RETROSPECTIVE STUDY COMPARING TWO EMBRYO TRANSFER VERSUS THREE EMBRYO TRANSFER AFTER IVF AND ICSI IN HOSPITAL SULTANAH NUR ZAHIRAH

BY

DR NOR AZIAN BINTI MOHD NOR

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CONTENT

TABLES AND FIGURES

LIST OF TABLES.

LIST OF FIGURES.

LIST OF ABBREVIATIONS

ART	Assisted Reproductive Technology
BMI	Body Mass Index
CPR	Clinical Pregnancy Rate
ET	Embryo Transfer
FH	Fetal Heart
GIFT	Gamete Intra –fallopian Transfer
HSNZ	Hospital Sultanah Nur Zahirah
HIS	Hospital Information System
IVF	In Vitro Fertilization
ICSI	Intra Cytoplasmic Sperm Injection
MCES	Mean Cumulative Embryo Score
Two ET	Two Embryo Transfer

Three ET	Three Embryo Transfer
UPT	Urine Pregnancy Test
USG	Ultrasonography
ZIFT	Zygote Intra-Fallopian Transfer

DEFINITION OF TERMINOLOGY

- Infertility

  Failure of conception despite having regular, unprotected sexual intercourses of one year duration.

- Assisted Reproduction

  The collective name for treatments designed to lead to conception by means other than sexual intercourse. Assisted reproduction techniques include intrauterine insemination, in vitro fertilization, intracytoplasmic sperm injection and donor insemination.

- Embryo

  Product of conception from time of fertilization to the end of embryonic stage 8 weeks after fertilization

- Embryo Transfer

  Procedure in which embryo(s) are placed in the uterus or fallopian tube

- Clinical Pregnancy

  Evidence of pregnancy by present of fetal heart or ultrasound visualization of gestational sac, embryonic pole with heart beat. It include ectopic pregnancy. Multiple gestational sac in one patient are counted as one clinical pregnancy

- Pregnancy Rate

  Number of clinical pregnancies per number of IVF/ICSI

- High-quality embryo features

  Included: (i) Four or five blastomeres on day 2;

  (ii) Seven or more blastomeres on day 3;

  (iii) No more than 20% fragmentation; and

  (iv) No observed multinucleation of blastomeres at any stage

ABSTRAK

Objektif:

Satu kajian retrospektif telah dijalankan di Hospital Sultanah Nur Zahirah, Kuala Terengganu untuk menentukan perbandingan jumlah kehamilan dan hasil daripada kehamilan tersebut terhadap pesakit yang menjalani prosedur persenyawaan in vitro selepas pemindahan dua atau tiga embrio.

Kaedah Kajian:

Kajian secara retrospektif dijalankan terhadap wanita-wanita yang mengalami masalah kesuburan dan menjalani prosedur persenyawaan in vitro di Unit Reproduktif dari bulan Januari 2010 sehingga bulan Mac 2015.

Rekod infertiliti dan data klinikal pesakit melalui sistem perisian berkomputer diteliti dan direkodkan ke dalam borang penyelidikan untuk dianalisis.

Keputusan:

Sebanyak 212 pesakit telah dipilih untuk menyertai kajian ini. Sejumlah 106 pesakit di bahagikan untuk setiap kumpulan.

Terdapat kaitan yang signifikan secara statistik diantara kadar kehamilan dan jumlah embrio dipindahkan.

Kadar kehamilan di dalam kumpulan yang menerima dua embrio hanyalah 30 pesakit (28.3%), sementara kadar kehamilan di dalam kumpulan yang menerima tiga embrio

adalah 49 pesakit (46.2%).Selain itu didapati kejadian kehamilan berganda meningkat secara signifikan apabila lebih embrio dipindahkan.

Kejadian untuk bayi kembar adalah 2 (1.9%) bagi pemindahan dua embrio,manakala

9 (8.5%) untuk pemindahan tiga embrio. transfer (p- value= 0.018).

Terdapat kandungan berkembar tiga di dalam kumpulan yang menerima tiga embrio, manakala tiada yang di rekodkan didalam kumpulan yang menerima dua embrio.

Sementara itu untuk kejadian keguguran dan kandungan di luar rahim tiada perbezaan secara statistik diantara dua kumpulan tersebut.

Kesimpulan:

Kadar kehamilan secara am didapati meningkat sejajar dengan jumlah embrio yang dipindahkan. Sementara itu, risiko untuk kehamilan berganda juga didapati meningkat sekiranya lebih embrio dipindahkan.

Tiada perbezaan untuk keguguran dan kandungan di luar rahim di antara dua kumpulan tersebut.

ABSTRACT

Objective:

A study was performed in Hospital Sultanah Nur Zahirah, Kuala Terengganu to determine the prevalence of clinical pregnancies, and the pregnancy outcomes in patients undergoing IVF after two or three embryo transfer.

Method:

All infertility patients subjected to IVF/ICSI from January 2010 – March 2015 in Reproductive Unit were retrospectively reviewed using infertility record and clinical data base via HIS computerized system. The details were entered into data collection form for analysis.

Result

A total of 212 patients were recruited in this study, with 106 patients in each group. There was a significant association between the pregnancy rate and the number of embryos transferred. Pregnancy rate after two embryo transferred group was 30(28.3%), while the pregnancy rate after three embryo transferred group was 49(46.2%).The incidence of multiple pregnancy increased significantly when higher number of embryos had been transferred. The incidence of twin pregnancy was 2(1.9%) when two embryos were transferred and 9 (8.5%) when three embryos were transferred. (p-value=0,018)

A triplet pregnancy was recorded in the three embryo transferred group while there was none in two embryo transferred group.

The incidence of miscarriage and ectopic pregnancy showed no difference statistically between the two groups.

Conclusion:

The clinical pregnancy rate increased with increased in the number of embryo transferred, however there also increased in the incidence of multiple pregnancy. Meanwhile, the incidence of miscarriage and ectopic pregnancy were not affected by the number of embryos transfer.

INTRODUCTION

The IVF/ICSI program was established in Hospital Sultanah Nur Zahirah in 2009. During the first establishment there were four staffs in the unit, with one specialist, one embryologist and two staff nurses. There were around 3000 to more than 4000 cases seen in the Infertility Unit every year, with the majority of cases were follow up cases.

The cycles of IVF per year in HSNZ Infertility Unit were between 67 to 84 cycles. The most IVF cycle performed was in 2010, with 84 cycles and the first successful IVF (live birth) was recorded in the same year. Meanwhile the pregnancy rates were between 11.9 % to 25% from 2009 until 2014. There were 70 live births/take home babies since 2010 until early 2015.

Today, HSNZ Infertility Centre became one of the most recognize ART Centre in the East Coast.

Infertility has become a significant issue in recent years. Among the contributing factors are career oriented women and delay in decision to start a family. These factors directly contribute to an increase in demand for assisted reproductive techniques (ART). Assisted reproductive techniques comprises of in-vitro fertilization (IVF), intra-cytoplasmic sperm injection (ICSI) and donor insemination (DI), gamete intra-fallopian transfer (GIFT), zygote intra-fallopion transfer (ZIFT) and tubal embryo transfer (TET) via laparoscopy.

ART involves the process of controlled ovarian hyper-stimulation with exogenous gonadotropin, oocytes retrieval, fertilization in laboratory and trans-cervical transfer of the embryo into the uterus.

Embryo transfer (ET) is one of the important steps in in-vitro fertilization (IVF), and failure in this process will lead to failure of the IVF procedure.

In July 1978, Louise Brown was the first live human birth following IVF and became the great marker in IVF history.

In the beginning of IVF, multiple numbers of embryos were transferred to increase the chance of pregnancies however this resulted in an increase in incidence of multiple pregnancies.

Subsequently there was an effort to reduce the number of multiple pregnancies which resulted from higher numbers of embryo transfer per cycle.

Many countries have their own regulations in regard to the number of embryos that can be transferred during an IVF cycle. For example in countries like Hungary, Switzerland, India and Italy, the number of embryos that can be transferred were limited to 3, whereas for Japan only one embryo should be transferred per cycle, as announced by Japan Society of Obstetrics and Gynaecology (JSOG) in 2008. China only allowed no more than 2 embryos transferred for women age <35 years and 3 embryos for women age ≥35 years who underwent second cycle IVF. United State and United Kingdom do not regulate the number of embryo transfer; instead they provide clinical recommendations regarding the issue.

American Society of Reproductive Medicine (ASRM) 2004 guideline recommended the number of embryos to be transferred was based on age and favourable prognosis such as first IVF cycle, previous successful IVF and availability of good quality embryos for cryopreservation. Meanwhile, women who had a history of failed IVF twice or more or had a less favourable prognosis, extra embryos may be transferred after a thorough consultation. While in case of the egg donation, the age of the donor was a factor that decided the number of embryos to be transferred(Technology, 2004)

All women undergoing ovulation induction should be informed regarding the risk of multiple pregnancy and ovarian hyper stimulation .Any infertility centres should have their own protocols to minimise the risk associated with infertility treatment.

According to the standard for assisted reproductive technology facility-embryology laboratory and operation theatre (medical development division ministry of health, Malaysia), data from the laboratory need to be regularly analysed so that minimum criteria are achieved: fertilization rate of at least 60% in couple without male factor infertility, fertilization of at least 60% following ICSI, a minimum pregnancy rate 25% per embryo transfer for IVF/ICSI. This study was conducted mainly to look for the local data/statistic

So far there is no law describing the acceptable number of transferred embryos available in Malaysia.

LITERATURE REVIEW

The number of embryo to be transferred has been considered as one of the predictor/factor to increase implantation rate and pregnancy rates.

The more embryos transferred, the higher the pregnancy rate achieved (Elsner *et al.*, 1997).

Shieve et al,1999 also mentioned that the chance of pregnancy increases along with the number of embryos transferred (Schieve *et al.*, 1999).

Instead a study by Ashrafi M et al, 2015 found that increasing the number of embryos transferred from two to three did not increase the pregnancy and live birth rates. Furthermore the results between two groups were found to be similar. However there was an increase in multiple pregnancies.

These results were similar to another study by Pandian Z et al, where the study mentioned that there was no significant live birth rate between two groups when comparing two embryo transfers with three embryos transfer, but multiple pregnancy rate was significantly lower in the first group.(Pandian *et al.*, 2005).

On the other hand, if single embryo transfer was compared to double embryo transfer, the on-going clinical pregnancy rates and live births was higher in double embryo transfer (Baruffi *et al.*, 2009).

Furthermore there also issue regarding increase in multiple pregnancy if more embryo to be transfer. Multiple pregnancies are associated with increased risk of maternal complication and morbidity (Walker et al., 2004) and also adverse neonatal outcomes, especially since there were more complications in higher order multiple gestation such as in triplet and quadruplet (Seoud et al., 1992).

Age of the women is known to affect the pregnancy rate and may influence the decision for number of embryo transfer per cycle. Other factors that may also affect successful outcome of IVF are duration of infertility, previous pregnancy and previous unsuccessful IVF attempt **(**Templeton *et al.*, 1996)

According to data by Setti et al, the reduction in the number of embryos transferred, from three to two in women <36 years of age, and from four to three in women ≥36 years of age, adversely affects the outcome of clinical pregnancy rate. The results also showed that a reduction in the number of embryos transferred was associated with a significant reduction of the general pregnancy rate with no significant effects in the rate of twin pregnancies, but the adverse outcome was in term of triplet pregnancy.

There also evidence that pregnancy rate increased if there was no reduction in embryo transfer (up to three in women <36 years old, and up to four in women age ≥36 years). (Setti *et al.*, 2005)

Meanwhile Widra et al, found that transferring more embryos in older women result in pregnancy rate that was not statistically different than in younger women.(Widra *et al.*, 1996)

The correlation between age and initial transfer preferences was found to be significant, as younger women favoured single embryo and double embryos transfer, whereas older women viewed multiple embryos transfer as desirable (Newton *et al.*, 2007).

A study by Md Latar IL and Razali N in UMMC revealed that women older than 35 years of age and had long duration of infertility viewed multiple pregnancy as acceptable even though they knew the risk associated with multiple pregnancies (Md Latar and Razali, 2014).

Furthermore for a large number of infertile couple, twin pregnancy is considered a desirable outcome(Gleicher *et al.*, 1995; Kalra *et al.*, 2003).

Embryo quality is among the variable that determine the pregnancy rate and outcome. The greater number of high grade embryo transferred resulted in higher clinical pregnancy rate (Burke *et al.*, 2000).

However, along with the significant increase of pregnancy rate and implantation rate, there was also a significant increase in multiple pregnancy if higher numbers of good quality embryos were transferred. (Staessen *et al.*, 1992) .Furthermore limiting two transfer from three transfer did not affect pregnancy rate in good prognosis patient (Staessen *et al.*, 1993).

Taşdemir M et al, in his study noted that if there was one good quality embryo present, transfer of either two or three embryos will not result in a statistically significant difference in pregnancy rate. Only when poor quality embryos were transferred that the number of embryos transferred per cycle will statistically influence the pregnancy rate.(Taşdemir *et al.*, 1995)

Another predictor for successful treatment of infertility is duration of infertility. Longer duration influenced the success rate of IVF even though in similar age group women. (Templeton *et al.*, 1996)

Embryo scoring criteria (based on Hospital Sultanah Nur Zahirah Reproductive and Infertility Protocol and Guideline)

Grade 1(excellent)	
1) Embryo at time of observation at least on:	a. Day 2: a 4cell b. Day 3:a 8 cell c. Day 4 :a compacted morula d. Day 5: a blastocyst with clear inner cell mass (ICM) and blastocoel cavity e. Day 6:a fully expanded blastocyst with clear ICM and blastocoel cavity
2) <10 %fragmentation 3) Blastomeres in proportion to each other with no sign of dark cytoplasm or poor blastomere membrane	
Grade 2(good)	Embryos exhibits 2 of the 3 characteristic for grade 1 embryos
Grade 3 (moderate)	Embryos exhibits 1 of the 3 characteristic for grade 1 embryos
Grade 4(poor)	Embryos exhibits 0 of the 3 characteristic for a grade 1 embryo

*Generally only embryo of grades 1 & 2 are chosen for Embryo Transfer, and grades 3 & 4 discarded

JUSTIFICATION OF STUDY

This study was chosen because:

- Hospital Sultanah Nur Zahirah, Kuala Terengganu is one of the established infertility centres in Malaysia and is the main centre for the East Coast region of Malaysia. Therefore, we need to explore and study various aspects on management of infertility problems/procedure in order to produce our own guidelines and protocols and to improve our reproductive services.

- To determine the outcomes of two versus three embryo transfer in term of the Clinical Pregnancy Rate (CPR) from January 2010 – March 2015 in Reproductive Unit, Hospital Sultanah Nur Zahirah, Kuala Terengganu.

- This study was also conducted to determine whether the number of embryos transferred of two versus three in assisted reproductive technology (ART) cycles can maintain acceptable pregnancy rates while reducing the overall incidence of multiple pregnancies.

RESEARCH OBJECTIVES

General Objective

- To determine the prevalence of clinical pregnancies after two versus three embryo transfer in patients undergoing IVF.

Specific Objective

- To compare the clinical pregnancy rate between two or three embryo transfer.
- To compare the pregnancy outcome between the two groups, in terms of:
    - Multiple pregnancies
    - Miscarriage
- To look for incident of and ectopic pregnancy following each embryo transfer

RESEARCH HYPOTHESIS

There was no association between numbers of embryos transfer with the number of clinical pregnancy.

METHODOLOGY

STUDY DESIGN, LOCATION AND PERIOD OF STUDY

• Retrospective study comparing number of embryos transfers (two vs. three) following IVF/ICSI. This study was conducted from January 2010– March 2015 in Reproductive Unit, Hospital Sultanah Nur Zahirah, Kuala Terengganu.

STUDY POPULATION

- All women subject to IVF/ICSI in Reproductive Unit, HSNZ

INCLUSION CRITERIAS

Infertility patients attending infertility clinic and subjected to IVF /ICSI were included in this study:

- Couple with two or more embryo available
- Couple with repeat IVF cycle

EXCLUSION CRITERIAS

- Couple with only one embryo available
- Age >42year