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Cohort Profile: Anhui Maternal-Child Health Study in China

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Abstract

Purpose The Anhui Maternal-Child Health Study (AMCHS) was designed to examine determinants of reproduction, pregnancy, and post-partum maternal and child health outcomes in Chinese women who received ART.

Participants AMCHS has been set up in Anhui, China. It recruits participants from all couples who sought ART treatment in the First Affiliated Hospital of Anhui Medical University starting from May 2017.The participants are interviewed to document baseline socio-demography, lifestyles, dietary intake and environmental exposure. Their clinical characteristics are obtained from hospital records and blood, follicular fluid and semen are collected. They are followed from study entry throughout each fertility treatment cycle, once per trimester of pregnancy (for those achieving pregnancy), and up to labour and delivery, or until they discontinue treatment or withdraw from the study. Details of their children are collected through a questionnaire focussing on developmental status and an examination of anthropometry which includes: height, weight, and head circumference.

Finding to date Until April 2021, 2042 couples were recruited in the study. 204 women failed to retrieve oocytes or withdrew for other reasons from the study. 1475 women confirmed pregnancies, of whom 1057 gave live birth to babies and 138 (9%) suffered from miscarriage or termination of pregnancy. Among 1057 families that gave birth to babies, 940 were followed-up until delivery, 576 were followed-up at age 30 - 42 days, 459 at 6 months, 375 at 12 months.

Future plans The AMCHS will identify comprehensive potential risk factors and explore any interaction effects between multiple factors for ART outcomes and maternal and child health. This is an on-going cohort study, it is anticipated that the findings will improve the risk assessment of ART to reduce adverse reproductive and pregnancy outcome.

Strengths and limitations of this study

- This is the first prospective reproductive cohort study conducted in Anhui, China.
- The study investigates a variety of risk factors for reproductive, pregnancy and child health outcomes over an extended time
- The study highly focused on the paternal impact on adverse reproductive and pregnancy outcomes
- The majority of participants in this study lived in urban areas.
- The rate of lost to follow up may rise with the cohort follow-up.

Introduction

There has been increased infertility in the population. Over the past two decades, the infertility rate has increased from 6.7% to 15.5% among women of reproductive age in the world [1, 2]. There are around 48 million infertility couples [3]. Infertility causes psychological distress, social stigmatization, and marital discord and heavy financial burden among couples [4-6]. The median per-person costs of In vitro fertilization (IVF) treatment is about $38,015 [7]. For these reasons, infertility has been identified as a public health priority in the world [8].

Assisted Reproductive Technology (ART) is an effective solution for infertility, allowing infertile couples to have a baby. Globally, approximatley 1.7% to 4% of all births occur through the use of ART [9]. While ART is important for infertility treatment, it could markedly increase the incidence of pregnancy complications (e.g., gestational diabetes mellitus (GDM), placenta previa, premature delivery) and poor neonatal outcomes (e.g., stillbirth) [9-12]. The reasons for adverse pregnancy outcomes remain unclear; however, there has been increasing research on whether infertility is caused by the technology of ART itself or by parental characteristics and their exposure to risk factors [13-15].

Prospective cohort studies are required to examine the risk factors and outcomes of couples who received ART [12, 16-18]. Previous studies showed that unhealthy lifestyles including cigarette smoking and physical inactivity adversely affects ART outcomes such as lower pregnancy rate, lower live birth rate and miscarriage [19, 20]. IVF treatment increases the risk of GDM [21], birth defects [22], and preterm birth compared to a natural pregnancy [23]. However, most studies have focused on maternal influences during pregnancy and the delivery process [24-27]. Few studies have examined multiple risk factors especially at a genetic level for adverse

pregnancy of ART, simultaneously. Little is known about how much the characteristics of a male spouse contributes to the infertility and fewer studies have collected such data from both men and women during the preconception period [28, 29]. Furthermore, current knowledge of risk factors for ART adverse outcomes are predominately derived from studies undertaken in high-income countries (HICs) [12, 17, 30, 31]. The findings of HICs' studies may not be applicable to those in low- and middle-income countries (LMICs), where the burden of infertility is higher [1]. Few prospective cohort studies have been undertaken in LMICs to assess risk factors among infertility couples who received ART and their prognosis [18, 32].

China is the largest and most populated LMIC in the world and around 15.5% of women have an infertility problem [33], which is higher than those in HICs (e.g., 12% in USA, 12.5% in UK) [1, 34]. Anhui, one of 34 provinces in China, is located in the mid-eastern region of China, and consists of 61 million residents [35]. The rate of infertility in Anhui is 14.5%, which is in the moderate level in China (15.5%) [36]. In spite of this, there has been little investigation on factors influencing infertility and maternal-child health of ART treatment, especially in Anhui. To address these omissions, we proposed and conducted the Anhui Maternal-Child Health Study (AMCHS). AMCHS is an ongoing prospective preconception cohort study of couples accepting ART treatment. Our cohort aims to examine (1) the comprehensive potential risk factors to early environment on health from maternal and paternal preconception to birth, (2) the impact of the interaction between genes and environment on infants' growth and development, and (3) the short-term and long-term implication of ART to the next generation compared with natural conceived pregnancy population.

Cohort description

Study design, setting, and participants

From May 2017, AMCHS recruited infertile couples who received medically assisted reproduction through IVF based technologies (i.e., fresh or frozen IVF protocols, including Intracytoplasmic sperm injection (ICSI)) in the First Affiliated Hospital of Anhui Medical University (AHMU), Hefei, Anhui province, China. The inclusion criteria for study participants were: (1) couples who were the first time to receives ART treatment; and (2) not accepting oocyte or sperm donation or intrauterine insemination.

AMCHS trained research coordinators registered sociodemographic information of eligible couples in the beginning of recruitment. When participants commenced IVF treatment, they were conducted through face-to-face interviews for baseline assessment, blood samples were also collected from infertile couples after 8 hours of fasting.

Informed consent was obtained from each couple, which allowed researchers to interview participants using a general health and risk factors questionnaire, access their medical records including cycle, pregnancy and delivery data, and take their long-term storage supplies of biological samples for solely medical research purposes. Ethics approval for the study was received from the ethics committee of Anhui Medical University (20160270).

During the period of May 2017 to December 2020, we recruited 2198 eligible couples consecutively and completed baseline assessments for the current paper data analysis. Figure 1 shows the flow chart of the processes of participants recruitment and follow-up through the study. Participants are followed up from the study entry throughout their fertility care, pregnancy, birth (for those achieving pregnancy) and children (until 3-year-old) or until they discontinue treatment or withdraw from the study. As of April 2021, 2042 couples have commenced IVF treatment, 1828 couples have received embryo transfer surgery (Fig. 1). Of

1475 confirmed pregnancies, 1057 gave live birth to babies. Of the 940 children followed up until delivery, 576 children completed the follow-up at age 30 - 42 days, 459 children completed the follow-up at age 6 months, 375 completed the follow-up at 12 months and 26 completed the follow-up at 36 months. In April 2021, 204 couples discontinued treatment or withdrew during the follow-up.

AMCHS is an ongoing cohort study and will continue to recruit the participants and follow up the remaining couples (n=1838) in the cohort (Fig. 1). During the period January to November 2021, AMCHC recruited and followed up 29 participants. The number of participants (n=2042) in the current dataset has shown enough study power for some outcome data analysis. For example, impact of maternal age on implementation. The study powers for other complicated data analysis for rare outcomes will be ensured in on-going recruitment and follow-up of the AMCHS cohort.

Patient and public involvement

Patients or the public were not involved in the design, conduct, reporting or dissemination plans of our research.

Data collection and follow up

The AMCHS was designed to examine exposures from preconception period throughout once per trimester of pregnancy (for those achieving pregnancy), and up to labour and delivery. Ultrasound monitoring of ovarian response was offered as an integral part of the cycle. When

the number of mature oocytes met the requirement of IVF/ICS, couples would be arranged to retrieve oocytes and sperm. Laboratory technicians in the IVF laboratory collected follicular fluid, sperm and seminal plasma. The fasting blood used to test for pregnancy was collected after two weeks of embryo transfer surgery. When a positive pregnancy test was obtained, pregnant women were invited to the reproductive center at gestational weeks 4, 24, 32 for a questionnaire interview and blood sample collection. Medical records at gestational weeks 4, 24, 32 about gestational clinical information were also accessed.

During the birth admission, maternal blood, and cord blood and tissue were collected by the nursery midwife. The child was examined at ages 30 - 42 days, 6 months, 1- and 3-year-old (during early childhood). At age 30 - 42 days, 6 months, 12 and 36 months after giving birth, mothers were approached by phone for a questionnaire interview and provided information about clinical physical examination at local community hospital.

Data quality was checked regularly by experienced researchers, where missing and unreasonable data was further collected by research coordinators through face-to-face interviews, telephone or by checking the electronic medical record system (figure 1).

Outcomes measures

We collected data from the interview questionnaires and extracted medical information from a fertility clinic and delivery records, blood samples, follicular fluid from the female partner, sperm and seminal plasma samples from male partner. A sample of cord blood and tissue was collected from the newborn.

Questionnaire survey

Both female and male participants were required to complete a baseline questionnaire which includes sociodemographic information (e.g., date of birth, sex, educational level, occupational class, income, and residential area), reproductive history, medical history, medication history and family history. Lifestyle questions (e.g., smoking habits, alcohol consumption, dietary intake, tea and coffee drinking habits, and physical-activities), environmental exposures (e.g., passive smoking, an dindoor air pollution), mental health (e.g., Chinese Perceived Stress Scale (CPSS, 0 - 40), The Centre for Epidemiologic Studies Depression Scale (CES-D, 0 - 60), Self-Report Anxiety Scale (SAS, 20 - 80) and sleep quality (Pittsburgh Sleep Quality Scale (PSQI, 0 - 20)) are assessed systematically in the baseline questionnaire and repeatedly at each visit. Based on the first questionnaire, questions about ART medications and pregnancy complication were added at later visits during each per trimester. Questionnaires about the newborn focused on food diaries, food frequency questionnaires intakes of dietary supplements, mothers' feeding practices, breast milk frequency, Brief Infant Sleep Questionnaire, anthropometry of infant, medical history, and medication history.

Electronic medical record abstraction— cycle, pregnancy and delivery data

The AMCHS team abstracted clinical medical records during each individual fertility treatment cycle and throughout follow up (up to the birth of an infant for those achieving pregnancy). Research coordinators abstracted clinical information in the First Affiliated Hospital of AMHU to ascertain the outcome of each cycle, including physician assigned infertility diagnosis, infertility medical history, blood pressure, BMI, medication regimen; hormone at HCG Day, luteal phase support after IVF, scoring for fertilization, embryo culture and transfer, biochemical pregnancy (with β-hCG measurements), clinical pregnancy (with ultrasound

assessment) and relevant data. Antenatal and neonatal hospital medical records were also accessed. Details of antenatal, intrapartum and postpartum events during the study period were captured from hospital medical records or phone interview.

The AMCHS Biobank

Blood samples were obtained from female partners at several times during the periods of preconception to delivery and from the male partner at preconception. We also collected blood samples from cord blood and tissue at birth. The collection of blood samples from couples to their babies enable us to explore any heredity influences and detect any potential biomarkers for disease aetiology. Follicular fluid, sperm and seminal plasma were also collected in order to evaluate the quality of gametes, which could be the key factors of IVF outcomes.

Plasma and blood cell samples were separated from the blood via centrifugation for 10 minutes at a relative centrifugal force of 3000 g at 4 ℃. Plasma was stored at a -80 ℃ refrigerator, and the blood cell was stored at -20 ℃ refrigerator. Cord blood and tissue were stored at 4 ℃ after a temporary delivery in the operation room and then transferred to a 20 ℃ refrigerator within 24 hours. Seminal plasma was centrifuged before it was sub-packaged. Finally, the sperm (1 ml) and seminal plasma (2 ml) was stored in a nitrogen canister in a -80 ℃ refrigerator. Follicular fluid was collected more than 5 ml on average. After being centrifuged, the sub package was stored in an -80℃ refrigerator.

The overview of data collection and timepoint of AMCHS are shown in Table 1 and Table 2(See Supplementary 1 and 2).

Statistical Analysis

Data were presented as mean (SD) for continuous variables and as number (percentage) for categoric variables. Multivariate adjusted regression models were used to examine the adverse outcomes in relation to baseline exposure variables. All analyses were performed using SPSS version 26.0 (SPSS Inc., Chicago, USA).

Finding to date

As this AMCHS is an on-going cohort study, including recruiting more participants, we have limited initial findings as described here.

Study participants and characteristics

Baseline characteristics of AMCHS women are shown in Table 3(See Supplementary 3). Of 2042 women, the average age at time of enrolment is 31.1 years old (SD 4.6) (range 20 - 48 years). The majority (50.3%) of women were in the age group 30 - 40 years. Business and services personnel were the main occupations of women (28.8%), and a high proportion of women were educated to college or university degree level. A majority of women (64.3%) had a normal pre-pregnancy BMI (18.5 -<23.9 g/ m^2), the mean BMI was 22.5 kg/m^2 (SD 3.1). 5.1% of women smoked or used to smoke before pregnancy, but more than two-thirds were exposed to passive smoking in daily life. About one-third of women consumed alcohol. 6.1% of women suffered anxiety, but 36.7% of women suffered from depression. None of the women had any sleeping problems. Most of the couples were from the city with a household income of 50,000 to 100,000 yuan (RMB) per year. Approximately four-fifths of women had a female infertility factor as their primary diagnosis. The main reason for infertility in these women was tubal factors (56.7%). About half of the women never had a history of pregnancy,

and 85.7% of women had never given birth. Of 291 women who used to have a pregnant history, very few participants had a history of hypertension or GDM. Of 1004 women who completed questions about pregnancy history, the rate of caesarean section history, adverse pregnancy history, artificial abortion were 47.1%, 44.4%, 63.6%, respectively, which are both higher than the average rate in China[37-39].

The characteristics of men who are spouses of the women are reported in Table 4 (See Supplementary 4). In terms of men at the time of enrolment, the average age was 32.5 years old (SD 5.3) (range 22-57 years), most of them were in the age group of 30 - 40 years. Most of the men worked as business and service personnel (26.2%) and were educated to degree level at college or university (56.1%). The percentage of overweight (BMI > 23.9 kg/ m^2) in men is the highest, and the mean BMI was 24.5 kg/ m^2 (SD 3.3). Only 4.6 % of men suffered anxiety, but 27.9% of men suffered depression. Very few of the men had a sleeping problem. Most men (54.3%) smoked or used to smoke, and more than 80% were exposed to passive smoking. The proportion of men consuming alcohol was 63.0%, and 33.8% had a male factor as their primary infertility diagnosis, with the main reason being abnormal progressive motility (37.7%).

Cycle endpoints

1828 couples have been followed for a total of 2757 cycles during follow-up in the MCHP Study. These cycles resulted in 1589 pregnancies of which 5.9% (n = 94) were only chemically detected by a β-hCG blood test and not clinically visualized on ultrasound (biochemical losses) (Figure 2)

Among the remaining 1475 ultrasound-confirmed pregnancies, 9.4% ended in spontaneous abortion before 20 gestational weeks, 0.58% ended in preterm birth, 9 pregnancies ended in stillbirth (loss on or after 20 weeks) (Fig. 2). There have been 1057 live birth. 117 of them need to be followed up. The remaining 940 pregnancies resulted in 1123 live births: 758 singletons and 363 multiples (181 pairs of twins, one set of triplets). To date, the overall live birth rate per initiated cycle is 38.3% (n = 940/2757), and the live birth rate among cycles achieving pregnancy is 71.6% (n = 1057/1475). (Figure 2)

Impact of female age on implantation failure

Table 5(See Supplementary 5) shows the results of logistic regression analysis for implantation failure according to maternal age. After adjustment for urban-rurality, education, income, female anxiety, ovulation factor, sexual abstinence, volume (ml), concentration (ml×10^6), progressive motility, infertility type, follicle-stimulating hormone, luteinizing hormone, estradiol, antral follicle counting. Increasing female age was associated with a higher likelihood implantation failure compared with female age 20-30 years: 31-35years OR 1.374 (95% CI 1.105-1.707), 35–39 years OR 1.793 (95% CI: 1.322-2.433) and ≥40 years OR 5.941 (95% CI: 3.046-11.590).

Strengths and limitation

The AMCHS is the first prospective reproductive cohort study conducted in Anhui, China. The main strengths of AMCHS are the data collected based on couples over an extended time. This allows us to evaluate the influence of specific sensitive windows towards reproductive and pregnancy outcomes, which are less possible to observe in most pregnancy cohorts [28, 29]. We investigate a variety of risk factors for reproductive, pregnancy and child health outcomes.

The in-person collection of multiple biospecimens from women, men, and their child was over an extended time, which enabled us to explore the relationship between risk factors and endpoints of interest at the molecule and genetic level. Using standardized questionnaires, our experienced interviewers had collected rich data from May 2017 to April 2021 and clinical abstraction from electricity medical records, which allowed us to examine lifestyle, nutritional, environmental exposure, mental status of infertile couples and provided us with comprehensive covariate data. The resourceful data allowed us to explore multiple interactions among risk factors. Furthermore, we highly focused on the paternal impact on adverse reproductive and pregnancy outcomes as the decline of sperm quality of male accounts for 30 - 50% of infertility factors [40] and contribute to miscarriage [41]. This is often ignored in most pregnancy cohort studies [28, 29].

The study has limitations. Firstly, the majority of participants in this study come from Anhui, and mainly in areas of Hefei; most of the participants lived in urban areas. They had a higher socioeconomic status and nutrition status, but less physical activities compared to rural residents. Thus, caution should be exercised in generalizing our findings to the whole Anhui province. As an on-going cohort study, we will recruit more participants, particularly from rural areas to examine some important factors (eg, indoor air pollution) associated with ART adverse outcomes. Secondly, although the participants' involvement is easy to sustain when they are in the middle of treatment for live birth, 62 of 940 families withdrew after their baby's birth and the rate of lost to follow up may rise with the cohort follow-up. Although the current withdrawal rate in our study is similar to those in Western countries [17, 18], we will take all measures to maintain the cohort members in the follow-up as many as possible and examine the effect of those lost to follow up on the results.

Collaboration

Protentional collaborators are invited to contact the corresponding author (caoyunxia6@126.com).

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Footnotes

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Author Contributions: The study protocol was designed by YC, RC and JY drafted the manuscript. CL, XP, XX and FT performed the data quality control and the statistical analysis. WZ and RK revised the manuscript. All authors participated in the study design, revised the article and approved the final version.

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Data sharing statement: Currently, the data are not available because it contains some sensitive information.

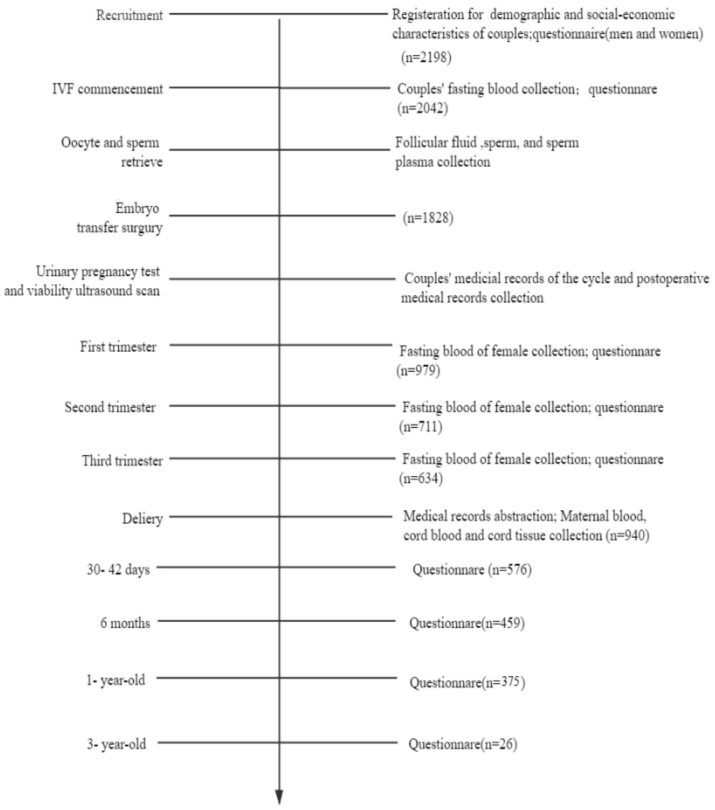

Figure 1. Flow Chart of participants and data collection at baseline and follow-up: the AMCHS

209x296mm (192 x 192 DPI)

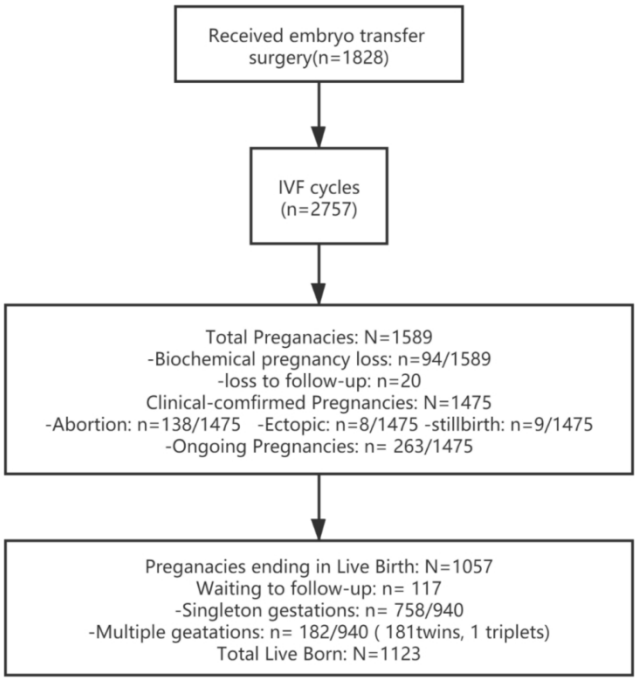

Figure 2. Flow Chart of participants who received the embryo transfer surgery: the AMCHS

279x262mm (144 x 144 DPI)

Supplementary Table 1. Data collected during the preconception and pregnancy period of the AMCHS

	Preconception period		Pregnancy period		
	Male	Female	Visit 1	Visit 2	Visit3
			(4 wks)	(24 wks)	(32 wks)
Socioeconomic data[a]	√	√			
Environmental exposure	√	√	√	√	√
Lifestyle questions[b]	√	√	√	√	√
Physical activities	√	√	√	√	√
Nutritional dietary	√	√	√	√	√
Health condition[c]	√	√	√	√	√
Sleep, Mood and Psychological Scale [d]	√	√	√	√	√
Medical records[e]	√	√	√	√	√
Biosample collection					
Blood (fasting)	√	√	√	√	√
Follicular fluid	√				
Sperm		√			
Sperm plasma		√			
Ultrasound scan			√	√	√

[a] Socioeconomic data includes: educational level; family income; occupation.
[b] Lifestyle questions includes: tea and coffee drinking habits, alcohol consumption, smoking status
[C] Health condition includes: medical history; medication history; IVF treatment history; pregnancy complication (during Pregnancy period); menstrual history; disease history.
[d] Sleep, Mood and Psychological Scale includes: Ten-Item Perceived Stress Scale; Self-Report Depression Scale; Self-Report Anxiety Report; Pittsburgh Sleep Quality Scale.
[e] Medical records about the cycle includes:
1 female: physician assigned infertility diagnosis; reproductive history; medication regimen; infertlity medical history; blood pressure; BMI; Outpatient preoperative routine examination before the cycle.
2 male: Semen Parameter; BMI; Outpatient preoperative routine examination before the cycle.
3 Procedures used for IVF treatment: medication regimen; hormone at HCG Day; Luteal phase support after IVF; Scoring for fertilization; Embryo culture and transfer.

Supplementary Table 2. Data collected at the post-delivery period of the AMCHS

	Delivery	Visit 1	Visit 2	Visit3	Visit4
		30 - 42 days	6 months	12 months	36 months
Interviews and self-administered questionnaires[a]		√	√	√	√
Medical records[b]	√				
Anthropometry[c]	√	√	√	√	√
Biosample collection					
Maternal blood	√				
Cord blood	√				
Cord tissue	√				

[a] Interviews and self-administered questionnaires focus on the child's developmental status includes: food diaries, food frequency questionnaires intakes of dietary supplements, mothers' feeding practices， Breast milk frequency; Brief Infant Sleep Questionnaire; Medical history; Medication history; physical activities.

[b] Medical records includes: gender of infant; number of gestation; type of delivery; Apgar score; congenital diseases

[c] Anthropometry includes: height; weight; head circumference.

Supplementary Table 3. Baseline characteristics of female participants: AMCHS

Variable	Female (n = 2042) N (%)
Age (years)	
Mean (SD)	31.1(4.6)
＜30, n(%)	926(45.4)
30-40, n (%)	1028(50.4)
＞40, n(%)	85(4.2)
Occupation	
Officers	541(26.5)
Technician	227(11.1)
Clerk	163(8.0)
Business and service personnel	589(28.8)
Agriculture, forestry, animal husbandry, fishery and water conservation personnel	15(0.7)
Production and shipping equipment operators and related personnel	76(3.7)
Solider	0(0.0)
Other practitioners that are inconvenient to be classified	67(3.3)
unemployed	361(17.7)
Education	
Under secondary	25(1.2)
Secondary	401(19.6)
High School	363(17.8)
College/university	1108(54.2)
Postgraduate	135(6.6)
District	
City	1559(76.5)
County	288(14.1)

Countryside	191(9.4)
Family annual income (RMB)	
<50 thousands	328(16.1)
50～100 thousands	760(37.2)
100～200 thousands	675(33.1)
200～300 thousands	167(8.2)
>300 thousands	104(5.1)
Body mass index (BMI) before pregnancy (kg/m2)*	
Mean (SD)	22.45(3.13)
Low (<18.5)	130(7.0)
Normal (18.5 -<23.9)	1192(64.3)
High (≥23.9)	532(28.7)
Smoking and drinking before pregnancy	
Active smoking (ever or current)	104(5.1)
Passive smoking	1249(61.5)
Drinking	687(33.8)
Anexity	124(6.1)
Depression	747(36.7)
Sleeping quality	
Bad	0(0.0)
Not bad	25(1.2)
Normal	547(26.9)
Good	1461(71.9)
Primary infertility diagnosis, n (%) (n=1866)	
Male factor	631(33.8)
Female factor	1568(84.0)
Ovulation disorders	267(14.3)
Uterine disorders	165(8.8)
Endometriosis	8(0.4)
Tubal factor	1059(56.7)

Unexplained	33(1.8)
Gravidity(n=2033)	
0	1029(50.6)
≥1	1004(49.4)
Parity(n=2033)	
0	1742(85.7)
≥1	291(14.3)
History of previous pregnancy (n=291)	
GDM	17(5.8)
Hypertension	6(2.1)
Caesarean section	137(47.1)
Adverse pregnancy history(n=1004)	446(44.4)
Artificial abortion(n=1004)	639(63.6)

Proportions were calculated excluding cases with missing data

Supplementary Table 4. Baseline characteristics of male participants: AMCHS

Variable	Men(n=2042) N (%)
Age (years)	
Mean (SD)	32.5(5.3)
＜30, n(%)	729(35.7)
30-40, n (%)	1139(55.8)
＞40, n(%)	174(8.5)
Occupation	
Officers	520(25.5)
Technician	461(22.6)
Clerk	71(3.5)
Business and service personnel	535(26.2)
Agriculture, forestry, animal husbandry, fishery and water conservation personnel	41(2.0)
Production and shipping equipment operators and related personnel	191(9.4)
Solider	11(0.5)
Student	3(0.1)
Other practitioners that are inconvenient to be classified	148(7.3)
Unemployed	58(2.8)
Education	
Under secondary	16(0.8)
Secondary	327(16.0)
High School	363(17.8)
College/university	1145(56.1)
Postgraduate	183(9.0)
Body mass index (BMI) before pregnancy (kg/m2)*	
Mean (SD)	24.5(3.3)

Low (<18.5)	41(2.2)
Normal (18.5 -<23.9)	812(44.0)
High (≥23.9)	994(53.8)
Smoking and drinking before pregnancy	
Active smoking (ever or current)	1091(54.3)
Passive smoking	1669(83.0)
Drinking	1265(63.0)
Anxiety	92(4.6)
Depression	561(27.9)
Sleeping quality	
Bad	2(0.1)
Not bad	12(0.6)
Normal	462(23.0)
Good	1532(76.3)
Sexual abstinence (n=1815)	
Mean (SD)	4.8(2.6)
Normal (2-7)	1740(95.9)
Abnormal (＜2 or ＞7)	75(4.1)
Volume (ml)（n=1822)	
Mean (SD)	3.7(1.9)
Normal (1.5-6)	1600(87.8)
Abnormal (＜1.5 or ＞6)	222(12.2)
Concentration(ml×106）(N=1789)	
Mean (SD)	72.5(73.2)
Normal (≥15)	1485 (83)
Oligozoospermic (＜15 and ＞0)	226(12.6)
Azoospermia (=0)	78(4.3)
Progressive motility (rapid + slow progression) (%) (n=1754)	
Mean (SD)	37.8(19.8)
Normal (≥32)	1093(62.3)

Abnormal (＜32）	661(37.7)
Morphology (%) (n=1453)	
Mean (SD)	5.0(6.0)
normal (≥4）	1037(71.4)
abnormal（<4）	416(28.6)

Proportions were calculated excluding cases with missing data

Supplementary Table 5. Associations of female age with implantation failure in Chinese women with ART treatment: AMCHS

Age (years)	Participants			Model 1			Model 2		
	All	Implantation failure (%)		*OR*	95% *CI*	*P*	*OR*	95% *CI*	*P*
20-30 (group 1)	852	314	36.9	Ref			Ref		
31-35(group 2)	675	303	44.9	1.29	1.02-1.64	0.032	1.37	1.11-1.78	0.004
36-40 (group 3)	237	124	52.3	1.61	1.10-2.35	0.015	1.79	1.32-2.43	<0.001
> 40 (group 4)	58	46	79.3	5.21	2.51-10.83	<0.001	5.94	3.05-11.59	<0.001

Model 1: adjusted for education, income and urban-rural living

Model 2: adjusted for urban-rurality, education, income, anxiety, depression, stress, tubal status, endometrium status, male age, male factor: sexual abstinence, volume (ml), concentration (ml×106), progressive motility, Infertility type, follicle-stimulating hormone, luteinizing hormone, estradiol and antral follicle counting.

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Cohort Profile: Anhui Maternal-Child Health Study in China

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Cohort Profile: Anhui Maternal-Child Health Study in China

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ABSTRACT

Purpose The Anhui Maternal-Child Health Study (AMCHS) aims to examine determinants of reproduction, pregnancy and post-partum maternal and child health outcomes in Chinese women who received assisted reproductive technology (ART).

Study design and Participants AMCHS is an on-going cohort study, starting from May 2017. It recruits participants from all couples who sought ART treatment in the First Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China. The participants are interviewed to document baseline socio-demography, lifestyles, dietary intake and environmental exposure. Their clinical characteristics are obtained from hospital records. Samples of blood, follicular fluid and semen are collected at clinic. Participants receive a standard long pituitary downregulation or a short protocol with an antagonist for the treatment. They are followed up from preconception to delivery, or discontinuation of ART treatment. Details of children health are documented through a questionnaire focussing on developmental status and anthropometry measurement.

Findings to date Until April 2021, AMCHS has recruited 2042 couples in the study. Of women, 111 withdrew from the study, 19 failed to retrieve oocytes. Among 1475 confirmed pregnancy, 146 had miscarriage or termination of pregnancy, 9 had stillbirth, and 263 were ongoing pregnancy. The implantation failure increased with maternal age; adjusted odds ratio was 1.43 (95% CI 1.16-1.77) in age of 31-35 years, 1.97 (95% CI 1.46-2.66) in 35–39 years and 6.52 (95% CI 3.35-12.68) in ≥40 years compared to those aged 20-30 years. Among the 1057 couples with

successful ART who were followed up for delivering baby, 576 had their children examined at age 30-42 days, 459 at 6 months, and 375 at 12 months.

Future plans The AMCHS will identify comprehensive risk factors for poor ART outcomes and explore potential interaction effects of multiple factors including socio-psychologic aspects environmental exposure, dietary intake and genetics on maternal and child health.

Strengths and limitations of this study

- The AMCHS is the first prospective reproductive cohort study conducted in Anhui, China, to examine factors influencing ART risk and outcomes.
- The study investigates a variety of risk factors, including BioBank data for reproductive, pregnancy and child health outcomes over time.
- The study includes and focuses on the paternal impact on adverse reproductive and pregnancy outcomes.
- The cohort may recruit more participants who are in high socioeconomic status (SES) and the findings would not be generalised to couples in low SES.
- The rate of lost to follow-up may rise with the cohort follow-up time, which could bring potential bias for the study findings.

INTRODUCTION

There has been increased infertility in the population. Over the past two decades, the infertility rate has increased from 6.7% to 15.5% among women of reproductive age in the world [1,2]. There are around 48 million infertility couples [3]. Infertility causes psychological distress, social stigmatization, marital discord and heavy financial burden in the families [4-6]. The median per-person cost of In vitro fertilization (IVF) treatment is about US$38,015 [7]. Infertility has been identified as a global public health priority [8].

Assisted Reproductive Technology (ART) is an effective solution for infertility, allowing infertile couples to have a baby. Globally, around 1.7% to 4% of all births are born in the use of ART [9]. While ART is important for infertility treatment, its outcomes vary with different ART procedures. In a cohort study of 631 singleton viable gestations at 19-36 weeks in Italy, Cavoretto, PI et al found that thawed blastocyst transfers were associated with greater estimated fetal weight and birth weight versus fresh blastocyst transfers[10]. There is evidence that ART could markedly increase the incidence of pregnancy complications (e.g., gestational diabetes mellitus (GDM), placenta previa, premature delivery) and poor neonatal outcomes (e.g., stillbirth) [9,11-13]. The reasons for adverse pregnancy outcomes remain unclear; however, there has been increasing research on whether infertility is caused by the technology of ART itself or by parental characteristics and their exposure to risk factors [14-16].

Previous studies showed that unhealthy lifestyles including cigarette smoking and physical inactivity adversely affects ART outcomes such as low pregnancy rate, low live birth rate and miscarriage [20,21] while IVF treatment increases the risk of GDM [22], birth defects [23], and preterm birth compared to a natural pregnancy [24]. However, most studies have focused on maternal influences during pregnancy and the delivery process [25-28]. Few studies have

examined multiple risk factors, especially at a genetic level for adverse pregnancy of ART, simultaneously. Little is known about how much the characteristics of a male spouse contributes to the infertility and fewer studies have collected such data from both men and women during the preconception period [29,30]. Furthermore, current knowledge of risk factors for ART adverse outcomes are predominately derived from studies undertaken in high-income countries (HICs) [13,18,31,32]. The findings of HICs' studies may not be applicable to those in low- and middle-income countries (LMICs), where the burden of infertility is higher [1]. Few prospective cohort studies have been undertaken in LMICs to assess risk factors among infertility couples who received ART and their prognosis [19,33].

China is the largest and most populated LMIC in the world and has around 15.5% of infertility in women (range 7.2%~26.7%)[34], which is higher than those in HICs (e.g., 12% in USA, 12.5% in UK) [1,35]. The cost of IVF and ART treatment is all out-of-pocket, with no government subsidy. Anhui, one of 34 provinces in China, is located in the mid-eastern region of China, and consists of 61 million residents [36]. The rate of infertility in Anhui is 14.5%, which is close to the national level of 15.5%, and many families cannot have their children due to infertility. While there has been increased number of infertility couples who take ART, there is little investigation on factors of ART treatment and outcomes in Chinese women. To address these omissions, we have recently proposed and conducted the Anhui Maternal-Child Health Study (AMCHS). AMCHS is an ongoing prospective preconception cohort study of couples accepting ART treatment. Our cohort study aims to examine (1) comprehensive risk factors of early environment from maternal and paternal preconception to birth influencing the development of embryo and fetal health, (2) the impact of the interaction between multiple environments and genes on infants' growth and development, (3) the short-term and long-

term implication of ART to the next generation compared with natural conceived pregnancy population, and (4) the impact of adverse pregnancy on children health.

COHORT DESCRIPTION

Study design, setting, and participants

From May 2017, we have invited all infertile couples who received medically assisted reproduction through IVF based technologies (i.e., fresh or frozen IVF protocols, including Intracytoplasmic sperm injection (ICSI)) in the First Affiliated Hospital of Anhui Medical University (AHMU), Hefei city, Anhui province, China to take part in AMCHS. Participants' recruitment in AMCHS is consecutive. The inclusion criteria for study participants are: (1) couples who are the first time to receives ART treatment, and (2) those who have not accepted oocyte or sperm donation or intrauterine insemination.

In the beginning of recruitment AMCHS research coordinators register sociodemographic information of eligible couples. Informed consent is obtained from each couple. This allows researchers to interview participants using a general health and risk factors questionnaire, access their medical records including cycle, pregnancy and delivery data, and take their long-term storage supplies of biological samples for solely medical research purposes. Ethics approval for the study has been received from the ethics committee of Anhui Medical University (20160270).

During the period of May 2017 to December 2020, we recruited 2198 eligible couples. The participants were interviewed for baseline assessment and their fasting blood samples were taken before commencing IVF treatment. Figure 1 shows the flow chart of the processes of participants' recruitment and follow-up through the study. Participants are followed up

from the study entry throughout their fertility care, pregnancy, birth (for those achieving pregnancy) and children (until 3-year-old) or until they discontinue treatment or withdrew from the study. As of April 2021, 2042 couples have had IVF treatment, of which 1828 couples received embryo transfer surgery (Fig. 1). Among 1475 women who confirmed pregnancies, 138 suffered from abortion, 8 suffered from ectopic, 9 suffered from stillbirth, 263 were ongoing pregnancies, and 1057 gave live birth to babies. There are 940 children followed up until delivery, 576 children completed the follow-up at age 30 - 42 days, 459 children completed the follow-up at age 6 months, 375 completed the follow-up at 12 months and 26 completed the follow-up at 36 months.

As an ongoing cohort study, AMCHS continues to recruit the participants and follow up the remaining couples in the cohort (Fig. 1). During the period of January to November 2021, AMCHS recruited and followed up 29 participants. The number of participants (n=2042) in the current dataset has shown enough study power for some outcome data analysis; for example, current analysis for the impact of maternal age on implementation. The study powers for other complicated data analysis for rare outcomes will be ensured in on-going recruitment and follow-up of the AMCHS cohort.

Patient and public involvement

Patients or the public are not involved in the design, conduct, reporting or dissemination plans of our research.

Questionnaire survey

Both female and male participants completed a baseline questionnaire survey. The questionnaire includes sociodemographic information (e.g., date of birth, sex, educational level, occupational class, income, and residential area), lifestyle questions (e.g., smoking habits, alcohol consumption, dietary intake, tea and coffee drinking habits, and physical-activities), environmental exposures (e.g., passive smoking, an indoor air pollution), reproductive history, medical history, medication history and family history. We assessed mental health via questionnaires of Chinese Perceived Stress Scale (CPSS, 0 - 40), Centre for Epidemiologic Studies Depression Scale (CES-D, 0 - 60), Self-Report Anxiety Scale (SAS, 20 - 80) and sleep quality (Pittsburgh Sleep Quality Scale (PSQI, 0 - 20)).

We include a questionnaire recording ART medications and pregnancy complication at later visits during each per trimester. To investigate the newborn health we use questionnaires to document food diaries of newborn in food frequency questionnaires including intakes of dietary supplements, mothers' feeding practices, breast milk frequency, sleep patterns in Brief Infant Sleep Questionnaire, anthropometry of infant, medical history, and medication history.

Data collection and follow-up of the cohort

The AMCHS is designed to examine exposures from preconception period throughout once per trimester of pregnancy (for those achieving pregnancy), and up to labour and delivery. Ultrasound monitoring of ovarian response was offered as an integral part of the cycle. When the number of mature oocytes met the requirement of IVF/ICS, couples would be arranged to retrieve oocytes and sperm. Laboratory technicians in the IVF laboratory collected follicular fluid, sperm and seminal plasma. The fasting blood used to test for pregnancy was collected

after two weeks of embryo transfer surgery. When a positive pregnancy test was obtained, pregnant women were invited to the reproductive center at gestational weeks 4, 24, 32 for a questionnaire interview and blood sample collection. Medical records at gestational weeks 4, 24, 32 about gestational clinical information were also accessed. The baseline questionnaire and mental health questionnaires are used at each visit.

During the birth admission, maternal blood, and cord blood and tissue were collected by the nursery midwife. The child was examined at ages 30 - 42 days, 6 months, 1- and 3-year-old (during early childhood). At age 30 - 42 days, 6 months, 12 and 36 months after giving birth, mothers were approached by phone for a questionnaire interview and provided information about clinical physical examination at local community hospital.

Data quality was checked regularly by experienced researchers; when missing and unreasonable data occurred, our research coordinators would correct them through face-to-face interviews for participants, telephone or by checking the electronic medical record system (figure 1).

Outcomes measures

We collected data from the interview questionnaires, extracted medical information from a fertility clinic and delivery records from hospital. Blood samples and follicular fluid were collected from the female partner. Sperm and seminal plasma samples were collected from male partner. A sample of cord blood and tissue was collected from the newborn.

Electronic medical record abstraction— cycle, pregnancy and delivery data

The AMCHS team abstracted clinical medical records during each individual fertility treatment cycle and throughout follow up (up to the birth of an infant for those achieving pregnancy). Research coordinators abstracted clinical information in the First Affiliated Hospital of AMHU to ascertain the outcome of each cycle, including physician assigned infertility diagnosis, infertility medical history, blood pressure, body mass index (BMI), medication regimen; hormone at HCG (Human chorionic gonadotropin) Day, luteal phase support after IVF, scoring for fertilization, embryo culture and transfer, biochemical pregnancy (with β-hCG measurements), clinical pregnancy (with ultrasound assessment) and relevant data. Antenatal and neonatal hospital medical records were also accessed. Details of antenatal, intrapartum and postpartum events during the study period were captured from hospital medical records or phone interview.

The AMCHS Biobank

Blood samples were obtained from female partners at several times during the periods of preconception to delivery and from the male partner at preconception. We also collected blood samples from cord blood and tissue at birth. The collection of blood samples from couples to their babies enable us to explore any heredity influences and detect any potential biomarkers for disease aetiology. Follicular fluid, sperm and seminal plasma were collected in order to evaluate the quality of gametes, which could be the key factors of IVF outcomes.

Plasma and blood cell samples were separated from the blood via centrifugation for 10 minutes at a relative centrifugal force of 3000 g at 4 ℃. Plasma was stored at a -80 ℃ refrigerator, and the blood cell was stored at -20 ℃ refrigerator. Cord blood and tissue were stored at 4 ℃ after a temporary delivery in the operation room and then transferred to a 20 ℃ refrigerator within 24 hours. Seminal plasma was centrifuged before it was sub-packaged.

Finally, the sperm (1 ml) and seminal plasma (2 ml) was stored in a nitrogen canister in a -80 ℃ refrigerator. Follicular fluid was collected more than 5 ml on average. After being centrifuged, the sub package was stored in an -80℃ refrigerator.

The overview of data collection and timepoint of AMCHS are shown in Table 1 and Table 2.

Table 1. Data collected during the preconception and pregnancy period of the AMCHS

	Preconception period		Pregnancy period		
Data variable	Male	Female	Visit 1 (4 wks)	Visit 2 (24 wks)	Visit 3 (32 wks)
Socioeconomic status[a]	√	√			
Lifestyles[b]	√	√	√	√	√
Environmental exposure	√	√	√	√	√
Physical activities	√	√	√	√	√
Nutritional dietary	√	√	√	√	√
Health condition[c]	√	√	√	√	√
Sleep, Mood and Psychological Scale [d]	√	√	√	√	√
Physician assigned infertility diagnosis	√	√			
Reproductive history		√			
Indications for IVF/ICSI treatment	√	√			
Outpatient preoperative routine examination before the cycle	√	√			
Semen Parameter	√				
ART medication regimen		√			
Luteal phase support after IVF		√			
Scoring for fertilization		√			
Hormone at HCG Day		√			
Embryo culture and transfer		√			
Ultrasound scan of pregnancy			√	√	√

Blood (fasting)	√	√	√	√	√
Follicular fluid		√			
Sperm	√				
Seminal plasma	√				

[a] Socioeconomic data include educational level, occupation and family income.
[b] Lifestyle questions include smoking status, alcohol consumption, tea and coffee drinking habits.
[c] Health condition include blood pressure，BMI， medical history, medication history, IVF treatment history, pregnancy complication during pregnancy period, menstrual history.
[d] Sleep, Mood and Psychological Scale consists of Ten-Item Perceived Stress Scale, Self-Report Depression Scale, Self-Report Anxiety Report, and Pittsburgh Sleep Quality Scale. The validity and reliability of Chinese version of above questionnaires have been verified[37,38.]

Table 2. Data collected at the post-delivery period of the AMCHS

	Delivery	Visit 1	Visit 2	Visit 3	Visit 4
Data variable		30-42 days	6 months	12 months	36 months
Interviews and self-administered questionnaires [a]		√	√	√	√
Medical records [b]	√				
Anthropometry measurement [c]	√	√	√	√	√
Biosample collection					
Maternal blood	√				
Cord blood	√				
Cord tissue	√				

[a] Interviews and self-administered questionnaires focus on the child's developmental status include food diaries, food frequency questionnaires intakes of dietary supplements, mothers' feeding practices，Breast milk frequency, Brief Infant Sleep Questionnaire, Medical history, Medication history and physical activities.
[b] Medical records include gender of infant; number of gestations; type of delivery; Apgar score; congenital diseases
[c] Anthropometry measurement includes height, weight, and head circumference.

Medical records are for antenatal, intrapartum and postpartum events of mothers and infants. Anthropometry is for children. The data of post-delivery period is for children.

Data presentation and statistical analysis

Data were presented as mean (SD) for continuous variables and as number (percentage) for categoric variables. The continuous variables include age, BMI, depression symptoms, anxiety symptoms, stress symptoms, sleep quality, , follicle-stimulating hormone, luteinizing hormone, estradiol. The categorical variables include district (city, country, countryside). Education level (Under secondary, Secondary, High School, College/university, Postgraduate), annual household income (<50 thousands CNY/year, 50 ～ 100 thousands CNY/year, 100 ～ 200 thousands CNY/year, 200～300 thousands CNY/year, >300 thousands CNY/year), occupation (officers, technician, clerk, business and service personnel, agriculture, forestry, animal husbandry, fishery and water conservation personnel, production and shipping equipment operators and related personnel, solider, other practitioners that are inconvenient to be classified, unemployed), smoking status (active smoking, passive smoking), tubal status (normal, abnormal), sexual abstinence (normal, abnormal), Infertility type (Primary infertility, secondary infertility), antral follicle counting (0, 1-5, 6-10, 11-15, >15) sperm volume (normal, abnormal). Multivariate adjusted regression models were used to examine the adverse outcomes in relation to baseline exposure variables. All analyses were performed using SPSS version 26.0 (IBM Co., Armonk, NY, USA).

FINDING TO DATA

As this AMCHS is an on-going cohort study, which recruit more participants and have longer time to follow up, we have limited initial findings as described here.

Study participants and characteristics

Table 3 shows baseline characteristics of AMCHS women and men. Most of the couples were from the urban area with a household income of 50,000 to 100,000 yuan (RMB) per year.

Of 2042 women, the average age at time of enrolment was 31.1 years old (SD 4.6) (range 20 - 48 years), with the majority being in the age group 30 - 40 years (50.4%), 60.8% have educational level at >=college, and 26.5% are working in office. While 5.1% of women smoked or used to smoke before pregnancy, 61.5% were exposed to passive smoking, and about one-third of women consumed alcohol. Majority of women (57.4%) had a normal pre-pregnancy BMI (18.5 -<23.9 g/ m^2) [the mean BMI was 22.5 kg/m^2 (SD 3.1)]. 6.1% of women suffered anxiety, but 36.7% of women suffered from depression. None of the women had any sleeping problems. Approximately four-fifths of women had a female infertility factor as their primary diagnosis. The main reason for infertility in these women was tubal factors (56.7%). 85.7% women never had a history of pregnancy, and 85.7% of women had never given birth. Of 291 women who used to have a pregnant history, very few participants had a history of hypertension or GDM. Of 1004 women who completed questions about pregnancy history, the rates of caesarean section history, adverse pregnancy history, artificial abortion were 47.1%, 44.4%, 63.6%, respectively, which are both higher than the average rate in China[39-41].

Of 2042 men, the average age at time of enrolment was 32.5 years old (SD 4.6) (range 22-57 years), and 55.8% were in the age group of 30 - 40 years. 65.1% of male participants have educational level >= college, and 26.2% are working in business and service personnel. 54.3% smoked or used to smoke, more than 80% were exposed to passive smoking ,63.3% consumed alcohol. The percentage of normal BMI (18.5 -<23.9 kg/ m^2) in men is the highest (%), [the mean BMI was 24.5 kg/ m^2 (SD 3.3)]. 27.9% of men suffered from depression, 4.6 % had anxiety, and 0.1% had a sleeping problem. 33.8% had a male factor as their primary infertility diagnosis, with the main reason being abnormal progressive motility (37.7%).

Table 3. Baseline characteristics of participants: AMCHS

Variable	Female (n = 2042) N (%)	Male (n = 2042) N (%)
Age (years)		
＜30, n(%)	923(45.2)	724 (35.5)
30-40, n (%)	1029(50.4)	1139(55.8)
＞40, n(%)	85(4.2)	174(8.5)
Missing data	5(0.2)	5(0.2)
District		
City (urban)	1559(76.5)	
County (suburban)	288(14.1)	
Countryside (rural)	191(9.4)	
Educational level		
Postgraduate	135(6.6)	183(9.0)
College/university	1108(54.2)	1145(56.1)
Secondary	401(19.6)	327(16.0)
Under secondary	25(1.2)	16(0.8)
Family annual income (RMB)		
<50 thousands	328(16.1)	
50～100 thousands	760(37.2)	
100～200 thousands	675(33.1)	
200～300 thousands	167(8.2)	
>300 thousands	104(5.1)	
Missing data	1(0.0)	
Occupation		
Officers	541(26.5)	520(25.5)
Technician	227(11.1)	461(22.6)
Clerk	163(8.0)	71(3.5)
Business and service personnel	589(28.9)	535(26.2)
Agriculture, forestry, animal husbandry, fishey and water conservation personnel	15(0.7)	41(2.0)
Production and shipping equipment operators and related personnel	76(3.7)	191(9.4)

Solider	0(0.0)	11(0.5)
Other practitioners that are inconvenient to be classified	67(3.3)	3(0.1)
unemployed	361(17.7)	148(7.2)
Missing data	2(0.1)	2(0.1)
Smoking and drinking before pregnancy		
Active smoking (ever or current)	104(5.1)	1091(54.3)
Passive smoking	1249(61.5)	1669(83.0)
Alcohol drinking	687(33.8)	1265(63.0)
Body mass index (BMI) before pregnancy (kg/m2)		
Mean (SD)	22.45(3.13)	24.5(3.3)
Low (<18.5)	130(6.4)	41(2.0)
Normal (18.5 -<23.9)	1173(57.4)	792(38.8)
High (≥23.9)	548(26.8)	728(35.7)
Missing data	191(9.4)	279(13.7)
Anxiety	124(6.1)	92(4.6)
Depression	747(36.7)	561(27.9)
Sleeping quality		
Bad	0(0.0)	2(0.1)
Not bad	25(1.2)	12(0.6)
Normal	547(26.9)	462(23.0)
Good	1461(71.9)	1532(76.3)
Missing data	1(0.0)	1(0.0)
Primary infertility diagnosis, n (%) (n=1866)		
Male factor	631(33.8)	
Female factor	1568(84.0)	
Ovulation disorders	267(17.3)	
Uterine disorders	165(10.5)	
Endometriosis	8(0.5)	
Tubal factor	1059(67.5)	
Unexplained	33(2.1)	
Missing data	36(2.3)	
Gravidity (n=2033)		

0	1029(50.6)
≥1	1004(49.4)
Parity (n=2033)	
0	1742(85.7)
≥1	291(14.3)
History of previous pregnancy (n=291)	
Gestational Diabetes Mellitus	17(5.8)
Hypertension	6(2.1)
Caesarean section	137(47.1)
Adverse pregnancy history(n=1004)	446(44.4)
Artificial abortion(n=1004)	639(63.6)
Sexual abstinence (n=1815)	
Mean (SD)	4.8(2.6)
Normal (2-7)	1740(95.9)
Abnormal (＜2 or ＞7)	75(4.1)
Sperm Volume (ml)（n=1822)	
Mean (SD)	3.7(1.9)
Normal (1.5-6)	1600(87.8)
Abnormal (＜1.5 or ＞6)	222(12.2)
Sperm Concentration(ml×106）(N=1789)	
Mean (SD)	72.5(73.2)
Normal (≥15)	1485 (83)
Oligozoospermic (＜15 and ＞0)	226(12.6)
Azoospermia (=0)	78(4.3)
Sperm Progressive motility (rapid + slow progression) (%) (n=1754)	
Mean (SD)	37.8(19.8)
Normal (≥32)	1093(62.3)
Abnormal (＜32)	661(37.7)
Sperm Morphology (%) (n=1453)	
Mean (SD)	5.0(6.0)
normal (≥4)	1037(71.4)
abnormal （<4)	416(28.6)

Cycle endpoints

1828 couples have been examined for a total of 2757 cycles during the follow-up time. These cycles resulted in 1589 pregnancies, of which 5.9% (n = 94) were only chemically detected by a β-hCG blood test and not clinically visualized on ultrasound (biochemical losses) (Figure 1)

Among these 1475 ultrasound-confirmed pregnancies, 9.4% ended in spontaneous abortion before 20 gestational weeks, 0.58% ended in preterm birth, 9 pregnancies ended in stillbirth (loss in or after 20 weeks) (Figure. 1). There have been 1057 live birth, of which 117 need to be followed up. The remaining 940 pregnancies resulted in 1123 live births: 758 singletons and 363 multiples (181 pairs of twins, one set of triplets). To date, the overall live birth rate per initiated cycle is 38.3% (n = 940/2757), and the live birth rate among cycles achieving pregnancy is 71.6% (n = 1057/1475) (Figure 1).

Data analysis for the impact of female age on implantation failure

This paper has shown our preliminary analysis examining the impact of maternal age on implantation failure based on the current follow-up data in AMCHS. Table 4 shows number and percentage of participants and implantation failure across four age groups, and adjusted odds ratio for implantation failure according to maternal age. The rate of implantation failure significantly increased with age. After adjustment for district, education and family income, odds ratio of implantation failure in women aged 30-34 years was 1.43 (95% CI 1.16-1.77), 1.97 (95% CI 1.46-2.66) in 35–39 years and 6.52 (95% CI 3.35-12.68) in ≥40 years compared to those aged 20-29 years. Further adjustment analysis for including clinical variables did not substantially change these ORs (see Model 2 in Table 4).

Table 4. Associations of female age with implantation failure: AMCHS

Age	Participants			Model 1			Model 2		
(years)	All	Implantation failure (%)		*OR*	95% *CI*	*P*	*OR*	95% *CI*	*P*
20-29 (group 1)	852	314	36.9	Ref		<0.001	Ref		<0.001
30-34 (group 2)	675	303	44.9	1.43	1.16-1.77	0.001	1.39	1.11-1.75	0.005
35-39 (group 3)	237	124	52.3	1.97	1.46-2.66	<0.001	1.65	1.20-2.30	0.002
>= 40 (group 4)	58	46	79.3	6.52	3.35-12.68	<0.001	4.99	2.47-10.08	<0.001

Model 1: adjusted for district (city, country, countryside), education level (under secondary, secondary, high school, college/university, postgraduate), annual household income (<50 thousands CNY/year, 50～100 thousands CNY/year, 100～200 thousands CNY/year, 200～300 thousands CNY/year, >300 thousands CNY/year).

Model 2: adjusted for male age, district (city, country, countryside), education level (under secondary, secondary, high School, College/university, Postgraduate), annual household income (<50 thousands CNY/year, 50～100 thousands CNY/year, 100～200 thousands CNY/year, 200～300 thousands CNY/year, >300 thousands CNY/year) anxiety, depression, stress, tubal status (normal, abnormal), , endometrium status (normal, abnormal), follicle counting (0, 1-5, 6-10, 11-15, >15), female Infertility type (Primary infertility, secondary infertility), follicle-stimulating hormone, luteinizing hormone, estradiol sexual abstinence (normal, abnormal), antral sperm volume (normal, abnormal), sperm concentration (normal, abnormal), sperm progressive motility (normal, abnormal).

STRENGTHS AND LIMITAIONS OF AMCHS

The AMCHS is the first prospective reproductive cohort study conducted in Anhui, China. The main strengths of AMCHS are the data collected based on couples from preconception period to 3-year-of child. This allows us to evaluate the influence of specific sensitive windows towards reproductive and pregnancy outcomes, which are less possible to observe in most pregnancy cohorts [29,30]. We investigate a variety of risk factors for reproductive, pregnancy and child health outcomes. The in-person collection of multiple biospecimens from women, men, and their child is over an extended time, enabling us to explore the relationship between

risk factors and endpoints of interest at the molecule and genetic level. Using standardized questionnaires, our experienced interviewers have collected rich data from May 2017 to April 2021 and clinical abstraction from electricity medical records, which allows us to examine lifestyle, nutritional, environmental exposure, mental status of infertile couples and provided us with comprehensive covariate data. The resourceful data allow us to explore multiple interactions among risk factors. Furthermore, we highly focus on the paternal impact on adverse reproductive and pregnancy outcomes as the decline of sperm quality of male accounts for 30 - 50% of infertility factors [42] and contribute to miscarriage[43]. This is often ignored in most pregnancy cohort studies [29,30].

The study has limitations. Firstly, the majority of participants in this study come from Anhui, and mainly from Hefei; 76.5% participants lived in urban areas. They were well-educated and had a higher socioeconomic status and nutrition status, but less physical activities compared to rural residents. Thus, caution should be exercised in generalizing our findings to the whole Anhui province. As an on-going cohort study, we will recruit more participants, particularly from rural areas to examine some important factors (eg, indoor air pollution) associated with ART adverse outcomes. Secondly, although the participants' involvement is easy to sustain when they are in the middle of treatment for live birth, 11.1% (117/1057) of families that gave birth to babies are lost to follow-up, and after their babies' birth 6.6% (62 of 940) families withdrew from the study. Although the current withdrawal rate in our study is similar to those in HICs [17, 18], it may increase with the follow-up time. We will take all measures to maintain the cohort members in the follow-up as many as possible and examine the effect of those lost to follow-up on the cohort study findings.

COLLABORATIONS

Protentional collaborators are invited to contact the corresponding author (caoyunxia5972@ahmu.edu.cn).

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Footnotes

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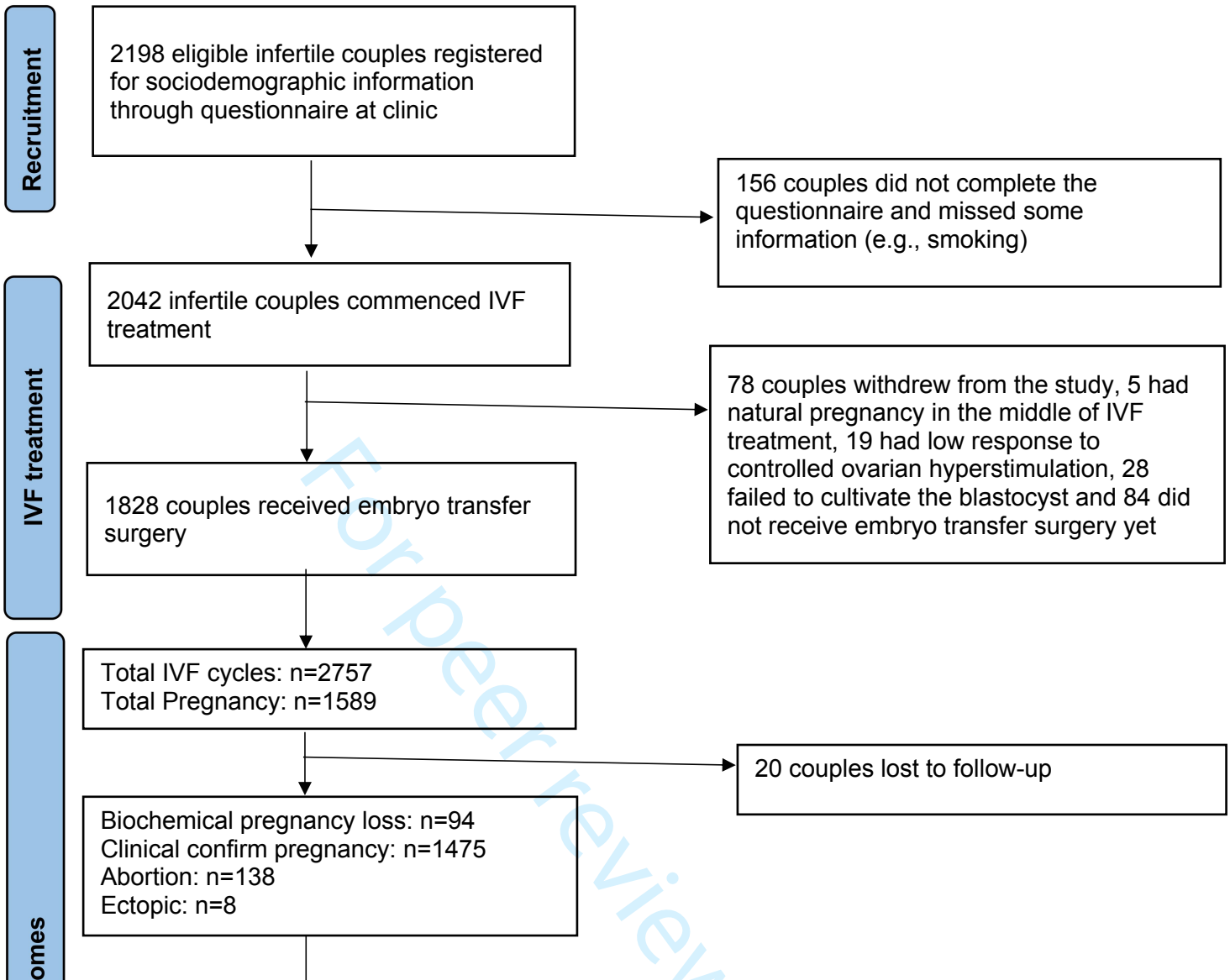
Data sharing statement: Currently, the data are not available because it contains some sensitive information.

Figure 1: Flow Chart for participants and updated outcomes in the follow-up of AMCHS



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Cohort Profile: Anhui Maternal-Child Health Study in China

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Cohort Profile: Anhui Maternal-Child Health Study in China

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ABSTRACT

Purpose The Anhui Maternal-Child Health Study (AMCHS) aims to examine determinants of reproduction, pregnancy and post-partum maternal and child health outcomes in Chinese women who received assisted reproductive technology (ART).

Study design and Participants AMCHS is an on-going cohort study starting from May 2017. AMCHS recruits participants from all couples who sought ART treatment in the First Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China. The participants are interviewed to document baseline socio-demography, lifestyles, dietary intake and environmental exposure. Their clinical characteristics are obtained from hospital records. Samples of blood, follicular fluid and semen are collected at the clinic. Participants receive a standard long pituitary downregulation or a short protocol with an antagonist for the treatment. They are followed up from preconception to delivery, or discontinuation of ART treatment. Details of their children's health are documented through a questionnaire focussing on developmental status and anthropometry measurement.

Findings to date Until April 2021, AMCHS had recruited 2042 couples in the study. 111 women withdrew from the study and 19 failed to retrieve oocytes. Among the 1475 confirmed pregnancies, 146 had miscarriages or terminated their pregnancies, 9 had stillbirths, and 263 were ongoing pregnancies. The implantation failure increased with maternal age; adjusted odds ratio was 1.43 (95% CI 1.16-1.77) in age of 31-35

years, 1.97 (95% CI 1.46-2.66) in 35–39 years and 6.52 (95% CI 3.35-12.68) in ≥40 years compared to those aged 20-30 years. Among the 1057 couples with successful ART who were followed up for delivering babies, 576 had their children examined at age 30-42 days, 459 at 6 months, and 375 at 12 months.

Future plans The AMCHS will identify comprehensive risk factors for poor ART outcomes and explore potential interaction effects of multiple factors including socio-psychologicial aspects of environmental exposure, dietary intake and genetics on maternal and child health.

Strengths and limitations of this study

- The AMCHS is the first prospective reproductive cohort study conducted in Anhui, China, to examine factors influencing ART risk and outcomes.
- The study investigates various risk factors, including BioBank data for reproductive, pregnancy and child health outcomes over time.
- The study includes and focuses on the paternal impact on adverse reproductive and pregnancy outcomes.
- The cohort may potentially recruit more participants who are in high socioeconomic status (SES) so and the findings might not be generalised to couples in low SES.
- The rate of lost to follow-up may rise with the cohort follow-up time, which could bring potential bias for the study findings.

INTRODUCTION

Infertility has increased in many populations and has been identified as a global public health priority[1]. The infertility rate has increased from 6.7% to 15.5% among women of reproductive age in the world over the past two decades[2,3], and there are around 48 million infertile couples[4]. Infertility causes psychological distress, social stigmatization, marital discord and heavy financial burden in families[5-7]. The median per-person cost of In vitro fertilization (IVF) treatment is about US$38,015[8].

Assisted Reproductive Technology (ART) is an effective solution for infertility. Globally, around 1.7% to 4% of all births are born using ART[9]. While ART is important for infertility treatment, its outcomes vary with different ART procedures. In a cohort study of 631 singleton viable gestations at 19-36 weeks in Italy, Cavoretto PI et al. found that thawed blastocyst transfers were associated with greater estimated fetal weight and birth weight versus fresh blastocyst transfers[10]. There is evidence that ART could markedly increase the incidence of pregnancy complications (e.g., gestational diabetes mellitus (GDM), placenta previa, premature delivery) and poor neonatal outcomes (e.g., stillbirth)[9,11-13]. The reasons for adverse pregnancy outcomes remain unclear; however, there has been increasing research on whether infertility is caused by the technology of ART itself or by parental characteristics and their exposure to risk factors[14-16].

Previous studies showed that unhealthy lifestyles including cigarette smoking and physical inactivity adversely affect ART outcomes such as low pregnancy rate, low

live birth rate and miscarriage[17,18], while IVF treatment increases the risk of GDM[19], birth defects[20], and preterm birth compared to a natural pregnancy[21]. However, most studies have focused on maternal influences during pregnancy and the delivery process[22-25]. Few studies have simultaneously examined multiple risk factors, especially at a genetic level for adverse pregnancy outcomes of ART. Little is known about how much the characteristics of a male spouse contribute to the infertility and fewer studies have collected such data from both men and women during the preconception period[26,27]. Furthermore, current knowledge of risk factors for ART adverse outcomes are predominately derived from studies undertaken in high-income countries (HICs)[13,28-30]. The findings of HICs' studies may not be applicable to those in low- and middle-income countries (LMICs), where the burden of infertility is higher[2]. Few prospective cohort studies have been undertaken in LMICs to assess risk factors among infertility couples who received ART and their prognosis[31,32].

China is the largest and most populated LMIC in the world and around 15.5% of women are infertile (range 7.2%~26.7%)[33], which is higher than those in HICs (e.g., 12% in the USA, 12.5% in the UK)[2,34]. The cost of IVF and ART treatment is personally funded, with no government subsidy. Anhui, one of 34 provinces in China, is located in the mid-eastern region of China, and consists of 61 million residents[35]. The rate of infertility in Anhui is 14.5%, which is close to the national level of 15.5%, and many families cannot have their children due to infertility. While there has been an increased in the number of infertile couples who take ART, there is little investigation in the factors of ART treatment and outcomes in Chinese women. To address these

omissions, we have recently proposed and conducted the Anhui Maternal-Child Health Study (AMCHS). AMCHS is an ongoing prospective preconception cohort study of couples accepting ART treatment. Our cohort study aims to examine (1) comprehensive risk factors of early environment from maternal and paternal preconception to birth influencing the development of embryo and fetal health, (2) the impact of the interaction between multiple environments and genes on infants' growth and development, (3) the short-term and long-term implication of ART to the next generation compared with natural conceived pregnancy population, and (4) the impact of adverse pregnancy on children health.

COHORT DESCRIPTION

Study design, setting, and participants

Since May 2017, we have invited all infertile couples who received medically assisted reproduction through IVF based technologies (i.e., fresh or frozen IVF protocols, including Intracytoplasmic sperm injection (ICSI)) in the First Affiliated Hospital of Anhui Medical University (AHMU), Hefei city, Anhui province, China to take part in AMCHS. Participants' recruitment in AMCHS is consecutive. The inclusion criteria for study participants are (1) couples who are receiving ART treatment for the first time, and (2) those who have not accepted oocyte or sperm donation or intrauterine insemination.

At the beginning of the recruitment cycle, AMCHS research coordinators register sociodemographic information of eligible couples. To obtain consent from the

participants, a fertility specialist would introduce the AMCHS to married couples who come to the clinic for IVF treatment consultations. Patients can fully consider from consultation to treatment. When married couples were both willing to participate in the study, they would sign an informed consent form. The discussion of the study and consent was carried out in a private room in the clinic. The couples would join in this cohort study for IVF treatment once they signed the consent form. We offered women free ultrasound monitoring throughout their IVF treatment as an incentive to taking part. Participants could withdraw at any time they wanted without giving any reasons, which would not affect their ongoing care. Researchers interviewed participants using a general health and risk factors questionnaire; these interviews were carried out in a private room in the clinic. Following participant consent, they also accessed participant's medical records, including cycle, pregnancy and delivery data, and took biological samples for long-term storage for solely medical research purposes. Ethics approval for the study has been received from the ethics committee of Anhui Medical University (20160270).

From May 2017 to December 2020, we recruited 2198 eligible couples. The participants were interviewed for baseline assessment and their fasting blood samples were taken before commencing IVF treatment. Figure 1 shows the flow chart of the participants' recruitment and follow-up processes throughout the study. Participants are followed up from the study entry throughout their fertility care, pregnancy, birth (for those achieving pregnancy) and children (until 3-year-old) or until they discontinue treatment or withdraw from the study. As of April 2021, 2042 couples

have had IVF treatment, of which 1828 couples received embryo transfer surgery (Fig. 1). Among 1475 women who confirmed pregnancies, 138 suffered from abortion, 8 suffered from ectopic, 9 suffered from stillbirth, 263 were ongoing pregnancies, and 1057 gave live birth to babies. There are 940 children followed up until delivery, 576 children completed the follow-up at age 30 - 42 days, 459 children completed the follow-up at age 6 months, 375 completed the follow-up at 12 months and 26 completed the follow-up at 36 months.

As an ongoing cohort study, AMCHS continues to recruit the participants and we conduct follow-up and interviews with the remaining couples in the cohort (Fig. 1). Initially, we planned to recruit 2000 couples for the outcome analysis and close the recruitment in June 2021. The sample size was considered according to the incidence of reproductive and pregnancy outcomes. The number of participants (n=2042) in the current dataset has shown enough study power for some outcomes based on the formula of the cohort sample size, for example, current analysis for the impact of maternal age on implantation failure. From January to November 2021, AMCHS recruited and followed up 29 participants. The study powers for other complicated data analysis for rare outcomes will be ensured in on-going recruitment and follow-up of the AMCHS cohort.

Patient and public involvement

Patients and the public involvement were considered, but neither are involved in the

design, conduct, reporting or dissemination plans of our research.

Questionnaire survey

Both female and male participants completed a baseline questionnaire survey. The questionnaire includes sociodemographic information (e.g., date of birth, sex, educational level, occupational class, income, and residential area), lifestyle questions (e.g., smoking habits, alcohol consumption, dietary intake, tea and coffee drinking habits, and physical activities), environmental exposures (e.g., passive smoking, or indoor air pollution), reproductive history, medical history, medication history and family history. We assessed mental health via questionnaires of Chinese Perceived Stress Scale (CPSS, 0 - 40), Centre for Epidemiologic Studies Depression Scale (CES-D, 0 - 60), Self-Report Anxiety Scale (SAS, 20 - 80) and sleep quality (Pittsburgh Sleep Quality Scale (PSQI, 0 - 20)).

We include a questionnaire recording ART medications and pregnancy complications at later visits during each per trimester. To investigate newborn health, we use questionnaires to document food diaries of newborn in food frequency questionnaires including intakes of dietary supplements, mothers' feeding practices, breast milk frequency, sleep patterns in Brief Infant Sleep Questionnaire, anthropometry of infant, medical history, and medication history.

Data collection and follow-up of the cohort

The AMCHS is designed to examine exposures from preconception period throughout once per trimester of pregnancy (for those achieving pregnancy), and up to labour and delivery. Ultrasound monitoring of ovarian response was offered as an integral part of the cycle. When the number of mature oocytes met the requirement of IVF/ICS, couples would be arranged to retrieve oocytes and sperm. Laboratory technicians in the IVF laboratory collected follicular fluid, sperm and seminal plasma. The fasting blood used to test for pregnancy was collected after two weeks of embryo transfer surgery. When a positive pregnancy test was obtained, pregnant women were invited to the reproductive centre at gestational weeks 4, 24, 32 for a questionnaire interview and blood sample collection. Medical records at gestational weeks 4, 24, 32 about gestational clinical information were also accessed. The baseline questionnaire and mental health questionnaires are used at each visit.

During the birth admission, maternal blood, and cord blood and tissue were collected by the nursery midwife. The child was examined at ages 30 - 42 days, 6 months, 1- and 3-year-old (during early childhood). At age 30 - 42 days, 6 months, 12 and 36 months after giving birth, mothers were approached by phone for a questionnaire interview and provided information about clinical physical examination at the local community hospital.

Electronic questionnaires were used for data collection. The original questionnaire data were encrypted and saved to the cloud every day. Quality control staff used pre-written programs to check the reliability and logic of the data. Results

would feed back to the research coordinators on the same day for verification and correction. When missing and unreasonable data occurred, our research coordinators would correct them through face-to-face interviews for participants, by telephone or by checking the electronic medical record system. (figure 1).

Outcomes measures

We collected data from the interview questionnaires, extracted medical information from a fertility clinic and delivery records from hospital. Blood samples and follicular fluid were collected from the female partner. Sperm and seminal plasma samples were collected from the male partner. A sample of cord blood and tissue was collected from the newborn.

Electronic medical record abstraction— cycle, pregnancy and delivery data

The AMCHS team abstracted clinical medical records during each individual fertility treatment cycle and throughout follow up (up to the birth of an infant for those achieving pregnancy). Research coordinators abstracted clinical information in the First Affiliated Hospital of AMHU to ascertain the outcome of each cycle, including physician assigned infertility diagnosis, infertility medical history, blood pressure, body mass index (BMI), medication regimen; hormone at HCG (Human chorionic gonadotropin) Day, luteal phase support after IVF, scoring for fertilization, embryo culture and transfer, biochemical pregnancy (with β-hCG measurements), clinical pregnancy (with ultrasound assessment) and relevant data. Antenatal and neonatal

hospital medical records were also accessed. During the study period, antenatal, intrapartum, and postpartum events were captured from hospital medical records or phone interviews.

The AMCHS Biobank

Blood samples were obtained from female partners at several times during the periods of preconception to delivery and from the male partner at preconception. We also collected blood samples from cord blood and tissue at birth. The collection of blood samples from couples to their babies enable us to explore any heredity influences and detect any potential biomarkers for disease aetiology. Follicular fluid, sperm and seminal plasma were collected in order to evaluate the quality of gametes, which could be the key factors of IVF outcomes.

Plasma and blood cell samples were separated from the blood via centrifugation for 10 minutes at a relative centrifugal force of 3000 g at 4 ℃. Plasma was stored at a -80 ℃ refrigerator, and the blood cell was stored at -20 ℃ refrigerator. Cord blood and tissue were stored at 4 ℃ after a temporary delivery in the operation room and then transferred to a 20 ℃ refrigerator within 24 hours. Seminal plasma was centrifuged before it was sub-packaged. Finally, the sperm (1 ml) and seminal plasma (2 ml) were stored in a nitrogen canister in a -80 ℃ refrigerator. Follicular fluid was collected more than 5 ml on average. After being centrifuged, the sub package was stored in an -80℃ refrigerator.

The overview of data collection and timepoint of AMCHS are shown in Table 1 and Table 2.

Table 1. Data collected during the preconception and pregnancy period of the AMCHS

	Preconception period		Pregnancy period		
Data variable	Male	Female	Visit 1	Visit 2	Visit 3
			(4 wks)	(24 wks)	(32 wks)
Socioeconomic status[a]	√	√			
Lifestyles[b]	√	√	√	√	√
Environmental exposure	√	√	√	√	√
Physical activities	√	√	√	√	√
Nutritional dietary	√	√	√	√	√
Health condition[c]	√	√	√	√	√
Sleep, Mood and Psychological Scale [d]	√	√	√	√	√
Physician assigned infertility diagnosis	√	√			
Reproductive history		√			
Indications for IVF/ICSI treatment	√	√			
Outpatient preoperative routine examination before the cycle	√	√			
Semen Parameter	√				
ART medication regimen		√			

Luteal phase support after IVF		√			
Scoring for fertilization		√			
Hormone at HCG Day		√			
Embryo culture and transfer		√			
Ultrasound scan of pregnancy			√	√	√
Blood (fasting)	√	√	√	√	√
Follicular fluid		√			
Sperm	√				
Seminal plasma	√				

[a] Socioeconomic data include educational level, occupation and family income.
[b] Lifestyle questions include smoking status, alcohol consumption, tea and coffee drinking habits.
[c] Health condition include blood pressure，BMI， medical history, medication history, IVF treatment history, pregnancy complication during pregnancy period, menstrual history.
[d] Sleep, Mood and Psychological Scale consists of Ten-Item Perceived Stress Scale, Self-Report Depression Scale, Self-Report Anxiety Report, and Pittsburgh Sleep Quality Scale. The validity and reliability of Chinese version of above questionnaires have been verified[36,37]

Table 2. Data collected at the post-delivery period of the AMCHS

	Delivery	Visit 1	Visit 2	Visit 3	Visit 4
Data variable		30-42 days	6 months	12 months	36 months

Interviews and self-administered questionnaires [a]		√	√	√	√
Medical records [b]	√				
Anthropometry measurement [c]	√	√	√	√	√
Biosample collection					
Maternal blood	√				
Cord blood	√				
Cord tissue	√				

[a] Interviews and self-administered questionnaires focus on the child's developmental status include food diaries, food frequency questionnaires intakes of dietary supplements, mothers' feeding practices， Breast milk frequency, Brief Infant Sleep Questionnaire, Medical history, Medication history and physical activities.

[b] Medical records include gender of infant; number of gestations; type of delivery; Apgar score; congenital diseases

[c] Anthropometry measurement includes height, weight, and head circumference.

Medical records are for antenatal, intrapartum and postpartum events of mothers and infants. Anthropometry is for children. The data of post-delivery period is for children.

Data presentation and statistical analysis

Data were presented as mean (SD) for continuous variables and as number (percentage) for categoric variables. The continuous variables include age, BMI, depression symptoms, anxiety symptoms, stress symptoms, sleep quality, follicle-stimulating hormone, luteinizing hormone, estradiol. The categorical variables include district (city, country, countryside). Education level (under secondary, secondary, high School, college/university, postgraduate), annual household income (<50 thousand CNY/year, 50～100 thousand CNY/year, 100～200 thousand CNY/year,

200 ～ 300 thousand CNY/year, >300 thousand CNY/year), occupation (officers, technician, clerk, business and service personnel, agriculture, forestry, animal husbandry, fishery and water conservation personnel, production and shipping equipment operators and related personnel, solider, other practitioners that are inconvenient to be classified, unemployed), smoking status (active smoking, passive smoking), tubal status (normal, abnormal), sexual abstinence (normal, abnormal), Infertility type (primary infertility, secondary infertility), antral follicle counting (0, 1-5, 6-10, 11-15, >15) sperm volume (normal, abnormal). Multivariate adjusted regression models were used to examine the adverse outcomes in relation to baseline exposure variables. All analyses were performed using SPSS version 26.0 (IBM Co., Armonk, NY, USA).

FINDING TO DATE

As this AMCHS is an on-going cohort study, which will recruit more participants and will have a longer time to follow up, our initial findings are limited in the followings.

Study participants and characteristics

Supplement table 1 shows baseline characteristics of AMCHS women and men. Most of the couples were from the urban areas with a household income of 50,000 to 100,000 yuan (RMB) per year. Of 2042 women, the average age at time of enrolment was 31.1 years old (SD 4.6) (range 20 - 48 years), with the majority being in the age group 30 - 40 years (50.4%), 60.8% are educated >=college, and 26.5% are working in office. While 5.1% of women smoked or used to smoke before pregnancy, 61.5% were

exposed to passive smoking, and about one-third of women consumed alcohol. The majority of women (57.4%) had a normal pre-pregnancy BMI (18.5 -<23.9 g/ m^2) [the mean BMI was 22.5 kg/m^2 (SD 3.1)]. 6.1% of women suffered anxiety, but 36.7% of women suffered from depression. None of the women had any sleeping problems. Approximately four-fifths of women had a female infertility factor as their primary diagnosis. The main reason for infertility in these women was tubal factors (56.7%). 85.7% women never had a history of pregnancy, and 85.7% of women had never given birth. Of 291 women who used to have a pregnant history, very few participants had a history of hypertension or GDM. Of 1004 women who completed questions about pregnancy history, the rates of caesarean section history, adverse pregnancy history, artificial abortion were 47.1%, 44.4%, 63.6%, respectively, which are both higher than the average rate in China[37-39].

Of 2042 men, the average age at time of enrolment was 32.5 years old (SD 4.6) (range 22-57 years), and 55.8% were in the age group of 30 - 40 years. 65.1% of male participants are educated >= college, and 26.2% are working in business and service personnel. 54.3% smoked or used to smoke, more than 80% were exposed to passive smoking ,63.3% consumed alcohol. The percentage of normal BMI (18.5 -<23.9 kg/ m^2) in men is the highest (%), [the mean BMI was 24.5 kg/ m^2 (SD 3.3)]. 27.9% of men suffered from depression, 4.6 % had anxiety, and 0.1% had a sleeping problem. 33.8% had a male factor as their primary infertility diagnosis, with the main reason being abnormal progressive motility (37.7%).

Cycle endpoints

1828 couples have been examined for a total of 2757 cycles during the follow-up time. These cycles resulted in 1589 pregnancies, of which 5.9% (n = 94) were only chemically detected by a β-hCG blood test and not clinically visualized on ultrasound (biochemical losses) (Figure 1)

Among these 1475 ultrasound-confirmed pregnancies, 9.4% ended in spontaneous abortion before 20 gestational weeks, 0.58% ended in preterm birth, 9 pregnancies ended in stillbirth (loss in or after 20 weeks) (Figure. 1). There have been 1057 live births, of which 117 need to be followed up. The remaining 940 pregnancies resulted in 1123 live births: 758 singletons and 363 multiples (181 pairs of twins, one set of triplets). To date, the overall live birth rate per initiated cycle is 38.3% (n = 940/2757), and the live birth rate among cycles achieving pregnancy is 71.6% (n = 1057/1475) (Figure 1).

Data analysis for the impact of female age on implantation failure

This paper has shown our preliminary analysis examining the impact of maternal age on implantation failure based on the current follow-up data in AMCHS. Table 3 shows number and percentage of participants and implantation failure across four age groups, and adjusted odds ratio for implantation failure according to maternal age. The rate of implantation failure significantly increased with age. After adjustment for district, education and family income, odds ratio of implantation failure in women

aged 30-34 years was 1.43 (95% CI 1.16-1.77), 1.97 (95% CI 1.46-2.66) in 35–39 years and 6.52 (95% CI 3.35-12.68) in ≥40 years compared to those aged 20-29 years. Further adjustment analysis for including clinical variables did not substantially change these ORs (see Model 2 in Table 3).

Table 3. Associations of female age with implantation failure: AMCHS

Age	Participants		Model 1			Model 2		
(years)	All	Implantation failure (%)	*OR*	95% *CI*	*P*	*OR*	95% *CI*	*P*
20-29 (group 1)	852	314 36.9	Ref		<0.001	Ref		<0.001
30-34 (group 2)	675	303 44.9	1.43	1.16-1.77	0.001	1.39	1.11-1.75	0.005
35-39 (group 3)	237	124 52.3	1.97	1.46-2.66	<0.001	1.65	1.20-2.30	0.002
>= 40 (group 4)	58	46 79.3	6.52	3.35-12.68	<0.001	4.99	2.47-10.08	<0.001

Model 1: adjusted for district (city, country, countryside), education level (under secondary, secondary, high school, college/university, postgraduate), annual household income (<50 thousands CNY/year, 50～100 thousands CNY/year, 100～200 thousands CNY/year, 200～300 thousands CNY/year, >300 thousands CNY/year).

Model 2: adjusted for male age, district (city, country, countryside), education level (under secondary, secondary, high School, College/university, Postgraduate), annual household income (<50 thousands CNY/year, 50～100 thousands CNY/year, 100～200 thousands CNY/year, 200～300 thousands CNY/year, >300 thousands CNY/year) anxiety, depression, stress, tubal status (normal, abnormal), , endometrium status (normal, abnormal), follicle counting (0, 1-5, 6-10, 11-15, >15), female Infertility type (Primary infertility, secondary infertility), follicle-stimulating hormone, luteinizing hormone, estradiol sexual abstinence (normal, abnormal), antral sperm volume (normal, abnormal), sperm concentration (normal, abnormal), sperm progressive

motility (normal, abnormal).

STRENGTHS AND LIMITAIONS OF AMCHS

The AMCHS is the first prospective reproductive cohort study conducted in Anhui, China. The main strengths of AMCHS are the data collected based on couples from preconception period to 3-year-of child. This allows us to evaluate the influence of specific sensitive windows towards reproductive and pregnancy outcomes, which are less possible to observe in most pregnancy cohorts[26,27]. We investigate a variety of risk factors for reproductive, pregnancy and child health outcomes. The in-person collection of multiple biospecimens from women, men, and their child is over an extended time, enabling us to explore the relationship between risk factors and endpoints of interest at the molecular and genetic level. Using standardized questionnaires, our experienced interviewers have collected rich data from May 2017 to April 2021 and clinical abstraction from electricity medical records, which allows us to examine lifestyle, nutritional, environmental exposure, mental status of infertile couples and provided us with comprehensive covariate data. The resourceful data allow us to explore multiple interactions among risk factors. Furthermore, we highly focus on the paternal impact on adverse reproductive and pregnancy outcomes as the decline of sperm quality of male accounts for 30 - 50% of infertility factors [40]and contribute to miscarriage[41]. This is often ignored in most pregnancy cohort studies[26,27].

The study has limitations. Firstly, the AMCHS cohort study was set up in only one Hospital in Anhui province, which may lack external validation. While there have

been more than 523 medical institutions approved to carry out human reproductive assisted technologies across China[42], we will set up a collaboration with other similarly designed maternal-child cohort studies from different provinces to enlarge the sample size by combining data sources to increase our study external validation. Furthermore, the majority of participants in the AMCHS cohort come from Hefei and 76.5% participants lived in urban areas. They were well-educated and had a higher socioeconomic status and nutrition status, but were less physically active compared to rural residents. Thus, caution should be exercised in generalizing our findings to the whole Anhui province. As an on-going cohort study, we will recruit more participants, particularly from rural areas to examine some specific factors (e.g., indoor air pollution) associated with ART adverse outcomes. Secondly, although the participants' involvement is easy to sustain when they are in the middle of treatment for live birth, 11.1% (117/1057) of families that gave birth to babies are lost to follow-up, and after their babies' birth 6.6% (62 of 940) families withdrew from the study. Although the current withdrawal rate in our study is similar to those in HICs[28,43], it may increase with the follow-up time. We will take all measures to maintain the cohort members in the follow-up as many as possible and examine the effect of those lost to follow-up on the cohort study findings.

COLLABORATIONS

Protentional collaborators are invited to contact the corresponding author (caoyunxia5972@ahmu.edu.cn).

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Footnotes

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Data sharing statement: Currently, the data are not available because it contains some sensitive information.

Figure 1: Flow Chart for participants and updated outcomes in the follow-up of AMCHS

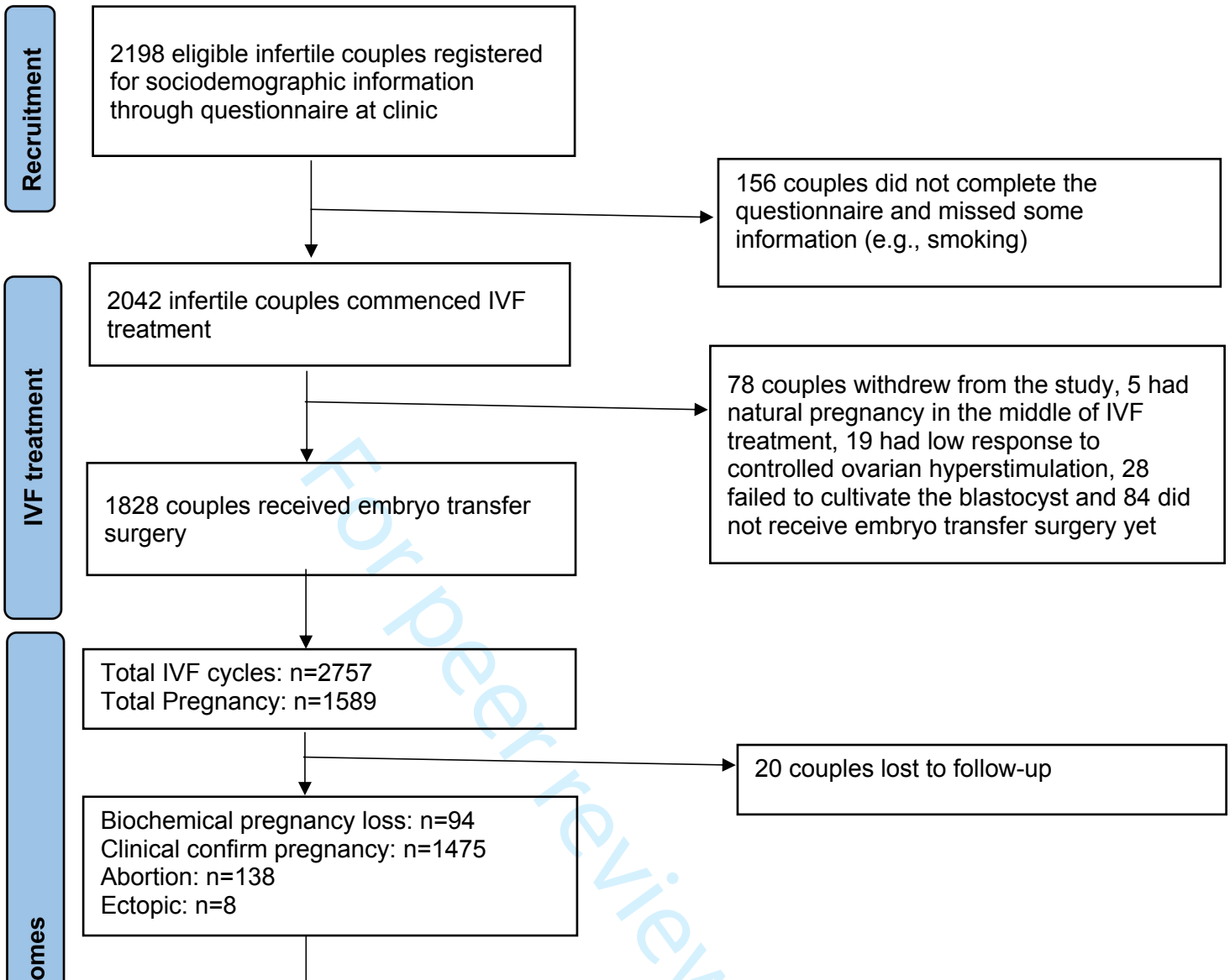

Figure 1: Flow Chart for participants and updated outcomes in the follow-up of AMCHS

Supplement Table 1: Baseline characteristics of participants: AMCHS

Variable	Female (n = 2042) N (%)	Male (n = 2042) N (%)
Age (years)		
＜30, n(%)	923(45.2)	724 (35.5)
30-40, n (%)	1029(50.4)	1139(55.8)
＞40, n(%)	85(4.2)	174(8.5)
Missing data	5(0.2)	5(0.2)
District		
City (urban)	1559(76.5)	
County (suburban)	288(14.1)	
Countryside (rural)	191(9.4)	
Educational level		
Postgraduate	135(6.6)	183(9.0)
College/university	1108(54.2)	1145(56.1)
Secondary	401(19.6)	327(16.0)
Under secondary	25(1.2)	16(0.8)
Family annual income (RMB)		
<50 thousands	328(16.1)	
50 ~ 100 thousands	760(37.2)	
100 ~ 200 thousands	675(33.1)	
200 ~ 300 thousands	167(8.2)	
>300 thousands	104(5.1)	
Missing data	1(0.0)	
Occupation		
Officers	541(26.5)	520(25.5)
Technician	227(11.1)	461(22.6)
Clerk	163(8.0)	71(3.5)

Business and service personnel	589(28.9)	535(26.2)
Agriculture, forestry, animal husbandry, fishery and water conservation personnel	15(0.7)	41(2.0)
Production and shipping equipment operators and related personnel	76(3.7)	191(9.4)
Solider	0(0.0)	11(0.5)
Other practitioners that are inconvenient to be classified	67(3.3)	3(0.1)
unemployed	361(17.7)	148(7.2)
Missing data	2(0.1)	2(0.1)
Smoking and drinking before pregnancy		
Active smoking (ever or current)	104(5.1)	1091(54.3)
Passive smoking	1249(61.5)	1669(83.0)
Alcohol drinking	687(33.8)	1265(63.0)
Body mass index (BMI) before pregnancy (kg/m2)		
Mean (SD)	22.45(3.13)	24.5(3.3)
Low (<18.5)	130(6.4)	41(2.0)
Normal (18.5 -<23.9)	1173(57.4)	792(38.8)
High (≥23.9)	548(26.8)	728(35.7)
Missing data	191(9.4)	279(13.7)
Anxiety	124(6.1)	92(4.6)
Depression	747(36.7)	561(27.9)
Sleeping quality		
Bad	0(0.0)	2(0.1)
Not bad	25(1.2)	12(0.6)
Normal	547(26.9)	462(23.0)
Good	1461(71.9)	1532(76.3)

Missing data	1(0.0)	1(0.0)
Primary infertility diagnosis, n (%) (n=1866)		
Male factor	631(33.8)	
Female factor	1568(84.0)	
Ovulation disorders	267(17.3)	
Uterine disorders	165(10.5)	
Endometriosis	8(0.5)	
Tubal factor	1059(67.5)	
Unexplained	33(2.1)	
Missing data	36(2.3)	
Gravidity (n=2033)		
0	1029(50.6)	
≥1	1004(49.4)	
Parity (n=2033)		
0	1742(85.7)	
≥1	291(14.3)	
History of previous pregnancy (n=291)		
Gestational Diabetes Mellitus	17(5.8)	
Hypertension	6(2.1)	
Caesarean section	137(47.1)	
Adverse pregnancy history(n=1004)	446(44.4)	
Artificial abortion(n=1004)	639(63.6)	
Sexual abstinence (n=1815)		
Mean (SD)		4.8(2.6)
Normal (2-7)		1740(95.9)
Abnormal (< 2 or > 7)		75(4.1)
Sperm Volume (ml)（n=1822)		
Mean (SD)		3.7(1.9)
Normal (1.5-6)		1600(87.8)

Abnormal (< 1.5 or > 6)	222(12.2)
Sperm Concentration(ml×106) (N=1789)	
Mean (SD)	72.5(73.2)
Normal (≥15)	1485 (83)
Oligozoospermic (< 15 and > 0)	226(12.6)
Azoospermia (=0)	78(4.3)
Sperm Progressive motility (rapid + slow progression) (%) (n=1754)	
Mean (SD)	37.8(19.8)
Normal (≥32)	1093(62.3)
Abnormal (< 32)	661(37.7)
Sperm Morphology (%) (n=1453)	
Mean (SD)	5.0(6.0)
normal (≥4)	1037(71.4)
abnormal (<4)	416(28.6)