Intravenous immunoglobulin treatment in women with four or more recurrent pregnancy losses: A double-blind, randomised, placebo-controlled trial

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Summary

Background There is no effective treatment for women with unexplained recurrent pregnancy loss (RPL). We aimed to investigate whether treatment with a high dose of intravenous immunoglobulin (IVIG) in early pregnancy can improve pregnancy outcomes in women with unexplained RPL.

Methods In a double-blind, randomised, placebo-controlled trial, women with primary RPL of unexplained aetiology received 400 mg/kg of IVIG daily or placebo for five consecutive days starting at 4–6 weeks of gestation. They had experienced four or more miscarriages except biochemical pregnancy loss and at least one miscarriage of normal chromosome karyotype. The primary outcome was ongoing pregnancy rate at 22 weeks of gestation, and the live birth rate was the secondary outcome. We analysed all women receiving the study drug (intention-to-treat, ITT) and women except those who miscarried due to fetal chromosome abnormality (modified-ITT). This study is registered with ClinicalTrials.gov number, NCT02184741.

Findings From June 3, 2014 to Jan 29, 2020, 102 women were randomly assigned to receive IVIG (n = 53) or placebo (n = 49). Three women were excluded; therefore 50 women received IVIG and 49 women received placebo in the ITT population. The ongoing pregnancy rate at 22 weeks of gestation (31/50 [62.0%] vs. 17/49 [34.7%]; odds ratio [OR] 3.07, 95% CI 1.35–6.97; p = 0.009) and the live birth rate (29/50 [58.0%] vs. 17/49 [34.7%]; OR 2.60, 95% CI 1.15–5.86; p = 0.033) in the IVIG group were higher than those in the placebo group in the ITT population. The ongoing pregnancy rate at 22 weeks of gestation (OR 6.27, 95% CI 2.21–17.78; p < 0.001) and the live birth rate (OR 4.85, 95% CI 1.74–13.49; p = 0.003) significantly increased in women who received IVIG at 4–5 weeks of gestation.

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as compared with placebo, but these increases were not evident in women who received IVIG at 6 weeks of gestation. Four newborns in the IVIG group and none in the placebo group had congenital anomalies ($p = 0.28$).

**Interpretation** A high dose of IVIG in very early pregnancy improved pregnancy outcome in women with four or more RPLs of unexplained aetiology.

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**Keywords:** Abortion; Intravenous immunoglobulin; Pregnancy outcome; Recurrent miscarriage; Recurrent pregnancy loss; Unknown aetiology

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**Research in context**

**Evidence before this study**

We searched PubMed up until May 23, 2022, using the search terms “unexplained recurrent pregnancy loss”, “therapy”, and “clinical trials” without language restrictions. No standard therapeutic modality for unexplained recurrent pregnancy loss (RPL) has been established. Randomised, double-blind, and placebo-controlled trials (RCTs) found no efficacy of paternal lymphocyte immunisation, prednisone, low dose aspirin and/or heparin, or vaginal progesterone, in women with unexplained RPL. Previous RCTs of intravenous immunoglobulin (IVIG) for unexplained RPL used medium-dose (20–50 g), but the efficacy of the medium-dose IVIG treatment remains unproved.

**Added value of this study**

This RCT enrolled more severe cases of primary RPL than previous RCTs, who experienced ≥4 miscarriages and at least one miscarriage of a fetus with normal chromosome karyotype. High dose of IVIG (100 g) was administered early in pregnancy starting at 4–6 weeks of gestation. Consequently, this study, for the first time, revealed that high-dose IVIG treatment in women with ≥4 RPLs of unexplained aetiology significantly increased rates of ongoing pregnancy at 22 weeks of gestation and live birth. However, the rates of preterm delivery and fetal growth restriction increased in the IVIG group.

**Implications of all the available evidence**

High dose of IVIG in very early pregnancy improved pregnancy outcome in women with ≥4 RPLs of unexplained aetiology. This new treatment will give courage and hope to women with severe unexplained RPL who wish to have children. Women who receive high-dose IVIG treatment should be carefully monitored for complications throughout their pregnancy periods. Large scale international clinical trials can be performed to confirm the efficacy of high-dose IVIG treatment on unexplained RPL.

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**Introduction**

Recurrent pregnancy loss (RPL) is defined as the loss of ≥2 or ≥3 pregnancies and affects 0.8%–1.4% of couples who attempt to have a baby. A variety of factors are involved in the pathogenesis of RPL, such as abnormal uterine morphology, thyroid dysfunction, antiphospholipid syndrome, thrombophilic disorder, and chromosome abnormality. However, the aetiology of >50% of RPL is unknown and is therefore designated as unexplained RPL. The mechanism underlying the pathology of unexplained RPL remains poorly understood. Recent studies have proposed immunological abnormalities, including natural killer (NK) cells, Th1/Th2 balance, cytokine, and regulatory T cells, for pathophysiology underlying unexplained RPL.

No standard therapeutic modality for unexplained RPL has been established. Randomised, double-blind, and placebo-controlled trials and systematic reviews have found no efficacy of paternal lymphocyte immunisation, prednisone, low dose aspirin and/or heparin, or vaginal progesterone, in women with unexplained RPL. Some studies have indicated that intravenous immunoglobulin (IVIG) may have therapeutic efficacy for unexplained RPL. Assessment of the efficacy of IVIG treatment in women with ≥2 miscarriages using randomised controlled trials (RCTs) were performed in the 1990s. These trials used a medium dose of IVIG, in which 20–50 g of immunoglobulin was infused once, weekly or every 2–4 weeks during follicular phase, early or mid-gestation. Conclusions drawn from these IVIG trials are controversial. Only one study found that IVIG was efficacious; however, other studies including RCTs in the 2000s did not. Systematic reviews suggested that medium-dose IVIG treatment was effective in women with secondary RPL, but another RCT refuted its efficacy on secondary RPL. There are several studies that report beneficial effects of medium-dose IVIG treatment; however, as most of the studies are not homogeneous in terms of the unexplained RPL definition, gestational age in which start and finish the
treatment, and design, they cannot be used together in
meta-analysis or systematic review to reach an evidence-
based level to be recommended in clinical practice.

It is acknowledged that high-dose IVIG treatment is
effective, and this therapy has long been applied to
a variety of immune-mediated diseases such as
immune thrombocytopenic purpura, Kawasaki’s disease,
Guillain–Barre syndrome, and myasthenia gravis. The
high-dose IVIG treatment (20 g daily for 5 days) in 4–6
weeks of gestation was first reported in 1998,25 and it
yielded a high live birth rate of 89% among women
with a history of ≥4 miscarriages of unexplained aetiology.26 However, these are observational studies, while no
RCTs have assessed whether high-dose IVIG treatment
improve pregnancy outcome in women with RPL. We
assumed that the immunomodulatory effects of high-dose
IVIG treatment in early pregnancy restore fecundity in
women with unexplained RPL. Therefore, this multicenter,
double-blind, randomised, placebo-controlled trial was
designed to investigate whether high-dose IVIG treat-
ment in 4–6 weeks of gestation can increase the rates of
ongoing pregnancy at 22 weeks of gestation and live birth among women with primary RPL of
unexplained aetiology who have a history of ≥4 miscarriages including at least one miscarriage of a fetus
with normal chromosome karyotype.

Methods

Study design and participants
The double-blind randomised placebo-controlled trial of
IVIG was conducted at 14 study sites including univers-
ity hospitals and national centres in Japan, wherein the
study protocol was approved by the institutional review
board of each institution and written informed consent
was obtained from all the participants. The pivotal
Phase II study was performed in accordance with the
principles of the Declaration of Helsinki. The authors
assume responsibility for the accuracy and complete-
ness of the data and analyses. The study was conducted
in accordance with the Good Clinical Practice guide-
lines. The monitoring services were outsourced to a
contract research organization, MEDISCIENCE PLAN-
ing Inc., Tokyo, Japan) independent of the medical
institution. Determination of eligibility, reliability of
data, and verification of safety were assured through
monitoring, including the inspection of source data at
least once every month and interviewing investigators
and clinical trial collaborators.

Women were eligible to participate if they met all of
the following criteria: women who have 1) primary RPL
and no children; 2) ≥4 RPLs excluding biochemical
pregnancy loss in the count of miscarriages; 3-a) no risk
factors of abnormal uterine morphology, thyroid dys-
function with abnormal levels of free T4 or TSH, chro-
mosome abnormality in a couple, a positive test of
antiphospholipid antibody (anti–cardiolipin antibody,
anti–β2-GPI antibody, and lupus anticoagulant), or
deficiencies of factor XII, protein S, and protein C; and
have experienced at least one miscarriage of a fetus with
normal chromosome karyotype; or 3-b) have experi-
enced at least one miscarriage of a fetus with normal
chromosome karyotype after having been treated for
risk factors as follows: surgical treatment of septate
uterus, medical therapy for thyroid dysfunction, and
combination therapy with low dose aspirin and heparin
for occasional positive of antiphospholipid antibody
test, deficiencies of factor XII, protein S, and protein C;
and 4) <42 years. Miscarriage was defined as pregnancy
loss before 22 weeks of gestation according to Japanese
law. At the start of the study in 2014, eligible women
were defined as <40 years, and the number of miscar-
riages with normal chromosome karyotype had to be at
least two for women with four or five RPLs and at least
one for women with ≥6 RPLs. As the number of partici-
pants was too small, the protocol was revised in
April 2015.

The exclusion criteria were the following: women
who have 1) chromosome abnormality in a couple,
antiphospholipid syndrome defined according to the
updated Sydney classification criteria,27 or the most
recent positive test of antiphospholipid antibody; 2) no
treatment despite having diabetes mellitus or impaired
glucose tolerance; 3) received IVIG for RPL; 4) a history
of stillbirth at ≥22 weeks of gestation; 5) treatment for
malignancy; 6) thromboembolism; 7) a history of shock
or hypersensitivity to immunoglobulin; or 8) IgA defi-
ciency or serum IgA level of <5 mg/dL.

Since no Japanese or South-East Asian has factor V
Leiden or prothrombin gene mutation, this study did
not assess these coagulation abnormalities for partici-
pants.

Randomisation and masking
Participants were randomly assigned in a 1:1 ratio to
either the active drug group or the placebo group. The
PLAN procedure in SAS was used to generate random-
isation codes, and a seed number was randomly speci-
fied by the allocation manager. The vials were wrapped
with an opaque seal to ensure indistinguishability by
pre-assigned physicians or pharmacists who were not
involved in drug distribution, administration, or evalua-
tion. Participants, physicians and nurses were blinded.
To equalize factors affecting miscarriage, randomisa-
tion was done by stratifying the participants on the basis
of the number of miscarriages (4 or 5 vs. ≥6) using the
minimization method for age (≥35 years vs. <35 years).

Procedures
The active drug used was 5% formulation of intact type
human immunoglobulin G (Kenketsu Venoglobulin
IH®, the Japan Blood Products Organization. Physiological saline was used as placebo. The active drug of 400 mg/kg or placebo of 8 mL/kg was administered by intravenous drip infusion for five consecutive days. Treatment was initiated at 4 to 6 weeks and 6 days of gestation after gestational sac was identified by ultrasonography. If miscarriages occurred, chromosome karyotype of the villi was performed wherever possible using G-banding or microarray methods.

Outcomes
Two populations were analyzed: all women who received the study drug (intention-to-treat, ITT) and women who received the study drug excluding those who miscarried due to fetal chromosome abnormality (modified-ITT). The primary outcome was the ongoing pregnancy rate at 22 weeks of gestation. The live birth rate was defined as secondary outcomes.

Statistical analysis
The sample size was calculated based on the results of an epidemiological survey of RPL in Japan. The live birth rates in women with four, five, and six prior miscarriages were 65.0%, 58.8%, and 34.2%, respectively. The live birth rate was 89.8% when 100 g of immunoglobulin was administered in women with ≥4 miscarriages. The sample size was calculated assuming live birth rates of 42%–48% (placebo) and 75% (IVIG) with \( \alpha = 0.05 \) and \( \beta = 0.20 \). A study with 40 women per group has 80% power. As miscarriages with fetal chromosome abnormality are determined ex post facto, enrollment was continued until the final number of participants increased by approximately 20%. The ongoing pregnancy rate at 22 weeks of gestation, live birth rate, and their 95% confidence interval [CI] were calculated. Fisher’s exact test was used to compare the two groups. To estimate ongoing pregnancy rates, Kaplan-Meier curves were plotted for the pregnancy period. Miscarriage and stillbirth were defined as events, and those who had a live birth were censored regardless of preterm or full-term delivery. The ongoing pregnancy rates at 12, 22, 28, and 34 weeks of gestation were estimated, and the IVIG-to-placebo hazard ratio was calculated. This study is registered with ClinicalTrials.gov number, NCT02184741.

Role of the funding source
This study was funded by the Japan Blood Products Organization. The study funder contributed in study design and data interpretation, but had no role in data collection, analysis, writing of the manuscript, or the decision to submit. The study funder remained blinded to individual treatment allocation throughout the study. All authors had full access to all the data in the study and accepted responsibility to submit for publication.

Results
From June 3, 2014 to Jan 29, 2020, 104 women were assessed for eligibility, and 102 women were randomly assigned to receive IVIG (n = 53) or placebo (n = 49); three women in the IVIG group withdrew consent or had an early miscarriage before infusion. Of the remaining 99 women, 50 women received IVIG and 49 women received placebo in the ITT population (Figure 1). These study drugs were started from 4 weeks and 3 days to 6 weeks and 6 days of gestation. The baseline characteristics and protocol adherence were similar in the two groups (Table 1).

After treatment, 19 women in the IVIG group and 31 in the placebo group had miscarriages. Of the 19 miscarriages in the IVIG group, 12 had normal chromosome karyotype, three had numerical chromosome abnormality, two were unknown due to inadequate specimen quality, and two were not analysed due to spontaneous evacuation of the abortus. Of the 31 miscarriages in the placebo group, 20 had normal chromosome karyotype, ten had numerical chromosome abnormality, and one was unknown.

One pregnancy with fetal anencephaly in the placebo group was terminated by induced abortion. The remaining 47 women in the IVIG group and 38 in the placebo group were included in the modified-ITT population (Figure 1).

The ongoing pregnancy rate at 22 weeks of gestation (31/50, 62.0%) in the IVIG group was higher than that (17/49, 34.7%) in the placebo group in the ITT population (odds ratio [OR] 3.07, 95% CI 1.35–6.97; \( p = 0.009 \)). The live birth rate (29/50, 58.0%) in the IVIG group was higher than that (17/49, 34.7%) in the placebo group (OR 2.60, 95% CI 1.15–5.86; \( p = 0.03 \)). The rates of ongoing pregnancy and live birth were not statistically different between IVIG (47 women) and placebo (38 women) groups in the modified-ITT population (Table 2).

The IVIG-to-placebo hazard ratios for the ongoing pregnancy were 0.47 (95% CI 0.26–0.82; \( p = 0.007 \)) in the ITT population and 0.52 (95% CI 0.27–0.98; \( p = 0.04 \)) in the modified-ITT population, respectively (Figure 2).

Among women who had live births, gestational age at delivery was earlier in the IVIG group than the placebo group, and the rates of preterm delivery (13/29 [44.8%] vs. 1/17 [5.9%]) and fetal growth restriction (10/29 [34.5%] vs. 0/17 [0%]) were higher in the IVIG group compared with the placebo group. The number of live births was 30 including one pair of twins in the IVIG group and 17 in the placebo group. In the IVIG group except twins, birth weight was lower, and the rate of small for gestational age (12/28 [35.7%] vs. 0/17 [0%]) was higher compared with the placebo group. The karyotypes of three miscarriages in the IVIG group and ten in the placebo group had numerical chromosome abnormalities, whereas those of four miscarriages in the IVIG group and one in the placebo group were...
The rates of normal/abnormal chromosome karyotypes of miscarriages or congenital anomalies were not different between the two groups. Four newborns in the IVIG group and none in the placebo group had congenital anomalies ($p = 0.28$). Normal chromosome karyotypes of a total of 32 miscarriages consisted of 46XX ($n = 23$) and 46XY ($n = 9$). The disproportion might be derived from contamination of maternal tissues to some extent. (Table 3). Although preeclampsia was observed in four (8.0%) of 50 women

| Participant characteristics | IVIG n=50 (%) | Placebo n=49 (%) |
|----------------------------|--------------|-----------------|
| Age, years                 | 35.2 ± 3.7   | 35.0 ± 4.0      |
| < 35                       | 21 (42.0)    | 22 (44.9)       |
| ≥ 35                       | 29 (58.0)    | 27 (55.1)       |
| Body weight, kg            | 56.3 ± 9.0   | 56.4 ± 11.0     |
| Number of prior miscarriage (range) | 5-1 ± 1.6 (4-11) | 5.2 ± 1.7 (4-11) |
| 4 or 5 times               | 36 (72.0)    | 34 (69.4)       |
| 6 times or more            | 14 (28.0)    | 15 (30.6)       |
| Latest weeks of gestation in past miscarriages |           |                 |
| < 12 weeks                 | 43 (86.0)    | 43 (87.8)       |
| ≥ 12 weeks and < 22 weeks  | 7 (14.0)     | 6 (12.2)        |
| Weeks of gestation when gestational sac was identified | 5 weeks, 0 day | 5 weeks, 1 day |
| ± 3 days                   | 5 weeks, 0 day | 5 weeks, 1 day |
| Weeks of gestation at the start of drug treatment | 5 weeks, 5 days | 5 weeks, 4 days |
| ± 4 days                   | ± 3 days     | ± 3 days        |
| 4 weeks                    | 3 (6.0)      | 3 (6.1)         |
| 5 weeks                    | 31 (62.0)    | 33 (67.3)       |
| 6 weeks                    | 16 (32.0)    | 13 (26.5)       |

Table 1: Baseline characteristics of study participants.
Plus–minus values are means ± SD. IVIG, intravenous immunoglobulin.
in the IVIG group and one (2·0%) of 49 women in the placebo group (p = 0·36), there were no thromboembolic events. Twenty-three (46·0%) of 50 women receiving IVIG had mild adverse events including elevated liver enzymes in nine (18·0%), headache in four (8·0%), skin rash in four (8·0%), and fever in two (4·0%) women, while a total of three (6·1%) of 49 women in the placebo group had adverse events.

To assess the relationship between the timing of treatment initiation and pregnancy outcomes, the subjects were divided into women who started treatment at 4 or 5 weeks of gestation and women who started at 6 weeks. In the ITT population, the rates of ongoing pregnancy at 22 weeks of gestation (OR 6·27, 95% CI 2·21–17·78; p < 0·001) and live birth (OR 4·85, 95% CI 1·74–13·49; p = 0·003); and the rates of ongoing pregnancy at 22 weeks of gestation (OR 5·40, 95% CI 1·79–16·30; p = 0·004) and live birth (OR 4·03, 95% CI 1·37–11·84; p = 0·02) in the modified-ITT population, were significantly higher in the IVIG group than the placebo group in women who started at 4 or 5 weeks of gestation, but not in women who started at 6 weeks (Table 4).

The subjects were also divided into women with four or five prior miscarriages and women with ≥6 prior miscarriages to assess the relationship between the number of miscarriages and pregnancy outcomes. In the ITT population, the rates of ongoing pregnancy at 22 weeks of gestation (OR 10·00, 95% CI 1·80–55·36; p = 0·009) and live birth (OR 7·20, 95% CI 1·35–38·32; p = 0·03); and the rates of ongoing pregnancy at 22 weeks of gestation (OR 8·33, 95% CI 1·47–47·23; p = 0·04) and live birth (OR 6·00, 95% CI 1·11–32·55; p = 0·05) in the modified-ITT population, were significantly higher in the IVIG group than the placebo group in women with ≥6 miscarriages (Table 5).

This study included participants who had experienced at least one miscarriage of a fetus with normal chromosome karyotype after having been treated for risk factors as follows: surgical treatment of septate uterus (IVIG 1/50 vs. placebo 1/49), medical therapy for thyroid dysfunction (IVIG 3/50 vs. placebo 4/49), and combination therapy with low dose aspirin and heparin for occasional positive of antiphospholipid antibody test (IVIG 0/50 vs. placebo 1/49), deficiencies of factor XII (IVIG 3/50 vs. placebo 0/49), protein S (IVIG 5/50 vs. placebo 3/49), and protein C (none). The other participants had no risk factors (IVIG 38/50 vs. placebo 40/49).

### Discussion

Previous RCTs for RPL of unexplained aetiology used medium-dose IVIG treatment (20–50 g, once, weekly or every 2–4 weeks) during follicular phase, early or mid-gestation in women with ≥2–3,15–20,22 or ≥4 prior miscarriages.15–24 However, the efficacy of these medium-dose IVIG treatments remains unproved.15–24 The present study enrolled more severe cases of primary RPL than previous RCTs, who experienced ≥4 miscarriages and at least one miscarriage of a fetus with normal chromosome karyotype. To make RPL participants more homogeneous, only primary RPL was enrolled. In addition, for the first time, high dose of IVIG (20 g daily for 5 days) was administered early in pregnancy starting at 4–6 weeks of gestation. Consequently, this RCT revealed that high-dose IVIG treatment in women with ≥4 RPLs of unexplained aetiology significantly increased rates of ongoing pregnancy at 22 weeks of gestation and live birth in the ITT population.
Figure 2. Kaplan-Meier curves of ongoing pregnancy rates. Kaplan-Meier curves of ongoing pregnancy rates for each of the IVIG and placebo groups in the intention-to-treat population (Panel A) and in the modified intention-to-treat population (Panel B). Miscarriage and stillbirth were defined as events, and pregnant women who had a live birth or an induced abortion due to fetal anomaly were censored and depicted as marks on the curve. IVIG, intravenous immunoglobulin.

Panel A: In the intention-to-treat population, Kaplan-Meier estimates of the ongoing pregnancy rates at 12, 22, 28, and 34 weeks of gestation were 38.8%, 36.7%, 36.7%, and 36.7% in the placebo group; and 68.0%, 62.0%, 60.0%, and 60.0% in the IVIG group, respectively. The IVIG-to-placebo hazard ratio for the ongoing pregnancy rate was 0.47 (95% CI: 0.26–0.82), and the log-rank test indicates a significant difference (p=0.007).

Panel B: In the modified intention-to-treat population, Kaplan-Meier estimates of the ongoing pregnancy rates at 12, 22, 28, and 34 weeks of gestation were 47.4%, 44.7%, 44.7%, and 44.7% in the placebo group; and 72.3%, 66.0%, 63.8%, and 63.8% in the IVIG group, respectively. The IVIG-to-placebo hazard ratio for the ongoing pregnancy rate was 0.52 (95% CI: 0.27–0.98), and the log-rank test indicates a significant difference (p=0.04).
The efficacy of IVIG treatment on the rates of ongoing pregnancy at 22 weeks of gestation and live birth was found in severe cases with ≥6 RPLs in the ITT and modified-ITT populations. These results suggest that severe cases of RPL may have undetermined aetiologies to a greater extent for which IVIG treatment could be effective. The time of treatment initiation was also associated with the efficacy of IVIG treatment. The efficacy on the rates of ongoing pregnancy at 22 weeks of gestation and live birth was more evident in women with administration started at 4 or 5 weeks of gestation, but not in women with administration started at 6 weeks of gestation in the ITT and modified-ITT populations. This RCT first demonstrated that high-dose IVIG treatment started at 4–5 weeks of gestation is especially effective on women with ≥4 RPLs of unexplained aetiology. A recent meta-analysis of RCTs found that IVIG treatment increased live birth rates when initiated prior to conception.\textsuperscript{28}

In the present study, high-dose IVIG treatment was well tolerated in most women, and none of them discontinued the treatment due to adverse effects. However, the gestational age at delivery was earlier, and the rates of preterm delivery and fetal growth restriction were higher in the IVIG group compared with the placebo group. Similarly, the birth weight was lower, and the gestational age at delivery was earlier, and the rates of preterm delivery and fetal growth restriction were higher in the IVIG group compared with the placebo group. There could be two main reasons for increased rates of preterm delivery and fetal growth restriction in

\begin{table}
\centering
\begin{tabular}{|l|l|l|l|}
\hline
Pregnancy outcomes & IVIG & Placebo & p-value \\
\hline
Ongoing pregnancy at 22 weeks of gestation & 31 (62.0) & 17 (34.7) & 0.009\textsuperscript{*} \\
Live birth & 29 (58.0) & 17 (34.7) & 0.03\textsuperscript{*} \\
Gestational age at delivery & 36 weeks, 1 day ± 4 weeks, 0 days & 39 weeks, 2 days ± 2 weeks, 0 days & 0.004\textsuperscript{**} \\
Mode: Vaginal delivery & 12 (41.4) & 7 (41.2) & 1.00 \\
Cesarean section & 17 (58.6) & 10 (58.8) & 1.00 \\
Preterm delivery (< 37 weeks) & 13 (44.8) & 1 (5.9) & 0.007\textsuperscript{*} \\
Fetal growth restriction & 10 (34.5) & 0 (0.0) & 0.006 \\
Miscarriage & 19 (63.3) & 31 (63.3) & 0.02 \\
Gestational age at miscarriage & 9 weeks, 2 days ± 2 weeks, 6 days & 8 weeks, 0 days ± 1 week, 2 days & 0.045\textsuperscript{**} \\
Time of miscarriage & < 12 weeks & 16 (84.2) & 30 (96.8) & 0.15 \\
≥ 12 weeks and < 22 weeks & 3 (15.8) & 1 (3.2) & 0.28 \\
Chromosome karyotype of miscarriage & & & \\
Normal & 12 (63.2) & 20 (64.5) & 0.49 \\
Abnormal & 3 (15.8) & 10 (32.3) & \\
Unknown\textsuperscript{1)} & 2 (10.5) & 1 (3.2) & \\
Not tested\textsuperscript{2)} & 2 (10.5) & 0 (0.0) & \\
Stillbirth & 1\textsuperscript{3)} (2.0) & 0 (0.0) & 0.39 \\
Unknown outcome due to discontinuation & 1\textsuperscript{4)} (2.0) & 2\textsuperscript{4)} (2.0) & 1.00 \\
\hline
Newborns & & & \\
\hline
Birth weight, g & 2246.4 ± 962.5 & 3071.6 ± 463.4 & 0.002\textsuperscript{**} \\
Apgar score at 5 minutes & 8.7 ± 1.1 & 9.1 ± 0.6 & 0.13 \\
Small for gestational age & 12 (35.7) & 0 (0.0) & 0.001 \\
Congenital anomaly & 4\textsuperscript{7)} (14.3) & 0 (0.0) & 0.28 \\
\hline
\end{tabular}
\caption{Comparison of pregnancy outcomes in intention-to-treat population.}
\end{table}
the IVIG group: 1) either the substantial doses of IVIG in very early pregnancy could negatively affect trophoblast invasion in the uterus, which will only be unveiled in the third trimester, or 2) alternatively, high-dose IVIG treatment may rescue some fetuses that would otherwise have been miscarried due to immune disturbances partially increasing the risk of later immune injury to the placenta and fetal growth restriction. A previous study also found the high rates of preterm delivery and fetal growth restriction in women with unexplained RPL who received high-dose IVIG.26 To clarify the reason for high rates of these adverse pregnancy outcomes in unexplained RPL women with high-dose IVIG treatment, further investigations are necessary.

High-dose IVIG treatment has long been applied to a variety of immune-mediated diseases. Many distinct but non-mutually exclusive mechanisms of action, including antiinflammation, suppression of autoantibodies and complements, blockade of FcRn and FcγRn, up-regulation of inhibitory FcγRIIB, modulation of monocytes, macrophages, dendritic cells, natural NK cells, T cells, B cells, and endothelial cells, account for the immunomodulatory effects of IVIG treatment.29-30 Aberrant immunities of NK cells, Th1/Th2 balance, cytokine, and regulatory T cells at the fetomaternal interface and/or in the maternal blood were proposed for aetiologies of unexplained RPL.6-10 In a mouse model of miscarriage induced by polyinosinic–polycytidylic acid, a high dose of intact type- immunoglobulin but not a medium dose or Fab- immunoglobulin restored fecundity through macrophages together with a reduction of TNF-α and IFN-γ expressions in the placenta.31 Similarly, a high dose of intact type- immunoglobulin in an early period reduced miscarriages through NK cells in a mouse model of miscarriage induced by lipopolysaccharide.32 High-dose IVIG treatment starting at 4–5 weeks of gestation might effectively restore normal immune environment, while the treatment starting at 6 weeks of gestation would be too late to yield the efficacy to restore fecundity. Modification of immune function by a high dose of IVIG during early pregnancy might reduce the number of miscarriages in humans, but the mechanism is still unknown and further investigation is required.

In the modified-ITT population, the efficacy of IVIG treatment on the rates of ongoing pregnancy at 22 weeks of gestation—no./total no. (%) and further investigation is required.
weeks of gestation or live birth did not reach statistical significance, since a total of 13 pregnancies were excluded due to chromosome abnormality of miscarriages. The ratio of miscarriage with abnormal chromosome karyotype in the IVIG group (35/50, 60%) was lower than that in the placebo group (24/33, 73%, p = 0.09). This is potentially a part of IVIG treatment effect, and the IVIG might have a preventative role in averting miscarriage with abnormal chromosome karyotype increasing the ratio of live birth, since transferred mosaic embryos with abnormal chromosome karyotype can develop into healthy euploid newborns in IVF. Further investigation is required.

In conclusion, high dose of IVIG in very early pregnancy improved pregnancy outcome in women with ≥4 RPLs of unexplained aetiology. This new treatment will give courage and hope to women with severe unexplained RPL who wish to bear children. However, this trial has several limitations and potential bias. To exclude women who occasionally experienced repeated miscarriages of abnormal chromosome karyotype as much as possible, this trial enrolled severe cases of primary RPL who experienced ≥4 miscarriages of unexplained aetiology and at least one miscarriage with normal chromosome karyotype. These inclusion criteria were most severe compared with previous trials of IVIG. Therefore, it took six years and three months to follow up pregnancies of 99 participants; however, this number was not large. The efficacy of high-dose IVIG treatment was not assessed for secondary RPL. Women who receive high-dose IVIG treatment in very early pregnancy should be carefully monitored for complications throughout their pregnancy periods. Large scale international clinical trials can be performed to confirm the efficacy of high-dose IVIG treatment in very early pregnancy on severe cases of unexplained RPL.

Table 5: Number of previous miscarriages and pregnancy outcome.

| Intention-to-treat population | Number of previous miscarriages | Administration | Ongoing pregnancy at 22 weeks of gestation–total no. (%) | 95% CI | Fisher’s exact test |
|------------------------------|---------------------------------|----------------|------------------------------------------------------|--------|--------------------|
| Ongoing pregnancy at 22 weeks of gestation | 4 or 5 times | Placebo | 14/34 (41.2) | 24–6–59 3 | 0.23 |
| | 6 times or more | Placebo | 3/15 (20.0) | 4.3–48 1 | 0.009 |
| Placebo | 21/36 (58.3) | 40–8–74 5 |
| | 10/14 (71.4) | 41–9–91 6 |

| Number of previous miscarriages | Administration | Live births–total no. (%) | 95% CI | Fisher’s exact test |
|------------------------------|----------------|--------------------------|--------|--------------------|
| Live birth | 4 or 5 times | Placebo | 14/34 (41.2) | 24–6–59 3 | 0.24 |
| | 6 times or more | Placebo | 3/15 (20.0) | 4.3–48 1 | 0.03 |
| Placebo | 20/36 (55.6) | 38–1–72 1 |
| | 9/14 (64.3) | 35–1–87 2 |

| Modified intention-to-treat population | Number of previous miscarriages | Administration | Ongoing pregnancy at 22 weeks of gestation–total no. (%) | 95% CI | Fisher’s exact test |
|------------------------------|---------------------------------|----------------|------------------------------------------------------|--------|--------------------|
| Ongoing pregnancy at 22 weeks of gestation | 4 or 5 times | Placebo | 14/25 (56.0) | 34–9–75 6 | 0.6 |
| | 6 times or more | Placebo | 3/13 (23.1) | 5–0–53 8 | 0.02 |
| Placebo | 21/33 (63.6) | 45–1–79 6 |
| | 10/14 (71.4) | 41–9–91 6 |

| Number of previous miscarriages | Administration | Live births–total no. (%) | 95% CI | Fisher’s exact test |
|------------------------------|----------------|--------------------------|--------|--------------------|
| Live birth | 4 or 5 times | Placebo | 14/25 (56.0) | 34–9–75 6 | 0.79 |
| | 6 times or more | Placebo | 3/13 (23.1) | 5–0–53 8 | 0.05 |
| Placebo | 20/33 (60.6) | 42–1–77 1 |
| | 9/14 (64.3) | 35–1–87 2 |

Contributors
H.Y., M.D., S.S., T.T., and H.Y. conceived and designed the study. M.D., S.S., T.T., M.M., T.S., T.N., K.T., M.N., S.Y., K.E., M.T., K.M., R.H., A.F., K.T., K.S., and T.E. acquired the data. H.Y., S.S., T.T., and H.Y. analysed and interpreted the data. H.Y., M.D., S.S., T.T., and H.Y. verified the underlying data. All authors had full access to all
the data in the study and accepted responsibility to submit for publication.

Data sharing statement
Data from this study will be available after approval of manufacturing and marketing from the Japanese regulatory authorities or three years after the completion of this study, whichever is later. Documentation including the protocol, statistical analysis plan, and informed consent document will be made available. Data will be made available to investigators after the proposal for use of the data has been approved. Proposals for the use of data should be addressed to the following yata-hiroaki@jbpo.or.jp.

Declaration of interests
We declare no competing interests.

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Supplementary materials
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