



Etiological evaluation of repeated biochemical pregnancy in infertile couples who have undergone *in vitro* fertilization

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Objective

This study aims to investigate whether there are any notable etiologies for repeated biochemical pregnancy (RBP) and, if so, to compare those etiologies associated with repeated spontaneous abortion in infertile couples who have undergone *in vitro* fertilization (IVF).

Methods

Forty-four infertile couples who underwent IVF and experienced RBP were included in this study. RBP was defined as more than 2 early pregnancy losses that occurred before the detection of a gestational sac, with ectopic pregnancies specifically excluded by serial serum beta human chorionic gonadotropin evaluation. Forty-three infertile couples who underwent IVF and experienced recurrent spontaneous abortion (RSA) were included as a control group. Karyotype analysis, anatomic evaluation of uterus, endocrine and immunological evaluation were performed. In addition, the number of pregnant women confirmed by 12 weeks' gestation was compared between groups.

Results

Immunological factors (RSA: 20.9% vs. RBP: 29.5%, $P=0.361$), diminished ovarian reserve (RSA: 10.9% vs. RBP: 17%, $P=0.552$), and parental chromosomal abnormalities (RSA: 18.6% vs. RBP: 9.1%, $P=0.218$) were not different between groups. Additionally, the incidence of uterine factors (RSA: 11.6% vs. RBP: 4.6%, $P=0.206$), unknown cause (RSA: 48.8% vs. RBP: 54.5%, $P=0.161$), and the pregnancy outcome identified until 12 weeks' gestation (RSA: 46.5% vs. RBP: 38.6%, $P=0.520$) did not differ between groups.

Conclusion

In the present study, the causes of RBP after IVF were similar to those of RSA. Accordingly, we suggest that efforts should be made to define the etiology of RBP, particularly for infertile couples, and that possible management strategies should be offered.

Keywords: Biochemical phenomena; Pregnancy; Habitual abortion; Etiology; *In vitro* fertilization

Introduction

ART can potentially cause significant psychological, emotional, physical, and financial difficulties for couples that pursue it. After embryo transfer (ET) is performed, the couple must wait for serum beta human chorionic gonadotropin (β -hCG) pregnancy test results. At this stage, it is possible that assisted conception could produce a positive pregnancy test that, in turn, can lead to one of several possible outcomes: A clinical pregnancy resulting in a live birth, a clinical pregnancy resulting in a miscarriage or a biochemical pregnancy. Early pregnancy loss occurs in approximately 15%–20% of pregnant

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women [1]. Of those, approximately 20% will experience at least 2 consecutive pregnancy loss events [1]. Miscarriage prevalence increases with increasing maternal age, and 57% of early pregnancy losses occur at less than 6 weeks of gestational age [2,3]. Early pregnancy loss is defined as a nonviable intrauterine pregnancy with either an empty gestational sac or a gestational sac containing an embryo or fetus but without heart activity within the first 13 weeks of gestation [4]. A biochemical pregnancy occurs when pregnancy is diagnosed from the detection of β -hCG in serum or urine, but does not develop into a clinical pregnancy [5]. Biochemical pregnancy is also commonly termed a "trophoblast in regression," "pre-clinical embryo loss," or "chemical pregnancy" [5,6].

Biochemical pregnancy is diagnosed based on a patient experiencing of the following: 1) a low β -hCG peak (<100 mIU/mL); 2) a rapid fall in urinary or serum β -hCG concentration; and 3) a short delay before the onset of the next menstrual period (which can differentiate this occurrence from a clinical pregnancy) [7]. The incidence of biochemical pregnancy is between 8% and 33% of all pregnancies and comprises 18%–22% of *in vitro* fertilization (IVF) pregnancies [3,8]. Recent work, however, has reported that the rate of biochemical pregnancy following IVF is lower than that of spontaneous pregnancy [9].

The etiology of a post-IVF biochemical pregnancy has not been clearly defined. Some studies have suggested that some potential causes of biochemical pregnancy include implantation failure due to chromosomal abnormalities and lack of endometrial receptivity due to anatomical, immunological or other reasons [11]. Another recent study, however, reported that biochemical pregnancy likely presents with similar etiology to that of recurrent spontaneous abortion (RSA) [10]. Unfortunately, the relatively small number of studies into these questions has left them unresolved.

We hypothesize that repeated biochemical pregnancy (RBP) presents with similar etiologies to those of RSA, including endometrial receptivity in patients who have undergone IVF. To assess this, we compare RBP etiologies to those of RSA that occurred following IVF, and we then investigate whether there are any notable RBP etiologies that suggest potential avenues to either reduce or prevent their occurrence.

Materials and methods

This study was approved by our institutional ethical board

(Cheil General Hospital and Women's Healthcare Center). We reviewed all medical records for those couples who underwent IVF at our center between January 2005 and December 2016. Patients who underwent etiological evaluations were selected. Forty-four couples that experienced more than 2 biochemical pregnancies following IVF were included, and comprised our study population. As a control group, we included 43 couples that experienced more than 2 spontaneous abortion events following IVF over the same period.

We found that RSA etiologies were associated with the following factors: immunological, anatomical, endocrine, parental chromosome abnormality, thrombophilia, fibrinolytic, and unknown etiology. Immunological factors include abnormal results from natural killer cell activity. Autoimmune factors include antinuclear antibody (ANA), anti-ANA, rheumatoid arthritis (RA) factor, antithyroglobulin antibody (Ab), lupus anticoagulant (LA), anticardiolipin immunoglobulin G (IgG), immunoglobulin M (IgM), anti-DNA, and antimicrosom Ab. Thrombophilia was evaluated by measuring the serum level of protein C, protein S, homocysteine, and antithrombin III. Anatomical factors include uterine synechia, as well as uterine anomalies such as uterine septum, unicornuate uterus and cervical insufficiency (as diagnosed by ultrasound, diagnostic laparoscopy, hysteroscopy, and magnetic resonance imaging). Endocrine factors include diabetes mellitus, polycystic ovary syndrome, thyroid disorders, hyperprolactinemia, and luteal phase defect. Genetic factors included aneuploidy of the abortus and parental chromosomal rearrangements.

Each of the above factors were analyzed and compared between the RBP and RSA groups. Statistical analysis was performed using the statistical package for social sciences (SPSS version 20; IBM Co., Armonk, NY, USA). The Student's *t*-test and Pearson χ^2 test was used to assess differences in the etiologies of the 2 groups. Statistically significant differences were defined as those with a *P*-value <0.05 .

Results

1. Comparison of clinical characteristics between groups

RBP patients were younger than RSA patients (34.3 ± 3.5 vs. 36.4 ± 3.7 , $P=0.005$). The body mass index did not significantly differ between groups (21.0 ± 2.9 vs. 21.9 ± 2.7 , $P=0.149$). The number of pregnancy events (1.5 ± 1.7 vs. 3.5 ± 1.3 , $P=0.000$)

Table 1. Comparison of clinical characteristics

Characteristics	RSA (n=43)	RBP (n=44)	P-value
Age (yr)	36.4±3.7	34.3±3.5	0.005
BMI (kg/m ²)	21.9±2.7	21.0±2.9	0.149
Gravida (excluding BP)	3.5±1.3	1.5±1.7	0.000
Gravida (including BP)	4.1±1.6	3.2±1.9	0.012
No. of previous miscarriages			
Excluding BP	2.6±0.8	0.5±0.5	0.000
Including BP	3.2±1.1	2.2±1.1	0.000
No. of previous live births	0.5±0.9	0.4±0.6	0.685
Serum TSH (uU/mL)	2.2±1.5	1.9±1.1	0.281
Serum prolactin (ng/mL)	10.6±5.5	11.3±2.8	0.627
Basal serum FSH (mIU/mL)	7.8±3.5	7.4±2.0	0.549
Basal serum LH (mIU/mL)	4.3±2.5	4.0±2.3	0.597
Basal serum estradiol (E ₂) (pg/mL)	30.1±19.6	37.4±35.0	0.268

Values are presented as the mean±standard deviation, or the *P*-value from the student's *t*-test.

RSA, recurrent spontaneous abortion; RBP, repeated biochemical pregnancy; BMI, body mass index; BP, blood pressure; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

and miscarriage events (0.5±0.5 vs. 2.6±0.8, *P*=0.000) (after excluding biochemical pregnancy events) were both significantly higher in the RSA group. If the blood pressure (BP) events are included, the number of pregnancy events (3.2±1.9 vs. 4.1±1.6, *P*=0.012) and miscarriage events (2.2±1.1 vs. 3.2±1.1, *P*=0.000) remained significantly higher in the RSA group. Serum levels of thyroid stimulation hormone, prolactin, follicular stimulation hormone, luteinizing hormone and estradiol on the 2nd or 3rd day of the menstrual cycle were similar in both groups (Table 1).

2. Comparisons of evaluated etiologies of early pregnancy losses between groups

The rate of immunological abnormalities such as elevated natural killer cell activity (>12%, [20]) and the rate of autoimmune factors including ANA, anti-ANA, RA factor, antithyroglobulin Ab, LA, anticardiolipin IgG, IgM, anti-DNA, antimicrosomal Ab, and thrombophilia did not significantly differ between groups (RSA: 20.9% vs. RBP: 29.5%, *P*=0.361). Diminished ovarian reserve (RSA: 10.9% vs. RBP: 17%, *P*=0.552) also exhibit no statistically significant differences between the groups. Also, the incidence of uterine factors (RSA: 11.6% vs. RBP: 4.6%, *P*=0.206) and unknown etiology (RSA: 48.8% vs. RBP: 54.5%, *P*=0.161) did not significantly differ between groups. Notably, the rate of chromosomal abnormalities for couples was similar between groups (RSA: 18.6%

vs. RBP: 9.1%, *P*=0.218) (Table 2).

3. Comparisons of pregnancy outcome between groups

The pregnancy outcome results are summarized in Table 3. Clinical pregnancy confirmed gestational sac by transvaginal ultrasound was found in 24 patients, or 55.8% of RSA group and 19 patients, or 43.1% of RBP group. Among them, the number of pregnancy maintenance until 12 weeks' gestation confirmed at out center was 20 patients in RSA group and 17 patients in RBP group. This difference between the 2 groups was not statistically significant (RSA: 46.5% vs. RBP: 38.6%, *P*=0.520).

Discussion

The development of *in vitro* fertilization and embryo transfer (IVF-ET) has allowed many couples to become pregnant who otherwise had no hope of doing so. After ET, the couple must wait for the results of a β-hCG pregnancy test. This may be associated with significant psychological morbidities, as it is the first decisive hurdle that must be confronted. One of the possible outcomes from a positive pregnancy test is a biochemical pregnancy, in which the initial positive pregnancy test fails to progress into a clinical pregnancy [6].

Table 2. Comparison of recurrent spontaneous abortion (RSA) and repeated biochemical pregnancy (RBP) etiology

Etiologies	RSA (n=43)	RBP (n=44)	P-value
Immunologic factor	9 (20.9)	13 (29.5)	0.361
Allo-immune factors			
NK cell elevation	4	6	
Autoimmune factors			
ACA antibody positive	2	3	
LA positive	2	2	
ATA positive	1	2	
Anatomic factor	5 (11.6)	2 (4.6)	0.206
Uterine septum	2	2	
Adenomyosis	2		
Submucosal myoma	1		
Parental chromosomal abnormality	8 (18.6)	4 (9.1)	0.218
Mosaicism	1 47,xxx[6]/45,x[3]/46,xx[91]	2 46,xx, fra(16)(q22)[29]/46,xx[41] 45,x[7]/47,xxx[5]/48,xxx[1]/46,xx[88]	
Inversion	1 46,x,inv(Y)(q11.222q12)	1 46,xy,inv(1)(p34.1q43)	
Balanced translocation	6 46,xx,t(2;11)(q36;p15.2) 46,xx,t(1;14)(p36;q24.1) 46,xx,t(14;20)(q31.2;q13.2) 45,xy,der(13;14)(q10;q10) 45,xx,der(13;14)(q10;q10) 46,xy,t(1;14)(q32.3;q24.1)	1 46,xx,t(4;10)(q28.2;q26.11)	
Unknown etiology	21 (48.8)	25 (54.5)	0.161

Values are presented as number (%).

NK, natural killer; ACA, antientromere antibody; LA, lupus anticoagulant; ATA, antithyroid antibody.

Table 3. Comparison of pregnancy outcome

Pregnancy and outcomes after next IVF cycle	RSA (n=43)	RBP (n=44)
Biochemical pregnancy	4 (9.3)	3 (6.8)
Clinical pregnancy	24 (55.8)	19 (43.1)
Pregnancy maintenance until 12 weeks' gestation	20 (46.5)	17 (38.6)
Delivery at term	13 (30.2)	9 (20.4)
Preterm delivery	2 (4.6)	3 (6.8)
Lost follow-up	7 (16.2)	6 (13.6)

Values are presented as number (%).

IVF, *in vitro* fertilization; RSA, recurrent spontaneous abortion; RBP, repeated biochemical pregnancy.

A biochemical pregnancy is diagnosed based on a positive β -hCG test in either serum or urine that does not develop into an intra- or extra-uterine (ectopic) gestational sac. In biochemical pregnancies, β -hCG levels usually decline without treatment [9]. Biochemical pregnancy rates have been reported to be between 18% and 22% of all IVF pregnancies [3]. One

potential cause of biochemical pregnancy after IVF is the use of gonadotrophins to induce hyper-stimulation in fresh IVF for endometrial gene and protein expression. Also of note is that estrogen and progesterone are taken to induce endometrial preparation (via gene and protein expression) during the frozen ET cycle. It is possible that these endometrial alterations

may prevent embryos from properly developing, and thereby increase the likelihood of biochemical pregnancy [9]. Biochemical pregnancies may occur at a higher rate during fresh cycles compared to frozen cycles, possibly due to alterations in endometrial receptivity [11].

Despite the fact that many IVF doctors have sought to investigate potential causes of biochemical pregnancy after IVF beyond drug-related alterations in endometrial receptivity, very few studies have been conducted. One exception evaluated the etiology of RSA and considered the number of previous biochemical pregnancies (with groups defined as having experienced of 0 or 1 vs. more than 2). This study reported that spontaneous abortion with a normal karyotype occurred more frequently in the RBP experience group [10]. Unfortunately, no previous studies have explored the etiology of RBP in comparison to RSA after IVF.

The present study investigated whether there are any notable etiologies for RBP in comparison to RSA in infertile couples who underwent IVF. We found that the incidence of parental chromosome abnormality was similar between RBP (9.1%) and RSA (18.6%) groups; however, it was higher than that in the general population (0.7%) [12]. When the etiology of RBP is a parental chromosomal anomaly, particularly a transmittable balanced translocation or inversion, then pre-implantation genetic diagnosis could help prevent the recurrence of RBP.

Among the evaluated etiologies, the frequency of unknown etiology of RSA and RBP after IVF was similar. We suspect that the etiology of RSA or RBP after IVF in these cases was related to decreased endometrial receptivity, which would be consistent with previous reports that found compromised embryo receptivity to be associated with biochemical pregnancy [13]. In such cases, various methods can be implemented that may improve endometrial receptivity, such as changes in the protocols for controlled ovarian stimulation, freezing and thawing embryos or endometrial stimulation before ET.

Uterine natural killer (uNK) cells in the endometrium are thought to support the remodeling of the uterine spiral arteries and to facilitate successful placentation by regulating trophoblast invasion [14]. Several previous studies have shown that a high density of uNK cells is associated with RSA [15]. An abnormal increase in the peripheral blood NK (pbNK) cell fraction is also associated with RSA and infertility [16]. Furthermore, the downregulation of NK cells has been associated with favorable pregnancy outcomes [17]. The exact pathogenic mechanism behind the role of NK cells in human

reproduction, however, remains unclear. Additional studies have found supporting evidence suggesting that an autoimmune mechanism plays a role in RSA. Pathogenic autoantibodies, such as antiphospholipid antibodies, antithyroid antibodies, and other autoimmune antibodies, have been shown to induce not only impaired blood circulation at the maternal-fetal interface, but also an inflammatory immune response, which is consistent with previous findings that inflammatory immune responses are related to RSA [18]. Moreover, women who experienced RSA were significantly more likely to have a positive test result for one or both thyroid antibodies (peroxidase and thyroglobulin) compared to a fertile control group [19]. We found that the incidence of immune factors was similar in RSA compared to RBP after IVF. Hence, we suggest that variable immune modulation treatment (which is already offered to RSA patients) should be offered to patients who have experienced repeated biochemical pregnancies after IVF and that are suspected having aberrant auto- or allo-immune responses.

Together with these prior studies, our work suggests that the causes of RBP after IVF are similar to those of RSA.

Consequently, we suggest that efforts should be made to define the etiology of RBP, especially for infertile couples pursuing IVF, and that management strategies that may prevent the recurrence of RBP should be explored.

The present study does have a limitation in that its study population included only infertile patients that underwent IVF and did not directly study a healthy control group. Hence, the rate of abnormal causative factors (particularly parental chromosome abnormalities) may be higher than that in the general population. As such, we suggest that large-scale prospective studies are needed to further substantiate our results. Additionally, further research on the outcomes of RBP management according to etiology is needed.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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