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The impact of fertility treatment on the neonatal respiratory outcomes and amniotic lamellar body counts in twin pregnancies

Hiroyuki Tsuda[a,b,*], Tomomi Kotani[b], Tomoko Nakano[b], Kenji Imai[b], Takafumi Ushida[b], Akihiro Hirakawa[c], Fumie Kinoshita[d], Yuichiro Takahashi[e], Shigenori Iwagaki[e], Fumitaka Kikkawa[b]

[a] *Department of Obstetrics and Gynecology, Japanese Red Cross Nagoya Daiichi Hospital, Nagoya, Japan*
[b] *Department of Obstetrics and Gynecology, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[c] *Department of Biostatistics and Bioinformatics, Graduate School of Medicine, The University of Tokyo, Japan*
[d] *Statistical Analysis Section, Center for Advanced Medicine and Clinical Research, Nagoya University Hospital, Nagoya, Japan*
[e] *Department of Fetal and Maternal Medicine, Nagara Medical Center, Gifu City, Japan*

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ABSTRACT

Background: To elucidate the impact of fertility treatment on neonatal respiratory outcomes and amniotic lamellar body counts (LBCs) in twin pregnancies.
Methods: One hundred ninety twin pairs, including 99 dichorionic twin (DCT) and 91 monochorionic twin (MCT) pairs were registered at our institutions. All amniotic fluid samples were obtained from each sac at cesarean section. Samples were analyzed immediately after arrival at the laboratory without centrifugation. We divided the patients into 3 groups: the no therapy group (natural conception), the induced ovulation group (with or without intrauterine insemination), and the assisted reproductive technology (ART) group (*in vitro* fertilization or intracytoplasmic sperm injection).
Results: No statistically significant associations between the fertility treatment and the rates of neonatal RDS/TTN were observed in the whole study population (odds ratio [OR], 0.95; 95% confidence interval [CI], 0.45–2.00), DCT (OR, 0.86; 95%CI, 0.30–2.47), and MCT (OR, 1.45; 95%CI, 0.41–5.11). In addition, there was no association between the fertility treatment and neonatal RDS/TTN in the propensity score analysis of the whole study population (OR, 1.25; 95%CI, 0.57–2.74).
Conclusions: None of the individual types of fertility treatment had a direct impact on respiratory disorders such as RDS and TTN in twin infants.

1. Introduction

Infertility is defined as the inability of a couple to conceive after 12 months of regular intercourse without the use of contraception in women of < 35 y; and after 6 months of regular intercourse without use of contraception in women of ≥35 y [1]. It is a common condition with important psychologic, economic, demographic, and medical implications. The prevalence of infertility is highest in Eastern Europe, North Africa/Middle East, Oceania, and Sub-Saharan Africa [2]. Worldwide, in 2010, 1.9% of women of 20–44 y who desired to have children were unable to have their first live birth and 10.5% of women with a previous live birth were unable to have an additional live birth.

Women who use fertility treatments (*in vitro* fertilization [IVF] or non-IVF) were reported to have a significantly higher risk of preterm birth and low birth weight [3–5]. The risk of preterm birth and low birth weight among untreated sub-fertile women has also been reported to be higher in comparison to the general population [5, 6]. The risk of preeclampsia, placental abruption, and fetal growth restriction have been reported to increase in singleton pregnancies after fertility treatments; however, the data on this topic are conflicting [3, 7–9]. In any case, when managing pregnant women after fertility treatment—especially assisted reproductive technology (ART)—attention should be paid to these obstetrical risks.

In infertile women using ART, the rate of multiple gestation increases due to an increased implantation rate. Twin pregnancies are associated with higher rates of preterm births and cesarean section (CS) delivery than singletons. Twin neonates are therefore at higher risk for respiratory disorders, such as respiratory distress syndrome (RDS) and transient tachypnea of the newborn (TTN) than singleton neonates. The rate of respiratory disorders in twin infants conceived after medically

* Corresponding author at: Department of Obstetrics and Gynecology, Japanese Red Cross Nagoya Daiichi Hospital, 3-35 Michishita-cho, Nakamura-ku, Nagoya 453-8511, Japan.
E-mail address: hirotty7099@yahoo.co.jp (H. Tsuda).

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assisted reproduction has been reported to be higher [10–13], or similar [14, 15] to that of infants conceived naturally. However, there is little evidence available on these relationships and the data mainly concern dichorionic twin (DCT) cases.

Several studies have shown that the lamellar body count (LBC) can be an accurate predictor of fetal lung maturity in a singleton pregnancy [16–18]. The LBC can be determined quickly and inexpensively, making it a more cost-effective predictor of RDS than the L/S ratio [16]. Recently, we reported that the LBC may be a potentially useful marker for predicting not only RDS, but also TTN in singleton and twin pregnancies [19–22].

The purpose of this study is to elucidate the impact of fertility treatment on neonatal respiratory outcomes and amniotic LBCs in twin pregnancies. In addition to evaluating these relationships in DCTs, we also evaluated them in monochorionic twins (MCTs). Finally, we further examined the relationship between fertility treatment and the rate of neonatal RDS/TTN using a multivariate logistic analysis and propensity scores composed of independent variables to adjust for the imbalance between the cases.

2. Materials and methods

2.1. Case registration

Data were collected from January 2010 to December 2016 at our hospital. We registered 380 neonates (190 twin pairs), including 99 DCT and 91 MCT pairs with no congenital abnormalities affecting the respiratory function, neonatal deaths, or complications such as twin-twin transfusion syndrome. Chorionicity was determined by early ultrasound findings and was confirmed by placental pathology following delivery. We divided the patients into 3 groups: the no therapy group (natural conception), the induced ovulation group (with or without intrauterine insemination), and the ART group (*in vitro* fertilization or intracytoplasmic sperm injection). This study was approved by the ethics committee of our hospital.

2.2. Amniotic fluid samples

All amniotic fluid (AF) samples were obtained from the sac at CS, which was performed at 27–38 weeks of gestation. We excluded the cases in which we could not collect AF samples from both sacs or in which the AF sample was contaminated with blood and/or meconium. The samples were analyzed immediately after arrival at the laboratory without centrifugation, according to the standard method for LBC reported by Neerhof et al. [17] The LBC (per microliter) was determined using a platelet channel on a Sysmex SF-3000 (Sysmex, Kobe, Japan); the procedure took no > 30 min [19–22].

2.3. Clinical characteristics

The neonatal data including gestational age at delivery, birth weight, sex, umbilical artery pH, and respiratory outcomes (RDS, TTN) were abstracted. The clinical characteristics of the mother, such as pregnancy-induced hypertension and diabetes mellitus (DM), including preexisting and gestational, were documented for assessment. Fetal growth restriction (FGR) was defined as a birth weight of < -1.5 SD from the gestational age in Japan [19]. The diagnoses of RDS and TTN were established by a neonatologist based on the combination of the clinical signs, clinical course, and chest x-ray findings. The radiological criteria for TTN were perihilar streaking, mild cardiomegaly, increased lung volumes with flat diaphragms, and fluid in the interlobar fissure [19]. The radiological criteria for RDS were a diffuse reticulogranular ground-glass pattern with air bronchograms and a decreased lung volume. The neonatologists were blinded to the LBC data.

2.4. Statistical analyses

The maternal and neonatal characteristics of the DCT and MCT groups were summarized. Continuous variables were shown as the mean and SD, while categorical variables were shown as the frequency and proportion. The distributions of continuous and categorical variables between the two groups were compared using Student's *t*-test and Fisher's exact test, respectively. Univariate and multivariate logistic regression analyses were performed to evaluate the associations between fertility treatment and neonatal RDS/TTN. The multivariate logistic model included the following eleven variables: maternal age, fetal sex, nulliparity, gestational age at delivery, birth weight SD, umbilical cord $pH < 7.15$, fetal growth restriction, pregnancy-induced hypertension, diabetes mellitus, maternal betamethasone, and premature rupture of the membranes (PROM). Furthermore, we examined the associations between fertility treatment and neonatal RDS/TTN based on the propensity score approach. We calculated propensity scores based on the results of the multivariate logistic regression analysis, which included the eleven above-mentioned variables and performed a regression adjustment (i.e., the inclusion of the propensity score as a linear predictor in the model). A $P < 0.05$ were considered to indicate statistical significance. All of the statistical analyses were performed using the SPSS for Windows (ver.24.0) and SAS (ver 9.4) software programs.

3. Results

3.1. Clinical background

The background information and characteristics of the DCTs and MCTs are shown in Table 1. Fifty neonates (13.2%) suffered from RDS/

Table 1
The maternal and neonatal characteristics in DCTs and MCTs.

	DCT (99 pairs)	MCT (91 pairs)	*P* value
Maternal age; y (range)	31.3 (± 4.2)	31.0 (± 4.6)	NS
Nulliparity	63/99 (63.6%)	47/91 (51.6%)	NS
Male neonate	101/198 (51.0%)	86/182 (47.3%)	NS
GAD; weeks (mean ± SD)	36.6 (± 1.8)	35.8 (± 2.2)	0.005
RDS/TTN	19/198 (9.6%)	31/182 (17.0%)	0.032
LBC; 10^4/μl (range)	9.4 (0.2–23.4)	8.1 (0.2–18.2)	NS
Fertility treatment	49/99 (49.5%)	16/91 (17.6%)	< 0.001
Umbilical artery pH	7.27 (± 0.05)	7.27 (± 0.05)	NS
Fetal growth restriction	51/198 (25.8%)	49/182 (26.9%)	NS
Maternal betamethasone	10/198 (5.1%)	6/182 (3.3%)	NS
Preterm rupture of membranes	5/99 (5.1%)	8/91 (8.8%)	NS
Pregnancy-induced hypertension	16/99 (16.2%)	18/91 (19.8%)	NS
Diabetes mellitus	6/99 (6.1%)	4/91 (4.4%)	NS

DCT, dichorionic twins; MCT, monochorionic twins; GAD, gestational age at delivery; SD, standard deviation; LBC, lamellar body count.