PETER OLANREWAJU SOLOMON

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DEPARTMENT OF MEDICAL GENETICS

MASTER'S PROGRAM IN BIOLOGY AND GENETICS

EVALUATING THE NUMERICAL ABNORMALITIES IN HUMAN EMBRYOS

M.Sc. THESIS

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Approval

We certify that we have read the thesis submitted by Peter Olanrewaju Solomon titled **"Evaluating the Numerical Abnormalities in Human Embryos"** and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Educational Sciences.

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Declaration

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

Peter Olanrewaju Solomon

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Peter Olanrewaju SOLOMON

Abstract

Evaluating the Numerical Abnormalities in Human Embryos

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Over time, advancements in assisted reproductive technologies (ART) have generated increasing interest in the impact of parental age on the chromosomal condition of embryos. This work provides a comprehensive evaluation utilizing a mathematical model to examine embryos with diverse chromosomal makeup in relation to parental age variances.

The objective of this study is to analyse a data-set by studying a diverse selection of embryos from individuals across various age groups. The mathematical model employs sophisticated statistical methods to quantify the relationship between parental age and the incidence of euploidy and aneuploidy in embryos.

The research reveals an intricate connection between the increasing age of parents and the frequency of chromosomal defects in embryos.

Ultimately, this study examines the practical consequences of these findings by making mathematical deductions, offering vital knowledge for healthcare practitioners specialising in reproductive medicine and couples seeking assisted reproductive technology. This research improves patient counselling, treatment options, and the overall enhancement of assisted reproductive technology (ART) success rates by investigating the correlation between parental age and embryo chromosomal status. Hence, the results obtained from this study have the potential to enhance the precision of embryo selection techniques, thereby resulting in improved outcomes in assisted reproductive operations.

Keywords: Aneuploidy, ART-assisted reproductive technologies, advance parental age, mathematical-model

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List of Abbreviations

ACGH:	Array Comparative Genomic Hybridisation
AMA:	Advanced Maternal Age
AOR:	Adjusted Odds Ratios
BMP:	Bone Morphogenetic Protein
CI:	Coincidence Intervals
DEGs:	Differential Expression Genes
DMCs:	Differential Methylation Cytosines
DNA:	Deoxyribonucleic Acid
FGF:	Fibroblast Growth Factor
FSH:	Follicle-Stimulating Hormone
GnRH:	Gonadotropin-Releasing Hormone
GO:	Gene Ontology
KEEG:	Kyoto Encyclopedia Of Genes and Genomes
LH:	Luteinizing Hormone
MI:	Meiosis 1
MII:	Meiosis 2
MtDNA:	Mitochondrial DNA
ND:	Non-Disjunction
IVF:	In Vitro Fertilization

PBs: Polar Bodies

PGC: Primordial Germ Cell

PSSC: Precocious Separation of Sister Chromatids

RS: Reverse Segregation

SCAs: Sex Chromosome Aneuploidies

DPI: Day Post Insemination

RNA: Ribonucleic Acid

TGF-β: Transforming Growth Factor-Beta

TSS: Transcription Start Site

ZP: Zona Pellucida

CHAPTER I

Introduction

The transmission of genetic material is ensured by essential mechanisms that are referred to as chromosomal replication, pairing, and separation. These systems are responsible for overseeing the process of cellular divisions that occur during the lifetime of an individual. These abnormalities are notably noticeable when they have an effect on meiosis, fertilisation, or the first stages of cleavage in embryogenesis (Magli et al., 2001).

As the age of the mother increases, the quality and quantity of the oocyte and follicle pool decrease. This results in a considerable decrease in human fertility, particularly in females. Ageing of the mother also leads to a rise in the proportion of oocytes that have defective chromosomes, which is a crucial factor in the formation of aneuploidy in the embryos that are produced as a result of this process (Rubio et al., 2020). Aneuploidy is the most common chromosomal abnormality observed in human embryos. Trisomic and monosomic embryos are responsible for at least 10% of human pregnancies. However, for women who are approaching the end of their reproductive years, this occurrence may surpass 50%. Furthermore, oocytes and embryos obtained from women who are of advanced maternal age (AMA) exhibit higher rates of aneuploidy, which can be attributed to meiotic recombination mistakes that are exacerbated by the process of ageing. Recent investigations on human and animal organisms have uncovered new findings regarding the intricacy of meiotic abnormalities (Rubio et al., 2020). Nagaoka et al. (2012) discovered that the rise in mistakes in females as they age is not attributable to a single source, but rather to a mix of several internal and external factors, as well as unique traits associated with oogenesis.

Roughly 0.3% of babies, 4% of stillbirths, and more than 35% of all spontaneous human abortions are found to be aneuploid. Process of gametogenesis. In humans, the process of gametogenesis is unique to each gender and is especially vulnerable to mistakes in chromosome segregation. Furthermore, aneuploidy has been found in 1%–4% of human sperm and up to 20% of oocytes, according to molecular cytogenetic research, the main contributing factor to human aneuploidy remains to be maternal age (Munné et al., 2003).

Additional chromosomes in trisomic kids are frequently inherited from the mother due to the failure of homologous chromosomes to separate appropriately during the initial meiotic division. The changes in recombination patterns between male and female meiosis divisions may potentially contribute to the distinct gender- and chromosome-specific variances in the development of human aneuploidy. In older oocytes, the failure of a whole chromosome to separate correctly during meiosis, as well as the early separation of sister chromatids or homologues before meiotic anaphase, can contribute to aneuploidy in hereditary situations. During the beginning of meiosis, geneticists have observed that there are checkpoints during the meiotic prophase and the spindle checkpoint at M-phase. These checkpoints have the capability to halt meiosis and initiate programmed cell death if there are any disturbances in the pairing/recombination or spindle attachment of chromosomes. There is a suggestion that differences in the number of abnormal chromosomes between genders may be due to variations in the strictness of checkpoints between females and males (Chiang et al., 2010).

Furthermore, it is important to mention that the decline in chromosome cohesion in oocytes, which happens as one ages, can potentially lead to aneuploidy. It is crucial to emphasise that this occurrence may be exclusive to females. Insufficient comparative evidence exists on the vulnerability of male and female germ cells in humans to substances that induce aneuploidy. Research has indicated that the occurrence of aneuploidy, a disorder characterised by an incorrect number of chromosomes, might increase in sperm after being exposed to medicinal medications, occupational agents, and lifestyle factors (Duncan et al., 2012).

Statement of Problem

There are some knowledge gaps in terms of unravelling the comprehensive relationship between advanced maternal and paternal age on embryo formation, especially in artificial reproductive mechanisms such as IVF (Invitro Fertilisation). According to Qi et al. (2014), the ageing of the mother causes an increase in the proportion of oocytes that have defective chromosomes. This is a significant factor in the development of aneuploidy in the embryos that are produced as a result of this process.

It is considered that embryonic aneuploidy is the key factor that contributes to the poor success of in vitro fertilisation (IVF) treatments. This not only makes it more difficult for embryos to successfully implant, but it also dramatically raises the likelihood of having a miscarriage (Qi et al., 2014).

Purpose of the study

The purpose of the study was to draw mathematical inferences and relative observations from the relationship of advanced maternal and parental age on embryo formation credibility in artificial reproductive mechanisms.

Moreso, the aim was to deduce and determine euploidy which is perfect for implantation, possible aneuploidy compatible with life and non-compatible with life. And to investigate and find possibilities (mathematical) explanations and correlations in the increase in maternal and paternal age with mathematical representation of possible outcomes. In literature, this study is the first to give this kind of mathematical approach to draw correlations and explanations for advanced maternal age with resulting euploids, aneuploids whose life compatibility was predetermined. On this basis, the research objectives for this study are:

1. To determine mathematically, the relationship between paternal and maternal age and embryo formation.
2. To evaluate mathematically the role of maternal age in aneuploid embryo abnormality.
3. To analyse the impact of abnormal human embryos in artificial reproductive mechanism such as IVF.

Research Questions

Based on the research on the numerical abnormalities in human embryos, the following research questions were addressed:

Q1. Is there a mathematical correlation between maternal and paternal age in terms of the possible outcome of embryo implantation?

Q2. Is aneuploidy more common in parents of advanced age or it is just a common phenomenon in regular couples and also in in vitro fertilisation (IVF)?

Significance of the Study

By doing an inquiry into the numerical abnormalities in human embryos, this study provides a viewpoint on the impact of ageing on aneuploidy for older mothers and how they impact embryo formation even in artificial reproductive mechanisms.

Recent research on model organisms and humans has provided a more comprehensive understanding of the intricacy of meiotic abnormalities. This study has demonstrated that the rise in errors among ageing female humans is not attributable to a single reason, but rather to a combination of several internal and external causes, together with specific traits connected to oogenesis. The occurrence of age-related anomalies decreases the ongoing success rates of implantation by elevating the occurrence of spontaneous abortions and increasing the frequency of aneuploidy in offspring (Nagaoka et al., 2012).

According to Rodrigo et al. (2011), aneuploidy may also be a factor in other groups of infertile individuals, such as couples who have experienced repeated losses or implantation failure. When the meiotic process is disrupted, it leads to a higher occurrence of chromosomal abnormalities in sperm, which is associated with male infertility.

Also, couples undergoing intracytoplasmic sperm injection (ICSI) have a higher incidence of aneuploidy in miscarriages due to male infertility (Campos-Galindo et al. 2015).

Limitation of the Study

Despite some reasonable number of studies done on existing abnormalities in human embryos such as aneuploidy, they still exist modern research gaps in the study of these abnormalities in human embryos. Not much has been done in recent times in attempting to provide improved research on embryo implantation in aged parents, especially from the maternal side and how to drastically reduce the occurrence of these abnormal embryos.

CHAPTER II

General Information

Normal Fertilization Process

Normal fertilization is a complex biological process that involves the fusion of a sperm cell with an egg cell (oocyte) to form a zygote. This process occurs in several stages (Fragouli et al., 2018). Sperm cells make their way through the female reproductive canal, with the assistance of cervical mucus and the contractions of the uterus, in order to reach the Fallopian tube, which is where the egg is located (Suarez & Pacey, 2006).

In the process of fertilization, sperm cells try to penetrate the outer layer of oocytes, any sperm cell that has been successful in penetrating the egg's protective covering (the zona pellucida) will activate alterations in the egg membrane that will prevent additional sperm cells from accessing the egg (Wassarman & Litscher, 2008).

Zygote production precedes fertilization, this involves the union of the nuclei of the egg and the sperm resulting in the production of a diploid cell known as a zygote. This cell carries an entire set of genetic information inherited from both of the parent cells. In the process of implantation, as it makes its way down the fallopian tube and eventually reaches the uterus, the zygote goes through a number of phases of cell division in preparation for the process known as implantation. Once it has reached the uterus, the developing embryo will go through the process of implantation into the lining of the uterus (Runft et al., 2004).

Gametogenesis: Spermatogenesis and Oogenesis

The process of gametogenesis holds significant importance in the life cycle of sexually reproducing animals, as it encompasses the development of specialized cells referred to as gametes. The gametes, namely sperm and eggs, possess 50% of the genetic material required for the formation of a novel organism. The complex mechanism further described in subsequent subtopics unfolds via a sequence of precisely controlled occurrences, thereby guaranteeing the transfer of hereditary material from one progeny to the subsequent generation (Ramaswamy et al., 2013).

The process of gametogenesis holds significant milestones in the area of reproductive biological sciences, as it serves as a paramount area of investigation for understanding the complexities and diversities involved in the creation of germ cells. This particular mechanism exhibits a significant level of conservation among various species, showcasing a noteworthy extent of similarities spanning from insects to mammals (Larose et al., 2019).

The process of gametogenesis can be classified into two main categories, namely spermatogenesis and oogenesis. Spermatogenesis is the biological process responsible for the manufacturing of male gametes, known as sperm cells, whereas oogenesis pertains to the development of female gametes, sometimes referred to as egg cells. Both processes have a shared core, although they display individual traits that are uniquely designed to meet the particular needs of each gender. Spermatogenesis is a biological process that predominantly occurs within the testes of male organisms. During this process, spermatogonia, the precursor cells for sperm, undergo a sequence of mitotic divisions and subsequent differentiation. This process leads to fully developed sperm cells that possess the essential anatomical features required for successful fertilization (Nishimura & L'Hernault, 2017).

Oogenesis, conversely, occurs within the ovaries of female organisms. Oocytes undergo an intricate sequence of divisions and differentiations, ultimately resulting in the generation of fully developed eggs. The temporal sequence and physiological processes related with oogenesis exhibit considerable variation among different species (Telfer, 2019).

Gametogenesis, at the molecular level, is regulated by a multitude of genes and signalling networks. The regulatory network in question carries out a vital role in making sure of the accurate timing and synchronization of events that are essential for the formation of fully functioning gametes. The key entities required in regulating the fate and functionality of germ cells include; transcription factors, growth factors, and a diverse array of signalling molecules, which collectively operate in a coordinated manner. Matson and Zarkower (2012), shed light and spotlight on the significance of specific transcription factors, namely SOX9 and DMRT1, in the process of determining the fate of male germ

cells. These factors are very important in the production of spermatogonia and coordinate the many stages of spermatogenesis.

The participation of growth factors, including bone morphogenetic proteins (BMPs) and transforming growth factor-beta (TGF-β), in the oogenesis process is particularly significant. These variables play a vital role in regulating folliculogenesis and facilitating the process of oocyte maturation. Although gametogenesis involves extensive regulatory processes, it is not immune to barriers. Deviations happening during this complex process can lead to reproductive genetic disorders, illnesses, and miscarriages. The occurrence of infertility or birth abnormalities can be attributed to mutations or disruptions in essential genes involved in gametogenesis, highlighting the vital importance of these biological processes in maintaining the general survival of the species (Castro et al., 2015).

Spermatogenesis is a biological process through which spermatogonial stem cells undergo differentiation, ultimately resulting in the formation of fully developed spermatozoa. The regulation of this complex process is governed by a multitude of chemical signals and cellular processes (Johnson et al., 2019). Understanding spermatogenesis holds great importance beyond the realm of reproductive biology, as it has been established that disturbances and interruptions in this intricate process are related to male infertility and various other reproductive illnesses (Du et al., 2021).

During the onset of puberty, the testes initiate the process of significantly augmenting the secretion of the steroid hormone known as testosterone. This hormone exhibits an array and wide range of effects.

More so, in addition to facilitating the development of other secondary sexual traits, it initiates the enlargement of the testes, maturity of the seminiferous tubules, and the onset of Spermatogenesis cells undergo differentiation into a complex network of seminiferous tubules in response to the presence of testosterone. The quiescent primordial germ cells (PGCs) undergo a resumption of development, thereafter undergoing multiple rounds of mitotic division, and ultimately differentiate into spermatogonia. The spermatogonia are located in close proximity to the basement membrane that encloses the seminiferous

tubules. Specifically, they reside inside and within the interstitial spaces formed by the Sertoli cells (Ramaswamy et al., 2013).

Throughout its journey from the seminiferous tubules to the ampulla of the oviduct, a sperm cell experiences a process of functional maturation that equips it with the necessary capabilities to successfully fertilize an oocyte. The production of sperm occurs within the seminiferous tubules, which are subsequently stored in the distal region of the epididymis. This elongated and intricately coiled duct, measuring approximately fifteen to twenty feet in length, is connected to the vas deferens in close proximity to its point of origin within the testis. During the process of ejaculation, spermatozoa are driven along the vas deferens and urethra, where they are combined with nutritive secretions originating from the seminal vesicles, prostate gland, and bulbourethral glands. Up to three hundred million spermatozoa have the potential to be introduced into the vagina with a single ejaculation. However, only a limited number, typically a few hundred, are able to successfully traverse the cervix, uterus, and oviduct, ultimately reaching the enlarged ampulla region. Spermatozoa remain viable and maintain their ability to fertilize an egg for a period of one to three days within the ampulla of the oviduct (Hunt & Hassold, 2008).

Capacitation, the ultimate phase of sperm maturation, mostly involves alterations in the acrosome, which prime it for the release of the enzymes necessary to enter the zona pellucida, a glycoprotein shell encompassing the egg. The process of capacitation occurs within the female reproductive system and is believed to necessitate exposure to oviductal secretions. Spermatozoa utilized in invitro fertilization (IVF) techniques undergo artificial capacitation (Chen et al., 2018).

The examination of spermatogenesis is essential in order to effectively handle matters pertaining to male infertility and reproductive illnesses, as disruptions can have a significant impact on male reproductive health. Disruptions in the process of spermatogenesis can arise due to genetic abnormalities, exposure to environmental contaminants, or the presence of underlying medical problems. The examination of these disturbances at the molecular level has the potential to facilitate the development of precise treatment solutions (Krausz & Riera-Escamilla, 2018).

A comprehensive comprehension of spermatogenesis is important in order to effectively treat matters pertaining to male infertility and reproductive diseases. Disruptions and interference in the process of spermatogenesis can arise due to genetic abnormalities, exposure to environmental contaminants, or the presence of underlying medical problems. The examination of these disruptions at the molecular level has the potential to facilitate the development of precise therapeutic approaches (Krausz & Riera-Escamilla, 2018).

Meiotic Divisions in Gametogenesis

In sexually reproducing organisms, the process of meiosis is an important stage. The product and outcome of this particular kind of cell division is the creation of haploid gametes, which have half as many chromosomes as the parental cell. For the number of chromosomes in a species to remain stable throughout generations, the whole meiotic division process is necessary (Lodish et al., 2000).

Meiosis I and II are the two successive rounds that make up the meiotic division. Meiosis I is especially important since it causes the number of chromosomes to decrease from diploid (2n) to haploid (n). To restore the diploid chromosomal number in the zygote during the subsequent fertilization process, two haploid gametes must unite. This reduction is necessary for this procedure.

During meiosis I, homologous chromosomes, one acquired from each parent, undergo recombination through a process known as crossing over. The genetic variety of kids is increased by this exchange of genetic material between homologous chromosomes. To ensure correct chromosomal segregation during meiosis I, chiasma physical linkages between homologous chromosomes must occur during crossing over (Castro et al., 2015).

Homologous chromosomes segregate into distinct daughter cells as meiosis I proceed, each with a distinct genetic makeup. In meiosis I, homologous chromosomes split, giving rise to cells with half the number of chromosomes, in contrast to mitosis, where sister chromatids separate. The positioning of the chromosomes at the metaphase plate and their subsequent separation during anaphase I control this process (Lodish et al., 2000).

The process of Meiosis II is very similar to a mitotic division, except for the fact that the initial cells are haploid. The sister chromatids of each chromosome, formed during

meiosis I, separate into distinct daughter cells during meiosis II. As a result, four distinct haploid cells are produced, each containing a distinct combination of genetic material. Errors in the meiotic division, like non-disjunction errors, can result in aneuploidy, which is characterized by cells having an atypical number of chromosomes. Imbalances in chromosome numbers can lead to significant impacts, such as developmental disorders and challenges with fertility. The precise control of meiotic events is therefore essential for the production of viable and diverse gametes (Snustad & Simmons, 2015).

Primordial Germ Cells (PGCs)

Cells that produce gametes in both male and female arise in 4^{th} week (hence embryonic sex mostly can be determined afterwards) and can be found in the extra-embryonic membrane. Primordial germ cells, otherwise known as PGCs, are a distinct population of germ cells. These cells are separate from somatic cells and are the progenitors to the gametes that are responsible for sexual reproduction. Somatic cells are the cells that make up the body's tissue. During embryonic development, the production and migration of PGCs is a process that is highly regulated, and the PGCs' ability to perform in an appropriate manner is needed for the continuation of the species (Matsui et al., 1992).

PGCs emerge during the early phases of embryonic development from a subset of cells that are referred to as the germ cell lineage. PGCs then undergo a series of differentiation processes that allow them to specialize into their final forms. PGCs are distinguished from somatic lineages through a sequence of molecular events that occur throughout the process of PGC specification. These processes lead to the separation of PGCs from somatic lineages. Research has demonstrated that numerous signalling pathways, such as bone morphogenetic protein (BMP), fibroblast growth factor (FGF), and Wnt signalling, play important roles in the specification and differentiation of PGCs (Ohinata et al., 2009).

PGCs, once they have been specified, proceed through a migratory phase in order to reach the developing gonads, which is where they will eventually differentiate into adult gametes (Runyan et al., 2006). This migration is a complicated process that is influenced by a variety of circumstances, both internal and external. Some studies have suggested

that chemotactic signals, adhesion molecules, and components of the extracellular matrix may play a role in directing the migration of PGCs (Molyneaux et al., 2001).

Primordial germ cells (PGCs) undergo substantial epigenetic reprogramming when they reach the gonadal ridges. This reprogramming involves the removal of epigenetic markers characteristic of somatic cells and the establishment of a unique epigenetic signature for germ cells (Hajkova et al., 2008). This phenomenon takes place to guarantee that every germ cell possesses its unique genetic composition. This mechanism is crucial for eradicating parental imprints and inducing totipotency in the germ cells that are undergoing development during foetal germination. Recent studies have revealed crucial epigenetic regulators that play a role in the process of reprogramming. Two examples of these epigenetic regulators are DNA methyltransferases and histone-modifying enzymes (Saitou et al., 2002).

The transformation of PGCs into either sperm or eggs is a critical stage in the development of sexual characteristics. According to research, the determination of sex in mammals takes place through a series of intricate genetic cascades, with PGCs responding to signals received from the environment (Koopman et al., 1990). The maturation of PGCs into either male or female germ cells is guided by the expression of genes that are particular to each gender as well as the activation of pathways that determine sex (McLaren, 2003). The process and stages of meiosis encompass two consecutive divisions, namely Meiosis I and Meiosis II, each characterized by discrete phases: prophase, metaphase, anaphase, and telophase. During the first stage of meiosis, known as Meiosis I, the process of homologous chromosomal separation occurs, leading to the formation of two cells that are haploid in nature. During the second stage of meiosis, known as Meiosis II, the process of segregating sister chromatids of each chromosome takes place, resulting in the production of four haploid daughter cells. The process encompasses distinct occurrences, such as homologous recombination, which entails the exchange of genetic material between chromosomes that are homologous to one other, hence playing a role in the augmentation of genetic variety (Snustad & Simmons, 2015).

Pre-Implantation Embryo Development

Preimplantation embryo development is a vital stage in the early phases of embryogenesis, happening shortly after fertilization and before implantation into the maternal uterus. This complex process entails a sequence of carefully coordinated events that ultimately lead to the formation of a blastocyst, a crucial multicellular structure necessary for successful implantation and subsequent embryonic development. During fertilization, the sperm enters the oocyte, resulting in the creation of a zygote. The zygote goes through a series of cleavage divisions, leading to a rapid growth in cell number while keeping a consistent overall size. The initial divisions play a crucial role in establishing the cells' totipotency, ensuring that each cell has the potential to develop into any cell type within the organism. Research has indicated that the early divisions in embryos are carefully controlled by factors passed down from the mother, and they have a crucial impact on the shaping of the developing embryo (Lamb et al., 2018).

As the cleavage divisions continue, the embryo undergoes a transformation into the morula stage, which is marked by a dense cluster of cells. By the fourth day after fertilization, the morula undergoes blastulation and develops into a blastocyst. During this stage, a fluid-filled cavity called the blastocoel is formed, and cells start to differentiate into two separate lineages; the outer trophectoderm and the inner cell mass. The trophectoderm is responsible for the development of extraembryonic tissues, including the placenta, while the inner cell mass undergoes differentiation to form the embryo itself. The segregation of cell fates plays a crucial role in the development of both embryonic and extraembryonic structures (Gardner et al., 2017).

The blastocyst is a well-structured entity, and its successful formation is a sign of a robust embryo. In recent studies, scientists have been exploring the intricate molecular processes that regulate the development of blastocysts. Through their investigations, they have revealed the significance of certain signalling pathways and transcription factors in this complex process. As an example, researchers have discovered that the Wnt and Hippo signalling pathways play crucial roles in determining the fate of the trophectoderm and inner cell mass, respectively (Morris et al., 2019). In maintaining pluripotency and

promoting cell fate decisions within the blastocyst, transcription factors like Oct4, Sox2, and Nanog have crucial roles (Niakan & Eggan, 2013).

The exploration of preimplantation embryo development has not only improved our comprehension of fundamental biological processes but has also opened doors for advancements in assisted reproductive technologies (ART). Manipulating and selecting embryos during the preimplantation stage is an important aspect of techniques like *in vitro* fertilization (IVF) and preimplantation genetic testing (PGT). These technologies have brought about a significant transformation in fertility treatments and have made it possible to prevent specific genetic disorders. This highlights the practical applications of comprehending preimplantation embryo development. Ultimately, the process of preimplantation embryo development is an intricate and meticulously controlled journey that lays the groundwork for a prosperous implantation and subsequent growth of the embryo. Continual research is revealing the intricate molecular and cellular processes that govern this crucial stage, providing valuable insights into fundamental biology and practical applications in reproductive medicine (Handyside, 2020).

Mitotic Division as Related to Preimplantation Embryonic Development

Mitotic division is an essential part of preimplantation embryo development, a highly intricate and carefully controlled process that results in the creation of a multicellular organism. The process of preimplantation embryo development starts soon after fertilization when a sperm combines with an egg to create a zygote. The zygote goes through a sequence of mitotic divisions, resulting in the creation of a blastocyst as described previously. The mitotic division plays a crucial role in ensuring the accurate distribution of genetic material to daughter cells during this critical stage of development (Ramaswamy et al., 2013).

The progression of the cell cycle is tightly regulated by the activity of cyclins and cyclin-dependent kinases (CDKs), ensuring the accurate timing of each phase. The S phase is of utmost importance, as it encompasses the duplication of DNA, guaranteeing that every offspring cell obtains a full and indistinguishable collection of genetic material. The accuracy of mitotic division during preimplantation embryo development is crucial for the creation of a robust and viable organism (Du et al., 2021).

Establishing cell polarity is a crucial aspect of mitotic division during preimplantation embryo development. As the zygote goes through successive rounds of mitotic division, cellular asymmetry starts to appear, resulting in the creation of different cell populations within the growing embryo. The early cell fate determination plays a critical role in the subsequent differentiation of cells into different lineages, including those involved in the formation of the inner cell mass and the trophectoderm in the blastocyst. The intricate processes governing cell polarity and fate determination during mitotic division are carefully controlled by a multitude of signalling pathways and transcription factors, guaranteeing the harmonious development of diverse cell lineages (Chen et al., 2018).

The mitotic spindle, a structure composed of microtubules, is crucial for ensuring the precise separation of chromosomes during mitotic division. Accurate spindle formation and function play a vital role in preventing chromosomal abnormalities and ensuring the accurate distribution of genetic material to daughter cells. Irregularities in spindle assembly or function can result in aneuploidy, a condition where cells have an abnormal number of chromosomes. Ensuring the proper function of the mitotic spindle during preimplantation embryo development is crucial for the generation of genetically stable cells, which greatly influences the overall success of embryogenesis (Marangos et al., 2007).

Recent research has provided new insights into the molecular mechanisms that regulate mitotic division in preimplantation embryos. Research has revealed important factors like Aurora kinases, Polo-like kinases, and microtubule-associated proteins that play a crucial role in the accurate control of mitotic events. In addition, recent advancements in live-cell imaging techniques have offered valuable insights into the dynamics of mitotic division, enabling researchers to observe and analyse the process as it happens. Such research enhances our comprehension of the fundamental biological mechanisms that influence the development of early embryos (Niakan & Eggan, 2013).

Evaluation of Euploid and Aneuploid Embryos in Accordance with Maternal and Paternal Age, Oocytes and Sperm Qualities

Euploidy and aneuploidy refer to the characterization of a cell's chromosomal composition. Euploids has a full complement of chromosomes, while aneuploids have an abnormal number of chromosomes, either more or less than the typical amount. These differences have a significant impact on genetics, development, and various illnesses.

For example, the human species is diploid, meaning it has 46 chromosomes organised into 23 pairs. An equilibrated chromosomal complement is crucial for proper cellular functioning, reproduction, and overall health. Genetic instability resulting from deviations from euploidy can have deadly effects, including developmental defects (Castro et al., 2015).

Spindle Formation and Chromosome Alignment

The process of capturing, aligning, and segregating chromosomes during mitosis is carried out by the spindle apparatus. Microtubules originate from centrosomes and create spindle fibers that connect to kinetochores, which are specific protein structures found on chromosomes. Accurate chromosome alignment at the metaphase plate is crucial for achieving euploidy, and it relies on the proper attachment of microtubules to kinetochores. Research has indicated that mistakes in spindle formation, like multipolar spindles or incorrect microtubule-kinetochore attachments, can result in chromosomes being misaligned and causing aneuploidy (Musacchio, 2015).

Spindle Checkpoints and Error Correction

In order to preserve the integrity of the genome, cells have developed surveillance mechanisms called spindle checkpoints. These checkpoints ensure the accuracy of spindle formation and chromosome attachment, pausing cell cycle progression until any mistakes are fixed. The mitotic checkpoint, also known as the spindle assembly checkpoint (SAC), ensures that all chromosomes are properly attached to the spindle before allowing the cell to proceed to anaphase. Spindle checkpoint dysregulation can have a negative impact on euploidy, resulting in the continued occurrence of chromosome segregation errors. As an

illustration, abnormalities in SAC components can lead to early initiation of anaphase, resulting in errors in chromosome distribution (Musacchio, 2015).

The presence of spindle defects can greatly affect the accuracy of chromosome segregation, which in turn has a significant impact on the maintenance of euploidy. One typical outcome is the production of daughter cells with an abnormal number of chromosomes, known as aneuploidy. Aneuploidy is associated with various pathological conditions, including developmental disorders and cancer. Research has shown that irregularities in spindle formation can lead to higher levels of chromosomal instability, highlighting the importance of proper spindle formation in preserving normal chromosome numbers (Bakhoum et al., 2009).

The process of spindle formation is regulated by a number of crucial molecular components, which work together to maintain its precision. The spindle apparatus relies on the collaboration of microtubule-associated proteins, motor proteins, and regulatory kinases to ensure its dynamic assembly and optimal functioning. As an example, the Aurora kinases have a vital role in controlling microtubule dynamics and centrosome maturation, which in turn affects spindle formation. Gaining a deep understanding of these intricate molecular mechanisms is crucial in order to develop precise therapies that can effectively address spindle defects and maintain the normal chromosomal content (Chu et al., 2023).

Aneuploidy and Causes of Aneuploidy: Nondisjunction

This syndrome is caused by errors in cell division, namely non-disjunction during meiosis or mitosis. Anomalies in the number of chromosomes, known as aneuploidy, are commonly associated with genetic abnormalities, including Turner syndrome (monosomy X) and Down syndrome (trisomy 21). Aneuploidy can result in a wide range of consequences, often leading to severe difficulties that might be life-threatening or cause deficits in development (Charalambous et al., 2022).

The average age of mothers giving birth has significantly increased worldwide, considering factors such as education, society, and the economy (Molina et al., 2019). In 2012, it was reported that there was a 13% increase in the proportion of women aged 35–

44 who decided to have their first child. Currently, there is a continuous increase in the ratio (Charalambous et al., 2022).

Aneuploidy is a disorder that often results in the failure of the embryo to implant properly and its development being halted. This can ultimately lead to either the spontaneous abortion of the fetus or the birth of offspring with abnormal numbers of chromosomes. The occurrence of aneuploidy in sperm cells is infrequent, happening in only 2% of cases (although still possible). On the other hand, numerical chromosomal aberrations are more common in female gametes, with 20% of oocytes being aberrant. These findings indicate that female gametes are more prone to chromosomal abnormalities. Studies have shown that the process of aging has a significant impact on oocyte aneuploidy, which can lead to female infertility. Based on a prior investigation of 15,000 embryo biopsies, it was found that women above the age of 42 have a high percentage (75%–100%) of embryos with aneuploidy. In contrast, women aged between 26 and 30 only had a rate of aneuploidy ranging from 20% to 27% (Sonowal et al., 2023).

The incidence of chromosomal segregation errors in oocytes of mice significantly rises with age, escalating from less than 5% at three months to a range of 30% to 50% at twelve months. Therefore, it is clear and well-recognized that rodents remain the preferred and most efficient model for studying the process behind chromosomal segregation in ageing oocytes. The mitochondria are the predominant organelles in oocyte plasma. Mitochondria stop reproducing in fully developed eggs from the moment of fertilization until the implantation of the blastocyst. These findings indicate that mitochondria located in the cytoplasm have a vital and indispensable function in the maturation of oocytes, fertilization, and the subsequent development of embryos. During the development and maturation of oocytes, mitochondria move along the spindle microtubules (MTs) in a dynamic manner. This movement is necessary to supply ATP and calcium ions, which are essential for the recruitment and assembly of internal organelles and the cytoskeleton (Zhang et al., 2019).

Zhang et al. (2019), studies have linked ovarian senescence to abnormal mitochondrial structure and function. Moreover, it was postulated that the collapse of the spindle and the subsequent occurrence of aneuploidy were a result of insufficient energy

supply during the process of spindle assembly and recruitment. This insufficiency was attributed to abnormal mitochondrial function and the metabolic changes associated with ageing oocytes.

Prior studies have recorded abnormalities in the shape of the spindle in oocytes of women of advanced maternal age (Battaglia et al., 1996), suggesting that age negatively affects microtubules. The impaired function of mitochondria in the eggs of older women may be caused by various factors, such as damage to mitochondrial DNA (mtDNA), disruption of mitochondrial gene expression, or a decrease in mitochondrial membrane potential (MMP) (Seidler and Moley, 2015). The changes in MMP were detected. The maturation progression of oocytes in the older group exhibited a substantial decline, as expected. An intact and functional mitochondrial membrane potential (MMP) is crucial for the upkeep of ATP-producing mitochondrial oxidative phosphorylation. Therefore, a decrease in the potential of the mitochondrial membrane could hinder the production of ATP, leading to insufficient energy for the spindle recruitment and assembly process. This finally results in the development of aneuploidy. Consequently, it was theorized that the dysfunctional spindle, which causes abnormal chromosome numbers in ageing egg cells, could be caused by a decrease in ATP due to mitochondrial failure (Chen et al., 2018).

Non-Disjunction in Meiosis I

Non-disjunction in meiosis I is a critical occurrence that can result in notable genetic abnormalities in offspring. The first division of meiosis, known as Meiosis I, is a crucial step in the cell division process that leads to the formation of gametes in sexually reproducing organisms. During the process of meiosis I, the homologous chromosomes undergo separation, leading to the formation of two haploid cells. Non-disjunction happens when homologous chromosomes do not separate correctly during this process (Bartoov et al., 2003).

The outcomes of non-disjunction in meiosis I have significant implications and can lead to aneuploidy. Imbalances in chromosome number caused by aneuploidy can result in genetic disorders and developmental abnormalities, disrupting the normal genetic makeup of an individual. The impact of the effects varies depending on the particular

chromosomes at play and the characteristics of the genetic material they carry (Bakhoum et al., 2009).

Research has uncovered various factors that play a role in non-disjunction during meiosis I. Age plays a crucial role, as the risk of non-disjunction events tends to rise with advanced maternal age. Research has indicated that there is an increased occurrence of non-disjunction in meiosis I among women who are over the age of 35. This is especially notable for certain chromosomes, like 21, which causes Down syndrome. The processes contributing to the age-related increase in non-disjunction are intricate and encompass a range of molecular and cellular mechanisms that gradually lose efficiency over time (Nagaoka et al., 2012).

Non-disjunction in meiosis I can lead to significant consequences for the developing embryo, particularly in terms of aneuploidy. Down syndrome, for example, is a widely recognized condition that occurs when there is an additional copy of chromosome 21 due to a non-disjunction event during meiosis I. Errors in chromosome segregation during meiosis I can lead to the development of other aneuploidies, such as Turner syndrome (monosomy X) or Klinefelter syndrome (XXY) (Nagaoka et al., 2012).

Gaining a deep understanding of the intricate molecular mechanisms that drive non-disjunction in meiosis I is of utmost importance in order to devise effective strategies to minimize its consequences. Recent research has been dedicated to the identification of particular genes and proteins that play a crucial role in the accurate separation of chromosomes during meiosis. For instance, research has emphasized the importance of microtubules and motor proteins in ensuring the precise separation of homologous chromosomes. Disruptions in these processes can result in non-disjunction events, which can cause aneuploidy (Hunt & Hassold, 2008).

Ultimately, the occurrence of non-disjunction during meiosis I can have profound implications for the genetic well-being of future generations. The connection between non-disjunction and aneuploidy highlights the significance of comprehending the molecular mechanisms that regulate chromosome segregation during meiosis I. Progress in this area has the potential to provide valuable insights into ways to decrease the occurrence of chromosomal abnormalities, particularly in older mothers. Further

exploration into the field of genetics and reproductive biology is crucial in order to better understand the intricacies of non-disjunction and its impact on human well-being (Bartoov et al., 2003).

Non-Disjunction in Meiosis II

Non-disjunction is a critical occurrence in meiosis II that can have profound effects on genetic stability and the occurrence of aneuploidy. During meiosis II, the main goal is to separate the sister chromatids of each chromosome, ensuring that each gamete receives a complete and accurate set of chromosomes. Meiosis II can experience non-disjunction, where sister chromatids don't properly separate and end up in the same daughter cell. Thus, one daughter cell ends up with an additional chromosome, while the other one is missing that specific chromosome. This results in an uneven distribution of chromosomal content in the resulting gametes (Hunt & Hassold, 2008).

The researchers discovered a notable rise in non-disjunction events during meiosis II in older women, resulting in a heightened risk of aneuploidy in their offspring. This link highlights the significance of comprehending the molecular mechanisms involved in non-disjunction during meiosis II in order to gain a deeper understanding of the factors that contribute to aneuploidy (Hunt & Hassold, 2008).

In addition, molecular studies have revealed the identification of distinct proteins and mechanisms that play a crucial role in maintaining accurate chromosome segregation during meiosis II. This valuable insight has provided a deeper understanding of the potential factors contributing to non-disjunction. Discovering these molecular players provides opportunities for creating interventions that can lower the occurrence of non-disjunction and, as a result, reduce the occurrence of aneuploidy. As an example, a study conducted by Nagaoka et al. (2012) shed light on the function of the centromeric protein Shugoshin in preventing early separation of sister chromatids during meiosis II, which could be a promising area for therapeutic interventions.

Ultimately, the occurrence of non-disjunction during meiosis II is a crucial occurrence that has the potential to result in aneuploidy, which can significantly affect the genetic stability of organisms (Bakhoum et al., 2009).

Non-Disjunction in Mitosis

Non-disjunction in mitosis can cause a disruption in the usual separation of chromosomes, resulting in an unequal distribution of genetic material among the daughter cells. This phenomenon can have significant implications for the organism, as it leads to an atypical number of chromosomes in one or more cells. Mitosis is a crucial process in the cell cycle, guaranteeing the accurate sharing of genetic material to daughter cells during cell division. The process consists of several stages, including prophase, metaphase, anaphase, and telophase. Disruptions in the precise segregation of chromosomes can occur at any of these stages. The outcomes of non-disjunction in mitosis can differ based on the timing and location of the occurrence (Duncan et al., 2012).

During prophase, chromosomes undergo condensation and become visible when observed under a microscope. At this stage, errors in chromosome alignment can occur, which can then lead to further errors in segregation. Scientists have discovered several factors that can play a role in prophase non-disjunction, including irregularities in chromosomal structure or malfunctions in the proteins that oversee chromosome organization.

Metaphase is a crucial stage in the cell cycle where chromosomes arrange and align themselves along the equator of the cell before they are separated. Disparate distribution of chromosomes to the daughter cells can occur due to non-disjunction during metaphase. Various factors, such as the incorrect connection of microtubules to chromosomes or problems with the spindle apparatus, can contribute to metaphase non-disjunction. Research has emphasized the significance of accurate spindle assembly and kinetochore-microtubule interactions in preventing errors during this phase (Chiang et al., 2010).

In anaphase, the stage of cell division, the sister chromatids are delicately separated and drawn towards opposite poles of the cell. Unequal distribution of chromatids during anaphase can result in daughter cells having an abnormal chromosome number due to non-disjunction. Through molecular studies, important proteins like separase and cohesin have been identified. These proteins have critical functions in ensuring the accurate separation of chromatids during anaphase (Seok et al., 2020).

Telophase is the concluding phase of mitosis, where two separate nuclei are formed in the daughter cells. Non-disjunction during telophase can have long-term impacts on the genetic composition of the resulting cells. Studies have revealed that issues with cytokinesis, the cell division process, can play a role in non-disjunction during telophase, resulting in the creation of cells with abnormal chromosome numbers. Gaining insight into the molecular mechanisms that contribute to non-disjunction in mitosis is of utmost importance in order to shed light on the origins of genetic disorders and developmental abnormalities. Several studies have utilized sophisticated molecular biology techniques, including live-cell imaging and genetic manipulations, to explore the factors involved in non-disjunction events. For example, a recent study conducted by Du et al. (2021), utilized CRISPR-Cas9 technology to alter essential proteins involved in cell division and examined the subsequent impact on the process of chromosome segregation.

Ultimately, the occurrence of non-disjunction during mitosis is a multifaceted process that carries important consequences for the stability of genetic material and the overall functioning of cells. Research efforts aimed at unravelling the molecular intricacies of non-disjunction events during various stages of mitosis enhance our comprehension of chromosome segregation and assist in identifying potential therapeutic targets for genetic disorders. As technology progresses, scientists will keep exploring the complexities of mitotic non-disjunction, offering valuable insights into the preservation of genomic integrity. (Du et al., 2021).

Other Relative Causes of Aneuploidy

There is evidence suggesting that exposure to specific environmental factors may elevate the chances of aneuploidy. Various environmental factors, like radiation, chemicals, and specific medications, have the potential to interfere with the usual DNA replication and repair mechanisms. Consequently, this disruption can result in chromosomal abnormalities. In addition, the generation of aneuploid cells has been linked to oxidative stress caused by environmental factors (Lamb et al., 2018).

Some individuals may have a predisposition to aneuploidy due to mutations or variations in genes involved in chromosome segregation and maintenance. As an illustration, alterations in the genes responsible for mitotic checkpoint proteins can

undermine the accuracy of cell division, resulting in a higher chance of aneuploidy. There is evidence linking certain lifestyle choices, such as smoking and alcohol consumption, to a higher likelihood of aneuploidy. Research has indicated that certain lifestyle choices have the potential to affect the integrity and repair mechanisms of DNA, which could potentially lead to abnormalities in chromosomes (Boitrelle et al., 2021).

Age-Related Decline in Oocyte Qualities

The decline in oocyte (egg cell) quality and function with age is a widely revered phenomenon in reproductive biology, and it has a significant impact on female fertility. As women get older, the quality of their eggs decreases, resulting in a decrease in their ability to reproduce. This decline is believed to be caused by a range of factors, such as abnormalities in the genetic makeup, dysfunction in the mitochondria, and changes in the microenvironment of the ovaries.

One of the main reasons behind the decrease in oocyte quality as one gets older is the higher occurrence of chromosomal abnormalities. Chromosomal errors, like aneuploidy, tend to be more common in the oocytes of older women. Research has indicated a gradual rise in the occurrence of chromosomal abnormalities as a woman's age increases (Hunt & Hassold, 2008). These abnormalities may result in unsuccessful fertilization, miscarriages, or the birth of offspring with chromosomal disorders, like Down syndrome.

Age-related decline in oocyte quality is closely linked to mitochondrial dysfunction. The role of mitochondria in energy production and regulation within the oocyte is of utmost importance. As individuals grow older, mitochondrial function tends to decline, resulting in reduced energy production and a diminished capacity to manage oxidative stress. Research has shown a correlation between mitochondrial dysfunction and suboptimal oocyte quality as well as impaired developmental potential (May-Panloup et al., 2016).

The ovarian microenvironment experiences alterations as one ages, which can affect the quality of oocytes. The process of ovarian aging involves a decrease in the quantity of primordial follicles and a rise in the proportion of atretic (degenerating)

follicles. Changes in the hormonal milieu occur as the ovarian reserve declines, with a decrease in anti-Müllerian hormone (AMH) levels and an increase in follicle-stimulating hormone (FSH) levels. These modifications are a factor in the general decrease in oocyte quality (Sunkara et al., 2011).

Maternal age plays a significant role in fertility and can have negative effects on reproductive outcomes. Multiple studies have provided evidence of a strong link between the age of the mother and a decrease in the quality of oocytes. This decline impacts both natural conception and the outcomes of assisted reproductive technologies (ART) (Te Velde and Pearson, 2002). ART procedures, such as *in vitro* fertilization (IVF), also demonstrate a decline in success rates as maternal age increases, emphasizing the significance of oocyte quality in achieving reproductive success (Sunkara et al., 2011).

In addition, the decline in oocyte quality with age is a complex process that is influenced by various genetic, mitochondrial, and environmental factors. Gaining insight into the mechanisms behind this decline is essential in order to devise strategies that can safeguard fertility and enhance reproductive outcomes for women as they grow older (Maiato & Silva, 2023).

Age-Related Decline in Sperm Qualities

The decline in sperm quality that occurs with age is a widely recognized phenomenon that has been thoroughly investigated in the field of reproductive medicine. Several scientific studies have presented evidence suggesting a link between increasing age and various sperm parameters. It's worth mentioning that while fertility is commonly linked to the age of women, the age of men also has a notable impact on reproductive outcomes (Jensen et al., 2015).

One important factor in the decline of sperm quality with age is the concentration of sperm. Research conducted by Jensen et al. (2015) has revealed a consistent decline in sperm concentration as men grow older. In this meta-analysis, data from over 15,000 men was examined, revealing a notable decrease in sperm concentration of about 1.4% annually (Jensen et al., 2015).

Also, it has been shown that sperm motility, a crucial aspect of male fertility, declines as individuals grow older. A previous study revealed a distinct correlation between advancing age and a decline in sperm motility. The scientists examined sperm samples from a substantial group of men and noticed a progressive decrease in motility and a corresponding rise in non-progressive motility as they grew older (Chiang et al., 2010).

As individuals grow older, the quality of sperm can be influenced, leading to morphological abnormalities such as misshapen or structurally defective sperm. A study conducted by Kidd et al. 2012 revealed a rise in the occurrence of sperm morphological abnormalities in men who are above the age of 40. The researchers proposed that these irregularities might play a role in reduced fertility and a heightened likelihood of miscarriage (Kidd et al., 2012).

Furthermore, researchers have explored genetic factors associated with sperm quality in relation to the ageing process, in addition to the conventional sperm parameters, they revealed a correlation between older paternal age and a higher likelihood of de novo mutations in the offspring. This highlights the significance of taking into account not just immediate reproductive outcomes, but also the potential long-term impacts on the health of the offspring.

Various mechanisms have been suggested to account for the decline in sperm quality that occurs with age. Oxidative stress arises from an imbalance between reactive oxygen species (ROS) and antioxidant defences, representing a significant mechanism. Previously conducted studies have shed light on the impact of oxidative stress on the deterioration of sperm DNA and membranes, which is believed to be a contributing factor to the decline in sperm quality associated with ageing (Chiang et al., 2010)

Changes in hormone levels that occur with age, specifically in relation to testosterone, could potentially contribute to a decrease in the quality of sperm. Previous research has examined the effects of age-related changes in serum testosterone on sperm parameters, indicating a possible connection between decreasing testosterone levels and impaired sperm quality.

It's important to recognize that there are differences among individuals, and not all men will see a major decrease in sperm quality as they get older. Nevertheless, the collective discoveries from numerous studies emphasize the significance of taking male age into account when it comes to reproductive health and family planning. It can be helpful for couples who are trying to conceive to gain insight into how the age of the father can affect fertility and the outcomes of reproduction. (Lim & Kaldis, 2013).

Maturation Of Oocytes: Classification Of Oocyte MI And MII Criteria for IVF Implantation

In the area of assisted reproductive technologies like in vitro fertilization (IVF), the maturation of oocytes plays a crucial role in determining the success of fertilization. There are two primary stages of oocyte maturation: the Germinal Vesicle (GV) stage, also referred to as M1, and the Metaphase II (MII) stage.

M1 Phase

During this stage, oocytes have a germinal vesicle that is visible in their nucleus. At this stage, the oocyte is in a state of meiotic arrest. This organism is not fully developed and is unable to undergo fertilization. In IVF procedures, oocytes are commonly collected during the M1 stage and then cultured until they reach the MII stage before fertilization can take place. A study conducted by Telfer et al. (2019) highlighted the significance of comprehending the elements that influence oocyte maturation, as it has a direct impact on the effectiveness of IVF procedures (Telfer, 2019).

M2 Phase

The M2 phase, represents the fully developed state of the oocyte, having gone through meiosis I and being prepared for fertilization. At this stage, oocytes have expelled the first polar body, which signals their preparedness for fertilization by a sperm. In IVF, mature M2 oocytes are carefully chosen for fertilization to enhance the likelihood of successful embryo development.

Research conducted by Ebner et al. (2016) has shed light on the importance of MII oocyte selection in IVF outcomes, underscoring the necessity for precise classification and evaluation. Having a clear understanding of the maturation status of oocytes is vital

when it comes to optimizing IVF protocols. Methods like controlled ovarian stimulation and precise timing for oocyte retrieval are crucial in maximizing the number of M2-stage oocytes available for fertilization.

Ultimately, the categorization of oocytes into M1 and M2 stages plays a crucial role in the realm of assisted reproductive technologies, specifically in the context of IVF. Accurate identification and careful selection of M2 oocytes play a crucial role in the success of IVF procedures, ultimately influencing the rates of pregnancy and live birth outcomes (Munné et al., 2003).

Intracytoplasmic Sperm Injection (ICSI)

An advanced assisted reproductive technology (ART) technique called Intracytoplasmic Sperm Injection (ICSI) was developed to help couples dealing with male infertility issues. In contrast to traditional in vitro fertilization (IVF), where the sperm and egg are combined in a dish and fertilization occurs naturally, ICSI entails the direct injection of a single sperm into an egg. This procedure is especially helpful for couples dealing with male-factor infertility, where the sperm may encounter challenges in entering the egg membrane (De Rycke et al., 2020).

Abnormal chromosome numbers in embryonic cells have been widely recognized as a contributing factor to infertility and recurrent pregnancy loss. Maternal age plays a crucial role in aneuploidy, especially when it comes to assisted reproductive technologies. Abnormal chromosomal content in embryos can have a significant impact on the success rates of ART, such as ICSI. This can result in implantation failure or miscarriage, affecting the overall outcome (Handyside, 2020).

Numerous studies have investigated the correlation between ICSI and aneuploidy. Although ICSI does not directly raise the risk of aneuploidy, the quality of sperm and the development of embryos can influence the chromosomal integrity of embryos created using ICSI. Considering the genetic health of embryos before transfer is of utmost importance for clinicians, as it greatly enhances the likelihood of a successful pregnancy (Gleicher et al., 2015).

There are various factors that can potentially contribute to the higher risk of aneuploidy in embryos conceived through ICSI. One possible factor to consider is the selection of sperm with compromised genetic integrity during the ICSI process. Abnormalities in sperm, such as fragmentation of DNA or defects in chromatin packaging, may play a role in the occurrence of aneuploidies in embryos.

A study conducted by Chen et al. (2018) examined the effects of sperm DNA fragmentation on the occurrence of aneuploidy in embryos obtained through ICSI. The researchers discovered a notable link between elevated levels of sperm DNA fragmentation and a higher occurrence of aneuploidy in embryos (Chen et al., 2018).

Pre-Implantation Genetic Testing for Aneuploids (PGT-A)

Pre-Implantation Genetic Testing for Aneuploids (PGT-A), previously referred to as Pre-Implantation Genetic Screening (PGS), is a reproductive technology that aims to detect numerical chromosomal abnormalities in embryos prior to their transfer to the uterus in in vitro fertilization (IVF) procedures (Harper et al., 2012). Understanding aneuploidy, which refers to an abnormal number of chromosomes in a cell, is essential in preventing miscarriages and developmental disorders in embryos. This is why preimplantation genetic testing for aneuploidy (PGT-A) is a vital tool in enhancing the effectiveness of assisted reproductive technologies (ART) (Gleicher et al., 2015).

Although ICSI has brought about a significant advancement in addressing male infertility, there are still lingering concerns about the potential risk of aneuploidy. Continual research is focused on improving sperm selection methods and optimizing laboratory conditions to reduce the potential genetic implications linked to ICSI. Scientists and medical professionals are constantly seeking ways to enhance the effectiveness of this method, guaranteeing the successful birth of healthy babies using assisted reproductive technologies (Bartoov et al., 2003).

Advancements in PGT-A technology

Over time, advancements in technology have greatly enhanced the precision and dependability of PGT-A. Conventional techniques, such as fluorescence in situ hybridization (FISH), had their limitations in terms of scope and accuracy. Modern

techniques like next-generation sequencing (NGS) and comparative genomic hybridization (CGH) have revolutionized the field of chromosomal analysis, enabling the detection of aneuploidies with unprecedented accuracy (Capalbo et al., 2017).

Using PGT-A helps in choosing embryos with a normal chromosomal makeup for transfer, which improves the chances of a successful pregnancy. This technology is especially advantageous for couples who have experienced multiple pregnancy losses, are older mothers, or have had repeated failures with IVF (Capalbo et al., 2017). Through the identification and transfer of euploid embryos, PGT-A strives to minimize the risk of implantation failure and miscarriage, leading to enhanced success rates for IVF procedures.

Some Drawbacks of PGT-A

Although PGT-A has the potential for numerous benefits, it has not been immune to criticism and controversy. There are differing opinions regarding the potential impact of the procedure on live birth rates. It has been suggested that false-positive results could potentially result in the disposal of viable embryos. In addition, there have been concerns regarding the potential adverse effects of biopsy procedures on the viability of embryos. There are ethical concerns surrounding the practice of selectively choosing embryos based on genetic information, as it may lead to unintended consequences and the creation of "designer babies" (Molyneaux et al., 2001).

Costs and Accessibility

Dealing with the expenses is a major hurdle when it comes to PGT-A. Using advanced sequencing techniques in IVF cycles can greatly impact the overall expenses. Therefore, certain couples may face limitations in accessing PGT-A, which could potentially worsen the disparities in fertility treatment access. Given the increasing prevalence of reproductive technologies, it is crucial to carefully consider the economic aspects of PGT-A (Fragouli et al., 2018).

Future Directions and Research

Continuing research is focused on tackling the restrictions and debates surrounding PGT-A. Research is currently focused on enhancing the precision of genetic

analysis, reducing the potential harm to embryos during biopsy procedures, and fine-tuning the criteria for embryo selection (Capalbo et al., 2017). Long-term studies examining the results of children born through PGT-A will offer valuable insights into the safety and effectiveness of this technology.

Pre-Implantation Genetic Testing for Aneuploids (PGT-A) is a remarkable breakthrough in reproductive medicine that brings hope to couples dealing with fertility issues. Just like a microbiologist, it's important to continue researching and considering the ethical implications of this technology to make sure it's used effectively and responsibly. With the ever-changing nature of the field, it is of utmost importance to adopt a well-rounded approach in order to guarantee the responsible and ethical utilization of PGT-A for the achievement of successful and healthy pregnancies (Capalbo et al., 2017).

Oocyte Quality and Integrity Criteria for Successful IVF Implantation

Thanks to advancements in assisted reproductive technologies, in-vitro fertilization (IVF) has become a game-changer for couples facing infertility, providing them with a renewed sense of hope. The outcome of IVF is heavily influenced by the quality of the oocytes utilized in the procedure. The integrity of oocytes plays a crucial role in determining the success of implantation, and it is vital to evaluate different criteria to achieve the best possible results.

Morphological characteristics

Assessing oocyte integrity in IVF primarily involves evaluating morphological characteristics. Healthy oocytes typically exhibit a round shape, uniform cytoplasm, and a clearly visible polar body. Having an intact zona pellucida and no cytoplasmic vacuoles are also signs of high-quality oocytes, as mentioned in a study by Jones et al. (2019). Assessing the physical characteristics of oocytes is an essential method used by embryologists to choose healthy ones for fertilization (Jones et al., 2020).

Maturation Stage

The maturation of oocytes plays a vital role in the success of IVF implantation. Immature oocytes collected during the retrieval process may have limited developmental potential. Thus, it is crucial to evaluate the stage of development. Assessing the

morphology of cumulus-oocyte complexes, evaluating nuclear maturation, and identifying the presence of the first polar body are crucial factors in determining the maturity of oocytes. Optimal maturation is linked to higher rates of fertilization and enhanced quality of embryos (Sirard, 2018).

Cytoplasmic Characteristics

The composition of the cytoplasm in an oocyte plays a crucial role in ensuring successful implantation. The development of embryos is influenced by factors such as mitochondrial function, cytoplasmic granulation, and the distribution of organelles (Sathananthan et al., 2020). The presence of large cytoplasmic vacuoles can have a significant impact on fertilization rates and embryo quality, highlighting the need for a comprehensive cytoplasmic evaluation.

Zona Pellucida Assessment

Ensuring the zona pellucida remains intact is crucial for the successful process of fertilization and implantation. An intact zona pellucida plays a crucial role in allowing sperm to penetrate during fertilization and also acts as a protective barrier for the developing embryo. Any irregularities or thinning of the zona pellucida can potentially affect the viability of the embryo. Thoroughly analysing and confirming the zona pellucida is crucial for maintaining the quality of oocytes (Castro et al., 2015).

Advancements in preimplantation genetic testing (PGT) have made it possible to assess the genetic integrity of oocytes. PGT can detect chromosomal abnormalities, aneuploidies, and genetic mutations, offering valuable insights for embryo selection. Incorporating genetic screening into the evaluation of oocyte quality improves the overall success rate of IVF implantation (De Rycke et al., 2020).

In addition, a thorough assessment of oocyte integrity is crucial for ensuring the successful implantation of IVF. By incorporating morphological assessments, maturation stage, cytoplasmic characteristics, zona pellucida integrity, and genetic screening, a holistic approach can be taken to choose superior oocytes. This comprehensive assessment not only increases the likelihood of successful fertilization but also improves embryo development and boosts the success rates of implantation in IVF (De Rycke et al., 2020).

Sperm Quality and Integrity Criteria for Successful IVF Implantation

The success of IVF heavily relies on the quality and integrity of the sperm utilized for fertilization. The viability and fertilization potential of sperm are influenced by a range of parameters. This review explores the essential factors involved in evaluating sperm integrity for IVF implantation, providing insights into the complex elements that influence positive results.

Sperm Morphology

Assessing sperm integrity in IVF involves considering morphology, which pertains to the size and shape of sperm cells. Irregular sperm shape can impede the process of fertilization and decrease the likelihood of successful implantation. Accurate assessment of sperm morphology is achieved through the use of established criteria, such as the Kruger strict criteria. Having sperm that are morphologically normal is essential for ensuring successful interaction with the egg, penetration, and ultimately, successful implantation (Lim & Kaldis, 2013).

Sperm Motility

Motility is an essential factor that greatly affects the quality of sperm in IVF. Progressive motility plays a crucial role in enabling sperm to successfully navigate the female reproductive tract, reach the egg, and facilitate the process of fertilization. Using computer-assisted sperm analysis (CASA), a quantitative assessment of sperm motility can be obtained, providing valuable information on the percentage of sperm that exhibit progressive movement. Understanding and analysing motility parameters is essential in predicting the potential for fertilization and the subsequent development of embryos (Lim & Kaldis, 2013).

Concentration of Sperm

The concentration of sperm plays a crucial role in determining the outcome of IVF procedures. A low sperm concentration can hinder the chances of successful fertilization as there may not be enough sperm available to fertilize the eggs. The World Health Organization (WHO) offers guidelines for normal sperm concentration, highlighting the significance of optimal levels for successful IVF outcomes. Assessing sperm

concentration assists in choosing the most promising sperm for fertilization (Boitrelle et al., 2021).

Integrity of DNA

Evaluating the integrity of sperm DNA is crucial in determining the quality of embryos and the likelihood of successful implantation in IVF. There are several factors that can lead to DNA fragmentation, such as oxidative stress, infections, and the natural process of ageing. Methods like the sperm chromatin structure assay (SCSA) and the terminal deoxynucleotidyl transferase dUTP nick-end labelling (TUNEL) assay are used to assess levels of DNA fragmentation. Preserving the integrity of sperm DNA is crucial in order to prevent any potential genetic abnormalities and to increase the chances of successful implantation (Evenson et al., 2002).

Sperm Viability

The viability of sperm, which refers to the ratio of live and dead sperm, plays a vital role in the success of IVF procedures. Healthy and functional sperm are crucial for successful fertilization, as they determine the overall viability and reproductive potential. Different staining techniques, like the eosin-nigrosin staining method, are used to distinguish between live and dead sperm cells. Choosing healthy sperm is crucial for ensuring successful implantation and the subsequent development of the embryo (Boitrelle et al., 2021).

Ultimately, the evaluation of sperm integrity is a complex procedure that encompasses the examination of various factors, including morphology, motility, concentration, DNA integrity, and viability. Thoroughly following strict criteria and utilizing cutting-edge laboratory techniques guarantees the choice of top-notch sperm for IVF procedures, ultimately impacting the success of implantation. Understanding and addressing these criteria can greatly enhance outcomes in assisted reproductive technologies, providing a renewed sense of hope for couples struggling with infertility (Boitrelle et al., 2021).

Mathematical models used in scientific research

Mathematical models, hypotheses and theorems are of paramount importance in diverse facets of medical study. These tools assist researchers in the description, analysis, and prediction of intricate biological systems and occurrences. In the field of medical research, a variety of mathematical models and theorems are frequently employed.

Some of these are highlighted below:

Epidemiological Models

SIR (Susceptible infectious removed)

The compartmental model is employed for the analysis of the transmission dynamics of infectious illnesses within a given population. The population is partitioned into three distinct compartments, namely susceptible, infectious, and removed, which encompasses individuals who have either recovered or perished (Panjeti & Real, 2011).

SEIR

Early models for studying diseases, classified infirmities under the “SEIR” model framework, is a widely used mathematical framework for studying the spread of infectious diseases. The compartmental model is employed for the analysis of the transmission dynamics of infectious illnesses within a given population. The population is partitioned into three distinct compartments, namely - Susceptible (S), Exposed (E), Infectious (I), and Recovered / Removed (R), (which encompasses individuals who have either recovered or perished) (Panjeti & Real, 2011).

The SEIR model, which is an expansion of the SIR model, incorporates an additional compartment known as the exposed compartment. This compartment accounts for individuals who have encountered the disease but have not yet reached the infectious stage.

Clinical Trials and Statistical Inference

Statistical methods and models play a crucial role in clinical trials when assessing the efficacy of medical interventions. Randomized Controlled Trials (RCTs) are carefully designed to ensure impartial estimates of treatment effects. Neyman and Pearson established the groundwork for contemporary statistical hypothesis testing (Panjeti & Real, 2011).

Survival Analysis

Survival analysis is frequently employed in medical research to examine the duration until a significant event takes place, such as patient survival or relapse. The Kaplan-Meier estimator and Cox proportional hazards model are commonly used in this field. In 1958, Kaplan and Meier introduced a non-parametric estimator for survival functions, while in 1972, Cox developed the proportional hazards model (Panagiotopoulou, 2009).

Mathematical Modelling in Imaging and Diagnostics

Mathematical models play a crucial role in improving image reconstruction, segmentation, and analysis in the field of medical imaging and diagnostics. The Radon transform, which is crucial in computed tomography (CT), was first introduced by Radon in 1917. In magnetic resonance imaging (MRI), the Fourier Transform is crucial for image reconstruction, as it was first introduced by Ernst and Lauterbur (Panagiotopoulou, 2009).

Pharmacokinetic and Pharmacodynamic Models

Mathematical models are utilized to explain the processes involved in drug absorption, distribution, metabolism, and excretion, as well as their impact on the body. The Hill equation is frequently employed in modelling dose-response relationships. The concept of the therapeutic window, first introduced by Paul Janssen in the 1960s, plays a crucial role in optimizing drug dosages (Panagiotopoulou, 2009).

Bio-mechanical models

These are computational tools utilized in the field of biomechanics to simulate and analyse the mechanical behaviour of biological systems.

Finite Element Analysis (FEA)

The FEA is a computational method widely utilized in the field of biomechanics to model and evaluate the mechanical response of biological structures, including bones and tissues, across various scenarios. Musculoskeletal Models: Mathematical models are employed for the purpose of comprehending the mechanical aspects of muscles, joints, and the skeletal system, hence facilitating the development of prosthetics and rehabilitation approaches (Panagiotopoulou, 2009).

Mathematical models in genetic research

Studies in genetics utilize a range of mathematical models to gain insights into the fundamental principles that govern inheritance, evolution, and the intricate interactions within biological systems. These models are essential for understanding the complexities of genetic information and making predictions in various situations. In this discussion, we will delve into various mathematical models utilized in genetic studies, offering valuable insights into their practical applications and overall importance.

An essential concept in genetic studies is the Hardy-Weinberg equilibrium (HWE), which was formulated independently by Godfrey Hardy and Wilhelm Weinberg in 1908. The HWE model explains the distribution of genetic variations in a population under certain conditions, including no selection, no mutation, no migration, random mating, and a large population size. Deviations from the Hardy-Weinberg equilibrium can reveal important information about population dynamics, such as the influence of natural selection, genetic drift, or migration (Slatkin, 2008).

Linkage disequilibrium (LD)

This is a vital concept in genetic modelling, as it refers to the non-random association of alleles at different loci. LD is shaped by various factors, including recombination, mutation, and selection. Several statistical measures, such as D' and r^2, are

used to assess the degree of linkage disequilibrium. LD plays a crucial role in comprehending the inheritance patterns of genes and identifying genetic markers linked to particular traits or diseases (Slatkin, 2008).

Models in population genetics, like the Wright-Fisher model and the coalescent theory, offer valuable insights into how genetic variation changes within populations as time goes on. The Wright-Fisher model explores the phenomenon of genetic drift in populations of limited size, while the coalescent theory delves into the historical lineage of gene copies by tracing them back in time. These models are crucial for gaining insights into the factors that influence the shaping of genetic diversity, including population size, migration, and mutation rates (Manolio et al., 2009).

Statistical models

These models are utilized in quantitative genetics to gain insights into the underlying genetic factors that contribute to complex traits. The twin study model, for example, assesses the heritability of traits by comparing the similarities between monozygotic and dizygotic twins. Structural Equation Modeling (SEM) and Genome-Wide Association Studies (GWAS) are also utilized to discover the genetic variants linked to complex traits and diseases (Manolio et al., 2009).

Evolutionary models

Like the Fisherian runaway model and the Price equation, provide valuable insights into the mechanisms that shape the evolution of phenotypic traits and social behaviors. The Fisherian runaway model elucidates the evolution of exaggerated traits through sexual selection, while the Price equation offers a framework for comprehending the changes in gene frequency over generations (Visscher et al., 2008).

Ultimately, the utilization of mathematical models in genetic studies provides a wide range of benefits, allowing for a deeper comprehension of inheritance, population dynamics, and evolution. These models allow researchers to make predictions, test hypotheses, and uncover the underlying factors of various traits and diseases. With the rapid progress of technology, the combination of mathematical modelling and empirical

data is constantly improving our grasp of genetics, leading to exciting new breakthroughs in this area of study.

CHAPTER III

Methodology

Materials and Methods

A retrospective analysis of data collected from individuals who had undergone in vitro fertilization therapy at the British Cyprus IVF Hospital between the years 2016 and 2021 was conducted for the purpose of this study. A total of 4123 embryos were gathered from 765 different couples, which resulted in the collection of information. These embryos were put through a series of tests that included preimplantation genetic diagnosis and next-generation sequencing before they were put through the vitrification process. Permission to proceed with the experiment was granted by the Institutional Review Board (IRB) of the Near East University.

Ovarian stimulation

Controlled ovarian hyperstimulation (COH) was performed using a GnRH antagonist method. On the day of the menstrual cycle, the patient received recombinant FSH (150–300 IU, Gonal F, Serono) and/or hMG (75–150 IU, Merional, IBSA). The ovarian response to stimulants was monitored using transvaginal ultrasonography and biochemical screening of blood progesterone (P4) and estradiol (E2) levels after the sixth day of stimulation. A daily dose of 0.25 mg of GnRH antagonist (Cetrotide, serono) was administered until the day of ovulation trigger, which is determined when the leading follicle reaches a size greater than 13 mm. Subjects were administered either 250 mg of hCG (Ovitrelle, serono) or 0.2 mg of triptorelin (Gonapeptyle, ferrin) in order to stimulate ovulation. Oocyte retrieval was planned to take place 35 hours after this dosage.

Oocyte retrieval and denudation

The follicular content was extracted on Day 0 (oocyte collection). This procedure was carried out as performed by Ahmed et al. (2023) in their study “Investigation of BAK, BAX and MAD2L1 Gene Expression in Human Aneuploid Blastocysts.

Semen Analysis, Intracytoplasmic Sperm Injection (ICSI), Embryo Culture, and Biopsy

The methodology for preparing semen samples followed the protocol outlined by Coban et al. (2020). A single sperm cell was identified for intracytoplasmic sperm injection (ICSI) using a 10μ PVP (Polyvinylpyrrolidone) solution containing 7% HSA (Human Serum Albumin), product number 90121, from Irvine Scientific in the United States.

During the fertilization check carried out 16-18 hours after the procedure, the presence of two polar bodies and two pronuclei was observed, which is considered a normal indication of fertilization. The assessment of the embryonic morphology on the third day was conducted utilizing a grading system as described by Ciray et al (2012). This approach considers many factors such as the blastomere count, blastomere uniformity, level of fragmentation, nucleus features, and cytoplasm properties in order to complete the assessment. A morphological examination of the embryos on days 5 and 6 was undertaken, adhering to the parameters established by Gardner and Schoolcraft (1999). This evaluation considered the level of development of the embryos, as well as the properties of the inner cell mass (ICM) and trophectoderm (TE) cells.

Parental Parameters

The maternal features of female partners were assessed based on the number of MI and MII oocytes extracted, referred to as MI oocyte number and MII oocyte number, respectively, as well as the number of follicles.

The current study considered sperm count, morphology, and motility as parameters related to male reproductive health. When determining the standards for different situations, the assessment of these elements was conducted in accordance with the latest guidelines from the World Health Organization (WHO).

Embryo Grading System

Two methodologies were employed in this study to assess the quality of e mbryos. A substantially altered iteration of Ciray and colleagues' method was utilized for the assessment of day 3 (d3) embryos (Ahmed et al., 2023). This module included five factors:

the total cell count, the condition of the nucleus, the rate of fragmentation, the symmetry of the cytoplasm, and the visual observant of the cytoplasm. More precisely, the total quantity of cells corresponds to the number of cells in the embryo on the third day following fertilization (Ahmed et al., 2023). The phrase "nuclei status" refers to the presence of either a single nucleus (T) or many nuclei (Y) within the cells of the embryo's morula. At this stage, it is preferable for each cell to have only one nucleus, therefore a score of T is preferred. In the current study, this variable is referred to as D3. Furthermore, the fragmentation ratio of the embryos was recorded. An embryologist assessed the quality of an embryo by quantifying the degree of fragmentation, which was expressed as a numerical value ranging from 0 to 70. Fragmentation is considered undesirable, and the optimal outcome for this attribute is indicated by a score of 0, whereas the lowest grade is indicated by a score of 70. Subsequently, a microscope was employed to observe the symmetry of the cytoplasm. The cytoplasmic distribution of an embryo was classified as U if it was irregularly distributed, but an even distribution of cytoplasm was denoted as E for morula cells. In this study, the criterion referred to as D3, which pertains to embryo quality, is associated with an equitable distribution of embryos. In the end, the cytoplasm's visual appearance was evaluated as an A grade due to its pristine condition, resulting in visibly brighter embryos. Conversely, embryos exhibiting cytoplasm with granules were categorized as having a K grade due to their darker appearance during microscopic analysis. Clear cytoplasm is the most favorable condition for day 3 embryos. Therefore, an embryo with a grade of A indicates higher quality. Considering all factors, 8TOEA would be an ideal grade on day three.

The Gardner criteria were employed to assess the quality of the embryos on days 5 (d5) and 6 (d6). Consequently, three characteristics were assessed in the embryos: expansion, the quality of the inner cell mass (ICM), and the trophectoderm quality (TE). The expansion's quality is assessed using a rating scale ranging from 1 to 6. An embryo with a grade of 6 is considered to be of superior quality, having successfully emerged from the zona pellucida layer. Conversely, an embryo with a grade of 1 is seen as being of inferior quality. Furthermore, the sequential letters A through C are employed for the evaluation of the trophectoderm's cellular composition. A grade A trophectoderm, also known as a high-quality trophectoderm layer, is distinguished by a profusion of cells on

the outer surface of the blastocyst. On the other hand, C represents a lower quality level of the trophectoderm. The assessment of the inner cell mass is conducted using a comparable approach as the final quality parameter. An A grade signifies a high-quality inner cell mass with numerous tightly concentrated cells, resembling TE grading, and is primed to initiate the development of the embryo itself. In addition, an ICM grade of C signifies a low-quality ICM with a limited quantity of cells. In general, embryos that have a rating of 6AA according to the Gardner criteria are considered to be of the best quality, whilst embryos with a rating of 1CC are considered to be of the lowest quality.

PGT-A Analysis

A private genetics laboratory using Next-Generation Sequencing (NGS) technology to conduct genetic analyses of the embryos, with the aim of detecting chromosomal abnormalities. The Ion ReproSeq PGS kit was used to perform PGT-A studies, screening all 24 chromosomes for aneuploidies.

Linear Regression Model

Through the use of linear regression analysis, which is dependent on the value of another variable, it is possible to make predictions regarding the value of a variable. The variable that you are attempting to forecast or predict is referred to as having a dependent variable. One of the variables that is used to make a prediction about the value of another variable is referred to as the independent variable (Glasserman, 2001).

One method of analysis involves identifying the coefficients of a linear equation by employing one or more independent variables that are most effective in predicting the value of the dependent variable. This method of analysis is also known as the linear regression method. Fitting a line or surface that minimizes the differences between the predicted and actual output values is the goal of linear regression, which entails fitting any line or surface. The "least squares" method is utilized by certain simple linear regression calculators in order to determine the ideal line of greatest fit for a given set of paired data from which to draw conclusions. Next, you will make an estimate of the value of X, which is the dependent variable, by using Y as the independent variable. This will allow you to make any necessary adjustments (Glasserman, 2001).

Chi-Square Test

The Chi-square ($\chi 2$) test is a valuable instrument that may be utilized to compare the outcomes of an experiment with the theoretical predictions that are derived from a hypothesis. The actual difference between the frequencies that were observed and those that were anticipated is what the Chi-square test measures. There is no doubt that the use of such a measure is necessary in sample research in order to conduct an in-depth analysis of the differences that exist between theoretical expectations and actual observations (Singhal & Rana, 2015).

Information on categorical variables, which may be thought of as a series of counts, is frequently gathered for the purpose of medical research. An arrangement of tabular data that is frequently used for the purpose of organizing and presenting counts is known as a contingency table. In order to determine whether or not there is a connection between the rows and columns of a contingency table, the chi-square test statistic can be utilized. To be more specific, this statistic can be applied to ascertain whether or not there are any differences in the proportions of the relevant risk factor between the study groups. The chi-square test and the methodology for evaluating hypotheses are both credited to Karl Pearson, who is also held responsible for their conception (Singhal & Rana, 2015).

CHAPTER IV

Results

A total of 4123 embryos were gathered from 766 different couples. The average maternal age was 43 and the average paternal age was 45years of age. A total of 639 MI and 25545 MII oocytes were obtained.

In the first part of the analysis, female and male age were investigated in relation to the euploidy and aneuploidy status (Equation 1). There was no significant difference in outcomes between groups (Chi-Squared Statistic: 1.9231; Degrees of Freedom: 1 and p-value: 0.83448, Figure 1). The correlation between female age and the euploidy status was − 0.20072 and the correlation between male age and outcome was 0.14376, respectively.

Equation 1

Female and Male age analysis in relation to euploidy and aneuploidy status

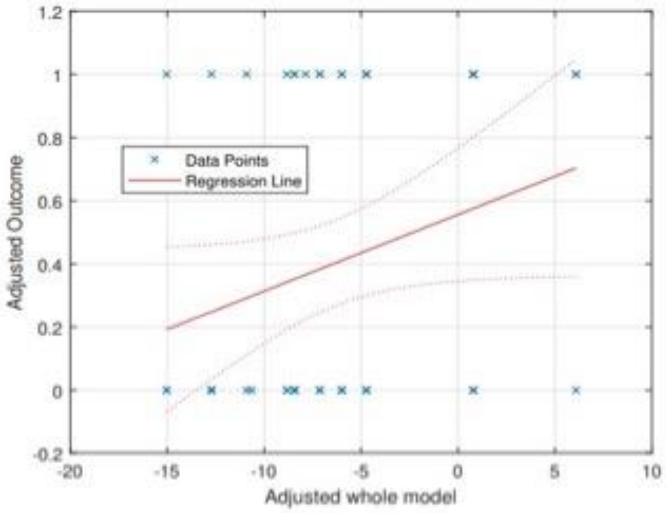
$$\chi^2 = \sum \frac{(O_{ij} - E_{ij})^2}{E_{ij}}$$

The linear regression analysis results showed that the intercept was at 0.55625. The intercept (0.55625) represents the estimated outcome when both Female Age and Male Age are zero. The negative coefficient for Female Age (-0.019974) suggests a decrease in the predicted outcome with an increase in female age. The positive coefficient for Male Age (0.013689) indicates a slight increase in the predicted outcome with an increase in male age. The model fit does not explain a significant proportion of the variability in the outcome (low R-squared). The p-value for the F-statistic suggests that the model as a whole is not statistically significant. Thus, from this model, age alone does not appear to be a strong predictor of IVF success based on these analyses. The chi-squared test indicates no significant difference in outcomes between groups. The

correlations and regression coefficients, while providing some associations, are weak. Additional variables and more extensive data may contribute to a more comprehensive understanding of the factors influencing IVF outcomes.

Figure 1

Regression analysis



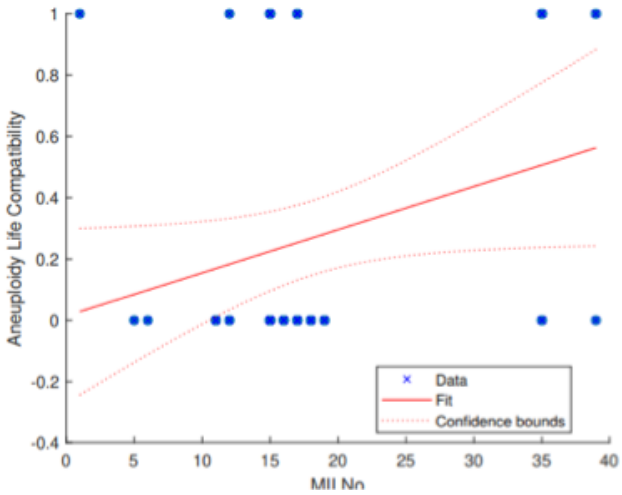
In the second part of the analysis, only the female age was investigated in relation to aneuploidy status. The negative coefficient (-0.033504) suggests that as the female age increases, the aneuploidy status also increases. Advanced maternal age is known to be associated with an increased risk of chromosomal abnormalities in embryos. The negative correlation aligns with this understanding, indicating a potential biological connection.

Further analysis was performed for the follicle number and MII number in correlation with aneuploidy status (Figure 2). The positive coefficient (0.01225) of follicle number and (0.014066) MII oocyte number suggests that an increase in follicle number is associated with euploidy, respectively. One possible explanation could be that more follicles are associated with higher chances of successful fertilization, leading to embryos

with better chromosomal compatibility. Furthermore, more mature oocytes may indicate better reproductive health, leading to higher chances of producing chromosomally normal embryos. The positive coefficient implies that as the number of follicles increases, there is a corresponding increase in the dependent variable. This is biologically meaningful, as a higher number of follicles could be associated with a greater number of available eggs, potentially enhancing the chances of success in IVF.

Figure 2

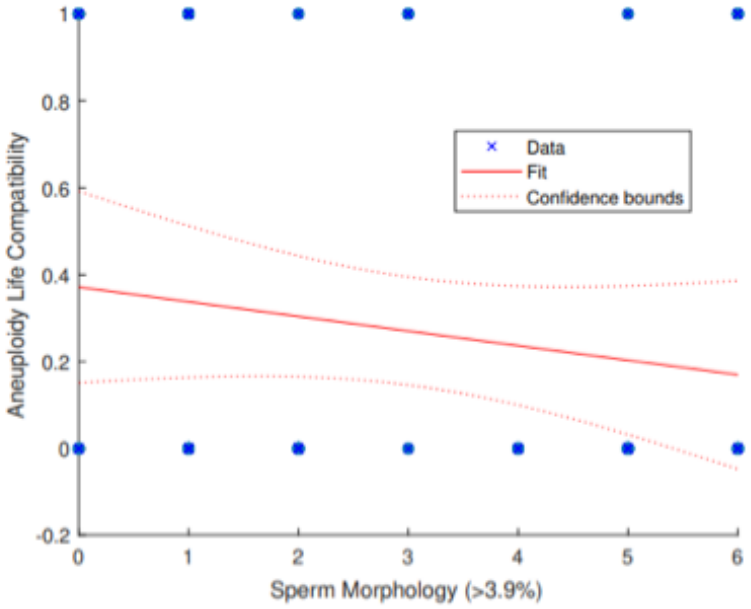
Regression analysis of MII number over aneuploidy



Analysis of the male parameters showed that the negative coefficient (-0.033665) suggests that an increase in abnormal sperm morphology is associated with lower euploidy status (Figure 3). Abnormal sperm morphology may indicate issues with sperm quality, potentially contributing to chromosomal abnormalities in embryos. The negative correlation supports the biological expectation.

Figure 3

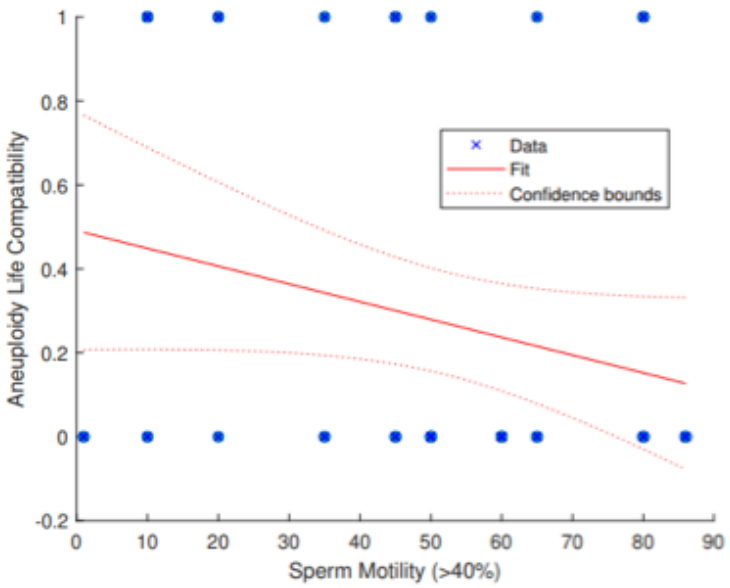
Regression analysis of sperm morphology over aneuploid life compatibility



The negative coefficient implies that as sperm motility increases, there is a decrease in the dependent variable. Again, this result is unexpected, as higher sperm motility is typically associated with better fertility. Further examination and consideration of other factors are needed to understand this relationship in the context of IVF.

Figure 4

Regression analysis of sperm motility over aneuploidy



The negative coefficient indicates that as sperm count increases, there is a decrease in the dependent variable (Figure 4). This result might be counterintuitive and warrants further investigation, as a higher sperm count is generally expected to be advantageous in IVF procedures.

Figure 5

Regression analysis of sperm number over aneuploidy

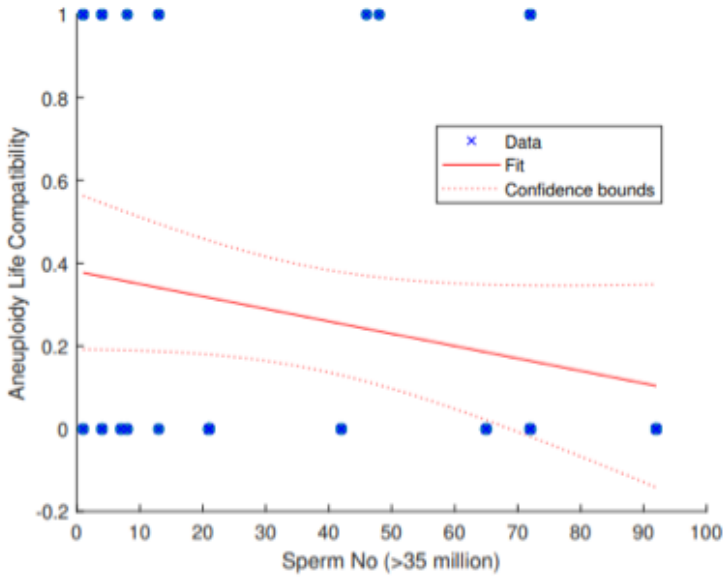
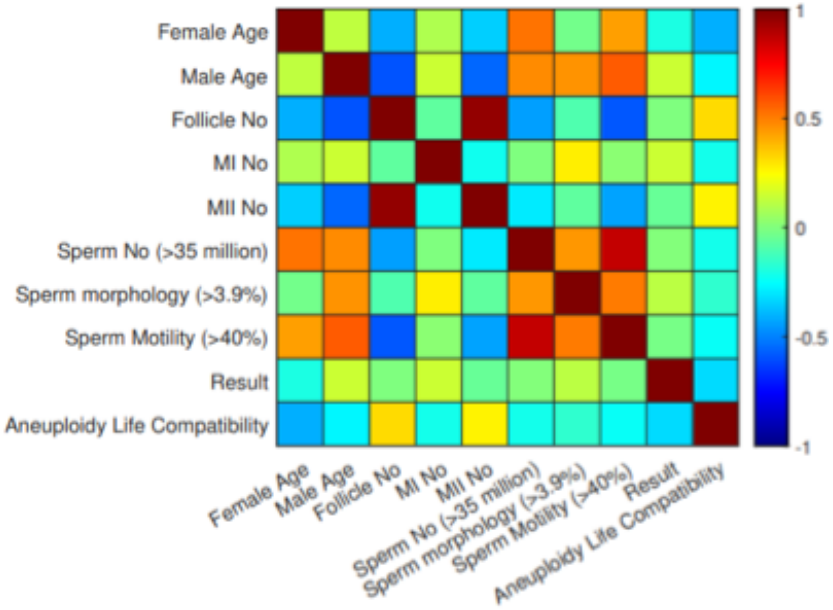


Figure 5 shows the regression analysis of sperm number and aneuploidy and figure 6 shows the correlation matrix of all the parameters that were investigated in this study.

Figure 6

Correlation matrix



CHAPTER V

Discussion

One of the known challenges that must be overcome in order to achieve successful reproduction is the documented increase in aneuploidy that occurs as the mother's age increases. It is possible for a woman's maternal aneuploidy to reach 80% by the time she reaches the age of 45. This is a large rise that occurs while she is getting older. Complete chromosomal aneuploidy is mostly brought on by mistakes that occur during the meiotic process in human embryos (Demko et al., 2016). The majority of these components are derived from the mother and emerge during the several stages of egg development that are quite complicated. These occurrences are largely caused by uneven predivision, which results in the early separation of sister chromatids and the subsequent missegregation of those chromatids. Nondisjunction, on the other hand, is the failure of homologous chromosomes or sister chromatids to separate from one another (Rabinowitz et al., 2012).

During the female foetal stage, the ovary contains approximately six to seven million oocytes. After maturity, only a small portion of oocytes, specifically 400-500, retain their fertility, since the remainder undergo apoptosis, a process of programmed cell death. During the process of reproduction, the loss of oocytes occurs at a constant rate, and the rate of decrease remains consistent with the period before menopause. As women become older, their ovarian reserve and oocyte quality decrease. Ovarian reserve refers to the number of eggs a woman has left, while oocyte quality refers to the ability of the egg to go through the necessary stages of development, be fertilised, and support the growth of a healthy embryo. This drop in ovarian reserve and oocyte quality affects a woman's ability to have a successful pregnancy and give birth to a healthy baby (Chu et al., 2023).

The impact of ageing on ovarian function is not fully elucidated by the age-related decrease in oocyte count. The fertility might also be influenced by an elevated occurrence of aneuploidy or a deterioration in the quality of follicles inside the microenvironment of the oocyte. The mentioned processes include the production of reactive oxygen species, harm to the mitochondria, reduction of telomeres, and changes in methylation. In general,

the process of ovarian ageing results in ovarian failure due to a decline in both the quality and number of oocytes (Seok et al., 2020).

In this study, the general outcome for female age showed a negative coefficient -0.033504 which suggests that as female age increases, the aneuploidy life compatibility decreases which is supported by different researches, revealing that advanced maternal age is associated with an increased risk of chromosomal abnormalities in embryos. This finding supports this claim indicating a biological correlation between maternal age and aneuploidy formation. The Chi-Squared test analysis however did not provide enough evidence for this but the regression analysis did give a much-needed insight to how parental age may impact embryo implantation in IVF and also in terms of aneuploid formation.

Chromosomal abnormalities in spermatozoa occur at a rate of 9%, with 7% being structural abnormalities and 1-2% being numerical abnormalities. These abnormalities are usually produced by meiotic mistakes that happen during the early stages of spermatogenesis. The mistakes encompass chromosomal abnormalities such as aneuploidy (abnormal number of chromosomes) and structural anomalies such translocations, inversions, and duplications. An increased prevalence of sperm carrying X Y aneuploidy, specifically 47, XYY Klinefelter syndrome and 47, XXY Klinefelter syndrome, is observed in older fathers (Luetjens et al., 2002). Furthermore, studies have shown a strong association between the frequency of sperm chromosomes with structural defects and the age of the father. The prevalence of structural chromosomal anomalies in spermatozoa has been determined to be 2.8% in males aged 20 to 24 and 13.6% in males aged 45 and above (Chu et al., 2023).

For this study, the Sperm Morphology (>3.9%) has a negative coefficient (-0.033665) which invariably suggests an increase in abnormal sperm morphology which likely indicate issues with sperm quality and has potential effect in chromosomal abnormalities in embryos. However, the observed higher number of Follicles suggests increased number of available eggs as well improve chance of IVF implantation as supported by other researches. There are negative relationships between male age, MI number, sperm count (>35 million), sperm morphology (>3.9%), sperm motility (>40%),

female age, and aneuploidy life compatibility. This means that higher values of these variables are often associated with poorer aneuploidy life compatibility. These findings provide evidence for the presence of biological factors that influence the quality of embryos. Chromosomal abnormalities in embryos can arise due to advanced age in both partners, irregular sperm characteristics, and improper egg maturation.

The MI and MII analysis for this study somehow failed to give proper correlation between the increased maturation index which would probably reflect mature oocytes which probably enhances the chances of improved embryos.

CHAPTER VI

Conclusion And Recommendations

Conclusion

Despite the fact that the findings of the study may have been a part of a whole, it can be deduced that the age of the parents plays a significant role in the aneuploidy rates that occur during embryo formation and implantation. The oocyte donors and sperm donors who participated in the in vitro fertilization (IVF) sample also show some link with an elevated risk of malformed embryos, which in many instances may demonstrate aneuploidy.

Sperm motility and sperm morphology are two examples of characteristics that have been demonstrated to have a positive link with the likelihood of producing healthy human embryos and have been shown to facilitate successful implantation in in vitro fertilization (IVF). However, scientists have also demonstrated that advanced paternal age (APA) may have a significant impact on the quality of the sperm and oocyte formation, which in turn has an impact on the appropriate creation of embryos and implantation.

Recommendation

This body of research contributes to the current body of knowledge regarding the impact of parental age on human embryo aneuploidy, particularly in patients who have had in vitro fertilization (IVF).

It is evident that there is a lack of medical study on modern causes of production of healthy embryos and also on successful implantation of embryos in IVF patients of different age categories, particularly those who are of advanced age. However, the research is in dire need of further investigations because it is obvious that there is a lack of medical research on these topics.

This will serve as a guiding light to ensure the appropriate treatment for in vitro fertilization (IVF) patients, particularly those of advanced age, in order to increase implantation rates and also drive down the number of children born with any form of chromosomal aberration. More studies on the role of paternal age in the formation of aneuploidy will also do a lot of good for the field of study.

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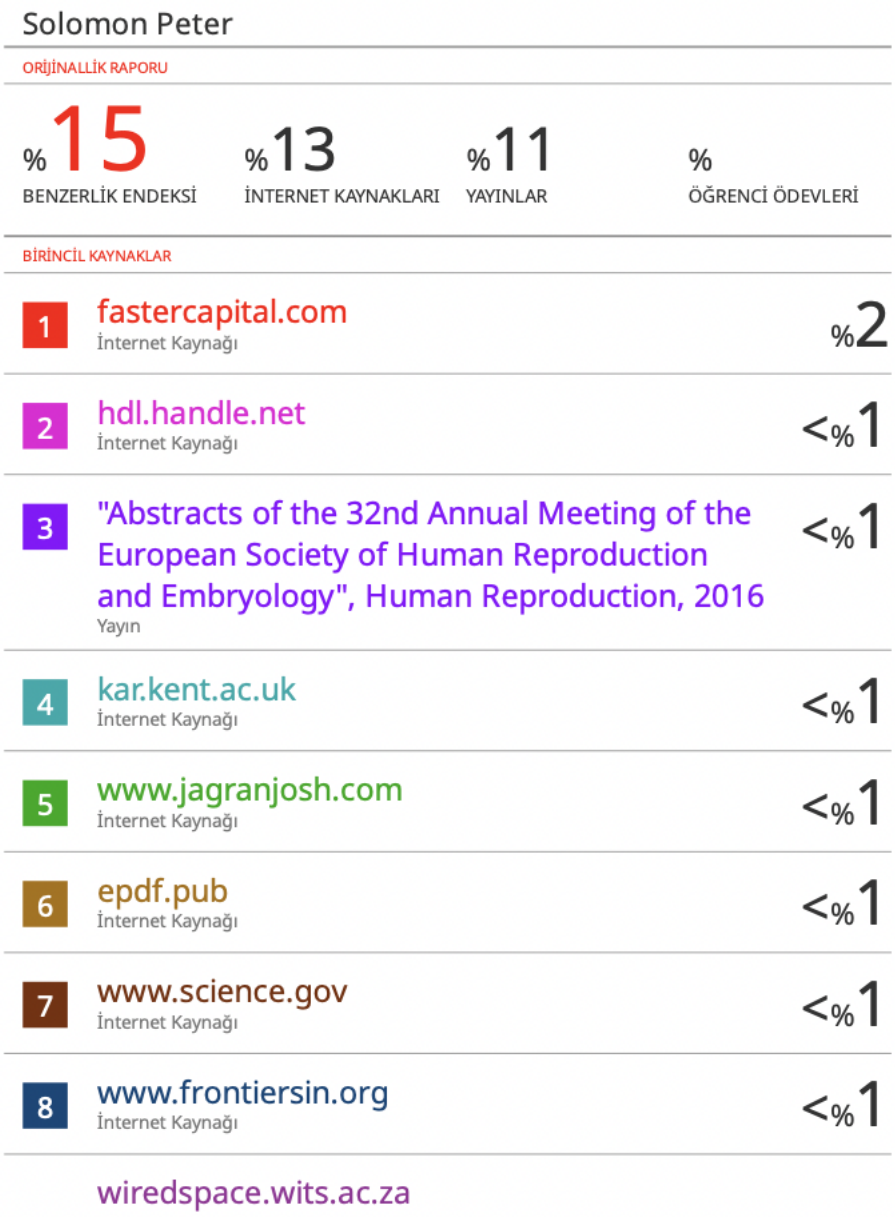
Appendices

Turnit In Report



Solomon Peter

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Ethical Approval

NEAR EAST UNIVERSITY
SCIENTIFIC RESEARCH ETHICS COMMITTEE

RESEARCH PROJECT EVALUATION REPORT

Meeting date :28.09.2023

Meeting Number :2023/116

Project number :1766

The project entitled **"The Molecular Regulation of Oocyte Formation and Preimplantation Embryo Development: Mathematical Modelling"** (Project no: NEU/2023/116-1766) has been reviewed and approved by the Near East University Scientific Research Ethical Committee.

Prof. Dr. Şanda Çalı
Near East University
Head of Scientific Research Ethics Committee

Committee Member	*Decision*	*Meeting Attendance*
	Approved (✓) / Rejected (X)	*Attended (✓) / Not attended(X)*
Prof. Dr. Tamer Yılmaz	✓	✓
Prof. Dr. Şahan Saygı	✓	✓
Prof. Dr. İlker Etikan	✓	✓
Doç. Dr. Mehtap Tınazlı	X	X
Doç. Dr. Nilüfer Galip Çelik	X	X
Doç. Dr. Dilek Sarpkaya Güder	✓	✓
Doç. Dr. Gulifeiya Abuduxike	✓	✓
Doç. Dr. Burçin Şanlıdağ	✓	✓