**GENERAL COMMENTS**

This paper presents a Cohort Profile initiated at Anhui Maternal-Child Health Study in China. I believe that this cohort profile presents potential scientific usefulness in order to advise other researchers of the existence of this precious dataset and to present an opportunity for scientific collaborations. The clinical protocol is scientifically sound and funding was disclosed appropriately. I endorse this cohort profile for publication after having resolved some minor issues listed below.

Clarify exactly how were patients recruited, please. Were they all consecutive cases accepting the research and seen at specific research centers? Please clarify and provide flow chart of excluded cases. Do they expect a certain proportion of patients refusing to be included?

Do they believe useful to introduce outcomes related to different ART procedures? E.g., low technology, high technology, fresh or frozen transfers, oocyte donations, etc. This is highly relevant in my view since ART is a heterogeneous group of procedures carried out on a very heterogeneous group of patients.

How do the authors believe the recruited participants to be representative were they of the wider population? Please add sterility indication and chronic medical problems in the datasets and please plan subgroup analyses in specific subgroups (tubal patency, maternal age, endometriosis, unexplained, male factor, mixed, etc).

Do the authors believe useful to add specific aims related to prematurity risk (1) and fetal growth? There were differences between fresh and frozen transfers which may be included in the study design (ref 2-3). I do recommend some recent publication from our group to be considered for assessment of intrauterine perfusion, fetal growth and risk of prematurity. These are essential study aims to be included in such an ambitious project.

References

1. Cavoretto PI, et al. IVF/ICSI treatment and the risk of iatrogenic preterm birth in singleton pregnancies: systematic review and meta-analysis of cohort studies. J Matern Fetal Neonatal Med. 2020 Jun
1. Cavoretto PI, et al. Greater fetal crown-rump length growth with the use of in vitro fertilization or intracytoplasmic sperm injection conceptions after thawed versus fresh blastocyst transfers: secondary analysis of a prospective cohort study. Fertil Steril. 2021 Jul;116(1):147-156. doi: 10.1016/j.fertnstert.2020.11.035. Epub 2021 Jan 23. PMID: 33500139.

2. Cavoretto P.I., et al. Greater estimated fetal weight and birth weight in IVF/ICSI pregnancies after thawed as compared with fresh blastocyst transfer: prospective cohort study with novel unified modeling methodology. Ultrasound Obstet Gynecol. Accepted Author Manuscript. https://doi.org/10.1002/uog.24806

REVIEWER Marly Cardoso
Universidade de São Paulo, Nutrition
REVIEW RETURNED 22-Jan-2022

GENERAL COMMENTS

The manuscript describes the cohort profile of the Anhui Maternal-Child Health Study (AMCHS). The ongoing cohort was set at the First Affiliated Hospital for Assisted Reproductive Technology (ART) of Anhui Medical University starting from May 2017. The AMCHS study was designed to examine determinants of reproduction, pregnancy, and post-partum maternal and child health outcomes in Chinese women who received ART. The scientific relevance of the study was well described in the Introduction. However, there are many important points for improvements for a better decision on its potential for publication, as follow:

1. Abstract: please describe first the meaning of the abbreviation ART.
2. Strengths and limitations: please describe the external validation of the study as an important limitation. The cohort was set up in only one Hospital in China - how many centers for ART are available in China? When describing "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" please provide quantitative values/estimates;
3. Data collection and follow-up: which measures will be collected for child follow-up? Please describe the quality control and validation of questionnaires or measures accuracy for the data collection.
4. Figures: Fig. 1 and 2 should be revised in just one. The current version of Figure 1 is difficult to understand - please follow Prisma guidelines for flow charts;
5. Tables: Suppl. Table 2, when presenting measures for post-delivery period of the AMCHS please indicate if the data refer to mother, father and/or children (for example, anthropometry from mothers/fathers/children?); SupplTables 3 and 4: female and male participants - mothers and fathers?

The writing deserves some editions for improvements.

REVIEWER Jacqueline Stephens
Flinders University, College of Medicine and Public Health
REVIEW RETURNED 27-Jan-2022

GENERAL COMMENTS

This large cohort study is collecting extensive peri- and postnatal data on fertility, ART and pregnancy success and has the potential to provide important insights on this topic. However, on reading this manuscript I have numerous questions about the study methods, which I list below. Overall, the paper is logical and easy to read,
however, I will highlight there are numerous spelling errors throughout which can be easily corrected by the copy editors during the proofing process.

Abstract:
Use of abbreviation “ART” without explanation.
Heading formatting needs fixing.

Introduction:
Line 17, a dollar amount is quoted without indication of the currency. Please specify if US$ or AUD$ etc?

The authors state the infertility rate in Anhui is 14.5% and that this is moderate compared to the rest of China. The authors then repeat the rate for China (15.5%) which was already reported in a previous sentence. Instead, can the authors provide a range of infertility rates for China provinces, e.g. (min%-max%)? This will give a better sense of how Anhui compares to other regions.

Methods:
Page9, Line 3: In describing the retention/attrition of the study cohort, it is critical that the reasons for attrition are listed. For example, of the 1475 confirmed pregnancies, only 1057 resulted in live births. What happened to the other 418 pregnancies? How many were unsuccessful or terminated, and how many were lost to follow-up or withdrew consent? This information might be best placed in the Figure 1.

It is not clear to me what the relevance is of reporting treatment discontinuation for specific timeframes is, eg. Discontinuation numbers for April 2021, or the recruitment number for Jan-Nov 2021. Can the authors please clarify the purpose of reporting these data for this timepoints, rather than for overall?

Do the authors have a target sample size, or a date when recruitment will close? What are these targets and why were they chosen?

What was the process for informed consent? What was the process if one person in the couple consented, but the other person did not? How long did participants have to consider participation before signing the form and data collection commencing? Did participants receive any incentive to participate, or reimbursement of costs incurred by participation (e.g. travel)? If so, how was coercion to participate avoided? Where was consent obtained, e.g. via telephone, in clinic waiting room, in a private clinic room?

A copy of the interview would be useful as a Supplementary Document. Was the interview questionnaire completed by the researchers, or by the participants? Was this a hardcopy form, or electronic? If hardcopy, who performed data entry and how were transcription errors minimised? Some of the data collected includes validated metrics (e.g. CES, PSQI); were these available to the authors in the main region language (mandarin), or did the authors translate these themselves? What about people who spoke other dialects, were translators used?

What were the processes for participants who were unsuccessful in achieving pregnancy and live birth; were they provided counselling or grief support?
Are the research coordinators who collected the sociodemographic and clinical data part of the authorship team? Are the midwives who collected tissue samples? If so, please indicate in the text which authors collected this data (or in a section describing what each author contributed to the manuscript/research). If not, please acknowledge in the acknowledgements section.

Where is the BioBank located? What security protocols exist to protect these samples? Are the data stored confidentially without patient data? Who has access to these samples?

Given the amount of data collected on the participants I have queries about the security and privacy of the data and its storage. Where is the cohort data stored? What security protocols exist to protect the participants data? How long will these data be retained and when will they be destroyed?

Results
IVF and ART are typically expensive procedures. What are the financial arrangements for accessing this procedure in China? The authors should include this background information in the introduction and make comment on this in the discussion with reference to the high proportion of participants with tertiary education and professional employment who are more likely to be able to afford this expensive treatment, as well as to comment on how this will be a limitation of the cohort data. Furthermore, that the well-educated/employed participants may bring other confounders into any future analyses should be commented on.

Page 14, Line 13: Please provide the previously reported average rates for China.

Was history of sleeping problems, depression, anxiety etc self-reported by participants, or confirmed via clinical/medical records review?

There are some variables included in the logistic regression analysis of age/implant failure which need further explanation, e.g. volume, concentration etc. Volume of what? Concentration of what? The variables included in the regression should be explained in the methods section, including the whether they are categorical or continuous variables, the levels for the categorical variables, and a brief explanation for any ambiguous variables which are not self-explanatory.

Figure 1:
This image is problematic in its current format. However, with some significant changes could be a very informative and crucial diagram for the readership to understand the recruitment, retention, and attrition of participants. I make some suggestions about how to improve this figure below:
1) The font is very small and hard to read.
2) There are multiple spelling errors, e.g. “surgery” should be “surgery”; “deliery” should be “delivery”.
3) The figure would benefit from having the attritions included in the diagram so readers can understand why the numbers decline across the timeline, e.g. exclusions (including reasons), lost to follow-up, withdrawn etc.
4) Make clear the number of couples who have completed each follow-up stage, and the number who are still being followed up.
5) Provide more detail description in the figure heading about the graphic. Do not use acronyms in the heading. The heading should provide enough detail for the Figure to be understood without needing to read the text.

Figure 2:
The exclusions are included in the main components of the flow chart. This chart would be better if modified to have the exclusions in a separate box (refer to the PRISMA flowchart for an example).

Tables:
On the whole, the tables are clear and self-explanatory. However, for brevity, Table 3 and 4 be combined.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Dr. Paolo Cavoretto, Univ Vita Salute San Raffaele

Comments to the Author:

This paper presents a Cohort Profile initiated at Anhui Maternal-Child Health Study in China. I believe that this cohort profile presents a potential scientific usefulness in order to advise other researchers of the existence of this precious dataset and to present an opportunity for scientific collaborations. The clinical protocol is scientifically sound and funding was disclosed appropriately. I endorse this cohort profile for publication after having resolved some minor issues listed below.

Response: We thank this reviewer for having valued our paper and given positive and very useful comments on it. We have revised the manuscript accordingly.

Comments: Clarify exactly how were patients recruited, please. Were they all consecutive cases accepting the research and seen at specific research centers? Please clarify and provide flow chart of excluded cases. Do they expect a certain proportion of patients refusing to be included?

Responses: Thanks for suggestion. In the revised manuscript we have added sentences to clarify the process of patients’ recruitment, Page 6, lines 8-11 “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.” Also, according to follow PRISAM guidelines we have made a new flow chart (Figure 1) to clarify excluded cases and the process. Initially, 2042 couples would like to participate in the study and then started IVF treatment, but 78 of them withdrew in the middle of IVF treatment. We hope that these could help better describe the process of participants’ recruitment in the paper.

Comments: Do they believe useful to introduce outcomes related to different ART procedures? Eg low technology, high technology, fresh or frozen transfers, oocyte donations, etc etc. This is highly relevant in my view since ART is a very heterogeneous group of procedures carried out on a very heterogeneous group of patients.

Responses: Yes, ART outcomes relate to different ART procedures. We have agreed on the idea that ART is a very heterogeneous group of procedures. We have added sentences in the revised manuscript, p5, l10-15 “While ART is important for infertility treatment, its outcomes vary
with different ART procedures. In a cohort study, 631 singleton viable gestations at 19-36 weeks were observed to compare growth trajectories between thawed and fresh blastocyst transfers in Italy from 2016 to 2020, Cavoretto, PI et al found that thawed blastocyst transfers were associated with greater estimated fetal weight and birth weight versus fresh blastocyst transfers10.".

Comments: How do the authors believe the recruited participants to be representative were they of the wider population? Please add sterility indication and chronic medical problems in the datasets and please plan subgroup analyses in specific subgroups (tubal patency, maternal age, endometriosis, unexplained, male factor, mixed, etc).

Responses: Thank the reviewer for comments. We cannot estimate the sample’s representatives since our study have not use a random sampling method to recruit participants from the general population. Thus we have discussed it as a weakness, p14, l20-24:”Firstly, the majority of participants in this study come from Anhui, and mainly in areas of 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.”

In our cohort study we have collected the data of sterility indication and detailed medical problems, which could affect ART outcomes. "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 (e.g., physician assigned infertility diagnosis; blood pressure; BMI; Outpatient preoperative routine examination before the cycle), medication history and family history” p10, l2-5.

We will set subgroup in the next study in future.

Comments: Do the authors believe useful to add specific aims related to prematurity risk (1) and fetal growth? There were differences between fresh and frozen transfers which may be included in the study design (ref 2-3). I do recommend some recent publications from our group to be considered for assessment of intrauterine perfusion, fetal growth and risk of prematurity. These are essential study aims to be included in such an ambitious project.

Responses: Thank the reviewer for suggestions. The relationship between prematurity risk and fetal growth is worth investigating. We have mentioned in the manuscript already, p5, l23—p6 lines 1-5 “Our cohort aims to examine (1) comprehensive and new risk factors to the development of embryo and fetal 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, (4) the impact of adverse pregnancy on health of children "

The articles listed blow provide a great perspective, and we will explore our cohort data to answer these important questions above in future.

References

1. Cavoretto PI, et al. IVF/ICSI treatment and the risk of iatrogenic preterm birth in singleton pregnancies: systematic review and meta-analysis of cohort studies. J Matern Fetal Neonatal Med. 2020 Jun 4:1-10. doi: 10.1080/14767058.2020.1771690. Epub ahead of print. PMID: 32498576.
2. Cavoretto PI, et al. Greater fetal crown-rump length growth with the use of in vitro fertilization or intracytoplasmic sperm injection conceptions after thawed versus fresh blastocyst transfers: secondary analysis of a prospective cohort study. Fertil Steril. 2021 Jul;116(1):147-156. doi: 10.1016/j.fertnstert.2020.11.035. Epub 2021 Jan 23. PMID: 33500139.

3. Cavoretto, P.I., et al. (2021), Greater estimated fetal weight and birth weight in IVF/ICSI pregnancies after thawed as compared with fresh blastocyst transfer: prospective cohort study with novel unified modeling methodology. Ultrasound Obstet Gynecol. Accepted Author Manuscript. https://doi.org/10.1002/uog.24806

Reviewer: 2

Dr. Marly Cardoso, Universidade de São Paulo

Comments to the Author:

The manuscript describes the cohort profile of the Anhui Maternal-Child Health Study (AMCHS). The ongoing cohort was set at the First Affiliated Hospital for Assisted Reproductive Technology (ART) of Anhui Medical University starting from May 2017. The AMCHS study was designed to examine determinants of reproduction, pregnancy, and post-partum maternal and child health outcomes in Chinese women who received ART. The scientific relevance of the study was well described in the Introduction. However, there are many important points for improvements for a better decision on its potential for publication, as follow:

Response: We thank this reviewer for having valued our paper and given positive and very useful comments on it. We have revised the manuscript accordingly.

Comments 1. Abstract: please describe first the meaning of the abbreviation ART.

Response: We have revised the manuscript accordingly, “……outcomes in Chinese women who received assisted reproductive technology (ART).” p2,l5.

2. Strengths and limitations: please describe the external validation of the study as an important limitation. The cohort was set up in only one Hospital in China - how many centers for ART are available in China? When describing "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" please provide quantitative values/estimates;

Response: Thanks for comments. As for external validation of the study, we have written something already in the manuscript, “Thus, caution should be exercised in generalizing our findings to the whole Anhui province”, p14, l23-24. According to data released by the National Health Commission, as of the end of June 2020, there were 523 medical institutions approved to carry out human reproductive assisted technologies in China (China news, 2021). We have provided the figure of participants in urban areas, “76.5% participants lived in urban areas” p14, l21.

Comments : 3. Data collection and follow-up: which measures will be collected for child follow-up? Please describe the quality control and validation of questionnaires or measures accuracy for the data collection.
Response: Thanks for the comments. In the manuscript, we have written these already, “The child was examined at age 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 hospitals” P9 l6-9. The data of Interviews and self-administered questionnaires were collected through phone call, and anthropometry of children is measured by clinician in the local community hospital. As the data collection of AMCHS is an ongoing process, we set real-time quality control for processing data collection and timed quality control check for methods of data collection. In brief, when we used electronic questionnaires 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. As for the measures accuracy, due to possible differences in testing methods, instruments and population characteristics between different local community hospitals, we collected clinical data results along with the corresponding key information, such as reference value ranges, instrument models and testing times to improve the reliability of the study results.

4. Figures: Fig. 1 and 2 should be revised in just one. The current version of Figure 1 is difficult to understand - please follow Prisma guidelines for flow charts;

Responses: Thank the reviewer for the comments. We have combined them into one and made revisions - see new Figure 1.

5. Tables: Suppl. Table 2, when presenting measures for post-delivery period of the AMCHS please indicate if the data refer to mother, father and/or children (for example, anthropometry from mothers/fathers/children?); SupplTables 3 and 4: female and male participants - mothers and fathers?

Responses: Thank the reviewer for the comments. The original Tables 3 and 4 referred to female and male participants’ characteristics. Now, we have put them together in Tables 3, which includes baseline characteristics of female and male participants. The infertility couples commenced to IVF treatment and some of them failed to have a baby or in the middle of treatment, while others gave birth to babies and then turned to parents. We have revised the footnote in Table “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.”

Comments: The writing deserves some editions for improvements.

Response: We have edited the manuscript in the revised version attached.

Reviewer: 3

Dr. Jacqueline Stephens, Flinders University

Comments to the Author:
Comments: This large cohort study is collecting extensive peri- and postnatal data on fertility, ART and pregnancy success and has the potential to provide important insights on this topic. However, on reading this manuscript I have numerous questions about the study methods, which I list below. Overall, the paper is logical and easy to read, however, I will highlight there are numerous spelling errors throughout which can be easily corrected by the copy editors during the proofing process.

Response: Thank the reviewer for interesting in our study and providing useful comments.

<b>Abstract: </b>

Comments: Use of abbreviation “ART” without explanation.

Heading formatting needs fixing.

Response: Thanks. In the revised paper, we have defined “ART” for its abbreviation for first use, Page 2 line 5, “…..outcomes in Chinese women who received assisted reproductive technology (ART).” Also, we have fixed the Heading formatting.

<b>Introduction:</b>

Comments: Line 17, a dollar amount is quoted without indication of the currency. Please specify if US$ or AUD$ etc?

Response: It is US$, and we have highlighted it in the revised manuscript

Comments: The authors state the infertility rate in Anhui is 14.5% and that this is moderate compared to the rest of China. The authors then repeat the rate for China (15.5%) which was already reported in a previous sentence. Instead, can the authors provide a range of infertility rates for China provinces, e.g. (min%-max%)? This will give a better sense of how Anhui compares to other regions.

Response: Thanks for suggestion. We have added in a sentence for it, p5, l17-18 “The rate of infertility in Anhui is 14.5%, which is closed to the average rate of 15.5% in China (range 7.2%~26.7%).”

<b>Methods:</b>

Comments: Page9, Line 3: In describing the retention/attrition of the study cohort, it is critical that the reasons for attrition are listed. For example, of the 1475 confirmed pregnancies, only 1057 resulted in live births. What happened to the other 418 pregnancies? How many were unsuccessful or terminated, and how many were lost to follow-up or withdrew consent? This information might be best placed in the Figure 1.

Response: Thank the reviewer for comments. In the revised paper, we have added the information, p7, l8-10 “Of 1475 confirmed pregnancies, 138 participants suffered from abortion, 8 suffered from ectopic, 9 suffered from stillbirth, 263 were ongoing pregnancies, 1057 gave live birth to babies”.

Comments: It is not clear to me what the relevance is of reporting treatment discontinuation for specific timeframes is, eg. Discontinuation numbers for April 2021, or the recruitment number for Jan-Nov 2021. Can the authors please clarify the purpose of reporting these data for this timepoints, rather than for overall?
Response: Our cohort participants’ recruitment started in May 2017 and initially, we planned to recruit 2000 participants for outcome analysis. Until December 2020, we have achieved the target number of participants, which could be analysed for baseline characteristics and outcomes. In April 2021 we drafted this paper and thus the numbers were discontinued for data analysis. As we have mentioned in the manuscript, p7, l14-15: “During the period of January to November 2021, AMCHS recruited and followed up 29 participants.” Although the speed of recruitment was slow down after December 2020, our recruitment and follow-up of the cohort continued to collect data for analysis. In brief, we reported the data according to the number of participants rather than specific time point.

Comments: Do the authors have a target sample size, or a date when recruitment will close? What are these targets and why were they chosen?

Response: As we said in the manuscript and mentioned above, initially we planned to recruit 2000 couple participants for the outcome analysis. We described the targeted sample size in our manuscript, p7, 15-6: “The number of participants (n=2042) in the current dataset has shown enough study power for some outcome analysis; for example, the association of maternal age with implementation”. The cohort is an on-going study. Its participant’s recruitment will continue in order to study rare outcomes, eg, pregnancy-induced hypertension syndrome, based on their study powers.

Comments: What was the process for informed consent? What was the process if one person in the couple consented, but the other person did not? How long did participants have to consider participation before signing the form and data collection commencing? Did participants receive any incentive to participate, or reimbursement of costs incurred by participation (e.g. travel)? If so, how was coercion to participate avoided? Where was consent obtained, e.g. via telephone, in clinic waiting room, in a private clinic room?

Response: Thank the reviewer for interesting in our study in China. To obtain the consents from the participants, our team clinicians in the First Affiliated Hospital of Anhui Medical University would introduce our cohort study, including the details of study to them when the married couples come to consult IVF treatment in our clinic. If they were willing to take part in the study, we would ask the couple to sign an informed consent form. The activity of discussing consent was in a private clinic room. After having the consent, the couples would join in this cohort study for IVF treatment. Participants would obtain free ultrasound monitoring throughout IVF treatment as incentive. Participants could withdraw at any time they want without any punishment. Consent was obtained in a private clinic room that was prepared for cohort recruitment.

Comments: A copy of the interview would be useful as a Supplementary Document. Was the interview questionnaire completed by the researchers, or by the participants? Was this a hardcopy form, or electronic? If hardcopy, who performed data entry and how were transcription errors minimised? Some of the data collected includes validated metrics (e.g. CES, PSQI); were these available to the authors in the main region language (mandarin), or did the authors translate these themselves? What about people who spoke other dialects, were translators used?

Response: Thank the reviewer for comments. Our interview included a number of questionnaires. They are in Chinese, which are validated in Chinese population and some of
Electronic questionnaires were completed by participants through mobile electronics. Our trained researchers in the team converted the data into the database and checked any possible errors. No matter which dialects the participants speak, all participants understand Chinese in writing.

Comments: What were the processes for participants who were unsuccessful in achieving pregnancy and live birth; were they provided counselling or grief support?

Response: When participants failed to achieve pregnancy or live birth, they would suspend IVF treatment for recover physical and mental well-being. This suspension is normally for 2 months or more which depends on participants’ willingness. We would like to provide counselling support to them if participants asked for it. After the suspension, the participants would continue IVF treatment.

Comments: Are the research coordinators who collected the sociodemographic and clinical data part of the authorship team? Are the midwives who collected tissue samples? If so, please indicate in the text which authors collected this data (or in a section describing what each author contributed to the manuscript/research). If not, please acknowledge in the acknowledgements section.

Response: Thanks for suggestions. The research coordinators are not part of the authorship team in this paper. However, we have appreciated their work in the paper, p19, l4-6: “We thank research coordinators for their valuable work to help collect data of sociodemographic, lifestyle, mental health and etc in questionnaire and connect all participants”. We have midwives in the research team to collect tissue samples and laboratory technicians to collect data of follicular fluid. In the revised manuscript, we have made the acknowledgement, p19, l6-7: “We thank laboratory technicians to collect data of follicular fluid, sperm and seminal plasma, and midwives to collect data of maternal blood, and cord blood and tissue.”

Comments: Where is the BioBank located? What security protocols exist to protect these samples? Are the data stored confidentially without patient data? Who has access to these samples?

Response: The BioBank data is located in the National Health Commission Key Laboratory of Study on Abnormal Gametes and Reproductive Tract (Anhui Medical University), Hefei city, Anhui province, China. In processing biologic samples, the team colleagues need to record the sample information (eg, sample collection time, fasting, haemolysis, etc) into the management system. Staff can view samples in real-time through the system to identify problems in time and then feedback to the site for improvement. We use video to record the entire sample operation and arrange quality control staff for random checks of the video to detect irregularities on time. All data are anonymous. The samples are stored confidentially without patient id data. The research coordinators and quality control staff can access these samples data. Researchers who want to use the cohort BioBank data can access these samples data after approved by PI and research panel.

Comments: Given the amount of data collected on the participants I have queries about the security and privacy of the data and its storage. Where is the cohort data stored? What security protocols exist
to protect the participants data? How long will these data be retained and when will they be destroyed?

Response: Thank the reviewer for inquiries. Our cohort study has been funded by a prestigious national research grant in China. It has required a high standard for data security and privacy. In brief, we store the data of questionnaire, clinic record and laboratory experiment in locked offices in Reproductive Medicine Center, Department of Obstetrics and Gynecology, the First Affiliated Hospital of Anhui Medical University. We enter the data into electronic datasets using patient’s study number id. The datasets are anonymous. They are stored in password-protective computers in Reproductive Medicine Center, Department of Obstetrics and Gynecology, the First Affiliated Hospital of Anhui Medical University. The data will be retained at least for five years but will be destroyed at any time when participants request.

Results

Comments: IVF and ART are typically expensive procedures. What are the financial arrangements for accessing this procedure in China? The authors should include this background information in the introduction and make comment on this in the discussion with reference to the high proportion of participants with tertiary education and professional employment who are more likely to be able to afford this expensive treatment, as well as to comment on how this will be a limitation of the cohort data. Furthermore, that the well-educated/employed participants may bring other confounders into any future analyses should be commented on.

Response: Thank the reviewer for comments. The cost of treatment is all out-of-pocket, with no government subsidy. In the revised manuscript, we have added it in Introduction, “The cost of treatment is all out-of-pocket, with no government subsidy”, p5, l15-16, and in Discussion “Firstly, the majority of participants in this study come from Anhui, and mainly in areas of Hefei city; 76.5% of participants live in urban areas. They are well-educated and have higher levels of socioeconomic status and nutrition status, but probably lower level of physical activities compared to rural counterparts. It is important to consider these for interpreting the findings of the study. Thus, caution should be exercised in generalizing our findings to the general population of infertility couples in Anhui” p14, l20-24.

With regards to “the well-educated/employed participants may bring other confounders into”, our data analysis will include all important covariables, including socio-demographic factor and lifestyles in multiple adjusted regression models and thus the residual effects of “other confounders” should be minimised.

Comments: Page 14, Line 13: Please provide the previously reported average rates for China.

Response: The reported averaged rate of infertility in China is 15.5% (range 7.2%-26.7%). We have put in the revised manuscript, p5, l13-14.

Comments: Was history of sleeping problems, depression, anxiety etc self-reported by participants, or confirmed via clinical/medical records review?

Response: We document the histories of sleeping problems, depression, and anxiety in participants using validated questionnaires, eg, sleep quality (Pittsburgh Sleep Quality Scale
PSQI (0 - 20) and The Centre for Epidemiologic Studies Depression Scale (CES-D, 0 - 60), Self-Report Anxiety Scale (SAS, 20 - 80). We have written these in the manuscript, p8, l7-10.“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”.

Comments: There are some variables included in the logistic regression analysis of age/implant failure which need further explanation, e.g. volume, concentration etc. Volume of what? Concentration of what? The variables included in the regression should be explained in the methods section, including the whether they are categorical or continuous variables, the levels for the categorical variables, and a brief explanation for any ambiguous variables which are not self-explanatory.

Response: Thanks for comments. In the revised manuscript, we have added in Supplementary Table 4, which include those explanations. In supplementary table 4: “sperm volume, sperm concentration, Sperm Progressive motility (rapid + slow progression), Sperm Morphology”. Also, we have added sentences in Methods, “As for confounders of regression models, 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). 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), sperm concentration (normal, abnormal), sperm progressive motility (normal, abnormal), continuous variables: anxiety, depression, stress, male age, follicle-stimulating hormone, luteinizing hormone, estradiol.” p11, l5-17.

Comments: Figure 1: This image is problematic in its current format. However, with some significant changes could be a very informative and crucial diagram for the readership to understand the recruitment, retention, and attrition of participants. I make some suggestions about how to improve this figure below:

1) The font is very small and hard to read.
2) There are multiple spelling errors, e.g. “surgery” should be “surgery”; “deliery” should be “delivery”.
3) The figure would benefit from having the attritions included in the diagram so readers can understand why the numbers decline across the timeline, e.g. exclusions (including reasons), lost to follow-up, withdrawn etc.
4) Make clear the number of couples who have completed each follow-up stage, and the number who are still being followed up.
5) Provide more detail description in the figure heading about the graphic. Do not use acronyms in the heading. The heading should provide enough detail for the Figure to be understood without needing to read the text.

Figure 2: The exclusions are included in the main components of the flow chart. This chart would be better if modified to have the exclusions in a separate box (refer to the PRISMA flowchart for an example).
Response: Thank you for your suggestion. We have revised the figures according to your comments and combined original figure 1 and 2 into a new figure 1 – “Figure 1: Participant Flow Chart for the Anhui maternal child cohort Study”.

<b>Tables:</b>

Comments: On the whole, the tables are clear and self-explanatory. However, for brevity, Table 3 and 4 be combined.

Response: Thank you for your suggestion. We have merged these two tables into a new Table 3 in the revised manuscript.