of intravenous immunoglobulins. *Immunology* 2002;107:387–393
2. Daya S, Gunby J, Clark DA: Intravenous immunoglobulins therapy for recurrent spontaneous abortion: A meta-analysis. *Am J Reprod Immunol* 1998;39:69–76
3. Daya S, Gunby J, Porter F, Scott J, Clark DA: Critical analysis of intravenous immunoglobulin therapy for recurrent miscarriage. *Hum Reprod Update* 1999;5:475–482
4. Stephenson MS, Fluker MR: Treatment of repeated unexplained in vitro fertilization failures with intravenous immunoglobulins: A randomized, placebo-controlled Canadian trial. *Fertil Steril* 2000;74:1108–1113
5. McDonough PG: “In silico experiments”—yes, but the great western cowboy “random chance” is still alive. *Fertil Steril* 2001;76:639–640
6. Sher G, Fisch JD: “In silico experiments”—yes, but the great western cowboy “random chance” is still alive. *Fertil Steril* 2001;76:636–637
7. Stricker RB, Steinleitner A: “In silico experiments”—yes but the great western cowboy “random chance” is still alive. *Fertil Steril* 2001;76:637–639
8. Kleinstein J, Khanga O, Gips H, Kunzel W: Intravenous immunoglobulins increase pregnancy rate in an IVF program. In: *Scientific Program and Abstracts of the Society for Gynecological Investigation*, Chicago 1994;248 (Abstr #P108)
9. De Placido G, Zullo F, Mollo A, Cappiello F, Nazzaro A, Colacuri N, Palumbo G: Intravenous immunoglobulins (IVIG) in the prevention of implantation failures. *Ann NY Acad Sci* 1994;734:232–234
10. Sher G, Maassarani G, Zouves C, Feinman M, Sohn S, Matzner W, Chong P, Ching W: The use of combined heparin/aspirin and immunoglobulins G therapy in the treatment of in vitro fertilization patients with antithyroid antibodies. *Am J Reprod Immunol* 1998;39:223–225
11. Coulam CB, Roussev RG: Role of T cell activation and inhibition markers in diagnosing women experiencing immunological implantation failure after in vitro fertilization and embryo transfer. *Am J Reprod Immunol* 2002;48(Abstr # I12):138
12. Stricker RB, Steinleitner A, Winger EE: Intravenous immunoglobulins (IVIG) therapy for immunologic abortion. *Clin Appl Immunol Rev* 2002;2:187–199
13. Clark DA, Coulam CB, Daya S, Chaouat G: Unexplained sporadic and recurrent miscarriage in the new millennium: A critical analysis of immune mechanisms and treatment. *Hum Reprod Update* 2001;7:501–511
14. Clark DA, Chaouat G: Loss of surface CD200 on stored allogeneic leukocytes may impair anti-abortion effect in vivo. *Am J Reprod Immunol* 2005;53:13–20
15. American Society for Reproductive Medicine, Assisted Reproductive Technology Registry: Assisted reproductive technology in the United States: 1999 results generated from the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology Registry. *Fertil Steril* 2002;78:918–931
16. Early Breast Cancer Trialists’ Collaborative Group: Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: An overview of the randomized trials. *Lancet* 2005;365:1687–1717
17. The Recurrent Miscarriage Immunotherapy Trialists Group: Worldwide collaborative observational study and meta-analysis on allogeneic leukocyte immunotherapy for recurrent spontaneous abortion. *Am J Reprod Immunol* 1994;32:55–72
18. Clark DA, Gunby J, Daya S: The use of allogeneic leukocytes of IV IgG for the treatment of patients with recurrent spontaneous abortion. *Transfus Med Rev* 1997;11:85–94
19. Concato J, Shah N, Horowitz RI: Randomized, controlled trials, observational studies, and hierarchy of research designs. *N Engl J Med* 2000;342:1887–1892
20. Benson K, Hartz AJ: A comparison of observational studies and randomized, controlled trials. *N Engl J Med* 2000;342:1878–1886

21. Ioannidis JPA, Haidich AB, Pappa M, Pantazia N, Kokori SI, Tektonidou MG, Contopoulos-Ioannidis DG, Lau J: Comparison of evidence of treatment effects in randomized and nonrandomized studies. *JAMA* 2001;286:821–830
22. Coulam CB, Clark DA: Antiphospholipid antibody status and IVF-debate. *Fertil Steril* 2000;74:848–849
23. Sher G, Feinman M, Zouves C, Kuttner G, Maassarani G, Salem R, Matzner W, Ching W, Chong P: High fecundity rates following in-vitro fertilization and embryo transfer in antiphospholipid seropositive women treated with heparin and aspirin. *Hum Reprod* 1994;9:2278–2283
24. Sher G, Matzner W, Feinman M, Maassarani G, Zouves C, Ching P, Ching W: The selective use of heparin/aspirin therapy alone of in combination with intravenous immunoglobulin G, in the management of anti-phospholipid antibody-positive women undergoing IVF. *Am J Reprod Immunol* 1998;40:74–82
25. Kutteh WH, Yetman DL, Chantilis SJ, Crain J: Effect of antiphospholipid antibodies in women undergoing in-vitro fertilization: Role of heparin and aspirin. *Hum Reprod* 1997;12:1171–1175
26. Coulam CB, Roussev RG: Correlation of NK cell activation and inhibition markers with NK cytotoxicity among women experiencing immunological implantation failure after in vitro fertilization and embryo transfer. *J Assist Reprod Genet* 2003;20:58–62
27. Coulam CB, Roussev RG: Increasing circulating T-cell activation markers are linked to subsequent implantation failure after transfer of in vitro fertilized embryos. *Am J Reprod Immunol* 2003;50:340–345
28. Aoki K, Kajijura S, Matsumoto Y, Okada S, Yagami Y, Gleicher N: Preconceptional natural killer cell activity as a predictor of miscarriage. *Lancet* 1995;335:1340–1342
29. Yamada H, Morikawa M, Kato EH, Shimada S, Kobashi G, Minakami H: Preconceptional natural killer cell activity and percentage as predictors of biochemical pregnancy and spontaneous abortion with a normal karyotype. *Am J Reprod Immunol* 2003;50:351–354
30. Kwak-Kim JY, Chung-Bang HS, Ng SC, Ntrivalas EI, Mangubat CP, Beaman KD, Beer AE, Gilman-Sachs A: Increased T helper 1 cytokine responses by circulating T cells are present in women with recurrent pregnancy losses and in infertile women with multiple implantation failures after IVF. *Hum Reprod* 2003;18:767–773
31. Moffet A, Regan L, Braude P: Natural killer cells, miscarriage, and infertility. *Br Med J* 2004;329:1283–1285
32. Clark DA, Yu G, Levy GA, Gorczynski RM: Procoagulants in fetus rejection: The role of the OX-2 (CD200) tolerance signal. *Semin Immunol* 2001;13:255–263
33. Lachapelle MH, Miron P, Hemmings R, Roy DA: Endometrial T, B, and NK cells in patients with recurrent spontaneous abortion. *J Immunol* 1996;158:4886–4891
34. Coulam CB, Stephenson M, Stern JJ, Clark DA: Immunotherapy for recurrent pregnancy loss: Analysis of results from clinical trials. *Am J Reprod Immunol* 1996;35:352–359
35. Clark DA, Daya S, Coulam CB, Gunby J, and The Recurrent Miscarriage Immunotherapy Trialists Group: Implications of abnormal human trophoblast karyotype for the evidence-based approach to the understanding, investigation, and treatment of recurrent spontaneous abortion. *Am J Reprod Immunol* 1996;35:495–498
36. Laudenslager ML, Aasal R, Adler L, Berger CL, Montgomery PT, Sandberg E, Wahlberg LJ, Wilkins RT, Zweig L, Reit ML: Elevated cytotoxicity in combat-veterans with long-term post-traumatic stress disorder: Preliminary observations. *Brain Behav Immun* 1998;12:74–79
37. Clark DA, Croitoru K: TH1/TH2,3 imbalance due to cytokine-producing NK, $\gamma\delta$ T, and NK $\gamma\delta$ T cells in murine deciduas in success or failure of pregnancy. *Am J Reprod Immunol* 2001;45:108–115
38. Mincheva-Nilsson L, Kling M, Hammarstrom S, Nagaeva O, Sundqvist KG, Hammarstrom ML, Baranov V: $\gamma\delta$ T cells of human early pregnancy decidua: Evidence for local proliferation, phenotypic heterogeneity, and extrathymic differentiation. *J Immunol* 1997;159:3266–3277
39. Nagaeva O, Bondestam K, Olofsson J, Damber MG, Mincheva-Nilsson L: An optimized technique for separation of human decidual leukocytes for cellular and molecular analyses. *Am J Reprod Immunol* 2002;47:203–212
40. Nagaeva O, Jonsson L, Mincheva-Nilsson L: Dominant IL-10 and TGF- β mRNA expression in $\gamma\delta$ T cells of human early pregnancy decidua suggests immunoregulatory potential. *Am J Reprod Immunol* 2002;48:9–17
41. Szekeres-Bartho J, Barakonyi A, Miko E, Polgar B, Palkovics T: The role of $\gamma\delta$ T cells in the feto-maternal relationship. *Semin Immunol* 2001;13:229–233
42. Hill JA, Polgar K, Anderson DJ: T-helper 1-type immunity to trophoblast in women with recurrent spontaneous abortion. *JAMA* 1995;273:1933–1936
43. Hamai Y, Fujii T, Yamashita T, Miki A, Kozuma S, Geraghty DE, Taketani Y: Peripheral blood mononuclear cells from women with recurrent abortion exhibit an aberrant reaction to release cytokines upon the direct contact of human leukocyte antigen-G-expressing cells. *Am J Reprod Immunol* 1998;40:408–413
44. Ruiz JE, Kwak JY, Baum L, Gilman-Sachs A, Beaman KD, Kim YB, Beer AE: Intravenous immunoglobulins inhibits natural killer activity in vivo in women with recurrent spontaneous abortion. *Am J Reprod Immunol* 1996;35:370–375
45. Kwak JY, Kwak FM, Ainsbinder SW, Ruiz AM, Beer AE: Elevated peripheral blood natural killer cells are effectively downregulated by immunoglobulin G infusion in women with recurrent spontaneous abortions. *Am J Reprod Immunol* 1996;35:363–369
46. Ruiz JE, Kwak JY, Baum L, Gilman-Sachs A, Beaman KD, Kim YB, Beer AE: Effects of intravenous immunoglobulin G on natural killer cell cytotoxicity in vitro in women with recurrent spontaneous abortion. *J Reprod Immunol* 1996;31:125–141
47. Graphou O, Chioti A, Pantazi A, Tsukoura C, Kontopoulou V, Guorgiadou E, Balafoutas C, Koussoulakos S, Margaritis LH, Varla-Leftherioti M: Effect of intravenous immunoglobulins treatment on the Th1/Th2 balance in women with recurrent spontaneous abortions. *Am J Reprod Immunol* 2003;49:21–29
48. Perricone R, De Carolis C, Giacomelli R, Guarino MD, De Sanctis G, Fontana L: GM-CSF and pregnancy: Evidence of significantly reduced blood concentrations in unexplained recurrent abortion efficiently reversed by intravenous immunoglobulin treatment. *Am J Reprod Immunol* 2003;50:232–237
49. Sjoblom C, Wikland M, Robertson SA: Granulocyte-macrophage colony stimulating factor (GM-CSF) acts independently of the beta common subunit of the GM-CSF receptor to prevent inner cell mass apoptosis in human embryos. *Biol Reprod* 2002;67:1817–1823

50. Regan L, Backos MJ, Rai R: Immunological testing and interventions for reproductive failure. RCOG (UK) Scientific Advisory Committee Opinion Paper #5, 2003. Retrieved from <http://www.rcog.org.uk/resources/Public/SAC.Opinion.Paper.5.pdf>
51. Coulam CB, Krysa L, Stern JJ, Bustillo M: Intravenous immunoglobulin for treatment of recurrent pregnancy loss. *Am J Reprod Immunol* 1995;34:333–337
52. Kiproff DD, Nachtigall RD, Weaver RC, Jacobson A, Main EK, Garvoy MR: The use of intravenous immunoglobulin in recurrent pregnancy loss associated with combined alloimmune and autoimmune abnormalities. *Am J Reprod Immunol* 1996;36:228–234
53. Stricker RB, Steinleitner A, Bookoff CN, Weckstein LN, Winger EE: Successful treatment of immunological abortion with low-dose intravenous immunoglobulin. *Fertil Steril* 2000;73:536–540
54. Stephenson MD, Dreher K, Houlihan E, Wu V: Prevention of unexplained recurrent spontaneous abortion using intravenous immunoglobulin: A prospective, randomized, double-blind, placebo-controlled trial. *Am J Reprod Immunol* 1998;39:82–88
55. Anonymous: Intravenous immunoglobulin in the prevention of recurrent miscarriage. The German RSA/IVIG Group. *Br J Obstet Gynaecol* 1994;101:1072–1077
56. Christiansen OB, Mathiesen O, Huth M, Rasmussen KL, Ingerslev HJ, Lauritsen JG, Grunnet N: Placebo-controlled trial of treatment of unexplained secondary recurrent spontaneous abortions and recurrent late spontaneous abortions with i.v. immunoglobulin. *Hum Reprod* 1995;10:2690–2695
57. Christiansen OB, Pedersen B, Rosgaard A, Huth M: A randomized, double-blind, placebo-controlled trial of intravenous immunoglobulin in the prevention of recurrent miscarriage: Evidence for a therapeutic effect in women with secondary recurrent miscarriage. *Hum Reprod* 2002;17:809–816
58. Perino A, Vassiliadis A, Vucetich A, Colacurci N, Menato G, Cignitti M, Semprini AE: Short-term therapy for recurrent abortion using intravenous immunoglobulins: Results of a double-blind placebo-controlled Italian study. *Hum Reprod* 1997;12:2388–2392
59. Jablonowska B, Selbing A, Palfi M, Ernerudh J, Kjellberg S, Lindton B: Prevention of recurrent spontaneous abortion by intravenous immunoglobulin: A double-blind placebo-controlled study. *Hum Reprod* 1999;14:838–841
60. Ratko TA, Burnett DA, Foulke GE, Matczewski KA, Sacher RA, and the University Hospital Consortium Expert Panel for Off-Label use of Polyvalent Intravenously Administered Immunoglobulin Preparations: Recommendations for off-label use of intravenously administered immunoglobulin preparations. *JAMA* 1995;273:1865–1870
61. Clark DA: Is there any evidence for immunologically-mediated or immunologically-modifiable early pregnancy failure? *J Assist Reprod Genet* 2003;20:62–71
62. Munne S, Sultan KM, Weler HU, Grifo JA, Cohen J, Rosenwaks Z: Assessment of numeric abnormalities of X, Y, 18 and 16 chromosomes in preimplantation human embryos before transfer. *Am J Obstet Gynecol* 1995;172:1191–1199
63. Clark DA, Ding J, Yu G, Levy GA, Gorczynski RM: Fgl2 prothrombinase expression in mouse trophoblast and deciduas triggers abortion but may be countered by OX-2. *Mol Hum Reprod* 2001;7:185–194
64. Knackstedt M, Ding JW, Arck PC, Hertwig K, Coulam CB, August C, Lea R, Dudenhausen JW, Gorczynski RM, Levy GA, Clark DA: Activation of the novel prothrombinase fgl2 as a basis for the pregnancy complications spontaneous abortion and preeclampsia. *Am J Reprod Immunol* 2001;46:196–210
65. Clark DA, Foerster K, Fung L, He W, Lee L, Mendicino M, Markert U, Gorczynski RM, Marsden P, Levy GA: The fgl2 prothrombinase/fibroleukin gene is required for LPS-triggered abortions and for normal hemostasis during murine reproduction. *Mol Hum Reprod* 2004;10:99–108
66. Mellor AL, Sivakumar J, Chandler P, Smith K, Molina H, Mao D, Munn DH: Prevention of T cell-driven complement activation and inflammation by tryptophan catabolism during pregnancy. *Nat Immunol* 2001;2:64–68
67. Clark DA, Manuel J, Lee L, Chaouat G, Gorczynski RM, Levy GA: Ecology of danger-dependent cytokine-boosted spontaneous abortion in the CBA x DBA/2 model. I. Synergistic effect of LPS and (TNF- α + IFN- γ) on pregnancy loss. *Am J Reprod Immunol* 2004;52:370–378
68. Clark DA, Yu G, Arck PC, Levy GA, Gorczynski RM: MD-1 is a critical part of the mechanism causing Th-1 cytokine-triggered fetal loss syndrome. *Am J Reprod Immunol* 2003;49:297–307
69. Chaouat G, Ledee-Bataille N, Zourbas S, Ostojic S, Dubanchet S, Martal J, Frydman R: Cytokines, implantation and early abortion: Re-examining the Th1/Th2 paradigm leads to question the single pathway, single therapy concept. *Am J Reprod Immunol* 2003;50:177–186
70. De Carolis C, Greco E, Guarino MD, Perricone C, Dal Lango A, Giacomelli R, Fontana L, Perricone R: Anti-thyroid antibodies and anti-phospholipid syndrome: Evidence of reduced fecundity and of poor pregnancy outcome in recurrent spontaneous aborters. *Am J Reprod Immunol* 2004;52:263–266
71. Clark DA, Banwatt D, Chaouat G: Stress-triggered abortion in mice is prevented by alloimmunization. *Am J Reprod Immunol* 1993;29:141–147
72. Clark DA, Blois S, Kandil J, Handjiski B, Manuel J, Arck PC: Reduced indoleamine 2,3-dioxygenase versus increased Th1/Th2 cytokine ratios as a basis for occult and clinical pregnancy failure in mice and humans. *Am J Reprod Immunol* 2005;54:203–216
73. Simon HU, Saphth PJ: IVIG-mechanisms of action. *Allergy* 2003;58:543–552
74. Wright GJ, Cherwinski H, Foster-Cuevas M, Brooke G, Puklavec MJ, Bigler M, Song Y, Jenmalm M, Gorman D, McClanahan T, Lin M-R, Brown MH, Sedgwick JD, Phillips JH, Barclay AN: Characterization of the CD200 receptor family in mice and humans and their interaction with CD200. *J Immunol* 2003;171:3034–3046
75. Gorczynski RM, Lee L, Boudakov I: Augmented induction of CD4⁺CD25⁺ Treg using monoclonal antibodies to CD200R. *Transplantation* 2005;79:488–491
76. Aluvihare VR, Kallikourdis M, Betz AG: Regulatory T cells mediate maternal tolerance to the fetus. *Nat Immunol* 2004;5:266–271
77. Zenclussen AC, Gerlof K, Zenclussen ML, Sollwedel A, Bertoja AZ, Ritter T, Kotsch K, Leber J, Volk HD: Abnormal T cell reactivity against paternal antigens in spontaneous abortion. Adoptive transfer of pregnancy-induced CD4⁺CD25⁺ T regulatory cells prevents fetal rejection in a murine abortion model. *Am J Pathol* 2005;166:811–822