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Fertilization and Embryogenesis Success Rates in Morula IVF on *Normo-Responder* Group

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A couple with infertility is a couple who fails to achieve pregnancy after 12 months or more after regular sexual intercourse without using contraception. Infertility can be caused by a male factor or a female factor. In-vitro fertilization (IVF) is one of the assisted reproductive technology (ART) for a couple with infertility. IVF has a high rate for a woman to achieve a pregnancy. Its procedure is the fertilization of sperm and oocyte by intracytoplasmic sperm injection (ICSI), or conventional IVF.this study aims to determine the success rate of fertilization and embryogenesis based on sperm characteristics in IVF patients in Morula IVF Jakarta period January – December 2018. The results of this study were analyzed bivariate based on sperm morphology, motility, and concentration. Based on the analysis that has been carried out, it was found that there was no significant relationship between the success rate of fertilization and sperm characteristics, with a p-value <0.050.

Keywords: Infertility; in vitro fertilization; fertilization rate; embryo quality.

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1. INTRODUCTION

Reproduction is a physiological process to reproduce offspring in every living thing. The human reproductive system involves sperm and oocyte. There are several stages of embryogenesis in human reproduction, starting from fertilization, division, and implantation. After all, stages last until implantation. According to the World Health Organization (WHO), a woman will experience pregnancy and get the best results when the gestational age reaches 38 to 42 weeks. Several determinants of pregnancy are found in both women and men. These factors include the oocyte, sperm, or a person's fertility.

The history of in-vitro fertilization (IVF) was initially carried out with the natural cycle. Only one oocyte was taken at the time of IVF, so the success rate was meager, with a live birth rate of only 9.6%. This therapy is also limited to women with spontaneous ovulation. In 1981 started using drugs for ovarian stimulation, first with clomiphene citrate, then using gonadotrophins and GnRH agonists, thereby increasing the uptake of the number of oocytes and embryos available for transfer. At the same time, the indications for IVF are widespread for anovulatory patients with different etiologies. Furthermore, intracytoplasmic sperm injection (ICSI) is indicated for men with severe reproductive disorders [1].

Based to WHO, if a woman does not get pregnant after 12 months or more by having sex at least 2-3 times a week regularly without using contraception, it can be said that the woman is infertile [2]. Several factors in women who experience infertility are bilateral tubal obstruction, ovulation disorders, and mild to severe endometriosis. At the same time, the determining factor in men can be caused by sperm factors that cannot be cured or repaired with surgery or idiopathic infertility [3]. In vitro fertilization (IVF) therapy is effective for patients with fallopian tube obstruction, severe male infertility, and persistent infertility after the failure of conventional therapy. However, in vitro fertilization therapy is quite expensive because it requires highly trained personnel and quite expensive equipment.

One in eight infertile men has a condition that can be treated using assisted reproductive technologies (ART) such as IVF. However, these therapies do not help cure or treat the cause of male infertility. ICSI is also an ART, where a single sperm is injected directly into the oocyte. This therapy helps men with poor semen production. The sperm is collected from semen or carefully collected from the testes or epididymis. ICSI can result in pregnancy even with low sperm production [4].

It is interesting for us to look back for a moment and find that fertility and infertility were the thoughts of men in the past. For men in the past, breeding a race and surviving that race was an anxiety source, whereas a woman who failed conception and did not become pregnant was a terrible disgrace. According to WHO, based on data obtained from Indonesia's Demographic Health Survey-2002, the infertility rate (combining primary and secondary infertility) in married women aged 15-45 years is approximately 22.3% experiencing infertility.2 Approximately 15% of couples undergo involuntary infertility therapy and require fertility. After 30 years of the first baby's birth with in-vitro fertilization, the worldwide birth rate has increased to 3 million due to in-vitro fertilization, and there has also been a pregnancy rate increased up to 30% per birth. Therefore, in vitro fertilization is one method that plays an essential role in increasing the demographic index [5].

More than 186 million people worldwide have infertility, mostly in developing countries. Meanwhile, the most influential negative predictor of fertility is the woman's age at conception, and other factors, including lifestyle and environmental factors [5]. The woman's age is the most significant determinant of the success of in vitro fertilization therapy. Women tend to delay pregnancy until the third decade of life. In Finland, the number of women over 35 years of first time giving birth increased from 6% in 1987 to 18% in 2006.

In men, the determinant of fertility success can be seen from the characteristics of the sperm. Some characteristics of the sperm are semen analysis, sperm motility, sperm morphology, acrosome integrity, nuclear maturation, and sperm preparation. Sperm morphology affects in vitro fertilization in humans; therefore, a decrease in sperm quality can result in a decreased chance of pregnancy in some couples after in vitro fertilization, according to Mahadevan, Trenton & Leeton, confirmed by Cohen et al. [6].

Several researchers have researched the relationship between sperm characteristics with fertilization and embryogenesis. Avendano's

study [7] et al. stated that a decrease in the percentage of typical sperm DNA fragmentation has a higher probability of pregnancy. In this study, the characteristics of sperm based on typical sperm DNA fragmentation were 17.6% and >17.6%. The study by Steirteghem [8] et al. stated that severe motility disorders in men undergoing intracytoplasmic sperm injection (ICSI) therapy had a high fertilization rate. Palermo et al. stated that men with defects such as oligoasthenoteratospermia had significant changes after sperm retrieval techniques and assisted reproduction, especially ICSI [9].

The Morula IVF Jakarta clinic, established in 1997, has in-vitro fertilization therapy services using the ICSI method if the results of semen analysis show minimal sperm quality and number. However, at the clinic, no one has investigated how successful sperm quality is with fertilization and embryogenesis in these couples. Therefore, researchers are interested in conducting a study entitled fertilization success rates and embryogenesis in the normo-responder group undergoing in-vitro fertilization based on sperm characteristics of patients at Morula IVF Jakarta.

Based on this background, a problem can be formulated, how is the success rate of fertilization and embryogenesis in the normo-responder group based on the sperm characteristics of patients at Morula IVF Jakarta in 2018? The general objective of this study is to determine the success rate of fertilization and embryogenesis in the normo-responder group based on the sperm characteristics of patients at Morula IVF Jakarta in 2018.

2. LITERATURE REVIEW

Infertility is a clinical failure of pregnancy after 12 months of unprotected sexual intercourse by regularly having sex at least 2-3 times a week without using contraception due to the reproductive capacity of the partner (individual or both) [5,9,10]. Couples who have never had a pregnancy can be referred to as primary infertility, while couples who have had a previous pregnancy (or pregnancy that resulted in a miscarriage) can be considered secondary infertility [9]. According to WHO (World Health Organization), infertility is the inability of a sexually active person, a non-contraceptive partner, to get pregnant spontaneously [11].

Infertility is a problem in 15% to 30% of married couples. Men can cause infertility problems due to a lack of sperm count and motility disorders, while several causes cause infertility in women, such as uterine tube occlusion, "unfriendly" cervical mucus, and immunity to spermatozoa [12]. Thus, infertility can be caused by factors: female factors (age, ovulation disorder, endometriosis, and tubal disorders) and male factors (Testicular Deficiency, Post-Testicular Disorders, Sperm Dysfunction, and Spermatogenesis Agents) [13,14].

This group can be divided into unexplained male infertility, and idiopathic male infertility, a condition in which infertility occurs spontaneously or the cause is unknown. Table 1. Shows the distribution frequency of various causes of infertility in men. The average incidence of unexplained infertility is close to 15%, although some studies report a UMI of 6% to 37%. Possible causes are the presence of anti-sperm antibodies, sperm DNA damage, increased levels of Reactive Oxygen Species (ROS), and sperm dysfunction. However, there are other possibilities due to unexplained factors in women and coitus factors such as inappropriate timing of sexual intercourse (not during the woman's fertile window), erectile dysfunction, or anejaculation [15].

Men with idiopathic infertility have a decrease in semen quality for no known reason and without a history of fertility-related problems, and on physical examination as well as endocrine laboratory results are typical [13,14] in couples who do not find problems but still do not experience clinical pregnancy. A person is said to have unexplained infertility if the cause is not identified after a fertility check [16].

Autoimmune infertility describes infertility in the malefactor of about 4.5%. Several theories are hypothesized to cause autoimmune infertility in men [17]. The first statement says the absence of sperm during embryological development, during which the immune system tolerates self-antigens. The second opinion states that spermatozoa are haploid and have different chromosomes formed by somatic cells. Meanwhile, the third theory referred to as the 'immunosuppression theory,' states that suppressor T lymphocytes, an inhibitory immune response, are activated by a small number of spermatozoa antigens continuously released from the genital tract [17].

Conventional semen parameters such as sperm count, motility, vitality, and morphology are

inadequate in assessing sperm function and for use in markers of fertility potential. Instead, sperm function tests can be used for information prognosis and diagnosis. The test can be used to see between fertility and male infertility and can help find the cause of subfertility in men, and it can be used therapeutically. In infertile men, a semen analysis can be performed, which consists of microscopic examination, sperm count, sperm motility, sperm morphology, anti-sperm antibodies, white blood cell assay, genetic res, reactive oxygen species, DNA damage, and sperm function tests [15].

Infertility in men can be divided into three groups: inadequate sperm production, deficiency of sperm function, or insufficiency of sperm delivery. A critical laboratory examination is a semen analysis. It is recommended that at least two collections be carried out in a comparable period of 3 to 7 days. Several reports indicate that sperm density increased by 25% per day for the first four days [18]. WHO has published reference tables for semen analysis, including volume, pH, semen concentration, total sperm count, motility, morphology and other parameters.

Spermatozoa agglutination is the union or attachment of motile spermatozoa head-head, tail-tail, or a mixture (tail-head). Usually, the motility is vigorous with vibrating movements, but sometimes the agglutinated sperm have limited movement. All motile spermatozoa attached between the head, tail, or midsection should still be recorded. Agglutination is generally classified according to its degree (grades 1 to 4), and the site of attachment (grades A to E) should be reported [19]: a) Grade 1: isolated (< 10 spermatozoa per agglutination, many free spermatozoa); b) Grade 2: moderate (10-50 spermatozoa per agglutination, free spermatozoa); c) Grade 3: large (>50 spermatozoa, some spermatozoa are still free); and d) Grade 4: gross (all spermatozoa are agglutinated and agglutinated in connections).

In the United States, one percent of pregnancies occur through assisted reproductive technology (ART). However, children from conception on ART provided increased rates of prematurity (<37 weeks gestation), low birth weight (<2,500 g), and infant mortality, and several ART approaches, such as in vitro fertilization, intrafallopian gamete transfer (GIFT), and Zygote intrafallopian transfer (ZIFT) [20]. Initial indications for a patient to use in vitro fertilization are tubal abnormalities, infertility of unknown cause (idiopathic), male infertility, endometriosis, cervical factors or immunological infertility, and hormonal disorders. Several factors also determine the success of in-vitro fertilization, such as the woman's age, duration of infertility, and previous pregnancies [20].

Before in-vitro fertilization, married couples generally have a standard infertility evaluation, including semen analysis. There are many variations of ovarian response and fertility at each woman's age, so in general, ovarian retrieval tests are performed on couples undergoing IVF. In patients with low ovarian storage, it manifests a reduced ovulatory stimulation, resulting in less egg retrieval and low pregnancy rates [21]. In-vitro fertilization uses physical and emotional energy, time, and money. Thus, many married couples who experience infertility feel stressed and depressed.

Before in vitro fertilization therapy, couples will generally perform a standard infertility evaluation, including semen analysis; assess the female reproductive tract by hysterosalpingography, transvaginal ultrasonography, or both; and perform tests to detect ovulation [15]. Because of the significant variation in ovarian response and fertility with each age and individual, additional tests for ovarian reserve are usually performed on patients before IVF. Decreased ovarian reserve results from a reduced ovarian response to stimulation, less egg retrieval, few embryos, and a low pregnancy rate.

A woman taking fertility drugs can cause nausea, stomach pain, mood changes, headaches, and other side effects. Many in vitro fertilization treatments are given by injection over several days and repeatedly so that they can cause injection bruising [13]. In some sporadic cases, ovarian hyperstimulation syndrome (OHSS) may be a side effect when taking Fertility drugs. These symptoms can cause fluid accumulation in the abdomen and chest, causing abdominal pain, nausea, weight gain (4.5 kg in 3 to 5 days), decreased urine excretion, nausea, vomiting, and shortness of breath. In some cases, it can be managed with complete rest, and in other cases, it can be referred for fluid removal with a needle and indications for treatment [13]. All IVF clinics in the United States have reported to the Centers for Disease Control and Prevention that in 2013 the clinical pregnancy rate per IVF cycle with non-donor eggs was 34% in the United States. Due to miscarriage, the actual live birth rate is

28%. However, IVF therapy can be pretty effective, based on the effects of other treatments and results that keep improving over time [18].

Spermatozoa quality based on DNA damage or maturation when removed from ejaculatory semen may differ from that of testes.32-39 The relationship between poor semen quality and embryonic development and clinical outcomes in patients undergoing ICSI is uncertain [22,23]. It could be due to the limited number of spermatozoa and good sperm quality used during ICSI [24]. Although ICSI is starting to develop and is an effective therapy for the male infertility treatment. Due to the sperm natural selection lack in the ICSI procedure, researchers and clinicians have some concerns about the use and child health to be born with the technique [25]. According to Tsai et al., there was no difference in clinical outcome and child development after ICSI with sperm extracted from the testicular or sperm with severe oligoasthenoterato zoospermia (OAT). However, in men with severe OAT, it is still necessary to select good-quality sperm during ICSI [26].

The effect of sperm quality on embryonic development after ICSI was negatively associated, although prior to activation of the embryo genome, it was thought sperm quality could influence embryogenesis from early developmental stages. Most of the female-to-male ratios of offspring when embryo transfer was on the third day were not significantly different from the offspring when embryo transfer was in the blastocyst phase (fifth day) [27]. It is suspected that the male sex ratio results from selective embryo transfer to support good-quality embryos [28]. Men with teratozoospermia produce good fertilization in conventional IVF if sperm concentration and sperm motility are within normal limits based on WHO standards.

In Fan et al.'s study, there was no difference in the percentage of fertilized oocytes, implantation rate, pregnancy rate, embryo quality, and spontaneous abortion rate between conventional IVF therapy and ICSI with average sperm concentration, progressive sperm motility, and abnormal sperm morphology. Thus, this study states that morphology is not a critical sperm parameter for determining the success rate of fertilization in IVF [29]. However, in some studies, abnormal sperm morphology has a deleterious effect on fertilization and pregnancy rates per cycle after IVF embryo transfer [30].

Fertilization is a developmental process that starts from fertilization until the onset of clinical pregnancy, the union of male gametes (sperm) and female gametes (oocytes). Fertilization will occur if the number of sperm that enter the cervix during ovulation is adequate. The initial stage of fertilization begins with the maturation of male and female gametes. The initial process of oocyte gamete maturation starts from primordial germ cells, which differentiate into oogonia. These cells will undergo mitotic division, and at the end of the third month, the cells will be arranged in groups lined by flattened cells called follicular cells. Most oogonia continue to divide by mitosis, and some stop at the prophase stage of meiosis I and form the primary oocyte. The total number of germ cells in the ovary is around 7 million [11].

Sperm reaching the cervix must have the ability to swim along cervical mucus and be able to move ascending toward the upper genital tract, where the oocyte is fertilized. The cervix and cervical mucus protect the upper genital tract from infection, and at the time of ovulation, cervical mucus thins so that it affects the increase in estradiol levels which will make it easier for sperm to get to the upper genital tract [16]. When oocyte storage decreases, a woman will experience infertility, sterility, shortened cycles, irregular menstruation, and POI. In studies in Western countries, a woman will generally experience POI at the age of 51 years, and 1% will experience POI before the age of 40. Women who experience POI early will experience infertility. Approximately 10% of women will experience decreased ovarian function in their early 30s [11,17].

Irregular menstrual cycles will begin 6 to 7 years before POI, along with approximately 10,000 follicles remaining. A decrease in the number of follicles results in the rejection of inhibin B, produced by granulosa cells in the early follicular phase. Due to the inverse relationship between FSH and inhibin B, due to loss of negative feedback. An increase in FSH during the early follicular phase is an early sign of ovarian aging. However, it does not appear clinically or simply presents as infertility because ovarian hormones remain constant, and the woman continues to ovulate and have regular cycles. An early sign of ovarian aging is a shortened cycle due to a shortened follicular phase. The faster follicular development will lead to earlier retrieval of the dominant follicle. If these changes persist, a woman will notice that her cycles are

lengthy and irregular as they enter variable POI and ovulation [13,14].

3. RESEARCH METHODS

This study was non-experimental and used a cross-sectional design by evaluating the success of fertilization and embryogenesis in the normo-responder group by assessing sperm characteristics. The data was taken retrospectively by tracing medical records of patients who underwent in-vitro fertilization at Morula IVF Jakarta from January to December 2018. This study was conducted in May - October 2019 at Morula IVF Jakarta. The population of this study was all married couples who underwent infertility treatment with in-vitro fertilization at Morula IVF Jakarta for the period January - December 2018. The sample of this study was a married couple who underwent infertility treatment with in-vitro fertilization at Morula IVF Jakarta. This study's sampling technique was purposive sampling. The instrument used in this study was the medical records of female and male patients who underwent infertility treatment with in-vitro fertilization for January – December 2018 at Morula IVF Jakarta. Data was collected using secondary data, namely the medical records of female and male patients who underwent infertility treatment with in-vitro fertilization at Morula IVF Jakarta, and this study had passed the ethical clearance examination. Several steps of data processing are as follows: editing, coding, tabulation, and entry. The data were then entered into the statistical program SPSS for Windows 24.0. The data analysis of this research used univariate analysis, which aims to explain the characteristics of each variable to be studied. The data will be processed with the SPSS for Windows 24.0 program and entered into a table or graph.

4. RESULTS AND DISCUSSION

Research on the success rate of fertilization and embryogenesis in the normo-responder group undergoing an in vitro fertilization program based on sperm characteristics has been carried out with a sample of 475 couples at Morula IVF Jakarta from January to December 2018. Of the 475 couples who underwent the in vitro fertilization program, all couples underwent fertilization and embryogenesis.

Based on Table 1, the most significant frequency of husband age is 331 people in the age range of 26-35 years, and the lowest frequency is one person in the age range 56-65 years and over 65 years.

Table 2 shows that the average sperm motility is 40.30%, the average sperm morphology is 1.82%, and the average sperm concentration is 54.07 million/ejaculate.

Based on Table 3, it can be said that there is no significant relationship between sperm characteristics and fertilization rate with respective significance on motility (0.135), sperm morphology (0.797), and sperm concentration (0.079).

Based on Table 4, it can be said that there is a significant relationship between sperm motility and the third-day embryo with moderate quality. There was no significant relationship between the morphology of sperm and embryo on the third day (excellent, good, moderate, poor). There was a significant relationship between the concentration of sperm and embryos on the third day of moderate quality.

Based on Table 5, it can be said that there is no significant relationship between sperm motility and the fifth-day embryo (excellent, good, moderate poor). In sperm morphology, there was a significant relationship in the fifth-day embryo with moderate quality. There was no significant relationship on the fifth day between sperm and embryo concentrations (excellent, good, moderate, poor).

Fertilization is the union of male and female gametes. The union of these gametes will produce a zygote, a single-cell stage in which the pronuclear membrane will be damaged. After the zygote divides, it will become a cell called a "pre-embryo." After about 14 days after fertilization and development of the pre-embryo, primitive lines will become embryos [23]. Increasing age has a significant and weak positive effect on the percentage of sperm DNA fragmentation in the study of Mettler et al. As age increases, sperm DNA fragmentation increases. Researchers have hypothesized that sperm from older men will be damaged more and retain immature formation [31].

Table 1. Frequency of husband's age

Age	Frequency	%
≤25 years	3	0.6
26-35 years	331	69.7
36-45 years	123	25.9
46-55 years	16	3.4
56-65 years	1	0.2
>65 years	1	0.2
Total	**475**	**100.0**

Table 2. Average frequency of sperm characteristics

Sperm characteristics	Average
Sperm Motility (%)	40.30
Sperm Morphology (%)	1.82
Sperm Concentration (million/ejaculate)	54.07

Table 3. Chi-square test results sperm characteristics with fertilization rate

Sperm characteristics	Fertilization rate
	P value*
Sperm Motility	0.135
Sperm Morphology	0.797
Sperm Concentration	0.079

*Note: *Based on chi-square test, P < 0.050*

Table 4. Results of chi-square test of sperm characteristics with third day embryo

Sperm characteristics	Third-day embryo			
	Excellent	Good	Moderate	Poor
	P value	P value	P value	P value
Sperm Motility	0.158	0.211	0.042	0.194
Sperm Morphology	0.501	0.487	0.966	0.874
Sperm Concentration	0.228	0.182	0.012	0.236

Table 5. Chi-square test results of sperm characteristics with fifth day embryo

Sperm characteristics	Fifth day embryo			
	Excellent	Good	Moderate	Poor
	P value	P value	P value	P value
Sperm Motility	0.862	0.152	0.056	0.369
Sperm Morphology	0.803	0.416	0.033	0.558
Sperm Concentration	0.386	0.169	0.061	0.294

Based on the data processing results using the Chi-Square test with the help of the SPSS statistical program version 24.0. The significant result is 0.501, which means it is greater than 0.050. So it can be concluded that there is no relationship between sperm motility and fertilization rates. Turhan et al. studied no association in men with severe motility disorders when IVF therapy was carried out using the ICSI method [32]. In the relationship between sperm motility and the third-day embryo, it was found that there was no relationship between excellent, good, and poor embryo quality. On the fifth day of the embryo, there was no relationship between the four embryo qualities (excellent, good, moderate, poor). Anifandis et al. stated that there was no significant relationship between normal and abnormal DNA fragmentation with embryo quality [33].

In this study, there was no significant relationship between sperm morphology and fertilization rate. There was no relationship between sperm morphology and the third-day embryo on all

embryo quality (excellent, good, moderate, poor). On the fifth day of embryos, there was no relationship between excellent, good, and poor quality, while in moderate quality embryos, there was a significant relationship. The study by De Vos et al. stated that there was no relationship between sperm morphological abnormalities on fertilization rates and embryo development [34]. The study by Vanderzwalmen et al. stated a relationship between sperm morphology and embryo quality on the fifth day (blastocyst phase) [35].

In this study, there was no significant relationship between sperm concentration and fertilization rate. There was no relationship between concentration and embryos on the third day of excellent, good, and poor quality, while for embryos with moderate quality, there was a significant relationship. On the embryo's fifth day, there was no relationship between all embryo qualities (excellent, good, moderate, poor). Breznik et al. stated that sperm concentration did not have a significant relationship with fertilization rates and embryo development on the third and fifth days [36].

The limitations of this study are as follows: a) This study was only conducted in one year, so it cannot describe the overall influence of sperm on success rates and embryogenesis; b) This study only looked at three characteristics of sperm, did not look at other sperm factors contained in semen analysis; c) This study only looks at women in the normo-responder group, so they do not know how sperm affects the poor-responder group; and d) In this study, it was not possible to see how pregnancy rates and implantation rates might occur in women undergoing IVF therapy.

5. CONCLUSION

Fertilization success rate based on sperm characteristics of patients at Morula IVF Jakarta found no significant relationship, and embryogenesis on the third and fifth day based on sperm characteristics found no significant relationship. Thus, to determine the impact of other sperm characteristics such as sperm vitality and sperm DNA fragmentation on the success rate of fertilization and embryogenesis at Morula IVF Jakarta. In order to more accurately determine the impact of sperm characteristics on the success of fertilization and embryogenesis at Morula IVF Jakarta, it is necessary to conduct research over a more extended period. To find out how to treat infertility in women and men. It is necessary to examine semen analysis for all couples to increase the rate of fertilization and embryogenesis in couples undergoing the fertilization program at Morula IVF Jakarta.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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