Morphology-based Selection, Metabolic Profiling and Omics Technology Compared with the Classical Morphology-based Embryo Selection in In-Vitro Fertilization

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Abstract

Introduction: The medical industry is one of the dynamic industries with frequent innovations and discoveries, which in-vitro fertilization (IVF) technology being the recent change. IVF is an assisted reproductive technology that was invented to treat fertility and genetic problems associated with conception which involves implantation of good quality embryos in uterus. The success of IVF depends majorly on the embryo selection, the age of the gestational carrier and the cause of infertility. In IVF, more than one embryo can be implanted in uterus, which may result in multiple pregnancies about 30% of embryo resulting from IVF treatments have abnormal chromosome, and this percentage may rise up to 60% with poor embryo selection. This has led to many studies in the last decade on possible methods to replace the conventional morphological evaluations or complement with them. This paper, therefore, aims to assess the performance of morphology-based selection, metabolic profiling, and omics technology in IVF procedure when compared with the modern selection methods. Method: This study provides a review of literatures on the assessment of the effectiveness of the traditional embryo transfer methods when compared with the modern methods. The review was based on PubMed electronic database. Information on female patient, different intervention models, comparisons and study designs was also provided. Results: Out of the 58 studies that were evaluated, only ten were identified as eligible for the review because they provided substantial reports on morphology assessment, metabolic profiling, and omics technology. The selected articles proved to be significant since they provided a comparative analysis of the models and their outcomes. This review identified that the modern methods had positive results as opposed to the traditional methods that had less positive results. Conclusion: Modern methods have positive results due to their precision compared to morphological technique. The time lapse imaging technique has the highest accuracy, and it complements the morphological method by reducing chances of failure of implantation caused by exposing the embryo to conditions outside the incubator. Results from the other modern method including metabolic profiling, omics technology, whether they had lower pregnancy rates and significant implantation failure or not. From the results, one can judge that morpho kinetics is the best model due to its high precision. On the integration of the time-lapse technique with classical morphology assessment, there was no significant difference between the time-lapse technique and the morphology evaluation. Although morpho kinetics provides high-quality results, the traditional methods are continually being used because they provide an observational assessment of the embryo cells. Also, the morphology-based method issued worldwide due to the lack of consensus on the benefits of the novel embryo transfer techniques.

Keywords: Morphology-based selection, Metabolic profiling, Omics technology, In-vitro fertilization

Introduction

In the past two decades, several types of studies have been done on assisted reproductive technologies (ART) to help establish and maintain pregnancies. These studies have been geared to solve humanity reproductive problems such as the high number of implantation failures, endometriosis, low sperm counts, problems in the uterus and fallopian tubes, problems associated with ovulations, antibody problems that harm sperm or oocytes, the inability of sperm to penetrate the cervical mucus and other infertility problems. Therefore, to solve these problems, ARTs
such as in-vitro fertilization and intracytoplasmic sperm injection (ICSI) technologies have been developed. The IVF starts with hormonal stimulation of the ovaries to produce more follicles. These hormones stimulate follicle production for 8-10 days, then an ultrasound guidance is done to monitor the follicle development. At least three mature follicles are needed to proceed with the process.

When a sufficient number of follicles are deemed to be mature, then a transvaginal guided oocyte retrieval procedure is done to obtain the oocytes from the follicles. The oocytes are then fertilized in IVF laboratory. After fertilization, the embryos are incubated in the IVF laboratory for two to six days.[1] The embryos that have been implanted in uterus are then monitored and evaluated. The other embryos of sufficient quality are cryopreserved for future use.

The success of the IVF is achieved after twelve weeks if an ultrasound detects cardiac activity. It is more likely that a competent embryo will result in successful pregnancy; therefore, the success of IVF depends on the embryo selection. Although the more the number of the embryos transferred the higher the chances of successful pregnancies are, it also increases the chances of multiple pregnancies which are regarded as complications in the IVF. Embryologists condemn multiple pregnancies because they result in increased treatment costs as well as poor maternal outcomes. Currently, there is no concurrence on the clinical benefits of the embryo selection techniques in regards to their outcomes. As a result, most embryologists routinely use morphology-based criteria. Base on the situation and problem, explained in the previous paragraph, there is an interesting question about how the performance of many novel embryo selection approaches compares with the classical morphology-based embryo selection. Therefore, this paper aims to assess the performance of many novel technological embryo selections, such as morphology-based selection, metabolic profiling, and omics technology, in IVF procedure when compared with the modern methods.

Method of Literature Selection

The paper was categorized into four different stages as seen in figure 1. The first was the research question that was designed in regard to the objectives of the study. The second was the search criteria which were intended to enhance the access to genuine, comprehensive and reliable medical information. Likewise, the search criteria were designed to obtain information that was relevant to the research question through the use of specific keywords and inclusion and exclusion criteria. Lastly, the method utilized various quality assessment methods to ensure valid data sources for the selected studies.

Pub Med electronic database was the credible source of articles used in the survey. Keywords were actually used to narrow down the search and enhance access to relevant information. Since the primary goal of the research was to evaluate the morphological systems of embryo assessment, a well-developed conclusion was drafted from the reports on different assessment models.

The assessment models were compared to the conventional morphological models. The keywords used in the search included embryo development, embryo assessment, embryo selection, non-invasive embryo selection, prognostic factors, and morphological time-lapse imaging. The use of many keywords proved to be effective as they provided a comprehensive approach to the evaluation of
different embryo assessment models. The study was conducted from the beginning of January 2000 to the end of January 2016. Study selection. The approach enabled the inclusion of appropriate sources of information and exclusion of unnecessary sources. Below is a brief description of this model. In this study the population is only couples who undergo IVF. However, the study also analyzed animal fertility. Under this model, interventions were also utilized. The embryo selections evaluated in this study had to be conventional morphology approaches, no morphological techniques, and morphological models. [2]

Under outcomes, the principal validation metric for the success of IVF treatment is the enhancement in implantation and gestation rates. Therefore, the studies used in this review had to verify the success rate by examining outcomes such as live implantation rate, pregnancy rate, multiple pregnancy rates and birth rate. The studies also had to include a comparative result between traditional morphology-based model and emerging embryo selection and assessment techniques. Notably, the studies that failed to provide either of the mentioned outcomes were excluded from the review. In the study design, each of the articles used in this review had to present either a prospective cohort study or a randomized control trial.

The article only made use of original and translated English papers. Papers were also selected depending on their format and relevance. The title and abstract were first scrutinized for relevance and once that failed to meet the requirements of the study, questions were excluded. Inclusion and exclusion criteria results. The articles used were obtained from PubMed electronic database; the items have to be screened to ensure they range between the year of study, that is between January 1st 2000 to January, 31st 2016.

Those that were not within this range of time were excluded. A total of 58 articles were extracted from PubMed database in the first data retrieval search. After data review, eight of these articles did not show enough relevance and so they were excluded. The next level of selection included the carefully reading abstracts for the remaining tables and 21 more papers did not meet eligibility; hence, they were eliminated. Lastly, the remaining 29 articles were eligible and complied with the requirements of the research question; they, therefore, were scrutinized. Nineteen articles were biased and so they were excluded. They were considered biased, and they only provided information on morphologically-biased embryo evaluation technique. Ultimately, ten publications were selected for this study as they met the eligible criteria.

From the discussion provided above concerning the articles that were considered for the study, it can be concluded that the small portions of the embryo selection studies provided a comparative analysis of different embryo selection models. Only a few studies provided an all-inclusive analysis of the outcomes observed in every selection technique.

Result of Literature Study

Morphological Evaluation

For the last two decades, the quality of embryos has been based on morphological assessments. The criteria that have been used over the years are the rate of cell division, pronuclear morphology, and progression to the blastocyst stage. The first cleaving embryos tend to implant at a higher rate. However, none of these criteria has been accurate in predicting the success of pregnancy, and this has been evident for the occurrence of situations in which poor quality embryos have been implanted and yielded multiple pregnancies, while in other cases there was no conception at all despite the implantation of quality embryos. To reduce cases of multiple pregnancies, a fine selection procedure has been developed to ensure that only one embryo is implanted. Embryologists have been using the retrospective scoring methods such as pronuclear scoring system where embryo quality is assessed after fertilization and before cleavage to assess nucleoli size, number, and distribution.

A strong correlation has been found to exist between the pronuclear scoring system and successful implantation. Morphological evaluation of the pre-implantation embryos’ viability has resulted in increased implantation and pregnancy rates. However, with the recent significant technological advancements, experts have developed more embryo selection technologies that either
enhance or substitute the conservative morphology-based embryo selection. Concerning the above discussions, we shall focus on the most recent embryo assessment methods and evaluate whether they relate to the IVF effectiveness when compared with the conventional morphological systems. [3] Below, in Table 1, are the most recent approaches used in the selection of embryos and oocytes in the IVF laboratories. The first is referred to as Morphological evaluation. The method wholly depends on a light microscope for morphological evaluation. [4] However, most researchers work towards the establishment of additional parameters to enhance the extrapolative power of this model. During the early stages of cleavage, it is possible to obtain information on the oocyte – sperm interaction as it is provided in the pronuclear morphology. [5] The second and third days involve the assessment of the number, proportion and size of the blastomeres. During this period, the level of fragmentation, multinucleation, and cytoplasmic appearances are also observed and scored. [6]

Table 1: Morphological Evaluation

<table>
<thead>
<tr>
<th>Stages</th>
<th>Observation</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Early stage</td>
<td>Oocyte – sperm interaction as it is provided in the pronuclear morphology.</td>
<td>[5]</td>
</tr>
<tr>
<td>The second and third days</td>
<td>number, proportion and size of the blastomeres, level of fragmentation, multi nucleation, and cytoplasmic appearances</td>
<td>[6]</td>
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</tbody>
</table>

Omics Technology

The second method is Omics technology of the embryo selection; the model improves on the examination of the embryonic molecular composition as well as the no morphological markers of embryo visibility. [7]. The preimplantation genetic screening model (PGS), which involves the genetic profiling of the blastomeres, is utilized to detect chromosomal defects. With the help of this process, embryos with genetic defects are removed from the IVF transfer process. [8]

In omics technology, such as proteomics approach is also used. This approach profoundly depends on the protein constitution of the embryo. It screens the secretome to analyze the quantity of the proteins that have been released to the neighboring culture media. The simple concept utilized in this approach is that viable embryos secrete more protein. [9] The last recent approach in this study is morphokinetics; this technology uses powerful instruments that are composed of an incubator and a built-in camera. The incubator provides a conducive space for embryo placement, and the camera records its gradual growth rate. [10]

This approach is more accurate and reliable compared to the use of static observation as it provides the exact time when particular embryo changes occurred. It provides timing details for the pronuclear formulation, early cleavage, initiation of blastulation as well as the division cell intervals. The objectives of the IVF process are considered successful if the implantation outcomes were positive. Implantation primarily involves both the embryo and the female. However, implantation depends on a well-functioning endometrium. The success of this process depends on the level of protein and its competence as well.

To enhance the implantation outcomes, scientists have managed to manipulate the endometrium embryo interaction; hence, some factors that prevent female infertility and implantation among women can be prevented. According to the conducted surveys on implantation and fertility, the rates have been improved over the past decade. According to researches, this increase is ascribed to the enhanced morphological embryo assessment techniques. As noted earlier, effective embryo selection models usually lead to successful IVF treatments since only competent embryos are chosen for the implantation. [11]

There is a limited relationship between the implantation rates, pregnancy, and live birth rates. The fact that the implantation rates had been improved has not led to the increase in pregnancy and birth rates. There has been a slight increase of 3 percent for the patients of ≤ 42 years of age. Meanwhile, there has been a slight decrease of 9.8 percent for > 42-year-old patients (3). Live birth rates have been constant for the > 42-year-old patients during the past 8-10 years. For the patients in the < 35 age group, the pregnancy and live birth rates has increased by 48 percent and 42 percent respectively.
The process of embryo selection is critical in the IVF treatment process. Static morphological assessment technique has been widely used by clinicians to select and evaluate the most viable embryo in regard to its quality and competence. However much the diversities of embryo selection techniques have been developed, there has been no accord on the clinical significance and benefits they pose in comparison to the conventional morphological method. Out of the 58 studies reviewed, only 10 were identified as eligible for this systematic review.

Among those studies, only one provided the clinical benefits of the morph kinetics. This review established that the use of a light microscope in the typical laboratory setting allowed clinicians to conduct adjunct morphological assessments. Traditionally, the quality of embryos was assessed based on their morphology. This allowed embryologists to select the most viable embryo for transfer. In this method, it has been agreed internationally that the first assessment of embryo should be conducted on the zygote, after ICSI, insemination or pronuclear appearance.

The presence of two pronuclei should be the first sign of successful fertilization. Although this method is used worldwide, it is challenging to validate the outcome of the morphological test because of the variation in scoring schemes in different fertility clinics; there has been a creation of an algorithm in the morphology-based assessment, and thus, aneuploidy can be detected in a noninvasive manner. In some instances, time-lapse imaging has been integrated into this model to make it efficient. This technological advancement improves zygote morphology in ways that the single and static monitoring system failed. [12]

Transcriptomics approach is important in assessing oocytes and embryos. [13] This method requires the clinicians to identify particular gene markers by evaluating the gene expression levels in granulosa and cumulus cells. The gene markers define the embryos’ eligibility. Similarly, proteomics and metabolomics are noninvasive models for embryo’s assessment and selection. Unlike most approaches, these methods assess the interaction between the embryo and its culture media. Embryos that show positive interaction in their culture media are considered eligible for transfer. However, metabolomics and proteomics are not commonly used due to the intense technology they require. Lack of rapid technology and bench-top instruments inhibits the efficacy of the mentioned approaches in the IVF laboratories. [14] Morpho kinetics allows continuous observation and analysis of cellular dynamics of the embryo for a considerable time in an embryo scope. This method improves the sensitivity of the morphologic system as well as reduces chances of failure caused by the exposure of embryos to conditions outside the incubators during monitoring.

The shortcoming of this method is that it may be affected by blastocyst overlapping, misleading the embryologist because it does not allow rotation to separate the blastomeres. Genomics, proteomics, metabolomics, and transcriptomics are involved in assessing the embryo’s viability. Genomics provides information on which genes are turned on or off, enabling embryologists to distinguish between normal and abnormal embryos.

Proteomics provides information on secreted factors that reflect developmental success and viability. On the other hand, transcriptomics provides information on gene expression as well as some RNA involved. Metabolomics is important in determining when metabolic activities in the embryo start, which is an essential criterion for selecting competent embryos. This technique, therefore, improves the precision of the morphological assessment procedure.

**Metabolite Profiling to Determine Embryo Viability**

Transcriptomics technique is important in assessing oocytes and embryos. This method requires the clinicians to identify particular gene markers by evaluating the gene expression levels in granulosa and cumulus cells. The gene markers define the embryos’ eligibility. Similarly, proteomics and metabolomics are noninvasive models for embryo’s assessment and selection. Unlike most approaches, these methods assess the interaction between the embryo and its culture media. Embryos that show positive interaction in their culture media are considered eligible for transfer.
However, metabolomics and proteomics are not commonly used due to the intense technology they require. Lack of rapid technology and bench-top instruments inhibits the efficacy of the mentioned approaches in the IVF laboratories the metabolic process begins at the pronuclear stage during the embryo’s development; during the compacting of the embryo, ATP synthesis from lactate and carboxylic acid pyruvate is the source of energy. This trend of energy synthesis continues until glucose becomes the primary metabolite which is the normal occurrence at the blastocyst stage. During development, the embryo absorbs certain substances from its surrounding environment and excretes some other products.

Ultra-micro fluorescence is then used to observe this environmental change. Other separation techniques are also used to determine the substances that have been released or consumed by the spent media; the information obtained is then used to evaluate the cellular activities and potential development of the embryo during the culture period. The table represents an analysis of other researchers regarding the use of metabolites to determine the embryo viability.

According to Hardy, an experiment conducted concerning the uptake of glucose and pyruvate by an embryo and blastocyst within a span of six days yielded the following results. Increased pyruvate uptake on the first four days indicated embryo development. Up to day four, glucose concentration remained unchanged and on day 5, both glucose and pyruvate uptake increased showed blastocyst development.

On the other hand, Turner focused on a two days experiment that analyzed pyruvate uptake to test pregnancy. According to him, embryos with good morphology had high chances of implantation if they showed an intermediate pyruvate uptake.

Conagahan [15] also presented an implantation test for the second and third days by evaluating pyruvate uptake; according to him, decreases pyruvate uptake was a sign of implantation. Gott et al [16] reflected-on pyruvate, glucose, and lactate uptake for 3 to 6 days to test embryo’s development. According to him, increased pyruvate uptake on days 2-4 indicated embryo development. Up to day four, glucose concentration remained unchanged, which meant there were very minimal metabolic activities and no embryo development. On day 5, both glucose and pyruvate uptake increased, which showed blastocyst development. And lastly, increased lactate uptake on day 3 to 5 indicated blastocyst development. All the above researchers used ultra-micro fluorescence to observe this environmental change.

They also used other separation techniques to determine the substances that had been released or consumed by the spent media. Houghton et al.,[17] presented an analysis of 18 amino acids which included glutamine, alanine, Arginine, Methionine, asparagine to test blastocyst development.

According to him, reduced glutamine, methionine, and arginine uptake indicated blastocyst development; while reduced alanine and asparagine production showed blastocyst development. He used the HPLC techniques for separation. He too presented an analysis of 18 Amino acids, including alanine, serine, and glycine during the compacting eight cells; as well determined blastocyst development. In this research, the followings were his findings. Decreased serine uptake indicated blastocyst development, whereas reduced glycine and alanine uptake showed blastocyst development.

Lastly, Briston and Sell presented a test for live birth and pregnancy during the second day, with a primary focus on leucine, glycine, glutamate and asparagine. According to Briston et al. [18] increased asparagine uptake, reduced glycine and leucine uptake indicated pregnancy and live birth, while according to Sell et al, [19] increased glutamate uptake showed pregnancy and live birth. Lack of rapid technology and bench-top instruments inhibited the efficacy of the techniques used by the above researchers.

Positive implantation outcome was a sign of a successful IVF process; implantation involves an embryo and a woman. However, implantation depends on a well-functioning endometrium and the level of protein as well as its competence. To enhance the implantation outcomes, scientists have managed to manipulate the endometrium.
embryo interaction; hence, some factors that prevent female infertility and implantation among women can be prevented.

Table 2: Gives an idea about and evaluation of preimplantation embryo metabolism as an indicator of reproductive viability

<table>
<thead>
<tr>
<th>Examination stage</th>
<th>Analyzed metabolite</th>
<th>Assessed outcome</th>
<th>Technology</th>
<th>Findings</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days 1 to 6</td>
<td>Glucose and pyruvate</td>
<td>Development of embryo and blastocyst</td>
<td>Ultra-microfluorescence assay</td>
<td>Increased pyruvate uptake on the first four days indicating embryo development</td>
<td>[20]</td>
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<td></td>
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<td></td>
<td>Up to day four glucose concentration remained unchanged</td>
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<td></td>
<td></td>
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<td></td>
<td>On day 5, both glucose and pyruvate uptake increased showing blastocyst development</td>
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</tr>
<tr>
<td>Day 2</td>
<td>Pyruvate</td>
<td>Pregnancy</td>
<td>Ultra-microfluorescence assay</td>
<td>Embryos with good morphology had high chances of implantation if they showed an intermediate pyruvate uptake</td>
<td>[21]</td>
</tr>
<tr>
<td>Day 2 to day 3</td>
<td>Pyruvate</td>
<td>Implantation</td>
<td>Ultra-microfluorescence assay</td>
<td>Decrease in pyruvate uptake</td>
<td>[15]</td>
</tr>
<tr>
<td>Day 3 to day 6</td>
<td>Pyruvate, lactate, and glucose</td>
<td>Embryo development</td>
<td>Ultra-microfluorescence assay</td>
<td>Increased pyruvate uptake on days 2-4 indicating embryo development</td>
<td>[16]</td>
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<td>Increased lactate uptake on day 3 to 5 indicating blastocyst development</td>
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<tr>
<td>Days 4 to day 6</td>
<td>Pyruvate and glucose</td>
<td>Development and quality of blastocyst</td>
<td>Ultra-microfluorescence assay</td>
<td>High glucose and pyruvate and glucose uptake on day 4 indicating blastocyst development</td>
<td>[22]</td>
</tr>
<tr>
<td>Day 2-3</td>
<td>18 amino acids which include glutamine, alanine, Arginine, Methionine, asparagine.</td>
<td>Development of blastocyst</td>
<td>High-performance liquid chromatography (HPLC)</td>
<td>Reduced glutamine, methionine and arginine uptake indicating blastocyst development</td>
<td>[17]</td>
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<td></td>
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<td>Reduced alanine and asparagine production showing blastocyst development</td>
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<tr>
<td>Compacting eight cells</td>
<td>18 Amino acids including, alanine, serine glycine</td>
<td>Development of blastocyst</td>
<td>High-performance liquid chromatography (HPLC)</td>
<td>Decreased serine uptake indicating blastocyst development, reduced glycine and alanine uptake showing blastocyst development</td>
<td>[17]</td>
</tr>
<tr>
<td>Day 2</td>
<td>Leucine, glycine, and asparagine</td>
<td>Live birth, pregnancy</td>
<td>High-performance liquid chromatography (HPLC)</td>
<td>Increased Asparagine uptake and reduced glycine and leucine uptake which indicated pregnancy and live birth</td>
<td>[18]</td>
</tr>
<tr>
<td>Day 2</td>
<td>Leucine, glutamate, Glycine, and asparagine</td>
<td>Pregnancy, live birth</td>
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</table>

Conclusion

This paper aims to review the studies on clinical results and benefits of using embryo selection technologies. Presently, there exists no consensus on the medical advantages of the mentioned techniques in regards to their outcomes. As a result, most embryologists routinely use morphology-based criteria. Selected articles agree that IVF has improved the pregnancy and child rates for the past decade. As a result, each study in this systemic review provided a considerable explanation of the different models and their clinical results. The three core models as specified by the articles are the morphological evaluation model, omics technologies, and morphokinetics. Morphological evaluation wholly depends on a light microscope for morphological evaluation. During the early stages of cleavage, it is possible to obtain information on the oocyte–sperm interaction as it is provided in the pronuclear morphology. The subsequent stages involve the assessment of the number, proportion and size of the blastomeres. On the other hand, omic technology improves on the examination of the embryonic molecular composition as well
as the non-morphological markers of embryo visibility. The preimplantation genetic screening model (PGS), which involves the genetic profiling of the blastomeres is utilized in detection of chromosomal defects. Proteomics approach is also used in this model. Lastly, morphokinetics technology uses powerful instruments that are composed of an incubator and a built-in camera. The incubator provides a space for embryo placement and the camera records its gradual growth rate. [23]

This approach is more accurate and reliable compared to the use of static observation as it provides the exact time this particular embryo changes occur. It provides timing details for the pronuclear formulation, early cleavage, initiation of blastulation as well as division cell intervals. The objectives of the IVF process are considered successful if the implantation outcomes are positive. Implantation primarily involves both an embryo and a woman. However, implantation depends on a well-functioning endometrium. The success of this process also depends on the level of protein and its competence. To enhance the implantation outcomes, scientists have managed to manipulate the endometrium embryo interaction; hence, some factors that prevent female infertility and implantation among women can be avoided. [24] It is also important to note that single and static morphological observations are significantly misleading, as they do not show the fate of embryos shortly after cleavage. Conventional models have therefore been considered significantly effective compared to novel models sure a high-quality product, diagrams and lettering MUST be either computer-drafted or drawn using India ink.

Acknowledgements

Special thanks to Dr. Santos Gunardi, MS, PA (K) from Department Anatomy Faculty of Medicine who always give support and suggestions. Special thanks too to Dr. Dr. Ninik Mujihartini, MS for explaining the metabolite examination regarding the IVF mentioned in this manuscript.

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