

1950s and continues to have an important role in fertility treatment. Currently, follicle-stimulating hormone (FSH) is used in mild ovarian stimulation either by itself or in combination with clomiphene citrate.¹ Required doses of FSH vary considerably, depending on whether it is used in ovarian stimulation alone or associated with assisted reproductive technology. While exogenous FSH administration does not lead to the marked anti-oestrogenic effects of clomiphene, it has been shown to have several effects other than stimulation of ovaries.^{2,3}

We have previously shown that children conceived following mild ovarian stimulation (clomiphene citrate with/without FSH, and without undergoing IVF treatment) were 0.53 SDS shorter than naturally conceived controls.⁴ However, it is unclear whether those findings were affected by the administration of additional FSH injections. Therefore, we aimed to assess whether the observed shorter stature was due to maternal treatment with clomiphene citrate alone or due to additional FSH injection(s).

Ethics approval for this study was provided by the Northern Y Regional Ethics Committee (Ministry of Health, New Zealand). Participants were healthy prepubertal children of New Zealand European ethnicity, born at term (37–41 weeks of gestation) after singleton pregnancies, and of higher socio-economic status.⁴

Fertility treatment methods were as previously described.⁴ In brief, mothers of participants received a standard stimulation regimen of clomiphene citrate for days 3–7, followed by 50–150 IU FSH on alternate days from day 9 if the oestradiol level on this day was <2000 pmol/l. The starting dose of clomiphene was 50 mg/day, subsequently increased to 100 mg/day if response was low in the first cycle or reduced to 25 mg/day if the initial response was high. For patients who received FSH, the mean total dose was 360 IU. All children were conceived using sperm from the mother's partner, so that the offspring of donor sperm were not included.

Clinical assessments were performed at the Paykel Clinical Research Unit as per Savage *et al.*⁴ Each child's birthweight, height and BMI were transformed into standard deviation scores (SDS); mid-parental height SDS (MPHSDS) and mean parental BMISDS (MPBMISDS) were calculated.⁴ Children's heights SDS were then individually adjusted for their genetic potential (parents' heights), using the formula: child's height SDS minus MPHSDS. Fasting blood samples were drawn for the assessment of lipid profile, growth factors, glucose and insulin (with assays as previously described⁴).

Study participants were allocated into three groups, according to the drug treatment received by the mother for ovarian stimulation: CC (clomiphene citrate alone), CCFSH_{SING} (clomiphene citrate with a single FSH injection) and CCFSH_{MULT} (clomiphene citrate with two or more FSH injections). Two sets of comparisons were carried out: CCFSH_{SING} vs CCFSH_{MULT}, and combined CCFSH vs CC. Groups were compared using linear mixed models with maternal code as random factor, and important confounders adjusted for (e.g. sex, birthweight SDS, gestational age, birth order and maternal age).

The addition of FSH to clomiphene citrate for ovarian stimulation does not affect offspring stature but may alter body composition in childhood

Clomiphene citrate (a nonsteroidal oestrogen antagonist) has been used for ovarian stimulation since its discovery in the late

Table 1. Study outcomes among children whose mothers underwent ovarian stimulation with clomiphene citrate alone (CC), clomiphene citrate and a single FSH injection (CCFSH_{SING}) or clomiphene citrate and two or more FSH injections (CCFSH_{MULT}). For comparison with CC group, CCFSH_{SING} and CCFSH_{MULT} were combined into CCFSH

	CCFSH _{SING}	CCFSH _{MULT}	<i>P</i>	CC	CCFSH	<i>P</i>
<i>N</i>	32	21		26	53	
Anthropometry						
Height SDS	0.86 (0.54–1.17)	0.70 (0.31–1.09)	0.54	1.01 (0.69–1.32)	0.78 (0.56–0.99)	0.24
Adjusted height SDS	−0.01 (−0.34–0.32)	−0.15 (−0.56–0.26)	0.60	−0.09 (−0.42–0.25)	−0.09 (−0.33–0.14)	0.97
BMI SDS	−0.28 (−0.70–0.13)	−0.18 (−0.68–0.32)	0.76	−0.30 (−0.71–0.12)	−0.18 (−0.47–0.11)	0.65
Total body fat (%)	17.5 (15.5–19.7)	17.0 (14.6–19.8)	0.77	14.8 (13.2–16.6)	17.6 (16.2–19.1)	0.019
Bone mineral density (g/cm ²)	0.847 (0.829–0.864)	0.846 (0.824–0.869)	0.99	0.820 (0.801–0.840)	0.845 (0.831–0.860)	0.049
Lipid profile						
Total cholesterol (mmol/l)	4.13 (3.84–4.43)	4.21 (3.87–4.55)	0.71	4.43 (4.08–4.79)	4.15 (3.90–4.41)	0.19
LDL-C (mmol/l)	2.37 (2.12–2.61)	2.43 (2.15–2.72)	0.71	2.66 (2.35–2.96)	2.39 (2.18–2.60)	0.15
HDL-C (mmol/l)	1.41 (1.31–1.52)	1.38 (1.25–1.51)	0.72	1.49 (1.33–1.64)	1.40 (1.30–1.50)	0.38
Triglycerides (mmol/l)	0.75 (0.66–0.85)	0.63 (0.51–0.74)	0.08	0.76 (0.63–0.88)	0.71 (0.62–0.80)	0.55
Glucose homeostasis						
Glucose (mmol/l)	4.71 (4.59–4.83)	4.59 (4.44–4.74)	0.22	4.53 (4.39–4.67)	4.66 (4.57–4.76)	0.13
Insulin (mmol/l)	5.08 (4.42–5.74)	5.42 (4.62–6.22)	0.52	4.61 (3.84–5.38)	5.26 (4.77–5.76)	0.16
HOMA-IR	1.07 (0.92–1.22)	1.14 (0.94–1.30)	0.68	0.94 (0.77–1.12)	1.10 (0.99–1.21)	0.13
Growth factors						
IGF-I (µg/l)	90 (77–106)	113 (92–137)	0.11	107 (90–127)	97 (87–109)	0.32
IGFBP-3 (µg/l)	2588 (2321–2854)	2637 (2411–2862)	0.78	2453 (2161–2746)	2625 (2436–2815)	0.33

Data are means and 95% confidence intervals adjusted for confounding factors in the multivariate models.

We studied 79 children (49% boys) aged 7.2 ± 2.2 years (range 3–11 years), including 26 CC, 32 CCFSH_{SING} and 21 CCFSH_{MULT}. CCFSH_{SING} and CCFSH_{MULT} children were of similar age, birthweight, gestational age and sex ratio (data not shown). Children in both groups were also anthropometrically similar, and biochemical analyses showed no differences in lipid profiles, growth factors or glucose homeostasis (Table 1).

Participants in CC and combined CCFSH groups were also of similar age, birthweight, gestational age and sex ratio (data not shown). There were no differences in height or BMISDS between groups, but CCFSH children had more total body fat ($P = 0.019$) and greater bone mineral density ($P = 0.049$) than CC children (Table 1). There were no differences in lipid profiles, parameters of glucose homeostasis or blood concentrations of growth factors between CC and CCFSH groups (Table 1).

Our findings suggest that the previously observed reduction in height in children born following maternal ovarian stimulation⁴ was not associated with additional FSH injection(s). Rather, the shorter stature seen in children born after ovarian stimulation alone⁴ was most likely associated with the administration of clomiphene citrate.

Clomiphene may possibly alter the methylation of imprinted and nonimprinted genes, have subtle effects in the developing oocyte or embryo and/or have effects on the endometrium leading to subsequent phenotypic changes.⁴ Additionally, there may be a role of maternal oestrogen levels on offspring height, possibly through epigenetic mechanisms. For example, IVF children are exposed to high oestrogen levels, children from frozen embryo transfers are exposed to normal oestrogen levels, while

children whose mothers receive clomiphene are exposed to low oestrogen levels.

Nonetheless, FSH appears to have some effect on body composition, being associated with increased adiposity in the offspring. Evidence from assisted reproductive technology suggests that exogenous FSH administration is associated with changes in oocyte DNA methylation.^{5,6} However, with the exception of one human study,⁶ the evidence of an effect of FSH on oocyte or embryo epigenetics has been extrapolated from animal models (reviewed by van Montfoort *et al.*⁷). Still, the observed changes associated with FSH administration included alterations in DNA methylation and gene expression, as well as changes in the methylation of H19, an imprinted gene involved in postnatal growth.⁵ As a result, it is possible that additional administration of FSH during ovarian stimulation may affect the offspring, leading to increased fat mass as observed in our cohort.

A limitation of this study was the relatively small sample size, which was only powered to detect a difference in adjusted height of 0.57 SDS between CC and CCFSH groups (with $\alpha = 0.05$ and 80% power). Importantly, however, this limitation did not affect our main conclusions, as the adjusted height SDS in CC and CCFSH groups was virtually identical (both -0.09 SDS; Table 1).

In conclusion, our preliminary findings indicate that exogenous maternal FSH treatment did not contribute to the shorter stature seen in children born following maternal ovarian stimulation. However, findings on body composition suggest that larger studies are needed to assess possible long-term risks of obesity in the offspring in association with exogenous FSH treatment during ovarian stimulation.

Funding

This work was supported by Gravida: National Centre for Growth and Development.

Disclosure statement

TS, JGBD, PLH and WSC have nothing to declare. JCP is a shareholder and scientific director of Fertility Associates. The authors have no other competing interests to declare.

Author contributions

WSC, TS, JCP, PLH and JGBD conceived and designed the study. TS collected the data, which were analysed by JGBD. JGBD and TS wrote the manuscript with input from WSC, PLH and JCP.

Tim Savage^{*†}, José G.B. Derraik^{*}, John C. Peek[‡],
Paul L. Hofman^{*†} and Wayne S. Cutfield^{*†}
^{*}Liggins Institute, University of Auckland, [†]Gravida: National
Centre for Growth and Development, [‡]Fertility Associates,
Auckland, New Zealand
E-mail: w.cutfield@auckland.ac.nz

doi: 10.1111/cen.12805

References

- Berker, B., Kahraman, K., Taskin, S. *et al.* (2011) Recombinant FSH versus clomiphene citrate for ovarian stimulation in couples with unexplained infertility and male subfertility undergoing intrauterine insemination: a randomized trial. *Archives of Gynecology and Obstetrics*, **284**, 1561–1566.
- Macklon, N. & Fauser, B. (2000) Impact of ovarian hyperstimulation on the luteal phase. *Journal of Reproduction and Fertility. Supplement*, **55**, 101–108.
- Broekmans, F., Soules, M. & Fauser, B. (2009) Ovarian aging: mechanisms and clinical consequences. *Endocrine Reviews*, **30**, 465–493.
- Savage, T., Peek, J.C., Robinson, E.M. *et al.* (2012) Ovarian stimulation leads to shorter stature in childhood. *Human Reproduction*, **27**, 3092–3099.
- Market-Velker, B., Zhang, L., Magri, L. *et al.* (2010) Dual effects of superovulation: loss of maternal and paternal imprinted methylation in a dose-dependent manner. *Human Molecular Genetics*, **19**, 36–51.
- Sato, A., Otsu, E., Negishi, H. *et al.* (2007) Aberrant DNA methylation of imprinted loci in superovulated oocytes. *Human Reproduction*, **22**, 26–35.
- van Montfoort, A., Hanssen, L., de Sutter, P. *et al.* (2012) Assisted reproduction treatment and epigenetic inheritance. *Human Reproduction Update*, **18**, 171–197.