EFFECT OF ENDOMETRIAL RCEPTIVITY ON IN VITRO FERTILIZATION/ INTRACYTOPLASMIC SPERM INJECTION OUTCOME IN HUSM

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1.0   PRELIMINARIES

1.1   TABLE OF CONTENTS

1.2 ACKNOWLEDGEMENT

Last but not least, I like to forward my gratitude to my colleagues and all staff whose help made the completion of this study possible.

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1.3 ABSTRAK

OBJEKTIF: Untuk menentukan kaitan di antara kesediaan endometrium dari segi ketebalan endometrium, rupa bentuk endometrium dan tahap progesteron darah dengan kebolehan mengandung pesakit-pesakit yang menjalani kitaran in vitro fertilization/ intracytoplasmic sperm injection (IVF/ ICSI).

KAEDAH KAJIAN: Seramai 50 orang pesakit yang menjalani kitaran IVF/ ICSI di Unit Rawatan Masalah Kesuburan Hospital USM telah menyertai kajian yang dijalankan selama 18 bulan ini. Pesakit-pesakit telah menjalani stimulasi ovari terkawal sehingga folikal bersaiz matang. Proses pengambilan benih wanita (oocyte retrieval) dilakukan dan seterusnya benih-benih ini telah disenyawakan secara IVF/ ICSI. Pemindahan janin dilakukan semasa peringkat blastocyst. Kejayaan mengandung ditentukan dengan melihat tahap beta hCG di dalam darah yang diambil 14 hari selepas pemindahan janin. Analisa untuk melihat kaitan di antara ketebalan endometrium, rupa bentuk endometrium dan tahap progesteron semasa hari suntikan hCG dengan kebolehan mengandung telah dilakukan.

KEPUTUSAN: 10 daripada 50 orang pesakit (20.0%) telah didapati positif mengandung. Purata ketebalan endometrium di kalangan mereka yang mengandung adalah 9.86 ±1.68 mm, manakala purata ketebalan endometrium bagi kumpulan tidak mengandung adalah 9.77± 2.53 mm. 7 daripada 10 orang pesakit yang mengandung mempunyai rupa bentuk endometrium trilaminar. Purata tahap progesteron semasa hari suntikan hCG adalah 3.717 ± 1.071 nmol/L bagi kumpulan yang mengandung dan 1.408 ± 4.691 nmol/L bagi

kumpulan yang tidak mengandung. Kesemua perbezaan ini didapati tidak signifikan dari segi statistik.

KESIMPULAN: Tiada kaitan didapati di antara ketebalan endometrium, rupa bentuk endometrium dan tahap progesteron darah semasa hari suntikan hCG dengan hasil kitaran IVF/ ICSI. Walau bagaimanapun, keputusan ini tidak muktamad disebabkan oleh saiz sampel yang kecil.

1.4   ABSTRACT

OBJECTIVE: To evaluate the association of endometrial receptivity by means of endometrial thickness, endometrial morphology and the level of serum progesterone with the outcome of in vitro fertilization/ intracytoplasmic sperm injection (IVF/ ICSI) cycles.

STUDY METHOD: This prospective study was performed over a period of 18 months in Hospital USM Fertility Unit. A total of 50 patients going to receive IVF/ICSI cycles participated in the study. 50 subjects had undergone controlled ovarian hyperstimulation protocol until mature follicles were observed. On the day when the patient is to receive hCG injection, the endometrial thickness, endometrial morphology and the level of serum progesterone were determined. Oocyte retrieval was done which was followed by IVF or ICSI. The developed embryos were transferred into the uterus at blastocyst stage. Serum beta hCG was measured 14 days after embryo transfer. The association between endometrial thickness, endometrial morphology and level of serum progesterone with pregnancy was analyzed.

RESULT: Pregnancy was achieved in 10 out of 50 patients (20.0%). The mean endometrial thickness for the pregnant group was 9.86 ± 1.68 mm, whilst 9.77 ± 2.53 mm in the non-pregnant group. 7 out of 10 pregnant patients had trilaminar endometrium. The mean progesterone level during the hCG day was 3.717 ± 1.071 nmol/L for the pregnant group and 1.408 ± 4.691 nmol/L for the non-pregnant group. All differences were not statistically significant.

CONCLUSION: Endometrial thickness, endometrial morphology and the level of serum progesterone on the hCG day was not significantly associated with the outcome of IVF/ ICSI cycles. However, this finding was guarded by the small sample size.

2.0   INTRODUCTION

2.1   Literature Review

Successful implantation requires good harmony between the endometrium with the embryo. Uterine receptivity accounts for about 31-64% of implantation. A blastocyst can implant into the endometrium only during short period of time called window of implantation. It is believed that it lasts about 48 hours, beginning 6-10 days after the LH surge in a spontaneous cycle. The window is advanced in clomiphene citrate or gonadotropin stimulated cycles and can delay in steroid hormone replacement cycles for donor recipients. There are many methods can be used to determine good endometrial receptivity, such as hormones manipulation, glycodelin, IGFBP-I, intergrin, myometrium, morphology and endometrial thickness (ET), and of recent is the progesterone level right after the LH surge.

2.1.1 Endometrial thickness as an indicator for good endometrial receptivity

Transvaginal ultrasound has been widely used to measure the endometrial thickness (ET) and this has been accepted as indicator for endometrial receptivity. It is cost effective and easily done by operator and widely available in all infertility unit.

Although endometrial thickness is a predictor to the degree of endometrial maturation, there is no ET value that confirms pregnancy in IVF/ ICSI cycle. Glissant et al in 1985

concluded that there was a significantly greater thickness in pregnant cycles, but did not indicate the cut off value which could enhance implantation (1).

A retrospective study on ICSI by Bozdag et al in 2009 have shown that ET of less than 7mm on the day of hCG is associated with lower pregnancy rate compare with group with ET between 7-14 mm. The pregnancy rate (PR) for those whose ET more than 14mm was shown to be comparable to the 7-14mm group. In contradiction to Bozdag et al, Weissman et al in 1999 reported a reduction in pregnancy and implantation rate when ET was more than 14mm on the day of hCG administration (40).

The frequently reported cut-off of 7 mm is related to a lower chance of pregnancy, but occurs infrequently. Several studies suggest a thickness less than 8mm is associated with lower pregnancy rate. Some other authors indicate thickness of 9-14mm is associated with higher implantation rate compared to those with 7-8mm. A study by Geyter et al, 2000 revealed that PR in patient with a thin endometrium was equal to those with normal endometrium. Based on previous studies, the lowest significant cut off value of ET was varied from 6-9mm. Due to the conflicting data on the best ET, the use of ET alone as a tool to decide on cycle cancellation, freezing of all embryos or refraining from further IVF treatment seems not to be justified.

2.2   Source population and sampling frame

All patients subjected to receive IVF/ICSI-ET cycles.

2.2.1 Endometrial morphology as indicator for good endometrial receptivity

Endometrial morphology is described as, homogenous or presence of trilaminar layer. It is found that trilaminar pattern was predictive of pregnancy. However pregnancies can occur in absence of this pattern, albeit at a lower frequency (Leibovitz at el 1999). Failure to establish a homogenous hyperchogenic pattern is associated with lower pregnancy rate.

Chen et al, 2010, conducted combined analysis of endometrial thickness and pattern in predicting the outcome of in vitro fertilization and embryo transfer (24). They concluded that the combination of ET with trilaminar pattern is associated with higher implantation rate. This information may be more helpful for patient counselling than the separate analyses.

2.2.2 Effect of serum progesterone on endometrial receptivity

Progesterone is a hormone of secretory phase that increases uterine receptivity by various mechanisms which include mast cell maturation, degranulation, production of cytokines and growth factors which are responsible for blastocysts implantation, successful conception and continuation of pregnancy. It has been debated whether the increment of

progesterone level in late follicular phase of controlled ovarian hyperstimulation cycle has a detrimental effect on the outcome of IVF (9-12).

Rehana Rehman et al in 2014 (14) reported that high progesterone level has deleterious effect on endometrial secretory transformation by causing early closure of implantation window. They indicated its level of more than 0.784ng/ml is associated with lower pregnancy rate. Other studies which are in agreement that high progesterone has reversal effect on successful outcome gave different cut off level. Other studies reported cut off level of 6.0nmol/L while some have taken 3.9nmol/L as the cut of value (11-17).

The difference in the minimum progesterone value may be contributed by different patient characteristic and protocol. Therefore, it is important to determine the minimum level for each center to improve the pregnancy rate.

2.3 Study Justification

The high cost and relatively low implantation and pregnancy rate in any ART treatment cycles have led to the need for evaluation of the predictors in these patients. Although many studies were previously done to evaluate the endometrial thickness during IVF/ ICSI cycles to predict the outcome, no conclusive cut off value of endometrial thickness could be obtained. In addition, the knowledge on the effect and cut off value of progesterone level in the serum in determining endometrial receptivity is rather new. Knowing the endometrial predictive factors for the success of IVF/ ICSI may not only could enhance the success of IVF/ ICSI cycles, the prognosis of the treatment could be discussed with the patients, thus helping them in making decision whether or not to proceed with the

treatment. Besides, having a proper understanding of the about to received treatment and its prognosis could reduce patient's anxiety and stress. Anxiety and stress has been shown to be an independent contributory factor for failure of any fertility treatment. Because of these reasons, this study, which is a part of a bigger study which looks into all the contributory factors for the success of IVF/ ICSI cycles, was performed.

3.0   OBJECTIVES

GENERAL OBJECTIVE

To evaluate the endometrial receptivity among patients receiving IVF/ ICSI cycles

SPECIFIC OBJECTIVES

1. To determine the pregnancy rate of patients going IVF/ ICSI cycles

2. To evaluate the association between endometrial thickness and morphology with the outcome of IVF/ICSI cycles

3. To evaluate the association between the level of serum progesterone on hCG injection day with the outcome of IVF/ICSI cycles

4.0   METHODOLOGY

4.1   Study design, setting and duration

This study was a prospective cohort study, which was done in the Hospital USM Fertility Unit of the Department of Obstetrics and Gynaecology, Hospital USM. It was performed for a period of eighteen months, from 1st February 2016 until 31st July 2017.

4.2   Reference population

The study includes all patients attending the Hospital USM Fertility Unit.

4.4   Sample size calculation

The sample size was calculated using the PS software, as in Table 1 and Table 2.

Table 1: Sample size calculation for endometrial thickness and level of serum progesterone

t-test	A	M	Power	δ	σ	Sample size
Endometrial thickness (43)	0.05	1	0.8	4	1.6	4
Age (43)	0.05	1	0.8	31.8	4.8	4

Progesterone level on day of hCG injection (31)	0.05	1	0.8	1.48	0.9	16
Duration of stimulation (36)	0.05	1	0.8	10.2	1.9	2

Table 2: Sample size calculation for endometrial morphology

Dichotomous	A	Power	Po	P1	m	Sample size
Endometrial morphology on day of hCG injection (19)	0.05	0.8	0.38	0.78	1	23

A minimum of 23 patients need to be recruited to meet 80% of statistical power. 50 patients were recruited.

4.5 Ethics and consent

The conduct of this study was approved by the Human Medical Research and Ethics Committee of USM dated 30th June 2015.

4.6 Inform consent

All patient attending the Hospital USM Fertility Unit and were subjected to IVF/ICSI cycle were invited to participate in this study.

The counselling on the IVF/ ICSI procedure was conducted by the Fertility Consultant. The indication of the IVF/ ICSI cycle, the ovulation induction protocol, the IVF/ ICSI procedure and its complication was explained in detail. Written consent was subsequently obtained from the couple. The couple was also informed that they have the right to withdraw from the study at any time without penalty.

4.7 Inclusion and exclusion criteria

4.7.1 Inclusion criteria

All patients must fulfil the below inclusion criteria before being enrolled into the study:

1. Agreed to receive IVF-ET/ICSI- ET in Hospital USM
2. Patients with acceptable ovarian reserved (serum FSH <10miu/ml and/or antral follicles >10)
3. Undergoing 1st cycle of fresh embryo transfer

4.7.2 Exclusion criteria

1. Patients with no developing embryo, or only Grade C embryos available
2. Patients with uncontrolled diabetes mellitus

3. Patients with underlying psychiatric disorder

4. Recurrent miscarriage

5. Patients diagnosed with antiphospholipid syndrome

6. Past or family history of inherited congenital disorders

7. Patients with active pelvic inflammatory disease

8. Patients with underlying TB endometritis

4.8 Sampling method and subject recruitment

All patients who attended the Hospital USM Fertility Unit, agreed to undergo the IVF/ICSI cycle and fulfilling the inclusion and exclusion criteria were invited to participate in the study. The consent was taken from the patient upon her enrollment by the Fertility Consultant. Sampling of these patients was done according to systematic random sampling, by alternating sampling.

4.9 Study method

4.9.1 Stimulation protocol

All patients were stimulated using the short GnRH antagonist protocol, using subcutaneous folitrophin beta (Puregon®) at the dose of 300iu daily, starting from the second day of menses of the treatment cycle. A transvaginal ultrasound was performed after 5 days of stimulation. Once the size of the follicles reached 13- 14mm, GnRH antagonist, garnirelix (Orgalutron®) at the dose 250mcg, was added. The transvaginal ultrasound scan was repeated every other day of stimulation, from that day onwards.

Once at least three follicles with the diameters of 18mm were observed, hCG (Pregnyl®) 10,000iu, which acts as LH surrogate to trigger ovulation, was administered. If less than three follicles were seen, the cycle was cancelled and was considered as failed stimulation. This patient dropped from the study.

The endometrial receptivity was determined by looking at the thickness and morphology of the endometrium, together with the level of progesterone on the day when the hCG was administered. For this purpose, 5cc of blood was withdrawn from the patient to determine the progesterone level. An ultrasound scan to determine the number of follicles stimulated, the thickness and morphology of the endometrium was done on the same day. The ultrasound scan was done by the consultant of the Hospital USM Fertility Unit. The endometrial thickness was determined by placing the electronic calipers on the outer walls of the endometrium at its widest diameter as seen in the sagittal axis of the uterine fundus. Endometrium pattern was graded as 'trilaminar', where triple lines, where the endometrium was hypoechoic with well- defined outer wall and a central echogenic line, was seen. The terminology of 'homogenous endometrium' was used when the endometrium was homogenously echodense with no visualization of central echogenic line as describe earlier. Endometrial evaluation was a routine part of monitoring at any stimulation cycles.

4.9.2 Oocyte retrieval

This procedure was performed under general anaesthesia. The transvaginal oocyte retrieval was performed by the consultant of the Fertility Unit, 36 hours after the administration of hCG. The presence of oocytes was obtained by one embryologist. Three times of follicular

flushing was done when no oocytes obtained at the first attempt. The number and quality of oocyte retrieved from each ovary was determined.

4.9.3 Embryo culture

All oocytes which were obtained were fertilised either by IVF or ICSI, 17 hours after the retrieval. The embryos were cultured in Vitrolife media. The presence of pronuclear cleavage was determined 24 hours later. The development of the embryo was monitored and graded on daily basis, with Grade A was considered as good quality embryo and Grade B and C were taken as poor quality embryo. The Grade A and B embryos were transferred at the blastocyst stage, i.e. on the 5th day of development, whilst Grade C embryo was not transferred as it might not be compatible to life.

4.9.4 Embryo transfer

The embryo was transferred transcervically into the uterus, under ultrasound guided. In accordance to the Malaysian government regulation of ART, not more than 2 embryos were transferred at a time. The good quality embryo has the best chance for implantation compared to the poor quality embryo. Only Grade A and Grade B embryo, to a maximum of two embryos, was transferred in this study. The remaining available embryos were cryopreserved for future usage.

The luteal phase was supported with vaginal gel 90mg micronized progesterone (Crinone 8%), which was administered on daily basis.

4.9.5 Determination of pregnancy

Three weeks after the fresh embryo transfer, 3cc of blood was taken to determine the level of beta hCG. Beta hCG of more than 0.2iu/L was taken as successful IVF cycle (biochemical pregnancy). An ultrasound scan of the uterus was performed two weeks later to determine the presence of gestational sac, which taken as successful clinical pregnancy.

4.10 Statistical analysis

The complete clinical research forms (CRFs) were checked and compiled. The information obtained in the CRFs was transferred to the computer data base file. The data was analyzed using statistical package SPSS version 23.0. All the variables were read and frequency runs were performed on them to check for errors, including coding error and missing values.

The Chi-square test and Fisher's exact test were used to analyze nominal variables in the form of frequency tables (endometrial morphology). Independent T test were used to analyze the numerical data, such as the endometrial thickness. Logistic regression analysis was performed to discriminate the independent data that would predict pregnancy rate. A p value of <0.05 was considered as statistically significant.

4.11 Definition of operational term

- Endometrial thickness- the maximal distance between echogenic interfaces of the myometrium and the endometrium and measured in the mid sagittal plane by two dimensional trans vaginal ultrasound

- In vitro fertilization (IVF)- insemination in vitro. The capacitated sperm were placed into culture media containing oocyte within 4-6 hours post retrieval.

- Intracytoplasmic sperm injection (ICSI)- one single spermatozoa was injected directly into the cytoplasm of an oocyte by micropuncture of zona pellucida under high quality inverted operating microscope.

- Oocyte retrieval- a procedure done to retrieve oocyte via vaginal needle under ultrasound guidance 36 hours after hCG injection

- Embryo transfer- fertilized ova at blastocyst stage were place into uterine cavity close to fundus , on the fifth day of fertilization, through a fine flexible soft catheter transcervically

- High quality embryo- grade A embryo- with even blastomeres and fragmentation

- Grade B embryo-even blastomere and less than 20% fragmentation

- Fresh embryo- embryo from fertilized ova at blastocyst stage

- hCG- human chorionic gonadotrophin

- Ovolution induction- process where drugs or hormone given to enhance the number and development of follicles

- Aspermia- failure of emission of semen (no ejaculate)

4.12 Study flow chart

The flow chart of the study is as shown in Figure 1.

Women attending Hospital USM Fertility Clinic and planned to receive IVF/ ICSI treatment

Inclusion criteria

Exclusion criteria

Consent

Ovarian hyperstimulation with monitoring

hCG administration

Assessment of endometrial receptivity

Oocyte retrieval

- Number of oocytes retrieved
- Quality of oocytes
- Number of embryos
- Quality of embryo

Serum progesterone

Endometrial thickness and morphology

Ultrasound to assess endometrial thickness and morphology

Assessment for chemical pregnancy (serum β-hCG)

Serum β-hCG >2miu/L

Serum β-hCG <2miu/L

Assessment for clinical pregnancy (ultrasound)

Presence of gestational sac

Absence of gestational

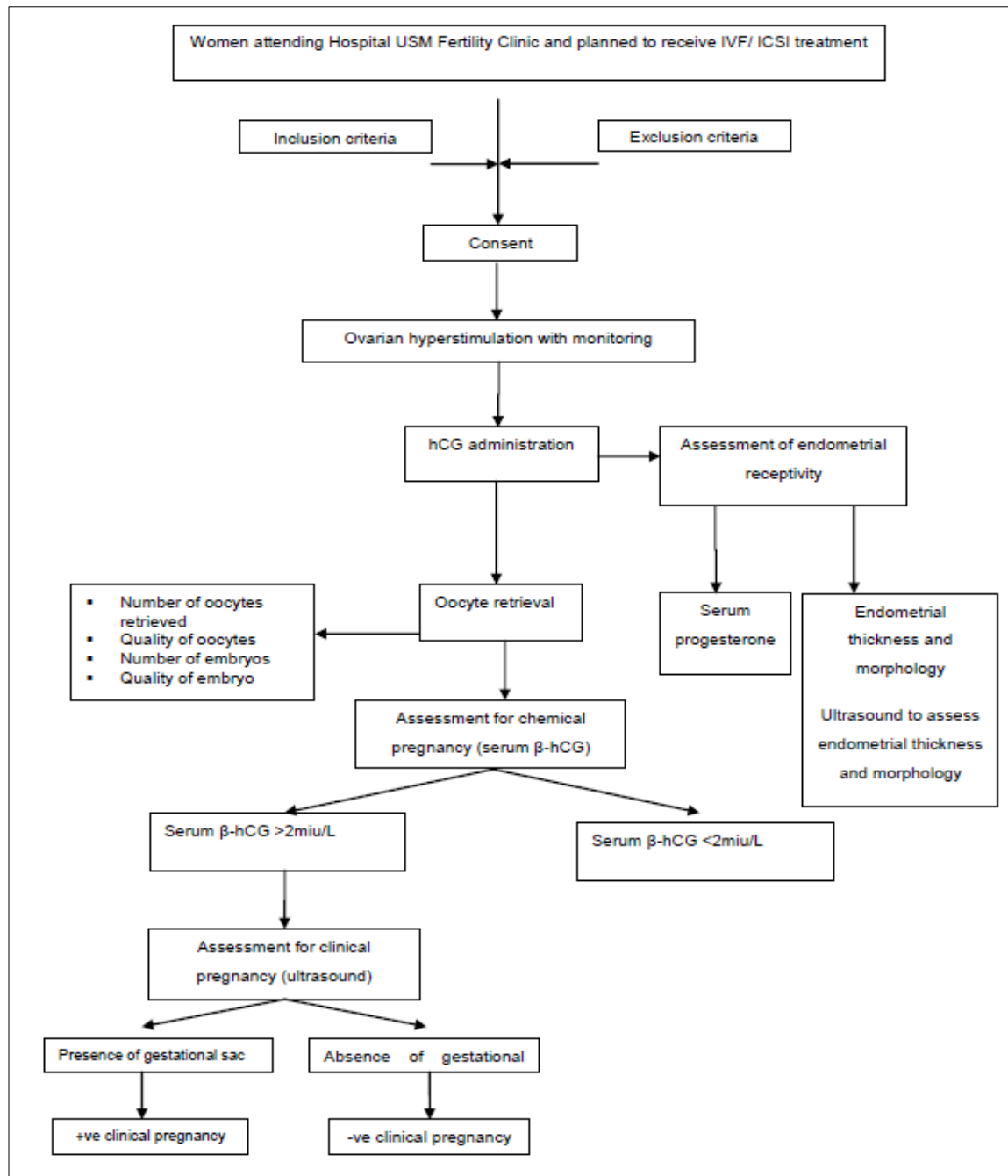+ve clinical pregnancy

-ve clinical pregnancy

Figure 1: Study Flow Chart

5.0   THE MANUSCRIPT

Effect of endometrial receptivity on in vitro fertilization/ intracytoplasmic sperm injection outcome in HUSM

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ABSTRACT

OBJECTIVE: To evaluate the association of endometrial receptivity by means of endometrial thickness, endometrial morphology and the level of serum progesterone with the outcome of in vitro fertilization/ intracytoplasmic sperm injection (IVF/ICSI) cycles.

STUDY METHOD: This prospective study was performed over a period of 18 months in Hospital USM Fertility Unit. A total of 50 patients going to receive IVF/ICSI cycles participated in the study. 50 subjects had undergone controlled ovarian hyperstimulation protocol until mature follicles were observed. On the day when the patient is to receive hCG injection, the endometrial thickness, endometrial morphology and the level of serum progesterone were determined. Oocyte retrieval was done followed by in-vitro fertilization or intracytoplasmic sperm injection. The developing embryos were transferred into the uterus at blastocyst stage. Serum beta hCG was measured 14 days after embryo transfer. The association between endometrial thickness, endometrial morphology and level of serum progesterone with pregnancy was analysed.

RESULT: Pregnancy was achieved in 10 out of 50 patients (20.0%). The mean endometrial thickness for the pregnant group was 9.86 ± 1.68 mm, whilst 9.77 ± 2.53 mm in the non-pregnant group. 7 out of 10 pregnant patients had trilaminar endometrium. The mean progesterone level during the hCG day was 3.717 ± 1.071 nmol/L for the pregnant group and 1.408 ± 4.691 nmol/L for the non-pregnant group. All differences were not statistically significant.

CONCLUSION: Endometrial thickness, endometrial morphology and the level of serum progesterone on the hCG day was not significantly associated with the outcome of IVF/ ICSI cycles. However, this finding was guarded by the small sample size.

Keywords: Endometrial receptivity, endometrial thickness, progesterone level, IVF/ ICSI cycles

INTRODUCTION

The advancement of assisted reproductive techniques has led to the improvement of pregnancy rates with in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) cycles. However, despite the advance techniques used, there were lots of failed cycles which have led to disappointment and more stressful condition among the couples who sought the treatment. The stressful condition has virtuously caused an increasing rate of failed cycle. For decades, researchers have tried to look at the factors which may contribute to the failure of IVF/ ICSI cycles. Endometrial receptivity has been shown to be one of the factors.

Endometrial thickens during the proliferative phase of the menstrual cycle in response to the oestrogen secretion by the maturing follicles. The thickened endometrium provides a site for embryo implantation during the first few weeks until placenta develops. Although there is little doubt that the physiological thickness of the endometrium is critical in the implantation of a successful pregnancy, there is still a controversy on the variation of thickness contributing to the success of IVF/ ICSI cycles.

Several studies have suggested a correlation between greater endometrial thickness (ET) for pregnant versus non-pregnant of IVF/ ICSI cycles (1-7). Some studies have reported non-successful pregnancies with ET below 6-9mm, even though the number of transfers to patients below this threshold were small (5-10). In contrast, many studies reported that there was no correlation between ET with pregnancy rates (9-10).

The current study was designed to determine the correlation between endometrial receptivity, which include ET and endometrial morphology and level of progesterone during hCG injection day with pregnancy in IVF/ ICSI cycles. Should it be significantly correlated, to identify the thickness required for successful implantation.

METHODOLOGY

This study was conducted over a period of 18 months, from 1st February 2016 until 31st July 2017, in the Hospital USM Fertility Unit. Approval to conduct this study was obtained from the Human Medical Research and Ethics Committee of USM. All patients who were subjected for IVF/ ICSI cycle were recruited using the systematic random sampling method. Patients with medical illnesses such as uncontrolled diabetes mellitus, antiphospholipid disorders, active pelvic inflammatory disease, tuberculosis, psychiatric illness and recurrent miscarriages were excluded.

Patients were stimulated using the short antagonist protocol. Subcutaneous follitrophin beta (Puregon®) at 300iu was administered on daily basis starting from the second day of menstrual cycle. Every other day of transvaginal scan were performed starting from day seventh of the cycle to determine the number and size of growing follicles. When at least three dominant follicles reached 13 to 14mm in diameter, additional 250mg of GnRH antagonist (Orgalutron®) injections were added, to prevent from premature LH surge which may cause premature ovulation, until the growing follicles mature at sizes of 16-18mm in diameter. At this stage, the number of mature follicles, endometrial thickness and morphology, as well as the level of serum progesterone were determined, and10000iu of hCG injection was administered at 2200hour on the same day.

Ultrasound guided oocyte retrieval was performed 36 hours later with the patient put under general anaesthesia. The number of the oocyte obtained was documented. The oocyte was then inseminated either using the IVF or ICSI as appropriate, using the Vitrolife® culture media. The success of fertilization was determined 17 hours later by looking at the presence of pronuclear body. Subsequently the embryos were cultured until they reached blastocyst stage. The blastocyst –stage embryos were transferred into the uterus five days after fertilization, under ultrasound guidance. Prior to the transfer, the quality of the embryos were determined. Only two embryos were transferred at a time. The remaining embryos were frozen in liquid nitrogen. Progesterone supplementation was given to all patients after the transfer.

The serum level of beta hCG was determined three weeks after the transfer. Patients with serum beta hCG more than 2miu/L was labeled as having biochemical pregnancy. Clinical pregnancy was defined by visualization of gestational sac on ultrasound 3 weeks after embryo transfer.

The relationship between patients' age, stimulation length, embryo- quality score, endometrial thickness, morphology and serum progesterone on hCG injection day with success of implantation was determined by t-test. The primary infertility diagnosis was compared by chi-square analysis. Receiver operating characteristic (ROC) analysis was use to evaluate the discriminatory ability of endometrial thickness.

RESULTS

Out of 58 patients initially enrolled, only 50 patients completed the cycle until embryo transfer. 2 patients whose age 39 and 42 years had empty oocyte syndrome, 4 patients developed ovarian hyperstimulation syndrome for which the embryo transfer were deferred, and 1 patient had all Grade C embryos and another patient had arrested embryo. The flow chart of the patients' recruitment is as shown in Figure 3.

Patients ages ranged from 26 – 43 years (mean age of 34.74 ± 4.34 years). 41 patients (82.0%) were having primary infertility while the remaining 9 patients (18.0%) were secondary infertility. The mean duration of subfertility was 7.70 ± 2.95 years. Endometrial thickness on day of hCG administration ranged from 6.1 to 14mm (mean endometrial thickness 9.6 ± 2.36 mm). The endometrium during the hCG day was noted to be trilaminar in 25 patients (50.0%) and another half was having homogenous morphology of endometrium. The mean level of serum progesterone on hCG day was 6.20 ± 11.49 nmol/L.

Out of 50 patients who had completed the study, pregnancy was achieved in ten patients (20.0%). Among them, two patients were delivered at term, one patient had preterm prelabour rupture of membranes at 30 weeks, one patient with ongoing pregnancy while the rest aborted during the first trimester. The other 40 patients (80.0%) failed to achieve pregnancy after the IVF/ ICSI procedure.

Table 3 describes the patients' and cycle characteristic between the pregnant and non-pregnant group. There was no difference in the age of the patients in either group. Patients

achieving pregnancy seems to have longer duration of subfertility. Female factor remains the leading cause of subfertility in the pregnant group (60.0%) whilst combination of female and male factors is the leading cause for the non-pregnant group (35.0%). Patients who managed to achieve pregnancy have shorter stimulation days (10.44 ± 0.88 days) as compared to the non-pregnant group (11.59 ± 1.69 days).

When looking at the ET, most patients have endometrium of more than 8mm thick on the hCG day, regardless of the outcome of pregnancy (9.86 ± 1.68mm and 9.77 ± 2.53mm for pregnant and non-pregnant group respectively). However, patients achieving pregnancy have thicker mean ET. None of the pregnant patients have ET of less than 7mm or more than 14mm.

Most of the pregnant patients have trilaminar endometrium on the hCG day (n=7, 70.0%). 55.0% of the non-pregnant group were noted to have homogenous pattern while only 45.0% of them have trilaminar pattern.

The mean serum progesterone on the hCG day in the pregnant group was 3.717 ± 1.071 nmol/L and 1.408 ± 4.691 nmol/L for the non-pregnant group. None of the pregnant patients has serum progesterone more than 6.0 nmol/L. The lowest serum progesterone in the pregnant group was 1.29 nmol/L and the highest was 5.39 nmol/L.

Due to the imbalance number between the pregnant and non-pregnant group, significant comparison could not be made. However, when the number is balanced and compared, none of the variables to test for endometrial receptivity is significant.