**Table 1** (*Continued*)

| Carrier Status | Prior Pregnancies | Subsequent Pregnancies |
|---|---|---|
| 46, XX, t(1;10)(q21;p11.2) | SA, SA, SA, SA | SA |
| 46, XX, t(1;10)(q42.1;q24.3) | SA, SA | SA |
| 46, XX, t(1;11)(p11;q13) | SA, SA, SA | SA (47, XY, +4) |
| 46,XX, t(1;15)(q32.1;q23) | SA, SA, SA | Term |
| 46, XX, t(2;15)(p23;q15) | SA, SA, SA | Term |
| 46, XX, t(2;15)(q31;q21.2) | SA, SA, SA, SA, SA, SA | Term |
| 46, XX, t(2;18)(q33;p11.3) | SA, SA, SA | SA |
| 46, XX, t(3;5)(p13;q33) | SA, SA, SA | Term |
| 46, XX, t(3;7)(p25;p13) | SA, SA, SA | Term |
| 46, XX, t(4;21)(p15.1;q22.2) | SA, SA | Term |
| 46, XX, t(5;13)(p15.3;q21.2) | SA, SA, SA | Term |
| 46, XX, t(6;7)(q25.1;p21) | SA, SA, SA | SA (46, XY, der(6)t(6;7)(q25.1;p21)) |
| 46, XX, t(6;8)[b] | SA, SA, SA, SA, SA, IUFD | SA (46, XX, del(6)(q23)) |
| 46, XX, t(6;8)(q23;p23) | SA, SA, SA, SA, SA, SA, IUFD | SA (46, XX, t(6;8)(q23;p23) |
| 46, XX, t(7;11)(p13;q21) | SA, SA | Term |
| 46, XX, t(7;18)(p15.3;p11.32) | SA, SA, SA | SA |
| 46, XX, t(7;18)(q32;q13) | SA, SA, SA, SA | SA |
| 46, XX, t(9;13)(q12;p12) | SA, SA, SA, SA | Term |
| 46, XX, t(10;17)(q26;p12) | SA, SA, SA | SA (46, XX, der(17)t(10;17)(q26;p12)mat) |
| 46, XX, t(10;21)p10;q10) | SA, SA, SA, SA | Term (46, XY, t(10;21)(p10;q10)) |
| 46, XX, t(11;22)(q23.3;q11.2) | SA, SA | Term |
| 46, XX, t(11,22)(q23;q11.2) | SA, SA, SA | SA (46, XX [25]/46, XX, del(5)(p14) [5]) |
| 46, XX, t(17;20)(p13;q13.1) | SA, SA, SA, Term | SA |
| 46, XX, t[b] | SA, SA | SA (47, XX or XY, +14) |
| 46, XX, t[b] | SA, SA, SA | Term |
| 46, XX, t[b] | SA, SA, SA | Term |
| 46, XY, t(1;10)(p32;q26) | SA, SA, SA, SA | Term |
| 46, XY, t(1;11)(p32.1;p15.1) | SA, SA | Term |
| 46, XY, t(2;7)(p10;q10) | SA, SA, SA | Term |
| 46, XY, t(3;7)(q25.3;q21.2) | SA, SA | Term |
| 46, XY, t(3;7)(q25.3;q21.1) | SA, SA, SA, SA | Term |
| 46, XY, t(3;15)(p22;q26.2) | SA, SA, SA | Term |
| 46, XY, t(4;10)(q34;q21.2) | SA, SA | Term |
| 46, XY, t(5;6)(q33.1;p11.2) | SA, SA, SA | Term |
| 46, XY, t(5;9)[b] | SA, SA, IUFD, IUFD | Term |
| 46, XY, t(5;10)(q22;q22) | SA, SA, SA | Biochemical |
| 46, XY, t(7;8)(q21;q22) | SA, SA | SA (46, XX) |
| 46, XY, t(7;8)(q32;q22) | SA, SA | Biochemical |
| 46, XY, t(7;17)(q11.23;q23.3) | SA, SA, SA, SA, SA | Term |
| 46, XY, t(8;12)(p21.3; q12) | SA, SA, SA, SA, Term | Term |
| 46, XY, t(10;13)(q24;q34) | SA, SA, SA, Term | Term |
| 46, XY, t(11;22)(q23.3;q11.2) | SA, SA, SA, Term | Term |
| 46, XY, t[b] | SA, SA | SA (46, XX) |
| Pal et al, 2009[22] | | |
| 46, XX, t(9;14)(q34;31) | SA, SA, SA | SA, SA, SA, hydatidiform mole |
| 46, XX, t(5;11)(q35;q13–25) | SA, SA, SA | Term |

SA, spontaneous abortion or miscarriage; ET, ectopic; IUFD, intrauterine fetal demise; GT, genetic termination.
[b]Unknown specific translocation.
In the Sugiura-Ogasawara et al study,[4,5] only the number of prior miscarriages were included, not prior ongoing pregnancies. Additional subsequent outcomes or new information about pregnancies are in boldface type.

**Table 2 Comparison of Prior and Subsequent Pregnancy Outcomes in Carriers of a Reciprocal Translocation Ascertained on the Basis of Recurrent Pregnancy Loss: Medically Managed**

| | No. of Carriers | Prior Pregnancies | | Subsequent Pregnancies | |
|---|---|---|---|---|---|
| | | Unsuccessful | Successful | Unsuccessful | Successful |
| Sugiura-Ogasawara et al, 2004[5] | 43 | 124 miscarriages | Not stated | 65 miscarriages, 1 fetal demise | 29 |
| Jobanputra et al, 2005[17] | 1 | 8 miscarriages | 1 | 1 miscarriage | 1 |
| Stephenson and Sierra, 2006[7] | 20 | 67 miscarriages, 3 ectopics | 9 | 13 miscarriages | 21 |
| Sugiura-Ogasawara et al, 2008[4] | 43 | 169 miscarriages, 4 fetal demises | 7 | 17 miscarriages | 29 |
| Pal et al, 2009[22] | 2 | 6 miscarriages | 0 | 3 miscarriages, 1 molar pregnancy | 1 |
| **Totals** | **109** | **94%** 374 miscarriages, 4 fetal demises, 3 ectopics | **6%** 17 successful pregnancies | **55%** 99 miscarriages, 1 fetal demise, 1 molar pregnancy | **45%** 81 successful pregnancies |

translocations were reported in 48 of the 50 male carriers. Of the male carriers, chromosome 7 was the most frequent chromosome involved in the reciprocal translocations ($n = 14$), followed by chromosome 10 ($n = 10$); chromosomes 5 and 8 ($n = 8$); chromosomes 3, 6, and 13 ($n = 7$, each); chromosome 11 ($n = 5$); chromosomes 1, 2, 4, 12, and 15 ($n = 4$, each); chromosomes 9 and 14 ($n = 2$, each), and chromosomes 16, 18, 21, and 22 ($n = 1$, each).

## Description of Medical Management Cases (Table 1)

Between 1986 and 2002, Sugiura-Ogasawara et al[5] evaluated 47 carriers of a balanced reciprocal translocation with a history of two or more consecutive first trimester miscarriages. Evaluation was performed for concomitant endocrine, uterine, autoimmune, infectious, and natural killer cell factors. Close monitoring included hospitalization for ~1 month, starting at 4 weeks of gestation, with ultrasonography twice weekly. The mean age at time of subsequent pregnancies was 30.7 years (standard deviation [SD]: 3.4). At least one subsequent pregnancy was reported in 43 of the 47 cases.

In 2005, Jobanputra et al[17] reported on a 34-year-old female carrier of a balanced reciprocal translocation with a history of recurrent pregnancy loss and a strong family history of breast cancer.

Between 1992 and 2005, Stephenson and Sierra[7] evaluated 28 carriers of a balanced reciprocal translocation and a history of recurrent pregnancy loss. The mean age at time of prior and subsequent miscarriages was 29.8 years (SD: 5.0) and 34.0 (SD: 4.3), respectively. Couples were screened for concomitant endocrine, uterine, autoimmune, and infectious factors. If one or more of the miscarriages was a fetal miscarriage (10 to 20 weeks of gestation), an inherited thrombophilia screen was also performed. Concomitant factors were identified in 12 cases; 8 women met criteria for the antiphospholipid syndrome and were treated with aspirin and heparin, two women had a luteal phase deficiency and were treated with progesterone suppositories or clomiphene citrate, and one woman had factor V Leiden and was treated with heparin. One woman had Crohn's disease and gestational diabetes and was treated with prednisone and insulin. Three women were treated empirically with aspirin and/or vaginal progesterone suppositories. Close monitoring consisted of serial βhCGs, starting within 1 week of a missed menses, endovaginal ultrasound at least at 6 and 10 weeks of gestation, and 24-hour physician coverage. At least one subsequent pregnancy was reported in 20 of the 28 cases.

Between 2003 and 2005, Sugiura-Ogasawara et al[4] evaluated 70 carriers of a balanced reciprocal translocation and a history of two or more consecutive miscarriages, of which 68 were treated medically. Evaluation was performed for concomitant autoimmune, endocrine, and uterine factors. With at least one positive antiphospholipid antibody, consisting of either the lupus anticoagulant, anticardiolipin, or β2-glycoprotein-I-dependent anticardiolipin antibodies, the patient was treated with low-dose aspirin and heparin. Supportive psychotherapy was provided. At least one subsequent pregnancy was reported in 43 of the 68 cases. The mean age at time of subsequent pregnancy was 31.4 years (SD: 4.4).

In 2005–2006, Pal et al[22] identified two carriers of a balanced reciprocal translocation with a history of three prior miscarriages, both of whom were referred to genetics for karyotyping. The mean age of the carrier at diagnosis was 27.0 years (SD: 2.8). Subsequent pregnancy outcomes were obtained by the medical record and phone interviews 12 to 24 months following

karyotyping. Evaluation and treatment of concomitant factors were not reported in the manuscript.

### Summary of Medical Management (Table 2)

A total of 109 cases from five publications met the criteria for the medical management group, which consisted of evaluation and management of concomitant factors associated with recurrent pregnancy loss, followed by close monitoring of subsequent pregnancies. There were at least 397 prior pregnancies in the medical management group, of which 374 were miscarriages <10 weeks, which equates to a mean of 3.4 prior miscarriages (range: 2 to 8). In addition, there were 17 live births, 3 ectopics, and 4 fetal deaths.

Using the first subsequent pregnancy only, the subsequent live birthrate was 60% (65 of 109). If the carrier was female, the subsequent live birthrate was 52% (34 of 65), and if the carrier was male, 75% (33 of 44). Using all subsequent outcomes, the cumulative live birthrate was 74% (81 of 109 cases) in the medical management group.

### Description of In Vitro Fertilization/ Preimplantation Genetic Diagnosis Management (Table 3)

In 1998, Munné et al[19] published three cases that met the criteria, with a mean maternal age of $33.7 \pm 4.9$ years. Methodology was limited to the description of the PGD. Polar body analysis following fluorescent in situ hybridization (FISH) with whole-chromosome painting DNA probes was performed. In addition, a telomeric probe was used in one of the cases. In the first case, three of eight polar bodies were balanced, of which two embryos were transferred; twins were delivered. In the second case, three of five polar bodies were normal, of which one embryo was transferred; pregnancy was ongoing at publication. In the third case, two of five polar bodies were either normal or balanced, but the transfer was cancelled because the embryos were developmentally abnormal.

Later in 1998, Munné et al[21] published one case that met the criteria, consisting of a woman 37 years of age. Six first polar bodies were analyzed following FISH with chromosome-painting probes. Three of the polar bodies were unbalanced, two were balanced, and one did not have a result because the polar body degenerated. Two embryos were transferred; pregnancy ended in miscarriage with a balanced translocation.

In 1999, Willadsen et al[25] published two cases that met the criteria, with a mean maternal age of $34 \pm 4.2$ years. Polar body analysis was performed on five oocytes following FISH and chromosome-painting probes. One embryo, with unclear FISH signals on first polar body analysis, was biopsied on day 3, fused with a frozen-thawed bovine M-II oocyte, which was followed by FISH using whole chromosome-painting probes. Repeat analysis of the metaphase-transformed blastomere nucleus was performed using spectral karyotyping (SKY), after transfer. The embryo was transferred on day 4; a preterm delivery resulted with normal female chromosomes. The newborn had a ventricular septal defect and underwent surgery without complication.

In the second case, first polar body analysis was informative for 7 of 11 oocytes; all were unbalanced or when balanced or normal, the oocytes did not fertilize. Three of the four oocytes with uninformative results fertilized and one or two blastomeres were biopsied and fused with bovine oocytes; analysis revealed the blastomeres were anuclear or unbalanced. Thus there were no embryos for transfer.

Munné et al[20] published three cases that met the criteria in 2000, with an average maternal age of $32.3 \pm 2.5$ years. Polar body analysis followed FISH with centromeric and telomeric probes for the chromosomes involved in the translocations. One to three embryos were transferred; pregnancy did not occur.

In 2000, Escudero et al[16] published two cases that met the criteria, with an average maternal age of $30 \pm 4.2$ years. Polar body biopsy, followed by FISH, using a combination of whole-chromosome painting and telomeric probes, was performed. Twelve polar bodies were obtained in the first case, of which nine were analyzed. One was balanced and the subsequent embryo was transferred; the pregnancy resulted in monozygotic (46, XY) twins. In the second case, eight polar bodies were obtained and analyzed. One was balanced and the subsequent embryo was transferred; pregnancy did not occur.

In 2000, Coonen et al[15] published two cases that met the criteria; maternal ages were not provided. In the first case, 18 embryos were biopsied, with one or two blastomeres aspirated on day 3. Following multitarget FISH, using three DNA probes, one embryo with a balanced reciprocal translocation was transferred on day 4. Pregnancy resulted in a successful term delivery with a balanced reciprocal translocation.

With the second case, the woman underwent three IVF cycles. The first cycle was cancelled due to poor ovarian stimulation. The second cycle yielded two embryos with normal and/or a balanced karyotype. Both karyotypically normal embryos were transferred, but pregnancy did not occur. In the third cycle, three embryos were biopsied; two were unbalanced and the results inconclusive in the third; no transfer was performed.

In 2003, Simopoulou et al[23] published four cases that met the criteria; mean maternal age was $33.3 \pm 1.9$ years. One or two blastomeres were aspirated on day 3, according to the developmental stage and

**Table 3 Carriers of a Reciprocal Translocation with a History of Recurrent Pregnancy Loss, with Subsequent IVF/PGD Management ($n=20$)**

| Carrier Status | Prior Pregnancies | Treatment | Subsequent Pregnancies |
|---|---|---|---|
| Munné et al, 1998[19] | | | |
| 46, XX, t(7;20)(q22;q11.2) | SA, SA, SA, SA (balanced t(7;20)(q22,q11.2)mat) | IVF/±ICSI/PGD | Twins (balanced t(7;20)(q22;q11.2)mat) |
| 46, XX, t(9;11)(p24 ;q12) | 5 pregnancy losses and a healthy baby from a natural cycle | IVF/±ICSI/PGD | Ongoing pregnancy (normal) |
| 46, XX, t(14;18)(q22;q11) | 5 pregnancy losses | IVF/±ICSI/PGD | No transfer |
| Munne et al, 1998[21] | | | |
| 46, XX, t(4:14)(p15.3;q24) | Repeated pregnancy loss; one was balanced t(4:14)(p15.3;q24)mat | IVF/±ICSI/PGD | SA (balanced t(4:14)(p15.3;q24)mat) |
| Willandsen et al, 1999[25] | | | |
| 46, XX, t(9;11)(p24;q12) | History of habitual abortion | IVF/ICSI/PGD | Preterm (200 g) newborn (46, XX) with a ventricular septal defect, which was repaired at 5 mo |
| 46, XX, t(11;16)(q21;q22) | History of recurrent miscarriage | IVF/ICSI/PGD | No transfer |
| Munné et al, 2000[20] | | | |
| 46, XY, t(10;13)(q22.3;q14) | SA, SA, SA, SA, SA | IVF/PGD | Did not conceive |
| 46, XX, t(10;14)(q26.1)(q22.1) | SA, SA | IVF/PGD | No transfer |
| 46, XY, t(10;18)(q24.1;p11.2) | SA, SA, SA, SA, SA, SA, SA, SA, SA (unbalanced), SA (unbalanced), SA (unbalanced), SA (unbalanced), SA (unbalanced), SA (unbalanced), 1 failed IVF cycle | IVF/PGD | Did not conceive |
| Escudero et al, 2000[16] | | | |
| 46, XX, t(2;14)(q23;q24) | SA, SA, SA | IVF/±ICSI/PGD | Monozygotic twins (46,XY), with hydronephrosis |
| 46, XX, t(2 ;14)(q31 ;q24) | Livebirth, SA, SA | IVF/±ICSI/PGD | Did not conceive |
| Coonen et al, 2000[15] | | | |
| 46, XY, t(3;11)(q27.3;q24.3) | SA, SA, SA, SA, GT (unbalanced, 46, XX, -11, +der(11) t(3;11)(q27.3;24.3)pat), SA, SA, SA | IVF/PGD | Term (balanced 46, XX, t(3;11)(q27.3;q24.3)pat) |
| 46, XX, t(3;11)(q27.3;q24.3) | SA, SA, fetal demise (24 wk, hydropic (46, XX, -11, +der(11) t(3 ;11)(q27.3 ;q24.3), SA, SA, SA, SA | IVF/PGD × 3 cycles | Did not conceive, No transfer done × 2 cycles |
| Simopoulou et al, 2003[23] | | | |
| 46, XX, t(16;17)(p13,3;p11,1) | SA, SA, SA, SA | IVF/PGD | Biochemical SA |
| 46, XX, t(8;12)(q11.2;q12) | SA, SA, SA, SA, SA | IVF/PGD | Term |
| 46,XY, t(1;18)(p32;q23) | SA, SA | IVF/PGD | Did not conceive |
| 46, XX, t(1;2)(q42.1;p23) | SA,SA,SA | IVF/PGD | Did not conceive |
| Sugiura-Ogasawara et al, 2008[4] | | | |
| 46, XX, t(6;8)(q23;p23) | SA, SA | IVF/PGD | SA |
| 46, XY, t(10;16)(p14;q12.2) | SA, SA | IVF/PGD | Did not transfer (single embryo unbalanced) |
| Wiland et al, 2008[24] | | | |
| 46, XY, t(2;7)(p11.2;q22.1) | SA, SA | IVF/PGD | Triplet pregnancy (monochorionic diamniotic twins and a singleton) selective reduction of twins (46, XY, t(2;7)(p11.2;q22.1), term (46, XX) |

SA, spontaneous abortion or miscarriage; GT, genetic termination.

embryo morphology. Following FISH, chromosomally balanced embryos were transferred on day 4. For the first case, seven embryos were biopsied, four were balanced, and three were transferred; a biochemical pregnancy loss resulted. For the second case, 13 embryos were biopsied, 2 were balanced, and 1 was transferred; a normal live birth resulted. For the third case, 11 were biopsied, 2 were balanced and both transferred; pregnancy did not occur. For the fourth case, 12 embryos were biopsied, 1 was balanced and frozen due to hyperstimulation during the IVF cycle. The embryo was subsequently transferred in a frozen cycle; pregnancy did not occur.

In 2008, Suguira-Ogasawara et al[4] published two cases that met criteria for the IVF/PGD group; mean maternal age was 35.5 ± 7.8 years. IVF/PGD details are not provided. The first case was noted to be a PGD failure. The second case did not result in an embryo transfer because the single embryo was unbalanced.

In 2008, Wiland et al[24] published one case that met criteria; maternal age was 29 years. Following a standard FISH protocol, a rehybridization occurred screening for the common aneuploidies of chromosomes 21, X, and Y. Nine embryos were biopsied and three were balanced, of which two were transferred. A triplet pregnancy resulted, with monozygotic twins and a singleton pregnancy. CVS was performed and then twins who were males were balanced chromosome carriers. Selective reduction to singleton was performed, and a live birth resulted.

### Summary of In Vitro Fertilization/ Preimplantation Genetic Diagnosis Management (Table 4)

Twenty cases from nine publications met the criteria for the IVF/PGD group. There were an estimated 86 prior pregnancies, of which 82 were miscarriages <10 weeks, which equates to a mean of 4.1 miscarriages per carrier (range: 2–15). In addition there were two live births, one fetal demise, and one genetic termination.

Using the first subsequent pregnancy only, the subsequent live birth was 35% (7 of 20) per carrier and 32% (7 of 22) per IVF cycle starts. If the carrier was female, the subsequent live birthrate was 33% (5 of 15), and if the carrier was male, 33% (2 of 6). The cumulative live birthrate was the same as the first subsequent pregnancy rate. Of note, the cumulative live birthrate per transfer rate was 41% (7 of 17).

### Cytogenetic Analyses of Prior and Subsequent Miscarriages

Only 21 miscarriages were karyotyped before medical or IVF/PGD management. In the medical management group, 33% (4 of 12) were unbalanced. In the IVF/PGD group, 78% (7 of 9) were unbalanced.

Fifty subsequent miscarriages were karyotyped. In the medical management group, 55% (27 of 49) were unbalanced, 29% (14 of 49) were 46,XX, 46,XY or balanced, and 16% (8 of 49) were trisomic, monosomic, or polyploid. There was only one subsequent miscarriage karyotyped in the IVF/PGD group; it was balanced.

## DISCUSSION

RPL is a devastating problem affecting ~5% of couples trying to conceive. Pathogenesis of RPL is believed to be multifactorial including genetic, anatomical, autoimmune factors, alloimmune, infection, endocrine disturbances, and idiopathic.[6,26,27] Genetic factors include numeric chromosome errors in miscarriages, which are usually random events that increase in frequency with advancing maternal age.[28] Parental carriers of a structural chromosome rearrangement account for 2.5 to 7.8% of couples with RPL.[2–6,27,29] Reciprocal translocations is estimated to represent 60% of the structural chromosome rearrangement cases.[30] Yet whether an unbalanced translocation is the cause of a miscarriage is often not known because chromosome testing of miscarriages is not performed routinely.

Published data suggest that carriers of a reciprocal translocation may have poorer pregnancy outcomes than carriers of a Robertsonian translocation.[5,7] Therefore, it is of utmost importance to have evidence-based management strategies for such carriers.

This systematic review of carriers with a reciprocal translocation, ascertained on a history of RPL, suggests that the cumulative live birth is higher with medical management than IVF/PGD. It is difficult, however, to compare pregnancy outcomes directly.[31] With medical management, pregnancy is usually defined by a positive hCG. With IVF/PGD, pregnancy is usually defined by the presence of a gestational sac on ultrasound, which would result in an underestimation of miscarriage rate because biochemical pregnancy losses would not be included. In addition, with IVF/PGD, studies often report pregnancy outcome per embryo transfer, rather than per cycle started. With reporting per cycle started, the time and cost of repeated IVF/PGD cycles, in which embryo transfer did not occur, is not taken into consideration.

Despite the widespread use of IVF/PGD for carriers of a translocation,[32–34] we were only able to identify 20 cases that met inclusion criteria in this systematic review. Individual obstetric histories were inconsistently reported in the IVF/PGD articles. Surrogate end points, such as miscarriage rate, rather than live birthrate, were often reported in the IVF/PGD studies, which again is problematic.[35,36] Simply comparing miscarriages rates, before ascertainment of carrier status (which would be

**Table 4 Comparison of Prior and Subsequent Pregnancy Outcomes in Carriers of a Reciprocal Translocation Ascertained on the Basis of Recurrent Pregnancy Loss: In Vitro Fertilization/Preimplantation Genetic Diagnosis**

| | | Prior Pregnancies | | IVF/PGD Outcomes | | | |
|---|---|---|---|---|---|---|---|
| | No. of Carriers | Unsuccessful | Successful | No. of Cycles | Did Not Conceive | Unsuccessful Pregnancies | Successful Pregnancies |
| Munné et al, 1998[19] | 3 | 14 miscarriages | 1 | 3 | 1 | 0 | 2 (twins; ongoing pregnancy) |
| Munné et al, 1998[21] | 1 | 2 miscarriages* | 0 | 1 | 0 | 1 miscarriage | 0 |
| Willadsen et al, 1999[25] | 2 | 6 miscarriages† | 0 | 2 | 1 | 0 | 1 (preterm) |
| Munné et al, 2000[20] | 3 | 22 miscarriages | 0 | 3 | 3 | 0 | 0 |
| Escudero et al, 2000[16] | 2 | 5 miscarriages | 1 | 2 | 1 | 0 | 1 (monozygotic twins, hydronephrosis) |
| Coonen et al, 2000[15] | 2 | 13 miscarriages, 1 fetal demise, 1 genetic termination | 0 | 4 | 3 | 0 | 1 (term) |
| Simopoulou et al, 2003[23] | 4 | 14 miscarriages | 0 | 4 | 2 | 1 miscarriage (biochemical) | 1 (term) |
| Sugiura-Ogasawara et al, 2008[4] | 2 | 4 miscarriages | 0 | 2 | 1 | 1 miscarriage | 0 |
| Wiland et al, 2008[24] | 1 | 2 miscarriages | 0 | 1 | 0 | 0 | 1 (triplet reduction, singleton term) |
| **Totals** | **20 carriers** | **98% total prior pregnancies** (82 miscarriages, 1 fetal demise, 1 genetic termination) | **4% total prior pregnancies** (2 successful pregnancies) | **22 cycles** | **12 did not conceive** | **3 miscarriages** | **7 successful pregnancies** |

*Estimated, based on "repeated pregnancy loss."
†Estimated, based on "habitual abortion" and "recurrent miscarriage."
IVF/PGD, in vitro fertilization/preimplantation genetic diagnosis.

100% for women with primary RPL) to subsequent,[10,12] is highly biased in favor of intervention, and therefore it is inappropriate to conclude benefit. The IVF/PGD studies were often descriptive, with reporting of embryo number, chromosomal determination, and only successful transfers.[20,33] Other recent systematic reviews of IVF/PGD for carriers of a structural chromosome with a history of recurrent miscarriage[37] and unexplained recurrent miscarriage[38] have not shown benefit with this strategy, compared with medical management.

There remains concern for carriers of a reciprocal translocation of having an ongoing pregnancy or live birth with an unbalanced rearrangement. In this review, only 1 of >100 patients had a ongoing pregnancy with an unbalanced reciprocal translocation[5]; other pregnancies with unbalanced reciprocal translocations ended in miscarriage. Therefore, the reason for ascertainment appears to be important. In this systematic review, the ascertainment was RPL, which appears to have a more favorable prognosis than a history of an ongoing pregnancy or live birth with an unbalanced translocation.

Goddijn et al,[3] in a retrospective analysis of 1324 Dutch couples, identified 51 carriers of structural chromosome rearrangement, of which 63% were reciprocal translocation. A nested case-control study of 41 of the 51 couples revealed no unbalanced structural chromosome rearrangements in subsequent ongoing pregnancies. Amniocentesis was performed on 26 of the 41 ongoing pregnancies; all were euploid, with 58% 46,XX or 46,XY, and 42% balanced structural chromosome rearrangements.

This study was designed to review systematically the effectiveness of management strategies for carriers of a reciprocal translocation involving two chromosomes, ascertained on the basis of RPL. We identified a total of 129 carriers who met the entry criteria. In the medical management group, using the first pregnancy after evaluation, the subsequent live birthrate was 60% (65 of 109). Using all subsequent outcomes, the cumulative live birthrate was 74% (81 of 109 cases) in the medical management group. In the IVF/PGD group, the subsequent live birthrate per cycle started was 35% (7 of 20); the cumulative live birthrate was the same.

Unfortunately, the published data are limited, and there are differences in the reporting of the data in each group, as previously discussed. It is difficult to directly compare outcomes for these two management strategies because of the different end points reported. Understanding the differences is essential for effective counseling. Until a well-designed study comparing the two strategies is performed, or at least prospective cohort studies with strict entry criteria and definitions, the cumulative experience and success of both medical management and IVF/PGD must be used for counseling of patients who are carriers of a reciprocal translocation, ascertained on the basis of RPL.

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# Increase in the plasma levels of protein Z-dependent protease inhibitor in normal pregnancies but not in non-pregnant patients with unexplained recurrent miscarriage

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## Summary

Protein Z (PZ)-dependent protease inhibitor (ZPI) is a serine protease inhibitor which efficiently inactivates activated factor X, when ZPI is complexed with PZ in plasma. Reduced plasma levels of ZPI and PZ have been reported in association with thrombosis. It has also been reported that PZ increases during pregnancy and that its partial deficiency is related to early pregnancy loss or recurrent miscarriage (RM). However, until now there has been no report on ZPI in pregnancy. To explore the possible role(s) of ZPI in the maintenance of pregnancy, we studied 42 non-pregnant normal women, 32 women with normal pregnancies, and 134 cases of unexplained RM in Japan, as well as 64 non-pregnant normal German females. Plasma ZPI was measured by in-house ELISA. There were significantly higher concentrations of plasma ZPI in normal pregnancies compared to non-pregnant women. The present study also confirmed that both factor X, the major target of ZPI, and protein Z increased during normal pregnancies. This increased ZPI and PZ may counteract the increased activated factor X, which may in turn contribute to the maintenance of normal placental circulation. Plasma ZPI levels were unchanged in non-pregnant RM women, while the plasma PZ level was slightly reduced, a finding consistent with existing reports. The exact relationship between RM and this unaltered ZPI with mild PZ reduction relative to normal pregnancies warrants further investigation.

## Keywords

Anti-coagulation system, protein Z-dependent protease inhibitor, protein Z, pregnancy, recurrent miscarriage

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## Introduction

Protein Z (PZ) is one of seven vitamin K-dependent coagulation proteins (VKDPs) with structural homology to coagulation factors VII, IX (FIX), and X (FX), and protein C (1). It serves as a cofactor for the inactivation of activated FX (FXa) by a serine protease inhibitor called PZ-dependent protease inhibitor (ZPI) (2). Since normal plasma contains excess ZPI relative to the amount of PZ, all the PZ is bound in a complex with ZPI (3); thus there is some free ZPI in normal plasma. While the ZPI-PZ complex efficiently inhibits FXa (4–6), free ZPI inactivates activated factor XI (FXIa) (5) as well as activated FIX (FIXa) to some extent (7).

PZ is considered to be synthesised mainly by the liver since its plasma levels were reduced in patients with chronic liver disorders (8). In addition, a liver-enriched transcription factor plays a crucial role in human *PZ* gene expression (9). The liver is also a major source of ZPI, the mRNA of which is most abundant in this organ (10). To our knowledge, the regulation of human *ZPI* gene expression has not been reported as yet.

Although the ZPI-PZ system seems to play a role(s) in anti-coagulation via the inactivation of FXa (and FXIa and FIXa) *in vitro*, the clinical significance of PZ for venous and arterial thrombosis, *in vivo*, remained controversial (11,12). Recently, a meta-analysis showed that low PZ levels were associated with an increased risk of thrombosis such as arterial vascular disease and venous thromboembolic disease, as well as with pregnancy complications (13).

The first patient with severe congenital PZ deficiency developed not only deep-vein thrombosis but also miscarriage (14). Recently, a relative/partial deficiency of PZ (PZ levels below 1.0–1.2 μg/ml=16–19 nM, in general) has been linked to serious complications with pregnancy (15, 16), such as early fetal loss, preterm delivery, etc.

In contrast to the numerous reports on PZ levels, there are only three existing publications describing plasma ZPI levels: one recent

report cited a significant relation between low ZPI levels and peripheral arterial disease (17), while another failed to find an association between low ZPI levels and venous thrombosis (18); still another found no difference in ZPI levels between normal controls and patients with anti-phospholipid antibodies (19).

In the present study, we have explored the possible contribution of ZPI (and PZ) to the stages of gestation in humans, as well as to recurrent miscarriage (RM).

## Methods

### Subjects

This study was performed with the approval of our Institutional Review Board. The work was conducted entirely in accordance with the Declaration of Helsinki. Informed consent was obtained from all individuals. One-hundred thirty-four non-pregnant Japanese women who had experienced RM, defined as two or more consecutive miscarriages (less than 22 weeks of gestation), participated in the present study. None of these patients had any readily identifiable causes of RM, such as uterine anomaly or chromosomal abnormality in either partner; however, there were five cases with anti-phospholipid syndrome. None had a history of thrombosis. We also studied 32 normal pregnant Japanese women without pregnancy complications or a history of RM, as well as 42 non-pregnant Japanese women with a history of normal pregnancy and without a history of apparent pregnancy losses, as controls, and 64 non-pregnant normal German females. None of these subjects was using contraceptives or hormone-containing drugs including oestrogen or progesterone. Demographical data of individuals involved in this study are shown in Table 1.

### Blood

Blood samples were collected and anticoagulated with 3.8% sodium citrate in a ratio of anticoagulant to blood of 1:9, and plasma samples were obtained by centrifugation at 1,000g for 10 minutes (min) at 4°C, and stored at –80°C until analysis.

### ELISA

An immunoassay for ZPI was performed using an in-house ELISA system. A rabbit anti-human ZPI polyclonal antibody was coated in a microtitre plate. Wells were washed with Tris-buffer, and plasma samples diluted 1/250 and 1/500 were applied and incubated for 120 min. After washing, a biotin-conjugated rabbit anti-human ZPI polyclonal antibody was added and incubated for 90 min. After washing, streptavidin-horseradish peroxidase (GE Healthcare, Little Chalfont, UK) was added and incubated for 60 min. After final washing, 3, 3', 5, 5'-tetramethylbenzidine (Bio-Rad, Hercules, CA, USA) was added, and the reaction was stopped after 10 min by adding 1 M $H_2SO_4$. Absorbance at 450 nm was recorded by a microtitre plate reader Biolumin 960 (Molecular dynamics, San Diego, CA, USA) and compared to a standard curve, using recombinant ZPI expressed in baby hamster kidney cells transfected with a ZPI cDNA. Recombinant ZPI was purified as reported by Han et al. (4), and its amount was determined by both biuret reaction using a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA) and densitometry of Coomassie brilliant blue staining of a sodium dodecyl sulfate-polyacrylamide gel after electrophoresis using bovine serum albumin as a standard protein. The ZPI concentration of a pooled normal plasma (obtained from seven healthy individuals) was determined using recombinant ZPI, and a standard curve was made every time using the pooled plasma as a reference for this assay (the range of 9–36 fmol, precision; ± 7%, lower limit of detection; 7 fmol, co-efficient of variation; 13.6%).

PZ antigen levels in plasma were measured by commercial sandwich ELISA methods, ZYMUTEST RK031A (Hyphen Biomed, Eragny, France), according to the manufacturer's instructions.

### FX assay

FX in plasma was determined using a Thrombocheck FACTOR X kit (Sysmex, Kobe, Japan), according to the manufacturer's instructions.

### Statistical analysis

Results are presented as medians and inter-quartile ranges in nM of three assays, and were analysed using the software program JMP ver. 6.0.3 (SAS Institute, Cary, NC, USA) by non-parametric (Wilcoxin/Kruskal-Wallis or Mann-Whitney) tests. A p-value less than 0.05 was considered statistically significant.

## Results

### Plasma concentrations of ZPI and PZ among non-pregnant normal Japanese females

Since what constitutes normal concentrations of both ZPI and PZ in plasma has not been determined among Japanese, we first developed an ELISA system for ZPI, as described in *Methods*. Medians (interquartile ranges, IQR) of plasma ZPI and PZ levels were 51.8 (45.1–59.6) and 29.9 (23.3–41.0) nM, respectively (Fig. 1A, B), among 42 healthy non-pregnant Japanese females aged 34.3 ± 4.3 years (mean ± SD).

Sixty-four non-pregnant healthy German females aged 28.1 ± 6.7 showed ZPI and PZ levels as 59.5 (54.4–63.3) and 35.8 (27.1–49.7) nM, respectively. Accordingly, the plasma ZPI and PZ levels in normal Japanese females were lower than those in German females ($p<0.001$ for ZPI and 0.058 for PZ, respectively; Fig. 1 A, B), among those individuals whose data we examined. No effect of age was seen on either ZPI or PZ levels in both normal Japanese females ($R^2=0.05$, $p=0.67$; $R^2=0.08$, $p=0.076$, respectively; see Suppl. Fig. 1A, available online at www.thrombosis-online.com) and normal German ($R^2=0.004$, $p=0.62$; $R^2=0.005$, $p=0.58$, respectively; Suppl. Fig. 1B, available online at www.thrombosis-online.com).

Since all the PZ is bound in a complex with ZPI in the circulation (2, 3), the concurrent change in concentrations of plasma ZPI and PZ suggest that levels of these two proteins are related (17–19). As expected, regression analysis demonstrated a linear relationship between plasma ZPI levels and PZ levels in normal non-pregnant Japanese controls ($R^2=0.28$, $p<0.001$; Fig. 2A). This is also true in normal non-pregnant German females ($R^2=0.23$, $p<0.0001$; Fig. 2B).

## Plasma levels of ZPI and PZ during normal pregnancy

Blood samples were obtained from 32 women with normal pregnancy (aged 32.8 ± 4.9 years) during the 1st (9.1 ± 1.4 weeks of gestation, WG), the 2nd (22.3 ± 1.4 WG), and the 3rd trimesters (32.8 ± 1.8 WG), as well as during the puerperal period (Puerp.; 5.3 ± 6.0 weeks; 3.8 ± 1.5 weeks, when two individuals are excluded). To the best of our knowledge, there has been no report on plasma ZPI levels during pregnancy.

Plasma ZPI levels significantly increased in the 1st trimester when compared with 42 non-pregnant normal controls (Fig. 3A). When these pregnant individuals were recruited in the longitudinal study, blood samples of all 32 participants were available for all study time points, i.e. the 1st, 2nd and 3rd trimesters, and the puerperal period. The median ZPI value increased significantly from the 1st to 2nd trimesters (Fig. 3A). Following delivery (Puerp.), ZPI concentrations fell significantly ($p<0.01$), although the median value of this period did not return to that of non-pregnant controls, most likely because blood samples of the participants were collected too soon after delivery (3.8 weeks for 30 individuals).

When each individual's plasma ZPI level was consecutively measured, 29 among 32 women demonstrated a steady increase with gestational age (Suppl. Fig. 2A, available online at www.thrombosis-online.com). However, the remaining three showed reduced ZPI levels, i.e. the average of ZPI values of the 2nd and 3rd trimesters was lower than that of the 1st trimester for these three subjects.

Plasma concentrations of PZ significantly increased from the 2nd trimester and continued rising throughout the rest of the pregnancy (Fig. 3B). When individuals' plasma PZ levels were consecutively measured, 31 among 32 women demonstrated a steady increase in these levels in relation to their gestational age (see Suppl. Fig. 2B, available online at www.thrombosis-online.com).

**Table 1: Demographical data of individuals involved in the present study.**

| | Controls | Normal pregnancies | RM cases |
|---|---|---|---|
| No. of participants | 42 | 32 | 134 |
| Age (mean ± SD) | 34.2 ± 4.3 | 32.8 ± 4.9 | 32.1 ± 4.1 |
| Age (range) | 24–42 | 22–41 | 24–42 |
| Overall No. of pregnancies | -- | 38 | 369 |
| No. of pregnancy losses | 0 | 9 | 327 |
| No. of pregnancy loss per participant | 0 | 0.28 | 2.4 |
| Partus per participant | 1.7 | 0.56 | 0.18 |

No., number.

Maternal age did not influence ZPI and PZ levels ($R^2=0.023$, $p=0.09$; $R^2=0.0001$, $p=0.90$, respectively; data not shown), as in non-pregnant normal controls (see Suppl. Fig. 1A, B, available online at www.thrombosis-online.com).

A linear regression analysis demonstrated a significant relationship between levels of plasma ZPI and plasma PZ during normal pregnancy ($R^2=0.27$, $p<0.001$; see Fig. 3A, available online at www.thrombosis-online.com), which is similar to the non-pregnant normal women as described above. When stratified by gestation stages, there was also a significant correlation during the 2nd trimester and Puerp. ($R^2=0.23$, $p=0.005$ and $R^2=0.31$, $p=0.001$, respectively; see Suppl. Fig. 3A, available online at www.thrombosis-online.com), while a trend toward a positive correlation was observed during the 1st and 3rd trimesters ($R^2=0.12$, $p=0.052$ and $R^2=0.11$, $p=0.059$, respectively; Suppl. Fig. 3A, available online at www.thrombosis-online.com).

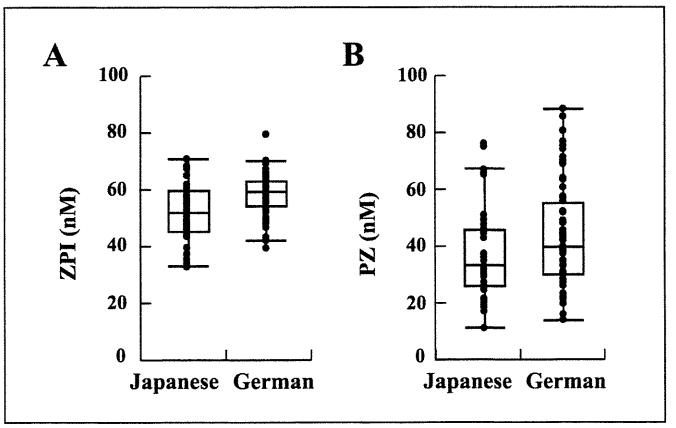


**Figure 1: Plasma ZPI (A) and PZ (B) levels in non-pregnant normal Japanese and German female controls.** Plasma concentrations of ZPI and PZ were measured by ELISA methods. Data are presented as box-plots of medians with ranges of 25–75% and whiskers for ranges of 10–90%, and outliners are also included. A statistically significant difference was observed for ZPI between normal Japanese (n=42) and German (n=64) controls ($p<0.001$), while a trend toward a PZ lower than German was found in Japanese ($p=0.058$).

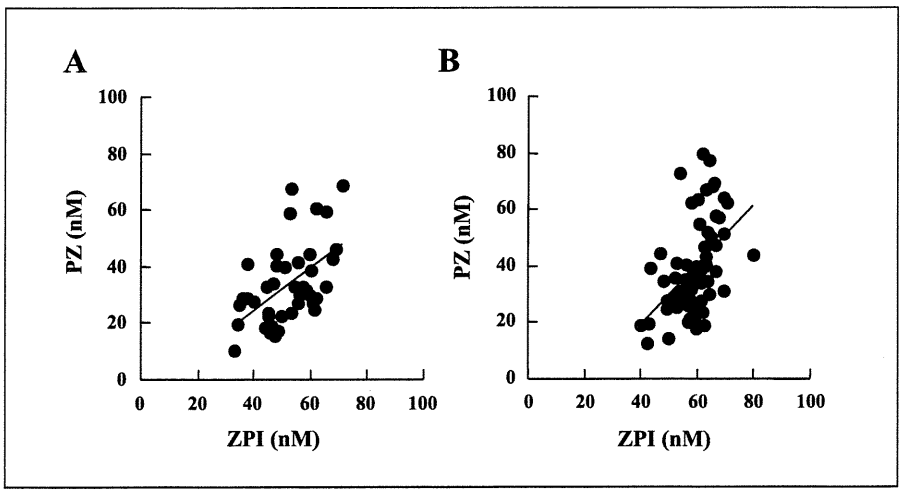


**Figure 2: Correlation analysis between plasma ZPI and PZ levels in non-pregnant Japanese (A) and German (B) controls.** Statistically significant correlations were obtained for normal Japanese (n=42) and German (n=64) controls ($R^2$=0.28 and 0.23, respectively; solid lines; p<0.001 and <0.0001, respectively).

## Plasma ZPI levels and PZ concentrations in patients with RM

ZPI levels in non-pregnant patients with RM were similar to those in non-pregnant normal women (p=0.30) (Fig. 4A). In addition, plasma PZ levels in non-pregnant patients with RM were significantly lower, when compared with non-pregnant normal women (p=0.03) (Fig. 4B). Nevertheless, a strong linear relationship between the plasma levels of ZPI and PZ was demonstrated in patients with RM ($R^2$=0.34, p<0.0001; Suppl. Fig. 3B). Age did not affect ZPI and PZ levels (data not shown).

## Discussion

Our present results indicate that the plasma ZPI levels are slightly lower in Japanese than in German individuals. In contrast, PZ levels were similar in Japanese and German individuals. It is important to note that the range of ZPI levels in the plasma samples of both Japanese and German control subjects was much narrower than the results for PZ ([20]; and the current study).

The strong positive correlation between ZPI and PZ levels was confirmed both in normal non-pregnant Japanese and German individuals by the current study. It was reported that the increase in plasma PZ after discontinuation of warfarin therapy was associated with a rise in the ZPI level (18). Al-Shanqeeti, et al. postulated that the rise in ZPI levels following discontinuation of warfarin may be related to a possible effect(s) of PZ, one of the vitamin K-dependent proteins, on ZPI catabolism including clearance of the ZPI-PZ complex, or the synthesis, secretion, or extra-vascular compartmentalisation of ZPI may be affected by PZ (18), or both. The exact mechanism of the inter-dependence of the plasma levels of ZPI and PZ will be explored in the future.

It is likely that the newly discovered increase in concentrations of ZPI during normal pregnancy is caused by the enhanced biosynthesis of this protein by the liver and/or its retarded clearance from the circulation, which might be related to a change in its post-

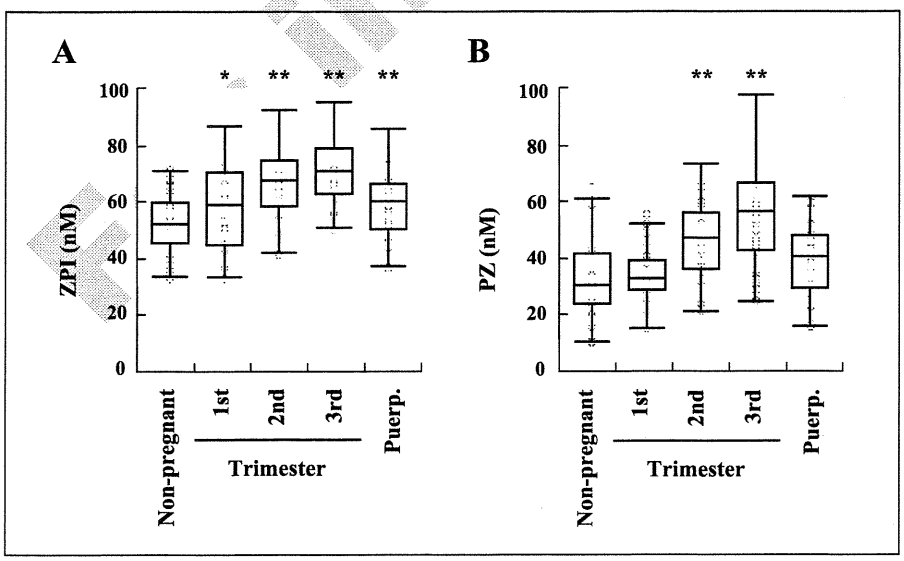


**Figure 3: Plasma ZPI (A) and PZ (B) levels in Japanese women with normal pregnancy and in non-pregnant controls.** Data are presented as described in Figure 1. Statistically significant differences were observed for both ZPI and PZ between each gestational period of women with normal pregnancy versus non-pregnant controls. *, p<0.05; **, p<0.01 (vs. non-pregnant control).

translational modification. The suggestion that the placenta could be the source of increased ZPI was excluded because no ZPI mRNA was detected by Northern blot analysis (10). Hormones which increase during pregnancy, such as oestrogen, progesterone, human placental lactogen, prolactin, etc., may enhance the production of ZPI. The placenta could be important as a source of placental hormones but not of ZPI itself, as discussed above.

Plasma concentrations of PZ also significantly increased as pregnancy advanced in the present study, which is in good agreement with previous reports in Caucasians (21, 22). A positive correlation between increased levels of plasma ZPI and PZ was also observed during all periods of normal pregnancy. It was reported that significantly higher levels of both ZPI and PZ were observed in women taking oral contraceptives (18), suggesting that oestrogen (and progesterone) has a positive effect on the synthesis of both ZPI and PZ, or that one of these two proteins affects the other. In our hands, however, hormone replacement therapy did not significantly influence either ZPI or PZ concentrations four weeks after the treatment in 15 cases (unpublished data).

In addition to their increased biosynthesis, the parallel change in ZPI and PZ levels may be attributable to the body's concomitant consumption of ZPI and PZ associated with the hyper-coagulability of normal pregnancy (19, 23). ZPI is consumed by FXa during coagulation, at least *in vitro*, and PZ is also digested by thrombin (20).

Nevertheless, our present study also demonstrates a more abrupt increase in ZPI over PZ, suggesting that distinct mechanisms are, at least in part, responsible for the elevation of these two gene products during normal pregnancy.

Since normal pregnancy is accompanied by increases in a number of coagulation factors including FX (24), which is one of the VKDPs and highly homologous to PZ (1), it was measured in pregnant and non-pregnant Japanese individuals. FX significantly increased during the 2nd and 3rd trimesters (see Suppl. Fig. 4, available online at www.thrombosis-online.com), as reported previously by Stirling et al. (25), the pattern of which closely resembles that of PZ. In contrast, FX did not significantly alter in RM cases (see Suppl. Fig. 5, available online at www.thrombosis-online.com). Accordingly, the increased ZPI and PZ levels during normal pregnancy may counterbalance the increased coagulation factors, especially FX, and regulate the hyper-coagulable state in pregnancy (24).

The fairly unchanged ZPI levels and slightly reduced PZ levels in non-pregnant RM was found in the present study. Inter-villous and/or spiral-artery thrombosis caused by ZPI or PZ deficiency may be associated with inadequate placental perfusion, resulting in complications of pregnancy. It is noteworthy that 35% of the offspring of ZPI-null were lost when their heterozygous adults were mated (26). These results suggest that ZPI deficiency would be a modest risk factor for miscarriage, at least in mice. These studies implicate the role(s) of the ZPI in the maintenance of normal pregnancy, in particular concerning placental function.

Enhanced clearance and/or hyper-consumption of ZPI and PZ caused by their autoantibodies could also lead to their secondary deficiency. Along this line, it is interesting that there was a good inverse correlation between anti-PZ IgM antibody levels and PZ concentrations in a subgroup of patients with recurrent embryo losses and PZ deficiency (27). However, anti-PZ antibody levels did not correlate with plasma PZ concentrations in controls and patients with pathologic pregnancies (27, 28). Most recently, it has also been reported that elevated anti-PZ IgG and IgM titers were seen in patients with idiopathic RM (29). In the case of ZPI, there is no report on its auto-antibodies, to our best knowledge.

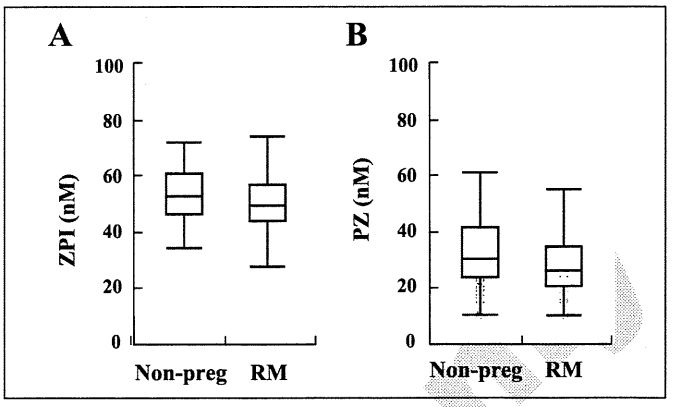


**Figure 4: Plasma ZPI (A) and PZ (B) levels in patients with RM.** No statistically significant difference was observed for ZPI (p=0.30), while there was a statistical difference in PZ (p=0.03) between patients with RM (n=134) vs. non-pregnant controls (n=42).

The limitations of this study include the relatively small number of RM patients evaluated. Therefore, further studies are needed to elucidate the relevance of relative ZPI deficiency in abnormal pregnancy, e.g. by recruiting many more RM cases.

In conclusion, this is the first report on ZPI levels during normal pregnancy (as well as in cases of non-pregnant RM), and we postulate that the elevated plasma concentrations of ZPI may be important for restoring the shifted balance of fibrinolysis and coagulation toward hemostasis during normal pregnancy.

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## What is known about this topic?

- Protein Z (PZ)-dependent protease inhibitor (ZPI) is a serine protease inhibitor which inactivates activated factor X.
- However, the clinical significance of low plasma ZPI levels in thrombosis and pregnancy abnormalities remains to be established.

## What does this paper add?

- Plasma ZPI increases during normal pregnancies and quickly decreases after delivery.
- Plasma ZPI remains unaltered in non-pregnant women who had experienced recurrent miscarriage.

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### Conflict of interest

None declared.

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# *SYCP3* mutation may not be associated with recurrent miscarriage caused by aneuploidy

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**BACKGROUND:** *SYCP3* mutations have been shown to generate an aberrant synaptonemal complex in a dominant-negative manner and to contribute to abnormal chromosomal behavior that might lead to recurrent miscarriage. We examined whether *SYCP3* mutation is associated with recurrent miscarriage caused by embryonic aneuploidy.

**METHODS:** The *SYCP3* 657T>C mutation was examined using PCR and sequencing in 101 patients with a history of three or more unexplained recurrent miscarriages and 82 fertile controls with no history of miscarriage. The embryonic karyotype in the aborted conceptus was analyzed.

**RESULTS:** The 657T>C mutation of *SYCP3* was identified in one patient with a history of six recurrent miscarriages with embryonic euploidy and one fertile woman in the control group. Patients with abnormal and normal chromosome were found to repeat miscarriage with abnormal and normal chromosome, respectively.

**CONCLUSIONS:** The 657T>C mutation of *SYCP3* may not be associated with recurrent miscarriage caused by aneuploidy. We found no clinical significance of routine examination of the *SYCP3* mutation because only one benign mutation was ascertained in 101 patients.

**Key words:** SYCP3 / recurrent miscarriage / fetal chromosome / meiosis / polymorphism

## Introduction

*SYCP3* mutations in women were found to generate an aberrant synaptonemal complex in a dominant-negative manner and to contribute to abnormal chromosomal behavior that might lead to recurrent miscarriage (Bolor *et al.*, 2009). Bolor *et al.* (2009) found *SYCP3* mutations in 2 of 26 (7.7%) patients with recurrent miscarriage. SYCP3 is a DNA-binding protein and a structural component of the synaptonemal complex, which mediates the synapsis or homologous pairing of chromosomes during meiosis of the germ cells. Male mice homozygous for the null mutation of the *Sycp3* gene are sterile as a result of massive apoptotic cell death in the testis during meiotic prophase (Yuan *et al.*, 2000). *Sycp3*-deficient female mice are subfertile with a severely reduced oocyte pool. Although two-thirds of mouse offspring are healthy, one-third is affected by aneuploidy and succumbs during development *in utero* (Yuan *et al.*, 2002). This is consistent with the observations that in humans, a mutation in *SYCP3* was identified in two patients with azoospermia (Miyamoto *et al.*, 2003), and that the lack of *SYCP3* gene expression in human testis may have a negative effect on spermatogenesis and male fertility (Aarabi *et al.*, 2006).

The identifiable causes of recurrent miscarriage may include abnormal chromosomes in either partner (particularly translocations), antiphospholipid antibodies (aPL) and uterine anomalies (Farquharson *et al.*, 1984; Sugiura-Ogasawara *et al.*, 2004, 2010). A currently prevailing hypothesis is that recurrent miscarriage may be a polygenetic disorder associated with both genetic and environmental determinants. Polymorphisms related to thrombophilia, such as Leiden mutation and prothrombin mutation, are known to be associated with recurrent miscarriage, although the mutations are not found in the Asian population (Nelen *et al.*, 1996; Rey *et al.*, 2003; Rai and Regan, 2006; Suzumori and Sugiura-Ogasawara, 2010). However, whether Factor V Leiden and Factor II prothrombin polymorphisms are risk factors for recurrent miscarriage is controversial (Coulam *et al.*, 2006; Goodman *et al.*, 2006).

An abnormal embryonic karyotype causes not only sporadic spontaneous abortion but also recurrent miscarriage because it was found in 51% of recurrent cases (Ogasawara et al., 2000; Carp et al., 2001). Bolor et al. (2009) could not prove an association between the *SYCP3* mutations found in 7.7% of patients and embryonic aneuploidy.

Preimplantation genetic screening (PGS) for aneuploidy has been performed widely; however, there is no evidence of its ability to improve delivery rates (Platteau et al., 2005; The ACOG., 2009; Harper et al., 2010). The *SYCP3* mutation might be a candidate for selection of cases for PGS if an association between the mutation and aneuploidy is established. Also, the 7.7% frequency of *SYCP3* mutation is relatively high because the frequency of translocations, aPL and major uterine anomalies is 4.5% (Sugiura-Ogasawara et al., 2004), 10.7% (Balasch et al.,1990) and 3.2% (Sugiura-Ogasawara et al., 2010), respectively. Here we investigate whether *SYCP3* mutations may be associated with recurrent miscarriage caused by aneuploidy.

# Materials and Methods

## Patients

All patients underwent a systematic examination, including hysterosalpingography, chromosome analysis for both partners, determination of aPL, including lupus anticoagulant and β2glycoprotein I-dependent anticardiolipin antibodies (Ogasawara et al., 1996), and blood tests for hyperthyroidism, diabetes mellitus and hyperprolactinemia before subsequent pregnancy in Nagoya City University Hospital between 2007 and 2010. A blood sample was taken at the examination and frozen at −70°C before analysis. Patients with identifiable causes of miscarriage, such as translocations, aPL and uterine anomalies, were excluded. The 81 patients for whom a previous or subsequent embryonic (or fetal) karyotype was ascertained at least one time were studied. A further 20 patients for whom the embryonic karyotype was unknown were added.

In Japan, miscarriage is defined as loss within 22 weeks gestation and stillbirth is defined as loss at 22 or more weeks of gestation. Stillbirths after 22 weeks gestation were included in the present study and shown as prior history in Table I.

A total of 101 patients with a history of three or more (3–16) unexplained consecutive first-trimester miscarriages were examined. Subsequent pregnancies were followed up until October 2010. The mean age of participants at examination was 34.4 ± 3.8 years, and the average number of previous miscarriages was 3.8 ± 2.7. Twenty-four patients had a history of live birth and two patients experienced recurrent miscarriage after changing partner, having had a live birth by a previous partner. The mean number of previous live births was 0.27 ± 0.5.

Gestational age was calculated based on basal body temperature charts. Ultrasonography was performed once a week from 4 to 8 weeks of gestation. Dilation and curettage was carried out when miscarriages were diagnosed, and the karyotypes of aborted conceptuses were determined using a standard G-banding technique.

The 82 fertile women with no history of recurrent miscarriage and complications of pregnancy were examined as controls. The fertile controls were recruited in Nagoya City University Hospital and Asamoto Women's Clinic. The mean age of women in the control group was 32.3 ± 6.2 years, and the average number of deliveries was 1.53 ± 0.6.

The study was approved by the Research Ethics Committee of Nagoya City University Graduate School of Medical Sciences. Each patient provided their written consent after full disclosure about the purpose and methods to be employed.

## DNA analysis

Genomic DNA was extracted from peripheral blood samples with the Midi Blood DNA Extraction kit (Qiagen, Tokyo, Japan). Oligonucleotide primers were designed to amplify each coding sequence, as well as exon–intron boundaries of the human *SYCP3* gene, encompassing exons 7–9 (GenBank accession number NM_153694). The sense and antisense PCR and sequence primers for *SYCP3* were, respectively, 5′-GATGGCGTG TGCCTATAATCCAAG-3′ and 5′-CGTCTTTATTTAATTGACAGTGT TAG-3′. Additional direct sequence primers were 5′-GTCAT GTTGCTCAGGCTGGTC-3′, 5′-TCTGTGGATTGATAATTATCTACT G-3′, 5′-TCCAATGCTCTGAGAACC-3′ and 5′-TCACCACAGC AAGTTGTG-3′. The coding exons 7–9 and exon–intron boundaries of human *SYCP3* gene were amplified by PCR and sequenced using the Big Dye Terminator v3.1 Cycle Sequencing kit (ABI Prism, Applied Biosystems, Foster City, CA, USA) on a 3100 automated sequencer.

# Results

Heterozygous 657T>C mutation in exon 8 of *SYCP3* was ascertained in one of 101 patients who had had six recurrent miscarriage and in one of the 82 fertile controls (Fig. 1). The IVS7-16_19 delACTT previously reported in one patient with recurrent miscarriage or 643delA previously reported in two patients with azoospermia was not found in any patients with recurrent miscarriage or in fertile controls. No other new mutation was found in patients with recurrent miscarriage or controls.

Thirty-two patients experienced miscarriage with a normal embryonic (fetal) chromosome karyotype, and 47 patients presented an abnormal embryonic (fetal) karyotype (Table I). Two patients had miscarriages with both normal and abnormal karyotype.

Patient No. 77 with the 657T>C mutation was 31 years old and experienced a total of six miscarriages and no live birth. Available fetal karyotypes were shown as 46, XX and 46, XY. The control with the 657T>C mutation had a history of one live birth and no miscarriage.

Nine patients (No. 39–47) had repeated miscarriage with an abnormal karyotype, and seven patients (No. 75–81) had repeated miscarriage with a normal karyotype. Only 2 out of 18 (11.1%) patients had experienced miscarriages with both abnormal and normal embryonic karyotypes.

# Discussion

In the present study, we found a heterozygous 657T>C mutation in exon 8 of the *SYCP3* gene in one patient and one fertile control. We could not find the IVS7-16_19 delACTT reported in one patient with recurrent miscarriage or 643delA reported in two patients with azoospermia in any patients with recurrent miscarriage or in fertile controls (Miyamoto et al., 2003; Bolor et al., 2009). No other new mutation was found in patients with recurrent miscarriage or controls.

Bolor et al. (2009) reported that 7.7% (2 of 26) patients with unexplained recurrent miscarriage were found to carry independent heterozygous nucleotide alterations, IVS7-16_19delACTT and 657T>C in *SYCP3*, neither of which was present among a control group of 150 fertile women. They also reported that analysis of transcripts from minigenes harboring each of these two mutations revealed that both mutations affected normal splicing, possibly resulting in the

**Table I Previous miscarriage, live birth and subsequent pregnancy outcome with karyotype analysis ($n$ = 101 patients).**

| Pt. | Prior history | | | Age (year) | Subsequent pregnancy | | | Karyotype | Cumulative live birth |
|---|---|---|---|---|---|---|---|---|---|
| | No. of P.M. | No of L.V. | Karyotype of previous miscarriage | | Outcome | | Karyotype of miscarriage | | |
| 1 | 2 | 2 | | 37 | f | | 47,XX,+16 | A | y |
| 2 | 3 | 0 | #3 47,XY,+16 | 34 | s | s | | A | y |
| 3 | 4 | 1 | | 39 | f | | 47,XX,+22 | A | y |
| 4 | 2 | 1 | | 37 | f | s | 47,XX,+21 | A | y |
| 5 | 2 | 0 | | 38 | f | s | 48,XX,+8,+22 | A | y |
| 6 | 3 | 0 | | 28 | f | s | 47,XY,+16 | A | y |
| 7 | 3 | 0 | #3 69,XXY | 34 | s | | | A | y |
| 8 | 4 | 1 | #3 47,XY,−13,+i(13)(p10),+i(13)(q10)[11]/46,XY,−13,+i(13)(q10)[19] | 33 | no | | | A | y |
| 9 | 4 | 1 | #2 47,XY,+18 stillbirth 32w | 32 | s | | | A | y |
| 10 | 2 | 0 | | 31 | f | s | 47,XX,+22 | A | y |
| 11 | 3 | 0 | #3 45,X | 42 | s | | | A | y |
| 12 | 4 | 1 | #4 48,XY,+10,+13[12]/47,XY,+13[8] | 35 | no | | | A | y |
| 13 | 3 | 0 | #3 47,XX,+12 | 40 | s | | | A | y |
| 14 | 4 | 0 | #4 46,XY,5cenh+,add(8)(p23)[7]/46,XY,5cenh+[13] | 32 | s | | | A | y |
| 15 | 4 | 1 | #3 47,XY,+7 | 38 | s | | | A | y |
| 16 | 4 | 1 | #4 47,XY,+3 | 34 | no | | | A | y |
| 17 | 3 | 1 | #3 47,XY,+16 | 36 | s | | | A | y |
| 18 | 4 | 0 | 46,XX,del(6)(q27)[12]/46,XX,add(6)(q27)[3]/46,XX,add(6)(q27)[2]/ | 37 | s | | 46,XX,der(6)t(1;6)(q11;q27)[2]/46,XX,der(6)t(6;9)(q27;q12)[1] | A | y |
| 19 | 2 | 0 | | 33 | f | s | 48,XX,+15,+20 | A | y |
| 20 | 3 | 0 | #3 45,XY, −21 | 30 | f | s | ND* | A | y |
| 21 | 3 | 1 | #3 45,X | 32 | no | | | A | y |
| 22 | 2 | 0 | #2 47,XX,+22 | 32 | f | s | | A | y |
| 23 | 3 | 1 | | 40 | f | s | 48,XX,+14,+15 | A | y |
| 24 | 3 | 0 | #3 47,XY,+16 | 31 | s | | | A | y |

*Continued*

**Table I** *Continued*

| Pt. | Prior history | | | Age (year) | Subsequent pregnancy | | | Karyotype | Cumulative live birth |
|---|---|---|---|---|---|---|---|---|---|
| | No. of P.M. | No of L.V. | Karyotype of previous miscarriage | | Outcome | | Karyotype of miscarriage | | |
| 25 | 4 | 0 | #3 47,+22 | 39 | s | | | A | y |
| 26 | 2 | 0 | | 37 | f | | 47,XY,? | A | n |
| 27 | 3 | 0 | | 34 | f | | 47,XY,+16 | A | n |
| 28 | 2 | 0 | | 39 | f | | 47,XX,+16 | A | n |
| 29 | 2 | 0 | | 32 | f | | 47,XY,+16 | A | n |
| 30 | 5 | 0 | #5 46,X,+16 | 27 | f | | Bio Misc | A | n |
| 31 | 3 | 0 | | 28 | f | | 47,XX,+13 | A | n |
| 32 | 5 | 0 | | 46 | f | | 47,XX,+22 | A | n |
| 33 | 3 | 0 | #3 47,XX,add(2)(q37),+20 | 42 | no | | | a | n |
| 34 | 4 | 0 | #1 stillbirth 16w; #4 47,XY,+15 | 39 | no | | | a | n |
| 35 | 3 | 0 | | 41 | f | | 47,XX,+16 | a | n |
| 36 | 4 | 0 | | 43 | f | | 48,XY,+16,+21 | a | n |
| 37 | 4 | 0 | | 38 | f | | 46,X,+3 | a | n |
| 38 | 6 | 0 | #1 stillbirth 33w; #4 45,X | 37 | no | | | a | n |
| 39 | 7 | 0 | #5 47,XX,+16; #7 45,X | 30 | s | | | aa | y |
| 40 | 6 | 0 | #6 47,XY,+16 | 32 | f | s | 47,XX,+13 | aa | y |
| 41 | 2 | 1 | 47,XY,+21*** | 30 | f | | 47,XX,+5 | aa | y |
| 42 | 3 | 0 | #3 47,XX,+16 | 29 | f | s | 47,XX,+3 | aa | y |
| 43 | 6 | 1 | #6 47,XX,+16 | 41 | f | | 47,XX,+12 | aa | y |
| 44 | 2 | 0 | #2 46,XY,add(8)(p23) | 33 | f | | 47,XY,+16 | aa | n |
| 45 | 4 | 0 | #2 45,X; #4 47,XX,+idic(8)(q?21.2) | 35 | no | | | aa | n |
| 46 | 2 | 0 | | 30 | f | f | 47,XX,+15;45,X | aa | n |
| 47 | 2 | 0 | #2 47,XX,+15 | 35 | f | | 45,X | aa | n |
| 48 | 14 | 2 | #12 47,XX,+16 #14 46,XY, 2 children with previous husband | 45 | no | | | an | y |
| 49 | 4 | 1 | #3 47,XY,+16;#4 46,XX | 30 | f | | 46,XY | ann | y |
| 50 | 4 | 0 | #4 46,XY | 35 | s | s | | n | y |
| 51 | 4 | 0 | #1 Stillbirth 28w; #4 46,XX | 28 | s | | On-going preg.EDC06/27/11 | n | y |
| 52 | 4 | 0 | #3 Stillbirth 18w | 35 | f | s | 46,XY stillbirth 33w | n | y |
| 53 | 3 | 0 | #3 46,XY | 33 | s | | | n | y |
| 54 | 5 | 1 | #5 46,XY | 36 | s | | | n | y |
| 55 | 4 | 1 | #3 46,XY | 36 | no | | | n | y |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 56 | 3 | 0 | #3 46,XX | 30 | s | | | | n | y |
| 57 | 3 | 0 | #3 46,XX | 32 | s | | | | n | y |
| 58 | 2 | 0 | | 34 | f | s | | 46,XX | n | y |
| 59 | 3 | 1 | #3 46,XX | 35 | no | | | | n | y |
| 60 | 3 | 1 | #3 46,XX | 33 | s | | | | n | y |
| 61 | 4 | 0 | #4 stillbirth 15w | 37 | f | s | | 46,XX stillbirth 13w | n | y |
| 62 | 3 | 0 | #3 46,XX | 33 | s | | | | n | y |
| 63 | 3 | 1 | #3 46,XY | 37 | s | | | | n | y |
| 64 | 3 | 0 | | 26 | f | | | 46,XX | n | n |
| 65 | 3 | 0 | | 34 | f | | | Normal Karyotype | n | n |
| 66 | 5 | 0 | 46,XY | 40 | no | | | | n | n |
| 67 | 2 | 0 | | 32 | f | | | 46,XX | n | n |
| 68 | 3 | 0 | #3 46,XY | 35 | f | | | NT** | n | n |
| 69 | 2 | 0 | | 39 | f | | | 46,XX | n | n |
| 70 | 3 | 0 | #3 46,XX | 28 | no | | | | n | n |
| 71 | 2 | 0 | | 36 | f | | | 46,XX | n | n |
| 72 | 2 | 0 | | 30 | f | | | 46,XY | n | n |
| 73 | 3 | 0 | #3 46,XX | 34 | no | | | | n | n |
| 74 | 2 | 0 | | 31 | f | | | 46,XY | n | n |
| 75 | 3 | 0 | #2 46,XX; #3 46,XY | 31 | s | | | | nn | y |
| 76 | 2 | 0 | #2 46,XY | 22 | f | s | | 46,XY; On-going preg.EDC 05/27/11 | nn | y |
| 77 | 5 | 0 | #5 46,XY | 31 | f | | | 46,XY | nn | n |
| 78 | 5 | 0 | #3 46,XX; #4 46,XY; #5 46,XY | 36 | f | f | s | 46,XX,t(11;19)(q21;q13.1) [4]/46,XX[26], Bio Misc | nnnn | y |
| 79 | 6 | 0 | #3#,4#,6 46,XX,inv(9); #5 46,XY,inv(9) | 31 | s | | | On-going preg.EDC 02/23/11 | nnnn | y |
| 80 | 9 | 0 | #5 46,XX;#6 46,XX;#8 46,XY | 38 | f | | | 46,XX | nnnn | n |
| 81 | 13 | 0 | #4 46,XY; 46,XX; 46,XX; 46,XX | 33 | f | f | f | 46,XX; 46,XX; 46,XX | Nnnnnnn | n |
| 82 | 3 | 0 | | 31 | s | | | | | y |
| 83 | 3 | 0 | | 39 | s | | | | | y |
| 84 | 3 | 0 | | 26 | s | | | | | y |
| 85 | 3 | 0 | | 38 | s | | | | | y |
| 86 | 23 | 0 | | 33 | s | | | | | y |
| 87 | 3 | 1 | | 35 | f | | | Bio Misc | | y |
| 88 | 3 | 0 | | 28 | s | | | | | y |
| 89 | 3 | 0 | #2 stillbirth 15w | 30 | s | | | | | y |
| 90 | 3 | 1 | | 35 | no | | | | | y |

*Continued*

**Table I** *Continued*

| Pt. | Prior history | | | Age (year) | Subsequent pregnancy | | Karyotype | Cumulative live birth |
|---|---|---|---|---|---|---|---|---|
| | No. of P.M. | No of L.V. | Karyotype of previous miscarriage | | Outcome | Karyotype of miscarriage | | |
| 91 | 3 | 2 | 2 children with previous husband | 34 | s | | | y |
| 92 | 3 | 1 | | 38 | s | On-going preg.EDC03/24/11 | | y |
| 93 | 3 | 0 | | 36 | s | | | y |
| 94 | 5 | 0 | | 34 | f | ND* | | n |
| 95 | 2 | 0 | | 28 | f | Bio Misc | | n |
| 96 | 4 | 0 | | 41 | no | | | n |
| 97 | 4 | 0 | | 42 | no | | | n |
| 98 | 3 | 0 | | 33 | no | | | n |
| 99 | 4 | 0 | | 31 | no | | | n |
| 100 | 3 | 0 | #1 stillbirth 18w; #3 stillbirth 23w | 32 | no | | | n |
| 101 | 4 | 0 | | 37 | f | Bio Misc | | n |
| | 3.782178 | 0.267327 | | 34.347 | | | | |
| | 2.681805 | 0.507762 | | 4.4033 | | | | |

*ND, not detected; **NT, not tested; ***Live birth, He is 8 years old; Pt., patient; P.M., previous miscarriage; L.V., Live Birth; Bio Misc, biochemical miscarriage, decreasing hCGs < 1500 mIU/ml; age, age at examination karyotype; a, aneuploidy; n, normal karyotype (euploidy) from Pt. 1–81, karyotype were knowed in proir history or subsequent pregnancy; 'Outcome' reflect the conclusion of subsequent pregnancy; 's' means success in live birth delivery; 'f' means failure, miscarriage.