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ORIGINAL ARTICLE *Reproductive epidemiology*

Cardiovascular health of 9-year-old IVF offspring: no association with ovarian hyperstimulation and the *in vitro* procedure

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STUDY QUESTION: Are the *in vitro* procedure, ovarian hyperstimulation or a combination of these two associated with blood pressure (BP) of 9-year-old IVF children born to subfertile couples?

SUMMARY ANSWER: Our study demonstrates that ovarian hyperstimulation and the *in vitro* procedure are not associated with BP values in 9-year-old children born to subfertile couples.

WHAT IS KNOWN ALREADY: Possible long-term effects of IVF on child health and development have been studied relatively little. This is surprising, as it is known that environmental conditions may influence embryonic and foetal development which may result in health related problems in later life. Some studies suggested that IVF is associated with higher BP at pre-school age. Yet, it is unclear whether this may be also true for older children and if so, which component of IVF, i.e. the ovarian hyperstimulation, the embryo culture or a combination of these, attributes to this potentially less favourable BP.

STUDY DESIGN, SIZE, DURATION: The Groningen Assisted Reproductive Technology cohort-study is a prospective assessor-blinded study of children followed from before birth onwards. In total, 170 children were assessed at the age of 9 years. The attrition rate up until the 9-year-old assessment was 21%.

PARTICIPANTS/MATERIALS, SETTING, METHODS: We evaluated cardiovascular health, focusing on BP (in mmHg and the internationally recognized percentiles of the US National High BP Education Program), heart rate and anthropometrics of 57 children born following controlled ovarian hyperstimulation-IVF/ICSI (COH-IVF/ICSI); 47 children born after modified natural cycle-IVF/ICSI (MNC-IVF/ICSI); and 66 children who were conceived naturally by subfertile couples (Sub-NC). Cardiovascular parameters were measured multiple times on one day. In addition, anthropometric data, including BMI and skinfold thickness, were collected.

MAIN RESULTS AND THE ROLE OF CHANCE: Systolic BP in mmHg did not differ between the COH-IVF/ICSI (mean 106.9, SD 6.7), MNC-IVF/ICSI (mean 104.8, SD 5.9) and Sub-NC (mean 106.3, SD 5.3) groups. In addition, systolic BP percentiles did not differ between the groups: COH-IVF/ICSI (mean 62.4, SD 20.2); MNC-IVF/ICSI (mean 56.3, SD 19.3); and Sub-NC (mean 62.3, SD17.8). Also, after adjustment for confounders BP in the three groups was similar. Heart rate and anthropometric values in the three groups did not differ.

For instance, BMI values in the COH-IVF/ICSI-children were 16.3 (median value, range 13.0–24.7), in MNC-IVF/ICSI-children 16.1 (range 12.7–22.5) and in Sub-NC children 16.3 (range 12.7–24.0).

LIMITATIONS, REASONS FOR CAUTION: The size of our study groups does not allow for pertinent conclusions on the effect of ovarian hyperstimulation and the *in vitro* procedure. The lack of a fertile control group may be regarded as another limitation.

WIDER IMPLICATIONS OF THE FINDINGS: Our study suggests that ovarian hyperstimulation and *in vitro* procedures are not associated with cardiovascular health in 9-year-old. Yet, BP percentiles of the three groups were higher than the expected 50th percentile. This might indicate that children of subfertile couples have a higher BP than naturally conceived children.

STUDY FUNDING/COMPETING INTEREST(S): The study was financially supported by the University Medical Center Groningen (UMCG), the two graduate schools of the UMCG, BCN, SHARE and the Cornelia Stichting. The sponsors of the study had no role in study design, data collection, data analysis, data interpretation or writing of the report. The authors have no conflicts of interest to declare.

Key words: IVF / blood pressure / ovarian hyperstimulation / *in vitro* procedure / school age

Introduction

Subfertility is a widespread problem for which assisted reproductive techniques (ARTs) are used increasingly (Human Fertilisation and Embryology Authority, 2013). In western industrialized countries nowadays 1–6% of all newborn children are conceived with the help of IVF, with or without ICSI (Calhaz-Jorge *et al.*, 2016). This number is high when considering the fact that little is known about the long-term effects of IVF on child development and health.

Studies on the short-term effects of IVF have shown that IVF in singleton pregnancies is associated with worse perinatal outcome, e.g. preterm birth and low birthweight (Pinborg *et al.*, 2013). It is known that preterm birth and low birthweight both have a negative influence on cardiometabolic health. Children born with a low birthweight have a higher tendency to be obese or have hypertension in late adulthood (Law *et al.*, 1993; Prokopec and Bellisle, 1993). IVF offspring have not yet reached late adult age, but multiple studies indicated that IVF offspring have a reduced insulin sensitivity, vascular dysfunction (such as increased right atrial size and thicker aorta intima thickness) and higher blood pressure (BP) levels at pre-school and school age (Belva *et al.*, 2007; Ceelen *et al.*, 2008; Sakka *et al.*, 2010; Scherrer *et al.*, 2012; Valenzuela-Alcaraz *et al.*, 2013; Chen *et al.*, 2014; Seggers *et al.*, 2014). These cardiometabolic impairments are associated with increased accumulation of Advanced Glycation End products (AGEs), which arise from chronic metabolic stress (de Vos *et al.*, 2013). The studies indicated that the higher BP in IVF offspring could not be explained by preterm birth and low birthweight alone and suggested an adverse effect of factors related to IVF itself, in particular of ovarian hyperstimulation (La Bastide-Van Gemert *et al.*, 2014; Seggers *et al.*, 2014). However, another study suggested that the higher BP levels should rather be attributed to the underlying subfertility than to the ART-procedures itself (Pontesilli *et al.*, 2015).

The primary aim of the present study is to disentangle the effects of ovarian hyperstimulation, the *in vitro* procedure and a combination of these two on the child's cardiovascular health at age 9. The secondary aim is to evaluate whether the severity of subfertility, evaluated with the proxy time to pregnancy (TTP), influences the child's cardiovascular health at 9 years. Our primary outcome measures are systolic blood pressure (SBP) and diastolic blood pressure (DBP) in mmHg. We hypothesize that cardiovascular outcome of IVF singletons is worse than that of naturally conceived singletons of subfertile couples, and—in line

with our results at the 4 years—that this putative worse outcome can be attributed to ovarian hyperstimulation (Seggers *et al.*, 2014).

Materials and Methods

Ethical approval

Parents gave written informed consent and the study design was approved by the ethics committee of the UMCG.

Study design

Participants were the now 9-year-old singletons of the Groningen ART cohort-study. The Groningen ART cohort-study is a prospective, assessor-blinded, longitudinal follow-up study of children born to subfertile couples, i.e. couples who were not able to conceive within 12 months from the start of unprotected intercourse, who eventually conceived either naturally of after ART. Pregnant subfertile couples with an expected delivery date between March 2005 and December 2006 were invited during the third trimester of pregnancy at the Department of Reproductive Medicine of the University Medical Center Groningen (UMCG) to participate in the study. Their children formed the following three groups: (i) singletons born after controlled ovarian hyperstimulation-IVF/ICSI (COH-IVF/ICSI), (ii) singletons conceived with modified natural cycle-IVF/ICSI (MNC-IVF/ICSI) and (iii) naturally conceived singletons of subfertile couples (Sub-NC). Placement in the COH-IVF/ICSI group depended on the presence of ovarian hyperstimulation. Criteria for inclusion in the MNC-IVF/ICSI group were female age between 18 and 36 years, no previous unsuccessful COH-IVF/ICSI treatment or first IVF/ICSI treatment after pregnancy, regular ovulatory menstrual cycle of 26–35 days and a BMI of 18–28 kg/m^2. Couples who conceived naturally after 1 year after the start of unprotected intercourse formed the Sub-NC group.

With these three groups the study investigates the independent effects of ovarian stimulation and the *in vitro* laboratory procedures on the offspring's health and development: comparison of COH-IVF/ICSI with MNC-IVF/ICSI reveals the effect of ovarian hyperstimulation; differences between the MNC-IVF/ICSI and Sub-NC group can be attributed to the *in vitro* procedure; comparing the COH-IVF/ICSI group with the Sub-NC group uncovers the combined effect of ovarian hyperstimulation and the *in vitro* procedure. Children born after oocyte cryopreservation and oocyte or embryo donation were excluded as were all twins.

Settings

Information on socioeconomic status and the prenatal, perinatal and neonatal period was collected on standardized charts. High level of education was defined as a higher vocational education or university education. We evaluated the effect of the severity of subfertility with the proxy TTP. In case of miscarriage TTP can be <1 year, as TTP has a new onset (for details see Middelburg *et al.*, 2009).

Follow-up examination

The follow-up assessment at 9 years took place between March 2014 and May 2016 at the UMCG or at the child's home. Assessors were blinded to the mode of conception. The assessment started with a brief introduction on the tests to be performed (cognitive, neurological, cardiovascular and anthropometric assessment). After the introduction BP was measured twice and neuropsychological development was evaluated (further description of the BP-measurement will follow). Next an intelligence test was performed and BP was measured twice for the second time. After the second BP-measurement, a neurological examination was performed. Finally, anthropometric data were collected and BP was assessed twice for the third time. In total, BP was measured three times in *duplo*. In the present paper, cardiovascular and anthropometric outcomes are reported.

Outcomes

Blood pressure

BP (mmHg) was measured using an automated BP monitor (Datascope Accutorr plus, Mahwah, NJ, USA), at the non-dominant arm while the child was seated, with the arm on the lap. It was measured in 6-fold: twice at the beginning of the assessment, twice after 2 h and twice at the end. Mean BP at each of the three measuring moments was used to calculate the overall mean BP. The overall means were used to calculate BP percentiles based on the standards of the US National High BP Education Program (Falkner and Daniels, 2004). The BP percentiles take sex, height and age in months into account. Hypertension is defined as a SBP- or DBP percentile above the 95th percentile. The overall mean BP was also used to calculate the pulse pressure (SBP minus DBP) (O'Rourke and Frohlich, 1999).

Anthropometrics

Standing height (in cm) was measured using a stadiometer (Seca Deutschland, Hamburg, Germany) and body weight (in kg) with an electronic weighing scale (Radweg, Radom, Poland). BMI; kg/m^2 was calculated. The proportion of children with a height below −1SD and of children with a BMI above 25, taking into account sex and age in months, was calculated (Dutch reference values, Schönbeck and van Buuren, 2010). Occipitofrontal head circumference and waist circumference (both in cm) were measured with a non-stretchable 'lasso' tape.

Biceps, triceps, subscapular and suprailiac skinfold thickness (in mm) were each measured three times, on the non-dominant side of the child, using a Servier caliper. The mean of the three measurements was used for further calculations. As a parameter of peripheral fat distribution the mean of biceps and triceps skinfold thickness was used; as a parameter of central fat distribution that of subscapular and suprailiac skinfold. The sum of the four means is an indicator of total body fat (Weststrate *et al.*, 1988).

Advanced glycation end products

AGEs are metabolic or stress-derived end products of sugars that play a key role in the pathogenesis of cardiovascular disease (de Vos *et al.*, 2013). The assessment of AGE accumulation in children born after IVF may provide insight into their risk for cardiovascular disease (Hegab *et al.*, 2012). AGE's were assessed with an AGE Reader (DiagnOptics Technologies BV, Groningen, The Netherlands). This is a non-invasive desk-top device that uses the characteristic fluorescent properties of AGEs to estimate the level of AGEs accumulation in the skin. This fluorescence assessment is validated and correlates with individual AGE compounds measured in skin biopsy (Smit and Gerrits, 2010). Technical details are described elsewhere (Mulder *et al.* 2006). Children had to position their forearm on top of the device. A series of three times two (right and left) consecutive measurements were carried out. Mean AGE was calculated on the basis of these six measurements and used in the outcome analyses. Mean AGE was classified as high if it exceeded +1SD of the mean reference values of AGEs of healthy Caucasian age-matched control subjects (Koetsier *et al.*, 2010).

Statistical analysis

To estimate differences in background and outcome characteristics the Fisher's exact test, Mann–Whitney U-test and Student's t-test were used. To assess potential differences in the continuous outcome variables between the three groups, multivariable linear regression analyses were performed while correcting for the following set of confounders: gestational age, TTP, maternal diabetes/hypertension/heart disease, pregnancy-induced hypertension, high maternal education, child's age and sex. Logistic regression analysis was used for dichotomous variables, while adjusting for the same set of confounders.

To study the effect of TTP on the outcome variables the three groups of the cohort were pooled to form one subfertile group. Pearson's correlations coefficients were determined and multivariable linear regression analyses were performed while adjusting for the same set of confounders except TTP and—when a group effect was found, with the inclusion of the ART-group status (COH-IVF/ICSI; MNC-IVF/ICSI and/or Sub-NC). Additional analyses were performed regarding the effect of TTP in the Sub-NC group alone. Note, when analysing BP percentiles age, height and sex are already taken into account.

A two-sample t-test power analysis for BP was performed. Assuming a SD of 8 mmHg based on previous publications (Seggers *et al.*, 2013, 2014), 42 children had to be included in each group in order to detect a difference of 5 mmHg to reach a power of 80%.

Results are expressed as regression coefficients (B) or odds ratio with 95% confidence intervals (95% CI). Probability values of <0.05 are considered statistically significant. Statistical analyses were performed using the IBM Statistical Package for the Social Sciences 20.0 for Windows.

Results

Participation

During prenatal inclusion, 89 COH-IVF/ICSI singletons, 79 MNC-IVF/ICSI singletons and 143 Sub-NC singletons were eligible for the study. Parents of 68 (76%), 57 (72%) and 90 (63%) singletons agreed to partake (numbers for the same three groups, respectively). At 9 years, 57 (83%), 47 (82%) and 66 (73%) children participated. Postnatal attrition after 9 years of follow-up was 21%. Background characteristics of participants and non-participants were similar (data not shown).

Parental and infant characteristics

The demographic characteristics of the ART groups are displayed in Table I. Paternal age at conception was higher in the COH-IVF/ICSI group (36.4 years) than in the MNC-IVF/ICSI group (34.0 years). TTP was longer in the two IVF groups (COH-IVF/ICSI 4.0 years; MNC-IVF/ICSI 3.8 years) compared to the Sub-NC group (2.0 years). The rate of folic acid use during gestation was higher in the MNC-IVF/ICSI group (100%) than in the Sub-NC group (86%). Gestational age was shorter in the COH-IVF/ICSI group (39.4 weeks) compared to

Table I Characteristics of participating parents and children.

Characteristics	COH-IVF/ICSI n = 57	MNC-IVF/ICSI n = 47	Sub-NC n = 66
Parental characteristics			
Maternal age at conception, median (range)	33.2 (27.0–40.9)	32.8 (26.2–37.5)	33.7 (23.1–40.3)
Paternal age at conception, median (range)	**36.4 (27.5–56.1)[#]**	**34.0 (28.3–47.8)[#]**	35.4 (25.5–48.7)
Education level mother high, n (%)[a,b]	20 (35)	21 (45)	31 (47)
Education level father high, n (%)[a,b]	26 (46)	16 (35)	25 (38)
Maternal BMI before pregnancy, median (range)[b]	23.9 (18.3–42.5)	23.1 (16.8–30.6)	23.0 (18.0–46.7)
Parental diabetes/heart/ vascular disease, n (%)[c]	3 (5)	1 (2)	2 (3)
Fertility parameters			
TTP in years, median (range)	**4.0 (0.1–13.3)[#]**	**3.8 (0.1–7.5)[^]**	**2.0 (0.1–11.3)[#/^]**
ICSI, n (%)	37 (65)	22 (47)	n.a.
Gestational characteristics			
Smoking during pregnancy, n (%)	6 (11)	5 (11)	6 (9)
Use of folic acid during pregnancy, n (%)[b]	50 (93)	**47 (100)[#]**	**57 (86)[#]**
Gestational diabetes, n (%)	0 (0)	1 (2)	2 (3)
Pregnancy-induced hypertension, n (%)	6 (11)	3 (6)	13 (20)
Caesarean section, n (%)	15 (26)	8 (17)	19 (29)
Birth characteristics			
Gestational age in weeks, median (range)	**39.4 (33.4–42.3)[#]**	39.7 (34.6–42.6)	**40.1 (30.1–42.6)[#]**
Preterm birth (<37 weeks), n (%)	6 (11)	6 (13)	4 (6)
Birthweight in grams, mean (σ)	**3340 (563)[#]**	**3383 (598)[^]**	**3594 (517)[#/^]**
Low birthweight, n (%)	3 (5)	4 (9)	2 (3)
Small-for-gestational age, n (%)	0 (0)	3 (6)	1 (2)
Neonatal characteristics			
NICU admission, n (%)	1 (2)	2 (4)	4 (6)
Apgar score at 5 min <7, n (%)[a]	0 (0)	0 (0)	0 (0)
Breastfeed for >6 weeks, n (%)[a]	29 (52)	22 (47)	33 (51)
Child characteristics			
Male sex, n (%)	32 (56)	22 (47)	33 (50)
Firstborn, n (%)[b]	38 (67)	34 (72)	39 (59)
Age at examination in months, median (range)	110.4 (108.5–126.5)	110.3 (108.2–131.8)	109.9 (100.7–118.5)
Sport club member, n (%)	50 (88)	40 (85)	58 (88)

Mann–Whitney U-test, student t-test and Fisher's exact tests were used to estimate group differences for background characteristics and fertility parameters. Statistically significant differences ($P < 0.05$) are displayed in bold; the symbols denote which groups differ significantly from each other. Values are number (percentage), mean (standard deviation [σ]) or median (range). COH-IVF, controlled ovarian hyperstimulation-IVF; MNC-IVF, modified natural cycle-IVF; n.a., not available; NICU, neonatal intensive care unit; Sub-NC, naturally conceived children born to subfertile couples; TTP, time to pregnancy.
[a]Missing data in the COH-IVF group: breastfeed for >6 weeks n = 1; education level father high n = 3; the use of folic acid during pregnancy n = 3; maternal BMI n = 1. Missing data in the MNC-IVF group: Apgar score 5 min < 7 n = 1. Missing data in the Sub-NC group: Apgar score 5 min < 7 n = 1; breastfeed for >6 weeks n = 1; education level father high n = 1.
[b]Higher vocational education or University education.
[c]Heart and vascular disease including known or treated hypertension.

the Sub-NC group (40.1 weeks). Birthweight was lower in the IVF groups (COH-IVF/ICSI 3340 grams; MNC-IVF/ICSI 3383 grams) than in the Sub-NC group (3594 grams). Neonatal and child characteristics were similar in the three groups.

BP and anthropometrics

Table II provides the 9-year BP and anthropometric data. Absolute SBP and DBP values in mmHg did not show statistically significant differences between the groups (SBP: COH-IVF/ICSI: 106.9; MNC-IVF/ICSI: 104.8; and Sub-NC: 106.3; DBP: COH-IVF/ICSI: 65.5; MNC-IVF/ICSI: 64.1; and Sub-NC: 66.0). Also, BP percentiles, pulse pressure and heart rate of the three groups were similar. Adjustment for confounders did not alter the results: all cardiovascular parameters including absolute BP values and BP percentile of the three groups remained comparable (Table III).

Anthropometric values in the three groups did not differ. Weight in kilograms (COH-IVF/ICSI: 31.7; MNC-IVF/ICSI: 33.7 and Sub-NC: 31.6) and standing height in centimetres (COH-IVF/ICSI: 140.9; MNC-IVF/ICSI: 141.6; and Sub-NC: 140.6) in the three groups were similar. The same held true for skinfold thickness. The similarities in anthropometric values of the three groups remained after adjustment

Table II BP and anthropometrics.

Outcome measurements	COH-IVF/ICSI *n* = 57	MNC-IVF/ICSI *n* = 47	Sub-NC *n* = 66
BP and heart rate			
SBP in mmHg, mean (σ)	106.9 (6.7)	104.8 (5.9)	106.3 (5.3)
DBP in mmHg, mean (σ)	65.5 (5.7)	64.1 (7.4)	66.0 (5.5)
SBP percentile, mean (σ)	62.4 (20.2)	56.3 (19.3)	62.3 (17.8)
DBP percentile, mean (σ)	63.2 (17.1)	58.8 (23.1)	64.7 (16.2)
High BP, *n* (%)[b]	3 (5)	0 (0)	1 (2)
Pulse pressure, mean (σ)	41.4 (4.7)	40.7 (5.0)	40.3 (5.5)
Heart rate in beat/min, mean (σ)	81.8 (9.4)	80.5 (9.5)	81.8 (10.6)
Anthropometrics			
Weight in kg, median (range)	31.7 (24.7–56.0)	33.7 (22.5–48.1)	31.6 (20.0–53.0)
Standing height in cm, mean (σ)	140.9 (6.0)	141.6 (7.0)	140.6 (6.0)
Standing height below −1SD, *n* (%)[c]	5 (9)	3 (6)	6 (9)
BMI, median (range)	16.3 (13.0–24.7)	16.1 (12.7–22.5)	16.3 (12.7–24.0)
BMI > 25, *n* (%)[d]	12 (21)	5 (11)	12 (18)
Biceps skinfold in cm, median (range)	0.60 (0.13–1.90)	0.57 (0.10–1.63)	0.57 (0.10–2.80)
Triceps skinfold in cm, median (range)	1.05 (0.33–2.43)	1.11 (0.30–2.33)	1.03 (0.27–2.27)
Subscapular skinfold in cm, median (range)	0.52 (0.20–2.20)	0.50 (0.13–3.30)	0.53 (0.20–3.20)
Suprailiac skinfold in cm, median (range)[a]	0.95 (0.10–3.00)	0.97 (0.20–3.00)	0.93 (0.30–3.40)
Total of skinfold in cm, median (range)[a]	0.83 (0.27–2.13)	0.82 (0.23–2.58)	0.75 (0.33–2.25)
Central/peripheral skinfold ratio, mean (σ)[a]	1.04 (0.32)	0.99 (0.38)	1.02 (0.31)
Waist circumference in cm, median (range)	61.1 (50.2–86.8)	61.3 (51.5–83.8)	61.5 (51.0–83.3)
Head circumference in cm, mean (σ)[a]	53.3 (1.83)	53.0 (1.79)	53.3 (1.56)
AGEs, median (range)[a]	1.15 (0.82–1.63)	1.12 (0.87–1.80)	1.10 (0.80–1.57)
AGE above +1SD, *n* (%)[a,e]	26 (49)	20 (44)	25 (40)

Mann–Whitney *U*-test, student *t*-test and Fisher's exact tests were used to estimate group differences. Values are number (percentage), mean (standard deviation [σ]) or median (range). AGEs, advanced glycation end products; COH-IVF, controlled ovarian hyperstimulation-IVF; DBP, diastolic blood pressure; MNC-IVF, modified natural cycle-IVF; SBP, systolic blood pressure; Sub-NC, naturally conceived children born to subfertile parents.

[a]Missing data in the COH-IVF group: AGE's *n* = 4; AGE high *n* = 4; central/peripheral skinfold ratio *n* = 1; suprailiac skinfold *n* = 1; total skinfold *n* = 1. Missing data in the MNC-IVF group: AGE's *n* = 2; AGE high *n* = 2. Missing data in the Sub-NC group: AGE's *n* = 4; AGE high *n* = 4; head circumference *n* = 1.

[b]High BP is defined as a SBP- or DBP percentile above the 95th percentile according to the standards of the US National High BP Education Program. The BP percentiles take sex, height and age into account.

[c]Scoring below −1SD on age based Dutch norm values.

[d]Scoring above a BMI of 25 based on international norm values.

[e]Scoring above +1SD on age based international norm values.

for confounders (Table III). Furthermore, AGE-values of the three groups did not differ, neither did the frequency of AGE-values +1SD: COH-IVF/ICSI 49%; MNC-IVF/ICSI 44%; and Sub-NC 40%.

TTP was significantly longer in the two IVF groups than in the Sub-NC group (Table I). Yet, the univariable and multivariable analyses in the pooled data showed that TTP was not correlated with BP and anthropometrics (Table IV). Subanalyses in the Sub-NC group only—in which TTP was not truncated by IVF—demonstrated that TTP was also not associated with cardiovascular health (data not shown).

Discussion

This prospective follow-up study indicated that BP and anthropometrics of 9-year-old singletons born following COH-IVF/ICSI, MNC-IVF/ICSI and Sub-NC were similar. In addition, our study demonstrated that a prolonged TTP, a proxy for the severity of subfertility, was not associated with adverse cardiovascular and anthropometric outcome.

Our results seem to contradict the findings of a recent review on 19 studies that compared cardiometabolic health of IVF offspring with that of naturally conceived offspring. The review concluded on the basis of studies with children of varying age, that BP of children conceived by IVF/ICSI is slightly (but statistically significant) higher than that of naturally conceived children (Guo *et al.*, 2017). However, it is not clear if a certain component of IVF, a combination of components, or subfertility causes this less optimal cardiovascular health. Previously, the Groningen ART cohort-study indicated that 4-year-old IVF children born following COH-IVF/ICSI have a higher SBP than children born following MNC-IVF/ICSI (Seggers *et al.*, 2014). This suggested a specific unfavourable effect of ovarian hyperstimulation (La Bastide-van Gemert *et al.*, 2014). The current study indicates that this adverse effect could not be replicated when the children were 9 years. Yet, the current findings may be better comparable with those of the studies of Belva *et al.* that suggested that elevated SBP levels disappear with increasing age (Belva *et al.*, 2007, 2012a). Belva *et al.* reported higher

Table III Multiple regression analyses of the effect of ovarian hyperstimulation, the *in vitro* procedure, or a combination of both on BP and anthropometrics.

Linear regression	Adjusted B (95%CI)		
	COH-IVF/ICSI vs MNC-IVF/ICSI	MNC-IVF/ICSI vs Sub-NC	COH-IVF/ICSI vs Sub-NC
SBP mmHg	1.99 (−0.35, 4.34)	−1.19 (−3.53, 1.15)	0.83 (−1.42, 3.09)
DBP mmHg	1.31 (−1.10, 3.71)	−1.59 (−3.99, 0.81)	−0.19 (−2.50, 2.12)
SBP percentile	5.20 (−2.27, 12.68)	−5.23 (−12.68, 2.21)	0.20 (−6.99, 7.38)
DBP percentile	3.51 (−3.71, 10.72)	−4.64 (−11.82, 2.54)	−0.75 (−7.68, 6.18)
Pulse pressure	0.68 (−1.35, 2.72)	0.40 (−1.64, 2.44)	1.02 (−0.94, 2.98)
Heart rate in beat/min	1.17 (−2.57, 4.92)	−1.12 (−4.86, 2.62)	0.06 (−3.54, 3.66)
Weight	0.02 (−0.05, 0.10)	−0.02 (−0.09, 0.06)	0.01 (−0.06, 0.08)
Standing height	−0.21 (−2.64, 2.21)	0.93 (−1.49, 3.35)	0.62 (−1.71, 2.95)
BMI	0.03 (−0.03, 0.08)	−0.03 (−0.08, 0.02)	0.00 (−0.05, 0.05)
Biceps skinfold	−0.02 (−0.22, 0.26)	−0.10 (−0.34, 0.14)	−0.07 (−0.30, 0.16)
Triceps skinfold	−0.03 (−0.20, 0.14)	−0.05 (−0.21, 0.12)	−0.07 (−0.23, 0.09)
Subscapular skinfold	0.03 (−0.21, 0.26)	−0.08 (−0.31, 0.15)	−0.12 (−0.36, 0.12)
Suprailiac skinfold	0.08 (−0.19, 0.35)	−0.15 (−0.43, 0.12)	−0.07 (−0.33, 0.20)
Total of skinfold	0.01 (−0.20, 0.21)	−0.12 (−0.32, 0.08)	−0.10 (−0.30, 0.09)
Central/peripheral skinfold ratio	0.09 (−0.05, 0.22)	−0.06 (−0.19, 0.07)	0.03 (−0.10, 0.16)
Waist circumference	0.01 (−0.04, 0.06)	−0.02 (−0.07, 0.03)	−0.01 (−0.06, 0.04)
Head circumference	0.36 (−0.32, 1.03)	−0.30 (−0.98, 0.37)	0.04 (−0.61, 0.69)
AGEs	−0.01 (−0.07, 0.05)	0.02 (−0.05, 0.06)	−0.01 (−0.07, 0.05)
Logistic regression	Adjusted B (95%CI)		
AGE above +1SD[b]	1.06 (0.43, 2.61)	1.32 (0.56, 3.12)	1.47 (0.65, 3.30)
BMI > 25[c]	2.48 (0.76, 8.15)	0.39 (0.12, 1.29)	1.07 (0.41, 2.83)
Standing height below −1SD[e]	1.49 (0.31, 7.07)	0.80 (0.16, 3.92)	0.83 (0.20, 3.47)

Multiple linear and logistic regression analyses were performed. In the multiple analyses, we corrected for gestational age, TTP, maternal diabetes/hypertension/heart disease, pregnancy-induced hypertension, high maternal education, age and sex. BP percentiles already take age in months, standing height in cm and sex into account.
[a]Missing data in the COH-IVF group: AGE's $n = 4$; AGE high $n = 4$; central/peripheral skinfold ratio $n = 1$; suprailiac skinfold $n = 1$; total skinfold $n = 1$. Missing data in the MNC-IVF group: AGE's $n = 2$; AGE high $n = 2$. Missing data in the Sub-NC group: AGE's $n = 3$; AGE high $n = 3$; head circumference $n = 1$.
[b] Scoring above +1SD on age based international norm values.
[c] Scoring above a BMI of 25 based on international norm values.
[d] High BP is defined as a SBP- or DBP percentile above the 95th percentile according to the standards of the US National High BP Education Program. The BP percentiles take sex, height and age into account.
[e] Scoring below −1SD on age based Dutch norm values.

BP levels in ICSI-conceived children compared to naturally conceived children aged 8 years. However, the differences between the two groups had disappeared at 14 years (Belva *et al.*, 2007, 2012a). Yet, others reported that cardiovascular condition in IVF/ICSI offspring over the age of 7 years was less optimal than that of controls. Scherrer *et al.* demonstrated that IVF/ICSI-children in a condition of environmental stress, i.e. high-altitude, showed in comparison to non-IVF/ICSI controls an increased rate of pulmonary hypertension, an increased right ventricle end-diastolic area and diastolic dysfunction (Scherrer *et al.*, 2012; von Arx *et al.*, 2015). The results of Chen *et al.* pointed in the same direction: they showed that IVF offspring with a mean age of 21.5 years had higher levels of SBP compared to controls after being exposed to three days of overfeeding (Chen *et al.*, 2014). Overall, this suggests that IVF/ICSI offspring have a less optimal cardiovascular health, which is not visible during non-stressed situations, but gets expressed during stressed conditions. It is already known that fetuses, exposed to less favourable uterine conditions, show a greater

BP increase during stressful events in later life. The propensity to higher BP is however not expressed during non-stress conditions (Painter *et al.*, 2006).

Belva *et al.* suggested that the disappearance of the elevated BP levels in the ICSI-conceived children at 14 years may have been a temporary phenomenon, induced by the hormonal changes and growth of puberty (Shankar *et al.*, 2005; Belva *et al.*, 2012a). For the disappearance of the elevated BP in our COH-IVF/ICSI-children this explanation is not valid, as our 9-year-old had not entered puberty. It is conceivable that the BP-assessment at 4 years had functioned as a rather stressful condition, as at the age of 4 years the child's cognitive abilities to understand the strange situation of a BP-measurement are limited. At 9 years, children are presumably less stressed by a BP-measurement, as their cognitive abilities allow them to classify the measurement as a somewhat weird but harmless medical test.

The study of Pontesilli *et al.* suggested that the higher BP levels should be attributed to subfertility rather than to ART-procedures

Table IV Associations effects of TTP (in months) in the pooled groups and cardiometabolic outcomes (*n* = 170).

Linear regression	Adjusted B (95%CI)	*P*-value
SBP mmHg	−0.02 (−0.44–0.40)	0.927
DBP mmHg	−0.20 (−0.63–0.23)	0.361
SBP percentile	−0.35 (−1.69–1.00)	0.614
DBP percentile	−0.77 (−2.06–0.52)	0.242
Pulse pressure	0.18 (−0.18–0.54)	0.325
Heart rate in beat/min	−0.11 (−0.79–0.58)	0.756
Weight	0.01 (−0.01–0.02)	0.471
Standing height	0.10 (−0.34–0.53)	0.663
BMI	0.00 (0.00–0.01)	0.487
Biceps skinfold	0.00 (−0.04–0.04)	0.910
Triceps skinfold	0.02 (−0.01–0.05)	0.148
Subscapular skinfold	0.02 (−0.03–0.06)	0.445
Suprailiac skinfold	0.02 (−0.03–0.07)	0.502
Total of skinfold	0.01 (−0.02–0.05)	0.480
Central/peripheral skinfold ratio	0.00 (−0.02–0.03)	0.774
Waist circumference	0.01 (−0.01–0.02)	0.110
Head circumference	−0.06 (−0.18–0.07)	0.372
AGEs	0.00 (−0.01–0.01)	0.930
Logistic regression		
AGE above +1SD[b]	0.98 (0.85–1.14)	0.789
BMI > 25[c]	1.13 (0.94–1.35)	0.205
High BP[d]	0.79 (0.44–1.42)	0.789
Standing height below −1SD[e]	0.99 (0.76–1.28)	0.910

Multiple linear and logistic regression analyses were performed. In the multiple analyses, we corrected for gestational age, TTP, maternal diabetes/hypertension/heart disease, pregnancy-induced hypertension, high maternal education, age and sex. BP percentiles already take age in months, standing height in cm and sex into account.
[a]Missing data: AGE's *n* = 10; AGE high *n* = 10; central/peripheral skinfold ratio *n* = 1; head circumference *n* = 1; suprailiac skinfold *n* = 1; total skinfold *n* = 1.
[b]Scoring above +1SD on age based international norm values.
[c]Scoring above a BMI of 25 based on international norm values.
[d]High BP is defined as a SBP- or DBP percentile above the 95th percentile according to the standards of the US National High BP Education Program. The BP percentiles take sex, height and age into account.
[e]Scoring below −1SD on age based Dutch norm values.

([Pontesilli *et al.*, 2015]). Unfortunately, most studies dealing with IVF and cardiovascular outcome lack detailed information on subfertility or lack a control group with natural conceived children in subfertile couples; this also holds true for the studies that suggested that ART-offspring is at risk of impaired cardiovascular function in stressful conditions. Our study was not able to assess the effect of subfertility *per se*. But we did have information on the severity of subfertility in terms of TTP. TTP was not associated with BP. Still it is noteworthy that the mean BP percentiles of the three groups were higher than the expected 50th percentile. This might indicate that offspring of subfertile couples have a higher BP than children who are conceived naturally. The relatively high frequencies of children with AGE-values above +1SD also point to the possibility that the underlying subfertility plays a role in the less favourable cardiovascular outcomes of ART-offspring reported in the literature.

The anthropometric values of the three groups did not differ. At the age of 4, Seggers *et al.* found evidence for an adverse effect of COH-IVF/ICSI on subscapular skinfold thickness ([Seggers *et al.*, 2014]). However, only after correction for the specific subset of confounders called 'early life risk factors' and not after correction for other subsets of confounders. Belva *et al.* found higher sum of skinfolds in 14-year-old ICSI-girls compared to natural conceived girls. Yet, the BMI of the ICSI-girls was 19.7, i.e. in the normal range ([Belva *et al.*, 2012b]). Our group sizes did not allow for a separate subgroup analysis for boys and girls. The recent review of Guo *et al.* indicated that the BMI of IVF children did not differ from that of natural conceived children, without, however, performing a sex-specific subgroup analysis ([Guo *et al.*, 2017]).

A strength of our study is its design. The three groups allow to separately evaluate the effect of ovarian hyperstimulation and the *in vitro* procedure on child development and health. In addition, the presence of the subfertile control group (Sub-NC group) prevents overestimation of the effect of IVF. Other strengths are the blinding of the assessors to the mode of conception, the recruitment of couples during pregnancy, the use of AGEs to assess cardiovascular health, and the acceptable attrition (21%) after 9 years of follow-up ([Fewtrell *et al.*, 2008]).

The main limitation of our study is the size of the groups. Our group sizes allowed to detect a difference of 5 mmHg, but were not large enough to draw firm conclusions regarding BP percentiles, as is illustrated by their broad confidence intervals, and regarding pathological BP values. In addition, the sample size of the MNC-group is relatively small. This prevents firm conclusions on the effect of MNC-IVF/ICSI. However, other studies investigating the effect of IVF on BP do not have the ability to study the effect of ovarian hyperstimulation and the *in vitro* procedure separately ([Belva *et al.*, 2007]; [Ceelen *et al.*, 2008]; [Sakka *et al.*,2010]; [Scherrer *et al.*, 2012]; [Valenzuela-Alcaraz *et al.*,2013]; [Chen *et al.*, 2014]).

Another limitation is the absence of a fertile control group, precluding a conclusion on the effect of the absence or presence of subfertility. With the couples' TTP we have detailed information on the severity of subfertility, but we were not able to address the impact of subfertility *per se* on cardiovascular health.

In conclusion, the present study suggests that controlled ovarian hyperstimulation, the *in vitro* procedure and a combination of these two are not associated with higher BP and unfavourable anthropometrics in 9-year-old children born to subfertile couples. In addition, the severity of subfertility (TTP) was not associated with worse cardiometabolic outcome. On the other hand, BP percentiles of the three groups were higher than the expected 50th percentile. This may indicate that offspring of subfertile couples have a higher BP than children who are conceived naturally. Therefore a study at later age which compares the BP levels of offspring of fertile and subfertile couples is necessary.

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Authors' roles

All authors fulfil the criteria for authorship; M.H.A., M.H. and M.J.H. initiated the study; D.K. and J.S. collected the data; S.I.B-v.G and D.K.

analysed the data; D.K., D.M., A.H. and M.H.A. interpreted the data; D.K. and M.H.A. drafted the report. All authors commented on the drafts, and have seen and approved the final version.

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Conflict of interest

The authors have no conflict of interest to declare.

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