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Is ovarian hyperstimulation associated with higher blood pressure in 4-year-old IVF offspring? Part I: multivariable regression analysis

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STUDY QUESTION: Does ovarian hyperstimulation, the in vitro procedure, or a combination of these two negatively influence blood pressure (BP) and anthropometrics of 4-year-old children born following IVF?

SUMMARY ANSWER: Higher systolic blood pressure (SBP) percentiles were found in 4-year-old children born following conventional IVF with ovarian hyperstimulation compared with children born following IVF without ovarian hyperstimulation.

WHAT IS KNOWN ALREADY: Increasing evidence suggests that IVF, which has an increased incidence of preterm birth and low birthweight, is associated with higher BP and altered body fat distribution in offspring but the underlying mechanisms are largely unknown.

STUDY DESIGN, SIZE, DURATION: We performed a prospective, assessor-blinded follow-up study in which 194 children were assessed. The attrition rate up until the 4-year-old assessment was 10%.

PARTICIPANTS/MATERIALS, SETTING, METHODS: We measured BP and anthropometrics of 4-year-old singletons born following conventional IVF with controlled ovarian hyperstimulation (COH-IVF, n = 63), or born following modified natural cycle IV (MNC-IVF, n = 52), or born to subfertile couples who conceived naturally (Sub-NC, n = 79). Both IVF and ICSI were performed. Primary outcome measures were the SBP percentiles and diastolic BP (DBP) percentiles. Anthropometric measures included triceps and subscapular skinfold thickness. Several multivariable regression analyses were applied in order to correct for subsets of confounders. The value ‘B’ is the unstandardized regression coefficient.

MAIN RESULTS AND THE ROLE OF CHANCE: SBP percentiles were significantly lower in the MNC-IVF group (mean 59, SD 24) than in the COH-IVF (mean 68, SD 22) and Sub-NC groups (mean 70, SD 16). The difference in SBP between COH-IVF and MNC-IVF remained significant after correction for current, early life and parental characteristics (B: 14.09; 95% confidence interval (CI): 5.39–22.79), whereas the difference between MNC-IVF and Sub-NC did not. DBP percentiles did not differ between groups. After correction for early life factors, subscapular skinfold thickness was thicker in the COH-IVF group than in the Sub-NC group (B: 0.28; 95% CI: 0.03–0.53).

LIMITATIONS, REASONS FOR CAUTION: Larger study groups are necessary to draw firm conclusions. An effect of gender or ICSI could not be properly investigated as stratifying would further reduce the sample size. We corrected for the known differences between MNC-IVF and COH-IVF but it is possible that the groups differ in additional, more subtle parental characteristics. In addition, we measured BP on 1 day only, had no control group of children born to fertile couples (precluding investigating effects of the underlying subfertility) and included singletons only.
Introduction

The increasing use of IVF, with or without ICSI, over the past 30 years has allowed many thousands of subfertile couples to fulfil their wish to have a child. Nowadays, up to 4% of newborns in Europe are conceived with IVF (Andersen et al., 2009). Meanwhile, several reports have evoked concerns that IVF might be associated with suboptimal cardiometabolic health in the offspring (Belva et al., 2007, 2012a; Ceelen et al., 2007, 2008; Sakka et al., 2010; Scherrer et al., 2012; Hart and Norman, 2013; Yeung and Druschel, 2013). Thus far, three studies reported increased blood pressure (BP) levels in children born following IVF/ICSI (Belva et al., 2007; Ceelen et al., 2008; Sakka et al., 2010), whereas two studies did not find differences in BP between IVF/ICSI offspring and children who were conceived naturally (Belva et al., 2012b; Scherrer et al., 2012). The two studies that did not find differences in BP, however, did report adverse outcomes on other parameters of cardiometabolic health in the IVF/ICSI offspring: the study of Scherrer et al. found an increased risk of vascular dysfunction in the offspring, and another study of Belva et al. found that ICSI girls have more central, peripheral and total body fat than their naturally conceived counterparts (Belva et al., 2012a; Scherrer et al., 2012).

Poorer cardiometabolic outcome after IVF may be expected as such conceptions more often result in preterm birth and low birthweight (Helmerhorst et al., 2004), which are risk factors for hypertension and adiposity (Wells et al., 2007). However, previous studies indicated that poorer cardiometabolic outcome in IVF offspring could not be explained by these factors alone (Belva et al., 2007, 2012a; Ceelen et al., 2007, 2008; Sakka et al., 2010; Scherrer et al., 2012).

There is an increasing body of evidence suggesting that the early environment shapes an individual’s health later in life, and that IVF might compromise the environment of the early embryo (Watkins and Fleming, 2009). IVF-related procedures that may be involved include: artificial maturation of follicles using ovarian hyperstimulation, early embryonic development in vitro or an altered early intrauterine environment due to ovarian hyperstimulation. Another factor mediating poor cardiometabolic outcome after IVF may be the underlying subfertility, which is associated with obstetrical complications, poorer perinatal outcomes and birth defects in offspring (Draper et al., 1999; Davies et al., 2012).

The aim of this prospective assessor-blinded study was to examine the effect of ovarian hyperstimulation, the in vitro procedure and a combination of these two on BP and anthropometrics of 4-year-olds. Our primary outcome measures were systolic BP (SBP) and diastolic BP (DBP) percentiles.

To disentangle the effect of ovarian hyperstimulation from that of the in vitro procedure, we compared three groups: (i) singleton births conceived with IVF with controlled ovarian hyperstimulation (COH-IVF), (ii) singletons conceived with IVF in a modified natural cycle (MNC-IVF) and (iii) naturally conceived singleton births born to subfertile couples (Sub-NC). COH-IVF is the conventional form of IVF in which ovarian hyperstimulation induces the growth of multiple follicles. In MNC-IVF no ovarian hyperstimulation is performed and the one follicle that naturally developed to dominance is used (Pelinck et al., 2008). The comparison of health of COH-IVF children to that of MNC-IVF children reflects, in part, the effect of ovarian hyperstimulation, and the comparison of MNC-IVF and Sub-NC children that of the in vitro procedure.

Methods

Recruitment

This study is part of the Groningen assisted reproduction technology (ART) cohort study, a prospective, assessor-blinded, longitudinal study on developmental outcome of IVF singletons (Middelburg et al., 2009). Pregnant subfertile couples with an expected delivery date between March 2005 and December 2006, who consulted the Department of Reproductive Medicine of the University Medical Center Groningen (UMCG), were invited to participate during the third trimester of pregnancy. The singletons born formed three groups: COH-IVF, MNC-IVF and Sub-NC. Placement in one of the first two groups depended on the presence (COH-IVF) or absence (MNC-IVF) of ovarian hyperstimulation. Criteria for inclusion in the MNC-IVF group were: female age 18—36 years, first IVF treatment or first IVF treatment after a pregnancy, the presence of a regular and ovulatory menstrual cycle of 26–35 days and a BMI of 18–28 kg/m². Details on minimal hormone application in MNC-IVF have been described elsewhere (Pelinck et al., 2008). The Sub-NC group comprised couples who had tried to conceive for at least 1 year and finally conceived naturally while waiting for fertility evaluation or treatment. Parents gave written informed consent and the study design was approved by the ethics committee of the UMCG.

Examination

Prenatal, perinatal and demographic information was gathered using standardized questionnaires, 2 weeks after the expected delivery date.
Medical records provided information on fertility diagnosis and treatment, time to pregnancy, and self-reported maternal weight and height. When the children were 4 years of age, the follow-up assessment included measurement of BP and an assessment of anthropometrics by trained researchers who were blinded to mode of conception. Assessments were carried out between September 2009 and February 2011 at the Institute of Developmental Neurology at the UMCG. An automated BP monitor (Datascope Accutorr plus, Mahwah, Nj, USA) with an appropriate cuff size was used to measure BP twice at the non-dominant arm while the child was seated. The first measurement took place after the child had performed cognitive tests. Next, anthropometric data were collected and subsequently BP was measured again. We used the mean of the two readings to calculate BP percentiles. The percentiles are based on the fourth report of the U.S. National High Blood Pressure Education Program, and take age, gender and height into account (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004).

Body weight was measured using an electronic scale (Radwag, Random, Poland) and was recorded to the nearest 0.1 kg. Standing height was measured with a stadiometer (Seca, Germany) to the nearest 0.1 cm. BMI was calculated (weight/height²) and was classified as normal or obese using international cut off points that take into account gender and age (obese 4-year-old girls: BMI ≥ 17.28 kg/m², obese 4-year-old boys: BMI ≥ 17.55 kg/m²) (Cole et al., 2000). Triceps and subscapular skinfold thickness were measured three times, alternating between the two skinfolds, using a Harpenden calliper. The mean of the three measurements was used as outcome parameter. The triceps skinfold was used as a measure of periphera! fat, the subscapular skinfold as a measure for truncal fat and the sum of these two as an indicator of total body fat. The subscapular/triceps skinfold ratio was used as an index of fat distribution. Waist and occipitofrontal head circumference was measured in millimetres with a nonstretchable ‘lasso’ tape.

Statistical analysis
Power calculation of the Groningen ART cohort study was based on neurological outcome at 18 months (Middelburg et al., 2009). Group differences in background variables and outcome measures were investigated with Fisher’s exact test, one-way analysis of variance or Kruskal–Wallis test when appropriate. In case significant differences were found between the three groups, Student’s t-tests and Mann–Whitney U-tests were used to specify the pair-wise differences.

Multivariable regression analyses were performed to explore potential differences in BP percentiles and skinfold thickness between the groups while correcting for possible confounders. In line with other studies in the field (Ceelen et al., 2008; Belva et al., 2012b), we performed separate regression analyses to correct for current risk indicators (weight, height, gender, age and sum of skinfold thickness), for early life factors (gestational age, birthweight, parity, periconceptional use of folic acid and breastfed > 6 weeks), for parental characteristics (parental age, high parental education, maternal pre-pregnancy BMI, pregnancy-induced hypertension, gestational diabetes, parental diabetes or heart/vascular diseases, smoking during pregnancy and alcohol consumption during pregnancy) and finally for all current, early life and parental variables. In all of these regression analyses we corrected for fertility factors (time to pregnancy and ICSI). Since BP percentiles already take age, gender and height into account, these current risk indicators were not entered into the regression analyses. Skinfold thickness was log-transformed since it showed a skewed distribution. In addition, the sum of skinfold thickness was not entered into the regression analyses for skinfold thickness. Results are expressed as unstandardized regression coefficients (B) with their 95% confidence intervals (95% CI). The analyses were performed using the IBM Statistical Package for the Social Sciences version 20. Probability values of < 0.05 were considered statistically significant.

Results
Participation
During the prenatal inclusion period, 89 COH-IVF singletons, 79 MNC-IVF singletons and 143 singletons born after natural conception were eligible; parents of 68 (76%), 57 (72%) and 90 (63%) singletons, respectively, agreed to participate. Background characteristics of participants and non-participants were largely similar. Exceptions were: maternal age at conception was lower in non-participating Sub-NC mothers (31 years) than in participating Sub-NC mothers (33 years, P = 0.031), and non-participating MNC-IVF mothers more often had pregnancy-induced hypertension (24%) than participating MNC-IVF mothers (6%, P = 0.029) (Middelburg et al., 2009).

At the 4-year-old assessment, 5 COH-IVF (7%), 5 MNC-IVF (9%) and 11 Sub-NC children (12%) did not participate. One MNC-IVF child died of a congenital heart disorder, and all other dropout was due to logistical reasons or assessment burden. As some children did not co-operate, we obtained at least one BP measurements from 60 COH-IVF (95% of included children), 47 MNC-IVF (90%) and 77 Sub-NC (97%) children.

Infant and parental characteristics
Table I shows the background characteristics of the groups. Groups were largely comparable, with some exceptions: birthweight was higher in the Sub-NC group than in the COH-IVF group (P = 0.021), and as might be expected, COH-IVF and MNC-IVF couples had a longer time to pregnancy than Sub-NC couples (P < 0.001; P = 0.001).

BP and anthropometric data
An overview of all outcome measures for the three groups is presented in Table II. SBP percentiles were significantly lower in the MNC-IVF group (mean 59.1, SD 23.7) than in the COH-IVF (mean 68.2, SD 21.8), and Sub-NC group (mean 70.1, SD 16.5). DBP percentiles did not differ between groups.

Table III shows the multivariable regression analyses of SBP percentiles, DBP percentiles, subscapular skinfold thickness and triceps skinfold thickness. Children born following IVF with ovarian hyperstimulation had higher SBP percentiles than children born following IVF without ovarian hyperstimulation, also after correction for various sets of variables (Table III). Mode of conception was not associated with DBP percentiles. Skinfold thickness was largely similar between the groups. When corrected for early life factors, the subscapular skinfold was thicker in the COH-IVF group than in the Sub-NC group (Table III).

Discussion
Our data suggest that 4-year-old children born following COH-IVF (i.e. IVF that includes ovarian hyperstimulation) have higher SBP percentiles than children born following MNC-IVF (i.e. IVF without ovarian hyperstimulation). COH-IVF is the conventional form of IVF, which was shown to be associated with suboptimal cardiometabolic outcome in several other studies (Belva et al., 2007, 2012a; Ceelen et al., 2007, 2008; Sakka et al., 2010; Scherrer et al., 2012). Other studies did not include children born following MNC-IVF, and could therefore not investigate the effect of ovarian hyperstimulation per se. Our study, using measures of BP at 4 years of age, suggests, for the first time, that ovarian hyperstimulation as applied in IVF is involved in the poorer cardiometabolic outcome of 4-year-old children born following COH-IVF. This suggests that changes in BP and body composition in children born following COH-IVF may be due to ovarian hyperstimulation itself or to IVF in general, rather than to other factors such as maternal age or parental characteristics.
seen in IVF offspring. In addition, we obtained some evidence for an adverse effect of COH-IVF on subscapular skinfold thickness: after correction for early life factors the difference between COH-IVF and Sub-NC became statistically significant. As truncal fat is primarily associated with cardiovascular problems (Snijder et al., 2006), and as childhood BP is known to track into adulthood (Chen and Wang, 2008), COH-IVF may be associated with the cardiometabolic syndrome. On the other hand, caution in the interpretation of our results is deserved, as SBP percentiles did not differ between COH-IVF and Sub-NC.

How ovarian hyperstimulation might induce cardiometabolic alterations in offspring is unknown. The effect of ovarian hyperstimulation may be 2-fold: (i) it affects the endometrium: stimulation of cycles is associated with altered expression of endometrial genes and their secretions (Macklon et al., 2008) and (ii) the oocyte may be affected: high levels of FSH may predispose to oocyte aneuploidy (Vialard et al., 2008) and (ii) the oocyte may be affected: high levels of FSH may predispose to oocyte aneuploidy (Vialard et al., 2008) and (ii) the oocyte may be affected: high levels of FSH may predispose to oocyte aneuploidy (Vialard et al., 2008) and (ii) the oocyte may be affected: high levels of FSH may predispose to oocyte aneuploidy (Vialard et al., 2008). Perhaps ovarian hyperstimulation results in environmental changes of the oocyte and/or embryo that lead to epigenetic modifications of key metabolic systems associated with BP regulation (Fleming et al., 2004). Recent evidence shows that IVF is associated with widespread epigenetic modifications in phenotypically normal children (Batcheller et al., 2011). This hypothesis is supported by findings in.
mice where ovarian hyperstimulation resulted in methylation disturbances (Shi and Haaf, 2002; Sato et al., 2007). Studies comparing perinatal outcomes after fresh embryo transfer with those of children born following transfer of cryopreserved embryos found better outcomes after cryopreservation (Halliday et al., 2010; Pinborg et al., 2012). As cryopreservation is characterized by the absence of recent hormone exposure related to oocyte collection, and thus with a potentially better implantation environment, these studies may also reflect a negative effect of ovarian hyperstimulation. However, the better outcomes after cryopreservation may also be the result of the selection of more viable embryos that survive the thawing procedure.

A major strength of our study is the composition of the study groups. To our knowledge this is the first study to evaluate the effect of ovarian hyperstimulation on BP and anthropometrics separately from that of the in vitro procedure. The recent study of Scherrer et al. on vascular dysfunctions in COH-IVF offspring also attempted to disentangle effects of ovarian hyperstimulation and the in vitro procedure (Scherrer et al., 2012). They concluded that ovarian hyperstimulation is not the culprit, as vascular function in I6 naturally conceived children born following hormonal stimulation of ovulation was normal. However, in the latter small group lower doses of hormones are applied in order to stimulate the growth of only few follicles, while in COH-IVF higher doses of hormones are applied to stimulate the growth of multiple follicles. This implies that it is too early to refute a potentially adverse effect of ovarian hyperstimulation, as applied in COH-IVF, on vascular function.

Another strength is our control group of children born to subfertile couples, which minimizes the role of some potential confounders. Furthermore, the prospective and assessor-blinded design is a strength. Couples were recruited during pregnancy, thereby reducing selection bias. A final strength is the use of BP percentiles which take into account gender, age and height. As the percentiles are based on international standards by Cole taking age and gender into account. Some children (n = 35) partly co-operated and allowed one BP measurement instead of two. This single measurement was used for the analyses.

### Table II Blood pressure and anthropometrics in the three groups.

<table>
<thead>
<tr>
<th>Blood pressure, pulse pressure and heart rate</th>
<th>COH-IVF</th>
<th>MNC-IVF</th>
<th>Sub-NC</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP percentile&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n = 60</td>
<td>68.2 (21.8)</td>
<td>n = 47</td>
<td>59.1 (23.7)</td>
</tr>
<tr>
<td>DBP percentile&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n = 60</td>
<td>76.7 (18.4)</td>
<td>n = 47</td>
<td>71.9 (18.8)</td>
</tr>
<tr>
<td>SBP in mmHg&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n = 60</td>
<td>102.0 (8.0)</td>
<td>n = 47</td>
<td>98.7 (8.5)</td>
</tr>
<tr>
<td>DBP in mmHg&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n = 60</td>
<td>63.2 (8.5)</td>
<td>n = 47</td>
<td>61.2 (8.6)</td>
</tr>
<tr>
<td>Pulse pressure in mmHg&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n = 60</td>
<td>38.9 (8.2)</td>
<td>n = 47</td>
<td>37.5 (8.7)</td>
</tr>
<tr>
<td>Heart rate in beats/min&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n = 60</td>
<td>97 (10.3)</td>
<td>n = 47</td>
<td>95 (10.7)</td>
</tr>
</tbody>
</table>

Anthropometrics

| BMI (weight/length)<sup>a</sup>            | n = 63  | 15.8 (1.3)  | n = 52  | 15.3 (1.9)  | n = 79  | 15.57 (1.3) | 0.331 |
| BMI > 25 kg/m<sup>2</sup>                 | n = 63  | 4 (6%)      | n = 52  | 2 (4%)      | n = 79  | 8 (10%)     | 0.436 |
| Weight in kg<sup>a</sup>                  | n = 63  | 18.0 (13.9–27.3) | n = 52  | 17.8 (12.2–30.0) | n = 79 | 17.7 (13.0–24.6) | 0.551 |
| Standing height in cm<sup>a</sup>         | n = 63  | 107.9 (4.5) | n = 52  | 107.9 (4.4) | n = 79  | 107.5 (3.9) | 0.840 |
| Triceps skinfold in cm<sup>c</sup>        | n = 59  | 1.10 (0.37–2.07) | n = 49  | 1.10 (0.50–1.93) | n = 77  | 0.97 (0.43–1.57) | 0.093 |
| Subscapular skinfold in cm<sup>c</sup>     | n = 57  | 0.53 (0.23–1.23) | n = 49  | 0.40 (0.27–1.20) | n = 75  | 0.47 (0.30–1.17) | 0.194 |
| Total of skinfolds in cm<sup>c</sup>      | n = 57  | 1.63 (0.67–2.97) | n = 49  | 1.50 (0.80–3.03) | n = 75  | 1.43 (0.73–2.60) | 0.138 |
| Subscapular/triceps ratio<sup>c</sup>     | n = 57  | 0.50 (0.27–1.25) | n = 49  | 0.44 (0.26–0.88) | n = 75  | 0.50 (0.29–1.17) | 0.221 |
| Waist circumference in cm<sup>c</sup>     | n = 63  | 53.0 (45.5–64.8) | n = 50  | 52.0 (44.0–67.75) | n = 79  | 52.0 (44.3–61.8) | 0.376 |
| Head circumference in cm<sup>c</sup>      | n = 63  | 50.5 (46.5–55.0) | n = 51  | 50.0 (48.00–54.90) | n = 79  | 50.5 (46.5–53.5) | 0.484 |

Note: Fisher’s exact test, one-way ANOVA’s, and Kruskal–Wallis tests were used. In case significant differences were found (P < 0.05), pairwise comparisons were performed using Student’s t-tests and Mann–Whitney U-tests.

SBP percentiles differed between COH-IVF and MNC-IVF: P = 0.042, and between Sub-NC and MNC-IVF: P = 0.007. SBP in mmHg differed between COH-IVF and MNC-IVF: P = 0.040, and between Sub-NC and MNC-IVF: P = 0.040. Statistically significant results are displayed in bold numbers.


BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure. BP percentiles were determined taking height, age and gender into account. Pulse pressure: SBP minus DBP.

Total of skinsfolds: subscapular + triceps skinfold thickness, as an indicator of total body fat. Subscapular/triceps ratio: subscapular/triceps skinfold thickness, as an index of fat distribution.

BMI > 25 kg/m<sup>2</sup> was determined using international standards by Cole taking age and gender into account.

Some children (n = 35) partly co-operated and allowed one BP measurement instead of two. This single measurement was used for the analyses.

<sup>a</sup>Mean (standard deviation).

<sup>b</sup>Number (percentage).

<sup>c</sup>Median (range).
Table III  Multiple regression analysis of the effect of ovarian hyperstimulation, the in vitro procedure and a combination of these two on BP and skinfold thickness.

<table>
<thead>
<tr>
<th>Adjusted for</th>
<th>Covariate</th>
<th>Reference</th>
<th>SBP percentiles B (95% CI)</th>
<th>P-value</th>
<th>DBP percentiles B (95% CI)</th>
<th>P-value</th>
<th>Subscapular skinfold B (95% CI)</th>
<th>P-value</th>
<th>Triceps skinfold B (95% CI)</th>
<th>P-value</th>
<th>Represents the effect of</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>MNC-IVF</td>
<td>Sub-NC</td>
<td>−10.93 (−18.35 to −3.50)</td>
<td>0.004</td>
<td>−3.75 (−10.40 to 2.90)</td>
<td>0.267</td>
<td>0.01 (−0.15 to 0.13)</td>
<td>0.874</td>
<td>0.08 (−0.03 to 0.19)</td>
<td>0.167</td>
<td>IVF procedure</td>
</tr>
<tr>
<td></td>
<td>COH-IVF</td>
<td>MNC-IVF</td>
<td>9.08 (1.27 to 16.90)</td>
<td>0.023</td>
<td>4.86 (−2.14 to 11.86)</td>
<td>0.172</td>
<td>0.11 (−0.04 to 0.25)</td>
<td>0.152</td>
<td>0.02 (−0.09 to 0.03)</td>
<td>0.705</td>
<td>Ovarian hyperstimulation</td>
</tr>
<tr>
<td>Current risk indicators</td>
<td>COH-IVF</td>
<td>Sub-NC</td>
<td>−1.84 (−8.75 to 5.06)</td>
<td>0.599</td>
<td>1.11 (−5.08 to 7.30)</td>
<td>0.724</td>
<td>0.10 (−0.04 to 0.22)</td>
<td>0.155</td>
<td>0.10 (−0.00 to 0.20)</td>
<td>0.061</td>
<td>Combination</td>
</tr>
<tr>
<td></td>
<td>MNC-IVF</td>
<td>Sub-NC</td>
<td>−2.05 (−16.46 to 12.37)</td>
<td>0.780</td>
<td>−10.43 (−24.09 to 3.23)</td>
<td>0.134</td>
<td>0.13 (−0.10 to 0.36)</td>
<td>0.253</td>
<td>0.05 (−0.15 to 0.26)</td>
<td>0.614</td>
<td>IVF procedure</td>
</tr>
<tr>
<td></td>
<td>COH-IVF</td>
<td>MNC-IVF</td>
<td>10.91 (3.00 to 18.81)</td>
<td>0.007</td>
<td>5.78 (−1.71 to 13.28)</td>
<td>0.129</td>
<td>0.04 (−0.09 to 0.16)</td>
<td>0.537</td>
<td>0.00 (−1.11 to 0.11)</td>
<td>0.996</td>
<td>Ovarian hyperstimulation</td>
</tr>
<tr>
<td>Early life factors</td>
<td>COH-IVF</td>
<td>Sub-NC</td>
<td>8.86 (−4.13 to 21.86)</td>
<td>0.180</td>
<td>−4.64 (−16.96 to 7.67)</td>
<td>0.458</td>
<td>0.17 (−0.03 to 0.38)</td>
<td>0.100</td>
<td>0.05 (−0.13 to 0.24)</td>
<td>0.574</td>
<td>Combination</td>
</tr>
<tr>
<td></td>
<td>MNC-IVF</td>
<td>Sub-NC</td>
<td>−1.21 (−16.22 to 13.79)</td>
<td>0.873</td>
<td>−11.78 (−25.46 to 1.91)</td>
<td>0.091</td>
<td>0.20 (−0.08 to 0.48)</td>
<td>0.170</td>
<td>0.07 (−0.16 to 0.29)</td>
<td>0.066</td>
<td>IVF procedure</td>
</tr>
<tr>
<td></td>
<td>COH-IVF</td>
<td>MNC-IVF</td>
<td>8.92 (0.63 to 17.21)</td>
<td>0.035</td>
<td>5.48 (−2.08 to 13.04)</td>
<td>0.155</td>
<td>0.08 (−0.07 to 0.24)</td>
<td>0.293</td>
<td>0.02 (−0.10 to 0.14)</td>
<td>0.720</td>
<td>Ovarian hyperstimulation</td>
</tr>
<tr>
<td>Parental characteristic</td>
<td>COH-IVF</td>
<td>Sub-NC</td>
<td>7.71 (−5.68 to 21.10)</td>
<td>0.257</td>
<td>−6.30 (−18.51 to 5.91)</td>
<td>0.310</td>
<td>0.28 (0.03 to 0.53)</td>
<td>0.029</td>
<td>0.09 (−0.11 to 0.29)</td>
<td>0.381</td>
<td>Combination</td>
</tr>
<tr>
<td></td>
<td>MNC-IVF</td>
<td>Sub-NC</td>
<td>1.71 (−13.17 to 16.39)</td>
<td>0.821</td>
<td>−12.32 (−25.84 to 1.20)</td>
<td>0.074</td>
<td>0.12 (−0.17 to 0.40)</td>
<td>0.420</td>
<td>0.03 (−0.19 to 0.26)</td>
<td>0.778</td>
<td>IVF procedure</td>
</tr>
<tr>
<td></td>
<td>COH-IVF</td>
<td>MNC-IVF</td>
<td>9.30 (1.04 to 17.55)</td>
<td>0.027</td>
<td>7.15 (−0.34 to 14.65)</td>
<td>0.061</td>
<td>0.12 (−0.05 to 0.26)</td>
<td>0.178</td>
<td>0.05 (−0.07 to 0.18)</td>
<td>0.428</td>
<td>Ovarian hyperstimulation</td>
</tr>
<tr>
<td>Current, early life and parental characteristic</td>
<td>COH-IVF</td>
<td>Sub-NC</td>
<td>11.00 (−2.59 to 24.60)</td>
<td>0.112</td>
<td>−5.16 (−17.52 to 7.19)</td>
<td>0.410</td>
<td>0.22 (−0.03 to 0.48)</td>
<td>0.087</td>
<td>0.08 (−0.12 to 0.28)</td>
<td>0.425</td>
<td>Combination</td>
</tr>
<tr>
<td></td>
<td>MNC-IVF</td>
<td>Sub-NC</td>
<td>−4.95 (−20.27 to 10.36)</td>
<td>0.524</td>
<td>−13.00 (−27.72 to 1.71)</td>
<td>0.083</td>
<td>0.09 (−0.16 to 0.34)</td>
<td>0.479</td>
<td>0.05 (−0.17 to 0.27)</td>
<td>0.654</td>
<td>IVF procedure</td>
</tr>
<tr>
<td></td>
<td>COH-IVF</td>
<td>MNC-IVF</td>
<td>14.09 (3.59 to 22.79)</td>
<td>0.002</td>
<td>6.25 (−2.12 to 14.61)</td>
<td>0.142</td>
<td>0.07 (−0.08 to 0.21)</td>
<td>0.357</td>
<td>0.00 (−0.13 to 0.13)</td>
<td>0.994</td>
<td>Ovarian hyperstimulation</td>
</tr>
</tbody>
</table>

COH-IVF, children born following controlled ovarian hyperstimulation-IVF; MNC-IVF, children born following modified natural cycle-IVF; Sub-NC, naturally conceived controls born to subfertile parents; SBP, systolic blood pressure; DBP, diastolic blood pressure. Note that BP percentiles were determined taking height, age and gender into account.

Statistically significant results are displayed in bold numbers. In all multiple analyses we corrected for fertility factors (time to pregnancy and ICSI).

Current risk indicators: weight and sum of skinfolds for the BP analyses. Weight, gender, height and age for the skinfold analyses.

Early life factors: gestational age, birthweight, parity, periconceptional use of folic acid, breastfeed ≥6 weeks.

Parental characteristics: maternal and paternal age, high maternal and paternal education, maternal pre-pregnancy BMI, pregnancy-induced hypertension, gestational diabetes, parental diabetes or heart/vascular diseases, smoking during pregnancy, alcohol consumption during pregnancy.
and naturally conceived 14-year-old adolescents (Belva et al., 2012b). It could be that differences in the underlying causes of subfertility between couples requesting IVF (mostly female factor) and ICSI (mostly male factor) have different consequences for BP in their offspring. However, this does not clarify why the authors previously found higher BP levels in 8-year-old ICSI children (Belva et al., 2007). An explanation for the absence of an adverse effect of ICSI on BP in the Belva et al. (2012b) study could be that BP was studied during puberty. The pubertal growth phase may temporarily disturb the tracking of BP (Lever and Harrap, 1992). In another paper, Belva and colleagues report that 14-year-old ICSI girls were more prone to central, peripheral and total adiposity than naturally conceived adolescents (Belva et al., 2012a). In our data, an effect of gender or ICSI could not be properly investigated as stratifying would further reduce the sample size. Nevertheless, smaller subanalyses did not indicate an effect of gender or ICSI on our outcome measures (data not shown).

Another limitation is related to the use of multivariable regression analysis, which currently is the standard statistical approach for research questions like ours. In the multivariable regression analysis, we corrected for several sets of covariates, without knowing which covariates are true confounders, making it hard to draw conclusions with respect to any underlying causal mechanism. Moreover, this analysis does not allow for the distinction between direct and indirect effects, nor takes into account the influence of latent variables, such as an effect of socioeconomic status. Furthermore, our results revealed a difference in BP between the COH-IVF and MNC-IVF group, but not between the COH-IVF and Sub-NC group. Further exploration of our data is beneficial as we are currently unaware of the causal mechanisms underlying our findings. Is the effect of COH-IVF a direct or indirect (mediated) effect? Do latent variables or the selection criteria for MNC-IVF play a confounding role? Causal graphs and the associated theory can be of great use in studying the epidemiology of developmental disabilities in children (Day and Reynolds, 2013). In particular the application of causal inference search algorithms provides a means to tackle the hurdles we encounter here. We applied this technique in the accompanying paper (La Bastide-Van Gemert et al., 2013).

It should be noted that, in total, 311 couples were eligible for the study of which 215 (69%) agreed to participate, so selection bias could play a role. Non-participating Sub-NC mothers were younger than participating Sub-NC mothers, and non-participating MNC-IVF mothers more often had pregnancy-induced hypertension than participating MNC-IVF mothers. This selection could have resulted in suboptimal outcomes in the Sub-NC group and in better outcomes in the MNC-IVF group. However, as maternal age and the frequency of pregnancy-induced hypertension were similar between the three groups (Table I), we do not expect this to be of great influence on the results. The attrition rate from inclusion to the follow-up assessment at the age of 4 years was 10%. Furthermore, the use of self-reported maternal weight and height may be regarded a limitation. These measures are, however, considered to be reasonably reliable (Lin et al., 2012) and they were similar for the three groups. Other limitations were the fact that we measured BP on 1 day only, that we did not have an extra control group of children born to fertile couples (precluding us from investigating the effect of the underlying subfertility) and finally, that we included singletons only. COH-IVF is associated with multiple births due to double embryo transfer, whereas MNC-IVF and natural conceptions do not frequently result in a twin pregnancy. As it is known that twin pregnancies more often result in preterm birth and low birthweight, which are independent risk factors for hypertension and adiposity in later life (Wells et al., 2007), cardiometabolic problems after COH-IVF may be underestimated here. Keeping in mind that childhood BP tracks into adulthood, we suggest replication of this study with larger groups, inclusion of a fertile control group and with multiple BP measurements at older ages, or using a 24-h telemetric approach. Ideally, children of patients randomly assigned to COH-IVF or MNC-IVF who underwent single embryo transfer, as in the INeS study, should be followed (Bensdorp et al., 2009).

In conclusion, the results of the present study suggest that ovarian hyperstimulation, as applied in IVF, is associated with higher SBP percentiles in 4-year-old offspring. In addition, we found some evidence for an adverse effect of COH-IVF on subcutaneous skinfold thickness. Future research is needed to confirm the role of ovarian hyperstimulation in poorer cardiometabolic outcome and should investigate the underlying mechanisms. Our findings emphasize the importance of cardiometabolic monitoring of the growing number of children conceived with IVF worldwide.

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Authors’ roles


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Conflict of interest

The authors have no conflict of interest to declare.

References


Blood pressure in IVF offspring, Part I


