A COMPREHENSIVE ANALYSIS OF CHROMOSOMAL POLYMORPHIC VARIATIONS IN FEMALES AND MALES IN THE OUTCOME OF ICSI

by

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A thesis submitted to the University of Birmingham for the degree of

DOCTOR OF PHILOSOPHY

Institute of Metabolism and Systems Research
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University of Birmingham
April 2022

SYNOPSIS

Infertility affects approximately one in eight couples worldwide. Intra cytoplasmic sperm injection (ICSI) is an assisted reproduction procedure in which a single sperm cell is injected directly into the cytoplasm of an egg and is first line treatment for many infertile couples. The live birth rate per ICSI cycle remains low, with over half a million couples remaining childless. Chromosomal polymorphisms are up to five times more common in couples with infertility compared to the general population. Although chromosomal polymorphisms are considered normal variations, some studies suggest they may be associated with adverse fertility outcomes.

To synthesise the existing evidence, I carried out a systematic review and meta-analysis of ten observational studies. I found that there was a slightly higher miscarriage rate observed in female carriers of chromosomal polymorphic variations compared to male carriers. However, the review did not find any adverse effects on rates of pregnancy, clinical pregnancy, on-going pregnancy at study end, pre-term birth and live birth. My main study analysed the chromosomal polymorphic variations of 942 ICSI and frozen embryo transfer (FET) cycles in Sri Lanka. Further, an analysis of types of chromosomal polymorphic variations based on gender, number of polymorphisms and their variants appear to have no adverse effect on reproductive outcomes compared to the couples without chromosomal polymorphism. Hence, my work concluded that chromosomal polymorphisms are unlikely to be associated with adverse reproductive outcomes.

Apart from chromosomal polymorphic variations with routine karyotyping, I found structural chromosomal abnormalities that were important to diagnose before the ICSI

procedure. Hence, routine karyotyping before the ICSI procedure may still have a role outside the research context.

ACKNOWLEDGEMENT

Hereby I would like to say thank you and acknowledge many who encouraged and supported me through the journey of my PhD. Frist and foremost, I would like to thank both my supervisors. Professor Ioannis Gallos for being my supervisor and Professor Arri Coomarasamy for being my co-supervisor and both for giving me this unique learning opportunity. I am very grateful to Professor Vajira Dissanayake of Human genetic unit, Faculty of Medicine, University of Colombo for supervising my work in Lanka hospitals. I am very honoured to learn under their supervision the intricacies of academic research, statistical analysis and pursue a novel thinking during the three years of my PhD.

Secondly, heartfelt thank you to my family who have been exceptionally supportive throughout my studies, most notably my wife Dr. Indika, my daughter Savini and my son Nethum through their love, kindness and patience. My gratitude goes to my parents who were behind my initial steps of education. Many thanks to my siblings and in-laws.

Thirdly, I would like to thank Lanka Hospitals corporation Plc management for accepting and granting the ethical approval to share the database of the Fertility Centre. Especially to Dr. Wimal Karandagoda (DMS) and Dr. Deepthi De Silva Karunaratne (DDMS).

In addition, I render my gratitude to Ms. Sugandika Gajaweera, Fertility Counsellor of Fertility Centre for her tireless support throughout my PhD. She has invested her time giving me useful feedback on my PhD work.

I acknowledge all the staff of Lanka Hospitals Fertility Centre for maintaining the clinical

database and especially Ridma Chandani, Madu Sammani, Thilini Pabasara, Amilka Lasanthi, Menakadevi and Yogaletchami for their support to search and retrieve clinical data for the analysis.

I would like to thank Derick Yates (for search strategy on abstracts in the systematic review and meta-analysis), Dr Malcolm Price (expertise knowledge on Stata statistical analysis) and Dr Sayeed Haque (designing the confidence interval plots) for their contribution towards the manuscripts, in University of Birmingham, and Dr. Nishendra Karunaratne (Consultant O&G) in the Fertility Centre of Lanka Hospitals Corp. Plc.

Thank you to my fellow post graduates' colleagues Pedro Melo, Argyro Papadopoulou, Hajra Khattak and Jayasish Ghosh in IMSR, and Himashi Hapangama and Gimhani Weerakkody, IVF Scientists in Fertility centre, LHC for their advices and support. Thanking Hannah Jeffery in IMSR for the administrative advices.

Special thank you to Nishantha Weerasinghe, Damian Silva, Chrishanthe De Almeida, Anjelo Nicholas, Niranjan Anton, Chatura Cooray, W.M.D. Wanasinghe, S. Sivasupramanium and Wasitha Perera for their continuous support towards fertility centre.

Finally, warm hearted thank you to late Mr. Mahanama Dodampegama for your insight and guidance in my first steps in to the field of Embryology.

May the triple gem bless you all! (In Buddhism)

LIST OF PUBLICATIONS AND TRAINING

This thesis incorporates the three following publications and training:

Manuscript published in *Human Fertility*, (2022)

- **Ralapanawe M.S.B.,** Khattak H., Hapangama H.R., Weerakkody G.R., Papadopoulou A., & Gallos I. (2022). Chromosomal Polymorphisms in Assisted Reproduction: A Systematic Review and Meta-analysis. *Human Fertility* [Preprint]. Available at: https://doi.org/10.1080/14647273.2022.2051614.

Manuscript published and in Reproduction & Fertility, (2022)

- **Ralapanawe M.S.B.,** Gajaweera S.L., Karunaratne N., Price M.J., Melo P., Coomarasamy A., & Gallos I.D. (2022). Chromosomal polymorphisms in assisted reproduction - an analysis of 942 cycles. *Reproduction & Fertility.* 3 (3), pp. 133-139.

Manuscript submitted for publication and in review, (2022)

- **Ralapanawe M.S.B.,** Gajaweera S.L., Karunaratne N., Dissanayake V.H.W., Price M.J., Pedro M., Coomarasamy A., & Gallos I.D. A comprehensive analysis of chromosomal polymorphic variants on reproductive outcomes after intracytoplasmic sperm injection treatment (Under review).

Training:

- **Cochrane Interactive Learning: Conducting an intervention**
  Module 1-11, August 2020 to January 2021

- **Introduction to Epidemiology**
  Bristol Medical School, University of Bristol, Bristol, UK.
  February 22nd -26th, 2021

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LIST OF ABBREVIATIONS

AMH	- Anti Mullerian Hormone
ART	- Assisted Reproductive Techniques
BMI	- Body Mass Index
DNA	- Deoxyribonucleic Acid
ESHRE	- European Society of Human Reproduction and Embryology
FET	- Frozen Embryo Transfer
FSH	- Follicle-Stimulating Hormone
GTG	- Giemsa Trypsin Giemsa banding
GTL	- Giemsa Trypsin Leishman banding
HFEA	- Human Fertilisation & Embryology Authority
ICMART	- International Committee for Monitoring Assisted Reproductive Technology
ICSI	- Intra Cytoplasmic Sperm Injection
IVF	- In-vitro Fertilisation
LH	- Luteinising Hormone
NGS	- Next Generation Sequencing
NICE	- National Institute for Health and Clinical Excellence
PGT	- Pre-implantation Genetic Testing
T4	- Free Thyroxine
TSH	- Thyroid-stimulating Hormone
WHO	- World Health Organisation

CHAPTER 1:

GENERAL INTRODUCTION

Infertility is a critical component of reproductive health and a global public health issue prioritised by the World Health Organisation (WHO) (Mascarenhas *et al.,* 2012). Infertility affects 48.5 million couples worldwide and causes significant psychological and social distress (Mascarenhas *et al.,* 2012; Agarwal *et al.*, 2015; Martinez *et al.,* 2012), especially in developing countries where this social distress can lead to discrimination and ostracism (Rouchou, 2013).

According to the International Committee for Monitoring Assisted Reproductive Technology (ICMART) and WHO, infertility is the inability to conceive after 12 months of regular unprotected sexual intercourse (Datta *et al.,* 2016). The National Institute for Health and Clinical Excellence (NICE) concurs with the time-period and endorses that the clinical investigations for infertility should commence when a woman of reproductive age has not conceived after one year of unprotected intercourse (Datta *et al.,* 2016).

Assisted reproductive techniques (ART) such as in-vitro fertilisation (IVF) and intra cytoplasmic sperm injection (ICSI) are used as treatment options for couples with infertility (Sunderam *et al.,* 2017) (Figure 1 and 2).

There are multiple causes of infertility for males and females such as, age, endocrine disorders, infections, genetic abnormalities and environmental factors (Mascarenhas *et al.,* 2012; Unuane *et al.,* 2011). In terms of genetic abnormalities that cause infertility, most are due to chromosomal structural aberrations, aneuploidies and single gene disorders (Zorrilla and Yatsenko, 2013). Recently, chromosomal polymorphism has also been associated with infertility (Cheng *et al.,* 2017). However, the effects of polymorphic

variations in the reproductive outcomes after ICSI is a subject of controversy, hence this association requires further research.

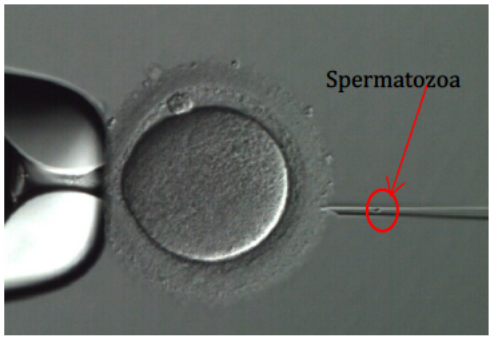

Figure 1: Injecting a spermatozoon into the oocyte (ICSI) (Lanka Hospitals Corporation PLC, 2018. Used with permission)

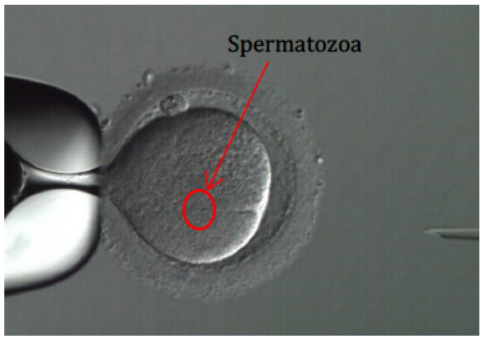

Figure 2: Spermatozoon seen inside the oocyte after injection (Lanka Hospitals Corporation PLC, 2018. Used with permission)

Chromosomal Polymorphic Variations

Chromosomal polymorphic variations are variants in heterochromatic regions of the chromosome (Wyandt and Tonk, 2011). Heterochromatic regions are the non-coding regions of tandem repeats of deoxyribonucleic acid (DNA) (Guo *et al.,* 2012). Variations in these regions of heterochromatin were considered normal as no significant phenotypic effects were observed, hence were termed polymorphisms (Cheng *et al.,* 2017). In the general population, these heritable variants include heterochromatic segments, satellites, satellite stalks, and certain inversions (Hong *et al.,* 2011).

The heterochromatic regions of the chromosome are non-coding regions, which have a functional impact although they were considered as "gene deserted" areas previously (Guo *et al.,* 2012). Functions of heterochromatin in polymorphic regions may suppress or silence gene expression, which could affect spermatogenesis and gametogenesis. This impact can play an important role in both male and female infertility (Xu *et al.,* 2016; Sipek Jr *et al.,* 2014).

Polymorphic variations are individually stable and mostly follow a Mendelian inheritance pattern from one generation to the other (Pokale, 2015). Occurrences of de novo polymorphic variations are rare and when they occur are due to unequal crossover between heterochromatic regions of homologous chromosomes in meiosis (Pokale, 2015). The de novo variants that are larger in size can be associated with different clinical conditions, such as infertility, recurrent miscarriages and even psychiatric disorders (Pokale, 2015).

The euchromatin and the heterochromatin regions of the chromosomes are visible through differential banding techniques in karyotyping (Wyandt and Tonk, 2011). One of the most commonly used banding technique is G-banding, where the euchromatin appears in lighter-coloured bands and the heterochromatin regions will appear in darker coloured bands on the karyotype report (Wyandt and Tonk, 2011) (Figure 3 and 4).

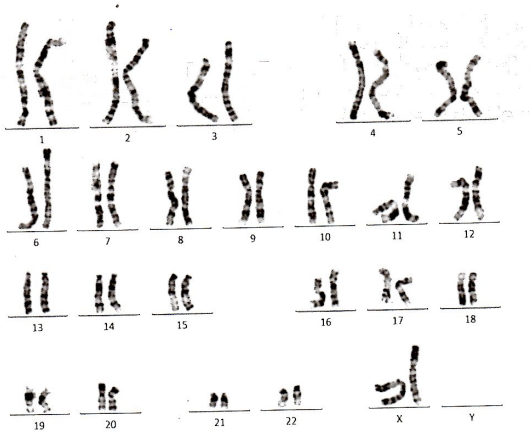

Figure 3: Karyotype of a normal female (Lanka Hospitals Corporation PLC, 2018. Used with permission)

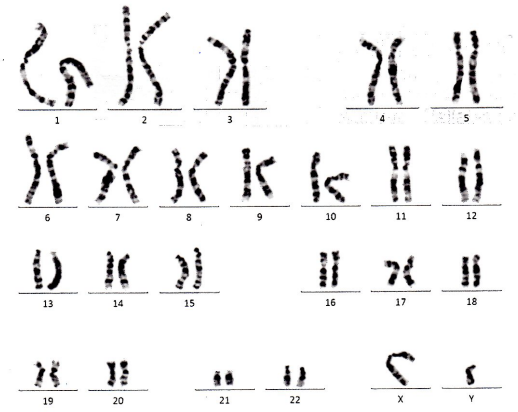

Figure 4: Karyotype of a normal male (Lanka Hospitals Corporation PLC, 2018. Used with permission)

Metacentric and sub-metacentric are the non-acrocentric chromosomes, in which on 1, 9 and 16, the polymorphic variations are usually visible on the long arm of the chromosome (Xu *et al.,* 2016). Here the polymorphic variations (1qh+, 9qh+, 16qh+) are visible as an increase of the length in the heterochromatin region of the long arm of these chromosomes (Liang *et al.,* 2014) (Figure 5).

a) 1qh+ b) 9qh+ c) 16qh+

Figure 5: non-acrocentric chromosomal polymorphic variants (Lanka Hospitals Corporation PLC, 2018. Used with permission)

On acrocentric chromosomes 13, 14, 15 21 and 22, polymorphic variations occur frequently on satellite stalks (pstk+) and satellites (ps+) on the short arms (Xu *et al.,* 2016). Variations in the length of stalks on the short arm of the chromosomes are depicted as 13pstk+, 14pstk+, 15pstk+, 21pstk+ and 22pstk+ (Liang *et al.,* 2014). The polymorphism on the distal arm of the Y chromosome (Yqh+/-) is also an acrocentric chromosome (Erwinsyah *et al*., 2017; Wang *et al.*, 2017) (Figure 6).

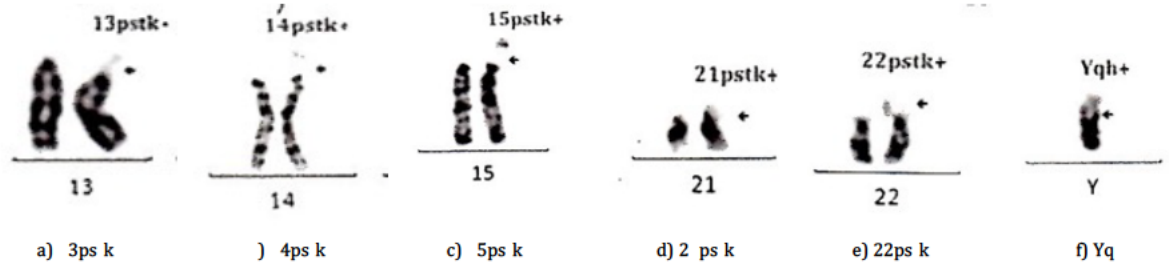

Figure 6: Satellite stalks seen in the acrocentric chromosomes (Lanka Hospitals Corporation PLC, 2018. Used with permission)

Variations in the size of satellites on the short arm of chromosome 13, 14, 15, 21 and 22 are depicted as 13ps+, 14ps+, 15ps+, 21ps+ and 22ps+ (Pokale, 2015) (Figure 7). Additionally, some polymorphic variations are visible as the presence of double satellites on the short arm of the chromosome 14 (14pss+) (Liang *et al.,* 2014).

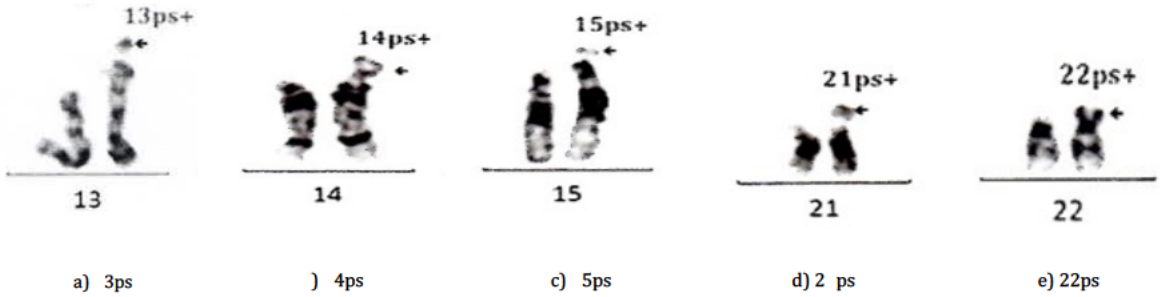

Figure 7: Satellites seen on the short arm of the acrocentric chromosomes (Lanka Hospitals Corporation PLC, 2018. Used with permission)

Pericentric inversions on chromosome 9 [inv (9)] are considered as polymorphic variation (Cheng *et al.,* 2017) (Figure 8). Inversion is a re-arrangement of the chromosome where it undergoes a breakage at two points and then the broken segment

is reinserted in reversed orientation (Kirkpatrick, 2010). There are two types of inversions; pericentric, which includes the centromere, and paracentric where the broken segment does not include the centromere (Kirkpatrick, 2010).

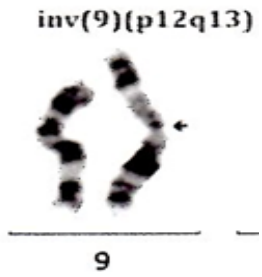

Figure 8: Pericentric inversions on chromosome 9 (Lanka Hospitals Corporation PLC, 2018. Used with permission)

Relationship between Chromosomal Polymorphic Variations and Infertility

It is estimated that the incidence of polymorphic variations in the infertile population is higher (approximately 10 15%) than the general population (2-5%), which indicates a possible association relationship between the polymorphism and infertility (Xu *et al.,* 2016). Certain genes associated with infertility are present in heterochromatin regions, which might undergo transcriptional activation in response to environmental stress (Madon *et al.,* 2005). Additionally, heterochromatin also has vital cellular functions in reproduction and polymorphic variations may have a deleterious effect on fertility (De la Fuente-Cortes *et al,* 2009).

Although published data in respect to occurrence of polymorphic variations in developing South Asian countries are lacking, it is interesting to notice the influence of polymorphic variations in Chinese population. Presence of polymorphic variations has different effects

on males and females (Xu *et al.*, 2016). A study of Chinese population indicated that polymorphic variations in females could be a contributing factor in causing lower embryo cleavage rates (Liang *et al.,* 2014). Another retrospective study showed a strong association between polymorphism and adverse pregnancy outcomes of females with unexplained infertility (Cheng *et al.,* 2017). According to this study, presence of polymorphic variations could be a contributing factor for unexplained infertility in females (Cheng *et al.,* 2017).

Among infertile couples, the occurrence of polymorphic variations was higher in males (Liang *et al.,* 2014). Presence of polymorphism affects spermatogenesis adversely and could affect the outcome of ICSI (Guo *et al.,* 2012). The incidence of polymorphic variations was higher in infertile males than in infertile females, which suggests that polymorphic variations in the Y chromosome can have an adverse effect in male infertility by affecting spermatogenesis and sperm quality (Xu *et al.,* 2016). Additionally, a higher incidence of polymorphic variations is observed in patients with severe oligozoospermia and azoospermia compared to those without it. (Guo *et al.,* 2012).

The centromere plays a vital role in cell division; hence the heterochromatin located at the centromere is equally important in cell division (Gerton and Hawley, 2005). When there is a variation in this region it leads to abnormal meiotic cell division such as flaws in centromere function, kinetochore assembly and difficulty in pairing of homologous chromosomes (Gerton and Hawley, 2005). This leads to disruption of cell division, which would damage the development of functional spermatozoa (Gerton and Hawley, 2005).

A recent study showed that the fertilisation, embryo cleavage and on-going pregnancy rates were low when polymorphic variations were present in males. Additionally, the quality of Day 3 embryos also was poor (Xu *et al.,* 2016). Another study showed that spermatozoa with polymorphic variations may result in abnormal embryonic development and low implantation rates after IVF/ICSI treatment (Guo *et al*., 2012). Furthermore, increased rates of recurrent miscarriage and other adverse obstetric outcomes have been associated with the occurrence of chromosomal polymorphism (Minocherhomji *et al.,* 2009). In addition to the effect of polymorphic variations in infertility, a study represents Turkish population suggested that polymorphic variations (9qh+) could be responsible for recurrent miscarriage (Ahmet Okay *et al.,* 2010). A study done in India revealed a high incidence of polymorphic variations in chromosome 1 and 9 in patients with recurrent miscarriages (Pokale, 2015). Another study also suggested that polymorphic variations could play a critical role in recurrent miscarriage (De la Fuente-Cortes *et al.,* 2009). Another study focused on the Chinese population showed a significant effect on the rate of miscarriages when both partners of the couple are carriers of chromosomal polymorphism (Xiaobin *et al.,* 2012).

Rationale for this PhD

Published data with respect to chromosomal polymorphism in developing countries are limited. Lanka Hospitals Fertility Centre is one of the largest fertility centres in Sri Lanka and performs more than four hundred ICSI cycles per year. I pioneered in establishing the fertility centre and the state-of-the-art IVF laboratory in Lanka Hospitals. I performed routine karyotyping in couples presenting with infertility and a considerable incidence of chromosomal polymorphism exists among our patients. I applied ICSI for all the patients

who have been investigated in routine karyotyping. A meta-analysis to investigate the association of chromosomal polymorphism in IVF or ICSI suggests that ICSI has a statistically significant fertilisation rate compared to standard IVF procedure (Ou *et al*., 2019). The effect of polymorphism on the outcome of ICSI in infertile patients in Sri Lanka is ambiguous, as there is a lack of studies done in regards to chromosomal polymorphism in Sri Lankan population. This thesis aims to answer whether the clinical application of parental karyotyping for the patients undergoing ICSI treatment is beneficial. Therefore, a comprehensive analysis of chromosomal polymorphism will enable us to provide patients with better counselling on the successful pregnancy rate and evaluate whether polymorphism could explain recurrent miscarriages. Thus, it will allow the patient to make better and informed decisions. It will be a bridge between genetics and reproductive outcomes. Lastly, this work would also help us incorporate the latest evidence regarding chromosomal polymorphism in our reproductive medicine practice.

Objectives

1. Review the evidence and examine the association of chromosomal polymorphisms and reproductive outcomes in in different geographical populations.

2. Identify and analyse comprehensively the effect of chromosomal polymorphisms in female, male and couples on reproductive outcomes of ICSI in the Sri Lankan population.

3. Identify and analyse comprehensively the effect of non-acrocentric, acrocentric and their combination of chromosomal polymorphic variants on reproductive outcomes of ICSI in Sri Lankan population.
4. Identify and analyse the effect of chromosomal polymorphism in female, male and couples on fertilisation and cleavage of ICSI in the Sri Lankan Population.

CHAPTER 2:

CHROMOSOMAL POLYMORPHISM IN ASSISTED REPRODUCTION: A SYSTEMATIC REVIEW AND META-ANALYSIS

Abstract

Research question: This systematic review and meta-analysis investigated the effects of chromosomal polymorphisms in reproductive outcomes following IVF or ICSI.

Design: I searched for literature in CENTRAL, CINAHL, EMBASE, and MEDLINE from inception to March 2020 with no language restrictions. I have analysed ten published cohort studies. Studies included females, males and couples undergoing assisted reproductive treatments with presence or absence of chromosomal polymorphisms and reporting on their reproductive outcomes.

Results: Ten studies matched the inclusion criteria and their quality was assessed using the Newcastle-Ottawa Quality Assessment Scale. Meta-Analysis of five cohort studies (9,659 participants) indicate that female carriers with chromosomal polymorphisms have a higher miscarriage rate when compared to non-carriers (risk ratio (RR) 1.54 (95% CI 1.19 to 1.98). No significant association was found for males (RR 0.96, 95% CI 0.64 to 1.43) and couples (RR 1.93, 95% CI 0.32 to 11.83) with chromosomal polymorphisms.

Conclusion: Chromosomal polymorphisms were not associated with a higher rate of biochemical, clinical, ongoing pregnancy at study end, and preterm and live birth. The effect of chromosomal polymorphisms on miscarriage appears to be gender-dependent given the higher miscarriage rate observed amongst female carriers with chromosomal polymorphisms compared to non-carriers.

Introduction

Infertility is considered a critical component of reproductive health and a global public health priority by the World Health Organisation (WHO) (Mascarenhas *et al*., 2012). It is estimated that 48.5 million couples were affected in 2010 (Agarwal *et al*., 2015; Martinez *et al*., 2012). Assisted reproductive techniques (ARTs), such as IVF and ICSI are offered as treatment solutions in couples with fertility issues (Sunderam *et al*., 2017). More than 1 million IVF or ICSI treatment cycles are carried out worldwide every year and this number is steadily increasing (European Society of Human Reproduction and Embryology [ESHRE], 2012; Mascarenhas *et al*., 2012). Despite several improvements in ARTs, the live birth rate for each cycle remains low at about 26% (Human Fertilisation and Embryology Authority [HFEA], 2019). This means that more than 50% of couples are left childless even after multiple treatment cycles (HFEA, 2019).

Chromosomal polymorphisms are variants in the heterochromatic regions of the chromosome (Wyandt & Tonk, 2011). Heterochromatic regions are the non-coding regions of tandem repeats of condensed DNA (Guo *et al*., 2012). Variations within these regions were thought to have no phenotypic effects and hence were characterised polymorphisms (Cheng *et al*., 2017; Serapinas *et al*., 2021). These inherited 'normal' variants include heterochromatic segments, satellites and satellite stalks, and certain inversions (Hong *et al*., 2011; Karaca *et al*., 2020). Further, heterochromatin of centromeric and pericentromeric which derived transcripts, nurture and widen the heterochromatin regions and its complexity invokes whether dynamics of heterochromatin includes inter-chromosomal interaction (Karaca *et al*., 2020). These DNA sequences are involved in gene suppression by silencing transcription of

euchromatin, which may lead to adverse phenotypic expressions (Madon *et al*., 2005; Sipek Jr *et al*., 2014).

The prevalence of chromosomal polymorphisms in the infertile population is up to three times higher than in the general population (Xu *et al*., 2016). Results from advanced molecular techniques, suggest that certain genes that are associated with infertility are present in heterochromatin regions and may undergo transcriptional activation in response to environmental stress (Madon *et al*., 2005). Recent clinical studies also support this association (Xu *et al*., 2016; Liang *et al*., 2014; Cheng *et al*., 2017). The aim of this review is to synthesise the existing evidence and investigate whether the presence of chromosomal polymorphisms adversely affects reproductive outcomes with IVF or ICSI.

Materials and Methods

This systematic review was designed to explore the association between the presence of chromosomal polymorphisms and infertility amongst females, males, and couples undergoing assisted reproductive treatments. However, this study was not pre-registered and it is important to pre-register the systematic review a priori to improve the transparency and the reliability of the study. The included polymorphic variations were heterochromatic segments (qh+/qh-) on the long arm of the chromosome 1, 9, 16 and distal region of the Y chromosome (Yqh+/Yqh-), satellites (ps+/pss), satellite stalks (pstk+) on the short arm of the acrocentric chromosomes 13,14,15,21,22 and peri-centric inversion of the chromosome 9, 16 [Inv(9), Inv(16)]. I compare the reproductive outcomes following IVF or ICSI between carriers and non-carriers of chromosomal polymorphisms.

Inclusion and Exclusion Criteria

Studies reporting results on reproductive outcomes of couples undergoing karyotyping analysis and assisted reproductive techniques (IVF or ICSI) were considered eligible for inclusion in this review. Studies involving participants who underwent treatment with donor oocytes or donor semen, or had known numerical and/or structural chromosomal abnormalities were excluded. Additionally, studies exclusively focusing on male infertility, occurrence of chromosomal polymorphism in patients opting for IVF/ICSI treatment, polymorphism as a form of epigenetic alterations in infertility, or studies focusing on embryo development and not on reproductive outcomes were also excluded from the review.

Literature Search

Literature searches were carried out by an information specialist on CENTRAL, Cochrane Library, CINAHL, EMBASE, and MEDLINE with no language restrictions (from inception to March 2020). The search strategy used the following keywords and/or medical subject headings (MeSH) terms: infertility, assisted reproduction, in-vitro fertilisation, intracytoplasmic sperm injection, chromosome, cytogenetic, karyotyping, polymorphism, polymorphic variants or variation, chromosome aberration or differentiation, chromosome anomaly or abnormality.

Study Selection and Data Synthesis

Two reviewers (M.R. and H.K.) screened all records for inclusion independently. Each study title and abstract were either included or excluded, and any disagreements were

resolved through discussion with a third reviewer (I.D.G.). The full manuscripts of the titles and abstracts pertaining to the inclusion criteria of this review were obtained. In case of duplication of articles, the most up to date and complete version was selected. Those studies that did not explicitly report any reproductive outcomes amongst carriers and non-carriers of chromosomal polymorphisms were excluded. Two reviewers (M.R. and H.K.) extracted outcome data in duplicate from the included studies.

Study Quality Assessment

Two reviewers (M.R. and H.K.) assessed the quality of the included studies using the Newcastle-Ottawa Quality Assessment Scales for observational studies independently (Wells *et al.*, 2019). This scoring scale ranges from zero to nine. One star is awarded for each of the following categories; case-cohort representative, ascertainment of exposure, outcome negative at commencement of study, outcome assessment, duration and adequacy of follow-up, whereas two stars can be awarded for comparability by design or analysis. Following the assumption that all items have equal weighting, an arbitrary score was assigned to each study, in order to give a quantitative appraisal of its overall quality. Assessment for publication bias could not be performed due to the limited number of available studies.

Statistical Analysis

Biochemical pregnancies, miscarriages, clinical pregnancies, ongoing pregnancies at study end, pre-term births and live birth rates were extracted from each of the included studies according to the presence or absence of chromosomal polymorphisms in females, males or couples undergoing IVF or ICSI treatment. The log of the ratio and its standard

error was computed for each of the studies included in the analysis. Pairwise meta-analysis with inverse variance weighting was performed in order to calculate the random-effects summary estimates. The square root of this number is the standard deviation (SD) of the underlying effects across all analysed studies. The estimated Confidence intervals (CIs) were centered on the natural logarithm of the pooled effect estimate, and the limits exponentiated to obtain an interval on the ratio scale. Forest plots, showing both individual study proportions with their respective CIs and the overall Der Simmonian & Laird pooled effect estimate according to presence of chromosomal polymorphisms, were computed for each of the pre-specified outcomes of interest. Heterogeneity of the effect estimates was assessed graphically using forest plots and was analysed statistically with the Chi-square test. All statistical analyses were performed using Review Manager 5.3 (Cochrane Community, London, UK).

Results

The literature search identified 2,787 study records, of which only 47 met the pre-specified inclusion criteria. Amongst these, I excluded 18 records that assessed ineligible research questions, 14 that were only available as oral communications, three that were case-reports, one study that reported on multinucleation of embryos rather than reproductive outcomes and one study that reported on IVF failure thus not reporting on any reproductive outcomes. Ten observational studies were included in the systematic review and the meta-analysis (Figure 9).

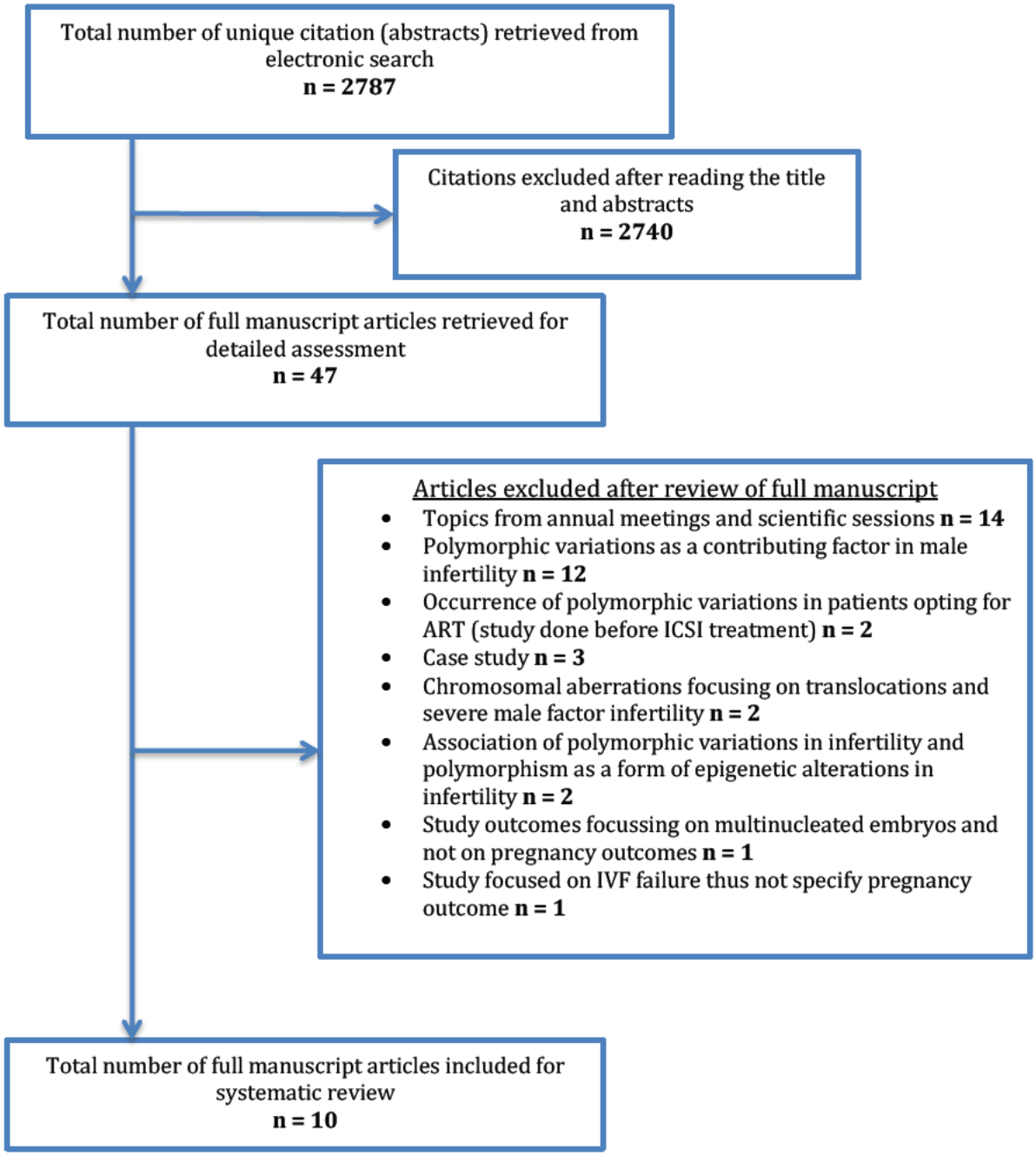

Figure 9: PRISMA flow chart

Study Characteristics

The study characteristics of the ten included studies are presented in Table 1. All included studies were retrospective cohorts, published between 2005 and 2017. The study sample sizes varied between 210 and 19,950. In all but one study, females were aged 40 years or

less. All protocols performed chromosome karyotyping prior to IVF or ICSI, using Giemsa-Trypsin-Giemsa (GTG) banding with peripheral blood. None of the studies used donor oocytes or sperms. Seven studies followed controlled ovarian stimulation protocol (long protocol), while two studies followed both long and short protocols. There was one study in which the stimulation protocol was not described. No conflicts of interest were identified and all ten studies scored high using the Newcastle-Ottawa Quality Assessment; nine studies obtained a score of 8 and one obtained a score of 7 (Table 2).

Biochemical Pregnancies

Six studies reported results by the presence of chromosomal polymorphisms for biochemical pregnancies following IVF or ICSI (Table 1). Three studies reported results for females and males individually; two focused exclusively on males, and one involved couple. Pairwise meta-analysis of the three studies involving females only (766 females) suggests that the presence of chromosomal polymorphisms makes no difference to biochemical pregnancy rates (RR 1.01, 95% CI 0.92 to 1.10, I^2=6%, *P*=0.35, Figure 10). Pairwise meta-analysis of the five studies involving males only (1,517 males) suggests that the presence of chromosomal polymorphisms makes little or no difference to biochemical pregnancy rates (RR 0.93, 95% CI 0.84 to 1.04, I^2= 59%, *P*=0.05, Figure 10). Only one study involved couples (1,108 couples), which also suggests that the presence of chromosomal polymorphisms makes no difference to biochemical pregnancy rates (RR 1.06, 95% CI 0.86 to 1.29, Figure 10).

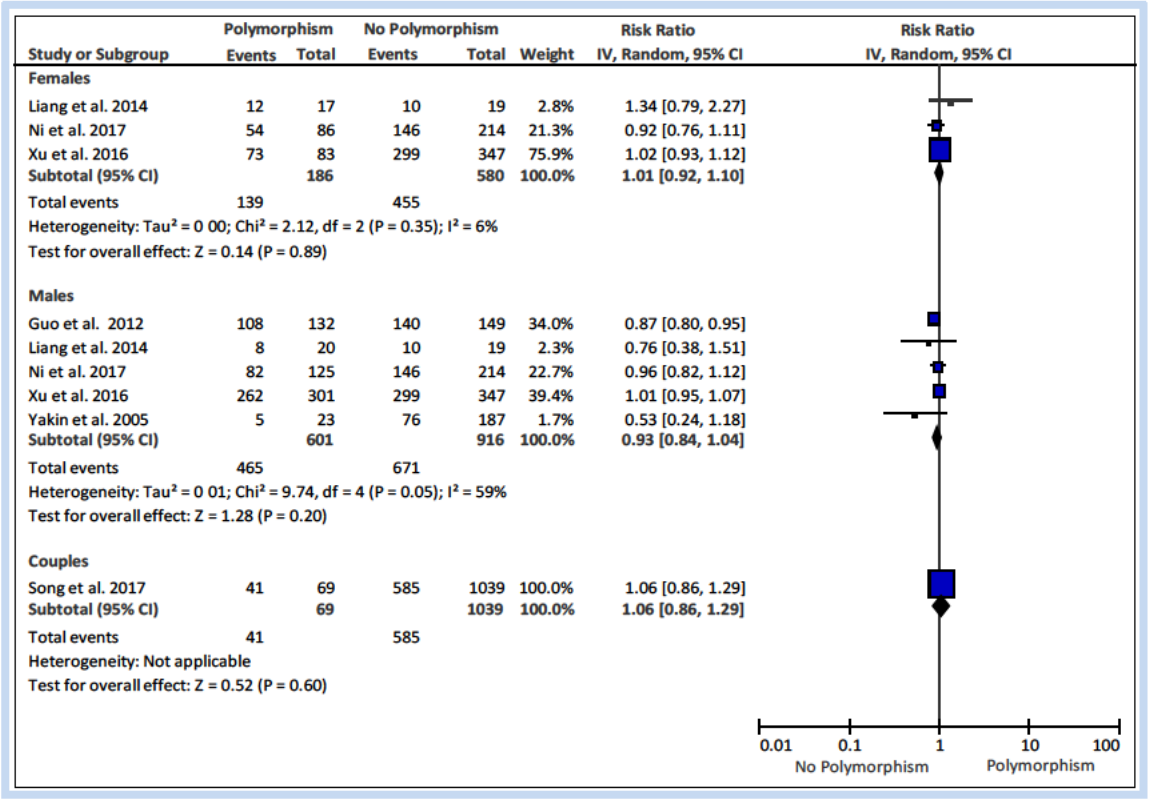

Figure 10: Forest plot with relative risk ratios and 95% CIs from pairwise meta-analysis of biochemical pregnancy by gender

Table 1: Characteristics of the included studies

Author (year)	Study design	Study population	Age of study Population	Patient type	Type of specimen	Method of Cytogenetic analysis	Laboratory protocol	Classification of chromosomal variations	Recorded Polymorphic variations	Stimulation protocol and ART technique used	Outcome assessed
Cheng *et al.* (2017)	Retrospective study	19,950 women 16,285 Infertile women, and 3,665 fertile women as the control group including 819 who are nullipara at the time of karyotype analysis in West China	Aged between 20 44 yrs Control group between 20 44yrs	Female	Peripheral blood	Karyotype analysis	GTG Banding	Based on international cytogenetic nomenclature	Stalks lengths on the short arm of acrocentric chromosomes (pstk+) Changes of satellites on the short arm of chromosomes 13/14/15/ 21/22 (pss/ps+) Pericentric Inversions of chromosomes 9/16[inv[9] and inv[16] Length of the pericentric heterochromatin on the long arm of chromosomes (qh+)	Controlled ovarian stimulation with Clomiphene, Letrozole, HCG, Gonadotrophins ART techniques including IUI, IVF, ICSI, ET, FER	Miscarriages Pre term birth
Guo *et al.* (2012)	Retrospective study	281 infertile couples underwent IVF/ICSI in China	Mean age of female control group 32.07 yrs Mean age of female in chromosomal polymorphism group 30.35 yrs	Male	Peripheral blood Peripheral blood	Karyotype analysis Azoospermic factor micro deletion of Y chromosome	GTG Banding M PCR	Based on international system for Chromosomal Nomenclature 2009 Based on EAA, EMQN and previous protocols	1, 9, 16qh+/ Y chromosome variation CPVs in D/G genomes Inversions of 9 and Y chromosome	Down regulation long protocol with rFSH and GnRH agonist ART Technique IVF/ICSI ET	Fertilisation rate Early miscarriage rate Pregnancy rate Clinical pregnancy rate Ongoing pregnancy rate

											Pre term birth rate
Hong *et al.* (2011)	Retrospective study	1,689 infertile couple undergoing IVF/ICSI treatments in China	Female mean age ranges from 29.47 to 29.78 yrs	Female and male	Peripheral blood	Karyotype analysis	GTG Banding	Based on international system for Chromosomal Nomenclature 2009	1, 9, 16qh+ Polymorphic variations in D/G genomes Inv(9) Y chromosome variation Multiple variation	Long and short down regulation protocol	Embryo implantation rate Clinical pregnancy rate Early miscarriage rate Ongoing pregnancy rate
Liang *et al.* (2014)	Retrospective study	614 Sub fertile couples in China	Mean age of female ranges from 31.40 to 33.18 yrs Mean age male ranges from 28.63 to 32.29 yrs	Female and male	Peripheral blood	Karyotype analysis	GTG Banding	Based on international cytogenetic nomenclature 2009	Yqh+ 1qh+, 9qh+, 16qh+ Double satellite on the short arm of chromosome 14 (14pss) Length of stalks on the short arm of chromosome 13/14/15/ 21/22 (pstk+/pstk) Inv(9)	Long luteal down regulation protocol Art technique IVF, ICSI	Fertilisation rate Cleavage rate Good quality embryo rate Pregnancy rate Biochemical pregnancy rate Clinical pregnancy rate Early spontaneous abortion rate Ongoing pregnancy rate Delivery rate
Ni *et al.* (2017)	Retrospective study	425 infertile couples who undergoing IVF in China	Female age between 20 40 yrs	Female and male	Peripheral blood	Karyotype analysis	GTG Banding	Based on international cytogenetic nomenclature 2009	1, 9, 16qh+ Polymorphic variations in D/G genomes Inv(9)	Controlled ovarian stimulation with GnRH agonist combined with r FSH	Biochemical pregnancy rate Clinical pregnancy rate Ongoing pregnancy rate

									Y chromosome variation	Fresh or frozen thawed embryo transfer	Early miscarriage rate Pre term birth rate Live birth rate Cumulative live birth rate
Song *et al.* (2017)	Retrospective study	1,108 infertile couples undergoing IVF/ICSI ET in China	Female age ≤38 yrs	Couples	Peripheral blood	Karyotype analysis	GTG Banding	Based on International Naming System for Human Cytology	1, 9, 16qh+/ Y chromosome variation Polymorphic variations in D/G genomes Inv(9)	Standard long and short protocol IVF/ICSI ET	Implantation rate Clinical pregnancy rate Early abortion rate Live birth rate
Xiao *et al.* (2012)	Retrospective study	72 Yqh+ carriers and 986 Yqh+ non carriers underwent IVF/ICSI ET in China	Mean age of female control group 31.87 yrs Mean age of female Yqh+ carrier group 31.41 yrs	Male	Peripheral blood	Karyotype analysis FISH analysis	GTG Banding	Based on international system for Chromosomal Nomenclature 2009	Yqh+	Down regulation protocol with rFSH and GnRH agonist ART Technique IVF/ICSI ET	Clinical pregnancy rate Fertilisation rate Cleavage rate Good quality embryo rate Implantation rate ‘ Cycle cancellation rate
Xiaobin, *et al.* (2012)	Retrospective study	1,584 infertile couples underwent IVF ET in China	Mean age of female ranges from 30.24 to 31.55yrs Mean age of male ranges from 33.51 to 34.29yrs	Couples	Peripheral blood	Karyotype analysis	GTG Banding	Based on international naming system for Human Cytogenetics 2005	1, 9, 16qh+/ Y chromosome variation Polymorphic variations in D/G genomes Inv(9)	Long protocol ART technique IVF ET	Fertilisation rate Effective rate of embryo Clinical pregnancy rate Early miscarriage rate

Xu *et al.* (2016)	Retrospective study	847 infertile couples undergoing IVF/ICSI treatments in China	No specific age range mentioned Female age <40 yrs	Female and male	Peripheral blood	Karyotype analysis	GTG Banding	Based on international system for Chromosomal Nomenclature 2013	1, 9, 16qh+ Polymorphic variations in D/G genomes Pericentric inversions of chromosomes 1, 9, and Y Y chromosome variation	Long luteal down regulation protocol Art technique IVF/ICSI ET	Fertilisation rate Cleavage rate Good quality embryo rate Pregnancy rate Biochemical pregnancy rate Clinical pregnancy rate Early spontaneous abortion rate, ongoing pregnancy rate Delivery rate
Yakin *et al.* (2005)	Retrospective study	210 infertile men with severe OATS in Turkey	Mean age control group 36.6 yrs Mean age of group with polymorphism 37.7 yrs	Male	Peripheral blood Semen sample	Karyotype analysis Y chromosome analysis FISH analysis	GTG Banding	Not reported	Heterochromatin polymorphism (1qh+, 9qh+, 16qh+, 18qh+, Yqh+) Sperm aneuploidy rate for chromosomes 13/18/21/X/Y	ART techniques	Implantation rate Clinical pregnancy rate

Table 2: Appraisal of methodological quality (Newcastle -Ottawa Scale) of included studies

Study	Representativeness of exposed cohort (S)	Selection of the non-exposed cohort (S)	Ascertainment of exposure (S)	Outcome negative at start (S)	Comparability by design/analysis (C)	Outcome assessment (O)	Duration of follow-up (O)	Adequacy of follow-up (O)	Score 9
Cheng *et al.* 2017	*	*	*	*	*	*	*	*	8
Guo *et al.* 2012	*	*	*	*	*	*	*	*	8
Hong *et al.* 2011	*	*	*	*	*	*	*	*	8
Liang *et al.* 2014	*	*	*	*	*	*	*	*	8
Ni *et al.* 2017	*	*	*	*	*	*	*	*	8
Song *et al.* 2017	*	*	*	*	*	*	*	*	8
Xiao *et al.* 2012	*	*	*	*	*	*	*	*	8
Xiaobin *et al.* 2012	*	*	*	*	*	*	*	*	8
Xu *et al.* 2016	*	*	*	*	*	*	*	*	8
Yakin *et al.* 2005	*	*	*	*	*	*	X	*	7

Miscarriages

Eight studies reported results by the presence of chromosomal polymorphisms for miscarriages following IVF or ICSI (Table 1). Miscarriage rate refers to both early and late pregnancy losses in the studies. Early miscarriage defines as a pregnancy loss before 12 weeks of gestational age and late miscarriage by the 20th week of the gestation. Four studies reported results for females and males individually; one focused exclusively on females; one focused on exclusively on males, and two involved couples. Pairwise meta-analysis of the five studies involving females only (9,659 females) suggests that the presence of chromosomal polymorphisms is associated with a higher miscarriage rate compared to females without polymorphisms (RR 1.54, 95% CI 1.19 to 1.98, I^2=0%, *P*=0.73, Figure 11). Pairwise meta-analysis of the five studies involving males only (2,896 males) suggests that the presence of chromosomal polymorphisms makes little or no difference to the miscarriage rates (RR 0.96, 95% CI 0.64 to 1.43, I^2= 31%, *P*=0.22, Figure 11). Two studies involved couples (2,044 couples), which also suggests that the presence of chromosomal polymorphisms makes no difference to miscarriage rates (RR 1.93, 95% CI 0.32 to 11.83, I^2 79%, *P*=0.03, Figure 11).

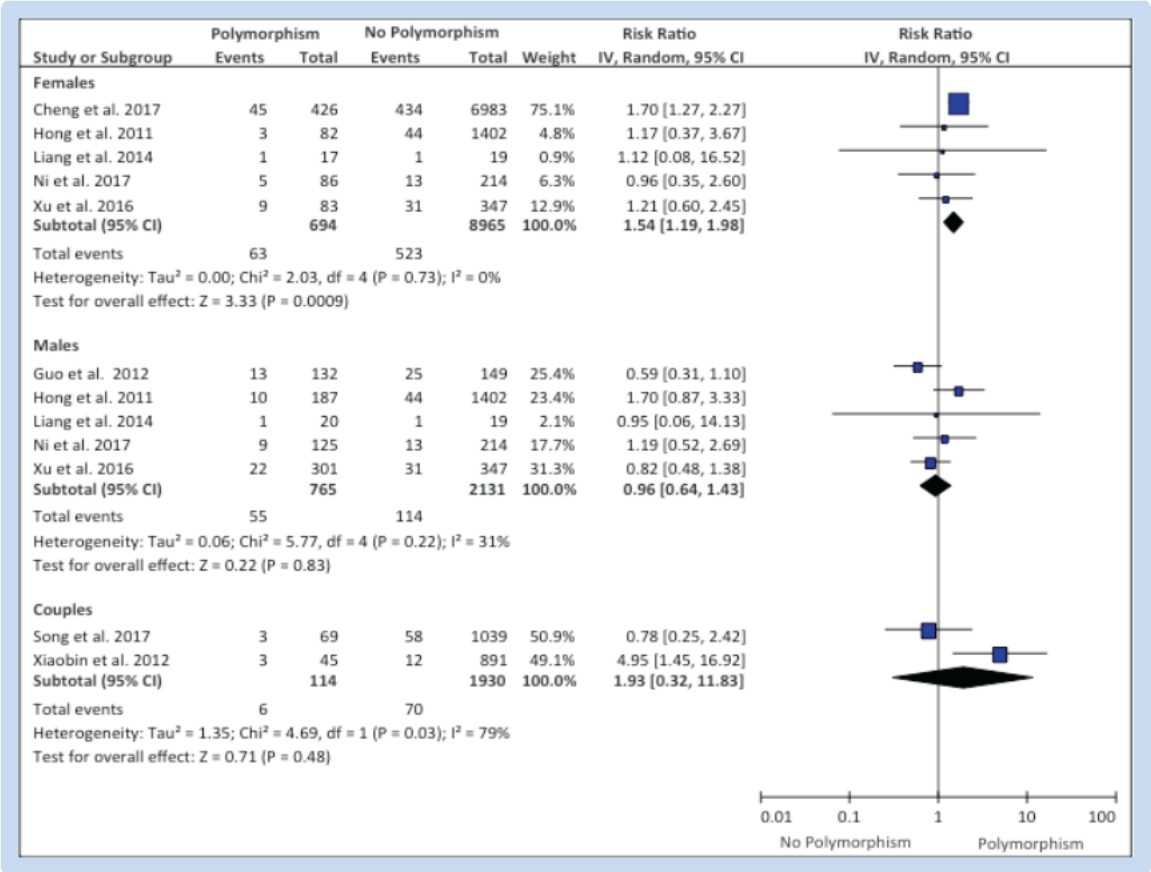

Figure 11: Forest plot with relative risk ratios and 95% CIs from pairwise meta-analysis of miscarriage by gender

Clinical Pregnancies

Nine studies reported results according to the presence of chromosomal polymorphisms for clinical pregnancies following IVF or ICSI (Table 1). Four studies reported results for females and males individually; three focused exclusively on males, and two involved couples. Pairwise meta-analysis of the four studies involving females only (9,655 females) suggests that the presence of chromosomal polymorphisms makes no difference to clinical pregnancy rates (RR 1.00, 95% CI 0.86 to 1.16, I2=27%, P=0.25, Figure 12). Pairwise meta-analysis of the seven studies involving males only (4164 males) suggests

that the presence of chromosomal polymorphisms makes little or no difference to clinical pregnancy rates (RR 0.90, 95% CI 0.78 to 1.03, I2=65%, P=0.008, Figure 12). Two studies involved couples (2,044 couples), which also suggests that the presence of chromosomal polymorphisms makes no difference to clinical pregnancy rates (RR 0.99, 95% CI 0.80 to 1.21, I2 0%, P=0.46, Figure 12).

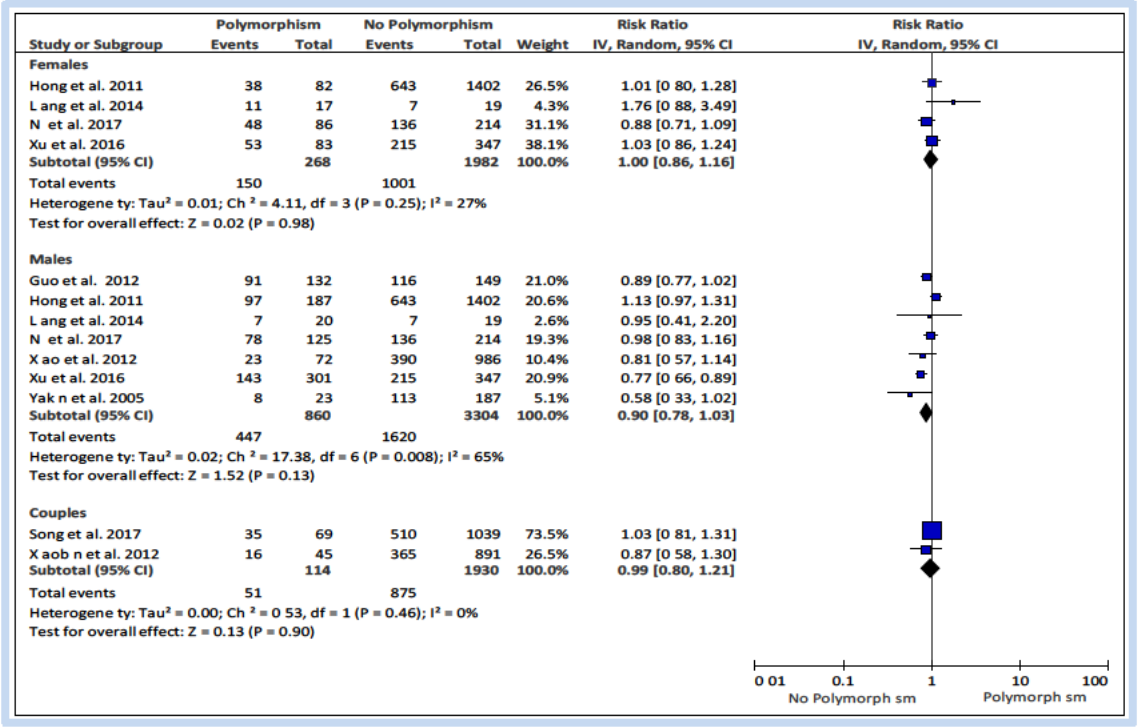

Figure 12: Forest plot with relative risk ratios and 95% CIs from pairwise meta-analysis of clinical pregnancy by gender

Ongoing Pregnancies

Five studies reported results by the presence of chromosomal polymorphisms for ongoing pregnancies following IVF or ICSI (Table 1). Four studies reported results for females and

males individually; one focused exclusively on males. Pairwise meta-analysis of the four studies involving females only (2,250 females) suggests that the presence of chromosomal polymorphisms makes no difference to ongoing pregnancy rates (RR 1.01, 95% CI 0.82 to 1.23, I^2=44%, *P*=0.15, Figure 13). Pairwise meta-analysis of the five studies involving males only (2,896 males) suggests that the presence of chromosomal polymorphisms makes little or no difference to ongoing pregnancy rates (RR 0.94, 95% CI 0.80 to 1.11, I^2= 63%, *P*=0.03, Figure 13).

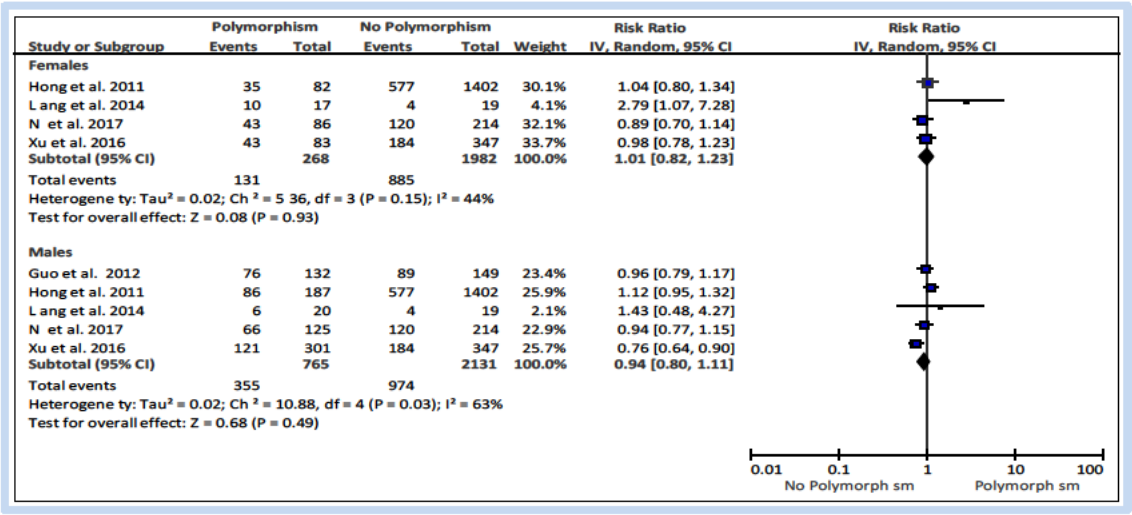

Figure 13: Forest plot with relative risk ratios and 95% CIs from pairwise meta-analysis of ongoing pregnancy at study end by gender

Preterm Births

Three studies reported results by the presence of chromosomal polymorphisms for preterm births following IVF or ICSI (Table 1). The preterm birth is defined as live birth before 37 weeks of the gestational age. One study reported results for females and males

individually; one focused exclusively on females, and one involved exclusively on males. Pairwise meta-analysis of the two studies involving females only (7,709 females) suggests that the presence of chromosomal polymorphisms makes no statistically significant difference to preterm birth rates (RR 2.28, 95% CI 0.70 to 7.45, I^2=82%, *P*=0.02, Figure 14). Pairwise meta-analysis of the two studies involving males only (620 males) suggests that the presence of chromosomal polymorphisms makes little or no difference to preterm birth rates (RR 1.34, 95% CI 0.78 to 2.29, I^2= 0%, *P*=0.54, Figure 14).

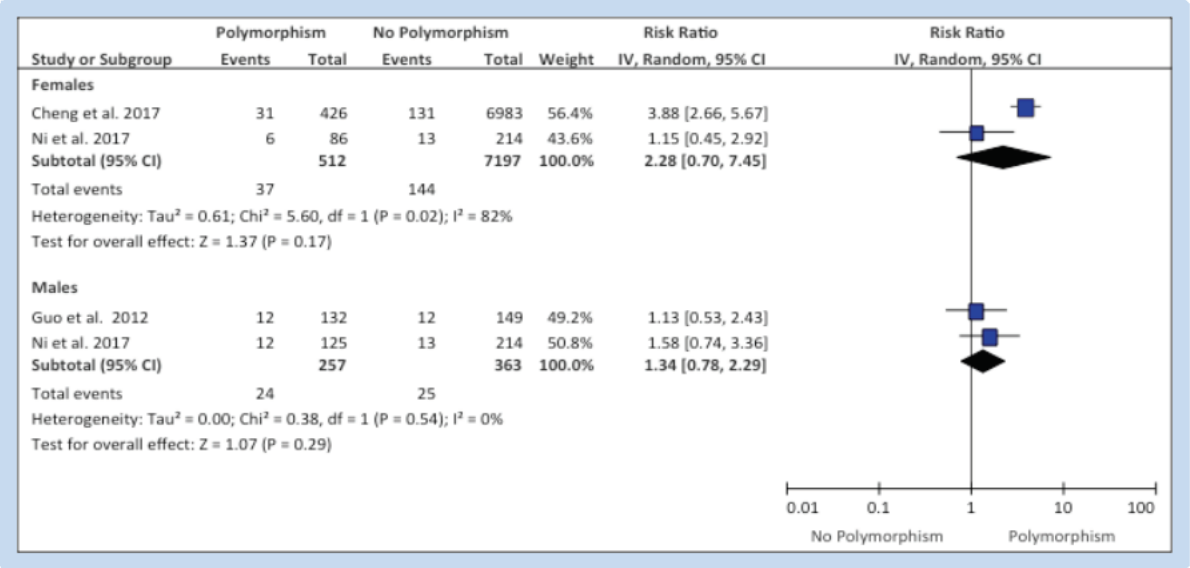

Figure 14: Forest plot with relative risk ratios and 95% CIs from pairwise meta-analysis of preterm birth by gender

Live Births

Four studies reported results by the presence of chromosomal polymorphisms for live births following IVF or ICSI (Table 1). Three studies reported results for females and males individually and one involved couple. Pairwise meta-analysis of the three studies

involving females only (766 females) suggests that the presence of chromosomal polymorphisms makes no difference to live birth rates (RR 1.02, 95% CI 0.80 to 1.28, I^2=33, *P*=0.22, Figure 15). Pairwise meta-analysis of the three studies involving males only (1,026 males) suggests that the presence of chromosomal polymorphisms makes little or no difference to live birth rates (RR 0.84, 95% CI 0.70 to 1.02, I^2= 29%, *P*=0.24, Figure 15). Only one study involved couples (1,108 couples), which also suggests the presence of chromosomal polymorphisms makes no difference to live birth rates (RR 1.04, 95% CI 0.79 to 1.38, Figure 15).

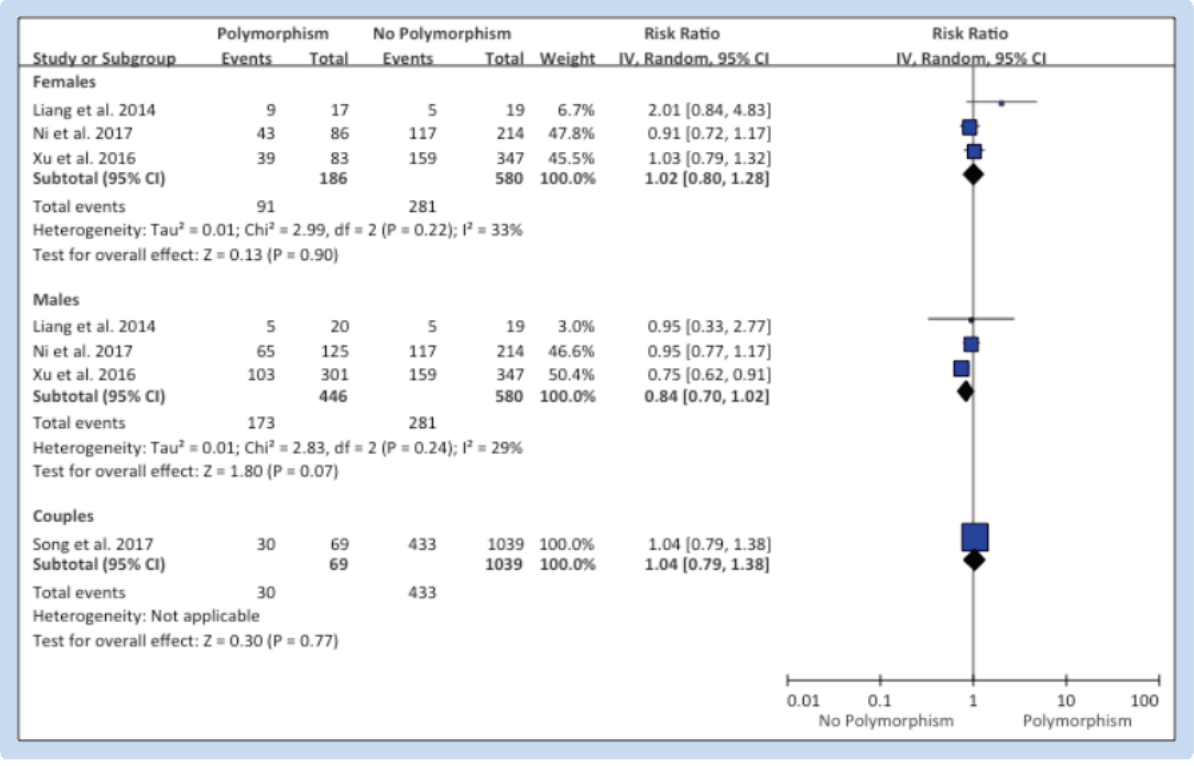

Figure 15: Forest plot with relative risk ratios and 95% CIs from pairwise meta-analysis of live birth by gender

Discussion

Ten observational studies were included in the review and the meta-analysis. I found a higher miscarriage rate when females are carriers of chromosomal polymorphisms, but males and couples with polymorphism showed no significant risk in miscarriage. There is little or no evidence that chromosomal polymorphisms in individuals (males or females) or couples that they adversely affect the chance of a pregnancy, clinical pregnancy, on-going pregnancy at study end, pre-term births and live births following IVF or ICSI. However, only a few studies were available for analysis resulting in a high degree of statistical uncertainty for most of the findings in this review.

I used a comprehensive search strategy to identify all relevant observational studies, with no language restrictions. I assessed the overall quality of the included studies using the Newcastle-Ottawa Quality Assessment Scale. The fact that all ten included studies scored high during this quality assessment suggesting most studies were at a low risk of bias. Most of the studies included populations of a similar age and ovarian reserve, but there was heterogeneity in the ovarian stimulation protocols used. Despite this, I encountered a low level of statistical heterogeneity in most analyses.

Caution is required when interpreting the findings as only a few studies were included in the meta-analysis and most of the estimates in this review have wide confidence intervals. Hence, there is still substantial uncertainty about how the presence of chromosomal polymorphisms affects reproductive outcomes after IVF or ICSI. Additionally, in several analyses of this review there was evidence of substantial heterogeneity. I expect this to be

a result of difference in the populations included and the assisted reproductive treatments used. However, in most cases, I observed low level of heterogeneity, which could be attributed to low power to detect substantial heterogeneity in view of the small number of included studies.

There is further uncertainty about which carrier is more important with some studies focusing on males, other studies on females and a few studies on couples. Recent studies suggest that recurrent pregnancy losses associated with acrocentric chromosomal polymorphism in Chinese women (Feng *et al*., 2021), while another study suggest Y chromosome in Chinese men also associated with recurrent miscarriages (Wang *et al*., 2017). Further, inversion in men and women if Chinese ethnicity adversely increase miscarriage rate (Li *et al*., 2020). The findings of my systematic review are not easily generalisable as most studies involved participants of Chinese origin and extrapolation to other populations may not be appropriate.

Overall, this systematic review found only a few studies contributing data to each analysis resulting in unstable estimates. Whilst there may be an association between female carriers with chromosomal polymorphism and miscarriage rate, this is due to one study with higher weight that does not report live births. Hence overall female polymorphism has no effect on live births. Further research is required to confirm the association of female carrier with chromosomal polymorphisms and miscarriage, and strengthen the certainty of the evidence for the other reproductive outcomes. For improving the generalisability of the evidence, more studies are required involving different ethnicities. Studies would need to examine polymorphism in both males and females to identify if

there is an association with adverse reproductive outcomes following IVF or ICSI, and explore if there is a biological gradient when both males and females are affected. I am addressing this research gap in Chapter 3. Furthermore, most of the studies examine the effect of different chromosomal polymorphisms pooled together; studies focusing on specific types of chromosomal polymorphisms may unearth new knowledge about the potential clinical effects of each individual polymorphism. I am addressing this need in Chapter 4.

CHAPTER 3:

CHROMOSOMAL POLYMORPHISMS IN OUTCOME OF ASSISTED REPRODUCTION: AN ANALYSIS OF 942 CYCLES

Abstract

Research Question: In this study, I investigate the association between chromosomal polymorphisms and reproductive outcomes in couples undergoing ICSI treatment.

Design: I analysed 942 ICSI and frozen embryo transfer cycles in 697 women who underwent karyotyping analysis using Giemsa Trypsin Leishman banding (GTL-banding) prior to ICSI treatment in the Fertility Centre of Lanka Hospitals, Sri Lanka between 2016 and 2018. The primary outcomes were pregnancy, miscarriage, and live birth rates. I compared outcomes according to the presence or absence of chromosomal polymorphism amongst females, males and couples.

Results: There were 294 pregnancies (31.2%) recorded in the study, 130 suffered a miscarriage (13.8%), 13 were ectopic pregnancies (1.3%), and 151 resulted in a live birth (16.0%). The evidence did not confidently identify a difference in pregnancy, miscarriage or live birth rates between couples with no chromosomal polymorphisms compared to couples where the female, male or both were carriers of a chromosomal polymorphism. This was the case in univariable and multivariable (adjusted for age, body mass index, ovarian reserve and treatment type) analyses.

Conclusion: The evidence did not identify a clear association between the presence of chromosomal polymorphism in females, males or couples and reproductive outcomes compared to couples without chromosomal polymorphism. Wide confidence intervals precluded the identification of clinically meaningful associations.

Introduction

Infertility is common, affecting one in eight couples (12%) worldwide (ESHRE, 2020). Assisted reproductive Technology, including in vitro fertilisation and intra cytoplasmic sperm injection, is the mainstay treatment for couples with infertility. More than 1 million ART cycles are performed worldwide every year and this number is steadily increasing (ESHRE, 2012; Mascarenhas *et al*., 2012). The use of ICSI has become particularly popular in recent years, with double the number of cycles globally compared to conventional IVF (ESHRE, 2018). However, the live birth rate per cycle relatively low (~30%) and over half of infertile couples remain childless (HFEA, 2016).

Chromosomal polymorphisms are normal variations that occur in 2-5% of the general population. These variations are seen in the genetically inactive heterochromatic regions of chromosomes (Wyandt *et al*., 2017) and have no clear impact on phenotype (Brothman *et al*., 2006). Chromosomal polymorphisms in many species result in reduced fertility (Kerkpatrick *et al*., 2010). In humans, they are also up to five times more common in couples with infertility compared to the general population (Xu *et al*., 2016). The presence of polymorphism affects spermatogenesis adversely and could be detrimental to the outcome of ICSI (Nakamura *et al*., 2001; Yakin *et al*., 2005). In addition, increased rates of recurrent miscarriages and other adverse obstetric outcomes have been associated with chromosomal polymorphism (Ahmet Okay *et al*., 2010; Minocherhomji *et al*., 2009; Pokale, 2015).

My systematic review in Chapter 2, found that chromosomal polymorphism in assisted reproduction is associated with higher rates of miscarriage. The findings were only

present in female carriers of chromosomal polymorphism compared to male carriers (Ralapanawe *et al.,* 2022a). The review did not find evidence that chromosomal polymorphisms have any adverse effects on rates of pregnancy, clinical pregnancy, on-going pregnancy at study end, pre-term birth and live birth after IVF or ICSI, irrespectively of whether the carrier was the female partner, the male, or both. Further research is necessary to confirm the association between polymorphic variations in females and miscarriage, and to strengthen the certainty of the evidence for other reproductive outcomes. If miscarriage rates are indeed higher; it is reasonable to hypothesise that there could be a knock-on effect on other pregnancy outcomes. This study explores the association between chromosomal polymorphisms and reproductive outcomes in couples undergoing ICSI treatment.

Materials and methods

Study Design

This was a prospective cohort study of couples undergoing a cycle of ICSI treatment and karyotyping analysis prior to IVF at Fertility Centre of Lanka Hospitals Corporation Plc, Sri Lanka, from January 2016 to December 2018. Pregnancy outcomes were collected until November 2019.

I excluded couples undergoing treatment with donor gametes, numerical or structural abnormalities in karyotyping or absence of karyotyping reports, poor follicular development, abnormal cleavage or blastocyst formation, freeze-all cycles and records where pregnancy outcomes had not been documented.

Karyotype Analysis

Karyotyping was performed on peripheral blood leukocytes. The standard laboratory protocol using Giemsa Trypsin Leishman - banding (GTL-banding) was followed for all samples. Twenty metaphases were counted and analysed. Four to five karyotypes were analysed at a banding resolution of 550x. The karyotyping results were reviewed by two analysts independently. The karyotyping grouped based on the international system for human cytogenetics nomenclature (ISCN) (Shaffer *et al,* 2013).

Ovarian Stimulation, ICSI and Embryo Culture

All female participants were stimulated with a long protocol using gonadotrophin-releasing hormone (GnRH) agonist 0.1mg (Decapeptyl, Ferring GmbH, Wittland, Germany) combined with recombinant FSH 150IU-450IU (Gonal F, Merck Serono, Modugno (BA), Italy) or short protocol with GnRH antagonist 0.25mg (Cetrotide, Baxter Oncology GmbH, Halle, Germany) combined with recombinant FSH 150IU 450IU. After the evaluation of estradiol level (1,000pg/ml to 5,000pg/ml) on the tenth day, hCG 250mcg (Ovidrel, Merck, Serono S.p.A., Modugno (BA), Italy) was administered. Oocyte recovery was performed 35 hours after the hCG injection. Following oocyte insemination with ICSI, embryos were cultured (Vitrolife Sweden AB, V.Frolunda, Sweden) up to three days. All embryos with more than six cells were selected. Two embryos were transferred per fresh cycle and the remaining embryos were vitrified. In women where a fresh transfer was not possible, I performed cryopreservation of all embryos and carried out frozen embryo transfer at a later date. Endometrial preparation proceeded with estradiol valerate (Progynova 2mg, Delpharm Lille sas, Lys-Lez-Lannoy, France) followed by a transvaginal scan on the 13th day of the estradiol tablets to evaluate the endometrial

thickness (More than 7mm). Luteal phase supported by Progesterone gel (Crinone 8%, Fleet laboratories Ltd, Hertfordshire, UK).

Embryo Transfer

Two cleavage stage fresh embryos were transferred per cycle, and the remaining embryos were vitrified. Subsequent frozen embryo transfer cycles involved warming and transfer at cleavage stage (6 to 8 cells) or further culture of embryos for two days until blastocyst formation.

Outcomes and Follow-up

Pregnancy was confirmed two weeks after embryo transfer (Serum β HCG >10mIU/ml). The primary outcomes included pregnancy rate (gestational age 4 to 6 weeks), miscarriage rate (gestational age less than 12 weeks) and livebirth rate (gestational age over 32 weeks). Outcome data were analysed with female, male and couple according to the presence or absence of chromosomal polymorphism. There were no missing data for demographic characteristics including age, body mass index (BMI), follicle-stimulating hormone (FSH), Luteinising hormone (LH), thyroxine-stimulating hormone (TSH), free thyroxine (T4) and Prolactin. The pregnancy rate refers to positive pregnancies for the cycles with embryo transfers. Miscarriage refers to pregnancy losses calculated from the total number of treatment cycles. Live birth rate refers to total number of live babies from the total number of fresh and frozen embryo transfers.

Statistical Analysis

Baseline characteristics and outcome data were described with proportions for binary data, or means with standard deviations or median and inter-quartile range for continuous variables, as appropriate. The rates of the reproductive outcomes were plotted graphically using proportions and 95% confidence intervals. A complete case analysis was adopted. Logistic regression models were fitted to estimate crude and adjusted odds ratio for confounding variables including age, FSH, luteinising hormone LH, body mass index BMI and type of treatment (fresh vs frozen). The confounding variables were chosen for clinical reasons as they were considered to determine the induction protocols and hormone doses. Thus, I did not adjust for previous miscarriages or previous failed IVF cycles as there were few participants with those outcomes in the study. All statistical analyses were done using STATA version 16.

Ethical Consideration

The ethics committee of Lanka Hospitals Corporation PLC granted permission for the use of patient record data database following the review of the study protocol (Refer to Appendix 3).

Results

There were 1,879 ICSI and frozen embryo transfer cycles performed at the Fertility Centre during the study period. In total, 937 ICSI and frozen embryo transfer cycles were excluded from the analysis due to use of donor gametes, absence of karyotyping reports, numerical and structural abnormalities in karyotyping, poor follicular development, abnormal cleavage and blastocyst formation, embryo vitrified without transfers and

records without pregnancy outcomes. Figure 16 shows the data selection process. There were 149 participants who underwent long (n = 114) or short (n = 35) protocol stimulation and, did not proceed with fresh embryo transfer due to hyperstimulation or any other factors, but went on to have frozen embryo transfer at a later date. In total, 942 treatment cycles (548 ICSI cycles and 394 frozen embryo transfer cycles) from 697 couples were included in the study.

From the excluded cycles with abnormal cleavage and blastocyst formation embryos (n=58), only four females were carriers of polymorphism (6.9%); only four males were carriers of polymorphism (6.9%); in 8 both the female and male were carriers of polymorphism (13.8%); in 23 neither partner carried a polymorphism (39.6%) and in 19 participants karyotyping was not performed (32.8%). Therefore, the comparison between the participants who had a normal cleavage and abnormal cleavage was not feasible (Supplementary Table 1).

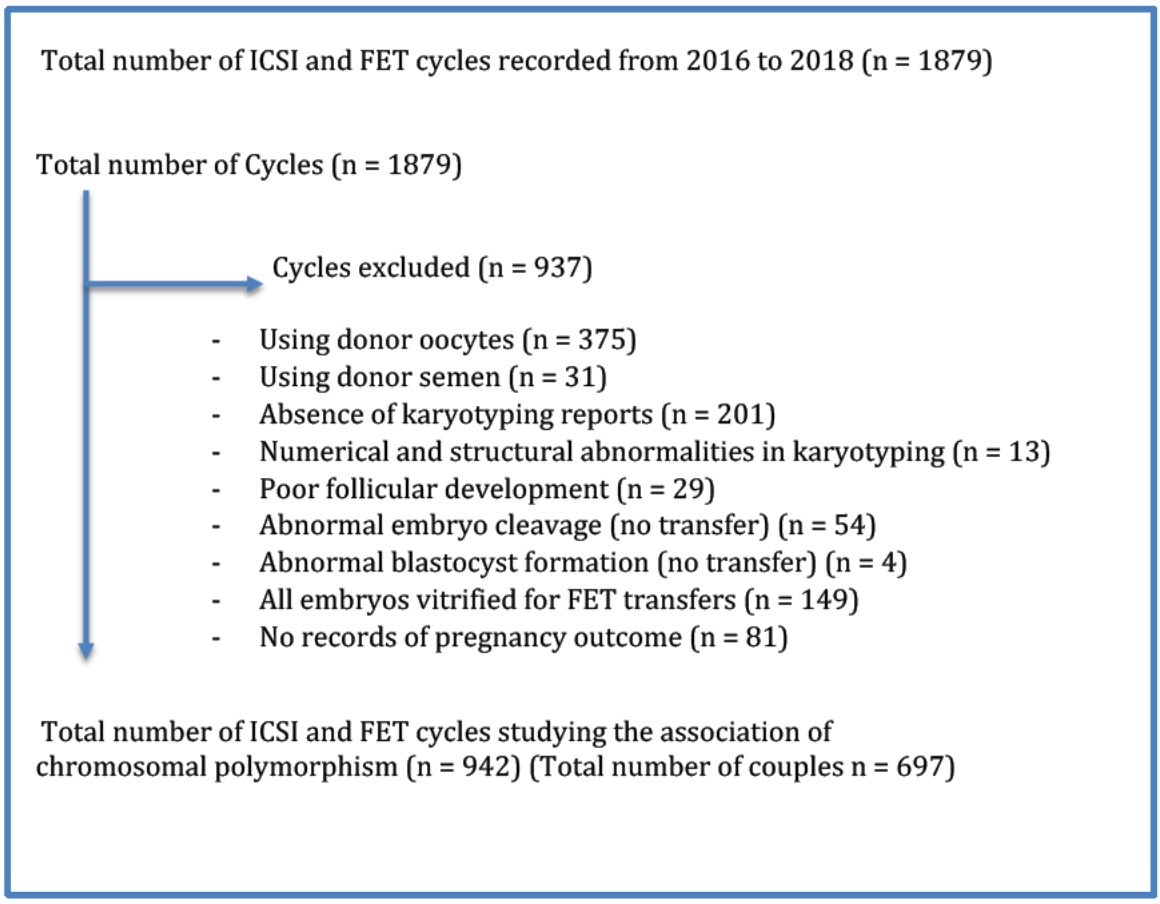

Figure 16: Flow chart of data selection process

Table 3 contains baseline characteristics of the study population.

Table 3: Baseline characteristics of the study population

Characteristics	Cohort n (%) or mean (SD) Whole data set (n=942)
Age	34 ± 4.1
BMI	24.0 ± 3.8
FSH	6.5 ± 1.8
LH	5.8±2.7
Treatment type	
ICSI cycles	
Long agonist	407 (43.2)
Short antagonist	141 (15.0)
FET cycles	
Cleavage stage transfers (Day 3)	219 (23.2)
Blastocyst stage transfers (Day 5)	175 (18.6)
Oocytes retrieved	15.5 ± 8.2
Mature oocytes	15.0 ± 8.2
Fertilised oocytes	11.2 ± 7.4
Cleavage embryos (Day 3)	7.5 ± 5.1
Chromosomal polymorphism	
Couples with polymorphism	144 (15.3)
Females with polymorphism	150 (15.9)
Males with polymorphism	200 (21.2)
Couples without polymorphism	448 (47.6)

BMI refers to body mass index, FSH refers to follicle stimulation hormone, LH refers to luteinising hormone
ICSI refers to intra cytoplasmic sperm injection, FET refers to frozen embryo transfer
All characteristics are of female participants

From the 942 cycles analysed, in 144 both the female and male were carriers of polymorphisms (15.3%); in 150 only the females were carriers of polymorphisms (15.9%); in 200 only the males were carriers of polymorphisms (21.2%); and in 448 cycles neither partner carried a polymorphism (47.6%).

There were 294 pregnancies (overall pregnancy rate 31.2%; ICSI pregnancy rate 24.3%, [133/548]; frozen embryo transfer 40.9% [161/394] recorded in 942 cycles in the study of which 130 suffered a miscarriage (overall miscarriage rate 13.8%; ICSI miscarriage rate 11.3% [62/548]; frozen embryo transfer 17.2% [68/394]), 13 had an ectopic pregnancy (1.3%; ICSI 1.5% [8/548]; frozen embryo transfer 1.3% [5/394]), and 151 had a live birth (overall live birth rate 16.0%; ICSI live birth rate 11.5% [63/548]; frozen embryo transfer

22.3% [88/394]). The total number of participants with chromosomal polymorphic variants was 494 (52.4%), while 448 (47.6%) did not exhibit any of the polymorphic variants. Table 4 shows details of pregnancy, miscarriage and livebirth rates according to the presence or absence of chromosomal polymorphism.

Table 4: Pregnancy, miscarriage and livebirth rates of carriers and non-carriers of chromosomal polymorphism

Polymorphism	Pregnancy Rate (%)	Miscarriage rate (%)	Live birth rate (%)
Females, males or couples with polymorphism (n=494)	156 (31.6)	73 (14.8)	79 (16.0)
Females with polymorphism (n=150)	36 (24)	19 (12.7)	16 (10.7)
Males with polymorphism (n=200)	68 (34)	28 (14)	38 (19)
Couples with polymorphism (n=144)	52 (36.1)	26 (18.1)	25 (17.4)
Couples without polymorphism (n=448)	138 (30.8)	57 (12.7)	72 (16.1)
Total (n=942)	**294 (31.2)**	**130 (13.8)**	**151 (16.0)**

Ectopic pregnancies (n=13, 1.3%) were excluded from the miscarriages

The crude and adjusted odds ratios for factors influencing the rates of pregnancy, miscarriage and live birth are presented in Table 5. I found no association between chromosomal polymorphisms and these reproductive outcomes.

Table 5: Crude and adjusted odds ratio for pregnancy, miscarriage and livebirth rates

Outcome	Crude OR		Adjusted OR	
	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
Pregnancy				
Females, males or couples with polymorphism	1.03 (0.78 to 1.36)	0.79	1.05 (0.79 to 1.39)	0.73
Females with polymorphism	0.70 (0.46 to 1.08)	0.11	0.70 (0.45 to 1.08)	0.10
Males with polymorphism	1.15 (0.81 to 1.64)	0.42	1.19 (0.82 to 1.71)	0.34
Couples with polymorphism	1.26 (0.85 to 1.88)	0.23	1.29 (0.86 to 1.93)	0.21
Miscarriage				
Females, males or couples with polymorphism	1.18 (0.81 to 1.72)	0.36	1.20 (0.83 to 1.73)	0.32
Females with polymorphism	0.99 (0.57 to 1.73)	0.98	1.00 (0.57 to 1.75)	0.99
Males with polymorphism	1.11 (0.68 to 1.81)	0.65	1.13 (0.69 to 1.86)	0.60
Couples with polymorphism	1.51 (0.90 to 2.51)	0.11	1.54 (0.92 to 2.57)	0.09
Live Birth				
Females, males or couples with polymorphism	0.99 (0.70 to 1.40)	0.97	1.00 (0.70 to 1.44)	0.95
Females with polymorphism	0.62 (0.35 to 1.10)	0.10	0.61 (0.34 to 1.10)	0.10
Males with polymorphism	1.22 (0.79 to 1.89)	0.35	1.28 (0.82 to 2.00)	0.27
Couples with polymorphism	1.09 (0.66 to 1.80)	0.71	1.10 (0.65 to 1.83)	0.71

The reference category is no chromosomal polymorphism in either partner
OR, odds ratio
CI, 95% confidence intervals

Figure 17 shows the point effect estimates and respective confidence intervals for outcomes of pregnancy, miscarriage, and live birth for the whole cohort and for female, male and couples with polymorphism.

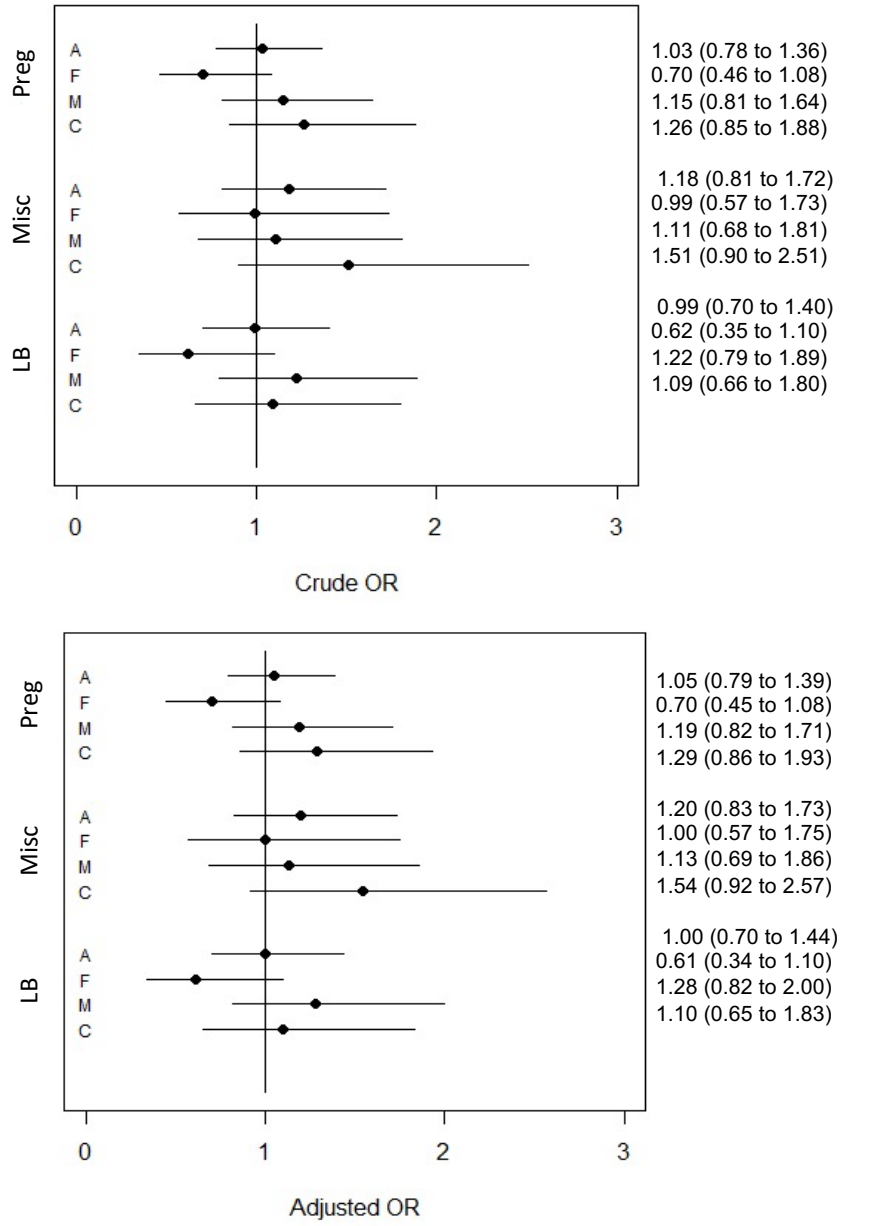

Figure 17: Confidence intervals of crude and adjusted odds ratios of pregnancy, miscarriage and live birth of female, male, and couples with chromosomal polymorphism

Discussion

In this prospective cohort study, the total study population is Sri Lankan ethnic origin. I found no evidence of a difference in pregnancy, miscarriage or live birth rates between couples without polymorphisms and those where one or both partners were carriers of a chromosomal polymorphisms. This was observed in the unadjusted univariate analysis and multivariate analysis adjusted for age, BMI, ovarian reserve markers and treatment type. Although, some of the point estimates suggest a clinically important impact, the confidence intervals were wide and cross the line of no effect.

In this study, all participant stimulation protocols were strictly monitored and all IVF procedures including ICSI conducted by myself as the sole clinical embryologist in the Lanka Hospitals fertility centre thus minimising the variations in the outcome. Some participants did not proceed with fresh embryo transfer due to hyperstimulation or other factors and underwent frozen embryo transfer instead. A small proportion of outcome data on pregnancy, miscarriage, and live birth were missing or not reported and were not included in the study. This study is large, but I cannot rule out a type II error. The attrition or loss to follow-up rate were low, and I was able to adjust the result for potential confounders.

My findings are consistent with the background literature summarised in my previous systematic review of observational studies (Ralapanawe *et al*., 2022a). The review suggested that there was a paucity of evidence of whether polymorphic variation in individuals (males or females) or couples adversely affects the chance of a pregnancy, miscarriage and live births following ICSI, except for miscarriages in the presence of

chromosomal polymorphism in females. However, nine studies in the systematic review involved participants of Chinese origin and extrapolation to other cohorts may not be appropriate.

The existing literature is conflicting, with some authors reporting that chromosomal polymorphisms are associated with adverse reproductive outcomes (Cheng *et al.*, 2017; Xiaobin *et al*., 2017) while others have identified no association (Hong *et al.*, 2011; Liang *et al*., 2014; Song *et al.,* 2017). It is possible that my study may have been underpowered to detect any differences. Further, a small adverse effect may exist for some populations, but not others. There is a need for additional prospective studies evaluating the association between chromosomal polymorphisms and reproductive outcomes in patients of multiple ethnicities.

Finally, future research should investigate whether there is an adverse effect from specific high-risk chromosomal polymorphisms on reproductive outcomes. There is evidence that specific types of polymorphisms including called non-acrocentric and Yqh in male, polymorphisms may exhibit a particularly strong association with reproductive outcomes (Sipek Jr *et al.,* 2014; Xu *et al.,* 2016; Yakin *et al.,* 2005). It remains unclear, however, whether these high-risk polymorphisms are associated with adverse outcomes following ART.

CHAPTER 4:

THE EFFECT OF TYPES OR NUMBER OF CHROMOSOMAL POLYMORPHIC VARIANTS (NON-ACROCENTRIC AND ACROCENTRIC) ON REPRODUCTIVE OUTCOMES AFTER ICSI: AN ANALYSIS OF 929 CYCLES

Abstract

Research Question: My systematic review of chromosomal polymorphism in assisted reproduction found an association with higher rates of miscarriages. My primary study of 942 ICSI cycles and found that the pregnancy, miscarriage and live birth rates did not confidently differ between couples with no polymorphisms compared to couples where the female and/or the male were carriers of a chromosomal polymorphism. In this study, I will be examining the effect of specific types or number of chromosomal polymorphic variants on reproductive outcomes of couples undergoing ICSI treatment.

Design: In this prospective cohort study, I analysed data from 929 ICSI and frozen embryo replacement cycles of 692 females who underwent karyotyping analysis GTL banding prior to the ICSI procedure. The outcomes of interest were the pregnancy rate, miscarriage rate and live birth rate per cycle.

Results: There were 281 pregnancies (30.2%) recorded in the study, 130 suffered a miscarriage (13.8%) and 151 resulted up in a live birth (16.0%) after further, excluding ectopic pregnancies. I did not identify a difference in the rates of pregnancy, miscarriage or live birth between participants with the types or number of chromosomal polymorphic variants (non-acrocentric and acrocentric) and those without chromosomal polymorphic variants. Similar to Chapter 3, multivariate regression (adjusted for age, BMI, FSH, LH and treatment type) verify my univariate analyses.

Conclusion: There was no difference in pregnancy, miscarriage, or live birth rates between participants with any types or number of chromosomal polymorphic variation

(female with non-acrocentric, acrocentric and their combinations, male with non-acrocentric, acrocentric and their combination, Yqh in male, couples with non-acrocentric and acrocentric polymorphic variations) and those with no chromosomal polymorphic variations. However, confidence intervals were wide.

Introduction

Infertility is considered a critical component of reproductive health and a global public health priority (Mascarenhas *et al*., 2012). In-vitro fertilisation and intracytoplasmic sperm injection are offered as treatment solutions in couples with fertility issues (Sunderam *et al*., 2017). More than one million IVF and ICSI treatment cycles are carried out worldwide every year (ESHRE 2012; Mascarenhas *et al*., 2012). Despite several improvements in these techniques, the live birth rate for each cycle remains low at about 26% (HFEA, 2019).

Chromosomal polymorphic variations occur in 2-5% of the general population and are considered variations of normal (Xu *et al.,* 2016). The incidence of polymorphic variations in the infertile population is higher (approximately 10 15%) comparing to the general population, suggesting an association with infertility (Luo *et al.,* 2020; Rawal *et al.,* 2020; Xu *et al.,* 2016). Chromosomal polymorphic variations are variants in the heterochromatic regions of the chromosome (Wyandt and Tonk, 2011). Heterochromatic regions are the non-coding regions of tandem repeats of DNA and variations in these regions do not result in different phenotypes (Cheng *et al.,* 2017, Guo *et al.,* 2012).

The genes necessary for fertility and viability reside in heterochromatin (Madon *et al.,* 2005). Heterochromatin contains in the long arm of the non-acrocentric chromosomes, and in the short arm and satellites of the acrocentric chromosomes (Gosden *et al*., 1981). The evidence suggest that heterochromatin is not inert and it is essential for cell and organisms' viability. Heterochromatin plays a role in spindle attachment, movements of chromosome, meiotic paring and cohesion of sister chromatid (Karpen and Endows,

1998). The functions of heterochromatin in polymorphic regions may suppress or silence gene expression, which could affect gametogenesis. This impact of polymorphic variations in chromosomes can play an important role in both male and female infertility (Xu *et al.,* 2016; Sipek Jr *et al.,* 2014).

These chromosomal polymorphic variations are divided into non-acrocentric, in which includes metacentric and sub-metacentric chromosomes, and acrocentric chromosomes. In the metacentric chromosomes, the centromere lies in the middle of the chromosome. Meanwhile, in the sub-metacentric chromosomes, the centrosome is deviated towards one end of the chromosome dividing the two arms into unequal lengths (Erwinsyah *et al*., 2017). According to the international system for human cytogenetics nomenclature (ISCN) standing committee recommendations, autosomes 1 to 3 are the large metacentric chromosomes, 4 and 5 are the large sub-metacentric chromosomes, 6 to 12 are the medium sizes metacentric and sub-metacentric chromosomes, and 16 to 20 are the relatively short metacentric and sub-metacentric chromosomes. In non-acrocentric chromosomes, polymorphic variations (heterochromatic segments) are visible on the long arm of the chromosome 1, 9 and 16 (Shaffer *et al*., 2013; Xu *et al*., 2016). Pericentric inversions on chromosome 9 [inv (9)] are also considered to be non-acrocentric polymorphic variations (Cheng *et al.,* 2017). In contrast, in the acrocentric chromosome's centromere lies near the end of the chromosome that one arm is short and the other arm is long (Erwinsyah *et al.,* 2017). According to the ISCN standing committee recommendations, medium sized acrocentric chromosomes with satellites are in chromosome 13, 14 and 15, short acrocentric chromosomes with satellites are in chromosome 21, 22 and Y chromosome without a satellite though heterochromatic

segment in the long arm. Satellite stalks and satellites are the acrocentric polymorphic variations frequently occur on the short arms of the chromosome 13, 14, 15, 21 and 22 (Shaffer *et al*., 2013; Xu *et al*., 2016).

There is evidence suggesting that specific types of chromosomal polymorphic variations or the presence of multiple chromosomal polymorphic variations influence reproductive outcomes of couples undergoing ART treatments (Li *et al*., 2020; Xu *et al*., 2016). A systematic review found that in women with any type or number of chromosomal polymorphic variations undergoing ART treatment, the risk of miscarriage was higher (relative risk [RR] 1.54, 95% confidence interval [CI] 1.19 to 1.98) than in women with no polymorphic variations undergoing ART treatment (Ralapanawe *et al.,* 2022a). However, studies suggest that specific types of chromosomal polymorphic variations such as non-acrocentric polymorphic variations could adversely affect reproductive outcomes more than other chromosomal polymorphic variations (Sipek Jr *et al.,* 2014; Xu *et al.,* 2016; Yakin *et al.,* 2005). The literature is not consistent, and another study suggests that perhaps inversions [Inv (9)] or acrocentric polymorphic variations might lead to lower cleavage rate and increased miscarriage risk (Li *et al*., 2020).

The current study aims to explore the effects of specific types or the presence of multiple chromosomal polymorphic variations in female partners, male partners and couples on the reproductive outcomes of patients undergoing ICSI treatment.

Materials and Methods

Study Design

The cohort study been described in Chapter 3. The difference is that I excluded couples who had an ectopic pregnancy for a more accurate understanding of the reproductive outcomes and has association of chromosomal polymorphic variations, especially in miscarriages and live births. The primary outcomes are the same as Chapter 3 and include the pregnancy rate per embryo transfer (gestational age 4 to 6 weeks); miscarriage per embryo transfer (gestational age less than 12 weeks); and live birth rate per embryo transfer (gestational age over 32 weeks). I compared outcomes according to the existence of different chromosomal polymorphic variations including non-acrocentric and acrocentric, a combination of polymorphic variations and the presence of number of chromosomal polymorphic variations in female or male partners and couples on their reproductive outcomes.

Statistical Analysis

Baseline characteristics and outcome data are described using means with standard deviations for symmetrically distributed continues data, means with interquartile range for skewed data, and proportions for binary data. I had no missing confounding variables or outcome data. Logistic regression models were fitted to estimate crude and adjusted odds ratio to examine the association of specific types and numbers of chromosomal polymorphisms and reproductive outcomes adjusting for confounding variables including age, BMI, serum FSH level, serum LH level, and type of treatment (fresh vs frozen).

Results

Data Selection

During the study period of January 2016 to December 2018, there were 1,879 fresh and frozen embryo transfer cycles performed at the Fertility Centre. Overall, 950 fresh and frozen embryo transfer cycles were excluded from the analysis due to ectopic pregnancies, chromosomal aberrations in karyotyping, absence of karyotyping reports, use of donor gametes, sub-standard follicle development, atypical cleavage of embryos and blastocysts, embryo cryopreservation without transfer and absence of recorded pregnancy outcomes (Figure I8). There were 149 patients who did not proceed with fresh embryo transfer due to hyperstimulation or any other factors, but went on to have subsequent frozen embryo transfer cycles whose outcomes were included in the study. A total of 929 treatment cycles (fresh cycles 540 and frozen embryo transfer cycles 389) from 692 couples were included in the study.

From the excluded cycles with atypical cleavage and blastocyst formation embryos (n=58), in two cases either the female or male were carriers of non-acrocentric polymorphism only (3.4%); in four cases either the female or male were carriers of acrocentric polymorphism only (6.9%); in six cases either the female or male were carriers with combination of non-acrocentric and acrocentric polymorphism (10.3%); in two cases only males were carriers of Yqh +/- (3.4%); in two cases both the female and male were carriers of non-acrocentric, acrocentric or combination of polymorphism (3.4%); in 23 neither partner carried a polymorphism (39.6%) and in 19 participants karyotyping was not performed (32.8%). Therefore, the comparison between the

participants who had a normal cleavage and abnormal cleavage was not feasible (Supplementary Table 2).

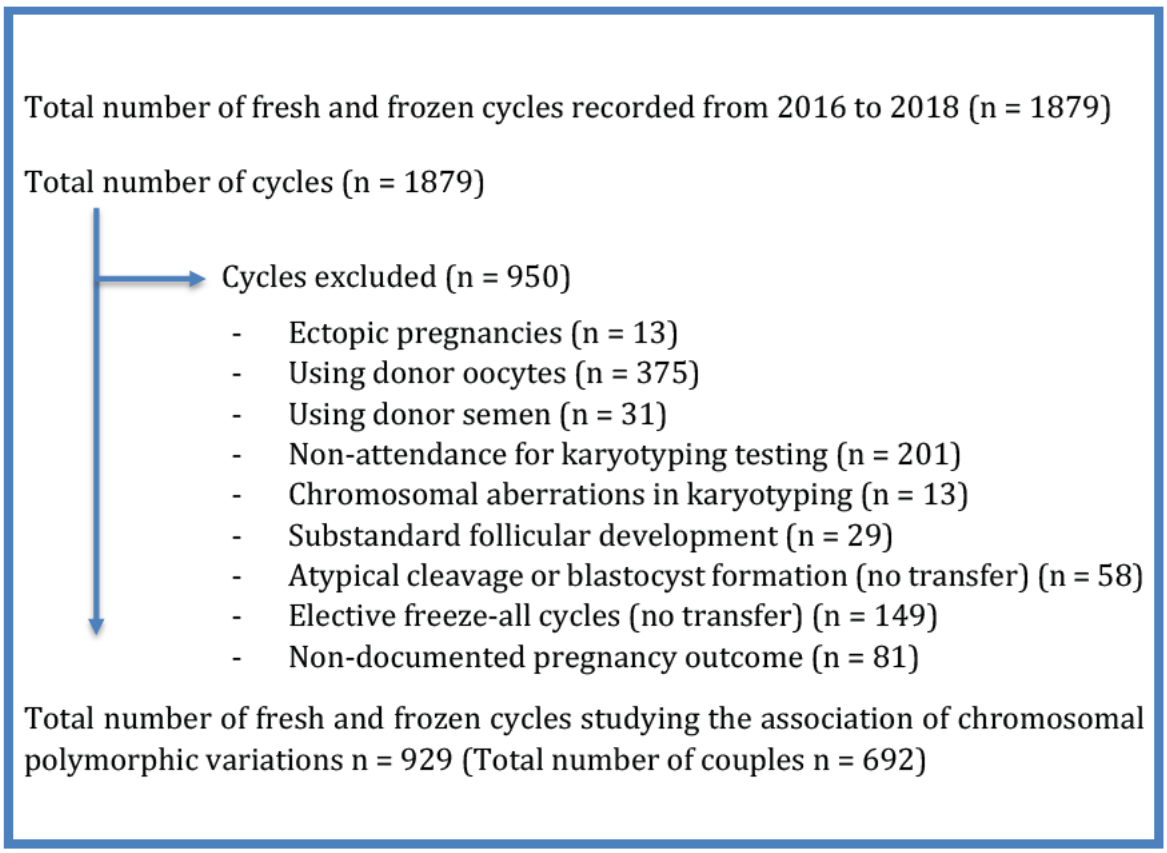

Figure 18: Flow chart of data selection process

Baseline Characteristics

Table 6 shows the baseline participant characteristics and the treatment types. The mean age, BMI, serum FSH and LH levels were similar in the carriers and non-carriers of non-acrocentric and acrocentric chromosomal polymorphic variations. The proportion of women undergoing long agonist and short antagonist protocols were similar between couples with chromosomal polymorphic variations compared to couples without. The proportion between these two groups of frozen embryo transfer cycles of cleavage stage

and blastocyst formation were also similar. Other parameters such as the mean number of oocytes retrieved, mature oocytes, fertilised oocytes and cleavage embryos (day 3) did not differ between the study groups.

Table 6: Baseline characteristics and treatment types of the study population

Characteristics	Couples with chromosomal polymorphism n (%) or mean (SD) (n=490)	Couples without chromosomal polymorphism n (%) or mean (SD) (n=439)
Age	33.7± 4.0	34.1±4.2
BMI	24.0±3.4	24.0±4.1
FSH	6.6±1.7	6.6±1.7
LH	5.8±2.8	5.7±2.6
Treatment type		
ICSI cycles		
Long agonist	216 (44.1)	186 (42.4)
Short antagonist	76 (15.5)	62 (14.1)
FET cycles		
Cleavage stage transfers (Day 3)	101 (20.6)	115 (26.2)
Blastocyst stage transfers (Day 5)	97 (19.8)	76 (17.3)
Oocytes retrieved	15.8±8.8	15.2±7.5
Mature oocytes	15.4±8.7	14.5±7.5
Fertilised oocytes	11.7±8.1	10.6±6.3
Cleavage embryos (Day 3)	7.8±5.4	7.0±4.7

BMI refers to body mass index, FSH refers to follicle stimulation hormone, LH refers to luteinising hormone
ICSI refers to intra cytoplasmic sperm injection, FET refers to frozen embryo transfer
All characteristics are of female participants

Types of Chromosomal Polymorphic Variations

The prevalence of non-acrocentric and acrocentric chromosomal polymorphic variations from the 929 cycles analysed are shown in Table 7. From the 929 cycles, in 48 either the female or male were carriers of non-acrocentric polymorphism only (5.2%), in 260 either the female or male were carriers of acrocentric polymorphism only (28%), in 10 either

the female or male were carriers with combination of non-acrocentric and acrocentric polymorphism (1.1%), in 29 only males were carriers of Yqh +/- (3.1%), in 143 both the female and male were carriers of non-acrocentric, acrocentric or combination of polymorphism (15.4%) and in 439 cycles none of the partners carried a chromosomal polymorphism (47.3%).

Table 7: Prevalence of non-acrocentric and acrocentric chromosomal polymorphic variants

Categories	n (%)
Non-acrocentric	48 (5.2)
Acrocentric	260 (28.0)
Combination of non-acrocentric and acrocentric	10 (1.1)
Yqh+/- in male	29 (3.1)
Couples with non-acrocentric and acrocentric	143 (15.4)
Couples without polymorphism	439 (47.3)

Distribution of chromosomal polymorphic variations

The distribution of the chromosomal polymorphic variations in females, males and couples of the study population are shown in Table 8.

Table 8: Distribution of chromosomal polymorphic variants in female and male in the study population

Classification	Female Karyotype	n	Male karyotype	n
Chromosomal polymorphic variants in female partners only (n=149)				
Non-acrocentric Polymorphic variants				
qh+	1qh+	1	46XY	1
	9qh+	20	46XY	20
	9qh-	2	46XY	2
	16qh+	1	46XY	1
Acrocentric Polymorphic variants				
One acrocentric polymorphic variant				
pstk+, ps+, cenh+	13pstk+	11	46XY	11
	14pstk+	15	46XY	15
	15pstk+	28	46XY	28
	21pstk+	15	46XY	15
	22pstk+	23	46XY	23
	13ps+	1	46XY	1
	15ps+	2	46XY	2
	22ps+	1	46XY	1
	15cenh+	1	46XY	1
Two acrocentric polymorphic variants				
pstk+, ps+	13pstk+, 14pstk+	1	46XY	1
	13pstk+, 15pstk+	1	46XY	1
	13pstk+, 21pstk+	4	46XY	4
	13pstk+, 22pstk+	2	46XY	2
	14pstk+, 15pstk+	1	46XY	1
	14pstk+, 21pstk+	2	46XY	2
	14pstk+, 22pstk+	2	46XY	2
	15pstk+, pstk+	4	46XY	4
	15pstk+, 22pstk+	4	46XY	4
	21pstk+, 22pstk+	2	46XY	2
	13ps+, 22pstk+	1	46XY	1
Combination of non-acrocentric and acrocentric polymorphic variants				
qh+, pstk+, ps+	9qh+, 13pstk+	1	46XY	1
	9qh+, 21pstk+	2	46XY	2
	9qh+, 14ps+, 21 ps+, 22ps+	1	46XY	1

Chromosomal Polymorphic variants in male partners only (n=198)				
Non-acrocentric Polymorphic variants				
qh+/qh-	46XX	2	1qh+	2
	46XX	1	1qh-	1
	46XX	17	9qh+	17
Inv(9)	46XX	4	Inv(9) (p12q13)	4
Acrocentric Polymorphic variants				
One acrocentric polymorphic variant				
pstk+, ps+, cenh+	46XX	12	13pstk+	12
	46XX	13	14pstk+	13
	46XX	26	15pstk+	26
	46XX	29	21pstk+	29
	46XX	19	22pstk+	19
	46XX	2	ps+	2
	46XX	4	13ps+	4
	46XX	2	14ps+	2
	46XX	1	15ps+	1
	46XX	3	21ps+	3
	46XX	6	22ps+	6
	46XX	1	15cenh+	1
Two acrocentric polymorphic variants				
pstk+, ps+	46XX	2	13pstk+, 14pstk+	2
	46XX	1	13pstk+, 21pstk+	1
	46XX	3	13pstk+, 22pstk+	3
	46XX	4	14pstk+, 21pstk+	4
	46XX	2	15pstk+, 21pstk+	2
	46XX	1	15ps+, 22pstk+	1
	46XX	1	14pstk+,21ps+	1
	46XX	3	21pstk+, 22pstk+	3
	46XX	1	22pstk+, pstk+	1
Three acrocentric polymorphic variants				
pstk+, ps+	46XX	1	13pstk+, 14pstk+, 21pstk+	1
	46XX	1	13pstk+, 15pstk+, 21pstk+	1
	46XX	1	14pstk+, 14pstk+, 22pstk+	1
Combination of non-acrocentric and acrocentric polymorphic variants				
qh+/-, pstk+	46XX	1	9qh+, 15pstk+	1
	46XX	1	9qh+, 21pstk+	1
	46XX	2	9qh-, 14pstk+	2
	46XX	2	9qh-, 21pstk+	2
Male Yqh				
Male Yqh only				

Yqh+/-	46XX	13	Yqh+	13
	46XX	5	Yqh-	5
Male Yqh and one acrocentric polymorphic variant				
Yqh+/-, pstk+	46XX	1	Yqh+, 15pstk+	1
	46XX	2	Yqh-, 14pstk+	2
	46XX	2	Yqh-, 21pstk+	2
	46XX	2	Yqh-, 22pstk+	2
Male Yqh and two acrocentric polymorphic variants				
Yqh+/-, pstk+	46XX	3	Yqh+, 13pstk+, 15pstk+	3
	46XX	1	Yqh-, 13pstk+, 15pstk+	1

Couples with chromosomal polymorphic variants (n=143)

Non-acrocentric polymorphic variant in female and acrocentric polymorphic variants in male				
qh+/-, pstk+, ps+	1qh+	1	13pstk+	1
	9qh+	2	13pstk+	2
	9qh+	1	14pstk+	1
	9qh+	2	15pstk+	2
	9qh+	1	22pstk+	1
	9qh-	2	22pstk+	2
	9qh+	2	13pstk+, 14pstk+	2
	9qh+	2	21ps+	2
	16qh+	1	14pstk+, 21pstk+	1
Non-acrocentric polymorphic variants in both female and male				
qh+/-	9qh+	1	9qh+	1
Non-acrocentric polymorphic variants in male and acrocentric polymorphic variants in female				
qh+/-, inv (9), pstk+, ps+	14pstk+	1	9qh-	1
	21pstk+	1	16qh+	1
	14pstk+, 15pstk+	1	9qh+	1
	13pstk+, 15pstk+, 22pstk+	2	9qh+	2
Combination of non-acrocentric and acrocentric polymorphic variants in female and male				
qh+/-, inv(9), pstk+, ps+	13pstk+	2	9qh-, 21pstk+	2
	14pstk+	1	9qh-, 13pstk+, 21pstk+	1
	15pstk+	1	9(inv), 13pstk+	1
	21pstk+	3	9qh+, 13pstk+	3
	22pstk+	2	9qh+, 13pstk+	2
	22pstk+	1	9qh-, 14pstk+	1
	22pstk+	1	9qh+, 21pstk+	1
	14pstk+, 15pstk+	1	9qh+, 13pstk+	1
	13pstk+, 15pstk+	1	9qh-, 13pstk+, 14ps+	1
	15pstk+, ps+	1	9qh+, 13pstk+, 22pstk+	1
	9qh-, 14pstk+	2	9qh+, 13pstk+, 21pstk+	2
	9qh+, 15pstk+	1	14pstk+	1

	9qh+, 22pstk+	1	9qh+	1
One acrocentric polymorphic variant in female and male				
pstk+, ps+	13pstk+	1	13pstk+	1
	13pstk+	1	14pstk+	1
	13pstk+	2	15pstk+	2
	13pstk+	4	21pstk+	4
	13pstk+	2	22pstk+	2
	14pstk+	1	13pstk+	1
	14pstk+	1	14pstk+	1
	14pstk+	3	15pstk+	3
	14pstk+	1	21pstk+	1
	15pstk+	1	14pstk+	1
	15pstk+	1	22pstk+	1
	21pstk+	1	14pstk+	1
	21pstk+	1	15pstk+	1
	21pstk+	3	21pstk+	3
	21pstk+	1	22pstk+	1
	22pstk+	2	13pstk+	2
	22pstk+	3	15pstk+	3
	21pstk+	1	13ps+	1
	22pstk+	1	13ps+	1
	14ps+	2	14pstk+	2
	14ps+	2	15pstk+	2
	14ps+	1	14ps+	1
One acrocentric polymorphic variant in female and two acrocentric polymorphic variants in male				
pstk+, ps+	13pstk+	1	13pstk+, 21pstk+	1
	13pstk+	2	14pstk+, 21pstk+	2
	13pstk+	1	21pstk+, 22pstk+	1
	14pstk+	3	13pstk+, 22pstk+	3
	14pstk+	2	14pstk+, 22pstk+	2
	14pstk+	1	13pstk+, 22ps+, pstk+	1
	15pstk+	1	15pstk+, 21pstk+	1
	15pstk+	2	14pstk+, 22pstk+	2
	15pstk+	2	22pstk+, ps+	2
	21pstk+	2	13pstk+, 15pstk+	2
	21pstk+	1	14pstk+, 15pstk+	1
	21pstk+	1	22pstk+, ps+	1
	22pstk+	1	14pstk+, 15pstk+	1
	22pstk+	2	15pstk+, 21pstk+	2
Two acrocentric polymorphic variants in female and one acrocentric polymorphic variant in male				
pstk+, ps+	13pstk+, 15pstk+	1	22pstk+	1
	13pstk+, 21pstk+	1	14pstk+	1

	13pstk+, 21pstk+	2	21pstk+	2
	13pstk+, 22pstk+	1	14pstk+	1
	13pstk+, 22pstk+	2	21pstk+	2
	13pstk+, 15pstk+	1	22ps+	1
	14pstk+, 15pstk+	1	22pstk+	1
	14pstk+, 21pstk+	1	13pstk+	1
	14pstk+, 21pstk+	3	15pstk+	3
	14pstk+, 21pstk+	2	21pstk+	2
	15pstk+, 22pstk+	4	14pstk+	4
Two acrocentric polymorphic variants in female and in male				
pstk+	14pstk+, 21pstk+	1	15pstk+, 22pstk+	1
	14pstk+, 21pstk+	1	14pstk+, 22pstk+	1
	15pstk+, 21pstk+	1	14pstk+, 21pstk+	1
	21pstk+, 22pstk+	1	13pstk+, 14pstk+	1
Three acrocentric polymorphic variants in female and one/two acrocentric polymorphic variants in male				
pstk+, ps+	14pstk+, ps+, 22pstk+	1	22pstk+, ps+	1
	14pstk+, 15pstk+, 21pstk+	1	14ps+, pstk+	1
	14pstk+, 15pstk+, 22pstk+	1	15pstk+	1
	14pstk+, 15pstk+, 22pstk+	1	13pstk+, 21pstk+	1
Non-acrocentric polymorphic variant in female and Male Yqh				
qh+/-, Yqh+/-	9qh-	1	Yqh-	1
Acrocentric polymorphic variant in female and Male Yqh				
pstk+, ps+, Yqh+/-	13pstk+	1	Yqh+	1
	14pstk+	1	Yqh-	1
	15pstk+	2	Yqh+	2
	21pstk+	2	Yqh+	2
	21pstk+	2	Yqh-	2
	14ps+	2	Yqh-	2
	22ps+	2	Yqh+	2
Combination of non-acrocentric and acrocentric polymorphic variants in female, male and male Yqh				
pstk+, Yqh+/-	14pstk+	1	Yqh-, 13pstk+	1
	15pstk+	1	Yqh-, 22pstk+	1
	15pstk+	1	Yqh+, 22pstk+	1
	22pstk+	2	Yqh+, 21pstk+	2
	9qh+, 22pstk+	1	Yqh-, 14pstk+	1
	15pstk+, 21pstk+	1	Yqh-	1

Reproductive Outcomes According to the Types of Polymorphic Variations

Table 9 shows details of pregnancy, miscarriage and live birth rates according to the presence or absence of chromosomal polymorphic variations.

Table 9: Percentages of pregnancy, miscarriage and livebirth rates of the non-acrocentric, acrocentric, Yqh in male and combination of polymorphic variants

Polymorphism	Pregnancy rate (%)	Miscarriage rate (%)	Live birth rate (%)
Females, males or couples with polymorphism (n=490)	152 (31.0)	73 (15.0)	79 (16.1)
Non-Acrocentric (n=48)	15 (31.2)	6 (12.5)	9 (18.7)
Acrocentric (n=260)	77 (29.6)	37 (14.2)	40 (15.4)
Combination of non-acrocentric & acrocentric (n=10)	1 (10.0)	1 (10.0)	0 (0.00)
Yqh in male (n=29)	8 (27.6)	3 (10.3)	5 (17.2)
Couples with non-acrocentric and acrocentric (n=143)	51 (35.7)	26 (18.2)	25 (17.5)
Couples without polymorphism (n=439)	129 (29.4)	57 (13.0)	72 (16.4)
Total (n=929)	**281 (30.2)**	**130 (14.0)**	**151 (16.2)**

There were 281 pregnancies (overall pregnancy rate 30.2%; non-acrocentric 31.2%, [15/48]; acrocentric 29.6% [77/260]; combination of non-acrocentric and acrocentric 10% [1/10]; Yqh in male 27.6% [8/29]; couples with non-acrocentric and acrocentric 35.7% [51/143]; couples without polymorphism 29.4% [129/439] recorded in 929 cycles in the study of which 130 suffered a miscarriage (overall miscarriage rate 14%; non-acrocentric 12.5%, [6/48]; acrocentric 14.2% [37/260]; combination of non-acrocentric and acrocentric 10% [1/10]; Yqh in male 10.3% [3/29]; couples with non-acrocentric and acrocentric 18.2% [26/143]; couples without polymorphism 13% [57/439] and 151 had a live birth (overall live birth rate 16.2%; non-acrocentric 18.7%, [9/48]; acrocentric 15.4% [40/260]; no live births in the combination of non-acrocentric and acrocentric

[0/10]; Yqh in male 17.2% [5/29]; couples with non-acrocentric and acrocentric 17.5% [25/143]; couples without polymorphism 16.4% [72/439]). The total number of participants with chromosomal polymorphic variants was 490 (52.7%), while the remaining 439 (47.3%) did not exhibit any of the polymorphic variants. Table 9 shows details of pregnancy, miscarriage and livebirth rates according to the presence or absence of chromosomal polymorphism.

There were 281 pregnancies (overall pregnancy rate 30.2%; ICSI pregnancy rate 23.1%, [125/540]; frozen embryo transfer 40.1% [156/389]) recorded in 929 cycles in which 130 suffered a miscarriage (overall miscarriage rate 13.9%; ICSI miscarriage rate 11.5% [62/540]; frozen embryo transfer 17.5% [68/389]) and 151 had a live birth (overall live birth rate 16.2%; ICSI live birth rate 11.7% [63/540]; frozen embryo transfer 22.6% [88/389]), while the remaining 439 (47.3%) did not exhibit any of the polymorphic variants.

Crudes and Adjusted Odds Ratios According to the Types of Chromosomal Polymorphic Variations

The crude odds ratio analysis for pregnancy, miscarriage and live birth rates according to the presence of non-acrocentric and acrocentric chromosomal polymorphic variations is presented in Table 10. The results show no evidence of an association between non-acrocentric and acrocentric chromosomal polymorphic variations and the rates of pregnancy, miscarriage, or live birth. However, confidence intervals tended to be wide

allowing for substantial possibility of an association of clinically important size (Table 10).

Table 10: Crude and adjusted odds ratio for pregnancy, miscarriage and livebirth

Outcome	Crude OR		Adjusted OR	
	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
Pregnancy				
Females, males or couples with polymorphism	1.08 (0.81 to 1.43)	0.58	1.09 (0.82 to 1.46)	0.52
Non-acrocentric	1.09 (0.57 to 2.07)	0.78	1.05 (0.54 to 2.04)	0.86
Acrocentric	1.01 (0.72 to 1.41)	0.94	1.01 (0.72 to 1.43)	0.91
Combination of acrocentric & non-acrocentric	0.26 (0.03 to 2.12)	0.21	0.22 (0.02 to 1.89)	0.17
Yqh in male	0.91 (0.39 to 2.12)	0.83	1.08 (0.46 to 2.56)	0.84
Couples with non-acrocentric or acrocentric	1.33 (0.89 to 1.98)	0.15	1.36 (0.90 to 2.06)	0.13
Miscarriage				
Females, males or couples with polymorphism	1.17 (0.80 to 1.70)	0.40	1.19 (0.81 to 1.73)	0.36
Non-acrocentric	0.95 (0.38 to 2.35)	0.92	0.94 (0.38 to 2.33)	0.90
Acrocentric	1.11 (0.71 to 1.73)	0.64	1.12 (0.71 to 1.76)	0.60
Combination of acrocentric & non-acrocentric	0.74 (0.09 to 5.98)	0.78	0.70 (0.08 to 5.77)	0.74
Yqh in male	0.77 (0.22 to 2.63)	0.68	0.82 (0.23 to 2.83)	0.75
Couples with non-acrocentric or acrocentric	1.48 (0.89 to 2.47)	0.12	1.52 (0.90 to 2.54)	0.11
Live Birth				
Females, males or couples with polymorphism	0.97 (0.69 to 1.38)	0.90	0.99 (0.69 to 1.42)	0.99
Non-acrocentric	1.17 (0.54 to 2.53)	0.78	1.16 (0.53 to 2.53)	0.71
Acrocentric	0.92 (0.60 to 1.41)	0.72	0.93 (0.60 to 1.43)	0.76
Yqh in male	1.06 (0.39 to 2.87)	0.90	1.35 (0.48 to 3.74)	0.56
Couples with non-acrocentric or acrocentric	1.07 (0.65 to 1.78)	0.76	1.09 (0.65 to 1.82)	0.73

The reference category is no chromosomal polymorphism in either partner
There were no live births recorded in combination of acrocentric & non-acrocentric group
Reproductive outcomes are adjusted for confounding variables including age, BMI, serum FSH, LH levels, and type of treatment
OR, odds ratio
CI, 95% confidence interval

In the study, outcome of crude and adjusted odds ratios of types of chromosomal polymorphic variations of pregnancy, miscarriage, live births of female, male and couples

compared to couples without chromosomal polymorphism does not reach statistical significance.

Number of Polymorphic Variations per Couple

Analysis of the number of chromosomal polymorphic variations in the 929 fresh and frozen embryo transfer cycles showed that either the female or male were carriers of one chromosomal polymorphic variation in 279 cycles (30.0%), two variations in 122 cycles (13.1%), three chromosomal polymorphic variations in 70 cycles (7.5%), four variations in 12 cycles (1.3%), and five variations in 7 cycles (0.7%). None of the partners carried a chromosomal polymorphic variation in 439 cycles (47.3%) (Table 11).

Table 11: Chromosomal polymorphic variations by number of variations per couple

Categories	n (%)
One chromosomal polymorphic variation	279 (30.0)
Two chromosomal polymorphic variations	122 (13.1)
Three chromosomal polymorphic variations	70 (7.5)
Four chromosomal polymorphic variations	12 (1.3)
Five chromosomal polymorphic variations	7 (0.7)
Couples without polymorphic variations	439 (47.3)

Reproductive Outcomes According to the Number of Polymorphic Variations

Table 12 shows the pregnancy, miscarriage and live birth rates according to number of (n = 490) either non-acrocentric or acrocentric polymorphic variations in study participants.

Table 12: Pregnancy, miscarriage and livebirth rates of the number of polymorphic variations in the study population

Chromosomal polymorphic variations	n	Pregnancy rate n (%)	Miscarriage rate n (%)	Live birth rate n (%)
Number of polymorphic variations of female, male and couples (n=490)				
One chromosomal polymorphic variation	279	80 (28.7)	38 (13.62)	42 (15.0)
Two chromosomal polymorphic variations	122	39 (32.0)	17 (13.9)	22 (18.0)
Three chromosomal polymorphic variations	70	24 (34.3)	15 (21.4)	9 (12.9)
Four chromosomal polymorphic variations	12	6 (50.0)	2 (16.7)	4 (33.3)
Five chromosomal polymorphic variations	7	3 (42.9)	1 (14.3)	2 (28.6)
Non-carriers of polymorphic variations	439	129 (29.4)	57 (13.0)	72 (16.4)
Total	**929**	**281 (30.2)**	**130 (14.0)**	**151 (16.2)**

Crude and Adjusted Odds Ratios According to the Number of Polymorphic Variations

Table 13 shows the crude and adjusted odds ratios for pregnancy, miscarriage and live birth rates according to number of polymorphic variations. I found no evidence of an association between number of chromosomal polymorphic variations and these reproductive outcomes. But again, confidence intervals are wide.

Table 13: Crude and adjusted odds ratio for pregnancy of the number of polymorphic variations in the study population

Outcome	Crude OR		Adjusted OR	
	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
Pregnancy				
One chromosomal polymorphic variation	0.96 (0.69 to 1.34)	0.83	0.96 (0.68 to 1.35)	0.85
Two chromosomal polymorphic variations	1.12 (0.73 to 1.73)	0.58	1.18 (0.75 to 1.83)	0.46
Three chromosomal polymorphic variations	1.25 (0.73 to 2.13)	0.40	1.25 (0.72 to 2.17)	0.42
Four chromosomal polymorphic variations	2.40 (0.76 to 7.58)	0.13	2.49 (0.75 to 8.20)	0.13
Five chromosomal polymorphic variations	1.80 (0.39 to 8.16)	0.44	2.20 (0.47 to 10.33)	0.31
Miscarriage				
One chromosomal polymorphic variation	1.05 (0.67 to 1.64)	0.80	1.05 (0.67 to 1.65)	0.79
Two chromosomal polymorphic variations	1.08 (0.60 to 1.94)	0.78	1.12 (0.62 to 2.01)	0.70
Three chromosomal polymorphic variations	1.82 (0.96 to 3.44)	0.06	1.89 (0.99 to 3.60)	0.05
Four chromosomal polymorphic variations	1.34 (0.28 to 6.27)	0.71	1.34 (0.28 to 6.40)	0.71
Five chromosomal polymorphic variations	1.11 (0.13 to 9.44)	0.91	1.10 (0.12 to 9.47)	0.92
Livebirth				
One chromosomal polymorphic variation	0.90 (0.59 to 1.36)	0.63	0.91 (0.60 to 1.40)	0.69
Two chromosomal polymorphic variations	1.12 (0.66 to 1.89)	0.67	1.17 (0.68 to 2.01)	0.55
Three chromosomal polymorphic variations	0.75 (0.35 to 1.58)	0.45	0.71 (0.33 to 1.52)	0.38
Four chromosomal polymorphic variations	2.54 (0.74 to 8.68)	0.13	2.57 (0.72 to 9.14)	0.14
Five chromosomal polymorphic variations	2.03 (0.38 to 10.71)	0.40	2.93 (0.53 to 16.12)	0.21

The reference category is no chromosomal polymorphic variations in either partner
Reproductive outcomes are adjusted for confounding variables including age, BMI, serum FSH, LH levels, and type of treatment
OR, odds ratio
CI, 95% confidence intervals

In the study, outcome of crude and adjusted odds ratios of number of chromosomal polymorphic variations of pregnancy, miscarriage, live births of female, male and couples compared to couples without chromosomal polymorphism does not reach statistical significance.

Discussion

I analysed data from 929 ICSI fresh and frozen embryo transfer cycles in 692 women who underwent karyotyping analysis prior to the ICSI procedure. I found no evidence of a difference in pregnancy, miscarriage, or live birth rates between participants with any type or number of chromosomal polymorphic variation (female with non-acrocentric, acrocentric and their combinations, male with non-acrocentric, acrocentric and their combination, Yqh in males, couples with non-acrocentric and acrocentric polymorphic variations) and those with no chromosomal polymorphic variations. However, the confidence intervals were often wide and allow for a substantial possibility of an association of clinically important size.

My study is unique in that I found no evidence of an association in the Sri Lankan population, and any effect on reproductive outcomes is likely to be minimal. I followed up all recruited participants up to live birth or and adjusted results for a number of potential confounders. However, there was a low prevalence of chromosomal polymorphic variations in this study, which may have led to insufficient power to detect clinically meaningful effects.

There is evidence suggesting that ICSI may lead to a better chance of a clinical pregnancy compared to conventional IVF. A recent study that concludes ICSI has a better chance of a clinical pregnancy compared to IVF in the presence of acrocentric chromosomal polymorphisms (Li *et al.*, 2020). The Intra cytoplasmic sperm injection has increased compared to standard IVF, with double the number of cycles globally. ICSI has an

advantage of selecting the progressive, morphologically normal spermatozoa and higher fertilisation rates than standard IVF procedures (Gleicher *et al*., 2019). However, it is not clear how ICSI could lead to better chance of a clinical pregnancy compared to conventional IVF in the presence of acrocentric chromosomal polymorphism (Li *et al.*, 2020). Future research could investigate whether there is an advantage in ICSI treatment compared to standard in vitro fertilisation in the presence of chromosomal polymorphic variations.

There were many previous studies of the Chinese population demonstrating an adverse effect between chromosomal polymorphisms and reproductive outcomes. The evidence suggests higher rate of pregnancy losses in the Chinese population in the presence of acrocentric chromosomal polymorphic variants. (Feng *et al.,* 2021). I found no association in the Sri Lankan population and any association with reproductive outcomes likely to be inconclusive. Finding of my systematic review in Chapter 2, only present that female carriers of chromosomal polymorphism in assisted reproduction are associated with higher rates of miscarriage compared to male carriers. Further research in my study investigates whether there is an adverse effect in different non-acrocentric, acrocentric and combination of chromosomal polymorphic variants, in which their types, numbers, gender of individuals and the couples on reproductive outcomes undergoing ICSI treatment in Chapter 5.

CHAPTER 5:

A DIFFERENT EXPLORATION OF THE VARIOUS TYPES OF POLYMORPHIC VARIANTS ON REPRODUCTIVE OUTCOMES AFTER ICSI

Abstract

Research Question: My primary study and the secondary study did not differ between couples with no chromosomal polymorphisms in their reproductive outcomes. Therefore, the aim of this study is to carry out an exploration of the data to explore for any other polymorphisms that could impact adversely reproductive outcomes. This exploratory analysis focused on the types, numbers, and gender of individuals with chromosomal polymorphisms.

Design: All the participants in this analysis are from the cohort described in Chapter 4. I analysed ICSI (929) and frozen embryo replacement cycles (692) of women who underwent karyotyping analysis prior to the ICSI procedure. The study examined comprehensively the types, numbers, gender of individuals and the couples of the non-acrocentric or acrocentric polymorphic variants or couples without polymorphic variants on reproductive outcomes of pregnancy rate, miscarriage rate and live birth rate.

Results: There was no evidence that pregnancy, miscarriage or live birth rates differ between the types, numbers, gender of individuals and the couples of the different variants of chromosomal polymorphism compared to couples without chromosomal polymorphism. The total of 281 pregnancies (30.2%) recorded in the study. Out of which 130 suffered a miscarriage (13.8%) and 151 resulted in a live birth (16.0%).

Conclusion: The various types, numbers, gender of individuals and the couples of chromosomal polymorphic variants are not associated with reproductive outcomes

undergoing ICSI treatments compared to the couples without chromosomal polymorphic variants. The results were consistent with the findings of Chapters 3 and 4.

Introduction

My systematic review of chromosomal polymorphism in assisted reproduction found an association with higher rates of miscarriage in female carriers of chromosomal polymorphism compared to male carriers. The review did not find evidence that chromosomal polymorphisms have any adverse effects on rates of pregnancy, clinical pregnancy, on-going pregnancy at study end, pre-term birth and live birth after IVF or ICSI, irrespective of whether the carrier was the female partner, the male, or both. In addition, the systematic review called for further research to confirm the association between polymorphic variations in females and miscarriage, and to strengthen the certainty of the evidence for other reproductive outcomes. I conducted such a study but found no evidence of a difference in pregnancy, miscarriage, or live birth rates between participants with any type or number of chromosomal polymorphic variation (female with non-acrocentric, acrocentric and their combinations, male with non-acrocentric, acrocentric and their combination, Yqh in males, couples with non-acrocentric and acrocentric polymorphic variations) and those with no chromosomal polymorphic variations.

Chromosomal polymorphisms are described more frequently in couples with infertility and recurrent miscarriage, but perhaps this link is specific to only some polymorphisms. The overall chromosomal abnormalities including chromosomal polymorphic variations within the patients with infertility vary from 1.05% to 17% (Pylyp *et al.,* 2015). Their clinical significance of heterochromatic regions of the chromosomes has a possible co-relation among the polymorphism and reproductive outcomes (Xu *et al.,* 2016). The importance of the effect of chromosomal polymorphism in the reproductive outcome

must be examined in depth to explore the adverse effect of specific polymorphic variant. In this study, I aim to comprehensively explore the dataset to explore the effects of different variants of chromosomal polymorphism, which includes the non-acrocentric and acrocentric and combination of polymorphic variants, their types, numbers, gender of individuals and the couples on reproductive outcomes undergoing ICSI treatment.

Materials and Methods

Study Design

This study is based on the cohort of women described in Chapter 4 and the same duration at the fertility centre of Lanka hospitals.

Statistical Analysis

The statistical analysis is similar to described in Chapter 4. Confounding variables including age, BMI, serum FSH level, serum LH level and type of treatment (fresh vs frozen) remains same. Logistic regression models were fitted to estimate crude and adjusted odds ratio to examine the association of chromosomal polymorphisms and reproductive outcomes.

Results

The total number of ICSI and frozen embryo transfer cycles performed were 1,879, at the Fertility Centre during the study period. Although 950 ICSI and frozen embryo transfer cycles excluded from the analysis due to ectopic pregnancies, numerical or structural abnormalities in karyotyping, absence of karyotyping reports, use of donor gametes, poor

follicular development, abnormal cleavage and blastocyst formation, embryo vitrified without transfers and records without pregnancy outcomes. Figure 18 shows the data selection process. There were 114 participants who underwent long protocol and 35 participants underwent short protocol stimulation did not proceed with the fresh embryo transfer. This was due to hyperstimulation or any other factors, though proceeded with the frozen embryo transfer cycles were included in the study (n=149). In total, 929 treatment cycles which consist of 540 ICSI cycles and 389 frozen embryo transfer cycles from of 692 couples were included in the study.

The pregnancy, miscarriage and live birth rates analysed according to the variability of each polymorphic variant. such as females with non-acrocentric or acrocentric polymorphic variants, males with non-acrocentric or acrocentric polymorphic variants, either female or male with combination of non -acrocentric and acrocentric polymorphic variants, males with Yqh, couples with one or more than one of non-acrocentric or acrocentric chromosomal polymorphic variants or a combination and couples without any of the polymorphic variants are in Table 14.

Table 14: Percentages of pregnancy, miscarriage and livebirth of participants with variability of chromosomal polymorphic variants

Polymorphism	n	Pregnancy rate (%)	Miscarriage rate (%)	Live birth rate (%)
Chromosomal Polymorphic variants in female only	**149**			
Non-acrocentric variants	24	7 (29.4)	4 (16.7)	3 (12.5)
Acrocentric variants	121			
One acrocentric variant	96	21 (21.9)	11 (11.5)	10 (10.4)
Two acrocentric variants	25	7 (28.0)	4 (16.0)	3 (12.0)
Combination of non-acrocentric and acrocentric	4	0 (00.0)	0 (0.00)	0 (0.00)
Chromosomal Polymorphic variants in male only	**198**			
Non-acrocentric variants	24	8 (33.3)	2 (8.3)	6 (25.0)
Acrocentric variants	139			
One acrocentric variant	118	40 (33.9)	19 (16.1)	21 (17.8)
Two acrocentric variants	18	8 (44.4)	2 (11.1)	6 (33.3)
Three acrocentric variants	3	1 (33.3)	1 (33.3)	0 (0.00)
Combination of non-acrocentric and acrocentric variants	6	1 (16.7)	1 (16.7)	0 (0.00)
Yqh in male	**29**			
Yqh in male only	18	6 (33.3)	3 (16.7)	3 (16.7)
Yqh in male and one acrocentric variant	7	2 (28.6)	0 (0.00)	2 (28.6)
Yqh in male and two acrocentric variants	4	0 (00.0)	0 (0.00)	0 (0.00)
Couples with chromosomal polymorphic variants	**143**			
Non-acrocentric variant in female and acrocentric variants in male	14	5 (35.7)	3 (21.4)	2 (14.3)
Non-acrocentric variants in female and male	1	0 (00.0)	0 (0.00)	0 (0.00)
Non-acrocentric variants in male and acrocentric variants in female	5	2 (40.0)	1 (20.0)	1 (20.0)
Combination of non-acrocentric and acrocentric variants in female and male	18	5 (27.8)	3 (16.7)	2 (11.1)
One acrocentric variant in female and male	36	11 (30.6)	6 (16.7)	5 (14.0)
One acrocentric variant in female and two acrocentric variants in male	22	5 (22.7)	2 (9.1)	3 (13.6)

Two acrocentric variants in female and one acrocentric variant in male	19	9 (47.4)	5 (26.3)	4 (21.0)
Two acrocentric variants in female and in male	4	3 (75.0)	1 (25.0)	2 (50.0)
Three acrocentric variants in female and one/two acrocentric variants in male	4	3 (75.0)	1 (25.0)	2 (50.0)
Non-acrocentric variant in female and Yqh in male	1	1 (100.0)	0 (0.00)	1 (100.00)
Acrocentric variant in female and Yqh in male	12	3 (25.0)	1 (8.3)	2 (16.7)
Combination of non-acrocentric and acrocentric in female, male and Yqh in male	7	4 (57.1)	3 (42.9)	1 (14.3)
Couples without polymorphic variants	**439**			
Non-carriers of polymorphic variants	439	129 (29.4)	57 (13.0)	72 (16.4)
Total	**929**	**281 (30.2)**	**130 (14.0)**	**151 (16.2)**

The number of pregnancies were 281 (overall pregnancy rate 30.2%; ICSI pregnancy rate 23.1%, [125/540]; frozen embryo transfers 40.1% [156/389]) recorded in 929 cycles in the study of which 130 were miscarriages (overall miscarriage rate 13.9%; ICSI miscarriage rate 11.5% [62/540]; frozen embryo transfers 17.5% [68/389]). The live births were 151 (overall live birth rate 16.2%; ICSI live birth rate 11.7% [63/540]; frozen embryo transfer 22.6% [88/389]). The total number of participants with chromosomal polymorphic variants was 490 (52.7%), though 439 (47.3%) did not show any of the polymorphic variants.

There were 149 females only participants with chromosomal polymorphic variants. The pregnancy rate of females only with non-acrocentric variants 29.4%, [7/24]; one acrocentric variant 21.9%, [21/96]; two acrocentric variants 28% [7/25] and no pregnancies recorded in the combination of non-acrocentric and acrocentric variant 0.00% [0/4]. The miscarriage rate of females only with non-acrocentric variants 16.7%, [4/24]; one acrocentric variant 11.5%, [11/96]; two acrocentric variants 16% [4/25] and

no miscarriages recorded in the combination of non-acrocentric and acrocentric variant 0.00% [0/4]. The live birth rate of females only with non-acrocentric variants 12.5%, [3/24]; one acrocentric variant 11.4%, [10/96]; two acrocentric variants 12% [3/25] and no live birth recorded in the combination of non-acrocentric and acrocentric variant 0.00% [0/4] presented in Table 14.

There were 198 males only participants with chromosomal polymorphic variants. The pregnancy rate of males only with non-acrocentric variants 33.3%, [8/24]; with one acrocentric variant 33.9%, [40/118]; with two acrocentric variants 44.4%, [8/18]; with three acrocentric variants 33.3%, [1/3] and with the combination of non-acrocentric and acrocentric variant 16.7%, [1/6]; In males with Yqh only 33.3%, [6/18]; Yqh in male and one acrocentric variant 28.6%, [2/7} and no pregnancies recorded in Yqh in male and two acrocentric variants 0.00%, [0/4]. The miscarriage rate of males only with non-acrocentric variants 8.3%, [2/24]; with one acrocentric variant 16.1%, [19/118]; with two acrocentric variants 11.1%, [2/18]; with three acrocentric variants 33.3%, [1/3] and with the combination of non-acrocentric and acrocentric variant 16.7%, [1/6]; In males with Yqh only 16.7%, [3/18]; no miscarriages recorded in Yqh in male and one acrocentric variant 00.0%, [0/7} and in Yqh in male and two acrocentric variants 0.00% [0/4]. The live birth rate of males only with non-acrocentric variants 25%, [6/24]; with one acrocentric variant 17.8%, [21/118]; with two acrocentric variants 33.3%, [6/18]; no live birth recorded with three acrocentric variants 0.00%, [0/3] and with the combination of non-acrocentric and acrocentric variant 0.00%, [0/6]; In males with Yqh in only 16.7%, [3/18]; Yqh in male and one acrocentric variant 28.6%, [2/7} and no pregnancies recorded in Yqh in male and two acrocentric variants 0.00% [0/4] presented in Table 14.

There were 143 couples with chromosomal polymorphic variants. The pregnancy rate with non-acrocentric variants in female and acrocentric variants in male 35.7%, [5/14]; no pregnancies recorded in non-acrocentric variants in female and male [0/1]; non-acrocentric variants in male and acrocentric variants in female 40%, [2/5]; combination of non-acrocentric and acrocentric variants in female and male 27.8%, [5/18], With one acrocentric variant in female and male 30.6%, [11/36]; one acrocentric variant in female and two acrocentric variants in male 22.7%, [5/22]; two acrocentric variants in female and one acrocentric variant in male 47.4%, [9/19]; two acrocentric variants in female and in male 75.0%, [3/4]; three acrocentric variants in female and one/two acrocentric variants in in male 75.0%, [3/4]. With non-acrocentric variant in female and Yqh in male 100.0%, [1/1]; acrocentric variant in female and Yqh in male 25.0%, [3/12]; combination of non-acrocentric and acrocentric in female, male and Yqh in male 57.1%, [4/7].

The miscarriage rate with non-acrocentric variants in female and acrocentric variants in male 21.4%, [3/14]; no pregnancies recorded in non-acrocentric variants in female and male [0/1]; non-acrocentric variants in male and acrocentric variants in female 20%, [1/5]; combination of non-acrocentric and acrocentric variants in female and male 16.7%, [3/18. With one acrocentric variant in female and male 16.7%, [6/36]; one acrocentric variant in female and two acrocentric variants in male 9.1%, [2/22]; two acrocentric variants in female and one acrocentric variant in male 26.3%, [5/19]; two acrocentric variants in female and in male 25.0%, [1/4]; three acrocentric variants in female and one/two acrocentric variants in in male 25.0%, [1/4]. There were no miscarriages reported with non-acrocentric variant in female and Yqh in male [0/1]; acrocentric

variant in female and Yqh in male 8.3.0%, [1/12]; combination of non-acrocentric and acrocentric in female, male and Yqh in male 42.9%, [3/7].

The live birth rate with non-acrocentric variants in female and acrocentric variants in male 14.3%, [2/14]; no pregnancies recorded in non-acrocentric variants in female and male [0/1]; non-acrocentric variants in male and acrocentric variants in female 20%, [1/5]; combination of non-acrocentric and acrocentric variants in female and male 11.1%, [2/18]. With one acrocentric variant in female and male 14.0%, [5/36]; one acrocentric variant in female and two acrocentric variants in male 13.6%, [3/22]; two acrocentric variants in female and one acrocentric variant in male 21.0%, [4/19]; two acrocentric variants in female and in male 50.0%, [2/4]; three acrocentric variants in female and one/two acrocentric variants in in male 50.0%, [2/4]. There was non-acrocentric variant in female and Yqh male 100.0%, [1/1]; acrocentric variant in female and Yqh in male 16.7%, [2/12]; combination of non-acrocentric and acrocentric in female, male and Yqh in male 14.3%, [1/7] presented in Table 14.

The crude and adjusted odds ratio analysis for pregnancies according to the non-acrocentric and acrocentric and combination of polymorphic variants, their types, numbers, gender of individuals and the couples on reproductive outcomes undergoing ICSI treatment is presented in Supplementary Table 3. There were no pregnancies reported in the females with a combination of non-acrocentric and acrocentric polymorphic variants, males with two acrocentric variants and Yqh and couples with non-acrocentric polymorphic variants.

The outcome of crude and adjusted odds ratios in the study of the non-acrocentric and acrocentric and combination of polymorphic variants, their types, numbers, gender of individuals and the couples on pregnancy of female, male and couples compared to couples without chromosomal polymorphism does not reach statistical significance.

The crude and adjusted odds ratio analysis for miscarriages according to the non-acrocentric and acrocentric and combination of polymorphic variants, their types, numbers, gender of individuals and the couples on reproductive outcomes undergoing ICSI treatment is presented in Supplementary Table 4. There were no miscarriages recorded in the males with one acrocentric polymorphic variant and Yqh, couples with non-acrocentric polymorphic variant in female and Yqh in male.

The outcome of crude and adjusted odds ratios in the study of the non-acrocentric and acrocentric and combination of polymorphic variants, their types, numbers, gender of individuals and the couples on miscarriages of female, male and couples compared to couples without chromosomal polymorphism does not reach statistical significance.

The crude and adjusted odds ratio analysis for live births according to the non-acrocentric and acrocentric and combination of polymorphic variants, their types, numbers, gender of individuals and the couples on reproductive outcomes undergoing ICSI treatment is presented in Supplementary Table 5. There were no livebirths recorded in the males with three acrocentric polymorphic variants. The single couple in the non-acrocentric polymorphic variant in female and Yqh in male resulted in a live birth.

The outcome of crude and adjusted odds ratios in the study of the non-acrocentric and acrocentric and combination of polymorphic variants, their types, numbers, gender of individuals and the couples on live births of female, male and couples compared to couples without chromosomal polymorphism does not reach statistical significance.

Discussion

This study described the effects of different variants of chromosomal polymorphism, which includes the non-acrocentric and acrocentric and combination of polymorphic variants, their types, numbers, gender of individuals and the couples on reproductive outcomes undergoing ICSI treatment. I did not find any evidence of a difference in pregnancy, miscarriage or live birth rates between couples without polymorphisms and those where one or both partners were carriers of a chromosomal polymorphisms. This was observed in the unadjusted univariate analysis and multivariate analysis adjusted for age, BMI, ovarian reserve markers and treatment type. Although, some of the point estimates suggest a clinically important impact, the confidence intervals were wide and cross the line of no effect.

The study limitations include a low prevalence of non-acrocentric and acrocentric chromosomal polymorphic variants. Therefore, a larger population would be preferable for more statistical power. Although, there were no statistical associations found, a type II error cannot be ruled out. The results from this exploratory study are meant only to be used for hypothesis generation in view of the multiple analyses carried out.

The evidence so far in this study associated with couples or female or male carriers with chromosomal polymorphic variants appear to have no adverse effect on reproductive outcomes of pregnancy, miscarriage and live births, compared to the couples without chromosomal polymorphism. There are similar studies been published that there were no adverse effect of chromosomal polymorphism of female or males with pregnancy. (Hong *et al.*, 2011; Liang *et al*., 2019).

The reports suggesting the ability of embryos to self-correct their minor genetical errors during their cleavage must be taken in to the consideration and examined. A recent study shed insight into human embryogenesis and the ability of self-correction, suggesting that genetic abnormalities may resolve during the initial stages of cell divisions up to implantation (Orvieto *et al.,* 2020). It is reasonable to postulate that through increased cell proliferation and cell death, mosaic embryos may be prone to self-correction in comparison to both euploidy and aneuploidy embryos (Santos *et al.,* 2010; Mantikou *et al.*,2012). Animal studies demonstrated that embryos during the preimplantation development encapsulate to overcome the chromosomal instability by chromosome containing fragments in to micronuclei and their elimination through cellular fragmentation (Daughtry *et al.*, 2019; Bolton *et al.,* 2016). The balance between cell survival and apoptosis is controlled by a cell death program that eliminates damaged cells in early embryo development (Orvieto *et al.,* 2021). Therefore, through self-correction, chromosomal polymorphic variants and indeed aneuploidy may lead to non-inferior treatment outcomes in carrier couples.

Similar to primary study in Chapter 3, secondary study in Chapter 4 and further analysis of Chapter 5, I found no association of chromosomal polymorphism with reproductive outcomes in the Sri Lankan population. Further analysis of the fertilisation and cleavage of gametes with chromosomal polymorphic variations and without polymorphism may shed light of the embryo development during embryo culture after ICSI procedure.

CHAPTER 6:

DOES CHROMOSOMAL POLYMORPHISM HAVE AN IMPACT ON FERTILISATION AND CLEAVAGE OF EMBRYOS? – AN ANALYSIS OF 540 ICSI CYCLES

Abstract

Research Question: Functions of heterochromatin in polymorphic regions may suppress or silence gene expression, which could affect spermatogenesis and oogenesis. This impact can play an important role in both male and female infertility. Although my primary and secondary studies did not differ the reproductive outcome, the aim of this study is to analyse the impact of chromosomal polymorphism in fertilisation and cleavage of the embryos in couples undergoing ICSI treatment.

Design: I analysed 540 ICSI cycles and excluded the frozen embryo transfer cycles at the fertility centre of the Lanka hospitals. Further, excluded participants were similar to Chapter 4. All participants of the ICSI cycles underwent karyotyping analysis using GTL-banding prior to the treatment. The primary outcomes were fertilisation and cleavage (Day 3) of embryos. I compared outcomes according to the presence or absence of chromosomal polymorphism amongst females, males and couples.

Results: There were 540 ICSI cycles analysed, in 86 both the female and male were carriers of polymorphisms (15.9%); in 87 only the females were carriers of polymorphisms (16.1%); in 119 only the males were carriers of polymorphisms (22.0%); and in 248 cycles neither partner carried a polymorphism (45.9%). I found there were low cleavage rate among female carriers (crude OR 1.50 [95% CI 1.14 to 1.97]; *P*= 0.00; adjusted OR 1.50 [95% CI 1.14 to 1.97]; *P*= 0.00) and female with acrocentric chromosomal polymorphism (crude OR 1.47 [95% CI 1.09 to 1.98]; *P*= 0.01; although

fertilisation in female, male and couples, and cleavage in males and couples does not differ in crude and adjusted odds ratios in the study.

Conclusion: The evidence identifies low cleavage in the females with chromosomal polymorphism and female with acrocentric chromosomal polymorphic variant, though there were no clear association between the presence of chromosomal polymorphism on fertilisation in female, male and couples, and cleavage in males and couples compared to couples without chromosomal polymorphism.

Introduction

Chromosomal polymorphic variations and their impact on fertilisation and cleavage is not yet clear. Although there are many studies suggesting chromosomal polymorphic variants have negative reproductive outcomes, predominantly reproductive failures and pregnancy losses (Ahmet Okay *et al.,* 2010; De la Fuente-Cortes *et al.,* 2009; Minocherhomji *et al.,* 2019; Pokale, 2015). Hence, chromosomal polymorphism and chromosomal composition of the embryonic genes remain very much speculative (Hernandez-Nieto *et al.,* 2021). The impact of the chromosomal polymorphism on gametes and the reproductive outcomes has not been widely examined and elucidated (Hernandez-Nieto *et al.,* 2021).

Low fertilisation and cleavage rate has been often linked to chromosomal polymorphism. Presence of chromosomal polymorphism showed that the fertilisation and embryo cleavage were low in males (Xu *et al.,* 2016). Additionally, the quality of cleavage (Day 3) embryos was not optimal (Xu *et al.,* 2016). Males with chromosomal polymorphism may result in abnormal embryonic development and low implantation rates after IVF or ICSI treatment (Guo *et al.,* 2012). A study of a Chinese population indicated that polymorphisms in females could be a contributing factor in causing lower embryo cleavage rates (Liang *et al,* 2014). Further, there are suggestions in the literature that large satellites in acrocentric chromosomes may develop improper chromosomal segregation, which leads to pregnancy losses (Hanif *et al.,* 2019).

Chromosomal polymorphism in males have been attributed to low fertilisation and cleavage rate in the recent systematic review. The review of a Chinese population

concluded there is a low fertilisation rate in male carriers of chromosomal polymorphism compared to male non-carriers of chromosomal polymorphism. Although there was no difference in the female carriers compared to female non-carriers of chromosomal polymorphism (Ou *et al.,* 2019). Further, males with chromosomal polymorphisms associated with lower cleavage rate compared to males without chromosomal polymorphisms, though females and couples with chromosomal polymorphism does not differ the cleavage rate compared to couples without chromosomal polymorphism (Ou *et al.,* 2019). The aim of this study is to analyse the impact of chromosomal polymorphism in fertilisation and cleavage of the embryos undergoing ICSI treatment.

Materials and Methods

Study Design

The study population is similar to participants of Chapter 4. I excluded the frozen embryo transfer cycles apart from the other cycles in the exclusion criteria at the fertility centre of the Lanka hospitals, since fertilisation and cleavage could be scored and evaluated in the fresh cycles only. All participants of the ICSI cycles underwent karyotyping analysis using GTL-banding prior to the treatment. The primary outcomes were fertilisation and cleavage (Day 3) of embryos. The total of 540 ICSI cycles were analysed in the study.

ICSI and Embryo Culture

Following oocyte insemination with ICSI, embryos were cultured (Vitrolife Sweden AB, V.Frolunda, Sweden) up to three days. All embryos were cultured in 6% CO_2 and 5% O_2 in incubators (Heracell, Germany). Fertilisation was observed 18 to 20 hours of intra

cytoplasmic sperm injection (Bhattacharya and Hamilton, 2021). Cleavage of embryos were evaluated and recorded daily for further 3 days. All embryos with more than six cells were selected. Two embryos were transferred per fresh cycle and the remaining embryos were vitrified.

Outcomes and Follow-up

The primary outcomes included fertilisation rate and cleavage rate. Outcome data were analysed with female, male and couple according to the presence or absence of chromosomal polymorphism. There were no missing data for demographic characteristics including age, BMI, FSH, LH, TSH, T4 and Prolactin. The fertilisation rate refers to total number of fertilised oocytes (2pn) from the total number of mature oocytes (MII). Cleavage rate refers to the total number of embryos cleaved up to six cells or more from the total number of fertilised oocytes.

Statistical Analysis

The fertilisation and cleavage rates were analysed on 540 ICSI cycles. All analysed data of 540 ICSI cycles were described with proportions for binary data, or means with standard deviations or median and inter-quartile range for continuous variables, as appropriate. Logistic regression models were fitted to estimate crude and adjusted odds ratio for confounding variables including age, BMI, FSH, LH hormones. All statistical analyses were done using STATA version 1.

Results

There were 1,879 ICSI and frozen embryo transfer cycles performed at the Fertility Centre during the study period. In total, 1339 ICSI and frozen embryo transfer cycles were

excluded from the analysis due to ectopic pregnancies, use of donor gametes, absence of karyotyping reports, numerical and structural abnormalities in karyotyping, poor follicular development, abnormal cleavage and blastocyst formation, embryo vitrified without transfers and records without pregnancy outcomes. Further, excluded 198 participants of frozen embryo transfer cycles who had a cleavage stage [(Day 3); (n=216)] or a blastocyst transfer (n=173). There were 540 participants who underwent long agonist (n=402) or short antagonist (n=138) protocol stimulation and, proceed with ICSI and embryo culture included in the study. In total, 292 treatment cycles with chromosomal polymorphism [292/540 (54.1)] and 248 treatment cycles without polymorphism [248/540 (46%)] included in the study (Figure 19).

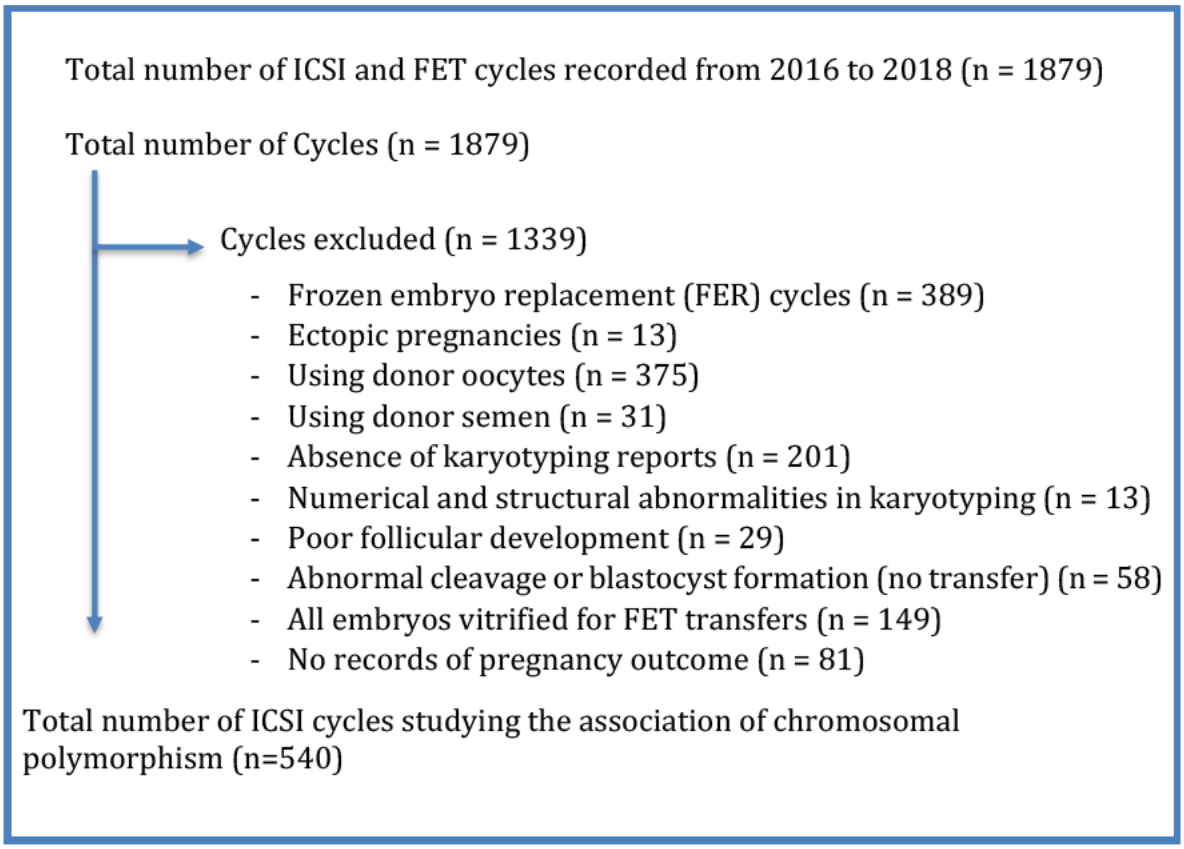

Figure 19: Flow chart of data selection process

Table 15 contains baseline characteristics of the study population.

Table 15: Baseline characteristics of the study population

Characteristics	Cohort with chromosomal polymorphism n (%) or mean (SD) (n=292)	Cohort without chromosomal polymorphism n (%) or mean (SD) (n=248)
Age	33.9± 3.9	34.1±4.2
BMI	23.8±3.1	24.0±4.1
FSH	6.6±1.6	6.6±1.7
LH	5.9±3.0	5.7±2.6
Treatment type		
ICSI cycles		
Long agonist	216 (74.0)	186 (75.0)
Short antagonist	76 (26.0)	62 (25.0)
Oocytes retrieved	15.9±8.8	15.2±7.5
Mature oocytes	15.5±8.8	14.5±7.5
Fertilised oocytes	11.9±8.2	10.6±6.3
Cleavage embryos (Day 3)	7.9±5.3	7.0±4.7

BMI refers to body mass index, FSH refers to follicle stimulation hormone, LH refers to luteinising hormone
ICSI refers to intra cytoplasmic sperm injection
All characteristics are of female participants

There were 248 ICSI cycles with non-carriers of chromosomal polymorphism (long agonist 75.0% [186/248]; short antagonist 25.0% [62/248]) recorded in 540 ICSI cycles in the study. The carriers of chromosomal polymorphism female only (long agonist 75.9% [66/87]; short antagonist 24.1% [21/87]), males only (long agonist 79.8% [95/119]; short antagonist 20.2% [24/119]), and couples with chromosomal polymorphism (long agonist 63.9% [55/86]; short antagonist 36.0% [31/86]). The total number of participants with chromosomal polymorphic variants was 292 (54.1%), while 248 (45.9%) did not exhibit any of the polymorphic variants. Table 15 shows details of fertilisation and cleavage according to the presence or absence of chromosomal polymorphisms.

The analysis of 540 ICSI cycles, in 86 both the female and male were carriers of polymorphisms (15.9%); in 87 only the females were carriers of polymorphisms (16.1%); in 119 only the males were carriers of polymorphisms (22.0%); and in 248 cycles neither partner carried a polymorphism (45.9%) presented in Table 16.

From the 540 ICSI cycles recorded in the study of which 292 were carriers of chromosomal polymorphism [(overall mean and SD of fertilisation 9.1±5.8; female only 8.9±5.6; male only 9.5±6.0; couples 8.9±5.9); (overall mean and SD of cleavage 6.0±4.2; female only 6.2±3.7; male only 6.2±4.6; couples 5.7±4.2)]. The total number of participants with chromosomal polymorphic variants was 292 (54.0%), while 248 (45.9%) did not exhibit any of the polymorphic variants. Table 16 shows details of means and standard deviations of fertilisation and cleavage according to the presence or absence of chromosomal polymorphism.

Table 16: Fertilisation and cleavage of carriers and non-carriers of chromosomal polymorphism

Polymorphism	Fertilisation mean ±SD	Cleavage mean ±SD
Females, males or couples with polymorphism (n=292)	9.1±5.8	6.0±4.2
Females with polymorphism (n=87)	8.9±5.6	6.2±3.7
Males with polymorphism (n=119)	9.5±6.0	6.2±4.6
Couples with polymorphism (n=86)	8.9±5.9	5.7±4.2
Couples without polymorphism (n=248)	8.9± 5.5	5.7±4.0
Total (n=540)	**9.0 ±5.7**	**5.9±4.1**

The crude and adjusted odds ratios of fertilisation and cleavage are presented in Table 17.

Table 17: Crude and adjusted odds ratios for fertilisation and cleavage rates

Outcome	Crude OR		Adjusted OR	
	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value
Fertilisation				
Females, males or couples with polymorphism	1.03 (0.88 to 1.21)	0.66	1.02 (0.87 to 1.20)	0.75
Females with polymorphism	0.99 (0.78 to 1.25)	0.96	0.98 (0.78 to 1.24)	0.89
Males with polymorphism	1.14 (0.93 to 1.40)	0.17	1.14 (0.93 to 1.39)	0.18
Couples with polymorphism	0.94 (0.73 to 1.19)	0.62	0.92 (0.72 to 1.17)	0.52
Cleavage				
Females, males or couples with polymorphism	1.12 (0.94 to 1.34)	0.18	1.14 (0.95 to 1.36)	0.13
Females with polymorphism	1.50 (1.14 to 1.97)	0.00	1.50 (1.14 to 1.97)	0.00
Males with polymorphism	0.97 (0.78 to 1.21)	0.82	0.99 (0.80 to 1.24)	0.98
Couples with polymorphism	1.05 (0.81 to 1.35)	0.69	1.07 (0.83 to 1.37)	0.58

The reference category is no chromosomal polymorphism in either partner
OR, odds ratio
CI, 95% confidence intervals

Overall, crude and adjusted odds ratios for the fertilisation outcome of female, male and couples [Crude OR 1.03 (95% CI 0.88 to 1.21), *P*=0.66; Adjusted OR 1.02 (95% CI 0.87 to 1.20), *P*=0.75], female with polymorphism [Crude OR 0.99 (95% CI 0.78 to 1.25), *P*=0.96; Adjusted OR 0.98 (95% CI 0.78 to 1.24), *P*=0.89], male with polymorphism [Crude OR 1.14 (95% CI 0.93 to 1.40), *P*=0.17; Adjusted OR 1.14 (95% CI 0.93 to 1.39), *P*=0.18], Couples with polymorphism [Crude OR 0.94 (95% CI 0.73 to 1.19), *P*=0.62; Adjusted OR 0.92 (95% CI 0.72 to 1.17), *P*=0.52] compared to couples without chromosomal polymorphism does not reach statistical significance.

Overall, crude and adjusted odds ratios of the cleavage outcome for male and couples [Crude OR 1.12 (95% CI 0.94 to 1.34), *P*=0.18; Adjusted OR 1.14 (95% CI 0.95 to 1.36), *P*=0.13], male with polymorphism [Crude OR 0.97 (95% CI 0.78 to 1.21), *P*=0.82; Adjusted OR 0.99 (95% CI 0.80 to 1.24), *P*=0.98], couples with polymorphism [Crude OR 1.05 (95% CI 0.81 to 1.35), *P*=0.69; Adjusted OR 1.07 (95% CI 0.83 to 1.37), *P*=0.58] compared to couples without chromosomal polymorphism does not reach statistical significance, except females with chromosomal polymorphism. I found there was a low cleavage rate among female with chromosomal polymorphism (crude OR 1.50 [95% CI 1.14 to 1.97]; *P*= 0.00; adjusted OR 1.50 [95% CI 1.14 to 1.97]; *P*= 0.00) compared to couples without polymorphism.

Further, analysis of fertilisation and cleavage according to the female carriers of non-acrocentric polymorphism only, female carriers of acrocentric polymorphism only, female carriers with both of non-acrocentric and acrocentric chromosomal polymorphism, male carriers of non-acrocentric polymorphism only, male carriers of acrocentric polymorphism only, male carriers with both of non-acrocentric and acrocentric chromosomal polymorphism, males with Yqh, couples with non-acrocentric or acrocentric chromosomal polymorphism and couples without any of the chromosomal polymorphisms displayed in Table 18.

From the analysis of 540 cycles of fertilisation and cleavage, in 11 the female were carriers of non-acrocentric polymorphism only (2.0%), in 74 the female were carriers of acrocentric polymorphism only (13.7%), in 6 the female were carriers with both non-acrocentric and acrocentric polymorphism (1.1%), in 17 the male were carriers of non-

acrocentric polymorphism only (3.1.%), in 77 the male were carriers of acrocentric polymorphism only (14.2%), in none of the male were carriers with both non-acrocentric and acrocentric polymorphism (0.0%), in 21 only males were carriers of Yqh (3.9%), in 86 both the female and male were carriers of non-acrocentric, acrocentric or combination of polymorphism (15.9%) and in 248 cycles none of the partners carried a chromosomal polymorphism (45.9%) are presented in Table 18.

Table 18: Mean and standard deviations of the non-acrocentric, acrocentric, male Yqh and combination of polymorphic variants in female, male and couples

Polymorphism	Fertilisation mean ±SD	Cleavage mean ±SD
Females, males or couples with polymorphism (n=292)	9.1±5.8	6.0±4.2
Non-Acrocentric female only (n=11)	11.6±6.9	8.0±4.5
Acrocentric female only(n=74)	8.6±5.3	6.0±3.5
Both non-acrocentric & acrocentric female only (n=6)	10.6±6.5	7.1±4.2
Non-Acrocentric male only (n=17)	9.0±5.3	5.6±4.8
Acrocentric male only (n=77)	9.6±6.1	6.1±4.6
Both non-acrocentric & acrocentric male only (n=0)	0.0±0.0	0.0±0.0
Yqh in male (n=21)	8.9±6.3	5.6±4.7
Couples with non-acrocentric and acrocentric (n=86)	8.9±5.9	5.7±4.2
Couples without polymorphism (n=248)	8.9±5.5	5.7±4.0
Total (n=540)	**9.0±5.7**	**5.9±4.1**

There was no fertilisation or cleavage recorded in both non-acrocentric & acrocentric male only group

There were 292 female, male and couples with chromosomal polymorphism (overall mean and SD of fertilisation 9.1±5.8; cleavage 6.0±4.2); non-acrocentric female only (mean and SD of fertilisation 11.6±6.9; cleavage 8.0±4.5); acrocentric female only (mean and SD of fertilisation 8.6±5.3; cleavage 6.0±3.5); both non-acrocentric and acrocentric female only (mean and SD of fertilisation 10.6±6.5; cleavage 7.1±4.2); non-acrocentric

male only (mean and SD of fertilisation 9.0±5.3; cleavage 5.6±4.8); acrocentric male only (mean and SD of fertilisation 9.6±6.1; cleavage 6.1±4.6); none in the both non-acrocentric and acrocentric male only; Yqh in male (mean and SD of fertilisation 8.9±6.3; cleavage 5.6±4.7); couples with non-acrocentric and acrocentric (mean and SD of fertilisation 8.9±5.9; cleavage 5.7±4.2); couples without polymorphism (mean and SD of fertilisation 8.9±5.5; cleavage 5.7±4.0) recorded in 540 cycles in the study. The total number of participants with chromosomal polymorphic variants was 292 (54.1%), while 248 (45.9%) did not exhibit any of the polymorphic variants. Table 18 shows details of fertilisation and cleavage according to the presence or absence of chromosomal polymorphism.

The crude odds ratio analysis for fertilisation and cleavage according to the presence of non-acrocentric and acrocentric chromosomal polymorphisms is presented in Table 19. I found no association between non-acrocentric and acrocentric chromosomal polymorphisms and outcome of fertilisation and cleavage of embryos. The adjusted odds ratio analysis, adjusted for age, BMI, FSH and LH and type of treatment (fresh vs frozen) also found no association between chromosomal polymorphisms and these reproductive outcomes (Table 19).

Table 19: Crude and adjusted odds ratio for fertilisation and cleavage

Outcome	Crude OR		Adjusted OR	
	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
Fertilisation				
Females, males or couples with polymorphism	1.03 (0.88 to 1.21)	0.66	1.02 (0.87 to 1.20)	0.75
Non-acrocentric female only	1.70 (0.95 to 3.05)	0.07	1.72 (0.95 to 3.11)	0.07
Acrocentric female only	0.95 (0.74 to 1.21)	0.39	0.94 (0.73 to 1.19)	0.62
Both acrocentric & non-acrocentric female only	0.99 (0.50 to 1.97)	0.99	0.96 (0.49 to 1.86)	0.91
Non-acrocentric male only	1.17 (0.77 to 1.76)	0.44	1.15 (0.76 to 1.74)	0.48
Acrocentric male only	1.15 (0.90 to 1.46)	0.25	1.15 (0.90 to 1.46)	0.25
Yqh in male	1.04 (0.73 to 1.47)	0.82	1.02 (0.73 to 1.44)	0.87
Couples with non-acrocentric or acrocentric	0.94 (0.73 to 1.19)	0.62	0.92 (0.72 to 1.18)	0.53
Cleavage				
Females, males or couples with polymorphism	1.12 (0.94 to 1.34)	0.18	1.14 (0.95 to 1.36)	0.13
Non-acrocentric female only	1.40 (0.78 to 2.53)	0.25	1.34 (0.73 to 2.44)	0.34
Acrocentric female only	1.47 (1.09 to 1.98)	0.01	1.48 (1.11 to 1.99)	0.00
Both acrocentric & non-acrocentric female only	1.54 (0.62 to 3.78)	0.34	1.56 (0.64 to 3.79)	0.31
Non-acrocentric male only	1.47 (0.79 to 2.74)	0.22	1.57 (0.85 to 2.89)	0.14
Acrocentric male only	0.91 (0.71 to 1.17)	0.49	0.92 (0.71 to 1.19)	0.54
Yqh in male	0.91 (0.61 to 1.34)	0.64	0.94 (0.62 to 1.40)	0.76
Couples with non-acrocentric or acrocentric	1.05 (0.81 to 1.35)	0.69	1.07 (0.83 to 1.37)	0.58

The reference category is no chromosomal polymorphism in either partner
There were no fertilisation or cleavage recorded in both non-acrocentric & acrocentric male only group
OR, odds ratio
CI, 95% confidence interval

Overall, crude and adjusted odds ratios for the fertilisation outcome of female, male and couples [Crude OR 1.03 (95% CI 0.88 to 1.21), *P*=0.66; Adjusted OR 1.02 (95% CI 0.87 to 1.20), *P*=0.75], non-acrocentric female only [Crude OR 1.70 (95% CI 0.95 to 3.05), *P*=0.07; Adjusted OR 1.72 (95% CI 0.95 to 3.11), *P*=0.07], acrocentric female only [Crude OR 0.95 (95% CI 0.74 to 1.21), *P*=0.39; Adjusted OR 0.94 (95% CI 0.73 to 1.19), *P*=0.62], both non-acrocentric and acrocentric female only [Crude OR 0.99 (95% CI 0.50 to 1.97), *P*=0.99; Adjusted OR 0.96 (95% CI 0.49 to 1.86), *P*=0.91], non-acrocentric male only [Crude OR

1.17 (95% CI 0.77 to 1.76), *P*=0.44; Adjusted OR 1.15 (95% CI 0.76 to 1.74), *P*=0.48], acrocentric male only [Crude OR 1.15 (95% CI 0.90 to 1.46), *P*=0.25; Adjusted OR 1.15 (95% CI 0.90 to 1.46), *P*=0.25], Yqh+/- in male [Crude OR 1.04 (95% CI 0.73 to 1.47), *P*=0.82; Adjusted OR 1.02 (95% CI 0.73 to 1.44), *P*=0.87] and couples with non-acrocentric and acrocentric chromosomal polymorphism [Crude OR 0.94 (95% CI 0.73 to 1.19), *P*=0.62; Adjusted OR 0.92 (95% CI 0.72 to 1.18), *P*=0.53] compared to couples without chromosomal polymorphism does not reach statistical significance.

Overall outcome of crude and adjusted odds ratios of cleavage of female, male and couples [Crude OR 1.12 (95% CI 0.94 to 1.34), *P*=0.18; Adjusted OR 1.14 (95% CI 0.95 to 1.36), *P*=0.13], non-acrocentric female only [Crude OR 1.40 (95% CI 0.78 to 2.53), *P*=0.25; Adjusted OR 1.34 (95% CI 0.73 to 2.44), *P*=0.34], both non-acrocentric and acrocentric female only [Crude OR 1.54 (95% CI 0.62 to 3.78), *P*=0.34; Adjusted OR 1.56 (95% CI 0.64 to 3.79), *P*=0.31], non-acrocentric male only [Crude OR 1.47 (95% CI 0.79 to 2.74), *P*=0.22; Adjusted OR 1.57 (95% CI 0.85 to 2.89), *P*=0.14], acrocentric male only [Crude OR 0.91 (95% CI 0.71 to 1.17), *P*=0.49; Adjusted OR 0.92 (95% CI 0.71 to 1.19), *P*=0.54], Yqh+/- in male [Crude OR 0.91 (95% CI 0.61 to 1.34), *P*=0.64; Adjusted OR 0.94 (95% CI 0.62 to 1.40), *P*=0.76] and couples with non-acrocentric and acrocentric chromosomal polymorphism [Crude OR 1.05 (95% CI 0.81 to 1.35), *P*=0.69; Adjusted OR 1.07 (95% CI 0.83 to 1.37), *P*=0.58] compared to couples without chromosomal polymorphism does not reach statistical significance except female with acrocentric chromosomal polymorphism. I found there was a low cleavage rate among female with acrocentric chromosomal polymorphism (crude OR 1.47 [95% CI 1.09 to 1.98]; *P*= 0.01; adjusted OR 1.48 [95% CI 1.11 to 1.99]; *P*= 0.00) compared to couples without polymorphism.

Discussion

In this analysis, I found there was a low cleavage rate among female with chromosomal polymorphism compared to couples without polymorphism. Further, I found there was a low cleavage rate among female with acrocentric chromosomal polymorphism compared to couples without polymorphism. There was no evidence of a difference in fertilisation and cleavage of male and couples, and among non-acrocentric female only, both non-acrocentric and acrocentric female only, non-acrocentric male only, acrocentric male only, Yqh in males, couples with non-acrocentric and acrocentric chromosomal polymorphism compared to couples without chromosomal polymorphisms. This was observed in the unadjusted univariate analysis and multivariate analysis adjusted for age, BMI, ovarian reserve markers and treatment type. Although, some of the point estimates suggest a clinically important impact, the confidence intervals were wide and cross the line of no effect.

The analysed study cohort underwent ICSI treatment cycles, but not all participants proceeded with fresh embryo transfer. Those who did not have a fresh embryo transfer due to ovarian hyperstimulation, not optimal endometrial preparation, personal reasons or any other nonspecific reason have been excluded from the study, since the reproductive outcome could not be evaluated. This study population is relatively large, but I cannot rule out a type II error. There were no missing data or the follow-up of fertilisation and cleavage rate, and I was able to analyse the result for potential confounders. Although there was a low cleavage rate among females with chromosomal polymorphic variations and with the multiple analysis of chromosomal polymorphism

does not differ the reproductive outcome. Therefore, the finding does not have any positive impact in reproductive outcome and should be interpreted with caution.

The findings are consistent with the background literature summarised in the single centre cohort study of the Chinese population (Li *et al*., 2020). The study suggested that there was a low 2pn cleavage rate in the females with 9(inv) and acrocentric chromosomal polymorphic variants in IVF cycles. Although, same as the background literature summarised in my systematic review there is no paucity of evidence of whether polymorphic variation in individuals (males or females) or couples adversely affects the chance of their clinical outcome (Ralapanawe *et al*., 2022a).

The outcome of fertilisation and cleavage of female, male and couples differ in different studies. The existing literature is conflicting, with some authors reporting that chromosomal polymorphisms are associated with low cleavage rate (Li *et al.*, 2020; Liang *et al.,* 2014), while others have identified there was a low fertilisation and cleavage rate or abnormal embryo development in males (Xu *et al.,* 2016; Guo *et al.,* 2012). Recent meta-analysis suggests there was a low fertilisation and cleavage rate in males in the Chinese population (Ou *et al*., 2019). It is possible that my study may have been underpowered to detect an any differences in males. Further, a small adverse effect may exist for some populations in males or couples, but not others.

The sequential culture media mimic physiological conditions for fertilisation and cleavage of embryos. The advances in sequential culture media, advent of vitrification and embryo culture techniques from cleavage stage to blastocyst formation led to better reproductive

outcome (Burks *et al.,* 2021). This will facilitate the gene expression, metabolic and enzyme activity, maintenance of the calcium levels and cytoskeleton and cell proliferation of the cleavage stage embryos (Bhattacharya & Hamilton, 2021). Further, more sophisticated time lapse imaging helps to assess the morphology and the cleavage of embryos without removing the embryos for the daily assessment. Though, still there is uncertainty that whether improvement of culture media and technology may have improved fertilisation and cleavage up to blastocyst formation leading to better reproductive outcome.

This study identified low cleavage in the females with chromosomal polymorphism and female with acrocentric chromosomal polymorphic variant, though there were no clear association between the presence of chromosomal polymorphism of fertilisation in female, male and couples, and cleavage in males and couples compared to couples without chromosomal polymorphism. Potential future studies could evaluate the association between chromosomal polymorphisms and outcomes of fertilisation and cleavage in patients of multiple ethnicities.

CHAPTER 7:

KARYOTYPING AND CHROMOSOMAL ABERRATIONS

Abstract

Research Question: The genetic factors attributed to fertility, apart from chromosomal polymorphic variations are chromosomal aberrations (numerical and structural abnormalities). The chromosomes of structural rearrangements interfere with the mechanism in meiotic segregation of chromosomal rearrangements which leads to the risk of miscarriages and birth defects. The aim of this study is to evaluate whether the routine karyotyping may still have a role outside the research context.

Design: There were 1,879 fresh and frozen embryo transfer cycles performed during the study period of January 2016 to December 2018. There were 13 couples (1.8%) with structural rearrangements, which, I have excluded from the cohort of the study.

Results: During the routine karyotyping of couples in this study, I discovered 13 couples (1.8%) with at least one structural abnormality or mosaicism that had clinical implication. Without knowledge of this structural abnormality or mosaicism, these couples would have undergone standard IVF or ICSI procedure.

Conclusion: Detecting the chromosomal aberrations in routine karyotyping testing could offer pre-implantation genetic testing with structural rearrangement to the couple during the genetic counselling. Pre-implantation genetic testing for structural chromosomal rearrangements (PGT-SR) and selecting the unaffected embryos to transfer will avoid passing the chromosomal abnormality to the offspring. Hence, routine karyotyping may still have a role outside the research context.

Introduction

The genetic factors attributed to fertility, apart from chromosomal polymorphic variations are chromosomal aberrations (numerical and structural abnormalities) (Yahaya *et al.,* 2021). The frequency of chromosomal aberrations in the general population estimated between 0.15 to 0.2% (Verdoni *et al.,* 2021). The prevalence of chromosomal structural rearrangements is 0.4% in prenatal samples and it is 25% higher in the infertile population compared to general population (Matau-Brull *et al.,* 2019). The common structural abnormalities are reciprocal and Robertsonian translocations (Lledo *et al.,* 2010). The phenotype of these translocations is usually normal; however, these carriers have relatively increased risk of producing unbalanced gametes which could lead to infertility and recurrent miscarriages (Boynukalin *et al*., 2021). Further, abnormal chromosomal segregation during the gametogenesis and parental balanced reciprocal translocations resulted in many of miscarriages. Hence, 1% accounts for the unbalanced translocation in spontaneous miscarriages (Pal *et al.,* 2018).

It is important to understand the meiotic segregation in the chromosomal rearrangement to predict the reproductive outcome. The chromosomes of structural rearrangements interfere with correct segregation of rest of the chromosomes by disrupting chromosomal alignment of the spindle during miosis-I (Mateu-brill *et al*., 2019). This phenomenon is well known as interchromosomal effect (ICE) observed among carriers of balanced translocations of the patients with trisomy 21 (Down's syndrome) children (Lejeune, 1963). Chromosomes involved in rearrangement of reciprocal translocations during meiosis-I, form a quadrivalent. Centromeres from different chromosomes separate and

mitigate in adjacent 1 segregation, in contrary homologous centromeres mitigate in adjacent-2 segregation. Complete or partial nondisjunction of the quadrivalent leads to 3.1 or 4.0 segregation (Scriven *et al.,* 1998). Thus, understanding the mechanism in meiotic segregation of chromosomal rearrangements is essential for the estimation of the risk of miscarriages and birth defects (Lledo *et al.,* 2010).

To overcome the negative reproductive outcomes due to chromosomal aberrations, there have been many methods introduced. Out of the several methods, one of the main methods is preimplantation genetic testing with structural chromosomal rearrangements (PGT-SR) analysis (Fiorentino *et al.,* 2011). Most recent analysis were whole genome amplification (WGA), microarray single-nucleotide polymorphisms (SNPs), array comparative genomic hybridisation (aCGH) and next generation sequencing (NGS).

Various Chromosomal Aberrations

Balanced or Unbalanced Translocations

In the gametogenesis, during the segregation of chromosomes the translocation can occur either balanced or unbalanced. Chromosomal aberrations such as reciprocal translocations been identified in karyotyping reports, which is characterised by balanced rearrangement of the chromosomes without any loss of DNA. Individuals with translocations are phenotypically normal and unless the translocation breakpoints disrupt the dominant genetic components (Verdoni *et al.,* 2021).

Female X Chromosome Mosaicism

One of the common abnormalities in women is X chromosome mosaicism such as 45X/46XX or 47XXX/46XX, which identifies in karyotyping reporting. Women with X chromosome mosaicism are at increased risk of chromosomal instability and aneuploidy gametes (Zachaki *et al*., 2020).

Male X Chromosome Mosaicism

Male X chromosome mosaicism has not been examined in large populations. In hemizygous males, the X chromosome may have a deleterious effect in cellular function (Zhou *et al.*, 2021). The most common chromosomal aberration in male infertility caused by Klinefelter syndrome (47, XXY), which also identifies in cytogenetic reports (Ozdermir *et al.,* 2020).

Deletions, Duplications and Insertions

Micro deletions, duplications and insertions which could result in recurrent miscarriages are also detected by preimplantation genetic testing. Identifying and defining the sequencing parameters for the applications in next generation sequencing and with minimal resolution detects the deletions, duplications and insertions ≥ 6Mb (Garcia-Pasqual *et al.*, 2020; Butler *et al.,* 2019).

Materials and Methods

There were 1,879 fresh and frozen embryo transfer cycles performed during the study period of January 2016 to December 2018. I excluded ectopic pregnancies, structural chromosomal rearrangements, absence of reports of karyotyping analysis, opted for donor gametes, sub-standard follicle development, abnormal cleavage of embryos and blastocysts, embryo vitrification without transfer and records without pregnancy outcomes, in which total of 950 fresh and frozen embryo transfer cycles were excluded (Figure I8). From the excluded cohort of structural rearrangement, there were 13 couples which could have undergone an ICSI cycle. They were counselled since preimplantation genetic testing were not available during the study period.

Results

Chromosomal Aberrations and Mosaicism in the Main Study

During the routine karyotyping of couples in this study, I discovered 13 couples (1.8%) with at least one structural abnormality or mosaicism that had clinical implications (Table 20). Without knowledge of this structural abnormality or mosaicism, these couples would have undergone standard IVF or ICSI procedure.

Table 20: Couples excluded from the study with Chromosomal aberrations

Female Karyotype	Male Karyotype
mos 47,XX, +mar[2 /46XX[41	46,XY
45,XX,rob,(13;14)(q10;q10	46,XY
46,XX, t(1:3)(q32;p12)	46,XY
46,XX,22pstk+	47,XXY(2)/46XY(33)
45,X, 9qh+(2)/ 46XX, 9qh+(38)	46,XY
46,XX,14pstk+,22pstk+	47,XXY (1)/ 46XY(19)
46,XX,21pstk+	46,XY, t(9;19)(p22;q13.2)
46,XX,13pstk+	46,XY, t(9;18)(q21.1:q22)
45,X[2 / 46XX[38	46,XY
46,XX, t(6;10)(p12;p12.1)	46,XY
47,XXX,9qh-,15pstk+[1 / 46XX, 9qh-,15pstk+[33	46,XY, Yqh-,21pstk+
46,XX,15pstk+,22pstk+	46,XY, t(13:15)(q31:q15)
47,XXX,[1 / 46XX,[19	46,XY, 15pstk+

There were three females and three males with translocations, in which one female with a balance translocation and a numerical abnormality. There were five females with mosaicisms and two males with mosaicism in karyotyping.

Fertility Treatment Options Offered with Chromosomal Aberrations

While the preimplantation genetic testing was not available during the study period, all female partners underwent an IVF cycle with donor egg programme and males were with donor semen programme. Although they were counselled to travel abroad for the treatments, they opted for donor gametes mainly for financial reasons and the relatively

low success rates with pre implantation genetic testing. Therefore, they have opted for donor gametes as alternative for their own gametes IVF cycles.

Discussion

From the total number of study population of 1,879 fresh and frozen embryo transfer cycles there were thirteen couples (1.8%), in which eight females and five males with structural abnormalities during the study period. There were both females and males with translocations and mosaicisms. One female had a balance translocation.

The study sample was large and the attrition was low. The study limitations include a low prevalence of structural aberrations. Thus, a larger population needed for more statistical power. However, I cannot rule out a type II error.

From the 942 treatment cycles (548 fresh ICSI cycles and 394 frozen embryo transfer cycles) 697 couples were included in the study. None of them were aware of having a chromosomal polymorphic variation in their karyotyping report. Apart from that there were thirteen couples with a structural aberration with a normal phenotypical appearance planning to undergo an ICSI cycle as well. Although a larger population were screened, they were limited to the attendees to the Lanka hospitals fertility centre. Therefore, prevalence of the structural aberrations may differ from the overall infertile population. Hence, generalisability may be limited.

The preimplantation genetic testing was not available during the study period; therefore, I could not offer such treatment for the couples during the study period. Although it is

offered at present. On the other hand, a smaller number of participants were found to have structural aberrations compared to the study population and after the counselling the couples opted for donor egg and donor semen treatment.

The structural rearrangements of preimplantation genetic testing by array comparative genomic hybridisation followed by next generation sequencing could enhance the evaluation of the chromosomal aberrations. The previous studies of chromosomal abnormalities were analysed using fluorescence in-situ hybridisation (FISH), though it can identify only the selected chromosomes rather than all of the chromosomes (Wilton et al., 2009). The chromosomes which are involved in the rearrangements may have a detrimental effect on normal chromosomes, which is defined as interchromosomal effect, though could not be identified by fluorescence in-situ hybridisation (Lejeune**,** 1963; Boynukalin *et al*., 2021). However, there are studies suggesting that array comparative genomic hybridisation could evaluate interchromosomal effect at cleavage stage (Mateu-Brull *et al*., 2019). Further, few studies suggest interchromosomal effect could evaluate at blastocyst stage. The factors affect the patterns of segregation of the euploidy embryos are the length of the translocated and interstitial segments, centromere position and the presence or absence of heterochromatic regions (Jalbert *et al*., 1980; Boynukalin *et al*., 2021). Therefore, PGT- SR with array comparative genomic hybridisation followed by next generation sequencing could evaluate the chromosomal aberrations in embryos.

The combined testing of pre-implantation genetic testing for aneuploidy (PGT-A) and structural chromosomal rearrangements (PGT-SR) will upgrade the role of genetic analysis of embryos. Pre-implantation genetic testing (PGT) is performed to analyse the

DNA of the blastomere or trophectoderm biopsies of an embryo to determine the genetic abnormalities (Coonen *et al.,* 2020; Viotti, 2020). Pre-implantation genetic testing for aneuploidy is to analyse the DNA for aneuploidy and preimplantation genetic testing for structural chromosomal rearrangements analyses the structural rearrangement of the DNA in an embryo, in which identifies chromosomal aberrations (Viotti, 2020; Zegers-Hochschild *et al.*, 2017). Pre-implantation genetic testing for structural chromosomal rearrangements gives an opportunity to identify the chromosomally unbalanced embryos. The comprehensive chromosomal screening done on blastomere or trophectoderm biopsies by using array array comparative genomic hybridisation and next generation sequencing (Boynukalin *et al*., 2021). Therefore, genetic testing for structural chromosomal aberrations will lead to select the euploidy embryo for the intra uterine transfer, which increase the pregnancy rate and decrease the miscarriage rate; the end result to achieve a normal pregnancy and a healthy child (Coonen *et al.*, 2020).

The karyotyping analysis as a standard method will provide valuable information of the parental cytogenetic aberrations, which will lead to genetic counselling process prior to IVF or ICSI procedure. Although, it is very stressful for the couples to consider their genetic history, financial difficulties and the physical impact of reproductive treatment and their outcome. By detecting the chromosomal aberrations in routine karyotyping testing, this could offer pre-implantation genetic testing with structural rearrangement to the couple during the genetic counselling. Pre-implantation genetic testing for structural chromosomal rearrangements testing and selecting the unaffected embryos to transfer will avoid passing the chromosomal abnormality to the offspring. Hence, routine karyotyping may still have a role outside the research context.

CHAPTER 8:

GENERAL DISCUSSION: INTERPRETATION OF THE FINDINGS, IMPLICATIONS AND FUTURE RESEARCH

Interpretation of the Findings

Summary of the Findings from My Research

The systematic review analyses the association of chromosomal polymorphism in the reproductive outcome in different geographical populations. There were observational studies were included in the review and the meta-analysis. I found a higher miscarriage rate when females are carriers of chromosomal polymorphisms. There is little or no evidence that chromosomal polymorphisms in individuals (males or females) or couples that they adversely affect the chance of a pregnancy, clinical pregnancy, on-going pregnancy at study end, pre-term births and live births following IVF or ICSI. Although, there was a higher miscarriage rate attributed to females with chromosomal polymorphism, this was due to the higher weight of one study, therefore overall female polymorphism makes little or no difference in the live birth rate.

The primary cohort study explores the association between chromosomal polymorphisms and reproductive outcomes in couples undergoing ICSI treatment in the population of Sri Lankan ethnic origin. Further, stimulation protocols of all the participants were strictly monitored and all ICSI were performed by myself as sole embryologist in the fertility centre, which minimised the variation of the outcome in the study. I found, there was no evidence of a difference in pregnancy, miscarriage or live birth rates between couples without polymorphisms and those where either partner or both partners were carriers of a chromosomal polymorphisms. Although, some of the point estimates suggest a clinically important impact, the confidence intervals were wide and cross the line of no effect. This study is large, but I cannot rule out a type II error.

A secondary analysis of the cohort explores the association between types and number of chromosomal polymorphic variants and their reproductive outcomes in couples undergoing ICSI treatment. This study describes the relationship between the types such as female or male carriers of non-acrocentric polymorphism only, female or male carriers of acrocentric polymorphism only, either female or male carriers with combination of non -acrocentric and acrocentric chromosomal polymorphism, males with Yqh, couples with one or more than one of non-acrocentric or acrocentric chromosomal polymorphism or a combination and couples without any of the chromosomal polymorphism. The pregnancy rates, miscarriage rates and live birth rates did not differ substantially between couples with no chromosomal polymorphisms compared to couples were the female or the male were carriers of a specific chromosomal polymorphism or both of them in the unadjusted univariate analysis or multivariate adjusted for age, body mass index, and ovarian reserve. Further, the confounding variables were considered according to induction protocols and hormone doses due to clinical reasons and did not adjust for previous miscarriages or IVF failures, since there were few participants with those outcomes in the study.

A comprehensive exploration of the dataset did not reveal any other high-risk groups focused on the types, numbers, gender of individuals and the couples with chromosomal polymorphic variants and couples without polymorphism on the reproductive outcomes of ICSI treatment. In the study of different exploration of each polymorphic variant (chapter 6) did not differ the outcome of pregnancy, miscarriage or live birth rates between couples without polymorphisms and those where one or both partners were

carriers of a chromosomal polymorphisms, which would have benefited from further hypothesis testing research.

The secondary analysis of the cohort of ICSI fresh treatment cycles only (n= 540) suggests low cleavage rate among females with chromosomal polymorphism and females with acrocentric chromosomal polymorphism compared to couples without polymorphism. There was no evidence of a difference in fertilisation and cleavage of male and couples, and among non-acrocentric female only, both non-acrocentric and acrocentric female only, non-acrocentric male only, acrocentric male only, male Yqh, couples with non-acrocentric and acrocentric chromosomal polymorphism compared to couples without chromosomal polymorphisms. However, this does not translate to a clinically significant difference in reproductive outcomes and should be interpreted with caution.

I was able to screen karyotyping of 1,879 ICSI fresh and frozen embryo transfer cycles during the study period and found a prevalence of 1.8% with structural rearrangements. These couples would have undergone an ICSI cycle procedure without the knowledge of this structural abnormality or mosaicism. Therefore, during genetic counselling couples could offer pre-implantation genetic testing with structural rearrangement and selecting the unaffected embryos to transfer.

Explanation of Findings from My Research

Different Ethnicities Could Have Different Effects

Many of the published studies were retrospective cohorts reported after a miscarriage or referred to infertility center for investigations and were in Chinese origin. Nine out of ten studies of my systematic review were also Chinese in origin. Although, the studies were conducted in different university infertility clinics in different provinces in China. My main research was based in Sri Lanka at Lanka Hospitals Fertility Centre. The aim was to investigate the effect of chromosomal polymorphic variations on reproductive outcome in Sri Lankan population. However, the evidence did not identify a clear association between the presence of chromosomal polymorphism in females, males or couples and reproductive outcomes compared to couples without chromosomal polymorphism.

Many studies focused on miscarriages and the karyotyping test was performed after recurrent miscarriages. Chromosomal polymorphic variations identified, though not been compared with the normal karyotyping infertile population. This may mislead the effects towards an adverse effect with chromosomal polymorphic variations. My study compares the effect of couples with chromosomal polymorphic variations and couples without chromosomal polymorphic vitiations and their reproductive outcome, instead of miscarriages only. Therefore, there may not have any significance in the overall reproductive outcome with or without chromosomal polymorphism.

Insufficient statistical power

In my systematic review and meta-analysis, there were few studies included for the interpretation of the findings (n=10) and most of the estimates in this review have wide confidence intervals. Overall, this systematic review found only a few studies contributing data to each analysis resulting in unstable estimates. There is substantial uncertainty about how the carriers of chromosomal polymorphisms affects reproductive outcomes after ART. Further, I was unable to divide into treatment groups such as intra uterine insemination (IUI), IVF or ICSI. The findings are not easily generalisable as most studies involved participants of Chinese origin and one study focused on Turkish origin. Therefore, extrapolation to other populations may not be appropriate.

For improving the generalisability of the evidence, more studies are required involving different ethnicities. Studies would need to examine polymorphism in both males and females to identify if there is an association with adverse reproductive outcomes following IVF or ICSI, and explore if there is a biological gradient when both males and females are affected. Furthermore, most of the study gives a cumulative effect of specific types of chromosomal polymorphisms.

Despite this, including 1879 ICSI and frozen embryo transfer cycles during the period of 2016 to 2018 and follow up until November 2019, I was able to study 942 ICSI and frozen embryo transfer cycles (total number of couples n =697) in my primary study. A small proportion of outcome data on pregnancy, miscarriage, and live birth were missing or not reported and were not included in the study. This study is large, but I cannot rule out a type II error. The attrition or loss to follow-up rate were low, and I was able to adjust the

result for potential confounders. There is uncertainty about which carrier is more important with some studies focusing on males, other studies on females and a few studies on couples.

Other studies were larger and for an example Cheng *et al*., 2017 analysed 19,950 female participants and two other studies also Chinese origin analysed 1689 (Hong *et al*., 2011), 1584 (Xiaobin *et al.*, 2012) respectively. Nevertheless, I believe my study was powered enough to detect a deference in reproductive outcome overall in the presence of chromosomal polymorphic variations vs without chromosomal polymorphic variations as large number contributed to this analysis though, the effect estimate was very close to no effect (Crude OR 0.99, 95% CI (0.70 1.40), *P*=0.97: Adjusted OR 1.00, 95% CI (0.70 - 1.44), *P*=0.95).

Secondary analyses, examining the types and number of either non-acrocentric or acrocentric chromosomal polymorphic variants or their combination are perhaps underpowered. Therefore, a larger sample is required with more statistical power for such analysis. There were no considerable differences noted and were not statistically significant. I had a good follow up rate, and were able to adjust the result for potential confounders and again no association was found.

Publication Bias

Publication bias is another possible explanation for the positive findings. The evidence of publication bias goes back to mid-1950's. Sterling revealed that published studies between 1955 to 1956, there were high proportions of statistically significant results

included in the four psychological journals (Dickersin, 1997). In these studies, about 97% hypothesis testing reportedly rejecting the null hypothesis (Dickersin, 1997). Justifying the reporting of meta-analysis towards the positive findings, publication bias could distort the results of the meta-analysis (Mathur & VanderWeele, 2020). The publication bias can be found in many stages, starting from author submission, peer review to editorial decisions (Song *et al.,* 2009). Authors' decisions not to submit negative results subjected to it may consider unimportant, fear of de-bunking previous theories or conclusions or rejection by the prestigious journals. This could mislead the statistical outcome of the results (Schroyens *et al.,* 2021).

There are methods which help to identify publication bias. A possible publication bias can be a larger effect in a smaller study. Therefore, funnel plots and Egger's linear regression test should be considered (Doleman, 2020). There is recent concern of using funnel plots to identify the publication bias in meta-analyses proportion outcomes (Hunter, 2014). *P* value-based tests also used to detect publication bias resulted in related asymmetry in meta-analysis (Furuya-Kanamori *et al.,* 2020).

The quality of the included studies of my systematic review and meta-analysis assessed using the Newcastle-Ottawa Quality Assessment Scales for observational studies independently (Wells *et al.*, 2019). Following the assumption that all items have equal weighting, an arbitrary score was assigned to each study, in order to give a quantitative appraisal of its overall quality. Assessment for publication bias could not be performed due to the limited number of available studies. Pairwise meta-analysis of the five studies involving females only (9,659 females) suggests that females with polymorphic variations

have comparable rates of miscarriages with females without polymorphism (RR 1.54, 95% CI 1.19 to 1.98, I^2=0%; P=0.0009) (Ralapanawe *et al.,* 2021a). The 75% of the weight of the female with miscarriages in the study concentrated to a one study (Cheng *et al.,* 2017) with carriers of chromosomal polymorphism (n=426) and much larger number of non-carriers of chromosomal polymorphism (n= 6983). I postulate that miscarriage rates are higher; it is reasonable to hypothesise that there could be a knock-on effect on other pregnancy outcomes. If the sensitivity analysis has been performed and this study has been excluded (Cheng *et al.,* 2017), that the pooled result may not differ with or without chromosomal polymorphism in miscarriage rate of the female participants. Therefore, the result would have suggested that the presence of chromosomal polymorphism in female makes little or no difference to miscarriage rate.

Biological Mechanism that Could Explain a Lower Cleavage but not An Association with Adverse Reproductive Outcome

Ability of Self-correction

In the early stages of embryogenesis there was a higher incidence of chromosomal abnormalities reported in human embryos. Rapid cell proliferation, that frequently leads to cell division mistakes that generate changes in the chromosomal content in the nucleus (Nagai *et al.,* 2021; Orvieto *et al.,* 2020). Non-disjunction or premature separation of a chromosome into sister chromatids results in aneuploidies. Errors in meiosis and mitotic errors after fertilisation and cell divisions (post zygotic) lead to aneuploidy. Mitotic errors occur during the cleavage after fertilisation in common in the early cleavage stage. The majority of human preimplantation embryos show aneuploidies as mosaicism. Mosaicism

remains fifty percent in cleavage stage and blastocyst stage IVF embryos. The percentage of mitotic errors increased up to 75% at the 9 16 cell stages, though the mitotic errors decreased to 64% at the morula stage and further decreased to 56% by the blastocyst formation. This concludes percentage of mitotic errors in blastocysts remains lower than in cleavage stage embryos (Mantikou *et al.,* 2013). This was observed at the cleavage stage embryos and detected while re-analysing the blastocysts (Li *et al.,* 2005). Although, these embryos have increased cell proliferation and cell death in comparison to euploidy embryos, which is suggestive of self-correction abilities of the both euploidy and aneuploidy embryos. The embryo self-correction results increased aneuploidy cell death or slow cell divisions (Santos *et al.,* 2010). The balance between the survival of cells and apoptotic signals controlled by a cell death program that eliminate of damaged cells in early embryo development (Orvieto *et al.,* 2021). This may be observed by activation of apoptotic pathways, which aneuploidy blastomeres leaving at the time of blastocyst formation (Mantikou *et al.,* 2012).

Animal models such as Rhesus embryos encapsulate the chromosome-containing cellular fragments into micronuclei during the preimplantation development and eliminate through cellular fragmentation (Daughtry *et al.,* 2019). Similarly, mouse embryo studies also show the ability of self-correction of embryos (Bolton *et al.,* 2016). Another study of bovine embryos suggests partial compaction which occurs in some blastomeres has an ability of self-correction (Nagai *et al.,* 2021). A recent study sheds insight on human embryogenesis and the capability of self-correction (Orvieto *et al.,* 2020). This may suggest that genetical abnormalities may self-correct during the cleavage stage to blastomere formation and that therefore pregnancy, miscarriage or the live birth rates

with carriers of chromosomal polymorphism may not differ from the non-carriers of chromosomal polymorphism in my primary and secondary studies on chromosomal polymorphism and reproductive outcomes (Ralapanawe *et al.,* 2022b; Ralapanawe *et al.,* 2022c in review).

Sequential Media and Vitrification

Newer methods such as sequential culture media and vitrification can augment the ability of self-correction which could also explain why the reproductive outcomes of my research were unaffected by chromosomal polymorphic variations. ESHRE has reported birth of 1.6million children from ART treatments during the period of 1997 to 2015. In 2015, assisted reproductive techniques achieved 7.3% of all the children born in Europe (ESHRE, 2020). Although a little known about the different induction protocols, culture methods and genetic biomarkers resulting in improved reproductive outcomes (Monge-Ochoa *et al.,* 2021). Apart from ICSI, other advances such as development of sequential culture media and embryo culture techniques up to blastocyst formation, advent of vitrification instead of slow freezing method, in part allowed for the successful cleavage of embryos leading to better clinical outcome of pregnancy (Burks *et al.,* 2021).

The sequential culture media mimic as close as possible the physiological conditions for fertilisation and cleavage of embryos. High tech incubators have the ability to maintain 37^0C temperature and a constant phase of CO_2 and O_2 gases to retain physiological pH in the bicarbonate-buffered culture medium. This will facilitate the gene expression, metabolic and enzyme activity, maintenance of the calcium levels and cytoskeleton and

cell proliferation of the cleavage stage embryos (Bhattacharya & Hamilton, 2021). Further, more sophisticated time lapse imaging helps to assess the morphology and the cleavage of embryos without removing the embryos for the daily assessment. Cryopreservation of embryos by vitrification has become the dominant method in many IVF laboratories worldwide. It has yielded higher success rates than slow freezing (Bhattacharya *et al.,* 2021). The improved protocols of frozen embryo transfers and vitrification has gain higher success rate in frozen embryo replacement cycles. The complex and state of art technology that has highly improved ART success rate also may attribute to achieve higher pregnancy rate, lower miscarriage and higher live birth rates, which may have hindered the impact of cytogenetic of the chromosomal polymorphism in embryo development. This may be another reason that the carriers and non-carriers of chromosomal polymorphism does not differ in the reproductive outcome in my primary study and secondary study on non-acrocentric and acrocentric chromosomal polymorphic variants in reproductive outcomes (Ralapanawe *et al.*, 2022b; Ralapanawe *et al.,* 2022c in review).

Luteal Phase Support

Similar rationale that improvement of the luteal support should have helped for better implantation in self-corrected embryos which led the reproductive outcome to remain unchanged. Successful implantation which leads to a pregnancy depend on the complex process involving interaction between embryo and endometrial receptivity (Craciunas *et al.,* 2019). The studies with comprehensive chromosomal screening show, even with a transfer of an euploidy embryo, the ongoing pregnancy rate remains 45% in infertile

population. Recently, more attention has focused on a so-called "implantation window" which defines the period of receptivity for the implantation of the embryo (Casper, 2020; Harper, 1992). One of the main factors for a successful pregnancy is optimal endometrial thickness and improvement of embryo freezing by vitrification. A recent study suggests the live birth rate with less than 5mm endometrial thickness was 15.6%, though gradually increased to 33.1% with over 10mm endometrial thickness. The miscarriage rate gradually decreased to 26.5% with more than 10mm endometrial thickness from the miscarriage rate of 41.7% with less than 5mm endometrial thickness (Gallos *et al.,* 2018). Second factor is the use of progesterone in the luteal phase to prepare the endometrium. The uterus prepares for implantation during the luteal phase. Progesterone plays the main role in luteal phase with the secretory endometrium, providing immunological tolerance favouring the embryo implantation. Exogenously administered progestogens have proven efficacy in luteal support in frozen thawed embryo transfer cycles (Labarta & Rodriguez, 2020). The study on the impact of low progesterone levels defines that less than 8.8ng/ml level of progesterone had significantly lowered the ongoing and live birth rates (Labarta *et al.,* 2021). During the period of 2013 to 2018 in the United Kingdom, the frozen thawed embryo transfer cycles has doubled the number up to 38%, where as fresh embryo transfers decreased to 11% (HFEA 2020). There are similar results reported from Europe, United States and around the world (ESHRE, 2020; Centers for Disease Control and Prevention, Society for Assisted Reproductive Technology [CDC], 2018). A recent systematic review suggests increased pregnancy rate and higher live birth rate with the luteal support with progesterone level more than 10ng/ml (Melo *et al.,* 2021). Further, when progesterone was administered vaginally, a steady concentration was achieved in 24-48 hours (Paulson *et al.,* 2014). In my primary and secondary study, all participants

had their endometrial thickness measured. All fresh and frozen embryo replacement (Hormone Replacement Therapy - HRT) was performed if the endometrial thickness was over 7mm in ultrasound scanning. Progesterone (Crinone gel 8%) was administered vaginally for all ICSI participants from the day after oocyte recovery and for all frozen embryo transfer participants from the fourteenth day of estradiol administered cycles, when the endometrial thickness more than 7mm in ultrasound scanning. The optimal luteal support also may have attributed for a better implantation rate that contributed to see little or no difference in the carriers of chromosomal polymorphism and the non-carriers of chromosomal polymorphism in their reproductive outcome (Ralapanawe *et al.,* 2022b; Ralapanawe *et al.,* 2022c in review).

IVF vs ICSI

Intra cytoplasmic sperm injection could have been another reason why reproductive outcomes are better in my research compared to the background literature. All ten studies of my systematic review had undergone IVF. Eight out of ten had ICSI procedure as well. In two studies that had IVF only, one study experienced low live births in male carriers of chromosomal polymorphism (Ni *et al.*, 2017) and the other study had a higher miscarriage rate when both partners were carriers of chromosomal polymorphism (Xiaobin *et al.,* 2012). A subgroup analysis was not possible since the results of the reproductive outcomes were not separately reported in these ten studies. Interestingly, there was a recent study that concluded ICSI will have a better chance of a clinical pregnancy compared to IVF in presence of acrocentric chromosomal polymorphism (Li *et al.*, 2020). Therefore, ICSI may have improved fertilisation and subsequent cleavage which leads to a better reproductive outcome. Further, the recent meta-analysis in association

of chromosomal polymorphism in IVF or ICSI suggests that there was a statistically significant fertilisation rate in ICSI compared to standard IVF although, the reproductive outcome remain similar whether the ART procedure remain in vitro fertilisation or intra cytoplasmic sperm injection (Ou *et al.,* 2019). However, it remains uncertain whether ICSI had improved fertilisation and subsequent cleavage, which led to better reproductive outcome in my study, in which the reproductive outcomes with carrier of chromosomal polymorphism and non-carriers does not differ following ICSI treatment (Ralapanawe *et al.,* 2021b). Future research should investigate whether there is an advantage in ICSI treatment compared to standard in vitro fertilisation and embryo culture with carriers of chromosomal polymorphism. ICSI has an advantage of selecting the progressive, morphologically normal spermatozoa and even having the advantage of fertilisation in males with severe oligozoospermia or astenozoospermia or teratozoospermia, which produces better quality embryos and does not have the similar potential in standard IVF.

Implication in Clinical Practice

Based on my research chromosomal polymorphic variants of female or male or couples should not be considered an adverse feature for the success of intra cytoplasmic sperm injection procedure. Even specific types of chromosomal polymorphic variants or multiple chromosomal polymorphic variants or chromosomal polymorphism in both female and male have no known adverse effect on reproductive outcomes of pregnancy, miscarriage and live births, compared to the couples without chromosomal polymorphism. A small difference in low cleavage rate among females with chromosomal polymorphism and females with acrocentric chromosomal polymorphism compared to couples without polymorphism are not translated to adverse reproductive outcomes. This

could be because of little effect of chromosomal polymorphism or better ICSI techniques or the ability of self-correction of the embryos. My research found no association with carriers or non-carriers of chromosomal polymorphic variations in the Sri Lankan population and any association with reproductive outcomes is likely to be non-influential.

In my research where I routinely karyotyped a larger number of couples with infertility. I found a prevalence of 1.8% of couples with structural rearrangements. These couples would benefit from preimplantation genetic testing as they would reduce the risk of chromosomal imbalances and potential disability to the offspring. The cost of karyotyping test compared to the total cost of IVF cycles borne by the couple is 2.5%, which identifies numerical and structural aberrations apart from chromosomal polymorphic variants prior to the IVF cycle. Therefore, it is still noteworthy to stipulate that karyotyping test is beneficial, since the cost of an IVF cycle is enormous and a failure has a tremendous impact on the couple both financially and emotionally.

Implication to Research

Further research is needed to improve the generalisability and more studies are required involving different ethnicities. Studies would need to focus on chromosomal polymorphism in both males and females to identify if there is an association with adverse reproductive outcomes following assisted reproductive technologies. Currently, many studies have examined the reproductive outcome with carriers of chromosomal polymorphism and non-carriers of chromosomal polymorphism of the Chinese origin (Ou *et al.,* 2019; Ralapanawe *et al.*, 2022a). Studies focusing on other ethnicities will elaborate

better clinical evidence and effect of chromosomal polymorphism on reproductive outcomes.

The evidence so far in many studies associated with chromosomal polymorphic variants of female or male or couples with chromosomal polymorphic variants appear to have no adverse effect on reproductive outcomes or most probably minimal if any adverse reproductive outcome compared to the couples without chromosomal polymorphism. Among the early pregnancy losses, which account 10-20% of clinical pregnancies occur in the first trimester, half of the miscarriages were related to chromosomal abnormalities (Kamar *et al*., 2021; Practice Committee of the American Society for Reproductive Medicine, 2012). There are many genes that regulate reproduction, though polymorphism is being recognised as one of the potential factors of influencing fertility (Haggarty *et al.,* 2006; Altmae *et al.*, 2010). Therefore, the concerns of early pregnancy losses related to chromosomal polymorphism must be investigated separately.

Further studies should be larger and adequately powered including larger number of participants with each specific chromosomal polymorphic variant. Since the pathogenesis of infertility, the role of genetic factors is becoming prominent among reproductive specialists (Stela *et al*., 2021). These studies should analyse specific types such as female or male carriers of non-acrocentric polymorphism only, female or male carriers of acrocentric polymorphism only, either female or male carriers with combination of non - acrocentric and acrocentric chromosomal polymorphism, males with Yqh, couples with one or more than one of non-acrocentric or acrocentric chromosomal polymorphism or a combination and couples without any of the chromosomal polymorphism.

Conclusion

My thesis analysed the effects of these chromosomal polymorphisms following ICSI in the Sri Lankan population. I have conducted a systematic review which showed higher miscarriage in the female partners with chromosomal polymorphisms undergoing assisted reproductive technology (ART). Although, there were no evidence that chromosomal polymorphisms affect the pregnancy, clinical pregnancy, on-going pregnancy at study end, pre-term birth and live birth after in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) treatment. My primary study and the secondary studies found that the pregnancy, miscarriage and live birth rates did not differ between carriers of chromosomal polymorphic variations and couples with no chromosomal polymorphic variations. I found there was a low cleavage rate among female with chromosomal polymorphism and acrocentric chromosomal polymorphic variants compared to couples without polymorphism and this does not translate to a clinically significant difference in reproductive outcomes. During the routine karyotyping of couples in this study, I discovered 13 couples (1.8%) with at least one structural abnormality that had clinical implications. Without knowledge of this structural abnormality, these couples would have undergone standard IVF or ICSI procedure. Instead, I was able to offer pre-implantation genetic diagnosis out to avoid passing the chromosomal abnormality to the offspring.

A recent analysis suggests that genetical abnormalities may self-correct during the cleavage stage to blastomere formation and that therefore the reproductive outcome of carriers of chromosomal polymorphism may not differ from the non-carriers of chromosomal polymorphism. It is noteworthy that recent studies suggest that ICSI

improves fertilisation and subsequent cleavage which leads to a better reproductive outcome. The sequential culture media which mimic the closest as possible physiological conditions will enhance further development of the embryos. Transferring an euploidy embryo on so-called “implantation window” which define the period of receptivity will also enhances the implantation. Another main factor for a successful pregnancy is optimal endometrial thickness. The improvement of embryo freezing by vitrification also brings successful preservation of self-corrected embryos for a later stage of transfer.

In my study that I found no association in the Sri Lankan population, and any association with reproductive outcomes is likely to be non-influential in the reproductive outcome. The karyotyping analysis as a standard method will provide valuable information of the parental cytogenetic abnormalities which will contribute to genetic counselling prior to IVF or ICSI procedure. Although, the chromosomal polymorphic variations do not influence the reproductive outcome of ICSI, routine karyotyping could detect other abnormalities such as chromosomal aberrations. Therefore, it facilitates counselling of the couples and to offer pre-implantation genetic testing with structural chromosomal rearrangements (PGT-SR) to avoid passing the chromosomal abnormality to the offspring. Hence, routine karyotyping may still have a role outside the research context. In addition, additional prospective, adequately powered studies, conducted in multiethnic populations, are required to further investigate whether the detection of chromosomal polymorphic variants prior to assisted conception may in fact be a futile diagnostic tool.

References

Agarwal, A., Mulgund, A., Hamada, A., and Chyatte M.R. (2015) 'A unique view on male infertility around the globe', *Reproductive Biology and Endocrinology,* 13 (1), pp. 1-9.

Ahmet Okay, C., Ozyazgan, I., Demiryilmaz, F., and Dundar, M. (2010) 'Cytogenetic Results of Patients with Infertility in Middle Anatolia, Turkey: Do Heterochromatin Polymorphisms Affect Fertility?', *Journal of Reproduction & Infertility*, 11 (3), pp. 179-181.

Altmae, S., Stavreus-Evers, A., Ruiz, J.R., Laanpere, M., Syvanen, T., Yngve, A., Salumets, A., and Nilsson, T.K. (2010) 'Variations in folate pathway genes are associated with unexplained female infertility', *Fertility and Sterility*, 94 (1), pp. 130-137.

Bhattacharya, S. and Hamilton, M. (eds) (2021) *Reproductive Medicine for the MRCOG*. Cambridge: Cambridge University Press.

Bolton, H., Graham, S.J., Van der Aa, N., Kumar, P., Theunis, K., Gallardo, E.F., Voet, T., and Zernicka-Goetz M. (2016) 'Mouse model of chromosome mosaicism reveals lineage-specific depletion of aneuploid cells and normal developmental potential', *Nature communications*, 7 (1), pp. 1-12.

Boynukalin, F.K., Gultomruk, M., Turgut, N.E., Rubio, C., Rodrigo, L., Yarkiner, Z., Ecemis, S., Karlikaya, G., Findikli, N., and Bahceci, M. (2021) 'The impact of patient, embryo, and translocation characteristics on the ploidy status of young couples undergoing preimplantation genetic testing for structural rearrangements (PGT-SR) by next generation sequencing (NGS)', *Journal of Assisted Reproduction and Genetics*, 38 (2), pp. 387-396.

Brothman, A.R., Schneider, N.R., Sajkevych, I., Cooley, L.D., Butler, M.G., Patil, S., Mascarello, J.T., Rao, K.W., Dewald, G.W., Park, J.P., Persons, D.L., Wolff, D.J., and Vance, G.H. (2006) 'Cytogenetic heteromorphisms; survey results and reporting practices of giemsa band regions that we have pondered for years', *Achieve of Pathology & Laboratory Medicine,* 130 (7), pp. 947-949.

Burks, C., Van Heertum, K., and Weinerman R. (2021) 'The Technological Advances in Embryo Selection and Genetic Testing: A Look Back at the Evolution of Aneuploidy Screening and the Prospects of Non-invasive PGT', *Reproductive medicine*, 2 (1), pp. 26-34.

Butler, R., Nakhuda, G., Guimond, C., Jing, C., Lee, N., Hitkari, J., Tallon, N., Taylor, B., and Yuzpe, A. (2019) 'Analysis of PGT-M and PGT-SR outcomes at a Canadian fertility clinic', *Prenatal diagnosis*, 39 (10), pp. 866-870.

Casper, R.F. (2020) 'Frozen embryo transfer: evidence-based markers for successful endometrial preparation', *Fertility and Sterility*, 113 (2), pp. 248-251.

Centers for Disease Control and Prevention, Society for Assisted Reproductive Technology (2018) *2016 Assisted Reproductive Technology National Summary Report*. US Department of Health and Human Service, Atlanta (GA). Available at: https://www.cdc.gov/art/pdf/2016-report/ART-2016-National-Summary-Report.pdf.

Cheng, R., Ma, Y., Nie, Y., Qiao, X., Yang, Z., Zeng, R., and Xu, L. (2017) 'Chromosomal polymorphisms are associated with female infertility and adverse reproductive outcomes after infertility treatment: a 7-year retrospective study', *Reproductive Biomedicine Online*, 35 (1), pp. 72-80.

Coonen, E., Rubio, C., Christopikou, D., Dimitriadou, E., Gontar, J., Goossens, V., Maurer, M., Spinella, F., Vermeulen, N., and De Rycke, M. (2020). ESHRE PGT Consortium good practice recommendations for the deletion of structural and numerical chromosomal aberrations', *Human Reproduction Open*, 2020 (3), pp. hoaa017.

Craciunas, L., Gallos, I., Chu, J., Bourne, T., Quenby, S., Brosens, J.J., and Coomarasamy, A. (2019) 'Conventional and modern markers of endometrial receptivity: a systematic and meta-analysis', *Human Reproduction update*, 25 (2), pp. 202-223.

Datta, J., Palmer, M.J., Tanton, C., Gibson, L.J., Jones, K.J., Macdowall, W., Glasier, A., Sonnenberg, P., Field, N., Mercer, C.H., Johnson, A.M., and Wellings K. (2016) 'Prevalence of infertility and help seeking among 15,000 women and men', *Human Reproduction*, 31 (9), pp. 2108-2118.

Daughtry, B.L., Rosenkrantz, J.L., Lazar, N.H., Fei, S.S., Redmayne, N., Torkenczy, K.A., Adey, A., Yan, M., Gao, L., Park, B., Nevonen, K.A., Carbone, L., and Chavez, S.L. (2019) 'Single-cell sequencing of primate preimplantation embryos reveals chromosome elimination via cellular fragmentation and blastomere exclusion,' *Genome Research,* 29 (3), pp. 367-382.

De la Fuente-Cortes, B.E., Cerda-Flores, R.M., Davila-Rodriguez, M.I., Garcia-Vielma, C., De la Rosa Alvarado R.M., and Cortes-Flores, E.I. (2009) 'Chromosomal abnormalities and polymorphic variants in couples with repeated miscarriage in Mexico', *Reproductive Biomedicine Online*, 18 (4), pp. 543-548.

Dickersin, K. (1997) 'How important is publication bias? A synthesis available data', *AIDS education and prevention*, 9, pp. 15-21.

Doleman, B., Freeman, S.C., Lund, J.N., Williams, J.P., and Sutton, A.J. (2020) 'Funnel plots may show asymmetry in the absence of publication bias with continuous outcomes dependent on baseline risk: presentation of a new publication bias test', *Research synthesis methods*, 11 (4), pp. 522-534.

Erwinsyah, R, Riandi, and Nurjhani M. (2017) 'Relevance of Human Chromosome Analysis Activities against Mutation Concept in Genetics Course', In *IOP Conference Series.: Materials Science and Engineering*, 180 (1), pp. 012285.

ESHRE Guideline Group on Ovarian Stimulation, Bosch E., Broer, S., Griesinger, G., Grynberg, M., Humaidan, P., Kolibianakis, E., Kunicki, M., Marca, A.L., Lainas, G., Clef, N.L., Massin, N., Mastenbroek, S., Polyzos, N., Sunkara, S.K., Timeva, T., Toyli, M., Urbancsek, J., Vermeulan N., and Broekmns F. (2020) 'ESHRE guideline: ovarian stimulation for IVF/ICSI', *Human Reproduction Open*, 2020 (2), pp. hoaa009.

European Society of Human Reproduction and Embryology. (2020) ART fact sheet. ESHRE; 2020.

European Society of Human Reproduction and Embryology. (2012) Focus on Reproduction. European society of human reproduction and embryology, 28th annual meeting.

European Society of Human Reproduction and Embryology. (2018). European society of human reproduction and embryology; 34th annual meeting.

Feng, X., Liu, J., Wang, Y., Fu, J., Cao, Y., and Wu, Y. (2021) 'Acrocentric chromosome polymorphic variants on Chinese female have possible association with unexplained recurrent pregnancy loss', *Reproductive Sciences*, 28 (2), pp. 575-584.

Fiorentino, F., Spizzichino, L., Bono, S., Biricik, A., Kokkali, G., Rienzi, L., Ubaldi, F.M., Iammarrone, E., Gordon, A., and Pantos, K. (2011) 'PGD for reciprocal and Robertsonian tranlocations using array comparative genomic hybridization', *Human Reproduction*, 26 (7), pp. 1925-1935

Furuya-Kanamori, L., Xu, C., Lin, L., Doan, T., Chu, H., Thalib, L., and Doi, S.A. (2020) '*P* value-driven methods were underpowered to detect publication bias: analysis of Cochrane review meta-analyses', *Journal of clinical epidemiology*, 118, pp. 86-92.

Gallos, I.D., Khairy, M., Chu, J., Rajkhowa, M., Tobias, A., Campbell, A., Dowell, K., Fishel, S., and Coomarasamy, A. (2018) 'Optimal endometrial thickness to maximize live births and minimize pregnancy losses: Analysis of 25,767 fresh embryo transfers', *Reproductive biomedicine online*, 37 (5), pp. 542-548.

Garcia-Pascual, C.M., Navarro-Sanchez, L., Navarro, R., Martinez, L., Jimenez, J., Rodrigo, L., Simon, C., and Rubio, C. (2020) 'Optimised NGS Approach for Detection of Aneuploidies and Mosaicism in PGT-A and Imbalances in PGT-SR', *Genes*, 11 (7), pp. 724.

Gerton, J.L., and Hawley, R.S. (2005) 'Homologous chromosome interactions in meiosis: diversity amidst conservation', *Nature Reviews,* 6, pp. 477-487.

Gleicher, N., Kushnir, V.A., and Barad, D.H. (2019) 'Worldwide decline of IVF birth rates and its probable causes', *Human Reproduction Open,* 2019(3), pp. hoz017.

Gosden, J.R., Lawrie, S.S., and Gosden, C.M. (1981) 'Satellite DNA sequences in the human acrocentric chromosomes: information from translocations and heteromorphisms ', *American Journal of Human Genetics*, 33 (2), pp. 243-251.

Guo, T., Qin, Y., Gao, X., Chen, H., Li, G., Ma, J., and Chen Z. (2012) 'The role of male chromosomal polymorphism played in spermatogenesis and the outcome of IVF/ICSI-ET treatment', *International Journal of Andrology*, 35 (6), pp. 802-809.

Haggarty, P., McCallum, H., McBain, H., Andrews, K., Duthie, S., McNeill, G., Templeton, N., Haites, D., Campbell, D., and Bhattacharya, S. (2006) 'Effect of B vitamins and genetics on success of in-vitro fertilisation: prospective cohort study', *The Lancet*, 367 (9521), pp. 1513-1519.

Hanif, M.I., Khan, A., Arif, A., and Shoeb, E. (2019) 'Cytogenetic investigation of couples with recurrent spontaneous miscarriages', *Pakistan Journal of Medical Sciences*, 35 (5), pp. 1422.

Harper, M.J. (1992) '10 The implantation window', *Bailliere's clinical obstetrics and gynaecology*, 6 (2), pp. 351-371.

Hernandez-Nieto, C., Gayete-Lafuente, S., Alkon-Meadows, T., Lee, J., Luna-Rojas, M., Mukherjee, T., Copperman, A.B., and Sandler, B. (2021) 'Parental chromosomal homeomorphisms are not associated with an increased risk of embryo aneuploidy', *JBRA Assisted Reproduction*, 25 (4), pp. 575.

Hong, Y., Zhou, Y.W., Tao, J., Wang, S.X., and Zhao, X.M. (2011) 'Do polymorphic variants of chromosomes affect the outcome of *in vitro* fertilisation and embryo transfer treatment?', *Human Reproduction,* 26 (4), pp. 933-940.

Human Fertilisation and Embryology Authority. (2016) Fertility-treatment-2014; trends and figures; Version 1 (2016).

Human Fertilisation and Embryology Authority. (2019) Fertility-treatment-2017; trends and figures. Version 1 (2019);23.

Human Fertilisation and Embryology Authority. (2020). Fertility Treatment 2018: trends and figures. HFEA; 2020.

Hunter, J.P., Saratzis, A., Sutton, A.J., Boucher, R.H., Sayers, R.D., and Bown, M.J. (2014) 'In meta-analyses of proportion studies, funnel plots were found to be an inaccurate method of assessing publication bias', *Journal of clinical epidemiology*, 67 (8), pp. 897-903.

Jalbert, P., Sele, B., and Jalbert, H. (1980) 'Reciprocal translocations: a way to predict the mode of imbalance segregation by pachytene-diagram drawing', *Human Genetics*, 55 (2), pp. 209-222.

Kamar, A., Turktekin, N., Oyzurt, R., Karakus, C., Saribal, D., and Hocaoglu-Emre, F.S. (2021) 'Cytogenetic analysis of early pregnancy loss after assisted reproduction treatment using intracytoplasmic sperm injection', *Ginekologia Polska*, 92 (7), pp. 475-480.

Karaca, Y., Pariltay, E., Mardan, L., Karaca, E., Durmaz, A., Durmaz, B., Aykut, A., Akin H., and Cogulu, O. (2020) 'Co-occurrences of polymorphic heterochromatin regions of chromosomes and effect on reproductive failure', *Reproductive biology*, 20 (1), pp. 42-47.

Karpen, G.H., and Endows, S. A. (1998) '71 Meiosis: chromosome behaviour and spindle dynamics' In Endow, S. A and Glover, D. M (eds) *Dynamics of Cell Division*, Oxford: OUP Oxford, pp. 203.

Kirkpatrick, M. (2010) 'How and why chromosome inversions evolve', *PLoS Biology,* 8 (9), pp. e1000501.

Labarta, E., and Rodriguez, C. (2020) 'Progesterone use in assisted reproductive technology', *Best Practice & Research Clinical Obstetrics & Gynaelcology*, 69, pp. 74-84.

Labarta, E., Mariani, G., Paolelli, S., Rodriguez-Varela, C., Giles, J., Bellver, J., Cruz, F., Marzal, A., Celada, P., Olmo, I., Alama, P., Remohi, J., and Bosch E. (2021) 'Impact of low serum progesterone levels on then day of embryo transfer on pregnancy outcome: a prospective cohort study in artificial cycles with vaginal progesterone', *Human Reproduction*, 36 (3), pp. 683-692.

Lanka Hospitals Corporation PLC. 2018. *Injection of spermatozoa into the oocyte*. [Microscopic image]. At: Colombo: Lanka Hospitals Corporation PLC, IVF Laboratory Data.

Lanka Hospitals Corporation PLC. 2018. *Spermatozoa inside the oocyte after injection*. [Microscopic image]. At: Colombo: Lanka Hospitals Corporation PLC, IVF Laboratory Data.

Lanka Hospitals Corporation PLC. 2018. *Karyotype of a normal female*. [Microscopic image]. At: Colombo: Lanka Hospitals Corporation PLC, Fertility Centre Data Base.

Lanka Hospitals Corporation PLC. *Karyotype of a normal male*. [Microscopic image]. At: Colombo: Lanka Hospitals Corporation PLC, Fertility Centre Data Base.

Lanka Hospitals Corporation PLC. 2018. *Non-acrocentric chromosomal polymorphic variants*. [Microscopic image]. At: Colombo: Lanka Hospitals Corporation PLC, Fertility Centre Data Base.

Lanka Hospitals Corporation PLC. 2018. *Satellite seen on the acrocentric chromosomes*. [Microscopic image]. At: Colombo: Lanka Hospitals Corporation PLC, Fertility Centre Data Base.

Lanka Hospitals Corporation PLC. 2018. *Satellites stalks seen on the short arm of the acrocentric chromosomes*. [Microscopic image]. At: Colombo: Lanka Hospitals Corporation PLC, Fertility Centre Data Base.

Lanka Hospitals Corporation PLC. 2018. *Pericentric inversions on chromosome 9*. [Microscopic image]. At: Colombo: Lanka Hospitals Corporation PLC, Fertility Centre Data Base.

Lejeune, J. (1963) 'Autosomal disorders. *Pediatrics*, 32 (3), pp. 326-337.

Li, S.J., Cheng, Y.X., Zhou, D.N., Zhang, Y., Yin, T.L., and Yang, J. (2020) 'Chromosomal polymorphisms associated with reproductive outcomes after IVF-ET', *Journal of Assisted Reproduction and Genetics,* 37, pp. 1703-1710.

Li, M., DeUgarte, C.M., Surrey, M., Danzer, H., DeCherney, A., and Hill, D.L. (2005) 'Fluorescent in situ hybridization reanalysis of day-6 human blastocysts diagnosed with aneuploidy on day 3', *Fertility and Sterility*, 84 (5), pp. 1395-1400.

Liang, J., Zhang, Y., Yu, Y., Sun, W., Jing, J., and Liu, R. (2014) 'Effect of chromosomal polymorphisms of different genders on fertilisation rate of fresh IVF-ICSI embryo transfer cycles', *Reproductive Biomedicine Online,* 29 (4), pp. 436-444.

Liang, S., Yang, J., Wu, H., Teng, X., and Duan, T. (2019) 'Effects of chromosome 9 inversion on IVF/ICSI: A 7-year retrospective cohort study', *Molecular genetics & genomic medicine*, 7 (9), pp. e856.

Lledo, B., Ortiz, J.A, Morales, R., Ten, J., de la Fuente, P.E., Garcia-Ochoa, C., and Bernabeu, R. (2010) 'The paternal effect of chromosome translocation carriers observed from meiotic segregation in embryos', *Human Reproduction*, 25 (7), pp. 1843-1848.

Luo, L.L., Hu, Z.M., Li, L.L., Zhang, H.G., Jiang, Y.T., Liu, R.Z., and Wang, R.X. (2020) 'Frequency and clinical manifestation of prenatal cytogenetic diagnosis of chromosomal polymorphisms in Northeast China', *Taiwanese Journal of Obstetrics and Gynecology*, 59 (6), pp. 910-915.

Madon, P.F., Athalye, A.S., and Parikh, F.R. (2005) 'Polymorphic variants on chromosomes probably play a significant role in infertility', *Reproductive Biomedicine Online,* 11 (6), pp. 726-732.

Mantikou, E., van Echten-Arends, J., Sikkema-Raddatz, B., van de Veen, F., Repping, S., and Mastenbroek, S. (2013) 'Temporal and developmental-stage variation in the occurrence of mitotic errors in trinuclear human preimplantation embryos', *Biology of reproduction*, 89 (2), pp. 42-1.

Mantikou, E., Wong, K.M., Repping, S., and Mastenbroek S. (2012) 'Molecular origin of mitotic aneuploidies in preimplantation embryos', *Biochimica et Biophysica Acta (BBA)-Molecular Basis Disease*, 1822 (12), pp. 1921-1930.

Mateu-Brull, E., Rodrigo, l., Peinado, V., Mercader, A., Campos-Galindo, I., Bromet, F., Garcia-Herrero, S., Florensa, M., Milan, M., and Rubio, C. (2019) 'Intrachromosomal effect in carriers of translocations and inversions assessed by preimplantation genetic testing for structural rearrangements (PGT-SR)', *Journal of Assisted reproduction and Genetics*, 36 (12), pp. 2547-2555.

Mathur, M.B., and VanderWeele, T.J. (2020) 'Sensitivity analysis for publication bias in meta-analysis', *Journal of the Royal Statistical Society: Series C (Applied Statistics),* 69 (5), pp. 1091-1119.

Martinez, G., Daniels, K., and Chandra, A. (2012) 'Fertility of men women aged 15-44 years in the United States: National Survey of Family Growth 2006 2010', *National Health Statistics Report*, 12 (51), pp. 1-28.

Mascarenhas, M.N., Flaxman, S.R., Boerma, T., Vanderpoel, S., and Stevens, G.A. (2012) 'National, Regional, and Global Trends in Infertility Prevalence Since 1990: A Systematic Analysis of 277 Health Surveys', *PLoS Medicine*, 9 (12), pp. e1001356.

Melo, P., Chung, Y., Pickering, O., Price, M.J., Fishel, S., Khairy, M., Kingsland, C., Lowe, P., Petsas, G., Rajkhowa, M., and Sephton, V. (2021) 'Serum luteal phase progesterone in women undergoing frozen embryo transfer in assisted conception: a systematic review and meta-analysis', *Fertility and Sterility*, 116 (6), pp, 1534-1556.

Minocherhomji, S., Athalye, A., Madon, P., Kulkarni, D., Uttamchandani, S.A., and Parikh, F. (2009) 'A case-control study identifying chromosomal polymorphic variations as forms of epigenetic alterations associated with the infertility phenotype', *Fertility and Sterility*, 92 (1), pp. 88-95.

Monge-Ochoa, B., Montoro, L., Gil-Arribas, E., Montoya, J., Ruiz-Pesini, E., Lopez-Perez, M.J., de Castro, F., and Diez-Sanchez, C. (2021) 'Variants Ala307Ala and Ser680Ser of 307 and 680 FSHr polymorphisms negatively influence on assisted reproductive techniques outcome and determine high probability of non-pregnancy in Caucasian patients', *Journal of Reproduction and Genetics*, pp. 1-11.

Nagai, H., Okada, M., Nagai, Y., Sakuraba, Y., Okae, H., Suzuki, R., and Sugimura, S. (2021) 'Abnormal cleavage is involved in the self-correction of bovine preimplantation embryos', *Biochemical and Biophysical Research Communications*, 562, pp. 76-82.

Nakamura, Y., Kitamura, M., Nishimura, K., Koga, N., Takeyama, M., Matsumiya, K., and Okuyama A. (2001) 'Chromosomal variants among 1970 infertile men', *International Journal of Urology*, 8 (2), pp. 49-52.

Ni, T., Li, J., Chen, H., Gao, Y., Gao, X., Yan, J., and Chen, Z. (2017) 'Male chromosomal polymorphisms reduce cumulative live birth rate for IVF couples', *Journal of Assisted Reproductive Genetics,* 34 (8), pp. 1017-1025.

Orvieto, R., Shimon, C., Rienstein, S., Jonish-Grossman, A., Shani, H., and Aizer, A. (2020) 'Do human embryo have the ability of self-correction?', *Reproductive Biology and Endocrinology*, 18 (1), pp. 1-6.

Orvieto, R., Aizer, A., and Gleicher, N. (2021) 'Is there still a rationale for non-invasive PGT-A by analysis of cell-free DNA released by human embryos into culture medium?', *Human reproduction*, 36 (5), pp. 1186-1190.

Ou, Z., Yin, M., Chen, Z., and Sun, L. (2019) 'Meta-analysis of association between chromosomal polymorphisms and outcomes of embryo transfer following in vitro fertilisation and/or intracytoplasmic sperm injection', *International Journal of Gynecology & Obstetrics*, 144 (2), pp. 135-142.

Ozdemir, T.R., Ozyilmaz, B., Cakmak, O., Kaya, O.O., Kose, C., Kirbiyik, o., Keskin, M.Z., Koc, A., Zeyrek, T., Kutbay, Y.B. and Erdogan, K.M. (2020). Evaluation of chromosomal abnormalities and Y-chromosome microdeletions in 1696 Turkish cases with primary male infertility: A single-centre study. *Turkish journal of urology*, 46 (2), pp. 95.

Pal, A.K., Ambulkar, P.S., Waghmare, J.E., Wankhede, V., Shende, M.R., and Tarnekar, A.M. (2018) 'Chromosomal aberrations in couples with pregnancy loss: a retrospective study', *Journal of Human Reproductive Science*, 11 (3), pp. 247.

Paulson, R.J., Collins, M.G., and Yankov, V.I. (2014) 'Progesterone pharmacokinetics and pharmacodynamics with 3 doses and 2 regimens of and effectivescent micronized progesterone virginal insert', *The Journal of Clinical Endocrinology and Metabolism*, 99 (11), pp. 4241 4249.

Pokale, Y.S. (2015) 'Does a Heterochromatic variant affect the Human Reproductive outcome?', *Research Journal of Recent Sciences,* 4, pp. 108-113.

Practice Committee of the American Society for Reproductive Medicine. (2012) 'Evaluation and treatment of recurrent pregnancy loss: a committee opinion', *Fertility and Sterility*, 98 (5), pp. 1103-1111.

Pylyp, L.Y., Spinenko, L.O., Verhoglyad, N.V., Kashevarova, O.O., and Zukin, V.D. (2015) 'Chromosomal abnormalities in patients with infertility', *Cytology and Genetics*, 49 (3), pp. 173-177.

Ralapanawe, M.S.B., Khattak, H., Hapangama, H.R., Weerakkody, G.R., Papadopoulou, A., and Gallos, I., (2022a) 'Chromosomal polymorphisms in assisted reproduction: a systematic review and meta-analysis', *Human Fertility* [Preprint]. Available at: https://doi.org/10.1080/14647273.2022.2051614.

Ralapanawe, M.S.B., Gajaweera, S.L., Karunarathne, N., Price, M.J., Pedro, M., Coomarasamy, A., and Gallos, I.D. (2022b) 'Chromosomal polymorphisms in assisted reproduction: an analysis of 942 cycles', *Reproduction and Fertility*, 3 (3), pp. 133-139.

Ralapanawe, M.S.B., Gajaweera, S.L., Karunarathne, N., Dissanayake, H.W.D., Price, M.J., Pedro, M., Coomarasamy, A., and Gallos, I.D. (2022c) 'A comprehensive analysis of chromosomal polymorphic variants on reproductive outcomes after intra cytoplasmic sperm injection treatment', [In review].

Rawal, L., Kumar, S., Mishra, S.R., Lal, V., and Bhattacharya, S.K. (2020). Clinical manifestations of chromosomal anomalies and polymorphic variations in patients

suffering from reproductive failure. *Journal of Human reproductive Sciences*, 13 (3), pp. 209-215.

Rouchou, B. (2013) 'Consequences of infertility in developing countries', *Perspective Public Health*, 133 (3), pp. 174-179.

Santos, M.A., Teklenburg, G., Macklon, N.S., Van Opstal, D., Schuring-Blom, G.H., Krijtenburg, P.J., de Vreeden-Elbertse, J., Fauser, B.C., and Baart, E.B. (2010) 'The fate of the mosaic embryo: chromosomal constitution and development of day 4, 5 and 8 human embryos', *Human Reproduction*, 25 (8), pp. 1916-1926.

Shaffer, L.G., McGovan-Jordan, L., and Schmid, M. (eds) (2013) *ISCN 2013: an international system for human cyctogenetic nomenclature.* Karger Medical and Scientific Publishers.

Scriven, P.N., Handyside, A.H., and Ogilvie, C.M. (1998) 'Chromosome translocations: segregation modes and strategies for preimplantation genetic diagnosis', *Prenatal Diagnosis: Published Affiliation with the International Society for Prenatal Diagnosis*, 18 (13), pp.1437-1449.

Schroyens, N., Sigwald, E.L., Van Den Noortgate, W., Beckers, T., and Luyten, L. (2021) 'Reactivation-dependent amnesia for contextual fear memories: Evidence for publication bias', *Eneuro.* 8 (1).

Serapinas, D., Valantinaviciene, E., Machtejviene, E., Bartkeviciute, A., & Bartkeviciene, D. (2021) 'Evaluation of chromosomal structural anomalies in fertility disorders', *Medicina,* 57 (1), pp. 37.

Sipek Jr, A., Mihalova, R., Panczak, A., Hrckova, L., Janashia, M., Kasprikova, N., and Kohoutova, M. (2014) 'Heterochromatin variants in human karyotypes: a possible association with reproductive failure', *Reproductive Biomedicine Online,* 29 (2), pp. 245-250.

Song, F., Parekh-Bhurke, S., Hooper, L., Loke, Y.K., Ryder, J.J., Sutton, A.J., Hing, C.B., and Harvey, I. (2009) 'Extent of publication bias in different categories of research cohorts: a meta-analysis of empirical studies', *BMC medical research methodology*, 9 (1), pp. 1-4.

Song, D., Yin, H., Zhang, H., Sun, Y., Shen, C., Du, H., Shi, M., Sun, Z., and Sun, F. (2017) 'Chromosomal polymorphisms do not affect outcome of in vitro fertilisation and embryo transfer treatment', *Academic Journal of Second Military Medical University,* 38 (7), pp. 836-841.

Stela, R., Veaceslav, M., Svetlana, C., Ana, M., and Mariana, S. (2021) 'Chromosomal abnormalities in men with azoospermia', *The Moldovan Medical Journal*, 64 (1), pp. 50-55.

Sunderam, S., Kissin, D.M., Crawford, S.B., Folger, S.G., Jamieson, D.J., Warner, L., and Barfield, W.D. (2017) 'Assisted Reproductive Technology Surveillance United States, 2014', *MMWR Surveillance Summaries*, 66 (6), pp. 1-24.

Unuane, D., Tournaye, H., Velkeniers, B., and Poppe, K. (2011) 'Endocrine disorders & female infertility', *Best Practice & Research Clinical Endocrinology & Metabolism*, 25 (6), pp. 861-873.

Verdoni, A., Hu, J., Surti, U., Babcock, M, Sheehan, E., Clemens, M., Drewes, S, Walsh, L., Clark, R., Katari, S. and Sanfilippo, J. (2021). Reproductive outcomes in individuals with chromosomal reciprocal translocations. *Genetics in Medicine*, (23), pp. 1753-1760.

Viotti, M. (2020) 'Preimplantation genetic testing for chromosomal abnormalities: aneuploidy, mosaicism, and structural rearrangements', *Genes*, 11 (6), pp. 602.

Wang, Y., Li, G., Zuo, M.Z., Fang, J.H., Li, H.R., Quan, D.D., Huang, L., and Peng, P.P. (2017) 'Y chromosome polymorphisms may contribute to an increased risk of male-induced unexplained recurrent miscarriage', *Bioscience reports*, 37 (2), pp. pBSR20160528.

Wells, G.A., Shea, B., O'Connell, D., Peterson, J., Welch, V., Locos, M., and Tugwell, P. (2019) 'The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses 2019'.

Wilton, L., Thornhill, A., Traeger-Synodinos, J., Sermon, K.D., and Harper, J.C. (2009) 'The causes of misdiagnosis and adverse outcomes in PGD', *Human Reproduction*, 24 (5) pp. 1221-1228.

Wyandt, H.E. and Tonk, V.S. (2011) *Human Chromosome Variation: Heteromorphism and Polymorphism*. Berlin: Springer.

Wyandt, H.E., Wilson, G.N., and Tonk, V.S., (2017) *Human Chromosome Variation: Heteromorphism, polymorphism and pathogenesis*. Singapore: Springer.

Xiao, Z., Zhou, X., Xu, W., and Yang J. (2012) 'A preliminary study of the relationship between the long arm of the Y chromosome (Yqh+) and reproductive outcomes in IVF/ICSI-ET', *European Journal of Obstetrics & Gynecology and Reproductive Biology,* 165 (1), pp. 57-60.

Xiaobin, Z., Yun, F., Qingqing, Y., Xiaowei, L., Weimin, F., Ling, W., Zhichao, L., and Aijun, Z. (2012) 'The effect of chromosomal abnormalities on the outcome of in vitro fertilisation and embryo transfer treatment', *Chinese Journal of Andrology,* 26 (10), 29-32.

Xu, X., Zhang, R., Wang,, W., Liu, H., Liu, L., Mao, B., and Zhang, X. (2016) 'The effect of chromosomal polymorphisms on the outcomes of fresh IVF/ICSI ET cycles in a Chinese population', *Journal of Assisted Reproduction and Genetics*, 33 (11), pp. 1481-486.

Yakin, K., Balaban, B., and Urman, B. (2005) 'Is there a possible correlation between chromosomal variants and spermatogenesis?', *International Journal of Urology,* 12 (11), pp. 984-989.

Yahaya, T.O., Oladele, O.E., Anyebe, D., Obi, C., Bunza, M.D.A., Sulaiman, R. and Liman, U.U. (2021) 'Chromosomal abnormalities predisposing to infertility, testing, and management: a narrative review', *Bulletin of the National Research centre,* 45 (1), pp. 1-15

Zachaki, S., Kouvidi, E., Pantou, A., Tsarouha, H., Mitrakos, A., Tounta, G., Charalampous, I., Manola, K.N., Kanavakis, E., and Mavrou, A. (2020). 'Low-level X Chromosome Mosaicism: A Common Finding in Women Undergoing IVF', *In Vivo*, 34 (3), pp. 1433-1437.

Zegers-Hochschild, F., Adamson, G.D., Dyer, S., Racowsky, C., De Mouzon, J., Sokol, R., Rienzi, L., Sunde, A., Schmidt, L., Cooke, I.D., and Simpson, J.L. (2017) 'The international glossary on infertility and fertility care', *Human Reproduction*, 32 (9), pp. 1786-1801.

Zhou, W., Lin, S.H., Khan, S.M., Yeager, M., Chanock, S.J. and Machiela M.J. (2021) 'Detectable chromosome X mosaicism in males is rarely tolerated in peripheral leukocytes', *Scientific report,* 11 (1), pp. 1-5.

Zorrilla, M., and Yatsenko, A.N. (2013) 'The Genetics of Infertility: Current Status of the Field', *Current Genetic Medicine Reports,* 1, pp. 247.

APPENDICES

APPENDIX 1: PROJECTED TIMELINE

	8/2018	3/2019	4/2019	5/2019	6/2019	7/2019	8/2019	9/2019	10/2019	11/2019	12/2019	1/2020	2/2020	3/2020
Project set up Documentation for registration process Submission of relevant documents or registration process including IELTS & other additional documents Visa invitation, visa application & obtaining of student visa														
Registration in Birmingham University Meeting the relevant officials of institute of IMSR in Birmingham women's hospital Discussion with the supervisor & formulating the strategy of systematic review Formulating the introduction of systematic review														
Discussion with the supervisor in IMSR Formulating of the systematic review Literature search for observational studies with an information specialist in Library & Knowledge Service - Birmingham women's hospital (Central Cochrane Library, CINAHL, EMBASE & MEDLINE)														
Gathering & reading the abstracts retrieved from the electronic search n=2739 Detailed assessment of full manuscript articles n=46 Excluding the articles after reviewing the full manuscript Selecting the relevant full manuscript article for the Systematic review n=10														
Data collection for the research Year 2016 Year 2017 Year 2018 Outcome up to year 2019														
Formulating the systematic review The introduction, methodology, study quality assessment with Newcastle-Ottawa scale PRISMA flow diagram Results analysis with forest plots followed by discussion														

Research data analysis Data analysing Dividing the data to sub-groups (with polymorphism & without polymorphism) Calculating the means of Age, FSH, LH, & BMI Structuring & processing of 1st year project report Preparation of PPT presentation	

	3/2020	4/2020	5/2020	6/2020	7/2020	8/2020	9/2020	10/2020	11/2020	12/2020	1/2021	2/2021	3/2021	4/2021
Completion of 1st year project report Editing & completing the 1st year project report														
Submission of 1st year project report Submission of 1st year report, GRS 1 & 3														
Analysis of frequency of the chromosomal polymorphic variants in females & males														
Annual progress review Preparation of presentation for APR APR Completed														
Second year project report Formulating the research module Analysis of the data of fresh ICSI & FER cycles Analysis of the effect of polymorphism in females and males and cumulative outcome of pregnancies														
Data analysis Clearing the database & Inviting a statistician														
Followed Cochrane interactive learning modules 1-11														
Submission of systematic review for publication														
Planning manuscript of the second year Introduction, methodology, results and Discussion														
Completion of the introduction of the manuscript														
Completion of the material & methods of the manuscript														
Followed online course on introduction to epidemiology (University of Bristol)														
Followed online course on statistics & theory (University of Birmingham)														

IMSR PGR Seminar Presented Systematic Review on Chromosomal polymorphism in assisted reproduction													
Completion of Data collection Data collection of pregnancy outcomes of non-acrocentric and acrocentric chromosomal polymorphism (ICSI cycles)													
Following the online Course on statistics & theory (Practical) (University of Birmingham)													

	5/2021	6/2021	7/2021	8/2021	9/2021	10/2021	11/2021	12/2021	1/2022	2/2022	3/2022
Data analysis of the effect acrocentric and non-acrocentric chromosomes in pregnancy outcomes											
Completion & submission of second-year project report Submission of 2nd year project report, GRS 1 & 3											
Annual Progress Review (2nd Year)											
IMSR PGR seminar Presentation of the findings: "Chromosomal Polymorphisms in Outcome of Assisted Reproduction - an analysis of 942 cycles"											
Completion of the manuscript "Chromosomal Polymorphisms in Outcome of Assisted Reproduction - an analysis of 942 cycles"											
Submission of the manuscript for publication "Chromosomal Polymorphisms in Outcome of Assisted Reproduction - an analysis of 942 cycles"											
Planning the final year project report Writing the introduction, materials and methods, results, and discussion of the effect acrocentric and non-acrocentric chromosomes											
Writing the thesis											
Completion of the manuscript "The effect of non-acrocentric and acrocentric chromosomal polymorphism on reproductive outcome of ICSI – an analysis of 942 cycles"											

Submission of the manuscript for publication "The effect of non-acrocentric and acrocentric chromosomal polymorphism on reproductive outcome of ICSI – an analysis of 942 cycles"											
Completion & submission of the thesis Submission of GRS 3 thesis awaited form & thesis awaited submission plan											
Review and submission of the thesis											

APPENDIX 2: SEARCH STRATEGIES OF THE SYSTEMATIC REVIEW

```
Search Name:    IVF and genetic polymorphism  - Madara Ralapanawe
Date Run:       12/06/2019 21:40:33
Comment:

ID      Search  Hits
#1      MeSH descriptor: [Infertility] explode all trees        2995
#2      infertility     8342
#3      #1 OR #2        8479
#4      MeSH descriptor: [Reproductive Techniques, Assisted] explode
all trees       3154
#5      assisted AND reproducti*        2650
#6      MeSH descriptor: [Fertilization in Vitro] explode all trees
        2040
#7      (in vitro OR in-vitro) AND Fertili?ation        4439
#8      MeSH descriptor: [Sperm Injections, Intracytoplasmic]
explode all trees       552
#9      Intracytoplasmic sperm injection*       2156
#10     #4 OR #5 OR #6 OR #7 OR #8 OR #9        7639
#11     (chromosom* OR cytogenetic OR karotyp*) AND (polymorphism*
or polymorphic or variation* or variant or differentiation or
aberration* or anomaly or anomalies or abnormalit*)     1882
#12     MeSH descriptor: [Polymorphism, Genetic] explode all trees
        3066
#13     MeSH descriptor: [Chromosomes] explode all trees and with
qualifier(s): [genetics - GE]   140
#14     #11 OR #12 OR #13       4872
#15     #3 AND #10 AND #14      49
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Search Name:    IVF and genetic polymorphism  - Madara Ralapanawe
Last Saved:     12/06/2019 13:17:43
Comment:

ID      Search
#1      MeSH descriptor: [Infertility] explode all trees
#2      infertility
#3      #1 OR #2
#4      MeSH descriptor: [Reproductive Techniques, Assisted] explode
all trees
#5      assisted AND reproducti*
#6      MeSH descriptor: [Fertilization in Vitro] explode all trees
#7      (in vitro OR in-vitro) AND Fertili?ation
#8      MeSH descriptor: [Sperm Injections, Intracytoplasmic]
explode all trees
#9      Intracytoplasmic sperm injection*
#10     #4 OR #5 OR #6 OR #7 OR #8 OR #9
#11     (chromosom* OR cytogenetic OR karotyp*) AND (polymorphism*
or polymorphic or variation* or variant or differentiation or
aberration* or anomaly or anomalies or abnormalit*)
#12     MeSH descriptor: [Polymorphism, Genetic] explode all trees
#13     MeSH descriptor: [Chromosomes] explode all trees and with
qualifier(s): [genetics - GE]
#14     #11 OR #12 OR #13
#15     #3 AND #10 AND #14
```

MY

EBSCOhost

Wednesday, June 12, 2019 8:42:34 AM

#	Query	Limiters/Expanders	Last Run Via	Results
S11	S9 AND S10	Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	82
S10	(chromosom* or cytogenetic* or karotyp*) and (polymorphism* or polymorphic or variation* or variant or differentiation or aberration* or anomaly or anomalies or abnormalit*)	Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	8,860
S9	S7 AND S8	Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	3,487
S8	S1 OR S2	Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	13,761
S7	S3 OR S4 OR S5 OR S6	Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	9,293

S6	Intracytoplasmic and sperm and injection*	Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	828
S5	SU fertilization in vitro	Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	5,443
S4	(in vitro or in-vitro or "test tube") and Fertili?ation	Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	6,618
S3	(assisted AND reproducti*) OR SU assisted reproduction	Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	3,595
S2	SU infertility	Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	9,981
S1	infertil*	Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	13,761

Cinahl search strategy (note cinhal is reproduced the opposite way round)

S11	S9 AND S10
S10	(chromosom* or cytogenetic* or karotyp*) and (polymorphism* or variation* or variant or differentiation or aberration* or anomaly or anomalies or abnormalit*)
S9	S7 AND S8
S8	S1 OR S2
S7	S3 OR S4 OR S5 OR S6
S6	Intracytoplasmic and sperm and injection*
S5	SU fertilization in vitro
S4	(in vitro or in-vitro or "test tube") and Fertili?ation
S3	(assisted AND reproducti*) OR SU assisted reproduction
S2	SU infertility
S1	infertil*

Database: Embase <1974 to 2019 June 11>
Search Strategy:
---
1 exp Infertility/ (115457)
2 infertil*.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (126046)
3 1 or 2 (149284)
4 exp infertility therapy/ (96196)
5 (assisted and reproducti*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (28071)
6 exp fertilization in vitro/ or exp in vitro fertilization/ (66485)
7 ((in vitro or in-vitro or "test tube") and Fertili?ation).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (60971)
8 exp intracytoplasmic sperm injection/ (19771)
9 (Intracytoplasmic and sperm and injection*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (21081)
10 4 or 5 or 6 or 7 or 8 or 9 (130093)
11 ((chromosom* or cytogenetic* or karotyp*) and (polymorphism* or polymorphic* or variation* or variant or differentiation or aberration* or anomaly or anomalies or abnormalit*)).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (229542)
12 exp chromosome polymorphism/ (2669)
13 11 or 12 (229542)
14 3 and 10 and 13 (2298)

****

<1>
Title
Improving fluorescence in-situ hybridization (FISH)-based preimplantation genetic diagnosis/ screening (PGD/PGS).
Source
International Journal of Reproductive BioMedicine. Conference: 7th Yazd International Congress and Student Award in Reproductive Medicine with 2nd Congress of Reproductive Genetics and Congress of Reproductive Immunology. Iran, Islamic Republic of. 15 (4 Supplement 1) (pp 30-31), 2017. Date of Publication: April 2017.
Author
Montazeri F.; Foroughmand A.M.; Kalantar S.M.; Aflatoonian A.; Khalili M.A.; Fesahat F.; Soleimani M.; Taheri F.
Publisher
Research and Clinical Center for Infertitlity
Publication Type
Conference Abstract

Chromosomal polymorphisms in IVF etc - EMBASE JUN 2019 - 2020

1. exp Infertility/

2. infertil*.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]

3. 1 or 2

4. exp infertility therapy/

5. (assisted and reproducti*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]

6. exp fertilization in vitro/ or exp in vitro fertilization/

7. ((in vitro or in-vitro or "test tube") and Fertili?ation).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]

8. exp intracytoplasmic sperm injection/

9. (Intracytoplasmic and sperm and injection*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]

10. 4 or 5 or 6 or 7 or 8 or 9

11. ((chromosom* or cytogenetic* or karotyp*) and (polymorphism* or polymorphic* or variation* or variant or differentiation or aberration* or anomaly or anomalies or abnormalit*)).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]

12. exp chromosome polymorphism/

13. 11 or 12

14. 3 and 10 and 13

15. (0? JUN 2019 or 1? JUN 2019 or 2? JUN 2019 or 3? JUN 2019).dp.

16. (0? JUL 2019 or 1? JUL 2019 or 2? JUL 2019 or 3? JUL 2019).dp.

17. (0? AUG 2019 or 1? AUG 2019 or 2? AUG 2019 or 3? AUG 2019).dp.

18. (0? SEP 2019 or 1? SEP 2019 or 2? SEP 2019 or 3? SEP 2019).dp.

19. (0? OCT 2019 or 1? OCT 2019 or 2? OCT 2019 or 3? OCT 2019).dp.

20. (0? NOV 2019 or 1? NOV 2019 or 2? NOV 2019 or 3? NOV 2019).dp.

21. (0? DEC 2019 or 1? DEC 2019 or 2? DEC 2019 or 3? DEC 2019).dp.

22. (0? JAN 2020 or 1? JAN 2020 or 2? JAN 2020 or 3? JAN 2020).dp.

23. (0? FEB 2020 or 1? FEB 2020 or 2? FEB 2020 or 3? FEB 2020).dp.

24. (0? MAR 2020 or 1? MAR 2020 or 2? MAR 2020 or 3? MAR 2020).dp.

25. 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24

26. 14 and 25

Database(s): **Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R)** 1946 to June 11, 2019

Search Strategy:

#	Searches	Results
1	exp Infertility/	63547
2	infertil*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	88676
3	1 or 2	92038
4	exp Reproductive Techniques, Assisted/	66471
5	(assisted and reproducti*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	21830
6	exp Fertilization in Vitro/	34346
7	((in vitro or in-vitro or "test tube") and Fertili?ation).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	42510
8	exp Sperm Injections, Intracytoplasmic/	6278
9	(Intracytoplasmic and sperm and injection*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	9720
10	4 or 5 or 6 or 7 or 8 or 9	83485
11	((chromosom* or cytogenetic* or karotyp*) and (polymorphism* or polymorphic or variation* or variant or differentiation or aberration* or anomaly or anomalies or abnormalit*)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	209422
12	exp Polymorphism, Genetic/	259162
13	*Chromosomes/ge [Genetics]	2396
14	11 or 12 or 13	425329
15	3 and 10 and 14	1189

Result 1.

Unique Identifier	31116011
Title	Decrease of spermatozoa with an unbalanced **chromosome** content after cell sorting **in** men carrying a structural **chromosomal abnormality**.
Source	Andrology. 2019 May 22.
Authors	El Fekih S; Tous C; Gueganic N; Brugnon F; Ali HB; Bujan L; Moinard N; Caire-Tetauru E; Ajina M; Douet-Guilbert N; Morel F; Perrin A.
Authors Full Name	El Fekih, S; Tous, C; Gueganic, N; Brugnon, F; Ali, H Ben; Bujan, L; Moinard, N; Caire-Tetauru, E; Ajina, M; Douet-Guilbert, N; Morel, F; Perrin, A.
Publication Type	Journal Article.

Medline Chromosomal polymorphisms search June 2019 to March 2020

1. exp Infertility/

2. infertil*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

3. 1 or 2

4. exp Reproductive Techniques, Assisted/

5. (assisted and reproducti*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

6. exp Fertilization in Vitro/

7. ((in vitro or in-vitro or "test tube") and Fertili?ation).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

8. exp Sperm Injections, Intracytoplasmic/

9. (Intracytoplasmic and sperm and injection*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

10. 4 or 5 or 6 or 7 or 8 or 9

11. ((chromosom* or cytogenetic* or karotyp*) and (polymorphism* or polymorphic or variation* or variant or differentiation or aberration* or anomaly or anomalies or abnormalit*)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

12. exp Polymorphism, Genetic/

13. *Chromosomes/ge [Genetics]

14. 11 or 12 or 13

15. 3 and 10 and 14

16. "201906*".ez. or "2019 jun **".dp.

17. "201907*".ez. or "2019 jul **".dp.

18. "201908*".ez. or "2019 aug **".dp.

19. "201909*".ez. or "2019 sep **".dp.

20. "201910*".ez. or "2019 oct **".dp.

21. "201911*".ez. or "2019 nov **".dp.

22. "201912*".ez. or "2019 dec **".dp.

23. "202001*".ez. or "2020 jan **".dp.

24. "202002*".ez. or "2020 feb **".dp.

25. "202003*".ez. or "2020 mar **".dp.

26. 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25

27. 15 and 26

APPENDIX 3: ETHICAL COMMITTEE APPROVAL – LANKA HOSPITALS

LANKA HOSPITALS

CARING CURING

12/12/2019

To whom it may concern

PGR's Name: Madara Sapumal Bandara Ralapanawe
ID Number: [redacted]
Programme: PhD Met+ Syst FT Non-Lab A300
Lead Supervisor: Dr. Ioannis Gallos
Co-Supervisor: Prof. Aravinthan Coomarasamy
Title of the research: A Comprehensive Analysis of Chromosomal Polymorphic Variations in Females and Males in the Outcome of ICSI.

Details pertaining to patients who opted to proceed with IVF/ICSI treatment at Lanka Hospitals Corporation Plc, Fertility Centre were collected from 1st January 2016 to 31st of December 2018, and the details of the outcome of these treatments of these patients were collected until 30th November 2019.

The hospital ethical committee has given ethical approval and clearance to Dr. Madara S.B. Ralapanawe to proceed with the research.

This is to confirm that Lanka Hospitals Corporation PLC has reviewed and granted permission to Dr. Madara S.B. Ralapanawe to submit the data for the purpose of research to the University of Birmingham,UK.

[redacted]

Dr. Wimal Karandagoda
Director Medical Services

Organization Accredited by Joint Commission International

THE LANKA HOSPITALS CORPORATION PLC (PQ 180) 578, Elvitigala Mawatha, Narahenpita, Colombo 5, Sri Lanka.
T : +94(0) 115 430000, +94(0) 115 530000 F : +94(0) 114 511199 E : info@lankahospitals.com

APPENDIX 4: STATA FILE OF DATA ANALYSIS (CHAPTER 3)

User: Madara Ralapanawe

```
  ___  ____  ____  ____  ____ (R)
 /__    /   ____/   /   ____/
___/   /   /___/   /   /___/   16.1   Copyright 19
> 85-2019 StataCorp LLC
  Statistics/Data analysis            StataCorp
                                      4905 Lakeway
>  Drive
                                      College Stat
> ion, Texas 77845 USA
                                      800-STATA-PC
>         https://www.stata.com
                                      979-696-4600
>         stata@stata.com
                                      979-696-4601
>  (fax)

Stata license: Single-user , expiring 18 Jul 2021
Serial number: 301609626577
  Licensed to: Madara Ralapanawe
               University of Birmingham

Notes:
      1. Unicode is supported; see help
         unicode_advice.

1 . use "/Users/madararalapanawe/Desktop/Madara fina
  > l.dta"

2 . ***F/M/C outcome

3 . tab poly

  Karyotype with
    Polymorphism |      Freq.     Percent        Cum.
-----------------+-----------------------------------
         Neither |        448       47.56       47.56
       Male only |        200       21.23       68.79
     Female only |        150       15.92       84.71
Male and Female  |        144       15.29      100.00
-----------------+-----------------------------------
           Total |        942      100.00

4 . tab researchtype_cc

       RECODE of
   researchtype_c
(Research Type) |      Freq.     Percent        Cum.
----------------+-----------------------------------
      FER CYCLES |        394       41.83       41.83
FRESH IVF CYCLES |        548       58.17      100.00
----------------+-----------------------------------
          Total |        942      100.00

5 . **** outcomes

6 . tab positivepregancytest_c

    Positive
   pregnancy
        test |      Freq.     Percent        Cum.
-------------+-----------------------------------
          No |        648       68.79       68.79
         yes |        294       31.21      100.00
-------------+-----------------------------------
       Total |        942      100.00

7 . tab miscarriage_c
```

Miscarriage	Freq.	Percent	Cum.
No	812	86.20	86.20
Yes	130	13.80	100.00
Total	942	100.00	

```
. tab livebirth_c
```

Live Birth	Freq.	Percent	Cum.
No	791	83.97	83.97
Yes	151	16.03	100.00
Total	942	100.00	

```
. ******* summary statistics

. summarize ageyears fshmiuml lhmiuml amhngml tshmicroiuml t4ngdl prolactinngml bmi cleavageday3 spermconce
> ntrationmillionml progressivemotility nonprogressivemotility normalmorphology, detail
```

Age (Years)

	Percentiles	Smallest		
1%	23	19		
5%	27	20		
10%	29	20	Obs	942
25%	31	20	Sum of Wgt.	942
50%	34		Mean	33.95223
		Largest	Std. Dev.	4.11483
75%	37	43		
90%	39	43	Variance	16.93183
95%	40	43	Skewness	-.3166296
99%	43	43	Kurtosis	3.23364

FSH (mIU/ml)

	Percentiles	Smallest		
1%	3.24	1.84		
5%	4.14	2.55		
10%	4.53	2.59	Obs	942
25%	5.47	2.8	Sum of Wgt.	942
50%	6.5		Mean	6.665499
		Largest	Std. Dev.	1.789872
75%	7.7	12.63		
90%	8.8	13.39	Variance	3.203642
95%	10.18	14.18	Skewness	.7764828
99%	12.23	14.24	Kurtosis	4.319247

LH (mIU/ml)

	Percentiles	Smallest		
1%	1.38	.07		
5%	2.4	.64		
10%	3	.64	Obs	942
25%	3.91	.81	Sum of Wgt.	942
50%	5.3		Mean	5.796433
		Largest	Std. Dev.	2.738047
75%	7.03	18.16		
90%	9.18	18.3	Variance	7.496902
95%	10.6	24.1	Skewness	1.663995
99%	14.65	24.1	Kurtosis	8.732794

AMH (ng/ml)

Percentiles	Smallest

1%	.3	.01		
5%	.87	.02		
10%	1.16	.02	Obs	543
25%	1.87	.26	Sum of Wgt.	543
50%	3.06		Mean	3.92553
		Largest	Std. Dev.	3.319573
75%	4.73	18.17		
90%	7.99	23.8	Variance	11.01956
95%	10.5	23.8	Skewness	2.564201
99%	17.33	24	Kurtosis	12.27338

TSH (microIU/ml)

	Percentiles	Smallest		
1%	.2	.021		
5%	.666	.037		
10%	.933	.113	Obs	927
25%	1.35	.129	Sum of Wgt.	927
50%	1.97		Mean	2.099612
		Largest	Std. Dev.	1.075426
75%	2.74	6.15		
90%	3.43	6.25	Variance	1.156541
95%	3.87	10.98	Skewness	1.652591
99%	4.83	10.98	Kurtosis	12.54264

T4 (ng/dl)

	Percentiles	Smallest		
1%	.82	.5		
5%	.95	.5		
10%	1	.793	Obs	924
25%	1.1	.793	Sum of Wgt.	924
50%	1.21		Mean	1.245992
		Largest	Std. Dev.	.2544233
75%	1.35	2.87		
90%	1.5	2.87	Variance	.0647312
95%	1.6	2.88	Skewness	2.625124
99%	2.56	2.88	Kurtosis	16.53983

Prolactin (ng/ml)

	Percentiles	Smallest		
1%	2.79	.45		
5%	6.44	.46		
10%	8.05	.46	Obs	932
25%	10.6	1.25	Sum of Wgt.	932
50%	14.705		Mean	15.71896
		Largest	Std. Dev.	6.824585
75%	20.29	40.9		
90%	24.81	40.9	Variance	46.57496
95%	27.4	41.4	Skewness	.7524003
99%	34.81	54.4	Kurtosis	4.228699

BMI

	Percentiles	Smallest		
1%	17	14.6		
5%	19	15		
10%	20	15.4	Obs	942
25%	21.3	15.8	Sum of Wgt.	942
50%	23.95		Mean	24.02822
		Largest	Std. Dev.	3.778522
75%	26	38.4		
90%	29	38.4	Variance	14.27723

95%	31	39	Skewness	.9291929
99%	35.8	42	Kurtosis	4.704358

Cleavage- Day 3

	Percentiles	Smallest		
1%	1	1		
5%	2	1		
10%	2	1	Obs	942
25%	4	1	Sum of Wgt.	942
50%	6		Mean	7.472399
		Largest	Std. Dev.	5.139167
75%	10	30		
90%	14	30	Variance	26.41103
95%	18	32	Skewness	1.2364
99%	22	34	Kurtosis	5.016284

Sperm Concentration (million/ml)

	Percentiles	Smallest		
1%	.001	.001		
5%	.5	.001		
10%	2.5	.001	Obs	942
25%	18	.001	Sum of Wgt.	942
50%	51.5		Mean	50.89772
		Largest	Std. Dev.	35.77305
75%	79.5	134		
90%	96	153	Variance	1279.711
95%	108	163	Skewness	.2247331
99%	132	163	Kurtosis	2.100592

Progressive Motility (%)

	Percentiles	Smallest		
1%	2	0		
5%	7	1		
10%	13	1	Obs	908
25%	30	1	Sum of Wgt.	908
50%	45		Mean	41.3293
		Largest	Std. Dev.	18.12209
75%	55	81		
90%	61	85	Variance	328.4102
95%	67	85	Skewness	-.4433097
99%	73	92	Kurtosis	2.438694

Non Progressive Motility (%)

	Percentiles	Smallest		
1%	1	1		
5%	3	1		
10%	4	1	Obs	847
25%	5	1	Sum of Wgt.	847
50%	6		Mean	7.874852
		Largest	Std. Dev.	5.987648
75%	8	54		
90%	14	56	Variance	35.85193
95%	18	60	Skewness	3.915996
99%	30	60	Kurtosis	27.00914

Normal Morphology (%)

	Percentiles	Smallest		
1%	1	0		
5%	3	1		
10%	6	1	Obs	856

25%	19	1	Sum of Wgt.	856
50%	34		Mean	32.20678
		Largest	Std. Dev.	17.40882
75%	43	95		
90%	53	95	Variance	303.0671
95%	58	96	Skewness	.0846984
99%	65	97	Kurtosis	3.085055

```
11 . *** descriptive statistics for continuous outcomes

12 . summarize fertilization fertilizationrate matureoocytes cleavageday3 cleavageday3rate, detail
```

Fertilization

	Percentiles	Smallest		
1%	1	1		
5%	2	1		
10%	3	1	Obs	942
25%	6	1	Sum of Wgt.	942
50%	10		Mean	11.25053
		Largest	Std. Dev.	7.398687
75%	15	41		
90%	22	41	Variance	54.74057
95%	25	41	Skewness	1.252425
99%	37	50	Kurtosis	5.164159

fertilizationrate

	Percentiles	Smallest		
1%	.25	.1428571		
5%	.3888889	.1666667		
10%	.4782609	.1666667	Obs	942
25%	.625	.1666667	Sum of Wgt.	942
50%	.75		Mean	.7331603
		Largest	Std. Dev.	.1841889
75%	.875	1		
90%	.9655172	1	Variance	.0339256
95%	1	1	Skewness	-.6591
99%	1	1	Kurtosis	3.034309

Mature Oocytes

	Percentiles	Smallest		
1%	2	1		
5%	4	1		
10%	6	1	Obs	942
25%	9	1	Sum of Wgt.	942
50%	14		Mean	14.99894
		Largest	Std. Dev.	8.19651
75%	20	42		
90%	26	42	Variance	67.18278
95%	30	45	Skewness	.8085389
99%	38	54	Kurtosis	3.623033

Cleavage- Day 3

	Percentiles	Smallest		
1%	1	1		
5%	2	1		
10%	2	1	Obs	942
25%	4	1	Sum of Wgt.	942
50%	6		Mean	7.472399
		Largest	Std. Dev.	5.139167
75%	10	30		

90%	14	30	Variance	26.41103
95%	18	32	Skewness	1.2364
99%	22	34	Kurtosis	5.016284

cleavageday3rate

	Percentiles	Smallest		
1%	.2	.1111111		
5%	.3125	.125		
10%	.4	.125	Obs	942
25%	.5263158	.1538462	Sum of Wgt.	942
50%	.7		Mean	.6921553
		Largest	Std. Dev.	.2142788
75%	.8571429	1		
90%	1	1	Variance	.0459154
95%	1	1	Skewness	-.2941222
99%	1	1	Kurtosis	2.306557

```
13 . sort poly

14 . ***** Outcomes

15 . * crosstabs with outcome

16 . tab2 poly positivepregancytest_cc, row chi exact

-> tabulation of poly by positivepregancytest_cc
```

Key
frequency *row percentage*

```
Enumerating sample-space combinations:
stage 4:  enumerations = 1
stage 3:  enumerations = 25
stage 2:  enumerations = 498
stage 1:  enumerations = 0
```

Karyotype with Polymorphism	Positive pregnancy test: No	yes	Total
Neither	310 69.20	138 30.80	448 100.00
Male only	132 66.00	68 34.00	200 100.00
Female only	114 76.00	36 24.00	150 100.00
Male and Female	92 63.89	52 36.11	144 100.00
Total	648 68.79	294 31.21	942 100.00

```
          Pearson chi2(3) =   6.0027   Pr = 0.111
           Fisher's exact =                 0.107

17 . tab2 poly miscarriage_c, row chi exact

-> tabulation of poly by miscarriage_c
```

Key
frequency *row percentage*

```
Enumerating sample-space combinations:
stage 4:  enumerations = 1
stage 3:  enumerations = 13
stage 2:  enumerations = 128
stage 1:  enumerations = 0
```

Karyotype with Polymorphism	Miscarriage No	Yes	Total
Neither	391 87.28	57 12.72	448 100.00
Male only	172 86.00	28 14.00	200 100.00
Female only	131 87.33	19 12.67	150 100.00
Male and Female	118 81.94	26 18.06	144 100.00
Total	812 86.20	130 13.80	942 100.00

Pearson chi2(3) = 2.7975 Pr = 0.424
Fisher's exact = 0.427

```
18 . tab2 poly livebirth_c, row chi exact

-> tabulation of poly by livebirth_c
```

Key
frequency *row percentage*

```
Enumerating sample-space combinations:
stage 4:  enumerations = 1
stage 3:  enumerations = 18
stage 2:  enumerations = 251
stage 1:  enumerations = 0
```

Karyotype with Polymorphism	Live Birth No	Yes	Total
Neither	376 83.93	72 16.07	448 100.00
Male only	162 81.00	38 19.00	200 100.00
Female only	134 89.33	16 10.67	150 100.00
Male and Female	119 82.64	25 17.36	144 100.00
Total	791 83.97	151 16.03	942 100.00

Pearson chi2(3) = 4.7064 Pr = 0.195

```
           Fisher's exact =                 0.181

19 . tab2 poly researchtype_cc, row chi exact

-> tabulation of poly by reSearchtype_cc

| Key             |
|-----------------|
| frequency       |
| row percentage  |

Enumerating sample-space combinations:
stage 4:  enumerations = 1
stage 3:  enumerations = 11
stage 2:  enumerations = 90
stage 1:  enumerations = 0

                  |  RECODE of researchtype_c (Research Type)
Karyotype with Po |  FER CYCLE  FRESH IVF |     Total
------------------+-----------------------+----------
          Neither |       194        254 |       448
                  |     43.30      56.70 |    100.00
------------------+-----------------------+----------
        Male only |        79        121 |       200
                  |     39.50      60.50 |    100.00
------------------+-----------------------+----------
      Female only |        63         87 |       150
                  |     42.00      58.00 |    100.00
------------------+-----------------------+----------
  Male and Female |        58         86 |       144
                  |     40.28      59.72 |    100.00
------------------+-----------------------+----------
            Total |       394        548 |       942
                  |     41.83      58.17 |    100.00

          Pearson chi2(3) =   0.9904   Pr = 0.804
           Fisher's exact =                 0.808

20 . * regression models unadjusted

21 . logistic positivepregancytest_cc i.poly, or

Logistic regression                             Number of obs     =        942
                                                LR chi2(3)        =       6.13
                                                Prob > chi2       =     0.1054
Log likelihood = -581.70192                     Pseudo R2         =     0.0052

------------------------------------------------------------------------------
positivepregancy~c | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------------+----------------------------------------------------------
              poly |
        Male only  |   1.157224   .2094347     0.81   0.420     .8116466    1.64994
      Female only  |   .7093822   .1538262    -1.58   0.113     .4637684    1.085074
    Male and Female|   1.269691   .2557491     1.19   0.236     .8555469    1.88431
                   |
             _cons |   .4451613    .045555    -7.91   0.000     .3642594    .5440314
------------------------------------------------------------------------------
Note: _cons estimates baseline odds.

22 . logistic miscarriage_c i.poly, or

Logistic regression                             Number of obs     =        942
                                                LR chi2(3)        =       2.65
                                                Prob > chi2       =     0.4492
Log likelihood = -376.72341                     Pseudo R2         =     0.0035
```

miscarriage_c	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
poly						
Male only	1.116687	.2772211	0.44	0.657	.6864644	1.81654
Female only	.9949109	.2820473	-0.02	0.986	.570791	1.734169
Male and Female	1.511448	.3913382	1.60	0.111	.9099207	2.510632
_cons	.1457801	.0206686	-13.58	0.000	.1104117	.1924779

Note: _cons estimates baseline odds.

```
23 . logistic livebirth_c i.poly, or
```

Logistic regression — Number of obs = 942; LR chi2(3) = 5.00; Prob > chi2 = 0.1721; Log likelihood = -412.13535; Pseudo R2 = 0.0060

livebirth_c	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
poly						
Male only	1.224966	.2712603	0.92	0.359	.7936539	1.890674
Female only	.6235489	.1834031	-1.61	0.108	.3503552	1.109769
Male and Female	1.097106	.2796044	0.36	0.716	.6657546	1.807934
_cons	.1914894	.0246334	-12.85	0.000	.1488145	.2464018

Note: _cons estimates baseline odds.

```
24 . * Missing data

25 . misstable summarize amhngml fshmiuml lhmiuml tshmicroiuml bmi ageyears fertilization poly cleavageday3 ma
   > tureoocytes positivepregancytest_cc overallfinaloutcome positiveoutcome_cc researchtype_cc
```

Variable	Obs=.	Obs>.	Obs<.	Unique values	Min	Max
amhngml	399		543	313	.01	24
tshmicroiuml	15		927	382	.021	10.98
overallfin-e	661		281	2	0	1

```
26 . mi set mlong

27 . mi register imputed amhngml
   (399 m=0 obs. now marked as incomplete)

28 . mi misstable summarize, all
```

Variable	Obs=.	Obs>.	Obs<.	Unique values	Min	Max
ivfno			942	>500	1397	7218
ageyears			942	24	19	43
fshmiuml			942	388	1.84	14.24
lhmiuml			942	456	.07	24.1
amhngml	399		543	313	.01	24
tshmicroiuml	15		927	382	.021	10.98
t4ngdl	18		924	120	.5	2.88
prolactinn-l	10		932	>500	.45	54.4
ivfcycle	(string variable)					
gonalfdoseiu	(string variable)					
karyotypef-e	(string variable)					
karyotypem-e	(string variable)					

bmi		942	78	14.6	42
spermconce-l		942	256	.001	163
progressiv-y	34	908	80	0	92
nonprogres-y	95	847	34	1	60
normalmorp-y	86	856	70	0	97
noofoocytes		942	41	1	60
matureoocy-s		942	42	1	54
fertilizat-n		942	38	1	50
cleavageday3		942	26	1	34
numberofem-n	5	937	2	1	2
frozenembr-s	6	936	23	0	28
remarksfre-e	(string variable)				
miscarriage	(string variable)				
livebirth	(string variable)				
poly		942	4	1	4
lnamhngml	400	542	313	-4.60517	3.178054
positivepr-c		942	2	0	1
miscarriag-c		942	2	0	1
livebirth_c		942	2	0	1
overallfin-e	661	281	2	0	1
fertilizat-e		942	151	.1428571	1
cleavageda-e		942	99	.1111111	1
positiveou-c		942	2	0	1
researchty-c		942	2	0	1

```
29 . mi impute regress amhngml fshmiuml lhmiuml tshmicroiuml bmi ageyears fertilization poly cleavageday3 matu
   > reoocytes positivepregancytest_cc positiveoutcome_cc, add(20) rseed(1234) force
   note: positiveoutcome_cc omitted because of collinearity

   Univariate imputation                       Imputations =       20
   Linear regression                                 added =       20
   Imputed: m=1 through m=20                       updated =        0
```

	Observations per m			
Variable	Complete	Incomplete	Imputed	Total
amhngml	543	399	386	942

```
   (complete + incomplete = total; imputed is the minimum across m
    of the number of filled-in observations.)

   Note: Right-hand-side variables (or weights) have missing values;
         model parameters estimated using listwise deletion.

30 . * regression models adjusted for Research Type, age, FSH, LH, BMI

31 . mi estimate, or: logistic positivepregancytest_cc i.poly i.researchtype_cc ageyears fshmiuml lhmiuml bmi

   Multiple-imputation estimates                 Imputations       =       20
   Logistic regression                           Number of obs     =      942
                                                 Average RVI       =   0.0000
                                                 Largest FMI       =   0.0000
   DF adjustment:   Large sample                 DF:     min       =        .
                                                         avg       =        .
                                                         max       =        .
   Model F test:       Equal FMI                 F(   8,      .)   =     5.42
   Within VCE type:          OIM                 Prob > F          =   0.0000
```

positivepregancytest_cc	Odds Ratio	Std. Err.	t	P>|t|	[95% Conf.	Interval]
poly						
Male only	1.193654	.2216519	0.95	0.340	.8295011	1.717669
Female only	.7008197	.15511	-1.61	0.108	.454164	1.081433
Male and Female	1.291051	.2672186	1.23	0.217	.8605258	1.936971

researchtype_cc						
FRESH IVF CYCLES	.4862963	.0712058	-4.92	0.000	.3649758	.6479446
ageyears	.9652349	.0175522	-1.95	0.052	.9314391	1.000257
fshmiuml	.9259815	.0405765	-1.75	0.079	.8497725	1.009025
lhmiuml	.9919926	.0272234	-0.29	0.770	.9400452	1.046811
bmi	.978493	.0194232	-1.10	0.273	.9411552	1.017312
_cons	6.348998	5.060731	2.32	0.020	1.331128	30.28241

Note: _cons estimates baseline odds.

```
32 . mi estimate, or: logistic miscarriage_c i.poly i.researchtype_cc ageyears fshmiuml lhmiuml bmi
```

Multiple-imputation estimates — Imputations = 20
Logistic regression — Number of obs = 942
Average RVI = 0.0000
Largest FMI = 0.0000
DF adjustment: Large sample — DF: min = 2.31e+63
avg = 5.01e+63
max = .
Model F test: Equal FMI — F(8, 4.2e+65) = 1.87
Within VCE type: OIM — Prob > F = 0.0600

miscarriage_c	Odds Ratio	Std. Err.	t	P>|t|	[95% Conf.	Interval]
poly						
Male only	1.137661	.2857123	0.51	0.608	.6954115	1.86116
Female only	1.003003	.2864656	0.01	0.992	.5730502	1.755546
Male and Female	1.543839	.4043335	1.66	0.097	.923998	2.579484
researchtype_cc						
FRESH IVF CYCLES	.6566056	.1272718	-2.17	0.030	.4490697	.9600535
ageyears	.9847153	.023381	-0.65	0.517	.9399393	1.031624
fshmiuml	.9006857	.0532787	-1.77	0.077	.8020875	1.011404
lhmiuml	1.040985	.0350114	1.19	0.232	.9745765	1.111918
bmi	1.019439	.025739	0.76	0.446	.9702196	1.071156
_cons	.2992723	.3089508	-1.17	0.243	.0395673	2.263581

Note: _cons estimates baseline odds.

```
33 . mi estimate, or: logistic livebirth_c i.poly i.researchtype_cc ageyears fshmiuml lhmiuml bmi
```

Multiple-imputation estimates — Imputations = 20
Logistic regression — Number of obs = 942
Average RVI = 0.0000
Largest FMI = 0.0000
DF adjustment: Large sample — DF: min = 6.99e+64
avg = 6.99e+64
max = .
Model F test: Equal FMI — F(8, 2.5e+67) = 4.52
Within VCE type: OIM — Prob > F = 0.0000

livebirth_c	Odds Ratio	Std. Err.	t	P>|t|	[95% Conf.	Interval]
poly						
Male only	1.281909	.2912685	1.09	0.274	.8212051	2.001071
Female only	.617206	.1842673	-1.62	0.106	.343798	1.108044
Male and Female	1.10049	.2873359	0.37	0.714	.6596888	1.835833
researchtype_cc						
FRESH IVF CYCLES	.4549133	.0843394	-4.25	0.000	.3163142	.6542423
ageyears	.9534267	.0217166	-2.09	0.036	.9117991	.9969547
fshmiuml	.9756145	.0537176	-0.45	0.654	.8758119	1.08679
lhmiuml	.9492444	.0352034	-1.40	0.160	.8826949	1.020811
bmi	.9352598	.0251115	-2.49	0.013	.8873148	.9857955
_cons	10.97294	11.2655	2.33	0.020	1.466989	82.07655

Note: _cons estimates baseline odds.

```
34 . **log close
35 .
```

User: Madara Ralapanawe

```
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1 . use "/Users/madararalapanawe/Desktop/Madara fina
  > l 1.1.dta"

2 . ***Overall poly or neither outcome

3 . tab poly

    Karyotype
         with
Polymorphis
           m |      Freq.     Percent        Cum.
-------------+-----------------------------------
     Neither |        448       47.56       47.56
  With poly  |        494       52.44      100.00
-------------+-----------------------------------
       Total |        942      100.00

4 . tab researchtype_cc

      RECODE of
  researchtype_c
(Research Type) |      Freq.     Percent        Cum.
----------------+-----------------------------------
      FER CYCLES |        394       41.83       41.83
FRESH IVF CYCLES |        548       58.17      100.00
----------------+-----------------------------------
           Total |        942      100.00

5 . **** outcomes

6 . tab positivepregancytest_c

    Positive
   pregnancy
        test |      Freq.     Percent        Cum.
-------------+-----------------------------------
          No |        648       68.79       68.79
         yes |        294       31.21      100.00
-------------+-----------------------------------
       Total |        942      100.00
```

```
 7 . tab miscarriage_c
```

Miscarriage	Freq.	Percent	Cum.
No	812	86.20	86.20
Yes	130	13.80	100.00
Total	942	100.00	

```
 8 . tab livebirth_c
```

Live Birth	Freq.	Percent	Cum.
No	791	83.97	83.97
Yes	151	16.03	100.00
Total	942	100.00	

```
 9 . sort poly

10 . * crosstabs with outcome

11 . tab2 poly positivepregancytest_cc, row chi exact

-> tabulation of poly by positivepregancytest_cc
```

Key
frequency *row percentage*

Karyotype with Polymorphism	Positive pregnancy test		
	No	yes	Total
Neither	310	138	448
	69.20	30.80	100.00
With poly	338	156	494
	68.42	31.58	100.00
Total	648	294	942
	68.79	31.21	100.00

```
          Pearson chi2(1) =   0.0658   Pr = 0.798
           Fisher's exact =                 0.833
   1-sided Fisher's exact =                 0.426

12 . tab2 poly miscarriage_c, row chi exact

-> tabulation of poly by miscarriage_c
```

Key
frequency *row percentage*

Karyotype with Polymorphism	Miscarriage		
	No	Yes	Total
Neither	391	57	448

	87.28	12.72	100.00
With poly	421	73	494
	85.22	14.78	100.00
Total	812	130	942
	86.20	13.80	100.00

Pearson chi2(1) = 0.8333 Pr = 0.361
Fisher's exact = 0.395
1-sided Fisher's exact = 0.207

```
13 . tab2 poly livebirth_c, row chi exact
```

-> tabulation of poly by livebirth_c

Key
frequency *row percentage*

Karyotype with Polymorphism	Live Birth		
	No	Yes	Total
Neither	376	72	448
	83.93	16.07	100.00
With poly	415	79	494
	84.01	15.99	100.00
Total	791	151	942
	83.97	16.03	100.00

Pearson chi2(1) = 0.0011 Pr = 0.973
Fisher's exact = 1.000
1-sided Fisher's exact = 0.522

```
14 . tab2 poly researchtype_cc, row chi exact
```

-> tabulation of poly by researchtype_cc

Key
frequency *row percentage*

Karyotype with Polymorphism	RECODE of researchtype_c (Research Type)		
	FER CYCLE	FRESH IVF	Total
Neither	194	254	448
	43.30	56.70	100.00
With poly	200	294	494
	40.49	59.51	100.00
Total	394	548	942
	41.83	58.17	100.00

Pearson chi2(1) = 0.7666 Pr = 0.381
Fisher's exact = 0.391
1-sided Fisher's exact = 0.209

```
15 . * regression models unadjusted

16 . logistic positivepregancytest_cc i.poly, or

Logistic regression                             Number of obs     =        942
                                                LR chi2(1)        =       0.07
                                                Prob > chi2       =     0.7975
Log likelihood = -584.73444                     Pseudo R2         =     0.0001

------------------------------------------------------------------------------
positivepregancytest_cc | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
------------------------+-----------------------------------------------------
                   poly |
             With poly |   1.036789   .1460402     0.26   0.798     .7866686    1.366436
                 _cons |   .4451613    .045555    -7.91   0.000     .3642594    .5440314
------------------------------------------------------------------------------
Note: _cons estimates baseline odds.

17 . logistic miscarriage_c i.poly, or

Logistic regression                             Number of obs     =        942
                                                LR chi2(1)        =       0.84
                                                Prob > chi2       =     0.3606
Log likelihood = -377.62937                     Pseudo R2         =     0.0011

------------------------------------------------------------------------------
miscarriage_c | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------+---------------------------------------------------------------
         poly |
   With poly |    1.18944   .2262293     0.91   0.362     .8193052    1.72679
       _cons |   .1457801   .0206686   -13.58   0.000     .1104117    .1924779
------------------------------------------------------------------------------
Note: _cons estimates baseline odds.

18 . logistic livebirth_c i.poly, or

Logistic regression                             Number of obs     =        942
                                                LR chi2(1)        =       0.00
                                                Prob > chi2       =     0.9735
Log likelihood = -414.63247                     Pseudo R2         =     0.0000

------------------------------------------------------------------------------
 livebirth_c | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        poly |
  With poly |   .9941098   .1767625    -0.03   0.973     .7015883    1.408596
      _cons |   .1914894   .0246334   -12.85   0.000     .1488145    .2464018
------------------------------------------------------------------------------
Note: _cons estimates baseline odds.

19 . * Missing data

20 . misstable summarize amhngml fshmiuml lhmiuml tshmicroiuml bmi ageyears fertilization poly cleavageday3 ma
> tureoocytes positivepregancytest_cc overallfinaloutcome positiveoutcome_cc researchtype_cc
                                                              Obs<.
                                                +------------------------------
                                                | Unique
     Variable |     Obs=.     Obs>.     Obs<. | values        Min        Max
--------------+--------------------------------+------------------------------
      amhngml |       399               543 |    313        .01         24
   tshmicroiuml |        15               927 |    382       .021      10.98
   overallfin~e |       661               281 |      2          0          1
------------------------------------------------------------------------------

21 . mi set mlong

22 . mi register imputed amhngml
   (399 m=0 obs. now marked as incomplete)
```

```
23 . mi misstable summarize, all
```

Variable	Obs=.	Obs>.	Obs<.	Unique values	Min	Max
			Obs<.			
ivfno			942	>500	1397	7218
ageyears			942	24	19	43
fshmiuml			942	388	1.84	14.24
lhmiuml			942	456	.07	24.1
amhngml	399		543	313	.01	24
tshmicroiuml	15		927	382	.021	10.98
t4ngdl	18		924	120	.5	2.88
prolactinn~l	10		932	>500	.45	54.4
ivfcycle	(string variable)					
gonalfdoseiu	(string variable)					
karyotypef~e	(string variable)					
karyotypem~e	(string variable)					
bmi			942	78	14.6	42
spermconce~l			942	256	.001	163
progressiv~y	34		908	80	0	92
nonprogres~y	95		847	34	1	60
normalmorp~y	86		856	70	0	97
noofoocytes			942	41	1	60
matureoocy~s			942	42	1	54
fertilizat~n			942	38	1	50
cleavageday3			942	26	1	34
numberofem~n	5		937	2	1	2
frozenembr~s	6		936	23	0	28
remarksfre~e	(string variable)					
miscarriage	(string variable)					
livebirth	(string variable)					
poly			942	2	1	2
lnamhngml	400		542	313	-4.60517	3.178054
positivepr~c			942	2	0	1
miscarriag~c			942	2	0	1
livebirth_c			942	2	0	1
overallfin~e	661		281	2	0	1
fertilizat~e			942	151	.1428571	1
cleavageda~e			942	99	.1111111	1
positiveou~c			942	2	0	1
researchty~c			942	2	0	1

```
24 . mi impute regress amhngml fshmiuml lhmiuml tshmicroiuml bmi ageyears fertilization poly cleavageday3 matu
> reoocytes positivepregancytest_cc positiveoutcome_cc, add(20) rseed(1234) force
note: positiveoutcome_cc omitted because of collinearity

Univariate imputation                       Imputations =       20
Linear regression                                 added =       20
Imputed: m=1 through m=20                       updated =        0
```

Variable	Observations per *m*			
	Complete	Incomplete	Imputed	Total
amhngml	543	399	386	942

```
(complete + incomplete = total; imputed is the minimum across m
 of the number of filled-in observations.)

Note: Right-hand-side variables (or weights) have missing values;
      model parameters estimated using listwise deletion.

25 . * regression models adjusted for Research Type, age, FSH, LH, BMI

26 . mi estimate, or: logistic positivepregancytest_cc i.poly i.researchtype_cc ageyears fshmiuml lhmiuml bmi
```

```
Multiple-imputation estimates                   Imputations       =         20
Logistic regression                             Number of obs     =        942
                                                Average RVI       =     0.0000
                                                Largest FMI       =     0.0000
DF adjustment:   Large sample                   DF:     min       =          .
                                                        avg       =          .
                                                        max       =          .
Model F test:       Equal FMI                   F(   6,      .)   =       6.29
Within VCE type:          OIM                   Prob > F          =     0.0000
```

positivepregancytest_cc	Odds Ratio	Std. Err.	t	P>|t|	[95% Conf.	Interval]
poly						
With poly	1.050861	.1513789	0.34	0.731	.7923695	1.393679
researchtype_cc						
FRESH IVF CYCLES	.4921949	.0717713	-4.86	0.000	.3698417	.6550257
ageyears	.9653181	.0174784	-1.95	0.051	.9316618	1.00019
fshmiuml	.9255647	.0405241	-1.77	0.077	.8494514	1.008498
lhmiuml	.9937346	.0267311	-0.23	0.815	.9426998	1.047532
bmi	.9780424	.0194001	-1.12	0.263	.9407485	1.016815
_cons	6.317161	5.028039	2.32	0.021	1.327463	30.06226

Note: **_cons** estimates baseline odds.

```
27 . mi estimate, or: logistic miscarriage_c i.poly i.researchtype_cc ageyears fshmiuml lhmiuml bmi

Multiple-imputation estimates                   Imputations       =         20
Logistic regression                             Number of obs     =        942
                                                Average RVI       =     0.0000
                                                Largest FMI       =     0.0000
DF adjustment:   Large sample                   DF:     min       =          .
                                                        avg       =          .
                                                        max       =          .
Model F test:       Equal FMI                   F(   6,      .)   =       2.18
Within VCE type:          OIM                   Prob > F          =     0.0422
```

miscarriage_c	Odds Ratio	Std. Err.	t	P>|t|	[95% Conf.	Interval]
poly						
With poly	1.209159	.2319812	0.99	0.322	.8301897	1.761121
researchtype_cc						
FRESH IVF CYCLES	.6595849	.1276903	-2.15	0.032	.4513204	.9639542
ageyears	.9838958	.023357	-0.68	0.494	.9391655	1.030756
fshmiuml	.9001943	.0535697	-1.77	0.077	.8010914	1.011557
lhmiuml	1.037669	.0345405	1.11	0.267	.9721318	1.107624
bmi	1.020649	.025842	0.81	0.420	.9712361	1.072576
_cons	.3051231	.315622	-1.15	0.251	.0401777	2.317209

Note: **_cons** estimates baseline odds.

```
28 . mi estimate, or: logistic livebirth_c i.poly i.researchtype_cc ageyears fshmiuml lhmiuml bmi

Multiple-imputation estimates                   Imputations       =         20
Logistic regression                             Number of obs     =        942
                                                Average RVI       =     0.0000
                                                Largest FMI       =     0.0000
DF adjustment:   Large sample                   DF:     min       =          .
                                                        avg       =          .
                                                        max       =          .
Model F test:       Equal FMI                   F(   6,      .)   =       5.25
Within VCE type:          OIM                   Prob > F          =     0.0000
```

livebirth_c	Odds Ratio	Std. Err.	t	P>|t|	[95% Conf.	Interval]

poly						
With poly	1.009729	.1830794	0.05	0.957	.7077319	1.440591
researchtype_cc						
FRESH IVF CYCLES	.4606195	.0851973	-4.19	0.000	.3205545	.6618851
ageyears	.9546017	.0216664	-2.05	0.041	.913067	.9980258
fshmiuml	.9740707	.053533	-0.48	0.633	.8746014	1.084853
lhmiuml	.9559692	.0343727	-1.25	0.210	.8909189	1.025769
bmi	.9333299	.0251096	-2.56	0.010	.8853909	.9838645
_cons	10.67543	10.94787	2.31	0.021	1.43041	79.67276

Note: **_cons** estimates baseline odds.

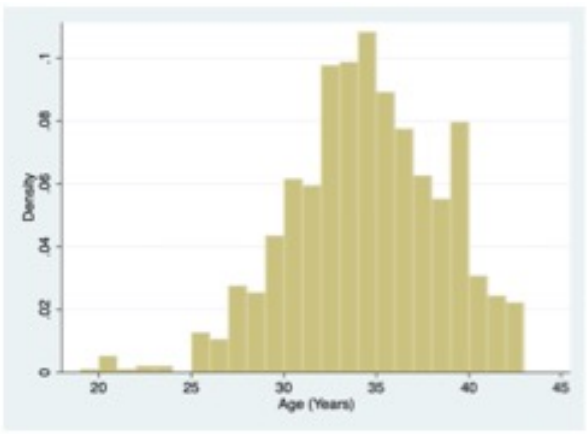
```
29 . **log close

30 .
31 .
```

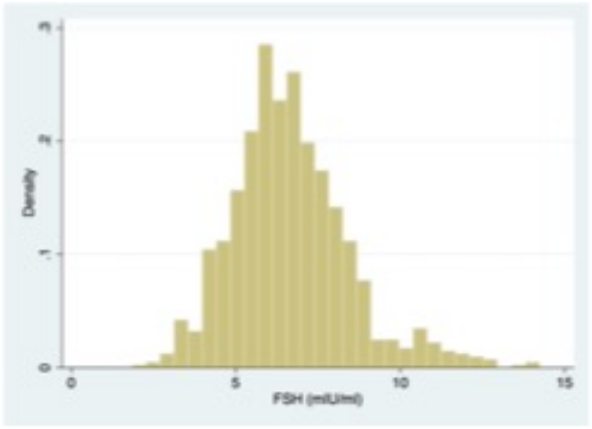
APPENDIX 5: STATA DO-FILE (CHAPTER 3)

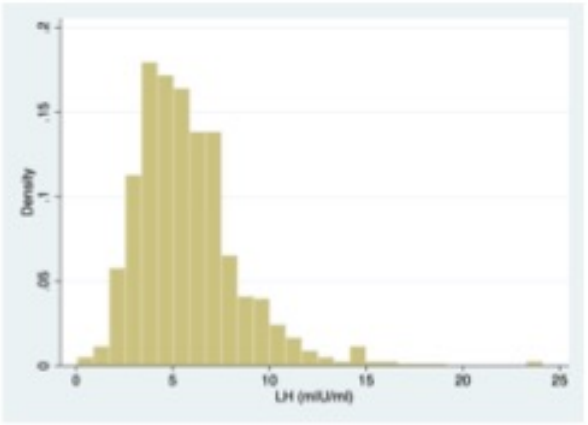

```
1  use "/Users/madararalapanawe/Desktop/Madara final.dta"
2  ***F/M/C outcome
3  tab poly
4  tab researchtype_cc
5  **** outcomes
6  tab positivepregancytest_c
7  tab miscarriage_c
8  tab livebirth_c
9  ******* summary statistics
10 summarize ageyears fshmiuml lhmiuml amhngml tshmicroiuml t4ngdl
    prolactinngml bmi cleavageday3 spermconcentrationmillionml
   progressivemotility nonprogressivemotility normalmorphology,
   detail
11 *** descriptive statistics for continuous outcomes
12 summarize fertilization fertilizationrate matureoocytes
   cleavageday3 cleavageday3rate, detail
13 sort poly
14 ***** Outcomes
15 * crosstabs with outcome
16 tab2 poly positivepregancytest_cc, row chi exact
17 tab2 poly miscarriage_c, row chi exact
18 tab2 poly livebirth_c, row chi exact
19 tab2 poly researchtype_cc, row chi exact
20 * regression models unadjusted
21 logistic positivepregancytest_cc i.poly, or
22 logistic miscarriage_c i.poly, or
23 logistic livebirth_c i.poly, or
24 * Missing data
25 misstable summarize amhngml fshmiuml lhmiuml tshmicroiuml bmi
   ageyears fertilization poly cleavageday3 matureoocytes
   positivepregancytest_cc overallfinaloutcome positiveoutcome_cc
   researchtype_cc
26 mi set mlong
27 mi register imputed amhngml
28 mi misstable summarize, all
29 mi impute regress amhngml fshmiuml lhmiuml tshmicroiuml bmi
   ageyears fertilization poly cleavageday3 matureoocytes
   positivepregancytest_cc positiveoutcome_cc, add(20) rseed(1234)
    force
30 * regression models adjusted for Research Type, age, FSH, LH,
   BMI
31 mi estimate, or: logistic positivepregancytest_cc i.poly i.
   researchtype_cc ageyears fshmiuml lhmiuml bmi
32 mi estimate, or: logistic miscarriage_c i.poly i.
   researchtype_cc ageyears fshmiuml lhmiuml bmi
33 mi estimate, or: logistic livebirth_c i.poly i.researchtype_cc
   ageyears fshmiuml lhmiuml bmi
34 **log close
```

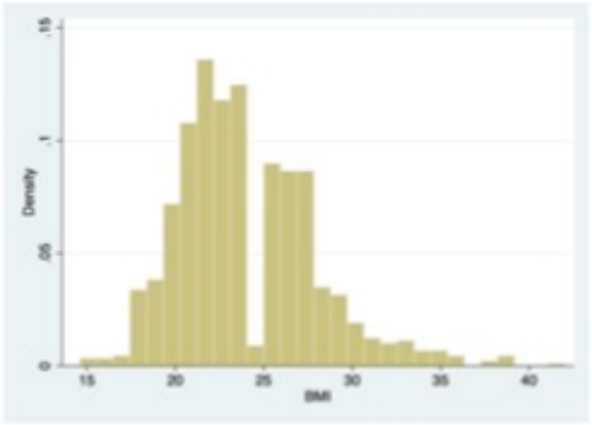
```
1  use "/Users/madararalapanawe/Desktop/Madara final 1.1.dta"
2  ***Overall poly or neither outcome
3  tab poly
4  tab researchtype_cc
5  **** outcomes
6  tab positivepregancytest_c
7  tab miscarriage_c
8  tab livebirth_c
9  sort poly
10 * crosstabs with outcome
11 tab2 poly positivepregancytest_cc, row chi exact
12 tab2 poly miscarriage_c, row chi exact
13 tab2 poly livebirth_c, row chi exact
14 tab2 poly researchtype_cc, row chi exact
15 * regression models unadjusted
16 logistic positivepregancytest_cc i.poly, or
17 logistic miscarriage_c i.poly, or
18 logistic livebirth_c i.poly, or
19 * Missing data
20 misstable summarize amhngml fshmiuml lhmiuml tshmicroiuml bmi
   ageyears fertilization poly cleavageday3 matureoocytes
   positivepregancytest_cc overallfinaloutcome positiveoutcome_cc
   researchtype_cc
21 mi set mlong
22 mi register imputed amhngml
23 mi misstable summarize, all
24 mi impute regress amhngml fshmiuml lhmiuml tshmicroiuml bmi
   ageyears fertilization poly cleavageday3 matureoocytes
   positivepregancytest_cc positiveoutcome_cc, add(20) rseed(1234)
    force
25 * regression models adjusted for Research Type, age, FSH, LH,
   BMI
26 mi estimate, or: logistic positivepregancytest_cc i.poly i.
   researchtype_cc ageyears fshmiuml lhmiuml bmi
27 mi estimate, or: logistic miscarriage_c i.poly i.
   researchtype_cc ageyears fshmiuml lhmiuml bmi
28 mi estimate, or: logistic livebirth_c i.poly i.researchtype_cc
   ageyears fshmiuml lhmiuml bmi
29 **log close
30
```

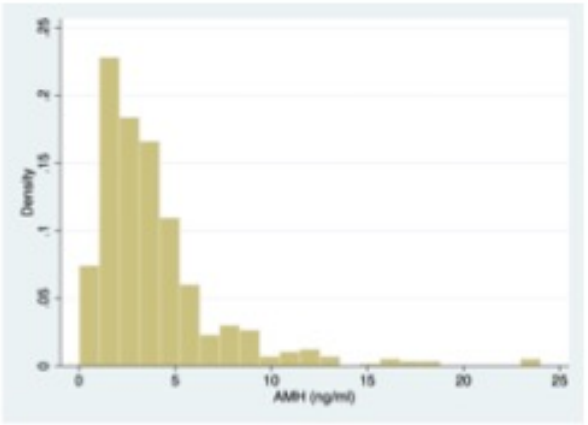
APPENDIX 6: HISTOGRAMS (CHAPTER 3)

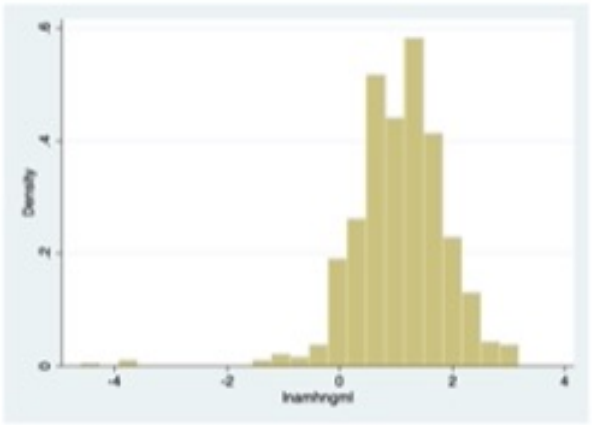


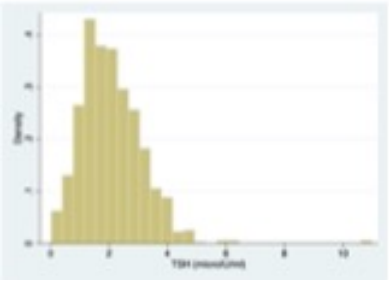


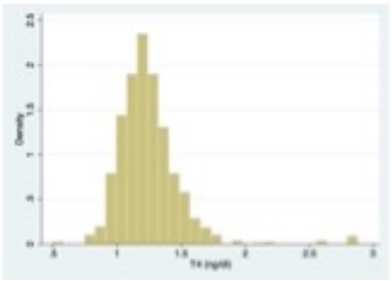


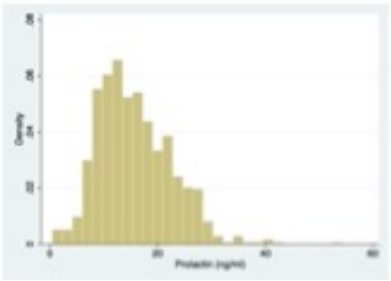


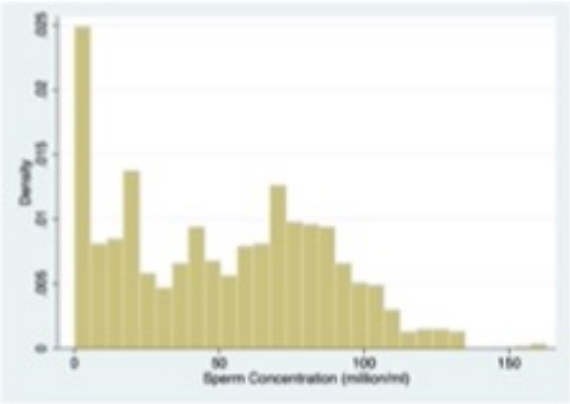


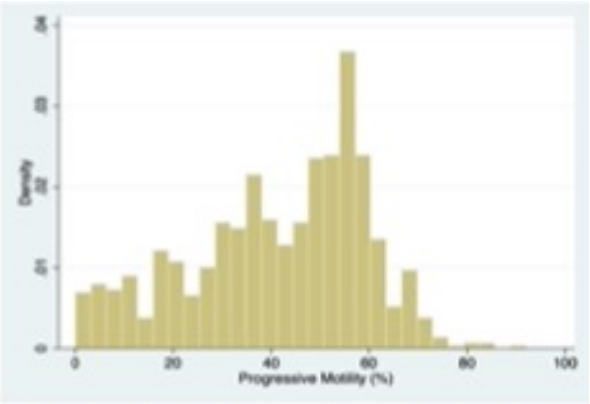


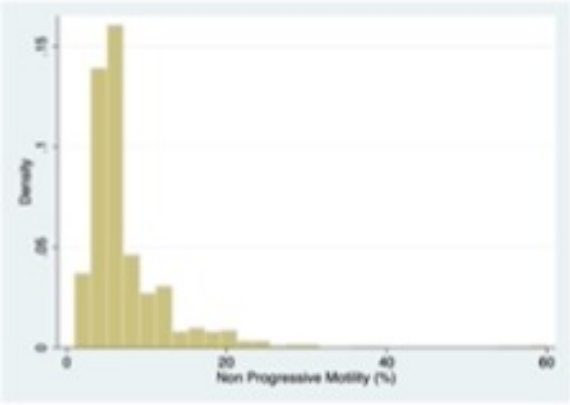


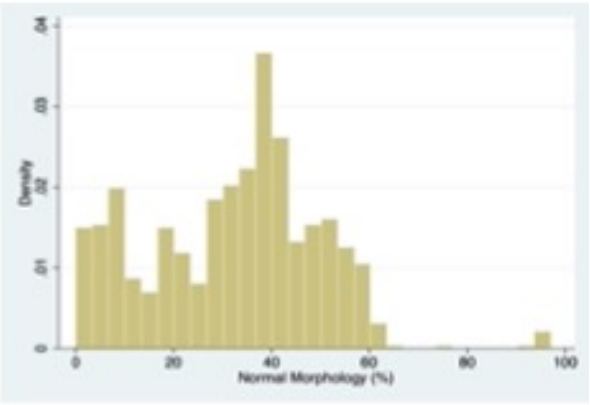


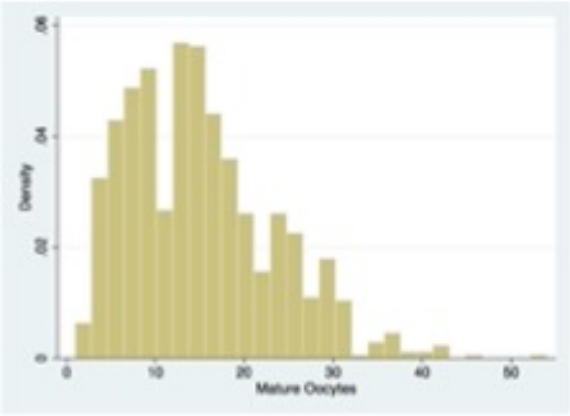


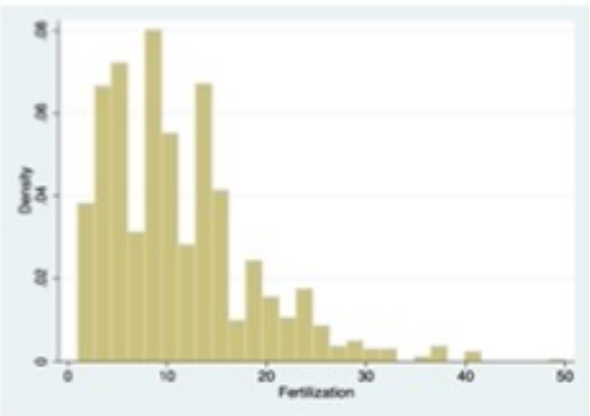

Density
Sperm Concentration (million/ml)

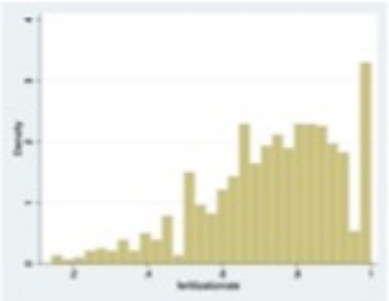

Density
Progressive Motility (%)

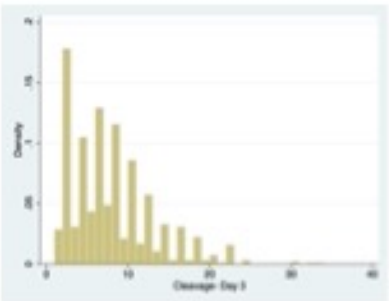

Density
Non Progressive Motility (%)

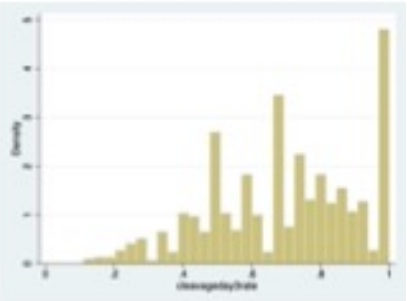

Density
Normal Morphology (%)


Density
Mature Oocytes


Density
Fertilization







APPENDIX 7: STATA FILE OF DATA ANALYSIS (CHAPTER 4)

User: Madara Ralapanawe

```
  ___  ____  ____  ____  ____ (R)
 /__    /   ____/   /   ____/
___/   /   /___/   /   /___/   16.1   Copyright 19
> 85-2019 StataCorp LLC
  Statistics/Data analysis            StataCorp
                                      4905 Lakeway
>  Drive
                                      College Stat
> ion, Texas 77845 USA
                                      800-STATA-PC
>         https://www.stata.com
                                      979-696-4600
>         stata@stata.com
                                      979-696-4601
>  (fax)

Stata license: Single-user , expiring 18 Jul 2021
Serial number: 301609626577
  Licensed to: Madara Ralapanawe
               University of Birmingham

Notes:
      1. Unicode is supported; see help
         unicode_advice.
      2. Your Stata license will expire on 18
         Jul 2021. Update your license.
      3. New update available; type -update all-

1 . use "/Users/madararalapanawe/Desktop/Madara fina
  > l 2.1.dta"

2 . ***Non-acrocentric and acrocentric outcomes

3 . tab poly2
```

Karyotype with Polymorphism	Freq.	Percent	Cum.
No Polymorphism in male or female (Cont	439	47.26	47.26
Only Non acrocentric ch.	48	5.17	52.42
Both Non-acro & Acro	10	1.08	53.50
only Acrocentric ch.	260	27.99	81.49
Male - Yqh	29	3.12	84.61
Couples with Ch. Polymorphism (both Fe	143	15.39	100.00
Total	929	100.00	

```
4 . tab poly2 researchtype_cc
```

Karyotype with Polymorphism	RECODE of researchtype_c (Research Type) FER CYCLE	FRESH IVF	Total
No Polymorphism in ma	191	248	439
Only Non acrocentric	20	28	48
Both Non-acro & Acro	4	6	10
only Acrocentric ch.	109	151	260
Male - Yqh	8	21	29
Couples with Ch. Pol	57	86	143
Total	389	540	929

```
5 . ******* summary statistics

6 . summarize ageyears fshmiuml lhmiuml amhngml tshmicroiuml t4ngdl prolactinngml bmi cleavageday3 spermconce
  > ntrationmillionml progressivemotility nonprogressivemotility normalmorphology, detail

                        Age (Years)
```

	Percentiles	Smallest		
1%	23	19		
5%	27	20		
10%	29	20	Obs	929
25%	31	20	Sum of Wgt.	929
50%	34		Mean	33.93757
		Largest	Std. Dev.	4.133596
75%	37	43		
90%	39	43	Variance	17.08662
95%	40	43	Skewness	-.3091794
99%	43	43	Kurtosis	3.21133

FSH (mIU/ml)

	Percentiles	Smallest		
1%	3.24	1.84		
5%	4.12	2.55		
10%	4.53	2.59	Obs	929
25%	5.47	2.8	Sum of Wgt.	929
50%	6.5		Mean	6.667901
		Largest	Std. Dev.	1.795731
75%	7.7	12.63		
90%	8.85	13.39	Variance	3.224648
95%	10.24	14.18	Skewness	.7790314
99%	12.23	14.24	Kurtosis	4.310105

LH (mIU/ml)

	Percentiles	Smallest		
1%	1.38	.07		
5%	2.41	.64		
10%	3	.64	Obs	929
25%	3.91	.81	Sum of Wgt.	929
50%	5.3		Mean	5.802831
		Largest	Std. Dev.	2.743695
75%	7.04	18.16		
90%	9.19	18.3	Variance	7.527861
95%	10.61	24.1	Skewness	1.668859
99%	14.65	24.1	Kurtosis	8.748494

AMH (ng/ml)

	Percentiles	Smallest		
1%	.3	.01		
5%	.87	.02		
10%	1.14	.02	Obs	537
25%	1.88	.26	Sum of Wgt.	537
50%	3.06		Mean	3.93157
		Largest	Std. Dev.	3.326232
75%	4.73	18.17		
90%	7.99	23.8	Variance	11.06382
95%	10.7	23.8	Skewness	2.567915
99%	17.33	24	Kurtosis	12.28286

TSH (microIU/ml)

	Percentiles	Smallest		
1%	.2	.021		
5%	.666	.037		
10%	.933	.113	Obs	914
25%	1.35	.129	Sum of Wgt.	914
50%	1.97		Mean	2.103664
		Largest	Std. Dev.	1.078511

75%	2.75	6.15		
90%	3.43	6.25	Variance	1.163186
95%	3.87	10.98	Skewness	1.655753
99%	4.83	10.98	Kurtosis	12.53879

T4 (ng/dl)

	Percentiles	Smallest		
1%	.82	.5		
5%	.947	.5		
10%	1	.793	Obs	911
25%	1.1	.793	Sum of Wgt.	911
50%	1.21		Mean	1.245299
		Largest	Std. Dev.	.2555726
75%	1.35	2.87		
90%	1.5	2.87	Variance	.0653174
95%	1.6	2.88	Skewness	2.632853
99%	2.56	2.88	Kurtosis	16.50149

Prolactin (ng/ml)

	Percentiles	Smallest		
1%	2.79	.45		
5%	6.44	.46		
10%	7.97	.46	Obs	919
25%	10.6	1.25	Sum of Wgt.	919
50%	14.72		Mean	15.73267
		Largest	Std. Dev.	6.83622
75%	20.3	40.9		
90%	24.81	40.9	Variance	46.7339
95%	27.41	41.4	Skewness	.7536616
99%	34.81	54.4	Kurtosis	4.231626

BMI

	Percentiles	Smallest		
1%	17	14.6		
5%	19	15		
10%	20	15.4	Obs	929
25%	21	15.8	Sum of Wgt.	929
50%	23.8		Mean	24.00805
		Largest	Std. Dev.	3.782752
75%	26	38.4		
90%	29	38.4	Variance	14.30921
95%	31	39	Skewness	.9412976
99%	35.8	42	Kurtosis	4.746414

Cleavage- Day 3

	Percentiles	Smallest		
1%	1	1		
5%	2	1		
10%	2	1	Obs	929
25%	4	1	Sum of Wgt.	929
50%	6		Mean	7.49085
		Largest	Std. Dev.	5.144483
75%	10	30		
90%	14	30	Variance	26.4657
95%	18	32	Skewness	1.239328
99%	22	34	Kurtosis	5.028253

Sperm Concentration (million/ml)

	Percentiles	Smallest
1%	.001	.001

5%	.5	.001		
10%	2.5	.001	Obs	929
25%	17.5	.001	Sum of Wgt.	929
50%	51.5		Mean	50.79188
		Largest	Std. Dev.	35.85426
75%	80	134		
90%	96	153	Variance	1285.528
95%	108	163	Skewness	.2308091
99%	132	163	Kurtosis	2.098486

Progressive Motility (%)

	Percentiles	Smallest		
1%	2	0		
5%	7	1		
10%	12	1	Obs	895
25%	29	1	Sum of Wgt.	895
50%	45		Mean	41.28827
		Largest	Std. Dev.	18.19541
75%	55	81		
90%	61	85	Variance	331.0728
95%	67	85	Skewness	-.4380593
99%	73	92	Kurtosis	2.425359

Non Progressive Motility (%)

	Percentiles	Smallest		
1%	1	1		
5%	3	1		
10%	4	1	Obs	835
25%	5	1	Sum of Wgt.	835
50%	6		Mean	7.900599
		Largest	Std. Dev.	6.011255
75%	8	54		
90%	14	56	Variance	36.13519
95%	18	60	Skewness	3.911229
99%	30	60	Kurtosis	26.89487

Normal Morphology (%)

	Percentiles	Smallest		
1%	1	0		
5%	3	1		
10%	7	1	Obs	844
25%	19	1	Sum of Wgt.	844
50%	34		Mean	32.13389
		Largest	Std. Dev.	17.27531
75%	43	95		
90%	53	95	Variance	298.4364
95%	57	96	Skewness	.0525877
99%	62	97	Kurtosis	3.00701

```
7 . sort poly2

8 . ***** Outcomes

9 . tab2 poly2 positivepregancytest_cc, row chi exact
```

-> tabulation of poly2 by positivepregancytest_cc

Key
frequency *row percentage*

```
Enumerating sample-space combinations:
stage 6:  enumerations = 1
stage 5:  enumerations = 5
stage 4:  enumerations = 40
stage 3:  enumerations = 348
stage 2:  enumerations = 4125
stage 1:  enumerations = 0
```

Karyotype with Polymorphism	Positive pregnancy test No	yes	Total
No Polymorphism in ma	310 70.62	129 29.38	439 100.00
Only Non acrocentric	33 68.75	15 31.25	48 100.00
Both Non-acro & Acro	9 90.00	1 10.00	10 100.00
only Acrocentric ch.	183 70.38	77 29.62	260 100.00
Male - Yqh	21 72.41	8 27.59	29 100.00
Couples with Ch. Pol	92 64.34	51 35.66	143 100.00
Total	648 69.75	281 30.25	929 100.00

```
          Pearson chi2(5) =   4.2561   Pr = 0.513
           Fisher's exact =                 0.549
```

```
10 . tab2 poly2 miscarriage_cc, row chi exact

-> tabulation of poly2 by miscarriage_cc
```

Key
frequency *row percentage*

```
Enumerating sample-space combinations:
stage 6:  enumerations = 1
stage 5:  enumerations = 4
stage 4:  enumerations = 18
stage 3:  enumerations = 89
stage 2:  enumerations = 630
stage 1:  enumerations = 0
```

Karyotype with Polymorphism	Miscarriage No	yes	Total
No Polymorphism in ma	382 87.02	57 12.98	439 100.00
Only Non acrocentric	42 87.50	6 12.50	48 100.00
Both Non-acro & Acro	9 90.00	1 10.00	10 100.00

only Acrocentric ch.	223 85.77	37 14.23	260 100.00
Male - Yqh	26 89.66	3 10.34	29 100.00
Couples with Ch. Pol	117 81.82	26 18.18	143 100.00
Total	799 86.01	130 13.99	929 100.00

Pearson chi2(5) = 3.0104 Pr = 0.698
Fisher's exact = 0.743

```
11 . tab2 poly2 livebirth_cc, row chi exact
```

-> tabulation of poly2 by livebirth_cc

Key
frequency *row percentage*

```
Enumerating sample-space combinations:
stage 6:  enumerations = 1
stage 5:  enumerations = 4
stage 4:  enumerations = 18
stage 3:  enumerations = 79
stage 2:  enumerations = 501
stage 1:  enumerations = 0
```

Karyotype with Polymorphism	Live birth No	yes	Total
No Polymorphism in ma	367 83.60	72 16.40	439 100.00
Only Non acrocentric	39 81.25	9 18.75	48 100.00
Both Non-acro & Acro	10 100.00	0 0.00	10 100.00
only Acrocentric ch.	220 84.62	40 15.38	260 100.00
Male - Yqh	24 82.76	5 17.24	29 100.00
Couples with Ch. Pol	118 82.52	25 17.48	143 100.00
Total	778 83.75	151 16.25	929 100.00

Pearson chi2(5) = 2.4912 Pr = 0.778
Fisher's exact = 0.827

```
12 . tab2 poly2 researchtype_cc, row chi exact
```

-> tabulation of poly2 by researchtype_cc

Key
frequency

row percentage

```
Enumerating sample-space combinations:
stage 6:  enumerations = 1
stage 5:  enumerations = 5
stage 4:  enumerations = 39
stage 3:  enumerations = 312
stage 2:  enumerations = 3515
stage 1:  enumerations = 0
```

Karyotype with Polymorphism	RECODE of researchtype_c (Research Type) FER CYCLE	FRESH IVF	Total
No Polymorphism in ma	191	248	439
	43.51	56.49	100.00
Only Non acrocentric	20	28	48
	41.67	58.33	100.00
Both Non-acro & Acro	4	6	10
	40.00	60.00	100.00
only Acrocentric ch.	109	151	260
	41.92	58.08	100.00
Male - Yqh	8	21	29
	27.59	72.41	100.00
Couples with Ch. Pol	57	86	143
	39.86	60.14	100.00
Total	389	540	929
	41.87	58.13	100.00

```
          Pearson chi2(5) =   3.1677   Pr = 0.674
           Fisher's exact =                0.679

13 . **Regression models unadjusted

14 . logistic positivepregancytest_cc i.poly2, or

Logistic regression                             Number of obs     =        929
                                                LR chi2(5)        =       4.63
                                                Prob > chi2       =     0.4627
Log likelihood = -567.11345                     Pseudo R2         =     0.0041
```

positivepregancytest_cc	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
poly2						
Only Non acrocentric ch.	1.092319	.3588851	0.27	0.788	.5736989	2.079767
Both Non-acro & Acro	.2670112	.2828415	-1.25	0.213	.033486	2.129101
only Acrocentric ch.	1.011141	.1734607	0.06	0.949	.7224177	1.415255
Male - Yqh	.915467	.3922611	-0.21	0.837	.3952921	2.120153
Couples with Ch. Polymorphism (both..)	1.332154	.2712337	1.41	0.159	.8938098	1.985471
_cons	.416129	.0435998	-8.37	0.000	.3388782	.51099

```
Note: _cons estimates baseline odds.

15 . logistic miscarriage_cc i.poly2, or

Logistic regression                             Number of obs     =        929
                                                LR chi2(5)        =       2.91
                                                Prob > chi2       =     0.7140
Log likelihood = -374.64756                     Pseudo R2         =     0.0039
```

miscarriage_cc	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
poly2						
Only Non acrocentric ch.	.9573935	.4393986	-0.09	0.924	.3894296	2.353705
Both Non-acro & Acro	.7446397	.7920084	-0.28	0.782	.0925971	5.988184
only Acrocentric ch.	1.11195	.2527651	0.47	0.641	.712185	1.736113
Male - Yqh	.7732795	.4841227	-0.41	0.681	.2266886	2.637809
Couples with Ch. Polymorphism (both..)	1.489279	.3859796	1.54	0.124	.8961243	2.475049
_cons	.1492147	.0211873	-13.40	0.000	.112966	.1970948

Note: **_cons** estimates baseline odds.

```
16 . logistic livebirth_cc i.poly2, or
note: 3.poly2 != 0 predicts failure perfectly
      3.poly2 dropped and 10 obs not used

Logistic regression                             Number of obs     =        919
                                                LR chi2(4)        =       0.52
                                                Prob > chi2       =     0.9716
Log likelihood = -410.30039                     Pseudo R2         =     0.0006
```

livebirth_cc	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
poly2						
Only Non acrocentric ch.	1.176282	.460655	0.41	0.678	.5459644	2.534303
Both Non-acro & Acro	1	(empty)				
only Acrocentric ch.	.9267677	.1991131	-0.35	0.723	.6082652	1.412046
Male - Yqh	1.061921	.5396821	0.12	0.906	.3921938	2.875305
Couples with Ch. Polymorphism (both..)	1.07992	.2755135	0.30	0.763	.6549824	1.780547
_cons	.1961853	.0252871	-12.64	0.000	.1523883	.2525697

Note: **_cons** estimates baseline odds.

```
17 . * Missing data

18 . misstable summarize amhngml fshmiuml lhmiuml tshmicroiuml bmi ageyears fertilization poly cleavageday3 ma
> tureoocytes positivepregancytest_cc positiveoutcome_cc researchtype_cc
```

			Obs<.			
Variable	Obs=.	Obs>.	Obs<.	Unique values	Min	Max
amhngml	392		537	313	.01	24
tshmicroiuml	15		914	381	.021	10.98

```
19 . mi set mlong

20 . mi register imputed amhngml
(392 m=0 obs. now marked as incomplete)

21 . mi misstable summarize, all
```

			Obs<.			
Variable	Obs=.	Obs>.	Obs<.	Unique values	Min	Max
ivfno			929	>500	1397	7218
ageyears			929	24	19	43
fshmiuml			929	387	1.84	14.24
lhmiuml			929	453	.07	24.1
amhngml	392		537	313	.01	24
tshmicroiuml	15		914	381	.021	10.98

t4ngdl	18	911	120	.5	2.88
prolactinn~l	10	919	>500	.45	54.4
ivfcycle	(string variable)				
gonalfdoseiu	(string variable)				
karyotypef~e	(string variable)				
karyotypem~e	(string variable)				
presenceof~m	(string variable)				
onlynonacr~h	(string variable)				
bothnonacr~o	(string variable)				
onlyacroce~h	(string variable)				
maleyqh	(string variable)				
nopolymorp~l	(string variable)				
coupleswit~m	(string variable)				
bmi		929	78	14.6	42
spermconce~l		929	256	.001	163
progressiv~y	34	895	80	0	92
nonprogres~y	94	835	34	1	60
normalmorp~y	85	844	70	0	97
noofoocytes		929	41	1	60
matureoocy~s		929	42	1	54
fertilizat~n		929	38	1	50
cleavageday3		929	26	1	34
numberofem~n	3	926	2	1	2
frozenembr~s	6	923	23	0	28
remarksfre~e	(string variable)				
miscarriage	(string variable)				
livebirth	(string variable)				
poly		929	4	1	4
lnamhngml	392	537	313	-4.60517	3.178054
positivepr~c		929	2	0	1
miscarria~_c		929	2	1	2
miscarria~cc		929	2	0	1
livebirth_c		929	2	1	2
livebirth_cc		929	2	0	1
misscarria~e		929	2	0	1
fertilizat~e		929	151	.1428571	1
cleavageda~e		929	99	.1111111	1
positiveou~c		929	2	0	1
researchty~c		929	2	0	1
presenceof~c		929	2	1	2
onlynonacr~c		929	2	1	2
bothnonacr~c		929	2	1	2
onlyacroce~c		929	2	1	2
maleyqh_c		929	2	1	2
nopolymorp~c		929	2	1	2
coupleswit~c		929	2	1	2
poly2		929	6	1	6

```
22 . mi impute regress amhngml fshmiuml lhmiuml tshmicroiuml bmi ageyears fertilization poly cleavageday3 matu
> reoocytes positivepregancytest_cc positiveoutcome_cc, add(20) rseed(1234) force
note: positiveoutcome_cc omitted because of collinearity

Univariate imputation                       Imputations =       20
Linear regression                                 added =       20
Imputed: m=1 through m=20                       updated =        0
```

	Observations per *m*			
Variable	Complete	Incomplete	Imputed	Total
amhngml	537	392	379	929

```
(complete + incomplete = total; imputed is the minimum across m
 of the number of filled-in observations.)

Note: Right-hand-side variables (or weights) have missing values;
      model parameters estimated using listwise deletion.
```

```
23 . **Regression models adjusted for age, FSH, LH, BMI, Research type

24 . mi estimate, or: logistic positivepregancytest_cc i.poly2 i.researchtype_cc ageyears fshmiuml lhmiuml bmi

Multiple-imputation estimates                   Imputations       =         20
Logistic regression                             Number of obs     =        929
                                                Average RVI       =     0.0000
                                                Largest FMI       =     0.0000
DF adjustment:   Large sample                   DF:     min       =   1.82e+64
                                                        avg       =   4.32e+67
                                                        max       =          .
Model F test:       Equal FMI                   F(  10, 1.5e+67)  =       4.40
Within VCE type:          OIM                   Prob > F          =     0.0000
```

positivepregancytest_cc	Odds Ratio	Std. Err.	t	P>|t|	[95% Conf.	Interval]
poly2						
Only Non acrocentric ch.	1.058974	.3568079	0.17	0.865	.5471211	2.049684
Both Non-acro & Acro	.2296452	.2470826	-1.37	0.172	.0278755	1.891875
only Acrocentric ch.	1.019512	.1792412	0.11	0.912	.7223407	1.438941
Male - Yqh	1.087155	.4755985	0.19	0.849	.4612281	2.56252
Couples with Ch. Polymorphism (both..)	1.369458	.2873042	1.50	0.134	.907761	2.065978
researchtype_cc						
FRESH IVF CYCLES	.4737294	.0705402	-5.02	0.000	.3538206	.6342749
ageyears	.9623659	.0177503	-2.08	0.038	.9281972	.9977924
fshmiuml	.9220045	.0413249	-1.81	0.070	.8444648	1.006664
lhmiuml	.9964066	.027297	-0.13	0.895	.9443164	1.05137
bmi	.971799	.019719	-1.41	0.159	.933909	1.011226
_cons	7.814961	6.35188	2.53	0.011	1.588852	38.43884

Note: **_cons** estimates baseline odds.

```
25 . mi estimate, or: logistic miscarriage_cc i.poly2 i.researchtype_cc ageyears fshmiuml lhmiuml bmi

Multiple-imputation estimates                   Imputations       =         20
Logistic regression                             Number of obs     =        929
                                                Average RVI       =     0.0000
                                                Largest FMI       =     0.0000
DF adjustment:   Large sample                   DF:     min       =   7.75e+63
                                                        avg       =   1.03e+68
                                                        max       =          .
Model F test:       Equal FMI                   F(  10, 6.9e+66)  =       1.52
Within VCE type:          OIM                   Prob > F          =     0.1265
```

miscarriage_cc	Odds Ratio	Std. Err.	t	P>|t|	[95% Conf.	Interval]
poly2						
Only Non acrocentric ch.	.9442488	.4368056	-0.12	0.901	.3813503	2.338023
Both Non-acro & Acro	.7065256	.7572535	-0.32	0.746	.0864591	5.773577
only Acrocentric ch.	1.127585	.2588525	0.52	0.601	.7190254	1.768293
Male - Yqh	.8234252	.5199158	-0.31	0.758	.2388748	2.838429
Couples with Ch. Polymorphism (both..)	1.52055	.3987534	1.60	0.110	.9094509	2.542273
researchtype_cc						
FRESH IVF CYCLES	.6604446	.1282607	-2.14	0.033	.4513672	.9663685
ageyears	.9860896	.0233963	-0.59	0.555	.9412836	1.033029
fshmiuml	.8978821	.053182	-1.82	0.069	.7994701	1.008408
lhmiuml	1.040465	.0348355	1.18	0.236	.9743801	1.111031
bmi	1.020253	.0256352	0.80	0.425	.9712257	1.071754
_cons	.2927886	.3023426	-1.19	0.234	.0386881	2.215804

Note: **_cons** estimates baseline odds.

```
26 . mi estimate, or: logistic livebirth_cc i.poly2 i.researchtype_cc ageyears fshmiuml lhmiuml bmi
```

```
Multiple-imputation estimates                 Imputations       =         20
Logistic regression                           Number of obs     =        919
                                              Average RVI       =     0.0000
                                              Largest FMI       =     0.0000
DF adjustment:   Large sample                 DF:     min       =          .
                                                      avg       =          .
                                                      max       =          .
Model F test:       Equal FMI                 F(   9,      .)   =       3.55
Within VCE type:          OIM                 Prob > F          =     0.0002
```

livebirth_cc	Odds Ratio	Std. Err.	t	P>|t|	[95% Conf.	Interval]
poly2						
Only Non acrocentric ch.	1.160007	.4628964	0.37	0.710	.5306303	2.535884
Both Non-acro & Acro	1	(omitted)				
only Acrocentric ch.	.936878	.20526	-0.30	0.766	.609809	1.439369
Male - Yqh	1.353085	.7020896	0.58	0.560	.4893878	3.74108
Couples with Ch. Polymorphism (both..)	1.092552	.2856244	0.34	0.735	.6545063	1.823772
researchtype_cc						
FRESH IVF CYCLES	.4568238	.0848436	-4.22	0.000	.3174383	.6574127
ageyears	.9544739	.021709	-2.05	0.040	.9128595	.9979855
fshmiuml	.970885	.0539154	-0.53	0.595	.8707604	1.082522
lhmiuml	.9546966	.0344583	-1.28	0.199	.8894932	1.02468
bmi	.9345468	.0251415	-2.52	0.012	.8865469	.9851454
_cons	10.97245	11.275	2.33	0.020	1.464306	82.21964

Note: **_cons** estimates baseline odds.

```
27 . **close log

28 .
```

```
  ___  ____  ____  ____  ____ (R)
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Notes:
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          unicode_advice.
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          Jul 2021. Update your license.
      3. New update available; type -update all-

1 . use "/Users/madararalapanawe/Desktop/Madara fina
  > l 2.pk4.dta"

2 . ***Overall outcomes

3 . tab poly2
```

Karyotype with Polymorphism	Freq.	Percent	Cum.
No Polymorphism in male or female (Cont	439	47.26	47.26
With polymorphism	490	52.74	100.00
Total	929	100.00	

```
4 . tab poly2 researchtype_cc
```

Karyotype with Polymorphism	RECODE of researchtype_c (Research Type)		Total
	PER CYCLE	FRESH IVF	
No Polymorphism in ma	191	248	439
With polymorphism	198	292	490
Total	389	540	929

```
5 . sort poly2

6 . ***** Outcomes

7 . tab2 poly2 positivepregancytest_cc, row chi exact

  -> tabulation of poly2 by positivepregancytest_cc
```

Key
frequency *row percentage*

Karyotype with Polymorphism	Positive pregnancy test No	yes	Total
No Polymorphism in ma	310	129	439
	70.62	29.38	100.00
With polymorphism	338	152	490
	68.98	31.02	100.00
Total	648	281	929
	69.75	30.25	100.00

Pearson chi2(1) = 0.2935 Pr = 0.588
Fisher's exact = 0.617
1-sided Fisher's exact = 0.319

```
8 . tab2 poly2 miscarriage_cc, row chi exact
```

-> tabulation of poly2 by miscarriage_cc

Key
frequency *row percentage*

Karyotype with Polymorphism	Miscarriage No	yes	Total
No Polymorphism in ma	382	57	439
	87.02	12.98	100.00
With polymorphism	417	73	490
	85.10	14.90	100.00
Total	799	130	929
	86.01	13.99	100.00

Pearson chi2(1) = 0.7047 Pr = 0.401
Fisher's exact = 0.449
1-sided Fisher's exact = 0.228

```
9 . tab2 poly2 livebirth_cc, row chi exact
```

-> tabulation of poly2 by livebirth_cc

Key
frequency *row percentage*

Karyotype with Polymorphism	Live birth No	yes	Total
No Polymorphism in ma	367	72	439
	83.60	16.40	100.00
With polymorphism	411	79	490
	83.88	16.12	100.00
Total	778	151	929
	83.75	16.25	100.00

Pearson chi2(1) = 0.0132 Pr = 0.909

```
          Fisher's exact =                 0.929
    1-sided Fisher's exact =               0.489

10 . tab2 poly2 researchtype_cc, row chi exact

-> tabulation of poly2 by researchtype_cc
```

Key
frequency *row percentage*

Karyotype with Polymorphism	RECODE of researchtype_c (Research Type) FER CYCLE	FRESH IVF	Total
No Polymorphism in ma	191 43.51	248 56.49	439 100.00
With polymorphism	198 40.41	292 59.59	490 100.00
Total	389 41.87	540 58.13	929 100.00

```
          Pearson chi2(1) =   0.9141   Pr = 0.339
          Fisher's exact =                 0.352
    1-sided Fisher's exact =               0.187

11 . **Regression models unadjusted

12 . logistic positivepregancytest_cc i.poly2, or

Logistic regression                             Number of obs     =        929
                                                LR chi2(1)        =       0.29
                                                Prob > chi2       =     0.5878
Log likelihood = -569.28132                     Pseudo R2         =     0.0003
```

positivepregancytest_cc	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
poly2						
With polymorphism	1.080684	.1547881	0.54	0.588	.8161688	1.430928
_cons	.416129	.0435998	-8.37	0.000	.3388782	.51099

```
Note: _cons estimates baseline odds.

13 . logistic miscarriage_cc i.poly2, or

Logistic regression                             Number of obs     =        929
                                                LR chi2(1)        =       0.71
                                                Prob > chi2       =     0.4005
Log likelihood =  -375.7488                     Pseudo R2         =     0.0009
```

miscarriage_cc	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
poly2						
With polymorphism	1.173209	.2233981	0.84	0.402	.8077792	1.703954
_cons	.1492147	.0211873	-13.40	0.000	.112966	.1970948

```
Note: _cons estimates baseline odds.

14 . logistic livebirth_cc i.poly2, or

Logistic regression                             Number of obs     =        929
```

```
                                                LR chi2(1)      =       0.01
                                                Prob > chi2     =     0.9086
Log likelihood = -412.33793                     Pseudo R2       =     0.0000
```

livebirth_cc	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
poly2						
With polymorphism	.979758	.1744547	-0.11	0.909	.6911221	1.388938
_cons	.1961853	.0252871	-12.64	0.000	.1523883	.2525697

Note: _cons estimates baseline odds.

```
15 . * Missing data

16 . misstable summarize amhngml fshmiuml lhmiuml tshmicroiuml bmi ageyears fertilization poly cleavageday3 ma
   > tureoocytes positivepregancytest_cc positiveoutcome_cc researchtype_cc
```

Variable	Obs=.	Obs>.	Obs<.	Unique values	Min	Max
amhngml	392		537	313	.01	24
tshmicroiuml	15		914	381	.021	10.98

```
17 . mi set mlong

18 . mi register imputed amhngml
   (392 m=0 obs. now marked as incomplete)

19 . mi misstable summarize, all
```

Variable	Obs=.	Obs>.	Obs<.	Unique values	Min	Max
ivfno			929	>500	1397	7218
ageyears			929	24	19	43
fshmiuml			929	387	1.84	14.24
lhmiuml			929	453	.07	24.1
amhngml	392		537	313	.01	24
tshmicroiuml	15		914	381	.021	10.98
t4ngdl	18		911	120	.5	2.88
prolactinn~l	10		919	>500	.45	54.4
ivfcycle	(string variable)					
gonalfdoseiu	(string variable)					
karyotypef~e	(string variable)					
karyotypem~e	(string variable)					
presenceof~m	(string variable)					
onlynonacr~h	(string variable)					
bothnonacr~o	(string variable)					
onlyacroce~h	(string variable)					
maleyqh	(string variable)					
nopolymorp~l	(string variable)					
coupleswit~m	(string variable)					
bmi			929	78	14.6	42
spermconce~l			929	256	.001	163
progressiv~y	34		895	80	0	92
nonprogres~y	94		835	34	1	60
normalmorp~y	85		844	70	0	97
noofoocytes			929	41	1	60
matureoocy~s			929	42	1	54
fertilizat~n			929	38	1	50
cleavageday3			929	26	1	34
numberofem~n	3		926	2	1	2
frozenembr~s	6		923	23	0	28
remarksfre~e	(string variable)					
miscarriage	(string variable)					

livebirth	(string variable)				
poly		929	4	1	4
lnamhngml	392	537	313	-4.60517	3.178054
positivepr~c		929	2	0	1
miscarria~_c		929	2	1	2
miscarria~cc		929	2	0	1
livebirth_c		929	2	1	2
livebirth_cc		929	2	0	1
misscarria~e		929	2	0	1
fertilizat~e		929	151	.1428571	1
cleavageda~e		929	99	.1111111	1
positiveou~c		929	2	0	1
researchty~c		929	2	0	1
presenceof~c		929	2	1	2
onlynonacr~c		929	2	1	2
bothnonacr~c		929	2	1	2
onlyacroce~c		929	2	1	2
maleyqh_c		929	2	1	2
nopolymorp~c		929	2	1	2
coupleswit~c		929	2	1	2
poly2		929	2	1	2

```
20 . mi impute regress amhngml fshmiuml lhmiuml tshmicroiuml bmi ageyears fertilization poly cleavageday3 matu
   > reoocytes positivepregancytest_cc positiveoutcome_cc, add(20) rseed(1234) force
   note: positiveoutcome_cc omitted because of collinearity

   Univariate imputation                       Imputations =       20
   Linear regression                                 added =       20
   Imputed: m=1 through m=20                       updated =        0
```

	Observations per *m*			
Variable	Complete	Incomplete	Imputed	Total
amhngml	537	392	379	929

```
   (complete + incomplete = total; imputed is the minimum across m
    of the number of filled-in observations.)

   Note: Right-hand-side variables (or weights) have missing values;
         model parameters estimated using listwise deletion.

21 . **Regression models adjusted for age, FSH, LH, BMI, Research type

22 . mi estimate, or: logistic positivepregancytest_cc i.poly2 i.researchtype_cc ageyears fshmiuml lhmiuml bmi

   Multiple-imputation estimates                 Imputations       =         20
   Logistic regression                           Number of obs     =        929
                                                 Average RVI       =     0.0000
                                                 Largest FMI       =     0.0000
   DF adjustment:   Large sample                 DF:     min       =          .
                                                         avg       =          .
                                                         max       =          .
   Model F test:       Equal FMI                 F(   6,      .)   =       6.73
   Within VCE type:          OIM                 Prob > F          =     0.0000
```

positivepregancytest_cc	Odds Ratio	Std. Err.	t	P>|t|	[95% Conf.	Interval]
poly2						
With polymorphism	1.098142	.1611915	0.64	0.524	.8235959	1.464207
researchtype_cc						
FRESH IVF CYCLES	.4765412	.0706855	-5.00	0.000	.356321	.6373228
ageyears	.9621138	.0176363	-2.11	0.035	.9281608	.9973088
fshmiuml	.9255467	.0411632	-1.74	0.082	.8482846	1.009846
lhmiuml	.9953052	.0271134	-0.17	0.863	.9435577	1.049891

```
                bmi |   .9731622   .0197354    -1.34   0.180     .9352401    1.012622
              _cons |   7.462732   6.040276     2.48   0.013     1.527363    36.4631
------------------------------------------------------------------------------

Note: _cons estimates baseline odds.

23 . mi estimate, or: logistic miscarriage_cc i.poly2 i.researchtype_cc ageyears fshmiuml lhmiuml bmi

Multiple-imputation estimates                   Imputations       =         20
Logistic regression                             Number of obs     =        929
                                                Average RVI       =     0.0000
                                                Largest FMI       =     0.0000
DF adjustment:   Large sample                   DF:     min       =          .
                                                        avg       =          .
                                                        max       =          .
Model F test:       Equal FMI                   F(   6,      .)   =       2.15
Within VCE type:          OIM                   Prob > F          =     0.0444

------------------------------------------------------------------------------
 miscarriage_cc | Odds Ratio   Std. Err.      t    P>|t|     [95% Conf. Interval]
----------------+-------------------------------------------------------------
          poly2 |
With polymorphism |   1.190818   .2287422     0.91   0.363     .8172209    1.735207
                |
  researchtype_cc |
 FRESH IVF CYCLES |   .6594047   .1277585    -2.15   0.032     .4510589    .9639862
        ageyears |   .9846405    .023287    -0.65   0.513     .9400405    1.031357
        fshmiuml |   .9002939   .0534354    -1.77   0.077     .8014247     1.01136
         lhmiuml |   1.037169   .0345107     1.10   0.273     .9716878    1.107063
             bmi |   1.022142   .0258057     0.87   0.386     .9727946    1.073992
           _cons |   .2948228   .3041307    -1.18   0.236     .0390379    2.226569
------------------------------------------------------------------------------

Note: _cons estimates baseline odds.

24 . mi estimate, or: logistic livebirth_cc i.poly2 i.researchtype_cc ageyears fshmiuml lhmiuml bmi

Multiple-imputation estimates                   Imputations       =         20
Logistic regression                             Number of obs     =        929
                                                Average RVI       =     0.0000
                                                Largest FMI       =     0.0000
DF adjustment:   Large sample                   DF:     min       =   9.71e+65
                                                        avg       =   9.71e+65
                                                        max       =          .
Model F test:       Equal FMI                   F(   6, 1.6e+68)  =       5.16
Within VCE type:          OIM                   Prob > F          =     0.0000

------------------------------------------------------------------------------
 livebirth_cc | Odds Ratio   Std. Err.      t    P>|t|     [95% Conf. Interval]
----------------+-------------------------------------------------------------
          poly2 |
With polymorphism |   .9978185   .1812033    -0.01   0.990     .6989947    1.424391
                |
  researchtype_cc |
 FRESH IVF CYCLES |    .46143   .0854331    -4.18   0.000     .3210016    .6632916
        ageyears |   .9555107   .0216158    -2.01   0.044     .9140699    .9988301
        fshmiuml |   .9735638   .0533924    -0.49   0.625     .8743446    1.084042
         lhmiuml |   .9559552    .034336    -1.25   0.210      .890972    1.025678
             bmi |   .9353139   .0250994    -2.49   0.013     .8873912    .9858246
           _cons |   10.0765   10.30197     2.26   0.024     1.358509     74.7407
------------------------------------------------------------------------------

Note: _cons estimates baseline odds.

25 . **close log

26 .
27 .
```

APPENDIX 8: STATA DO-FILE (CHAPTER 4)


```
1  use "/Users/madararalapanawe/Desktop/Madara final 2.2.dta"
2  ***Non-acrocentric and acrocentric outcomes
3  tab poly2
4  tab poly2 researchtype_cc
5  ******* summary statistics
6  summarize ageyears fshmiuml lhmiuml amhngml tshmicroiuml t4ngdl
    prolactinngml bmi cleavageday3 spermconcentrationmillionml
   progressivemotility nonprogressivemotility normalmorphology,
   detail
7  sort poly2
8  ***** Outcomes
9  tab2 poly2 positivepregancytest_cc, row chi exact
10 tab2 poly2 miscarriage_cc, row chi exact
11 tab2 poly2 livebirth_cc, row chi exact
12 tab2 poly2 researchtype_cc, row chi exact
13 **Regression models unadjusted
14 logistic positivepregancytest_cc i.poly2, or
15 logistic miscarriage_cc i.poly2, or
16 logistic livebirth_cc i.poly2, or
17 * Missing data
18 misstable summarize amhngml fshmiuml lhmiuml tshmicroiuml bmi
   ageyears fertilization poly cleavageday3 matureoocytes
   positivepregancytest_cc positiveoutcome_cc researchtype_cc
19 mi set mlong
20 mi register imputed amhngml
21 mi misstable summarize, all
22 mi impute regress amhngml fshmiuml lhmiuml tshmicroiuml bmi
   ageyears fertilization poly cleavageday3 matureoocytes
   positivepregancytest_cc positiveoutcome_cc, add(20) rseed(1234)
    force
23 **Regression models adjusted for age, FSH, LH, BMI, Research
   type
24 mi estimate, or: logistic positivepregancytest_cc i.poly2 i.
   researchtype_cc ageyears fshmiuml lhmiuml bmi
25 mi estimate, or: logistic miscarriage_cc i.poly2 i.
   researchtype_cc ageyears fshmiuml lhmiuml bmi
26 mi estimate, or: logistic livebirth_cc i.poly2 i.
   researchtype_cc ageyears fshmiuml lhmiuml bmi
27 **close log
28
```

APPENDIX 9: SUPPLEMENTARY TABLES (CHAPTER 3, 4 & 5)

Supplementary Table 1: Percentages of carriers and non-carriers of chromosomal polymorphism with abnormal cleavage and blastocyst formation

Polymorphism	n (%)
Females, males, or couples with abnormal embryo cleavage and blastocyst formation	
Females with polymorphism	4 (6.9)
Males with polymorphism	4 (6.9)
Couples with polymorphism	8 (13.8)
Couples without polymorphism	23 (39.6)
Abnormal cleavage & blastocyst formation (Karyotyping not done)	19 (32.8)
Total	**58 (100.0)**

Supplementary Table 2: Percentages of carriers of non-acrocentric and acrocentric chromosomal polymorphism and non-carriers of chromosomal polymorphism with abnormal cleavage and blastocyst formation

Categories	n (%)
Females, males, or couples with atypical cleavage and blastocyst formation	
Non-acrocentric	2 (3.4)
Acrocentric	4 (6.9)
Combination of non-acrocentric and acrocentric	6 (10.3)
Yqh+/- in male	2 (3.4)
Couples with non-acrocentric and acrocentric	2 (3.4)
Couples without polymorphism	23 (39.6)
Atypical cleavage & blastocyst formation (Karyotyping not done)	19 (32.8)
Total	**58 (100)**

Supplementary Table 3: Crude and adjusted odds ratio with the variability of chromosomal polymorphic variants for pregnancy

Outcome	Crude OR		Adjusted OR	
	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
Chromosomal Polymorphic variants in female only				
Non-acrocentric variants	0.98 (0.40 to 2.44)	0.98	0.83 (0.32 to 2.11)	0.69
Acrocentric variants				
One acrocentric variant	0.67 (0.39 to 1.13)	0.14	0.68 (0.40 to 1.17)	0.17
Two acrocentric variants	0.93 (0.38 to 2.29)	0.88	1.02 (0.41 to 2.57)	0.95
Chromosomal Polymorphic variants in male only				
Non-acrocentric variants	1.20 (0.50 to 2.87)	0.68	1.34 (0.55 to 3.27)	0.51
Acrocentric variants				
One acrocentric variant	1.23 (0.79 to 1.90)	0.34	1.19 (0.76 to 1.86)	0.43
Two acrocentric variants	1.92 (0.74 to 4.98)	0.17	2.04 (0.76 to 5.46)	0.15
Three acrocentric variants	1.20 (0.10 to 13.36)	0.88	1.50 (0.12 to 18.44)	0.75
Combination of non-acrocentric and acrocentric variants	0.48 (0.05 to 4.15)	0.50	0.45 (0.04 to 4.10)	0.48
Yqh in male				
Yqh in male only	1.20 (0.44 to 3.27)	0.71	1.58 (0.57 to 4.41)	0.37
Yqh in male and one acrocentric variant	0.96 (0.18 to 5.01)	0.96	0.91 (0.16 to 4.91)	0.91
Couples with chromosomal polymorphic variants				
Non-acrocentric variant in female and acrocentric variants in male	1.33 (0.43 to 4.06)	0.61	1.27 (0.40 to 4.02)	0.67
Non-acrocentric variants in male and acrocentric variants in female	1.60 (0.26 to 9.70)	0.60	1.71 (0.26 to 11.05)	0.57
Combination of non-acrocentric and acrocentric variants in female and male	0.92 (0.32 to 2.64)	0.88	0.88 (0.30 to 2.60)	0.82
One acrocentric variant in female and male	1.05 (0.50 to 2.21)	0.88	1.14 (0.53 to 2.43)	0.73
One acrocentric variant in female and two acrocentric variants in male	0.70 (0.25 to 1.95)	0.50	0.79 (0.28 to 2.24)	0.66
Two acrocentric variants in female and one acrocentric variant in male	2.16 (0.85 to 5.44)	0.10	1.85 (0.70 to 4.88)	0.21
Two acrocentric variants in female and in male	7.20 (0.74 to 69.95)	0.08	4.76 (0.48 to 46.94)	0.18
Three acrocentric variants in female and one/two acrocentric variants in male	7.20 (0.74 to 69.95)	0.08	10.57 (1.06 to 104.83)	0.04
Acrocentric variant in female and Yqh in male	0.80 (0.21 to 3.00)	0.66	0.87 (0.22 to 3.38)	0.84
Combination of non-acrocentric and acrocentric in female, male and Yqh in male	3.20 (0.70 to 14.51)	0.13	4.03 (0.86 to 18.93)	0.07

The reference category is no chromosomal polymorphism in either partner
OR, odds ratio
CI, 95% confidence intervals

Supplementary Table 4: Crude and adjusted odds ratio with the variability of chromosomal polymorphic variants for miscarriage

Outcome	Crude OR		Adjusted OR	
	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
Chromosomal Polymorphic variants in female only				
Non-acrocentric variants	1.34 (0.44 to 4.06)	0.60	1.25 (0.40 to 3.84)	0.69
Acrocentric variants				
One acrocentric variant	0.86 (0.43 to 1.72)	0.68	0.86 (0.43 to 1.72)	0.67
Two acrocentric variants	1.27 (0.42 to 3.85)	0.66	1.49 (0.48 to 4.56)	0.48
Chromosomal Polymorphic variants in male only				
Non-acrocentric variants	0.60 (0.13 to 2.66)	0.51	0.64 (0.14 to 2.82)	0.55
Acrocentric variants				
One acrocentric variant	1.28 (0.73 to 2.26)	0.38	1.29 (0.72 to 2.29)	0.37
Two acrocentric variants	0.83 (0.18 to 3.73)	0.81	0.83 (0.18 to 3.75)	0.81
Three acrocentric variants	3.35 (0.29 to 37.55)	0.32	3.20 (0.26 to 38.19)	0.35
Combination of non-acrocentric and acrocentric variants	1.34 (0.15 to 11.68)	0.79	1.27 (0.14 to 11.31)	0.82
Yqh in male				
Yqh in male	1.34 (0.37 to 4.77)	0.65	1.43 (0.39 to 5.21)	0.58
Couples with chromosomal polymorphic variants				
Non-acrocentric variant in female and acrocentric variants in male	1.82 (0.49 to 6.75)	0.36	1.80 (0.47 to 6.80)	0.38
Non-acrocentric variants in male and acrocentric variants in female	1.67 (0.18 to 15.25)	0.64	1.68 (0.18 to 15.59)	0.64
Combination of non-acrocentric and acrocentric variants in female and male	1.34 (0.37 to 4.77)	0.65	1.30 (0.36 to 4.67)	0.68
One acrocentric variant in female and male	1.34 (0.53 to 3.36)	0.53	1.34 (0.52 to 3.41)	0.53
One acrocentric variant in female and two acrocentric variants in male	0.67 (0.15 to 2.94)	0.59	0.73 (0.16 to 3.24)	0.68
Two acrocentric variants in female and one acrocentric variant in male	2.39 (0.83 to 6.89)	0.10	2.36 (0.80 to 6.96)	0.12
Two acrocentric variants in female and in male	2.23 (0.22 to 21.84)	0.49	1.79 (0.17 to 18.22)	0.61
Three acrocentric variants in female and one/two acrocentric variants in male	2.23 (0.22 to 21.84)	0.49	2.54 (0.25 to 25.33)	0.42
Acrocentric variant in female and Yqh in male	0.60 (0.07 to 4.80)	0.63	0.70 (0.08 to 5.69)	0.74
Combination of non-acrocentric and acrocentric in female, male and Yqh in male	5.02 (1.09 to 23.04)	0.03	5.55 (1.17 to 26.28)	0.03

The reference category is no chromosomal polymorphism in either partner
OR, odds ratio
CI, 95% confidence intervals

Supplementary Table 5: Crude and adjusted odds ratio with the variability of chromosomal polymorphic variants for livebirth

Outcome	Crude OR		Adjusted OR	
	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
Chromosomal Polymorphic variants in female only				
Non-acrocentric variants	0.72 (0.21 to 2.50)	0.61	0.50 (0.17 to 2.12)	0.43
Acrocentric variants				
One acrocentric variant	0.59 (0.29 to 1.19)	0.14	0.62 (0.30 to 1.27)	0.19
Two acrocentric variants	0.69 (0.20 to 2.38)	0.56	0.70 (0.20 to 2.47)	0.58
Chromosomal Polymorphic variants in male only				
Non-acrocentric variants	1.69 (0.65 to 4.42)	0.27	2.01 (0.75 to 5.35)	0.16
Acrocentric variants				
One acrocentric variant	1.10 (0.64 to 1.88)	0.71	1.06 (0.61 to 1.83)	0.83
Two acrocentric variants	2.54 (0.92 to 7.01)	0.07	2.85 (0.99 to 8.16)	0.05
Yqh in male				
Yqh in male only	1.01 (0.28 to 3.61)	0.97	1.52 (0.41 to 5.53)	0.52
Yqh in male and one acrocentric variant	2.03 (0.38 to 10.71)	0.40	2.05 (0.37 to 11.23)	0.40
Couples with chromosomal polymorphic variants				
Non-acrocentric variant in female and acrocentric variants in male	0.84 (0.18 to 3.87)	0.83	0.82 (0.17 to 3.83)	0.80
Non-acrocentric variants in male and acrocentric variants in female	1.27 (0.14 to 11.56)	0.82	1.37 (0.14 to 13.32)	0.78
Combination of non-acrocentric and acrocentric variants in female and male	0.63 (0.14 to 2.83)	0.55	0.60 (0.13 to 2.77)	0.51
One acrocentric variant in female and male	0.82 (0.30 to 2.18)	0.69	0.91 (0.33 to 2.49)	0.86
One acrocentric variant in female and two acrocentric variants in male	0.80 (0.23 to 2.79)	0.73	0.91 (0.25 to 3.23)	0.88
Two acrocentric variants in female and one acrocentric variant in male	1.35 (0.43 to 4.21)	0.59	1.00 (0.30 to 3.25)	0.99
Two acrocentric variants in female and in male	5.09 (0.70 to 36.77)	0.10	3.29 (0.45 to 24.97)	0.23
Three acrocentric variants in female and one/two acrocentric variants in male	5.09 (0.70 to 36.77)	0.10	8.55 (1.11 to 65.57)	0.03
Acrocentric variant in female and Yqh in male	1.01 (0.21 to 4.75)	0.98	1.00 (0.20 to 4.92)	0.99
Combination of non-acrocentric and acrocentric in female, male and Yqh in male	0.84 (0.10 to 7.16)	0.88	1.08 (0.12 to 9.54)	0.93

The reference category is no chromosomal polymorphism in either partner
OR, odds ratio
CI, 95% confidence intervals