# Phenotypic differences in children conceived from fresh and thawed embryos in in vitro fertilization compared with naturally conceived children

Mark P. Green, Ph.D.,[a,b] Fran Mouat, M.D.,[a] Harriet L. Miles, M.D., Ph.D.,[a] Sarah A. Hopkins, Ph.D.,[a,b] José G. B. Derraik, Ph.D.,[a] Paul L. Hofman, M.D., Ph.D.,[a,b] John C. Peek, Ph.D.,[c] and Wayne S. Cutfield, M.D., Ph.D.[a,b]

[a] Liggins Institute, University of Auckland; [b] Gravida (National Centre for Growth and Development), University of Auckland; and [c] Fertility Associates, Auckland, New Zealand

**Objective:** To determine whether anthropometric and biochemical features differ in in vitro fertilization (IVF) children conceived via fresh ($IVF_F$) or thawed ($IVF_T$) embryo transfer compared with naturally conceived controls.
**Design:** A cross-sectional controlled study.
**Setting:** University clinical research unit.
**Patient(s):** Healthy prepubertal children (3.5–11.0 years), singletons, born at term (>37 weeks), who were either naturally conceived (controls; n = 94) or IVF children conceived via the transfer of a fresh ($IVF_F$; n = 72) or thawed ($IVF_T$; n = 43) embryo.
**Intervention(s):** None.
**Main Outcome Measure(s):** Assessments of anthropometry (adjusted for parental variables), dual-energy X-ray absorptiometry–derived body composition, fasting plasma growth factors, lipids, and parameters of glucose regulation.
**Result(s):** The $IVF_F$ but not the $IVF_T$ children weighed less at birth than the control children. The $IVF_F$ children were taller than both the controls and $IVF_T$ children. Sex-specific analyses showed height differences among girls, with $IVF_F$ girls being taller than their control and $IVF_T$ counterparts. Taller stature in $IVF_F$ children was associated with increased insulin-like growth factor I (IGF-I) concentrations compared with controls, whereas the $IVF_T$ children displayed increased IGF-II and decreased insulin-like growth factor binding protein 3 (IGFBP-3) concentrations compared with the controls. More favorable lipid profiles were also evident in $IVF_F$ but not $IVF_T$ children compared with the control children.
**Conclusion(s):** These preliminary findings highlight that the transfer of a fresh versus a thawed IVF embryo affects height, plasma growth factor, and lipid profiles in childhood. Therefore, embryo derivation should be considered when assessing physical and biochemical phenotype of IVF children. (Fertil Steril® 2013;99: 1898–904. ©2013 by American Society for Reproductive Medicine.)
**Key Words:** BMI, child, growth, IGF, IVF

**Discuss:** You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/greenm-ivf-igf-children-bmi/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

The use of in vitro fertilization (IVF) technologies has been steadily increasing in Western countries, with an estimated 4 million IVF babies born worldwide since 1978 (1). The children of IVF now account for approximately 1% to 3% of current births in developed countries (2). Over the last decade, improved embryologic techniques and a move to transferring fewer fresh embryos has led to a substantial increase in the proportion of IVF children born from the transfer of a thawed rather than fresh embryo (3). Recent data from the United Kingdom (4), Europe (5), the United States (6), and elsewhere (7) show that approximately 20% of cycles involved thawed embryos.

Received November 2, 2012; revised January 21, 2013; accepted February 6, 2013; published online March 6, 2013.
M.P.G. received a fellowship from Fertility Associates. F.M. has nothing to disclose. H.L.M. has nothing to disclose. S.A.H. has nothing to disclose. J.G.B.D. has nothing to disclose. P.L.H. has nothing to disclose. J.C.P. is a shareholder of Fertility Associates. W.S.C. has nothing to disclose.
M.P.G. and F.M. contributed equally as first authors to the study.
Supported by grants from the Gravida: National Centre for Growth and Development (NRCGD) and the Australasian Paediatric Endocrine Group (APEG).
Reprint requests: Wayne S. Cutfield, M.D., Ph.D., Liggins Institute, University of Auckland, 85 Park Road, Grafton, Auckland 1023, New Zealand (E-mail: w.cutfield@auckland.ac.nz).

Fertility and Sterility® Vol. 99, No. 7, June 2013 0015-0282/$36.00
Copyright ©2013 American Society for Reproductive Medicine, Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.fertnstert.2013.02.009

Studies have shown that IVF children born after the transfer of a fresh embryo weighed less than naturally conceived children at birth, which was not observed among those born from the transfer of a thawed embryo (8–11). However, neonatal outcomes after transfer of a thawed compared with fresh embryo transfer were similar, including rates of prematurity, still birth, neonatal death, and major malformations (12–14). Nonetheless, few studies have investigated growth into childhood, and most have not excluded important confounding factors such as the use of gamete donors, "vanishing" twins, and premature and multiple births as well as small for gestation age (SGA) children (15–20). In addition, data were seldom corrected for parental (genetic) contributions (21, 22), and children from fresh and thawed embryos were not separated in the analyses. A recent review highlighted that these confounding factors may explain the conflicting reports as to whether differences exist between IVF and naturally conceived children (23).

Despite conflicting reports, a number of studies, including one from our own group, have detected distinct phenotypic differences before puberty in IVF children when compared with naturally conceived children (24–26). However, to date none have carefully compared the characteristics between children conceived from fresh and thawed embryos. We aimed to compare anthropometric and biochemical features between IVF children born after the transfer of a fresh versus a thawed embryo as well as to those of naturally conceived children. The current study uses data from our existing cohort as well as newly recruited children. Importantly, confounding factors (e.g., parental anthropometry) were taken into account.

## MATERIALS AND METHODS

### Participants

The study recruited healthy prepubertal children with no medically diagnosed disorders, who were taking no medications, who were between the ages of 3.5 and 11 years of age, and were born between September 1993 and July 2005 at term (>37 weeks' gestation) after singleton pregnancies. The IVF participants were living in Auckland and Hamilton (New Zealand) and were recruited from Fertility Associates (the largest IVF provider in the country) between March 2004 and November 2008, and their children were conceived via the transfer of either a fresh ($IVF_F$) or thawed ($IVF_T$) embryo. The IVF cycles in which a thawed embryo was transferred were undertaken between June 1994 and April 2003. An equal proportion of all children (50%) were derived by either conventional IVF or intracytoplasmic sperm injection (ICSI). The naturally conceived controls were school friends or siblings of the IVF participants so that the controls were matched as closely as possible for socioeconomic background (using school decile and residential address), age, sex, and ethnicity. Exclusion criteria were the use of donor gametes (sperm or egg), more than one fetal sac identified at 7 to 8 weeks' gestation, children born small for gestational age (SGA), and women with a known medical syndrome, a chronic illness, or who were receiving regular medications. Ethics approval (MEC/06/11/148) was provided by the Auckland Ethics Committee. Written and verbal informed consent was obtained from all participants.

### Descriptive and Anthropometric Measurements

All clinical assessments were performed at the Maurice & Agnes Paykel Clinical Research Unit (The Liggins Institute, University of Auckland). Gestation length, birth weight corrected to standard deviation scores (SDS) (27), and the child's age at assessment were recorded. Maternal age, height, and weight at conception were retrieved from IVF patient records, and paternal height and weight were obtained during the study. Body mass index (BMI) was calculated, and midparental height and midparental BMI were corrected to a SDS (27). Each child's weight, standing height (measured using a Harpenden stadiometer), and BMI were calculated as an SDS, and corrected for midparental height and midparental BMI (27, 28). Weight and body composition (percentage body fat and bone mineral density z-score) were assessed using whole-body dual-energy x-ray absorptiometry (DEXA) (Lunar Prodigy 2000; General Electric). Bone age was blindly assessed by a single pediatric radiologist using established standards (29) so that bone age and chronologic age could be compared. Parents were asked to complete a 3-day dietary record on behalf of their child before the anthropometric and endocrine study.

### Metabolic and Endocrine Measurements

After an overnight fast, an early morning blood sample was taken from each child. Plasma glucose, triglycerides, total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) concentrations were measured with commercial kits (Roche Diagnostics) on an autoanalyzer (Roche/Hitachi 9002 Analyzer; Hitachi). All assays had an intra-assay coefficient variation (CV) lower than 5%. Insulin concentrations were determined by enzyme immunoassay (IMX microparticle assay; Abbott Laboratories) with an intra-assay CV of 4.5%. Commercially available enzyme-linked immunosorbent assays (ELISA; Diagnostic Systems Laboratories) were used to evaluate plasma insulin-like growth factor I (IGF-I) (DSL-10–5600; intra-assay CV 5.8%, interassay CV 8.4%), IGF-II (DSL-10–9100; intra-assay CV 4.7%, inter-assay CV 6.2%), and insulin-like growth factor binding protein 3 (IGFBP-3) (DSL-10–6600; intra-assay CV 7.3%, interassay CV 8.2%).

Insulin sensitivity was assessed using the homeostasis model assessment resistance index (HOMA-IR) (30). This was calculated using the following formula: HOMA-IR = Fasting glucose (mM) × Fasting insulin (mIU/L)/22.5.

### Statistical Analysis

Descriptive, anthropometric, metabolic, and endocrine data were compared between [1] controls and IVF children ($IVF_F$ and $IVF_T$ combined), and [2] between all three groups (controls, $IVF_F$, and $IVF_T$) to identify overall IVF effects as well as specific group effects. A generalized linear mixed model followed by Tukey's post hoc comparison was used to assess

differences in response variables. Where appropriate, the child's age, standing height, percentage body fat, and sex were included in the model. Interactions between the main effects were also examined. All analyses were run using the Proc Mix Glimmix procedure of SAS software version 9.1 (SAS Institute). The sex ratio (proportion of males) within each group was compared with an expected 1:1 ratio by a corrected chi-square procedure as well as by using binomial analysis. The mean and standard error of the mean (SEM) are reported for descriptive data, and the mean and SEM adjusted for other variables in the model are reported for all other measures. $P<.05$ was considered statistically significant.

# RESULTS

## Participants

The study cohort consisted of 209 children, of which 115 were conceived using IVF and 94 conceived naturally. For the IVF group, 137 children were eligible for the study, of which 84% were recruited. Most of the $IVF_F$ (56 of 61) children had been previously studied, as were 71 of the 94 control participants (24). Thirteen of the original 69 $IVF_F$ from the cohort featured in the study by Miles et al. (24) were excluded as a result of refinement of inclusion/exclusion criteria. In addition, we recruited 43 $IVF_T$ children (and added a further five $IVF_F$ children who volunteered) as well as a further 23 approximately matched controls.

Gestation length, birth weight SDS, and maternal age were similar among the children recruited into the study and those who did not take part. Causes of infertility and the proportion of children conceived using ICSI were also similar. Thus, the IVF children recruited for this study are representative of the IVF cohort born during this time period. The IVF children were conceived via the transfer of fresh ($IVF_F$; n = 72; 38 males, 34 females) or thawed ($IVF_T$; n = 43; 21 males, 22 females) embryos. The naturally conceived children (controls; n = 94) comprised 49 males and 45 females.

Included in the study cohort were a number of sibling pairs (controls and IVF, n = 12, Same IVF group n = 6, Different IVF group n = 12). Compliance and completion of anthropometry and blood sampling was high (>91%), although 11 children (controls: n = 1; $IVF_F$: n = 4; $IVF_T$: n = 6) did not undergo DEXA analysis because of their geographic distance, and 18 children (controls: n = 3; $IVF_F$: n = 11; $IVF_T$: n = 4) chose not to provide a blood sample.

## IVF Children: Ovarian Stimulation Protocol, IVF, and Culture Data

The majority (92%) of mothers of IVF children received a standard gonadotropin-releasing hormone (GnRH) agonist down-regulation stimulation protocol, with the remainder receiving either stimulation without a GnRH agonist (2%) or a GnRH agonist flare stimulation protocol (6%). Fifty percent of cycles used ICSI. During this period, fresh embryos were usually transferred on day 2 after oocyte collection. Luteal support was provided by micronized progesterone (600 mg/day, Utrogestan; Pharmaco (NZ) Ltd.) until $\beta$ human chorionic gonadotropin ($\beta$-hCG) testing on day 18. Embryos were frozen using a standard propanediol method (31) on the day of embryo transfer. Embryos were thawed and transferred within a few hours in either a natural menstrual cycle (81%) tracked by daily measurement of blood luteinizing hormone (LH) or in a manufactured cycle (19%) using estradiol valerate (4 mg/day, Progynova; Bayer Schering Pharma) and micronized progesterone (600 mg/day Utrogestan; Pharmaco (NZ) Ltd.). The mean number of embryos transferred was $1.7 \pm 0.7$ for fresh and $2.3 \pm 0.5$ for thawed cycles.

## Participant Characteristics and Measures

Birth and parental measures for the three groups are summarized in Table 1. The maternal age at conception was higher ($P<.0001$) among mothers of IVF children ($IVF_F$ and $IVF_T$ combined) compared with mothers of the control children, but all other parental characteristics were similar (see Table 1). The $IVF_F$ children were lighter at birth than either the $IVF_T$ or the control children (based on both grams and SDS; see Table 1). There were no differences in dietary records among the groups. As expected, more IVF children were firstborns (76%) compared with the control children (49%), although birth order did not influence any of the outcome measures, including height SDS or metabolic or endocrine concentrations (data not shown). No differences were evident among the children derived by conventional IVF ($IVF_F$: n = 38; $IVF_T$: n = 20) or ICSI ($IVF_F$: n = 34; $IVF_T$: n = 23) (data not shown).

Anthropometric data are summarized in Table 2. The control children were slightly older ($P=.003$) than either the $IVF_F$ or $IVF_T$ children, but there were no differences between bone age and chronologic age among groups (see Table 2). When height was corrected for midparental height, the IVF children ($IVF_F$ and $IVF_T$ combined) were taller ($P=.004$) than the control children; however, when embryo treatment (fresh versus thawed) was accounted for, only the $IVF_F$ children were taller ($P=.002$) than the control children. The $IVF_F$ children were also taller ($P=.05$) than the $IVF_T$ children. When the boys and girls were examined separately, sexually dimorphic differences in height SDS were evident (Fig. 1). The height differences were only statistically significant ($P<.05$) in the girls, with $IVF_F$ girls being approximately 2.5 cm taller than the controls, and approximately 1.5 cm taller than the $IVF_T$ girls (see Fig. 1). There were no other differences in anthropometry among the groups (see Table 2).

The plasma IGF-I and IGF-II concentrations were higher in the IVF children ($IVF_F$ and $IVF_T$ combined) than in the control children (Table 3). However, in comparison with the controls, the IGF-I concentrations were only higher ($P<.04$) in the $IVF_F$ group, whereas the IGF-II concentrations were increased ($P<.03$) and IGFBP-3 decreased ($P<.002$) only in the $IVF_T$ children (see Table 3). Note that similar to the pattern observed for height, higher IGF-I concentrations were observed in the $IVF_F$ girls but not the boys ($P<.01$; $IVF_F$ girls: $116.87 \pm 7.38$ $\mu$g/L; $IVF_F$ boys: $81.95 \pm 6.24$ $\mu$g/L). Overall, there was an association between taller stature and higher circulating concentrations of IGF-I ($r^2 = 0.27$; $P<.0001$) and IGF-II ($r^2 = 0.14$; $P<.0001$). In contrast, differences in the plasma IGF-II and IGFBP-3 concentrations were evident in both $IVF_T$ boys and girls.

## TABLE 1

**Summary of birth and parental characteristics of control (CON), IVF combined, IVF fresh ($IVF_F$), and IVF thawed ($IVF_T$) children.**

| Characteristic | CON (n = 94) | IVF combined (n = 115) | $IVF_F$ (n = 72) | $IVF_T$ (n = 43) | *P* value: CON vs. IVF combined | *P* value: CON vs. $IVF_F$ | *P* value: CON vs. $IVF_T$ | *P* value: $IVF_F$ vs. $IVF_T$ |
|---|---|---|---|---|---|---|---|---|
| Midparental height SDS | 0.87 ± 0.09 | 0.86 ± 0.08 | 0.76 ± 0.10 | 1.02 ± 0.13 | .93 | .70 | .60 | .26 |
| Midparental BMI SDS | 0.95 ± 0.08 | 0.89 ± 0.07 | 0.90 ± 0.09 | 0.87 ± 0.12 | .60 | .92 | .87 | .98 |
| Maternal age (y) | 32.36 ± 0.45 | 35.38 ± 0.41 | 34.92 ± 0.52 | 36.15 ± 0.67 | <.0001* | <.0001* | <.0001* | .15 |
| Gestation (wk) | 39.65 ± 0.14 | 39.59 ± 0.12 | 39.47 ± 0.16 | 39.79 ± 0.21 | .76 | .69 | .84 | .45 |
| Birth weight (g) | 3,593 ± 48 | 3,501 ± 51 | 3,377 ± 64 | 3,708 ± 77 | .20 | .01* | .20 | .002* |
| Birth weight SDS | 0.34 ± 0.11 | 0.13 ± 0.10 | −0.08 ± 0.12 | 0.48 ± 0.15 | .15 | .03* | .72 | .01* |

*Note:* Data are mean ± standard error of the mean. BMI = body mass index.
* Statistical significance (*P*< .05).

*Green. Phenotypic differences in IVF children. Fertil Steril 2013.*

Glucose and insulin concentrations as well as HOMA-IR (insulin sensitivity) were similar among the groups (see Table 3). No differences in plasma cholesterol or LDL concentrations were identified, but HDL concentrations were higher (*P*<.001) in the $IVF_F$ and lower (*P*<.001) in the $IVF_T$ children compared with the controls (see Table 3). Further, $IVF_F$ children had lower (*P*<.05) triglyceride concentrations than both the $IVF_T$ and control children (see Table 3).

## DISCUSSION

The current study identified anthropometric, endocrine, and metabolic differences between IVF pre-pubertal children born following the transfer of a fresh compared to thawed embryo. In addition, differences were also observed between IVF and naturally conceived children.

At birth, $IVF_F$ children weighted approximately 250 g less than the $IVF_T$ children, who had a similar birth weight to naturally conceived children. This difference in birth weight among the three groups is comparable to those reported in large IVF cohort studies (9, 11, 32). However, despite weighing less at birth than $IVF_T$ and naturally conceived children, $IVF_F$ children appeared to have caught up in linear growth but without changes in their weight or BMI compared with either of the other groups. This contrasts with SGA children, whose catch-up growth is associated with an increase in fat mass by early childhood (33). The $IVF_F$ girls were in fact taller than the controls and $IVF_T$ girls in our study. Ceelen et al. (25) have suggested that the exaggerated growth of IVF children during infancy is a physiologic and compensatory process that promotes the restoration of the infants' genetic growth trajectory after a period of prenatal growth restraint due to unfavorable environmental conditions. Previous studies, both cross-sectional (17, 20, 34, 35) and longitudinal (25), have identified lighter birth weights and catch-up growth in IVF children but found no differences in height or weight compared with naturally conceived children. However, these studies did not have our strict inclusion criteria, and they did not exclude participants born SGA, premature, or from multiple births. All of these factors have been independently shown to constrain childhood growth, and thus might have masked any increase in the height of $IVF_F$ children (36, 37). Importantly, taller stature in $IVF_F$ children cannot be explained by earlier biologic maturation as assessed by bone age x-rays (38); consequently, our study provides strong evidence that the observed differences in height are likely to persist into adulthood.

Altered plasma endocrine (IGF) profiles were also observed in IVF children compared with naturally conceived children, with an increase in plasma IGF-I concentration

## TABLE 2

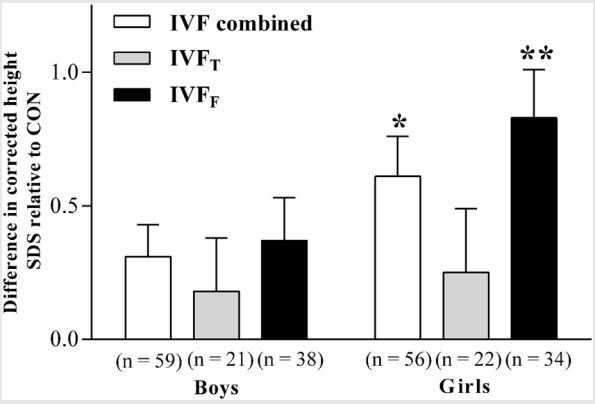
**Summary of anthropometric measures of control (CON), IVF combined, IVF fresh ($IVF_F$), and IVF thawed ($IVF_T$) children.**

| Characteristic | CON (n = 94) | IVF combined (n = 115) | $IVF_F$ (n = 72) | $IVF_T$ (n = 43) | *P* value: CON vs. IVF combined | *P* value: CON vs. $IVF_F$ | *P* value: CON vs. $IVF_T$ | *P* value: $IVF_F$ vs. $IVF_T$ |
|---|---|---|---|---|---|---|---|---|
| Sex M/F (proportion male) | 49/45 (52.1%) | 59/56 (51.3%) | 38/34 (52.7%) | 21/22 (48.8%) | .30 | .50 | .62 | .68 |
| Age (y) | 6.80 ± 0.18 | 6.04 ± 0.17 | 6.03 ± 0.21 | 6.07 ± 0.27 | .003* | .02* | .07 | .99 |
| Bone age (y) | 6.86 ± 0.19 | 6.11 ± 0.20 | 6.08 ± 0.25 | 6.16 ± 0.34 | .008* | .04* | .17 | .98 |
| Height SDS | 0.64 ± 0.09 | 1.08 ± 0.09 | 1.11 ± 0.11 | 1.01 ± 0.14 | .0008* | .004* | .08 | .83 |
| BMI SDS | 0.10 ± 0.10 | −0.03 ± 0.10 | −0.12 ± 0.13 | 0.12 ± 0.16 | .36 | .36 | .99 | .46 |
| DEXA fat %[a] | 17.9 ± 0.77 | 16.1 ± 0.70 | 16.7 ± 0.88 | 15.0 ± 1.14 | .08 | .58 | .09 | .45 |
| Corrected height SDS[b] | −0.22 ± 0.10 | 0.18 ± 0.10 | 0.32 ± 0.12 | −0.06 ± 0.16 | .004* | .002* | .67 | .05* |
| Corrected BMI SDS[b] | −0.84 ± 0.12 | −0.78 ± 0.11 | −0.88 ± 0.13 | −0.62 ± 0.17 | .72 | .98 | .56 | .48 |

*Note:* Data are as mean ± standard error of the mean. BMI = body mass index; DEXA = dual-energy x-ray absorptiometry; SDS = standard deviation score.
[a] CON (n = 93), IVF combined (n = 105), $IVF_F$ (n = 68), and $IVF_T$ (n = 37).
[b] Parameters corrected for midparental SDS.
* Statistical significance (*P*< .05).

*Green. Phenotypic differences in IVF children. Fertil Steril 2013.*

## FIGURE 1




Differences in corrected height standard deviation scores (SDS) (Child's height SDS − Midparental height SDS) relative to naturally conceived (control) children (CON: controls: n = 94) for IVF children born from the transfer of an IVF fresh (IVF$_F$: n = 72) or thawed (IVF$_T$: n = 43) embryo, according to sex. Data are also included for IVF combined (IVF$_F$ + IVF$_T$: n = 115). Data are the mean ± the standard error of the mean (SEM). *$P$<.05; **$P$<.01 for comparisons with control children within each sex.

*Green. Phenotypic differences in IVF children. Fertil Steril 2013.*

observed in IVF$_F$ girls. Insulin-like growth factors are well established as promoters of growth, hence the increase in circulating IGF-I would logically fit with the increased postnatal catch-up growth and height of IVF$_F$ girls (24). An increase in IGF-II coupled with a decrease in IGFBP-3 concentrations was also evident in the IVF$_T$ children. These particular changes are less easily explained and may involve different processes than those regulating increased IGF-I concentrations in IVF$_F$ girls.

Some metabolic parameters (HDL and triglyceride concentrations) also differed between the IVF$_F$ and IVF$_T$ children, with IVF$_T$ children having concentrations closer to those of naturally conceived children. Only IVF$_F$ children displayed a more favorable lipid profile with higher HDL and lower triglyceride concentrations. Conversely, Kanaka-Gantenbein et al. (39) found that IVF children had elevated triglyceride levels, but that study included a large proportion of SGA and pubertal children in whom higher triglyceride levels occur.

The factors leading to the observed phenotypic and biochemical differences in IVF$_F$ and IVF$_T$ children are unclear. Possible explanations include one or more of the following: [1] use of supraphysiologic doses of fertility drugs that may affect the oocyte and uterus; [2] the IVF culture process; [3] the freeze-thaw process; [4] embryo selection; and [5] parental genetic or epigenetic characteristics. Supraphysiologic doses of fertility drugs may alter the intrauterine environment, which may in turn affect embryo development, but this theory remains contentious (9, 40–43). The IVF$_F$ children were embryos transferred into a uterine environment exposed to fertility drugs, in contrast to IVF$_T$ children who were transferred into the uterus in a natural or manufactured cycle. Some researchers advocate that an embryo should be transferred in a natural cycle to avoid the impact of ovarian stimulation on the endometrium (44). Thawed embryo pregnancy rates appear to be lower compared with fresh embryo transfers (5, 8), possibly due to the selection of second-best embryo for freezing and/or a detrimental effect of the freeze-thaw process. However, recent studies have identified comparable or increased pregnancy rates after the transfer of a thawed embryo (45, 46). Parental characteristics (from mother and/or father) that are associated with subfertility and infertility as well as birth order may also influence the growth and metabolism of the offspring (40). However, recent studies accounting for all these factors have still identified differences in birth weight and anthropometric measures between IVF and naturally conceived children (18, 25).

Importantly, not only are the initiating factors unclear, but the subsequent mechanisms leading to the observed changes are also unknown. One possible mechanism that has been explored in animal studies is epigenetic alteration in imprinted and nonimprinted genes that can affect growth.

## TABLE 3

**Circulating endocrine and metabolic measures of control (CON), IVF combined, IVF fresh (IVF$_F$), and IVF thawed (IVF$_T$) children.**

| Characteristic | CON (n = 91) | IVF combined (n = 100) | IVF$_F$ (n = 61) | IVF$_T$ (n = 39) | *P* value: CON vs. IVF combined | *P* value: CON vs. IVF$_F$ | *P* value: CON vs. IVF$_T$ | *P* value: IVF$_F$ vs. IVF$_T$ |
|---|---|---|---|---|---|---|---|---|
| IGF-I (49–538 μg/L) | 94.0 ± 5.0 | 98.3 ± 5.1 | 100.0 ± 5.5 | 95.6 ± 9.9 | .06 | .04* | .92 | .24 |
| IGF-II (557–924 μg/L) | 765 ± 25 | 824 ± 28 | 785 ± 27 | 878 ± 55 | .02* | .23 | .03 | .52 |
| IGFBP-3 (1,000–8,400 μg/L) | 4,257 ± 127 | 3,911 ± 135 | 4,447 ± 135 | 3,169 ± 219 | .46 | .28 | .002* | <.001* |
| Glucose (2.6–5.7 mM) | 4.80 ± 0.05 | 4.66 ± 0.04 | 4.70 ± 0.06 | 4.61 ± 0.07 | .27 | .30 | .15 | .28 |
| Insulin (0.5–11.5 mIU/L) | 5.15 ± 0.50 | 5.47 ± 0.43 | 5.33 ± 0.78 | 5.67 ± 1.09 | .25 | .42 | .78 | .76 |
| HOMA-IR (0.14–5.54) | 1.17 ± 0.08 | 1.14 ± 0.06 | 1.07 ± 0.07 | 1.24 ± 0.09 | .47 | .99 | .45 | .58 |
| Triglycerides (0.2–2.1 mM) | 0.75 ± 0.03 | 0.69 ± 0.03 | 0.63 ± 0.03 | 0.79 ± 0.04 | .12 | .01* | .75 | .008* |
| Total cholesterol (1.8–5.6 mM) | 4.28 ± 0.06 | 4.22 ± 0.07 | 4.28 ± 0.09 | 4.12 ± 0.12 | .99 | .98 | .72 | .74 |
| HDL (0.6–2.0 mM) | 1.42 ± 0.04 | 1.43 ± 0.04 | 1.64 ± 0.04 | 1.11 ± 0.04 | .55 | <.001* | <.001* | <.001* |
| LDL (0.9–3.9 mM) | 2.43 ± 0.05 | 2.30 ± 0.06 | 2.33 ± 0.08 | 2.24 ± 0.10 | .21 | .46 | .35 | .82 |

*Note:* Data are mean ± standard error of the mean. The normal reference range for children is provided in parentheses. HOMA-IR = homeostasis model assessment resistance index; IGF = insulin-like growth factor; IGFBP-3 = insulin-like growth factor binding protein 3; HDL = high-density lipoprotein; LDL = low-density lipoprotein.
* Statistical significance ($P$< .05).

*Green. Phenotypic differences in IVF children. Fertil Steril 2013.*

For example, in vitro embryo culture and related procedures were shown to influence the expression and methylation status of the imprinted genes *IGF2*, *IGF2R*, and *H19* (47, 48), and hence the IGF-II concentrations.

Strengths of the present study over the majority of previous studies on IVF children are the use of strict inclusion criteria to remove multiple, premature, and SGA children, as well as the consideration given to a number of potential confounders of anthropometric data such as parental genetic contribution, socioeconomic background, nutrition, and puberty. These findings in $IVF_F$ children are an expansion on our previous study that examined a smaller number of $IVF_F$ children, which obtained similar results but did not include $IVF_T$ children (24). It is important to acknowledge that the observations we have made in IVF children may just reflect the specific IVF treatment at that time. In vitro fertilization techniques have since evolved, with the advent of sequential culture medium, widespread culture to day 5 before embryo transfer, and embryo vitrification. Hence, differences identified in children derived from embryos frozen on day 2 as in our study may not be applicable to the offspring born from blastocyst culture pregnancies, the stage to which embryos are commonly cultured at present.

It should also be noted that irrespective of the anthropometric and biochemical differences identified the parameters were within normal ranges for all children studied. Therefore, the long-term clinical relevance of these subtle differences are unclear. Conceivably, these changes could lead to different phenotypic and biochemical outcomes in IVF offspring. However, as this was a cross-sectional study, a longitudinal follow-up of this cohort into adulthood would be required to determine whether these changes persist or further evolve beyond childhood.

Embryo treatment for IVF affects height, growth factors, and lipid profile in childhood. Most notably, $IVF_F$ girls were taller than $IVF_T$ and control girls, and the observed differences in height are likely to persist into adulthood. Thus, whether a fresh or thawed IVF embryo is transferred during IVF treatment should be considered when assessing growth and metabolism in these offspring. Further research on larger cohorts is needed to better understand the long-term clinical relevance, initiating factors, and associated mechanisms of these findings in IVF children.

**Acknowledgments:** The authors thank the staff at the Maurice & Nessie Paykel Clinical Research Unit (The Liggins Institute, Auckland) and Fertility Associates clinics (Auckland and Hamilton) for their help with subject recruitment and testing, and the Paykel Trust for long-term funding of the Maurice & Agnes Paykel Clinical Research Unit at the Liggins Institute (University of Auckland).

## REFERENCES

1. Vogel G, Enserink M. Nobel prize: Honor for test tube baby pioneer. Science 2010;330:158–9.
2. Maher ER. Imprinting and assisted reproductive technology. Hum Mol Genet 2005;14:R133–8.
3. Kallen B, Finnstrom O, Lindam A, Nilsson E, Nygren KG, Olausson PO. Trends in delivery and neonatal outcome after in vitro fertilization in Sweden: data for 25 years. Hum Reprod 2010;25:1026–34.
4. Human Fertilisation and Embryology Authority. Fertility facts and figures 2008. In: HFEA, ed. London, England: HFEA, 2010:1–10.
5. De Mouzon J, Goossens V, Bhattacharya S, Castilla JA, Ferraretti AP, Korsak V, et al. Assisted reproductive technology in Europe, 2006: results generated from European registers by ESHRE. Hum Reprod 2010;25:1851–62.
6. Sunderam S, Chang J, Flowers L, Kulkarni A, Sentelle G, Jeng G, et al. Assisted reproductive technology surveillance—United States. 2006. Morb Mortal Wkly Rep Surveill Summ 2009;58:1–25.
7. Nygren KG, Sullivan E, Zegers-Hochschild F, Mansour R, Ishihara O, Adamson GD, et al. International Committee for Monitoring Assisted Reproductive Technology (ICMART) world report: assisted reproductive technology 2003. Fertil Steril 2011;95:2209–22.e1–17.
8. Belva F, Henriet S, Van den Abbeel E, Camus M, Devroey P, Van der Elst J, et al. Neonatal outcome of 937 children born after transfer of cryopreserved embryos obtained by ICSI and IVF and comparison with outcome data of fresh ICSI and IVF cycles. Hum Reprod 2008;23:2227–38.
9. Shih W, Rushford DD, Bourne H, Garrett C, McBain JC, Healy DL, et al. Factors affecting low birthweight after assisted reproduction technology: difference between transfer of fresh and cryopreserved embryos suggests an adverse effect of oocyte collection. Hum Reprod 2008;23:1644–53.
10. McDonald SD, Han Z, Mulla S, Murphy KE, Beyene J, Ohlsson A, et al. Preterm birth and low birth weight among in vitro fertilization singletons: a systematic review and meta-analyses. Eur J Obstet Gynecol Reprod Biol 2009;146:138–48.
11. Pinborg A, Loft A, Henningsen A-KA, Rasmussen S, Andersen AN. Infant outcome of 957 singletons born after frozen embryo replacement: the Danish National Cohort Study 1995–2006. Fertil Steril 2010;94:1320–7.
12. Wennerholm UB, Soderstrom-Anttila V, Bergh C, Aittomaki K, Hazekamp J, Nygren KG, et al. Children born after cryopreservation of embryos or oocytes: a systematic review of outcome data. Hum Reprod 2009;24:2158–72.
13. Aflatoonian A, Moghaddam FM, Mashayekhy M, Mohamadian F. Comparison of early pregnancy and neonatal outcomes after frozen and fresh embryo transfer in ART cycles. J Assist Reprod Genet 2010;27:695–700.
14. Finnstrom O, Kallen B, Lindam A, Nilsson E, Nygren K-G, Olausson PO. Maternal and child outcome after in vitro fertilization - a review of 25 years of population-based data from Sweden. Acta Obstet Gynecol Scand 2011;90:494–500.
15. Brandes JM, Scher A, Itzkovits J, Thaler I, Sarid M, Gershonibaruch R. Growth and development of childen conceived by in vitro fertilization. Pediatrics 1992;90:424–9.
16. Koivurova S, Hartikainen AL, Sovio U, Gissler M, Hemminki E, Jarvelin MR. Growth, psychomotor development and morbidity up to 3 years of age in children born after IVF. Hum Reprod 2003;18:2328–36.
17. Ludwig AK, Sutcliffe AG, Diedrich K, Ludwig M. Post-neonatal health and development of children born after assisted reproduction: a systematic review of controlled studies. Eur J Obstet Gynecol Reprod Biol 2006;127:3–25.
18. Ceelen M, van Weissenbruch MM, Roos JC, Vermeiden JPW, van Leeuwen FE. de Waal HAD-v. Body composition in children and adolescents born after in vitro fertilization or spontaneous conception. J Clin Endocrinol Metab 2007;92:3417–23.
19. Makhoul IR, Tamir A, Bader D, Rotschild A, Weintraub Z, Yurman S, et al. In vitro fertilisation and use of ovulation enhancers may both influence childhood height in very low birthweight infants. Arch Dis Child Fetal Neonatal Ed 2009;94:F355–9.
20. Basatemur E, Shevlin M, Sutcliffe A. Growth of children conceived by IVF and ICSI up to 12 years of age. Reprod Biomed Online 2010;20:144–9.
21. Saunders K, Spensley J, Munro J, Halasz G. Growth and physical outcome of children conceived by in vitro fertilization. Pediatrics 1996;97:688–92.
22. Olivennes F, Kerbrat V, Rufat P, Blanchet V, Fanchin R, Frydman R. Follow-up of a cohort of 422 children aged 6 to 13 years conceived by in vitro fertilization. Fertil Steril 1997;67:284–9.
23. Savage T, Peek J, Hofman PL, Cutfield WS. Childhood outcomes of assisted reproductive technology. Hum Reprod 2011;26:2392–400.

24. Miles HL, Hofman PL, Peek J, Harris M, Wilson D, Robinson EM, et al. In vitro fertilization improves childhood growth and metabolism. J Clin Endocrinol Metab 2007;92:3441–5.
25. Ceelen M, van Weissenbruch MM, Prein J, Smit JJ, Vermeiden JPW, Spreeuwenberg M, et al. Growth during infancy and early childhood in relation to blood pressure and body fat measures at age 8–18 years of IVF children and spontaneously conceived controls born to subfertile parents. Hum Reprod 2009;24:2788–95.
26. Belva F, Roelants M, Painter R, Bonduelle M, Devroey P, De Schepper J. Pubertal development in ICSI children. Hum Reprod 2012;27:1156–61.
27. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 1976;51:170–9.
28. Cole TJ, Freeman JV, Preece MA. Body-mass index reference curves for the UK, 1990. Arch Dis Child 1995;73:25–9.
29. Greulich W, Pyle S. Radiographic atlas of skeletal development of the hand and wrist. 2nd ed. Stanford, CA: Standford University Press; 1950.
30. Cutfield WS, Jefferies CA, Jackson WE, Robinson EM, Hofman PL. Evaluation of HOMA and QUICKI as measures of insulin sensitivity in prepubertal children. Pediatr Diabetes 2003;4:119–25.
31. Cohen J, DeVane GW, Elsner CW, Fehilly CB, Kort HI, Massey JB, et al. Cryopreservation of zygotes and early cleaved human embryos. Fertil Steril 1988; 49:283–9.
32. Pelkonen S, Koivunen R, Gissler M, Nuojua-Huttunen S, Suikkari AM, Hyden-Granskog C, et al. Perinatal outcome of children born after frozen and fresh embryo transfer: the Finnish cohort study 1995–2006. Hum Reprod 2010;25:914–23.
33. Ibanez L, Lopez-Bermejo A, Diaz M, de Zegher F. Catch-up growth in girls born small for gestational age precedes childhood progression to high adiposity. Fertil Steril 2011;96:220–3.
34. Bonduelle M, Wennerholm UB, Loft A, Tarlatzis BC, Peters C, Henriet S, et al. A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, in vitro fertilization and natural conception. Hum Reprod 2005;20:413–9.
35. Kai CM, Main KM, Andersen AN, Loft A, Chellakooty M, Skakkebaek NE, et al. Serum insulin-like growth factor-I (IGF-I) and growth in children born after assisted reproduction. J Clin Endocrinol Metab 2006;91:4352–60.
36. Peralta-Carcelen M, Jackson DS, Goran MI, Royal SA, Mayo MS, Nelson KG. Growth of adolescents who were born at extremely low birth weight without major disability. J Pediatr 2000;136:633–40.
37. Wehkalampi K, Hovi P, Dunkel L, Strang-Karlsson S, Jarvenpaa A-L, Eriksson JG, et al. Advanced pubertal growth spurt in subjects born preterm: the Helsinki study of very low birth weight adults. J Clin Endocrinol Metab 2011;96:525–33.
38. Tanner JM, Whitehouse RH, Marshall W, Carter B. Prediction of adult height from height, bone age, and occurrence of menarche, at ages 4 to 16 with allowance for midparent height. Arch Dis Child 1975;50:14–26.
39. Kanaka-Gantenbein C, Sakka S, Chrousos GP. Assisted reproduction and its neuroendocrine impact on the offspring. In: Martini L, Chrousos GP, Labrie F, Pacak K, Pfaff DW, editors. Neuroendocrinology: pathological situations and diseases, Vol. 182. Amsterdam: Elsevier Science; 2010:161–74.
40. Griesinger G, Kolibianakis EM, Diedrich K, Ludwig M. Ovarian stimulation for IVF has no quantitative association with birthweight: a registry study. Hum Reprod 2008;23:2549–54.
41. Henningsen AK, Pinborg A, Lidegaard O, Vestergaard C, Forman JL, Andersen AN. Perinatal outcome of singleton siblings born after assisted reproductive technology and spontaneous conception: Danish national sibling-cohort study. Fertil Steril 2011;95:959–63.
42. Pelinck MJ, Hadders-Algra M, Haadsma ML, Nijhuis WL, Kiewiet SM, Hoek A, et al. Is the birthweight of singletons born after IVF reduced by ovarian stimulation or by IVF laboratory procedures? Reprod Biomed Online 2010;21:245–51.
43. Santos MA, Kuijk EW, Macklon NS. The impact of ovarian stimulation for IVF on the developing embryo. Reproduction 2010;139:23–34.
44. Fauser BC, Nargund G, Andersen AN, Norman R, Tarlatzis B, Boivin J, et al. Mild ovarian stimulation for IVF: 10 years later. Hum Reprod 2010;25:2678–84.
45. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfer in normal responders. Fertil Steril 2011; 96:344–8.
46. Ku PY, Lee RK, Lin SY, Lin MH, Hwu YM. Comparison of the clinical outcomes between fresh blastocyst and vitrified-thawed blastocyst transfer. J Assist Reprod Genet 2012;29:1353–6.
47. Kholsa S, Dean W, Brown D, Reik W, Feil R. Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. Biol Reprod 2001;64:918–26.
48. Young LE, Fernandes K, McEvoy TG, Butterwith SC, Gutierrez CG, Carolan C, et al. Epigenetic change in IGF2R is associated with fetal overgrowth after sheep embryo culture. Nat Genet 2001;27:153–4.