# Implications of assisted reproductive technologies on term singleton birth weight: an analysis of 25,777 children in the national assisted reproduction registry of Japan

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**Objective:** To evaluate the implications of assisted reproductive technologies (ART) on neonatal birth weight.
**Design:** A retrospective study using analysis of covariance and multiple logistic regression analysis of the Japanese ART registry.
**Setting:** Japanese institutions providing ART treatment.
**Patient(s):** A total of 25,777 singleton neonates reaching term gestation following ART during the years 2007–2008, with 11,374 achieved through fresh embryo transfers (fresh ET) and 14,403 achieved through frozen-thawed embryo transfers (FET).
**Intervention(s):** None.
**Main Outcome Measure(s):** Birth weight.
**Result(s):** The mean birth weight after FET was significantly higher compared with fresh ET and all Japanese births (3,100.7 ± 387.2 g, 3,009.8 ± 376.8 g, and 3,059.6 ± 369.6 g, respectively). The risk for low birth weight in FET was significantly lower compared with fresh ET. In fresh ET, ovarian stimulations were associated with about twofold risk of low birth weight compared with natural cycle. Regarding to the duration of embryonic culture, the risks resulting from a shorter culturing time were significantly higher compared with a longer culturing time in fresh ET.
**Conclusion(s):** The best method of embryo transfer for fetal growth was FET after extended culturing until blastocyst stage. However, further investigations should be performed to understand the safety of ART treatment. (Fertil Steril® 2013;99:450–5. ©2013 by American Society for Reproductive Medicine.)
**Key Words:** Assisted reproductive technology, frozen-thawed embryo transfer, birth weight, ovarian stimulation, luteal support

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The development of assisted reproductive technologies (ART) has supported a large number of infertile couples over the past three decades. The current indications of infertility include tubal factors, male factors, unknown factors, malignant diseases, etc. These various situations require the appropriate protocols to achieve conception, including ovarian stimulation, insemination methods (i.e., in vitro fertilization [IVF] and intracytoplasmic sperm injection [ICSI]), cryopreservation, and luteal phase support. Regarding conception following ART treatment, the implications of the protocols used on the fetus and neonates should be considered. In the past

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decade, the influence of these treatments on neonatal and infant outcomes has been investigated (1, 2).

Several large-scaled studies describing the neonatal outcomes of ART have been reported in national registry systems in a few countries (3–8). It has been reported that ART treatment increases the rates of low birth weight and admission to neonatal intensive care units (4, 5, 9). A multiple pregnancy is considered to be one of the factors contributing to low birth weight as well as to preterm birth (4, 5, 10). Although the prevention of multiple pregnancies might reduce the rates of low birth weight and preterm birth, the rate of low birth weight for infants with ART treatment was reported to be higher compared with non-ART treatments in singleton pregnancies (3, 6). On the other hand, it was postulated that the mean birth weight after frozen-thawed embryo transfer (FET) resulted in heavier infants compared with fresh embryo transfer (3, 6, 11–13). The cause of this difference is not yet fully understood.

In the present study, the difference between the mean birth weight of ART and non-ART outcomes was analyzed based on data retrieved from the Japanese national registry of ART, as well as statistical data of the mean birth weight obtained from the Ministry of Health, Labor, and Welfare in Japan. In addition, factors implicated in low birth weight from singleton pregnancies following ART were analyzed, including ovarian stimulation, the number of retrieved oocytes, fertilization methods, the duration of embryo culture, luteal phase hormone therapy, and the timing of embryo transfer.

## MATERIALS AND METHODS

All of the data analyzed in the present study were provided by the Japan Society of Obstetrics and Gynecology (JSOG) and all of Japanese neonates data were provided by Ministry of Health, Labor, and Welfare in Japan. This study was approved by the Registration and Research Subcommittee of the JSOG Ethics Committee, and the data set was provided according to the guidelines determined. Institutional Review Board approval was not obtained, because the entire data set was collected by Japan Society of Obstetrics and Gynecology and does not include any identifiable parameters.

### Patients and the Registry System

Patients were treated by 603 ART institutes in Japan. These institutes were registered with the JSOG online registration system and required to record the patients' ages, locations (prefecture, institution), subvention in ART treatment, protocols used in the treatment, and obstetrical outcomes. A summary of these registered data are annually disclosed on the JSOG home page.

The patients' information and the outcomes of their treatments were extracted from the JSOG database during a period ranging from 2007 to 2008. There were 352,682 cycles of treatment over the 2-year period, including 111,289 cycles of IVF, 120,516 cycles of ICSI, 14,174 split ICSI, and 102,916 cycles of FET. Treatment cycles using previously frozen oocytes, gamete intrafallopian transfer, oocyte intrauterine transfer, two-step embryo transfer cycles, and cases with missing or incomplete data were excluded. Pregnancies were achieved for 59,927 cycles, and 46,454 cycles of them were singleton pregnancies. Based on these data, 25,777 cycles of singleton birth at term gestation were investigated in this study.

### ART Treatments

**Ovarian stimulations.** The ovarian stimulation regimens included natural menstrual cycle (no stimulation), clomiphene citrate, clomiphene citrate with gonadotropin, gonadotropin, gonadotropin with GnRH agonist, gonadotropin with GnRH antagonist, and other protocols. In the present study, the category of other protocols was excluded. The gonadotropins used for ovarian stimulation included recombinant FSH and hMG. For the natural menstrual cycle protocols, there was no ovarian stimulation with clomiphene citrate or gonadotropin until after oocyte retrieval. The GnRH agonist protocols included long protocol, short protocol, ultralong protocol, and ultrashort protocol. Each protocol included an injection of hCG or an LH surge flare-up by GnRH agonist to facilitate oocyte maturation before oocyte retrieval.

**Categories of ART treatment.** The duration of embryonic culture until embryo transfer was categorized into two groups: cleavage stage and blastocyst stage. Embryo transfer cycles were categorized into two groups: fresh ET and FET.

**Luteal phase support.** During the luteal phase, there were several types of hormone supplementation used for both fresh ET and FET cycles. In the JSOG registry system, luteal phase hormone support included six groups: nontreatment, progesterone supplementation, hCG injection, hCG injection with progesterone supplementation, estrogen with progesterone supplementation, and others.

During FET cycles, the ovulatory cycle regimens were divided into nontreatment and hormone supplementation during the luteal phase, as mentioned above. Meanwhile, during the hormone replacement cycle, the endometrium was prepared via an initial estrogen supplement and following progesterone administration.

### Outcomes of Measurement

Pregnancies were defined via detection of a gestational sac with the use of ultrasound, and cycles with a singleton fetus in the first trimester and delivery at term gestation were extracted. Twin pregnancies, including monozygotic and dizygotic twins, were excluded. Parameters such as the birth weight, gender, and gestational age of the singleton infants were extracted from the database.

The mean birth weight resulting from fresh ET and FET cycles were compared with all neonates registered with the Ministry of Health, Labor, and Welfare in Japan from October 2007 to September 2009.

### Statistical Analysis

The difference of the mean birth weights in fresh ET, FET, and all Japanese neonates was interpreted by analysis of covariance (ANCOVA). The rate of low birth weight (<2,500 g) at term gestation was analyzed by multivariable logistic regression for ART, fresh ET, and FET cycles. Variables extracted for

this study included the maternal age at the time of ART treatment, the ovarian stimulation protocols, the number of retrieved oocytes, the insemination procedures, the duration of in vitro culture, the luteal phase hormone therapy protocols, whether fresh ET or FET was used, the sex of the infant, and the gestational age at birth. These independent variables were selected by means of backward elimination in each analysis. All of the statistical analyses were performed with the use of SAS software: JMP version 9.0.2 and SAS version 9.1.3 SP4 (SAS Institute). A significant difference was defined as $P<.05$ in all analyses.

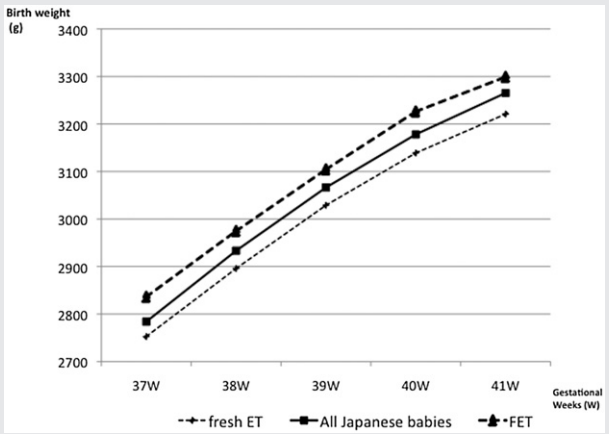
## RESULTS

For the present study, 1,842,598 Japanese singleton neonates and 25,777 singleton neonates following ART treatment were analyzed. The mean birth weight of neonates was 3,009.8 ± 376.8 g, 3,059.6 ± 369.6 g, and 3,100.7 ± 387.2 g in fresh ET cycles, all Japanese neonates, and FET cycles, respectively.

As seen in Figure 1, there was no interaction among the three groups regarding gestational age and birth weight. Therefore, ANCOVA was performed to verify differences in birth weights among these three groups. The average birth weight of the fresh ET group was lower and the average birth weight of the FET group significantly higher ($P<.0001$) after adjusting for gestational age. The birth weight after FET was approximately 90 g heavier compared with fresh ET in any of the gestational weeks (Fig. 1).

The risk of low birth weight was significantly lower in FET cycles according to multivariable logistic regression adjusted for maternal age, duration of embryo culture, gestational age, and sex of neonates (odds ratio [OR] 0.71, 95% confidence interval [CI] 0.63–0.80]).

**FIGURE 1**




The mean birth weight of three groups following term gestation. The mean birth weights of neonates were 3,009.8 ± 376.8 g, 3,059.6 ± 369.6 g, and 3,100.7 ± 387.2 g in fresh embryo transfer (ET) cycles, all Japanese neonates, and frozen-thawed ET (FET) cycles, respectively. The mean birth weight of fresh ET was lower compared with FET and all Japanese neonates. Among the three groups, the mean birth weight of FET was significantly higher ($P<.0001$) after adjusting for gestational age.

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### Risk for Low Birth Weight in Fresh ET Cycles

The relationship between birth weight and ART protocols used in fresh ET was analyzed. Various ovarian stimulation protocols were compared with the natural cycle protocol (Table 1). The odds ratios were adjusted for age, ovarian stimulation, duration of embryonic culture, gestational age, and sex of neonates. Other factors, which included the number of retrieved oocytes (<9 or ≥10), IVF, ICSI or split ICSI, and luteal phase hormone therapy, were eliminated owing to a low correlation with the risk of low birth weight ($P>.4$; data not shown). During fresh ET cycles, ovarian stimulation procedures were significantly correlated with birth weight. Ovarian stimulation with clomiphene citrate and clomiphene citrate with gonadotropins significantly increased the risk of low birth weight compared with the natural cycles (OR 2.09 [95% CI 1.34–3.33] and OR 1.88 [95% CI 1.22–2.98], respectively). In the GnRH agonist and GnRH antagonist protocols, the risk of low birth weight similarly increased (OR 1.72 [95% CI 1.17–2.62] and OR 1.60 [95% CI 1.05–2.50], respectively). There was a tendency for an increase in low birth weight during the gonadotropin alone cycle.

An extended embryonic culture for blastocysts decreased the risk of a low birth weight following the fresh ET cycle (OR 0.85 [95% CI 0.71–1.00]).

### Risk for Low Birth Weight in FET Cycles

The factors that correlated with a risk for low birth weight following FET cycles were analyzed. Patients' ages, duration of embryo cultures, luteal phase hormone therapies, sex of neonates, and gestational ages were adjusted for. The various luteal phase hormone therapies were compared with an ovulatory cycle without hormone supplementation. As presented in Table 2, the combined use of estrogen and progesterone for luteal phase hormone therapy significantly decreased the risk of low birth weight (OR 0.77 [95% CI 0.60–0.99]). There was no correlation between the duration of embryo culture and the risk of low birth weight.

### Risk for Low Birth Weight Based on Maternal Age Following Fresh ET and FET

The relative risk for low birth weight with an increase in maternal age is presented in Table 3. The risk for low birth weight following fresh ET resulted in slightly lower and/or no change with an advancing maternal age after the OR was adjusted for ovarian stimulation, duration of embryo culture, gestational age, and the sex of neonates (OR 0.98 [95% CI 0.96–1.00]). The risk for low birth weight following FET also showed slightly lower or no change regarding to maternal age (OR 0.96 [95% CI 0.94–0.98]).

### Risk for Low Birth Weight in the Sex of Neonates Following Fresh ET and FET

The risk of low birth weight following fresh ET and FET cycles was higher in female neonates compared with male neonates (OR 1.86 [95% CI 1.61–2.16] and OR 1.78 [95% CI 1.52–2.08], respectively), after ORs were adjusted for ovarian

## TABLE 1

**Mean birth weight and relative risk of low birth weight in each category and the variables used with fresh embryo transfer (ET).**

| Category | Variable | n (%) | Mean ± SD birth weight (g) | cOR (95% CI) | aOR (95% CI) |
|---|---|---|---|---|---|
| Ovarian stimulation protocols | Natural | 610 (5.4) | 3,043.5 ± 353.3 | Ref | Ref |
| | Clomiphene citrate | 981 (8.6) | 2,986.4 ± 382.3 | 1.19 (0.94–1.49) | 2.09 (1.34–3.33) |
| | Clomiphene citrate + gonadotropin | 1,237 (10.9) | 2,998.7 ± 386.1 | 1.11 (0.89–1.37) | 1.88 (1.22–2.98) |
| | Gonadotropin alone | 322 (2.8) | 3,001.9 ± 372.6 | 1.07 (0.70–1.58) | 1.65 (0.93–2.92) |
| | GnRH agonist | 6,336 (55.7) | 3,010.7 ± 378.3 | 1.02 (0.89–1.18) | 1.72 (1.17–2.62) |
| | GnRH antagonist | 1,888 (16.6) | 3,016.7 ± 370.0 | 0.93 (0.77–1.13) | 1.60 (1.05–2.50) |
| Duration of embryo culture | Cleavage stage | 7,994 (70.3) | 3,002.1 ± 377.2 | Ref | Ref |
| | Blastocyst stage | 3,380 (29.7) | 3,028.0 ± 375.3 | 0.56 (0.72–0.99) | 0.85 (0.71–1.00) |
| Sex of neonates | Male | 5,836 (51.3) | 3,050.9 ± 377.7 | Ref | Ref |
| | Female | 5,538 (48.3) | 2,966.4 ± 370.9 | 1.64 (1.43–1.89) | 1.86 (1.61–2.16) |

*Note:* In ovarian stimulation protocols, a natural cycle was used as the standard to measure the relative risks of clomiphene citrate, clomiphene citrate + gonadotropin, gonadotropin alone, GnRH agonist, and GnRH antagonist. During embryonic culture, the cleavage stage was set as the standard and the relative risk of the blastocyst stage was measured. When considering the sex of neonates, male neonates served as the standard to measure the relative risk of female neonates. aOR = adjusted odds ratio; cOR = crude odds ratio; CI = confidence interval.

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stimulation, the duration of embryo culture, gestational age, and maternal age (Tables 1 and 2).

## DISCUSSION

In the present study, the birth weight of 25,777 singleton neonates delivered at term following ART treatment was analyzed based on data retrieved from the national ART registry of Japan from 2007 and 2008. Cases of multiple neonates and pre- and postterm births were excluded because of differences in treatment strategies for obstetrical and neonatal management and various complications found in each of the institutes, as mentioned in several reports (4, 9, 10, 14, 15).

In the national ART registry in Japan, data were collected from all patients who underwent ART treatment and were enrolled through the JSOG online registration system. These data were mandatorily collected from all clinics and hospitals in Japan where ART treatment is provided. This registry system encourages the input of details for the ART procedures and perinatal and neonatal outcomes. The present study involved a large cohort (25,777 singleton neonates) of the neonatal outcomes after ART treatment, particularly singleton birth weights from term gestations.

The risk for low birth weight following fresh ET tended to be altered by the ovarian stimulation protocol, as presented in Table 1. Compared with the outcome of neonates in natural cycles, the birth weight in stimulated cycles was decreased. The risk of low birth weight in clomiphene cycles was two times higher compared with natural cycles. As mentioned above, a similar tendency was observed in cycles with the GnRH agonist and antagonist. These data may suggest that ovarian stimulation adversely affected the development of the placenta and fetal growth through the variation of the endometrial and uterine environment after implantation. There have been several reports regarding these speculations. It was reported that intrauterine insemination with ovulatory stimulation in infertile couples was associated with low birth weight infants compared with natural cycles (16). Ovulation induction in infertile women provoked the shortening of the crown-rump length measurement in the first trimester and increased the risk of low birth weight compared with fertile women (17). Regarding ART treatment, it was reported that the standard IVF with ovarian stimulation resulted in singleton birth weights that were significantly lower compared with birth weights following IVF in a modified natural cycle without ovarian stimulation (12). Additionally, the alteration of endometrial receptivity

## TABLE 2

**Mean birth weight and relative risk of low birth weight in each category and the variables used with frozen-thawed embryo transfer (FET).**

| Category | Variable | n (%) | Mean ± SD birth weight (g) | cOR (95% CI) | aOR (95% CI) |
|---|---|---|---|---|---|
| Duration of embryo culture | Cleavage stage | 2,946 (20.5) | 3,069.6 ± 384.7 | Ref | Ref |
| | Blastocyst stage | 11,457 (79.5) | 3,108.6 ± 387.5 | 0.81 (0.68–0.97) | 0.87 (0.72–1.05) |
| Luteal phase hormone supplementation | None | 875 (6.1) | 3,064.3 ± 393.1 | Ref | Ref |
| | hCG | 4,657 (32.3) | 3,102.1 ± 385.5 | 0.96 (0.82–1.13) | 0.84 (0.64–1.09) |
| | P + hCG | 377 (2.6) | 3,068.5 ± 371.9 | 1.05 (0.76–1.43) | 0.97 (0.68–1.35) |
| | P | 1,210 (8.4) | 3,061.1 ± 391.2 | 1.42 (1.11–1.78) | 1.08 (0.78–1.49) |
| | Estrogen + P | 6,115 (42.5) | 3,118.0 ± 387.1 | 0.81 (0.70–0.95) | 0.77 (0.60–0.99) |
| | Others | 1,169 (8.1) | 3,083.0 ± 385.9 | 1.11 (0.76–1.57) | 0.91 (0.60–1.35) |
| Sex of neonates | Male | 7,566 (52.5) | 3,151.9 ± 385.0 | Ref | Ref |
| | Female | 6,837 (47.5) | 3,044.0 ± 381.8 | 1.65 (1.41–1.92) | 1.78 (1.52–2.08) |

*Note:* During embryonic culture, the cleavage stage was set as the standard and the relative risk of the blastocyst stage was measured. During luteal phase hormone supplementation, no luteal phase hormone supplementation was the standard compared with the relative risks of hCG, P + hCG, P alone, estrogen + P, and others. When considering the sex of neonates, male neonates served as the standard to measure the relative risk of female neonates. aOR = adjusted odds ratio; cOR = crude odds ratio; CI = confidence interval.

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**TABLE 3**

**Relative risk of low birth weight based on maternal age during fresh fresh embryo transfer (ET) and frozen-thawed ET (FET).**

| | Fresh ET (n = 11,374) | | | FET (n = 14,403) | | |
|---|---|---|---|---|---|---|
| | Mean ± SD | cOR (95% CI) | aOR (95% CI) | Mean ± SD | cOR (95% CI) | aOR (95% CI) |
| Age (by 1 y) | 34.53 ± 3.75 | 1.01 (0.99–1.02) | 0.98 (0.96–1.00) | 34.63 ± 3.87 | 0.99 (0.97–1.01) | 0.96 (0.94–0.98) |

*Note:* aOR = adjusted odds ratio; cOR = crude odds ratio; CI = confidence interval.

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was implicated by controlled ovarian stimulation (18, 19). Analysis in the present study included the protocols for stimulation with the use of clomiphene, clomiphene with hMG/FSH, and hMG/FSH in addition to the GnRH agonist and antagonist compared with natural cycles. The present analysis demonstrated that all of these stimulation protocols increased the risk of low birth weight following fresh ET. The results support the findings of Haouzi et al. (18, 19).

In recent years, treatment with FET cycles has prevailed in Japan. The JSOG recommends single-embryo transfer to prevent multiple pregnancies, except for women >35 years old and/or with recurrent implantation failures. Additionally, the high quality of this procedure and a high assurance of the vitrification were developed in Japan (20–23).

In the present study, the adjusted mean birth weight of neonates after FET cycles was significantly higher compared with fresh ET cycles and all Japanese neonates at term gestation (3,100.7 ± 387.2 g vs. 3,009.8 ± 376.8 g and 3,059.6 ± 369.6 g, respectively). Similar results were reported in a Danish study reporting that the mean birth weight after FET cycles was 167 g higher than after fresh ET cycles (3).

There are several potential causes for the heavier birth weight found in neonates following FET cycles. In FET cycles included in the present study, it was clearly demonstrated that the mean birth weight increased with supplementation of estrogen and progesterone compared with natural cycles. The supplementation of estrogen and progesterone suggests the possible improvement of the uterine environment, leading to the development of the placenta, subsequent fetal growth, and heavier birth weight after implantation. Based on these results, one of the causes in the difference of the mean birth weight following fresh ET and FET was due to ovarian stimulation. Further study is required to understand the development of the endometrium during natural, stimulated, and hormone replacement cycles.

Other than the methods of embryo transfer, the duration of embryonic culture also may be one of the factors affecting the birth weight of neonates. Whereas the risk of low birth weight did not change in FET cycles, the risk decreased following a long culturing period extended to the blastocyst stage in fresh ET cycles. These results indicated that birth weight was also affected by the duration of embryonic culture, the medium for culture and freeze-thawing, and the manipulation of freezing and thawing. These processes in ART, including extended culture and cryopreservation, may induce epigenetic changes of the embryos and promote changes in birth weight. Recently, it was reported that the freezing and thawing processes affect DNA methylation (24). The alteration of epigenetic marks, including DNA methylation, was associated with fetal and child growth (25, 26). In the present study, the difference in birth weight was not critical when considering fetal abnormality. However, it was speculated that small changes of birth weight of neonates were most likely due to the epigenetic alteration mentioned above. Further studies are needed to determine the safety and effectiveness of ART treatment.

According to Nelson et al. (27), a higher maternal age carries a higher risk for low birth weight. The present study showed that there was no correlation with a risk for low birth weight and a high maternal age (Table 3). The possible causes for an increase in this risk may be due to parity, socioeconomic status, and paternal age (14, 28). Although these variables were not analyzed in the present study, it was speculated that pregnant women were generally encouraged to reduce their daily activities following ART treatment in Japan. This change in daily behavior may contribute to a reduction in the risk of low birth weight in women with a high maternal age.

In addition, the risk for low birth weight in both fresh ET and FET was higher in female than that in male neonates. These outcomes were similar to the results from earlier studies and the general population (29).

In summary, the results presented in this study demonstrated a meaningful impact on fetal growth following ART treatment. FET and extended culturing of embryos resulted in an increase in neonatal birth weight. Ovarian stimulation also affected neonatal birth weight compared with natural cycles. These findings may indicate that the uterine environment was affected by ovarian activity, with direct implications for fetal growth. The best method of embryo transfer for fetal growth was FET after extended culturing to blastocyst stage along with sufficient hormone supplementation. However, the influence of epigenetic alteration in these manipulations and culturing may also play a role in neonatal birth weight. Further studies should be performed to fully understand the safety and effectiveness of ART treatment.

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