Human Reproduction, Vol.37, No.11, pp. 2578–2588, 2022
Advance Access Publication on September 19, 2022 https://doi.org/10.1093/humrep/deac201

human reproduction

ORIGINAL ARTICLE *Infertility*

Even high normal blood pressure affects live birth rate in women undergoing fresh embryo transfer

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Submitted on March 11, 2022; resubmitted on August 13, 2022; editorial decision on August 31, 2022

STUDY QUESTION: Do differences in blood pressure within the normal range have any impacts on the live birth rate (primary outcome) or biochemical pregnancy rate (beta-hCG positivity), clinical pregnancy rate (heart beating in ultrasound), abortion rate and ectopic pregnancy rate (secondary outcomes) of fresh embryo transfer in women undergoing their IVF/ICSI treatment?

SUMMARY ANSWER: Even rather small differences in baseline blood pressure in women with normal blood pressure according to current guidelines undergoing fresh embryo transfer after IVF/ICSI affects substantially the live birth rate.

WHAT IS KNOWN ALREADY: Pre-pregnancy hypertension is a well-known risk factor for adverse pregnancy events such as preeclampsia, fetal growth restriction, placental abruption and adverse neonatal events. It is likewise well known that hypertension during pregnancy in women undergoing ART is associated with adverse pregnancy outcomes. However, whether blood pressure at the high end of the normal range has an impact on ART is unknown.

STUDY DESIGN, SIZE, DURATION: It is a prospective observational cohort study based on a single IVF center between January 2017 and December 2018.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Two thousand four hundred and eighteen women with normal blood pressure undergoing fresh embryo transfer after IVF/ICSI at the Reproductive and Genetic Hospital of CITIC-Xiangya were enrolled in this study.

MAIN RESULTS AND THE ROLE OF CHANCE: Blood pressure was measured at the first visit when women consulted the IVF center due to infertility. In women with a successful pregnancy outcome (1487 live births out of 2418 women undergoing fresh embryo transfer after IVF/ICSI), systolic blood pressure (SBP) (114.1 ± 9.48 mmHg versus 115.4 ± 9.8 mmHg, $P = 0.001$) and diastolic blood pressure (DBP) (74.5 ± 7.5 mmHg versus 75.3 ± 7.34 mmHg, $P = 0.006$) were lower than in those who did not achieve live births. Multivariate logistic regression analysis revealed that SBP (OR: 0.987, 95% CI: 0.979–0.996, $P = 0.004$) and DBP (OR: 0.986, 95% CI: 0.975–0.998, $P = 0.016$) were negatively associated with live birth. Similarly, SBP was significantly negatively related to clinical pregnancy rate (OR: 0.990, 95% CI: 0.981–0.999, $P = 0.033$), while for DBP the association was not statistically significant (OR: 0.994, 95% CI: 0.982–1.006, $P = 0.343$). However, both SBP and DBP were positively associated with miscarriage OR: 1.021 (95% CI: 1.004–1.037, $P = 0.013$) and

OR: 1.027 (95% CI: 1.005–1.049, $P = 0.014$), respectively. Both SBP and DBP were unrelated to biochemical pregnancy (hCG positivity), implantation and ectopic pregnancy rate.

LIMITATIONS, REASONS FOR CAUTION: Whether lowering blood pressure before initiating ART treatment in women with SBP or DBP higher than the thresholds defined in our study will confer a benefit is unknown. Also, we cannot exclude bias due to different ethnicities. Moreover, participants in our study only received fresh embryo transfer, whether the results could apply to frozen embryo transfer is unclear.

WIDER IMPLICATIONS OF THE FINDINGS: Our study challenges the current blood pressure goals in women undergoing fresh embryo transfer after IVF/ICSI. Further studies are needed to figure out the mechanism and effective approach to increase IVF/ICSI pregnancy outcomes.

STUDY FUNDING/COMPETING INTEREST(S): Hunan Provincial Grant for Innovative Province Construction (2019SK4012). The authors declare that there were no conflicts of interest in this study.

TRIAL REGISTRATION NUMBER: N/A.

Key words: IVF / intracytoplasmic sperm injection / artificial reproductive technologies / blood pressure / embryo transfer / live birth rate / pregnancy complication / perinatal outcomes

Introduction

ART has been developed rapidly over the past few decades. Currently, the overall clinical pregnancy rate of ART is over 50% (Niederberger *et al.*, 2018). Many factors influence the success of a clinical pregnancy such as maternal age, ovarian reserve, infertility duration and type, hormone levels and endometrial receptivity (Hu *et al.*, 2018; Hwang *et al.*, 2020; Xie *et al.*, 2020). However, there are still some unknown risk factors that could affect the pregnancy outcomes of ART.

Pre-pregnancy hypertension is a well-known risk factor for adverse pregnancy events such as preeclampsia, fetal growth restriction, placental abruption and adverse neonatal events (Bramham *et al.*, 2014; Magee *et al.*, 2016). A cohort study on 109 932 pregnancies including 1417 (1.3%) women with chronic hypertension reported that maternal hypertension at conception was associated with increased risk of stillbirth, small for gestational age (SGA), gestational diabetes mellitus, iatrogenic preterm birth (PTB) <37 weeks and elective cesarean section (CS), decreased risk of large for gestational age and had no significant effect on late miscarriage, spontaneous PTB or emergency cesarean section (Panaitescu *et al.*, 2017). Similarly, another study on 352 patients with chronic hypertension found that maternal chronic hypertension was also associated with lower birth weight, lower Apgar score and the number of intrauterine complications such as intrauterine growth restriction (IUGR), stillbirth and placental abruption (Akbar *et al.*, 2019).

It is likewise well known that hypertension during pregnancy in women undergoing ART is associated with adverse pregnancy outcomes. Women with hypertension who conceive may experience even more placental complications, in particular SGA, as well as other adverse maternal and neonatal outcomes (such as prematurity and cesarean delivery) than do similar women with unassisted conceptions (Dayan *et al.*, 2016). Pregnancy-induced hypertension after frozen embryo transfer (FET) and oocyte donation is associated with a substantially increased rate of PTB (Stern *et al.*, 2021). In addition, a meta-analysis including 66 longitudinal studies showed that all pregnancy-related hypertensive disorders were increased following any invasive ART (Thomopoulos *et al.*, 2017).

The standard definition of diagnostic criteria for hypertension does not consider outcome data of pregnancy. Hypertension was defined as systolic blood pressure (SBP) over 140 mmHg, diastolic blood pressure (DBP) over 90 mmHg or taking antihypertensive medicine (Liu, 2020). They are based on the relationship between cardiovascular/renal diseases and blood pressure. The optimal blood pressure at conception for major pregnancy outcomes such as live birth rate especially in women undergoing ART is simply unknown. The aim of our prospective observational study was thus to analyze the relationship between blood pressure before initiation of ART and major birth outcomes such as live birth rate.

Materials and methods

Study design and setting

We performed a prospective observational study on women undergoing IVF/ICSI and receiving fresh embryo transfer. The current study was approved by the Ethics Committee of the Reproductive and Genetic Hospital of CITIC-Xiangya (approval number: LL-SC-2018-014) and written consent was obtained from all participating patients.

Cardiovascular risk factors such as age, blood pressure, BMI, glucose and blood biochemical parameters (routine blood test, blood coagulation function, lipids, kidney function, thyroid function) were evaluated for every participant at the same time of blood pressure measurement.

The blood pressure was measured at the first visit when the woman came to the hospital (before the ovary stimulation) asking for support to get pregnant. Participants were required to avoid smoking, drinking coffee and strenuous exercise within half an hour before the blood pressure measurement, and they were advised to empty their bladder and rest for more than 5 min in a quiet environment to avoid an increase in blood pressure. All subjects were seated, and the sleeves were rolled up or removed before the blood pressure measurement. The cuff was placed on the right arm at the level of the heart and adjusted depending on the arm circumference (the cuff airbag covered at least 80% of the upper arm circumference) according to the Chinese guideline (Liu, 2020). A well-trained nurse took three blood pressure measurements using

an automatic blood pressure measuring system (Mibobo, Shenzhen Raycome Health Technology Co., Ltd. Shenzhen, China) with breaks of 5 min in between and we used the calculated mean values.

Participants

Women who came to our hospital to receive their first IVF/ICSI were eligible for enrollment. Inclusion criteria were: age between 18 and 40 years, first IVF/ICSI procedure and fresh embryo transfer.

Exclusion criteria were: abnormal uterine anatomy, endometriosis, intrauterine adhesion, untreated hydrosalpinx, uterine myoma (multiple, submucous, or intramural myoma >3 cm), women receiving oocyte donation, pre-implantation genetic test for aneuploid, adult-onset adrenogenital syndrome, Cushing syndrome, infertility caused by hypothalamic or pituitary diseases, SBP ≥140 mmHg or DBP ≥90 mmHg and women receiving anti-hypertensives treatment.

Participating women received pituitary down-regulation protocols exactly as described previously (Cai *et al.*, 2020; Chen *et al.*, 2020, 2022a,b).

The following protocols were used:

a. Long-acting gonadotropin-releasing–hormone agonist (GnRH-a) down-regulation protocol (n=1178). On the day of the mid-luteal phase of the month before ovulation induction, or the 21st day of the artificial cycle 1.5–1.875 mg GnRH-a (Diphereline, Ipsen Pharma Biotech, France) was injected i.m.
b. Short-acting GnRH-a down-regulation protocol (n=686). 0.05 mg GnRH-a (Ferring GmbH, Switzerland) was injected daily starting on the day of the mid-luteal phase of the month before with hCG.
   The time from receiving GnRH to start ovarian stimulation for long-acting GnRH-a and short-acting GnRH-a was the same.
c. Modified ultra-long protocol (n=554): as previously described (Chen *et al.*, 2022a,b) 1.5–1.875 mg GnRHa (Diphereline, Ipsen Pharma Biotech, France) was intramuscularly injected on Day 20 of the patient's menstrual cycle immediately preceding the treatment cycle. This was repeated on Day 21 of the treatment cycle.

After a period of 13–20 days, following confirmation of pituitary-ovarian suppression, recombinant follicle stimulating hormone (Gonal-F or Puregon; Merck Serono S.A., Coinsins, Switzerland) was administered in the long-term protocol patients and short-term protocol patients. Human menopausal gonadotropin (Menopur; Ferring Pharmaceuticals, Kiel, Germany) was administered in ultra-long protocol patients. Recombinant hCG (Serono, Switzerland) was injected when follicles ≥18 mm accounted for 60–70% of follicles >14 mm, or follicles ≥20 mm accounted for 40–50% of follicles >14 mm and estradiol (E2) per every 14 mm follicle was 200–300 pg/ml. Oocyte retrieval was performed 35–36 h following hCG injection.

Indications and techniques for oocyte aspiration, oocyte, and embryo culture, insemination, ICSI, assisted hatching and embryo transfer was performed based on the routine of the center (ISO 9001 Certification). Luteal phase supplementation was started immediately after oocyte retrieval for 4 weeks.

Embryo transfer was performed 3 or 5 days after the oocyte retrieval.

Outcome measurement

Biochemical pregnancy was defined as hCG ≥200 mIU/ml 17 days after oocyte retrieval, also named hCG positive. Live birth was defined as the birth of one or more live-born infants (at any gestational age). Delivery of multiple infants counted as one live-birth delivery, while clinical pregnancy was defined as the existence of gestational sac(s) with fetal heart activity by ultrasound at week 4 after embryo transfer. Implantation rate was defined as the total number of gestational sacs divided by the total number of embryos transferred. Thereafter, early miscarriage was defined as intrauterine pregnancy loss after confirmation of gestational sacs during the first trimester. (Wilkinson *et al.*, 2016).

Considering successful embryo transfer (biochemical pregnancy) as the first clinical endpoint (successful treatment), for the long protocol (both short-acting and long-acting GnRH-a protocol) the average elapsed time between blood pressure measurement at the initial visit and the first successful treatment was 50–60 days and 80–90 days for the ultra-long protocol.

Follow up

Participants were followed in our center until 70 days after embryo transfer, and then they were transferred to the primary obstetrics department. We called every participant during their mid-term and late-term pregnancy and after delivery to follow up on pregnancy complications and perinatal outcomes. Our participants obtained this follow-up information in other hospitals and provided this information to us since our center does not include an obstetrics department.

Data analysis

Statistical Package for Social Sciences for Windows, version 25.0 (SPSS Inc, Chicago, IL, USA) was used to perform data analyses. Homogeneity of variance and normality of data was estimated using the Levene and Kolmogorov–Smirnov tests, respectively. Values were expressed as means ± SD, or frequency (%). Comparisons of continuous variables between groups were done using the Kruskal–Wallis test or ANOVA according to the normality. Categorical variables were compared by the chi-square (χ^2) test or Fisher's exact test. Multivariate logistic regression analysis was performed to figure out the risk factors for pregnancy outcomes. Receiver-operating characteristic (ROC) analysis was performed to evaluate the association between blood pressure and live birth rate. The optimal cut-off value of SBP and DBP was defined as the value on the ROC curve that was associated with the minimum euclidean distance from the curve to the upper left corner of the graph (Hocher *et al.*, 2003). Data were considered statistically significant with a two-sided $P < 0.05$.

Results

The study enrollment was done between January 2017 and December 2018 in the Reproductive and Genetic Hospital of CITIC-Xiangya. Details of patient recruitment are shown in the flow chart (Supplementary Fig. S1). A total of 2418 women were included in our study. The main reason for infertility was fallopian tube abnormalities (n = 2043). There were 669 polycystic ovary syndrome (PCOS)

women included in our study and 294 women with mixed reasons. We first divided our participants into two groups according to the live birth: live birth (n = 1487) and non-live birth group (n = 931). The demographic and characteristics of participants at baseline are given in Table I and Supplementary Table SI. Age (29.1 ± 3.40 versus 29.6 ± 3.84, $P < 0.01$), menstrual cycle (the number of days of a typical menstrual cycle) (35.2 ± 16.77 versus 37.5 ± 21.57, $P < 0.01$), SBP (114.1 ± 9.48 versus 115.4 ± 9.80, < 0.01), DBP (74.5 ± 7.5 versus 75.3 ± 7.34, $P < 0.01$), the mean arterial pressure (87.7 ± 7.50 versus 88.7 ± 7.48, $P < 0.01$) (Table I), erythrocyte sedimentation rate (ESR) (13.6 ± 8.68 versus 14.7 ± 9.17, $P < 0.01$) and platelets (PLT) (222.7 ± 54.70 versus 227.6 ± 55.26, $P = 0.03$) were lower in women who achieved live birth (Supplementary Table SI).

Patients' characteristics during the ovarian hyperstimulation and laboratory outcomes are presented in Table II. The consumption of gonadotropin (Gn) (2226 ± 936.0 versus 2304 ± 918.8, $P = 0.03$) was lower in women who achieved a live birth while oocyte and embryo quality were higher metaphase II (MII) oocytes (11.0 ± 4.37 versus 10.3 ± 4.32, $P < 0.01$); two pronuclei (PN) zygotes (7.2 ± 3.40 versus 6.7 ± 3.50, $P < 0.01$) and blastocyst formation rate (35.7% versus 33.0%, $P = 0.01$). In addition, thicker endometrium was achieved before embryo transfer (13.5 ± 2.09 versus 13.1 ± 2.14, $P < 0.01$), and more top embryos were transferred (82.2% versus 78.0%, $P < 0.01$) in women who achieved live birth.

Next, a multivariate logistic regression analysis was performed. We included all baseline factors that were different between groups (age, menstrual cycle, SBP, DBP, mean arterial pressure, ESR, PLT) into a regression model (Tables III–V). SBP and DBP were included in the model separately, considering their interaction. This analysis revealed that SBP (OR: 0.99, 95% CI: 0.98–1.00, $P < 0.01$) and DBP (OR: 0.99, 95% CI: 0.985–1.00, $P = 0.02$) were negatively associated with live birth. Although the OR and CI are very close to one, the *P*-values were < 0.05, which means blood pressure was still a risk factor for live birth. Also, age, menstrual cycle and ESR negatively affected live birth. Similarly, SBP was negatively related to clinical pregnancy (OR: 0.99, 95% CI: 0.98–1.00, $P = 0.03$) and ongoing pregnancy rate (OR: 0.99, 95% CI: 0.98–1.00, $P = 0.01$), while DBP would affect only ongoing pregnancy (OR: 0.99, 95% CI: 0.98–1.00, $P = 0.03$). However, both SBP and DBP were positively associated with miscarriage with OR values of 1.02 (95% CI 1.00–1.04, $P = 0.01$) and 1.03 (95% CI 1.01–1.05, $P = 0.01$), respectively. Both SBP and DBP were unrelated to biochemical pregnancy (hCG positive), implantation rate and ectopic pregnancy (see Supplementary Tables SII, SIII, SIV, SV, SVI and SVII).

Thus, we analyzed the relationship between SBP, DBP and live birth. The distribution of blood pressure is shown in Supplementary Fig. S2. We plotted ROC curves and defined the cut-off value for SBP and DBP using these ROC curves (endpoint: live birth). The systolic cut-off value was 119.5 mmHg, and the diastolic cut-off value was 69.5 mmHg, respectively (Supplementary Fig. S3). Then we divided our participants into two groups according to the cut-off value: SBP < 119.5 mmHg (n = 1797), SBP ≥ 119.5 mmHg (n = 621); DBP < 69.5 mmHg (n = 557), DBP ≥ 69.5 mmHg (n = 1861). For SBP analysis, the results showed that the live birth rate (63.1% versus 57.0%, $P = 0.01$), clinical pregnancy rate (69.7% versus 64.7%, $P = 0.02$) and ongoing pregnancy rate (65.8% versus 60.6%, $P = 0.02$) were lower in higher SBP women, while the miscarriage rate was significantly higher (9.6% versus 14.2%, $P = 0.01$). There is no difference in hCG positive rate, implantation rate and ectopic pregnancy rate. Also, no difference was observed in pregnancy complications and neonatal outcomes (Supplementary Fig. S4A, Table VI).

For DBP analysis, the live birth rate (67.5% versus 59.7%, $P < 0.01$), clinical pregnancy rate (72.7% versus 67.1%, $P = 0.01$) and ongoing pregnancy rate (69.7% versus 62.9%, $P < 0.01$) were lower in higher DBP women, while miscarriage rate was significantly higher (6.7% versus 12.0%, $P < 0.01$). There is no difference in hCG positive rate, implantation rate and ectopic pregnancy rate. However, more low live birth weight deliveries (< 2500 g) (29.4% versus 23.3%, $P = 0.02$) were observed in lower DBP women (Supplementary Fig. S4B, Table VI).

Moreover, a multivariate regression analysis for live birth based on SBP and DBP categorized by cut-off value and other variables that were significantly associated with live birth rates showed that both SBP and DBP were independent risk factors for live birth (Supplementary Table SVIII).

Discussion

Numerous studies show that hypertension in pregnancy is associated with adverse events for both mother and child (Bramham *et al.*, 2014; Panaitescu *et al.*, 2017; Akbar *et al.*, 2019). However, it is important to emphasize that the definitions used for hypertension in pregnancy follow those used for hypertension in the general population (Gabb *et al.*, 2016). In particular, the importance of blood pressure with respect to the success of IVF/ICSI treatment has not been adequately studied. It is therefore by no means certain that the usual blood pressure criteria in the general population but also in women who became pregnant spontaneously also apply to women undergoing IVF/ICSI treatment.

Particularly large studies have not yet been conducted to determine the optimal blood pressure concerning live birth rates—the most important clinical parameter after IVF/ICSI treatment—in women undergoing ART. We suspect that different thresholds might apply for such sensitive parameters as the live birth rate after ART. We, therefore, prospectively followed women with normal SBP and normal DBP according to current guidelines who underwent ART. The primary endpoint was the live birth rate. In women who had completely normal blood pressure values according to the currently used guidelines at study entry (first examination in preparation for ART), a cut-off value of 119.5 mmHg SBP showed a 6% difference concerning the rate of live births (63.5% versus 57.00%, p = 0.01). For DBP (cut-off: 69.5 mmHg), the effect in terms of live birth rate is even more pronounced: 7.8% (67.5% versus 59.70%, p < 0.01). Our results regarding the clinically most important endpoint in reproductive medicine question the currently used guidelines for assessing optimal blood pressure in women seeking ART.

Hypertension is known to affect ART outcomes. Dayan *et al.* (2016) reported that hypertensive women with ART pregnancies were at higher risk of placental-mediated complications than women with unassisted pregnancies (adjusted risk ratio 1.48; 95% CI, 1.35–1.56). However, the mechanisms linking adverse pregnancy outcomes and hypertension are not fully understood. One study reported an imbalance in angiogenic regulators leading to placental bed hypoxia and a

Table I Demographic and baseline clinical characteristics of participants according to live birth.

	Live birth (n = 1487)	No-live birth (n = 931)	*P*-value
Age years, mean (SD)	29.1 (3.40)	29.6 (3.84)	<0.01
Menstrual cycle days, mean (SD)	35.2 (16.77)	37.5 (21.57)	<0.01
Infertility reason			
Fallopian tube abnormalities	92.3 (1372/1487)	90.8 (845/931)	0.19
Ovulation disorders (PCOS)	26.7 (397/1487)	29.2 (272/931)	0.18
Infertility type			
Primary	55.2 (821/1487)	48.9 (455/931)	<0.01
Secondary	44.8 (666/1487)	51.1 (476/931)	0.14
Parity	15.5 (230/1487)	17.7 (165/931)	
Pre-existing diseases			0.78
Diabetes	0.5 (8/1487)	0.4 (4/931)	0.67
HBV carrier	3.6 (54/1487)	4.0 (37/931)	
Previous history			0.94
Tuberculosis	2.1 (31/1487)	2.0 (19/931)	0.85
Heart diseases	0.1 (2/1487)	0.1 (1/931)	0.79
Urinary tract affection	0.3 (4/1487)	0.2 (2/931)	0.25
Treponema pallidum affection	0.8 (12/1487)	1.3 (12/931)	0.66
Condyloma acuminatum	0.3 (4/1487)	0.1 (1/931)	0.79
Pelvic inflammatory disease	8.5 (126/1487)	8.2 (76/931)	
BMI kg/m^2, mean (SD)	21.4 (2.4)	21.5 (2.39)	0.32
Waist circumference cm, mean (SD)	73.9 (7.71)	74.4 (7.74)	0.12
Hip circumference cm, mean (SD)	90.6 (7.05)	90.8 (6.68)	0.57
Waist-to-hip ratio, mean (SD)	0.8 (0.05)	0.8 (0.05)	0.08
Infertility duration years, mean (SD)	3.5 (2.28)	3.6 (2.40)	0.27
AMH ng/ml, mean (SD)	7.1 (4.79)	7.2 (5.26)	0.81
AFC	24.9 (11.66)	25.3 (12.73)	0.45
Basal FSH mIU/ml, mean (SD)	5.8 (1.63)	5.8 (1.50)	0.99
Basal LH mIU/ml, mean (SD)	4.5 (4.95)	4.3 (2.89)	0.27
Basal FSH/LH, mean (SD)	1.8 (1.79)	1.7 (0.93)	0.61
Basal E2 pg/ml, mean (SD)	40.1 (37.72)	38.6 (27.77)	0.30
Basal P ng/ml, mean (SD)	0.4 (1.28)	0.4 (1.19)	0.62
Basal T ng/ml, mean (SD)	0.5 (2.89)	0.3 (0.97)	0.08
Total 25(OH)D, ng/ml mean (SD)	20.1 (4.87)	19.9 (5.23)	0.48
Free 25(OH)D pg/ml, mean (SD)	4.8 (1.03)	4.8 (1.02)	0.36
Pulse beats/min, mean (SD)	81.9 (9.79)	81.6 (9.98)	0.52
Systolic blood pressure mmHg, mean (SD)	114.0 (9.48)	115.4 (9.80)	<0.01
Diastolic blood pressure mmHg, mean (SD)	74.5 (7.50)	75.3 (7.34)	<0.01
Pulse pressure mmHg, mean (SD)	39.6 (7.11)	40.1 (7.35)	0.11
Mean arterial pressure mmHg, mean (SD)	87.7 (7.50)	88.7 (7.48)	<0.01

PCOS, polycystic ovary syndrome; AMH, anti-Müllerian hormone; AFC, antral follicle count; E_2, estradiol; P, progesterone; T, testosterone.
Data are given as % (n/N) unless stated otherwise.
More baseline characteristics of the study population are given in in Supplementary Table SI.

subsequent endothelial dysfunction may have ultimately resulted in fetal growth restriction (Nzelu *et al.*, 2020). Another study assessed the correlation between angiogenic regulators and oxidation stress markers and adverse pregnancy outcomes among Ghanaian preeclamptic (PE) and gestational hypertensive (GH) women (Turpin *et al.*, 2015). They found an imbalance in angiogenic regulators, which was identified by an increase in soluble fms-like tyrosine kinase-1 (sFlt1) and a decrease in placental growth factor levels in PE and GH

Table II Controlled ovarian hyperstimulation characteristics and laboratory outcomes in participants according to live birth.

	Live birth (n = 1487)	No-live birth (n = 931)	*P*-value
Protocol			
Long protocol	77.34 (1150/1487)	76.69 (714/931)	0.71
Ultra-long protocol	22.63 (337/1487)	23.31 (217/931)	
Gn dosage IU, mean (SD)	2226.26 (935.95)	2304.30 (918.80)	0.03
Gn duration days, mean (SD)	10.76 (1.84)	10.83 (1.82)	0.46
E2 on hCG day pg/ml, mean (SD)[a]	3660.26 (1334.54)	3579.36 (1360.32)	0.14
P on hCG day ng/ml, mean (SD)[a]	0.64 (0.28)	0.64 (0.29)	0.65
LH on hCG day mIU/ml, mean (SD)[a]	1.70 (0.87)	1.68 (0.75)	0.70
hCG dosage for triggering IU, mean (SD)	6263.28 (1499.82)	6272.82 (1419.22)	0.21
No. of oocytes retrieved, mean (SD)	12.35 (4.68)	11.68 (4.72)	0.48
No. of MII oocytes[b], mean (SD)	10.98 (4.37)	10.31 (4.32)	<0.01
No. of 2PN zygotes, mean (SD)	7.16 (3.40)	6.71 (3.50)	<0.01
Fertilization methods			
IVF	68.33 (1016/1487)	71.43 (665/931)	0.22
ICSI	16.81 (250/1487)	15.90 (148/931)	
IVF+ICSI	14.86 (221/1487)	12.67 (118/931)	
Fertilization rate (%), mean (SD),	66.42 (20.30)	65.33 (20.82)	0.21
Day 3 good-quality embryo rate	65.38 (5905/9032)	64.49 (3355/5202)	0.29
Blastocyst formation rate	35.74 (1770/4952)	33.04 (1021/3090)	0.01
EM thickness before ET mm, mean (SD)	13.52 (2.09)	13.14 (2.14)	<0.01
Number of embryos transferred, mean (SD)	1.91 (0.29)	1.89 (0.32)	0.06
Day3 embryo	1.98 (0.13)	1.97 (0.18)	0.02
Blastocyst	1.56 (0.50)	1.55 (0.50)	0.90
Top embryo transfer rate (%)	82.22 (2335/2840)	77.96 (1369/1756)	<0.01

D3, Day 3; E_2, estradiol; Gn, gonadotropin; EM, endometrium; GV, germinal vesicle; MI, metaphase I; MII, metaphase II; 2PN, pronucleus; ET, embryo transfer; good-quality embryo, D3 embryo ≥7C-II, blastocyst ≥4BB; fair embryo, D3 embryo <7C-II, blastocyst <4BB.
[a]hCG day: the last day of ovarian stimulation, hCG was injected to trigger the final maturation of oocytes.
[b]Reflects oocytes quality, only MII oocyte can be fertilized.
Data are given as % (n/N) unless stated otherwise.

Table III Multivariate logistic regression analysis for live birth according to systolic blood pressure (stepwise regression).

	B	*P*-value	Odds ratio	95% CI of odds ratio	
				Lower bound	Upper bound
Age (years)	−0.03	<0.01	0.97	0.94	0.99
Menstrual cycle (days)	−0.01	0.03	0.99	0.99	1.00
Systolic blood pressure (mmHg)	−0.01	<0.01	0.99	0.98	1.00
ESR (mm/h)	−0.01	0.01	0.99	0.98	1.00
Endometrial thickness (mm)	0.08	<0.01	1.1	1.04	1.12
The number of top embryos transferred	0.14	<0.01	1.15	1.04	1.28
Constant	0.03	0.01	1.03	1.01	1.05

We added factors to the regression models that were significantly different in the baseline characteristics according to live birth yes/no (see also Tables I and II as well as Supplementary Table SI): age, menstrual cycle length, systolic blood pressure, diastolic blood pressure, mean arterial pressure, ESR, PLT, gonadotrophins dosage, the number of MII oocytes, the number of 2PN zygotes, endometrial thickness before embryo transfer, the number of Day 3 embryo transferred and the number of top embryos transferred into regression stepwise forward model. All these variables are continuous variables. Considering the interaction effect of systolic, diastolic blood pressure and mean arterial pressure we put them into the regression model separately.
ESR, erythrocyte sedimentation rate; PLT, platelets.

Table IV Multivariate logistic regression analysis for live birth according to diastolic blood pressure (stepwise regression).

	B	*P*-value	Odds ratio	95% CI of odds ratio	
				Lower bound	Upper bound
Age (years)	−0.04	<0.01	0.97	0.94	0.99
Menstrual cycle (days)	−0.01	0.02	1.00	1.00	1.00
Diastolic blood pressure (mmHg)	−0.01	0.02	0.99	0.98	1.00
ESR (mm/h)	−0.01	0.01	0.99	0.98	1.00
Endometrial thickness (mm)	0.08	<0.01	1.08	1.04	1.12
The number of top embryos transferred	0.14	0.01	1.15	1.04	1.28
MII oocytes	0.03	0.01	1.03	1.01	1.05
Constant	1.35	0.04	3.84		

ESR, erythrocyte sedimentation rate.

Table V Multivariate logistic regression analysis for live birth according to mean arterial pressure (stepwise regression).

	B	*P*-value	Odds ratio	95% CI of odds ratio	
				Lower bound	Upper bound
Age (years)	−0.04	<0.01	0.97	0.94	0.99
Menstrual cycle (days)	−0.01	0.03	1.00	0.99	1.00
Mean arterial pressure (mmHg)	−0.02	<0.01	0.98	0.97	1.00
ESR (mm/h)	−0.01	0.01	0.99	0.98	1.00
Endometrial thickness	0.08	<0.01	1.08	1.04	1.12
The number of top embryos transferred	0.14	0.01	1.15	1.04	1.28
MII oocytes	0.03	0.01	1.03	1.01	1.05
Constant	1.75	0.01	5.72		

ESR, erythrocyte sedimentation rate.

women compared to that in normal pregnant (NP) women (PE>GH>NP). In addition, oxidative stress was observed amongst the participants with GH, PE and PE co-existing with adverse pregnancy outcomes as depicted by the high lipid peroxidation and reduced total antioxidant capacity levels. Furthermore, angiogenic and oxidative stress biomarkers correlated significantly with IUGR, intrauterine fetal death, placental abruption, stillbirth and postpartum hemorrhage (Turpin *et al.*, 2015). Our study, however, indicates that undesirable ART pregnancy outcomes concerning blood pressure can occur at much lower thresholds for SBP and DBP as currently used in daily clinical practice. Our data indicate that the first step to getting pregnant (fertilization and implantation of the embryo) is less dependent on blood pressure since biochemical pregnancy rates (detection of beta-hCG in blood some days after IVF/ICSI) are not affected by blood pressure. However, our data showed that the next step, clinical pregnancy was associated with blood pressure. This finding supports the studies described above, showing that maternal blood pressure plays a role in the angiogenesis of the placenta and fetus and overall placental function (Troisi *et al.*, 2008; Atlass *et al.*, 2020; Workalemahu *et al.*, 2020). It is, however, important to note that the blood pressure threshold critical for these effects might be lower than currently assumed.

The Seventh Report of the Joint National Commission (JNC 7) on High Blood Pressure established a new concept of prehypertension (120–139 mmHg SBP or 80–89 mmHg DBP) as a new risk category (Joint National Committee on Prevention and Pressure, 1997). It was reported that subclinical and clinical target organ dysfunction and injury already exist during periods of prehypertension. The Framingham Heart Study indicated that a thicker arteriole wall, abnormal vascular endothelial function, activated renin–angiotensin system, increased excitability of sensory nerve and vasoconstriction was detected already in prehypertension (Vasan *et al.*, 2002). There is clear evidence that endothelial dysfunction is associated with miscarriage (Papazoglou *et al.*, 2005; Germain *et al.*, 2007; Lee *et al.*, 2010; Andraweera *et al.*, 2012). Women with placentation defects had a significant decrease in endothelium-dependent dilatation, a higher rate of endothelial dysfunction, lower serum nitric oxide and higher cholesterol as compared with control subjects, which contributed to miscarriage to some degree (Germain *et al.*, 2007). The Greek ATTICA study also showed that the level of c-reactive

Table VI Pregnancy outcomes and prenatal outcomes of participant according to systolic and diastolic blood pressure cut-off value.

	SBP < 119.5 mmHg (n = 1797)	119.5 ≤ SBP < 140 mmHg (n = 621)	*P*-value	DBP < 69.5 mmHg (n = 557)	69.5 ≤ DBP < 90 mmHg (n = 1861)	*P*-value
hCG positive rate	72.51 (1303/1797)	69.73 (433/621)	0.18	74.51 (415/557)	70.98 (1321/1861)	0.11
Clinical pregnancy rate	69.67 (1252/1797)	64.73 (402/621)	0.02	72.71 (405/557)	67.11 (1249/1861)	0.01
Implantation rate	52.56 (1799/3423)	50.81 (596/1173)	0.30	54.01 (572/1059)	51.54 (1823/3537)	0.16
Ectopic pregnancy rate	2.00 (25/1252)	1.74 (7/402)	0.75	1.98 (8/405)	1.92 (24/1249)	0.95
Ongoing pregnancy rate	65.83 (1183/1797)	60.55 (376/621)	0.02	69.66 (388/557)	62.92 (1171/1861)	<0.01
Early miscarriage rate	9.58 (120/1252)	14.18 (57/402)	0.01	6.67 (27/405)	12.01 (150/1249)	<0.01
Live birth rate	63.05 (1133/1797)	57.00 (354/621)	0.01	67.50 (376/557)	59.70 (1111/1861)	<0.01
Singleton	65.31 (740/1133)	66.10 (234/354)	0.79	65.96 (248/376)	65.35 (726/1111)	0.83
Twins	34.69 (393/1133)	33.90 (120/354)		34.04 (128/376)	34.65 (385/1111)	
Number of boys	52.10 (795/1526)	53.16 (252/474)	0.68	54.17 (273/504)	51.74 (774/1496)	0.35
Number of girls	47.90 (731/1526)	46.84 (222/474)		45.83 (231/504)	48.26 (722/1496)	
Birthweight (all live birth)						
<2500 g	24.57 (375/1526)	25.53 (121/474)		29.37 (148/504)	23.26 (348/1496)	0.02
2500–4000 g	72.87 (1112/1526)	70.68 (335/474)	0.31	67.66 (341/504)	73.93 (1106/1496)	
≥4000 g	2.56 (39/1526)	3.80 (18/474)		2.98 (15/504)	2.81 (42/1496)	
Birthweight (full-term live birth)						
<2500 g	12.27 (144/1174)	13.90 (51/367)		14.29 (53/371)	12.14 (142/1170)	0.49
2500–4000 g	84.41 (991/1174)	81.20 (298/367)	0.24	81.67 (303/371)	84.27 (986/1170)	
≥4000 g	3.32 (39/1174)	4.90 (18/367)		4.04 (15/371)	3.59 (42/1170)	
Gestation weeks						
<37 weeks	17.48 (198/1133)	17.80 (63/354)	0.89	19.95 (75/376)	16.74 (186/1111)	0.16
≥37 weeks	82.52 (935/1133)	82.20 (291/354)		80.05 (301/376)	83.26 (925/1111)	
Delivery methods						
Natural labor	29.21 (331/1133)	26.55 (94/354)	0.33	28.99 (109/376)	28.44 (316/1111)	0.84
Cesarean section	70.79 (802/1133)	73.45 (260/354)		71.01 (267/376)	71.56 (795/1111)	
Prenatal complications						
Gestational diabetes mellitus	10.07 (181/1797)	10.31 (64/621)	0.87	9.69 (54/557)	10..26 (191/1861)	0.70
Hypertensive disorders complicating pregnancy	1.73 (31/1797)	2.74 (17/621)	0.12	1.80 (10/557)	2.04 (38/1861)	0.71
Anemia	8.63 (155/1797)	6.44 (40/621)	0.09	10.05 (56/557)	7.47 (139/1861)	0.05
Edema	9.35 (168/1797)	8.05 (50/621)	0.33	11.13 (62/557)	8.38 (156/1861)	0.05
Polyhydramnios	0.17 (3/1797)	0	0.18	0.36 (2/557)	0.05 (1/1861)	0.07
Oligohydramnios	0.83 (15/1797)	0.48 (3/621)	0.36	0.90 (5/557)	0.70 (13/1861)	0.63
Placenta previa	0.39 (7/1797)	0.81 (5/621)	0.20	0.90 (5/557)	0.38 (7/1861)	0.12
Placenta abruption	0.11 (2/1797)	0.16 (1/621)	1.00	0	0.16 (3/1861)	0.79
Intrauterine hypoxia	0.39 (7/1797)	0.16 (1/621)	0.69	0.72 (4/557)	0.21 (4/1861)	0.16
Respiratory tract infection	0	0	NS	0	0	NS

Data are given as % (n/N).
Empty cells are the subtitle column.

protein, tumor necrosis factor-α, homocysteine and other markers of oxidative stress and inflammation associated with atherosclerosis were significantly higher in patients with prehypertension than in patients with normal blood pressure (Chrysohoou *et al.*, 2004). We did not measure such indicators at that time. however, ESR, a non-specific indicator of an inflammatory response, was significantly higher in women without live birth, which indicated a higher inflammatory status in such women. Our multivariate regression analysis also demonstrated an inverse relationship between ESR and live birth rate. There is clear evidence illustrating that pro-inflammatory status could affect the pregnancy process, leading to miscarriage, placenta dysfunction and other pregnancy complications, thus lowering the live birth rate (Nadeau-Vallee *et al.*, 2016; Brien *et al.*, 2020).

It is well-known that blood pressure definitions and guidelines were originally developed to prevent cardiovascular diseases and stroke. The accepted hypertension definition is BP ≥ 140/90 mmHg, which is an established risk factor for cardiovascular disease (Gabb et al., 2016). However, whether this threshold fits in assisted reproduction is unknown. Thus, we made this study to define an optimal BP related to pregnancy. To the best of our knowledge, the current study with a large sample size showed for the first time that maternal pre-pregnancy blood pressure is a risk factor for poorer pregnancy outcomes in women undergoing fresh embryo transfer, even in a non-hypertensive study population. It is of note that more careful consideration of apparently high-normal blood pressure is also needed in women getting pregnant naturally. A huge recent study likewise showed that among healthy low-risk women, small increases in preconception blood pressure were associated with developing pre-eclampsia and gestational hypertension and hence this will adversely influence the live birth rate (Nobles et al., 2020). It is of note that the blood pressure differences between the live birth group and the non-live birth group are small. First of all, this is because we excluded women with hypertension from the study. It was explicitly the aim of our study to understand better the impact of variation of blood pressure within normal ranges of blood pressure according to current guidelines on the live birth rate. Hence differences in a group of women with formally normal blood pressure must be small. Second—and this is more important—it is well known that even small differences in blood pressure treatment effects of antihypertensive drugs in special populations such as diabetic CKD patients translate to benefits in mortality (Marso et al., 2016; Wanner et al., 2016; Hocher and Tsuprykov, 2017; Mann et al., 2017). The hypothesis coming from our data that even small changes in blood pressure in women with high normal blood pressure according to current guidelines may affect ART outcomes such as live birth rate needs to be confirmed in a multicenter placebo-controlled study showing safety and efficacy. The blood pressure-lowering interventions should be done with lifestyle modification (physical activity and/or low salt diet etc.) and/or drugs proven to be safe in pregnancy.

Based on our data, we suggest an SBP cut-off value of 119.5 mmHg and a DBP cut-off value of 69.5 mmHg (Supplementary Fig. S3). Women with a blood pressure above this threshold might benefit from lifestyle modifications (e.g. bodyweight reduction, low salt a/o DASH diet, exercise) to decrease blood pressure. Before defining new blood pressure goals for women undergoing ART, however, independent confirmation is needed. Our data suggest that we currently might underestimate the adverse impact of elevated BP in reproductive medicine. The current thresholds, developed by expert boards of internal medicine, nephrology and cardiologists are presumably too high for young women in their reproductive period. It is however of note that the recent American Heart Association (AHA) guidelines refer to stage 1 hypertension already between 130 and 139 mmHg SBP for the general population (Flack and Adekola, 2020).

However, limitations also exist in the present study. Since it was a single-center observational study, we cannot answer whether lowering blood pressure before initiating ART treatment in women with SBP or DBP higher than the thresholds defined in our study will confer a benefit. Also, we cannot exclude bias due to different ethnicities, such as whether our data fit Caucasian women. A further multi-center randomized clinical trial would be needed for confirmation. Moreover, participants in our study only received fresh embryo transfers. Whether the results could apply to FET is unclear and needs further investigation. An important limitation of the study is the fact that we only took blood pressure measurements in one single arm—although it was the average of three independent measurements at the study entry visit, see methods. Recent guidelines such as The European Society of Hypertension 2021 recommend taking at least two readings and recommendation to take measurements of both arms. Finally, potential confounding factors such as physical activity and eating behoite—salt intake—were not reported, but might be of relevance: In addition, due to the nature of the study (observational study) also yet unknown confounding factors cannot be excluded.

Conclusion

The current study showed that even rather small differences in SBP and DBP in women without hypertension undergoing IVF/ICSI treatment are associated with the live birth rate. Such small differences in blood pressure have not been taken into account in the management of patients undergoing IVF/ICSI treatment so far. If independently confirmed, our study may stimulate a placebo-controlled double-blind clinical study to finally prove that even very small blood pressure effects are linked to major outcomes of IVF/ICSI treatment of infertility.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The data underlying this article are available in the article and in its online supplementary material.

Authors' roles

Conceptualization: B.H. Data curation: H.C., J.L., S.C. and S.T. Formal analysis: H.C. and B.H. Methodology: H.C. and B.H. Project administration: S.C. and S.T. Software: H.C. and X.Z. Supervision: L.H., G.L., F.G. and B.H. Validation: H.C., S.T. and B.H. Writing-original draft: H.C. and B.H. Writing—review and editing: H.C., J.L., C.-F.H., B.K.K., B.R., L.H. and G.L.

Funding

Hunan Provincial Grant for Innovative Province Construction (2019SK4012).

Conflict of interest

The authors declare that there were no conflicts of interest in this study.

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