Research Article

The effect of maternal age on the outcomes of in vitro fertilization Sulaimani region

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Abstract

Background In Vitro fertilization IVF can be a very effective fertility treatment. However, we need to have sufficient eggs of sufficient quality in order to make it successful. Both egg quantity and egg quality are very much related to female age A woman's ability to conceive a child reduces with age, on average, the younger the women have the higher chances of success.

Objectives This work is intended to study the impact of maternal age on the outcome of IVF resulting in biochemical pregnancy in Sulaimani region.

Methods This retrospective study has being done in Dwarozh IVF private center in Sulaimani, between December 2008 and January 2011 on 125 women. All of these women had undergone thorough IVF treatment according to established protocols. According to maternal age the women were divided in to group 1 below 35 years and group 2 from 36 years and above.

For each cycle number of retrieved oocyte, injected oocyte, fertilization, number of embryo transfer, quality and grading of the embryo with the result of biochemical pregnancy has been studied.

Then comparison between the two maternal age groups has been done to show the significance of maternal age on the outcome of IVF.

Results For the Group 1(G1), the effect of maternal age on Fertilizes oocytes is significant, as the T-value is 37.035577. The P-Value is < .00001. The result is significant at p < .05.and the percentage of positive biochemical pregnancies was **45.83%.**

While for Group 2 (G2) the effect of maternal age on fertilized oocyte is significant, as the T-value is 58.997473. The P-Value is < .00001. The result is significant at p < .05 and the percentage of biochemical pregnancies were **33.96%**

Conclusion Maternal age has significant effect on the number of fertilized oocytes and percentage of biochemical pregnancy for the IVF process. The percentage of biochemical pregnancy is more in age group less than 35 years.

ABBREVIATIONS: IVF: In vitro fertilization, Oocyte R: oocyte retrieval, MI: Meiosis I, MII: Meiosis II, GV: Germinal vesicle, ET: embryo transfer, Bhcg: Beta human chorionic gonadotropin.

Key words: In vitro fertilization, Maternal age, Fertilization, Biochemical pregnancy.

INTRODUCTION

Ovarian function declines as women approach their later reproductive years until menopause, and increasing age is associated with lowered fecundity and infertility. Women experience a decline in natural fertility that begins in the mid-30s, and they will often reach sterility many years before the complete cessation of menses. Although IVF may aid some couples who present with fertility issues, it

will not compensate for the decline in natural fertility that occurs with delayed child-bearing.IVF is also invasive, expensive, and not covered by most provincial health plans for this indication. In addition,

complications of pregnancy increase for both the mother and the offspring with advanced maternal age (1). The age at which women should be advised against proceeding with initial or further infertility treatment is one of the many unresolved questions in this area of women's health and was the subject of investigation in this study (2).

There is no universal definition of advanced reproductive age in women, in part because the effects of increasing age occur as a continuum, rather than as a threshold effect. Fertility clearly declines with advancing age, especially after the mid-30s, and women who conceive are at greater risk of pregnancy complications (3).Advanced maternal age, in a broad sense, is the instance of a woman being of an older age at a stage of reproduction, although there are various definitions of specific age and stage of reproduction. The variability in definitions regarding age is in part explained by the effects of increasing age occurring as a continuum rather than as a threshold effect. The most commonly used definition of advanced maternal age is 35 years or more at the time of childbirth. Assisted reproductive technologies have helped many families to have their healthy offspring during the past decades. *In vitro* fertilization and embryos transfer (IVF-ET) has become increasingly popular and the pregnancy rate was improved by the development of the technology (3, 4). Many women in advanced age want to have babies through IVF. However, it has been reported that one of the main limiting factors to fertility and reproductive outcomes was female's age (5, 6). Women over 38 years old had poor IVF outcomes, especially in women over 40 years old (7). Then which age period is the best period for pregnancy? Are IVF outcomes worse in women between 30 and 35 years old than those in women under 30 years old? Not many papers mentioned this.

In this study, we examined the difference of IVF outcomes between two maternal age groups, 35 and below (G1) with 36 years and above (G2). There are a lot of studies conducted on the effect of maternal age on the outcome of IVF, as a study done by YAN JunHao (2012) in China concluded that patients with higher maternal age had worse IVF outcomes. In women of fertile age, patients between 20 and 30 years old have the best IVF outcomes. Patients over 40 years old have poor IVF outcome and high miscarriage rate, which suggested the necessity of preimplantation genetic screening (PGS) (8).

In other study the results indicated that maternal age adversely affects both clinical pregnancy rates and rates of spontaneous abortion, when summed across treatments and stimulation protocols. (9).

Other study conclude that To determine true efficacy, success rates have to be related to patient characteristics, such as age and cause of infertility (10).

PATIENTS AND METHODS

All women undergoing IVF in Dwarozh IVF private center in Sulaimani, between December 2008 and January 2011 were included in the retrospective study. All of these women had undergone thorough IVF treatment according to established protocols.The protocol includes:

1. **Ovarian stimulation**: done by gynaecologist starting from the second day of cycle by transvaginal ultrasound and hormonal assay (FSH,LH).Induction by GnRH (Gonadotropin), then follow up by ultrasound, until it show more than 4 follicles with diameter of 16-17mm then administration of HCG (Human chorionic gonadotropin) that assists maturation of ova.
2. **Oocyte retrieval**: Done after 34-36 hours after HCG administration by transvaginal ultrasound ,the oocyte cumulus complex collected under stereo microscope, and washed with flushing media then placed in incubator 37 C and 6% CO2 for 1-2 hours.
3. **Denudation of the oocyte**: Removal of surrounding cumulus cells is accomplished by combined enzymatic exposure to hyaluronidase enzyme in buffered media and mechanical treatment under a stereo microscope. Each oocyte then examined under microscope to assess maturation stage and its integrity. Metaphase MII was assessed according to the absence the germinal vesicle (GV) and the presence of an extruded polar body . Metaphase MI was assessed according to the absence of both GV and extruded polar body and prophase PI when GV was obvious in the absences of extruded polar body.
4. **Sperm preparation:** the collected semen allowed to be liquefy for 15-30 minutes, followed by centrifugation and incubation.
5. **ICSI procedure (intracytoplasmic sperm injection):** was carried out on heated stage (37C) of an inverted microscope 40 hours after HCG trigger for MII stage retrieved oocytes and 64 hours after HCG trigger for immature oocytes that had undergone nuclear maturation.After injection ,the oocyte were washed and stored in culture media and kept in incubator at 37Cfor 16-18 hours.

6. **Assessment of fertilization, embryo quality and embryotransfer:** At 16-18 hours after microinjection, the oocytes were checked for survival and fertilization. The criteria for normal fertilization are the presence of two clearly visible pronuclei, embryo cleavage and quality are evaluated 2 days after ICSI. Embryo transfer was performed on day 2 or 3 .For each couple ,one to three embryos are transferred for female age below 35 years old and more than three embryos for females more than 35 years old. Then cycles followed by serum B-HCG and ultrasound for confirmation of Pregnancy. Biochemical pregnancy was verified by plasma -HCG>20 IU L 14 days after embryo transfer.

Patients

A total of 125 IVF-ET cycles were included in this study. The IVF outcomes were analyzed in two different groups sorted by the age period. The groups were G1 that include 35 years old and below (72 cycles), and G2 were 36 years and above (53 cycles).

IVF outcomes

The means of age, retrieved oocyte, MII, MI, injected and fertilized oocyte, and number of embryo transfer and rate of biochemical pregnancy by BHCG were compared between the two age groups.

Ethics

This study was a retrospective analysis on the clinical practice outcomes and the data of our study were approved by scientific and ethics committee of school of medicine in university of Sulaimani.

Statistical analysis

For G1, the T-value is 37.035577. The P-Value is < .00001. The result is significant at p < .05, between maternal age (less than 35) and the fertilized oocytes. Also the percentage of positive biochemical pregnancies (that are detected by BHCG) was 45.83%.For G2 the T-value is 58.997473. The P-Value is < .00001.

The result is significant at p < .05. between maternal age (more than 35) and the fertilized oocytes. Also the percentage of positive biochemical pregnancies (that are detected by BHCG) was 33.96%.

RESULT

A total of 125 IVF-ET cycles were included in this study. The IVF outcomes were analyzed in two age

Groups, G1: 35 years old and below (72 cycles), and G2 were 36 years and above (53 cycles).There is a significant effect of maternal age on numbers of fertilized oocytes which leads to high percentage of positive biochemical pregnancy in IVF procedure.

Table 1.Showing the results for Group 1 and Group 2

	Group 1 Mean +SD	Group 2 Mean +SD
Age	28.98+3.75	39.16+2.41
Oocyte Retrival	11.26+5.45	8.07+5.76
MII	8.37+4.81	5.94+4.8
MI	1.83+1.28	1.57+0.79
GV	2.09+1.42	1.77+1.26
Injected oocyte	8.37+4.81	5.94+4.8
Fertilized	5.77+3.77	4.2+3.56
No. of ET	3.08+1.12	2.73+1.3
Grade A	1.26+1.18	0.88+0.89
P-value	< .00001	< .00001
Bhcg	45.83%	33.96%

Table 2. Showing the sum and percentages of data for 35 years and below G1

N=72	Oocyte Retrived	MII	MI	GV	Injected Ovum	Fertilized	Bhcg
Sum	811	603	57	65	603	416	33
%		74.35	7.02	8.01		68.98	45.83

Table (3) Showing the Sum and percentage of data of 36 years and above

N= 53	Oocyte Retrived	MII	MI	GV	Injected Ovum	Fertilized	Bhcg
Sum	428	315	44	32	315	228	18
%		73.59	10.28	7.47		72.38	33.96

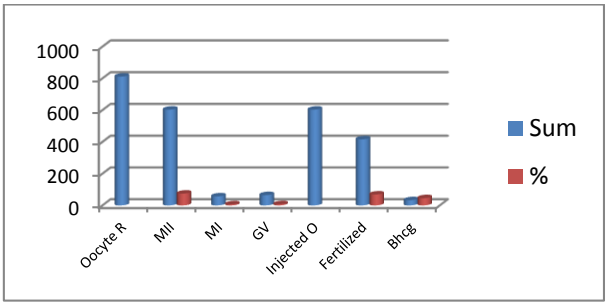

Figure 1. Showing the sum and percentages of the parameters for Group 1

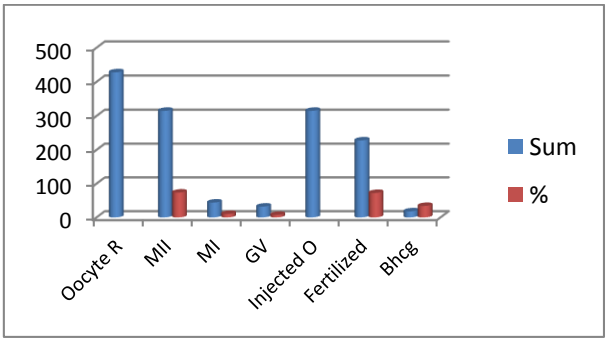

Figure 2. Showing the sum and percentages of the parameters for Group 2

DISCUSSION

Ovarian aging will have begun before women notice any clinical changes to their menstrual cycles; therefore, they are often unaware that they may be at greater risk of infertility.Ovarian reserve testing has been explored as a means to determine a woman's fertility potential and provide an assessment of ovarian aging. Although chronological age alone serves as a good marker of ovarian reserve, some women will experience a decline in their natural fertility sooner than average, while some older women may

maintain above average ovarian function. Identification of these two groups, in which ovarian reserve is inconsistent with chronological age, may be useful for counselling and planning treatment.

There are increased rates of obstetrical and maternal complications with increasing maternal age, including maternal death, hypertension, prematurity, fetal and neonatal death, and operative delivery (1).

It is recognized by many reporters that with the increasing of maternal age, the IVF outcomes become increasingly worse (4). Some IVF centers even limited the last age for IVF as 43 years old (8), Women with advanced maternal age will have poor ovary response during controlled ovary hyperstimulation (COH), low retrieved oocyte number, low oocyte fertilization rate, low good quality embryo rate,

low embryo implantation rate, low pregnancy rate, high miscarriage rate, high preterm delivery rate and high birth defect rate (9,12,13,14).

We found that first of all, in current study, the fertility significantly dropped in women over 35 years old, as shown in table 2 and 3.There is a slow decline in pregnancy rates in the early 30's, The decline is more substantial in the late 30's and early 40's, Very few women over 44 are still fertile. Miscarriage rates also increase as the mother ages. Although there are a high percentage of fertilized oocyte in G2 (72.38%) but the positive biochemical pregnancy percentage are lower than G1 (33.96%) as shown in the Fig.(1 and 2) .This result is going with other study done in China by Liu K, Case A (15).

Advanced maternal age may cause the aging of oocyte which will result in abnormal fertilization and development, such as polyspermy, division arrest, implantation failure and miscarriage.

In addition, It is reported that genetic abnormalities, uterine anatomical defects, antiphospholipid antibodies, environmental factors, advanced maternal age, obesity, endometriosis, etc. may be the possible causes of miscarriage in natural and assisted conceptions (16).

To draw the conclusion, patients with higher maternal age had worse IVF outcomes. Patients between 20 and 30 years old have the best IVF outcomes in women of fertile age. Patients over 40 years old have really poor IVF outcomes and high miscarriage rate, which suggested the necessity of preimplantation genetic screening (PGS) (9).

Conclusion

Maternal age has significant effect on the number of retrieved oocyte, and the outcome of Biochemical pregnancy in IVF process.

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