Comparison of birth weights in patients randomly assigned to fresh or frozen-thawed embryo transfer

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Objective: To estimate birth weight differences between patients randomized to fresh or thawed ET.
Design: Post hoc analysis of results from two similar randomized trials.
Setting: Private fertility center.
Patient(s): One hundred thirty-four first-time IVF patients, ages 18–40 years at oocyte retrieval, who had live birth.
Intervention(s): Patients were randomly assigned to have either fresh blastocyst transfer or all bipronuclear oocytes frozen followed by thaw, extended culture, and blastocyst transfer in a subsequent cycle. Preimplantation genetic screening was not allowed.
Main Outcome Measure(s): Mean birth weight.
Result(s): After allowing for the contributions of multiple significant variables (gestational age at birth, the presence of a vanished twin, number of infants delivered) in multiple linear regression, the adjusted mean birth weight was 166 g (95% confidence interval, 43–290 g) lower after fresh blastocyst transfer when compared with transfer of blastocysts derived from thawed bipronuclear oocytes.
Conclusion(s): Birth weights are lower in cycles with fresh blastocyst transfer after controlled ovarian stimulation than in transfers of frozen-thawed embryos in the absence of ovarian stimulation. This finding confirms similar results reported in many retrospective studies.
Clinical Trial Registration Numbers: NCT00963625 and NCT00963079. (Fertil Steril® 2016;106:317–21. ©2016 by American Society for Reproductive Medicine.)
Key Words: Assisted reproduction, ovarian stimulation, embryo cryopreservation, birth weight

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Controlled ovarian stimulation (COS) with gonadotropins is routinely used to obtain multiple oocytes to increase success rates of IVF as compared with natural ovulation cycles. However, COS and its associated supraphysiologic hormone levels may result in a suboptimal uterine environment for embryo implantation and growth.

COS exposure is associated with altered endometrial development when compared with natural cycles. Developmental differences associated with COS exposure include histologic advancement, premature down-regulation of the P receptor, an abbreviated luteal phase, glandular-stromal dyssynchrony, genomic dysregulation, altered leukocyte localization and activation, premature nucleolar channel formation, advanced angiogenesis, increased blood vessel density, and reduced endometrial blood flow (1–10). The degree of histologic advancement correlates with IVF outcome and is increased when P levels are prematurely elevated (1, 2, 11–13). One randomized trial found that embryos transferred in fresh autologous cycles with COS exposure are less likely to implant than their frozen-thawed counterparts transferred in cycles without COS exposure, suggesting a possible effect of reduced endometrial receptivity after ovarian stimulation (14).

It has been repeatedly observed in registry studies and meta-analyses that infants resulting from fresh autologous ET have reduced birth weight, increased risk of low birth weight, and other perinatal risks associated with birth weight when compared with infants resulting from the transfer of frozen-thawed embryos (15–29). For example, the reported birth weight differences, consistently greater with frozen embryos than with fresh, are 167–250 g in Denmark (16–18), 134 g in Finland (19), 133 g in combined

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Nordic registries (20), 156 g in the United States (21), 91–100 g in Japan (22, 23), 145 g in Australia and New Zealand (24), and 80 g among singletons in Latin America (25). Multiple retrospective clinical studies have also found greater birth weight with frozen-thawed ET (FET) than with fresh transfer, with reported birth weight differences ranging from 50 to 218 g (26–29).

One potential cause of birth weight differences is a suboptimal uterine environment after COS exposure (21). Alternatively, cryopreservation might alter birth weight through embryonic effects. However, the contrasting patterns in birth weight effects after autologous fresh, autologous FET, fresh cycles of oocyte donation, and FET cycles using embryos derived from donor oocytes have been used to examine the potential for embryonic or uterine effects of cryopreservation. An American registry study reported no significant difference between the incidence of low birth weight after fresh donor and donor FET cycles, while finding increased incidence of low birth weight after autologous fresh transfer when compared with autologous FET (21). Furthermore, a retrospective cohort study compared births from fresh donor cycles and donor FET cycles in a set of recipients with at least one live birth from each transfer type and found no significant differences in birth weight or perinatal outcomes (30). Each of these findings in oocyte donation cycles contradicts the hypothesis of a significant embryonic effect of cryopreservation on birth weight.

However, retrospective studies, including registry studies, have inherent potential confounding through lack of randomization, and registry studies typically lack detail regarding treatment protocols, such as embryo culture conditions, cryopreservation technique, endometrial preparation, luteal support, and COS protocol, among other potentially relevant parameters. For example, FET cycles have frequently used supernumerary embryos after the morphologically best (primary) embryos were transferred fresh, potentially creating confounding biases in embryo selection and patient selection in retrospective and registry studies. The multiple opportunities for confounding may cloud interpretation of results.

The current study is a post hoc analysis of two concurrent randomized trials, one in normal responders (14) and the other in high responders (31), in which patients were randomly assigned to fresh ET or FET at a single center. The two studies used identical staff, culture conditions, COS protocols and medications, cryopreservation methods, embryo selection and transfer techniques, and luteal support. The inclusion criteria were similar (first-time IVF patients, 18–40 years of age, day 3 FSH $<$10 IU/L, no preimplantation genetic testing) and differed only in the number of antral follicles so that the normal-responder study (14) specified 8 to 15 antral follicles and the high-responder study (31) specified $>$15 antral follicles. The purpose of the difference in antral follicle count was so that the normal-responder protocol could specify an ovulatory trigger of hCG alone, while, for safety reasons, the high-responder study specified a trigger of low-dose hCG in combination with GnRH agonist. However, both of these trigger types were allowed in both studies, based on physician discretion. Therefore the two studies form a continuum of subjects with normal to high ovarian response treated under consistent protocols and laboratory conditions.

Both studies used only primary embryos (no supernumerary embryos) regardless of whether the transfers were fresh or frozen-thawed. The transferred embryos in the FET arms were blastocysts derived from thawed bipronuclear oocytes, so that the morphologically best blastocysts in each study arm were selected for transfer.

A post hoc analysis of these two studies will therefore be useful in examining birth weight differences, if any, without potential confounding from unknown methodological differences or nonrandomized treatment assignment.

MATERIALS AND METHODS

The two randomized trials were performed from 2007 through 2010 and included 259 subjects randomized 1:1 to cohort cryopreservation or else fresh embryo culture at a single infertility center. The two trials were registered on ClinicalTrials.gov with trial numbers NCT00963625 (normal-responder study) and NCT00963079 (high-responder study). Both studies were Institutional Review Board approved and independently monitored. Each study specified an interim stopping point and stopping criteria. The normal-responder study was stopped at that point because the stopping criteria were met (a difference in clinical pregnancy rate with $P < .03$), while the high-responder study was halted at that same point for safety reasons (the routine transfer of two blastocysts in all patients could no longer be supported owing to excessive twins).

Inclusion criteria specified women age 18–40 years seeking their first IVF treatment, with 8–15 antral follicles in the normal-responder study and 16 or more antral follicles in the high-responder study. Cycle day 3 FSH $\geq$ 10 IU/L was exclusionary, as was any use of preimplantation genetic testing. For the current post hoc study of birth weights, only live births are included in the birth weight analyses.

After informed consent, all subjects underwent conventional COS with gonadotropins, including a combination of urinary FSH (Menopur, Ferring Pharmaceuticals Inc.) and recombinant FSH (Follistim, Schering-Plough Inc.) in all cases. Follicular development was ultrasonically monitored at 2- to 3-day intervals. On or about the sixth day of stimulation, GnRH antagonist (ganirelix acetate, Schering-Plough) was initiated in all patients and sustained until the completion of stimulation.

After at least three follicles reached 18 mm in mean diameter, an ovulatory trigger of either hCG alone or else a reduced dose of hCG in combination with 4 mg GnRH agonist (leuprolide acetate) was administered to promote final oocyte maturation. The high-responder study protocol specified a dual trigger, and the normal-responder study protocol specified a trigger of hCG alone, but variation in the triggers was allowed for safety reasons, and this variation is described in the Results section. For safety reasons, patients who had extreme response to COS (typically more than 30–40 developing follicles) received GnRH agonist trigger alone and were dropped from the studies. Retrieval was scheduled 35–36 hours after trigger. In both studies, randomization was performed by