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Original Article

Obstetrics and Gynaecology Section

Characteristics and Pregnancy Rate of IVF-ICSI Patients with Short Antagonist Protocols: A 4-Year Single Center Retrospective Study

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ABSTRACT

Introduction: In-vitro Fertilisation-Intracytoplasmic Sperm Injection (IVF-ICSI) is a modern technology where a sperm is injected into an ovum. IVF-ICSI is a solution to overcome infertility when all other assisted reproductive technique is not successful. The short antagonist protocol is the most commonly performed ovarian stimulation and the preference in many IVF clinics. The pregnancy rate for IVF-ICSI program in each clinic is influenced by variety of factors.

Aim: To study the success rates and patients characteristics that would increase the pregnancy rate in the IVF-ICSI patients with short antagonist protocols for ovarian stimulation.

Materials and Methods: Medical records of patients who participated in IVF-ICSI program in IVF clinic, from January 2014 to December 2017 were studied. The data were then analysed using Chi-square test.

Results: A total of 428 IVF-ICSI patients met the inclusion criteria; 191 (44.6%) had clinical pregnancies. The mean age of the study population was 33.06±4.529 (22-45) years, the mean maternal BMI was 24.49±4.193 (15.8-43.5) kg/m^2, and the mean duration of infertility was 6.03±3.594 (0-20) years. The number of oocytes obtained Ovum Pick Up (OPU) was 6.74±3.726 (1-22) and mean number of Embryo Transfer (ET) was 2.58±0.946 (1-4). The clinical pregnancy rates significantly increased with the maternal age below 35 years (p=0.017), normal BMI (p=0.016), infertility period less than six years (p=0.005), oocytes more than five obtained during OPU (p=0.001), and three embryos transferred during ET (p=0.003).

Conclusion: The pregnancy rate of IVF-ICSI patients with a short antagonist protocol and fresh ET was 44.6%. The number of oocytes more than five obtained during OPU and three embryos transferred during ET significantly increase the pregnancy rates, while the characteristics of patients who are more likely to be pregnant stastistically are maternal age less than 35 years, a normal BMI, and infertility period of less than six years.

Keywords: Infertility, In-vitro fertilisation, Intracytoplasmic sperm injection, Ovarian stimulation antagonist

INTRODUCTION

IVF-ICSI is a modern technology in which an ovum is injected with sperm outside the body. IVF-ICSI is a solution to overcome infertility when all other assisted reproductive methods are unsuccessful. IVF-ICSI is a complex and expensive procedure. Therefore, not all levels of people in the society can undertake such process [1]. The short antagonist protocol is the most commonly performed ovarian stimulation and the preference of many IVF clinics because it is safer, more convenient and more affordable [2,3].

Typically, IVF has a success rate of 35-42% in various IVF centers globally [4]. Among 32 IVF centers in Indonesia, the pregnancy rate is 28.57% [5]. The variation in the success rate of pregnancy in each clinic is also influenced by various factors other than the methods of ovarian stimulation e.g., age, maternal weight, infertility period, IVF history, pregnancy records, number of eggs, quantity, and quality of the transferred embryo [6]. By understanding these factors that could influence the pregnancy success rate in the short antagonist protocol, infertile couples who will undertake the IVF-ICSI procedure could be informed regarding the actions that need to be taken into account prior to undergoing such mechanisms [7].

MATERIALS AND METHODS

This study used retrospective analysis through Cohort method. Using consecutive sampling method, the samples in this study were taken from the medical records of infertile couples who undertook IVF program, IVF Clinic Prima Medika Hospital, Denpasar, Bali, Indonesia. The data were generated within the period of four years, that is, from January 2014 to December 2017. The Institutional Ethical Committee approval was obtained for the study (035/RSPM/DIR/III/2018).

The data for the following patients were included for the study-patients that followed the first IVF program, underwent all IVF processes through antagonist ovarian stimulation protocol, Intracytoplasmic Sperm Injection (ICSI), and fresh ET. Patients who postponed the above IVF procedures were categorised within exclusion criteria.

Ovarian stimulation: On day 2 of menstrual period, a basic evaluation was conducted by ultrasound examination and hormonal assay (LH, FSH, Estradiol and Prolactin). Ovarian stimulation began with the administration of recombinant FSH (Gonal-F, Merck Serono) injections on day 3, where younger patients (<35 years) were given two ampoules (150 IU) of Gonal-F every day, and elderly patients (≥35 years) were arranged for administration of a three-ampoule injection (225 IU) of Gonal-F every day. The same dose was maintained for the first 4 days of stimulation. On day 7, estradiol serum (E2) and transvaginal ultrasonography examinations were performed to monitor follicular development. The FSH dose was adjusted optimally based on the results of transvaginal ultrasonography for the number and size of developing follicles. The GnRH antagonist, cetrorelix, was then given daily by s.c. injection (0.25 mg/day) at night (6:00-8:00 PM) from day 7 of the stimulation cycle to the day of Human Chorionic Gonadotropin (hCG) administration (permanent antagonist). Additional transvaginal

ultrasound examinations were also performed after days 9 and 11 of treatment.

Procedures for oocyte retrieval: rFSH (Gonal-F, MerckSerono) and cetrorelix (Cetrorelix, MerckSerono) were administered continuously until three follicles reached ≥16 mm. The serum concentrations for Estradiol (E2) was tested and HCG (Ovidrel, MerckSerono) was then administered. Oocytes were retrieved 34-36 hour after HCG injection and were fertilised in vitro (ICSI) according to the standard procedures.

Embryo transfer: Embryo transfer was performed 72 hours after oocyte collection (embryo 3 days). A maximum of three embryos were transferred to each patient, except in special patient circumstances where four embryos could be tranferred. Progesterone (suppositaria) was given every day vaginally; (90 mg/day) from the first day after oocyte retrieval to maintain luteal function. Clinical pregnancy was defined as an increase in serum β-HCG 12 days after ET and the presence of a gestational sac by ultrasonography within 2 weeks after β-HCG examination.

STATISTICAL ANALYSIS

Statistical analysis was done using the SPSS 17.0. The maternal age, maternal BMI, duration of infertility, history of previous pregnancy were collected as part of the patients characteristics. The relationship of characteristics with the success of pregnancy was tested by Chi-square ($p<0.05$).

RESULTS

Based on the analysis, there were 569 records of married couples who took part in the IVF program at IVF Clinic Prima Medika Hospital, with antagonist protocols and 60 cycles were cancelled. There were 428 cycles which met the inclusion criteria (first IVF, short antagonist protocol, ICSI, and fresh ET). It was found that there were 245 chemical pregnancies, consisting of 191 clinical pregnancies and 54 miscarriages [Table/Fig-1]. Hence, it could be inferred that during the four years, the pregnancy rate was 44.6%. The mean of maternal age was 33.06±4.529 (22-45) years, BMI was 24.49±4.193 (15.8-43.5) kg/m^2, and the mean infertile duration was 6.03±3.594 (0-20) years. The mean IVF cycle that the patients undertook was 1.22±0.564 (1-5); number of oocytes obtained through OPU was 6.74±3.726 (1-22), and the mean number of embryos transferred through ET was 2.58±0.946 (1-4).

Year	Cycles	Cancelled cycles	Chemical pregnancy	Clinical pregnancy
2014	90	17	29	21
2015	138	15	57	42
2016	166	13	77	61
2017	175	15	82	67
Total	569	60	245	191

[Table/Fig-1]: Antagonist protocol, inclusion criteria, chemical and clinical pregnancy.

In this study, the pregnancy rate of patients who were less than 35 years old was higher 31.5% (135/428) than those who were above 35 years 13.1% (56/428). The relationship between pregnancy success and maternal age showed a significant difference ($p<0.05$) as shown in [Table/Fig-2].

Age (Year)	Pregnant n (%)	Not Pregnant n (%)	Total n (%)
<35	135 (31.5)	150 (35.1)	285 (66.6)
>35	56 (13.1)	87 (20.3)	143 (33.4)
Total	191 (44.6)	237 (55.4)	428 (100)

[Table/Fig-2]: Maternal age and pregnancy success. ($p=0.017$) n: number of cycles

The highest percentage of patients who became pregnant had normal BMI (27.1%). [Table/Fig-3] shows significant differences in normal BMI amongst others pertaining to pregnancy success ($p<0.05$).

BMI (Kg/m^2)	Pregnant n (%)	Not Pregnant n (%)	total n (%)
Underweight (<18.5)	18 (4.2)	9 (2.1)	27 (6.3)
Normal (18.5-24.9)	116 (27.1)	132 (30.8)	248 (57.9)
Overweight (25-30)	40 (9.3)	75 (17.6)	115 (26.9)
Obese (>30)	17 (4.0)	21 (4.9)	38 (8.9)
Total	191 (44.6)	237 (55.4)	428 (100)

[Table/Fig-3]: Maternal Body mass index (BMI) and pregnancy success. ($p=0.016$); n: number of cycles

A total of 342 (79.9%) primary infertile patients and 86 (20.1%) secondary infertile patients were included in this study. There was no significant difference between primary and secondary infertility to the success of pregnancy as shown in [Table/Fig-4] ($p>0.05$).

Type of Infertility	Pregnant n (%)	Not Pregnant n (%)	total n (%)
Primary infertility	152 (35.5)	190 (44.4)	342 (79.9)
Secondary infertility	39 (9.1)	47 (11.0)	86 (20.1)
Total	191 (44.6)	237 (55.4)	428 (100)

[Table/Fig-4]: Type of infertility and pregnancy success. ($p=0.88$); n: number of cycles

Ninety eight couples (22.9%) took IVF programs with infertility duration less than three years; 161 couples (37.6%) with infertility duration of three to six years; and 169 couples (39.5%) with infertility duration above six years. The relationship between infertility duration and pregnancy success showed a significant difference as shown in [Table/Fig-5] ($p<0.05$).

Infertility duration (year)	Pregnant n (%)	Not Pregnant n (%)	Sum n (%)
<3	39 (9.1)	59 (13.8)	98 (22.9)
3-6	88 (20.6)	73 (17.1)	161 (37.6)
>6	64 (14.9)	105 (24.5)	169 (39.5)
Total	191 (44.6)	237 (55.4)	428 (100)

[Table/Fig-5]: Infertility duration and pregnancy success. ($p=0.005$) n: number of cycles

In this study, the number of oocytes obtained during OPU had a significant difference in pregnancy success as displayed in [Table/Fig-6] ($p<0.005$). Similarly, the number of embryos implanted at ET on pregnancy success showed a significant difference as shown in [Table/Fig-7] ($p<0.005$).

Number of oocytes	Pregnant n (%)	Not Pregnant n (%)	Sum n (%)
<5	57 (13.3)	123 (28.8)	180 (42.1)
>5	134 (31.3)	114 (26.6)	72 (57.9)
Total	191 (44.6)	237 (55.4)	428 (100)

[Table/Fig-6]: Number of oocytes obtained during OPU and pregnancy success. ($p=0.001$); n: number of cycles

Number of embryo	Pregnant n (%)	Not pregnant n (%)	Sum n (%)
1	21(5.0)	49 (11.4)	70 (16.4)
2	43 (10.0)	66 (15.4)	109 (25.4)
3	86 (20.1)	94 (22)	180 (42.1)
4	41 (9.6)	28 (6.5)	69 (16.1)
Total	191 (44,6)	237 (55,4)	428 (100)

[Table/Fig-7]: Number of embryo implanted during ET and pregnancy success. ($p=0.003$); n: number of cycles

DISCUSSION

In this study, the pregnancy success of patients in the first IVF-ICSI program through short antagonist protocol and fresh ET was 44.6 %. According to the 2017 IVF Indonesia Report, from 32 IVF centers with 9,122 IVF unselected cycles (long agonist protocol, short antagonist protocol, mild stimulation, fresh ET and frozen ET), the pregnancy rate was at 28.57% [5].

Maternal Age

In this study, the pregnancy rate of patients aged <35 was higher than those above 35 (31.5% vs. 13.1%). The results of this study were in accordance with the work of Kirti R et al., found that pregnancy success in patients below 35-years-old was at 47.6%. Then it continued to decrease for patients aged 35-37 years (38.9%), 38-40 years (30.1%) and only 20.5% at the age of 41-42 years [1].

In IVF Indonesia Report 2017, with unselected IVF participants, the pregnancy rate below 35 years old was 17.47% and for those above 35 years old was 11.11% [5]. In the IVF cycle, older women tended to produce fewer oocytes and lower implantation potential embryos, which consequently decreased the pregnancy rate [8]. Women who follow an IVF cycle over 35 years have a lower clinical pregnancy rate; lower multiple pregnancy rates and a higher rate of spontaneous miscarriage [1]. In non-selected patients IVF-ICSI, the highest pregnancy rate was observed in women aged 26-30 years, with a gradual decrease according to increasing age [9].

In the case of a Poor Ovarian Response (POR), the women younger than 35 years old showed significantly higher number of mature oocytes, good embryo scores, and higher clinical pregnancy rates compared with POR women aged 35-40 years and above 40 years [10]. Hong-zi DU et al., concluded that embryo quality as a result of IVF-ET was affected by the age of infertile women [11]. Taylor TH et al., studied that there were no noticeable differences in the level of euploidy blastocyst between groups of fertile and infertile patients when classified by maternal age (<35 years and above 35 years). However, implantation and pregnancy rates were significantly higher in the population of fertile patients, although only by transferring euploidy blastocysts. These arguments indicate that infertility as a disease can include other aspects such as the uterus or other unknown embryological factors that can affect the IVF results [12]. Shapiro BS et al., stated that an increase in asynchrony between the embryo and endometrial receptivity in older women was due to ovarian stimulation, resulting in a decreased IVF-ICSI outcome [13].

Body Mass Index (BMI)

In this study, the highest pregnancy rate was found in patients who had normal BMI (18.5-24.9 Kg/m^2). Conversely, the percentage of chemically pregnant patients was lower in overweight and obese women. A higher BMI contributed to a significant reduction in pregnancy rates. This result was supported by Pinborg A et al., and Rittenberg V et al., who reported that there was a decrease in chemical pregnancies success in obese women compared to women with normal BMI [14,15].

Obesity has long been associated with a variety of sequelae of reproduction including anovulation, infertility and increased risk of miscarriage. The presence of infertility and obesity are some of the real challenges in the short and long term management of these patients [16]. Obesity is also affiliated with the increase in the number of gonadotropin doses, a worse stimulation response, to a higher incidence of spontaneous abortion. Obese pregnant women also have a risk of experiencing gestational diabetes, hypertension, thrombo-embolism, congenital abnormalities in infants, which could reduce live birth rates as a consequence [14]. In women who underwent IVF, pre-pregnancy BMI also affected pregnancy, and obstetric outcome. Those with less than normal BMI and obesity, negatively affected all ART outcomes [17,18]. Without damaging the embryo quality, obesity affects IVF results by reducing endometrial receptivity [19].

Duration and Type of Infertility

In this study, the success rate of pregnancy was higher for a fertile period of <3 years and three to six years compared to >6 years duration. The same results were found in a research conducted by The National Institute for Health and Clinical Excellent (NICE) which concluded that the duration of infertility had a significant influence on pregnancy success, where the success rate of pregnancies in the infertile duration of one to three years was 15.3% compared to infertile duration of 10-12 years at 12.4% [4]. Huang YL et al., discovered that fertilisation rates decreased for patients with an infertile duration longer than 6.4 years and for patients with primary infertility [20]. Pregnancy rates of primary infertile patients in the study by Ashrafi M et al., were reported to be lower than patients with secondary infertility thus not significantly different [6]. Smith ADAC et al., obtained an increase in pregnancy rates for primary infertile patients by repeating the IVF programs [21].

The Quantity of Oocyte during OPU and Amount of Embryo during ET

In this study, the number of oocytes greater than five obtained during OPU contributed to a higher pregnancy success than the number of oocytes less than or equal to five with significant differences ($p<0.005$). Moreover, the number of embryos implanted at the time of ET (three vs. less than three) was significantly different with $p<0.005$ on pregnancy success. These results are in accordance with a study conducted by Kara M et al., where the increasing number of oocytes obtained influenced the increase in pregnancy success [22]. Supporting the arguments, Ashrafi M et al., found a positive correlation between the number of embryos planted during ET and the success of pregnancy [6].

Pregnancy rate would increase following the rising number of oocytes until a total of fifteen oocytes and expected to be stable for oocytes higher than fifteen. However, the incidence of Ovarian Hyper-Stimulation Syndrome (OHSS) increases significantly if the number of oocytes are greater than fifteen [23]. Compared to a Single Embryo Transfer (SET), more than one embryo transfer (i.e., two embryos/Double Embryo Transfer/DET) would increase 47% of multiple pregnancy rates and decrease the pregnancy rate of live births by 10-15% [24]. Embryo transfer greater than three significantly increases Low Birth Weight (LBW) birth rates [25]. However, patients with inferior prognosis (Poor Ovarian Responders/POR) could still achieve the pregnancy level until the age of 40 with ET, with at least two and preferably three embryos [26,27].

LIMITATION

The limitations of this study is, its retrospective nature, it being single centric. Most of the patients came from distant or different districts, so the conditions of the pregnancies, for e.g., miscarriages and ectopic pregnancies after the first ultrasonography, live birth, birth weight were not recorded at this research clinic.

CONCLUSION

The pregnancy success of IVF-ICSI patients with short antagonist protocol and fresh ET was 44.6%. The number of oocytes more than five obtained during OPU and three embryos transferred during ET significantly increase the pregnancy rates, while the characteristics of patients who are more likely to be pregnant stastistically are maternal age less than 35 years, a normal BMI, and infertility period of less than six years.

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