Original Article

Oocyte Related Factors and Chance of Implantation

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Abstract

Background: In IVF/ICSI treatment, several attempts have been made to quantify the implantation potential of embryos in order to optimize the pregnancy rate. The objective was to determine the possible factors which might have positive impact on implantation.

Materials and Methods: We retrospectively analyzed 110 IVF- ICSI cycles as first trial for ART programs. Maternal and ovulation factors such as female age, body mass index, type of infertility, infertility diagnosis, duration of ovulation stimulation, numbers of aspirated and fertilized oocytes, day of ET, were compared in high (≥2 gestational sacs) and low implantation groups (one or no sac). All analyses were adjusted for age and PCO subgroups in line with the design of the study.

Results: Our results showed that there were significant differences in follicle size between high and low implantation groups (19.32±0.37 vs. 18.07±0.32 respectively) (p= 0.014). Mean number of grade (V) oocytes was also statistically greater in high implantation group (p=0.035). Our results were also compared based on age and PCO diagnosis. Women younger than 35 years old in high implantation group had higher number of grade (V) oocytes than the other group (p=0.038). Assessing our results based on PCO diagnosis, we found that the number of oocytes grade (IV) were significantly higher in high implantation group with non PCOs diagnosis (4.82±2.87 vs. 4.25±3.6) (p=0.043). Non PCO women in low implantation group had also greater number of grade II oocytes compared to the other group (p=0.017). The mean follicle size was significantly greater in high implantation group than the other group (19.32±2.17 versus 18.07±1.76) (p=0.014).

Conclusion: Follicle size, and oocyte quality have positive effect on high implantation potential.

Keywords: Implantation, Follicle Size, Oocyte Quality, Oocyte Number

Introduction

Despite advances in IVF procedures and the transfer of embryos with high morphological quality embryos, pregnancy rate from IVF has remained around 30% per embryo transfer procedure. Failure of the transferred embryo to implant remains the most important limiting factor determining success rate of in vitro fertilization (IVF) or intra cytoplasmic sperm injection (ICSI). In order to improve the present implantation rates, a better understanding of the factors which determine successful implantation is required while reducing the risk for multiple gestations.

New stimulation regimens, for example, using either highly purified or recombinant gonadotrophins influenced remarkably the quality and overall number of aspirated oocytes (1). Several studies have been performed to precisely define the morphology of these oocytes and correlate it with a higher incidence of fertilization, embryo development and pregnancy rates (2, 3).

Previous studies have shown different maternal and embryo variables that have positive influence on implantation. One prospective study showed that first IVF cycle, conventional IVF as fertilization method, 4-cell embryo, and ovarian sensitivity were important factors for predicting a

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high ongoing implantation rate (4). Interestingly, according to population-based studies, as much as two thirds of high-order gestation (≥triplet) may be attributable to ovulation-inducing drugs or related techniques (5).

Women older than 35 years of age and PCO diagnosis are two conflicts for ovulation induction programs. Engmann et al. 1999 showed that Women with PCO required lower dose of gonadotrophins for ovarian stimulation and produced more follicles and viable oocytes than women with normal ovaries (6). On the other hand, there is an expected decrease in ovarian response with increase in age (7). Tur et al. (5) showed that the risk of high-order multiple implantations was significantly correlated with increasing the total number of follicles and age of women ≤ 32 years. The risk of high order multiple pregnancies are highest in younger women with normal ovarian function (8).

These observations suggest that the current criteria for clinical monitoring of ovulation stimulation programs are inadequate to prevent a high incidence of high order multiple births and identification of reliable predictors of high implantation during these ovulation induction cycles is clearly necessary. Therefore, the present investigation was undertaken to determine whether there existed specific variables related to patient's clinical characteristics, treatment modality, and ovarian response that might be associated with the occurrence of high order implantation according to woman's age and PCOs diagnosis.

Materials and Methods

During 20 months (January 1, 2005 to August 31, 2006), a total of 110 IVF- ICSI cycles were evaluated in our retrospective study.

The data including patients' demography, infertility diagnoses, clinical information pertaining to the ART cycle, and information on resultant pregnancies and births were collected. Couples with primary or secondary infertility were accepted as candidates if their infertility was due to PCO or non-PCO causes. All patients who had their first trial for ART cycles were eligible to participate in this retrospective study.

Patients were treated with the long GnRH agonist desensitization protocol, starting in the midluteal phase with 5 × 100 μg of buserelin (Suprefact, Hoechst, Germany) for a period of 2 weeks. Thereafter, gonadotrophin stimulation (Gonal-F Serono, Geneva, Switzerland) was initiated. When at least three mature follicles with a diameter of 18 mm were present, 10 000 IU of HCG (Profasi; Serono, Geneva, Switzerland) was given.

Oocytes were retrieved 36–38 hr following transvaginal hCG using through sonographically guided puncture. After retrieval, the oocytes were incubated in G1.2 media (vitrolofe, motndalswage, Goteborg) under mineral oil in tissue culture dishes (Falcon 3001) at temperature of 31c. The presence of two pronuclei and two polar bodies were assessed 16-18 h after IVF or ICSI. Patients with a yield of at least seven normal fertilized oocytes were selected for transfer on either day 2 or day 3 after oocyte retrieval. Oocyte grading was performed as following: a germinal vesicle stage (grade I), a metaphase 1 stage (grade II), metaphase II stages (grade III), mature (grade IV), very mature (grade V) and post mature oocytes (grade VI). Embryos were classified based on morphological criteria as described previously (9).

Fertilization was performed by conventional IVF or ICSI, and following standard techniques. Commercial culture media were used according to local routines. Embryo transfer was performed 2 or 3 days after oocyte retrieval. Luteal support was given with progesterone, either intramuscularly or vaginally.

The demographic and oocyte related factors that might affect the probability of implantation rate after IVF-ICSI treatment were analyzed. The following variables were checked in this respect: female age, body mass index, type of infertility, infertility diagnosis, duration of ovulation stimulation, numbers of aspirated, fertilized oocytes, follicle size, oocyte grade, and day of ET.

Implantation was defined as number of gestational sacs with a fetus with heart activity, detected on sonography in gestational weeks 7-8. We defined the existence of two or more gestational sacs as high implantation and one or no sac as low implantation.

The protocol was approved by our ethical committee. All patients' signed consents were obtained on their initial visit as the permission to use their results without using their names in future studies.

Statistical analysis

All analyses were performed using the SPSS (version 13). All P-values were two sided and values <0.05 were taken to indicate statistical significance.

Age was analyzed both as a continuous variable and as a categorizing element. In this form, the age categorized to 35 years and less (≤35) and more than 35. Infertility diagnosis was also categorized as PCO and non-PCO.

All the variables were compared between high implantation and low implantation groups. The independent variables such as age, type of infertility (first or second), infertility diagnosis, Type of drug, the day of ET, and pregnancy were compared by chi-square (x^2) between groups. We used Mann Whitney-U test or t-test for comparing BMI, duration of ovulation stimulation, numbers of oocyte and embryo transferred, follicle size, and dosage of drug between groups.

All analyses were adjusted for age and PCO stratums in line with the design of the study. The data were expressed as mean± standard deviation (SD) or mean with ranges when SD was higher than mean values. The categorized data were expressed as percentage (%).

Results

Our results indicated there was no significant difference in patient's characteristics between low and high implantation groups, including age, BMI, type of infertility, infertility diagnosis, stimulation duration, and embryo transfer day (Table 1).

Ovulatory factors compared between two groups consisted of number of retrieved oocytes, oocyte quality, and follicle size. Our results showed that mean number of retrieved oocytes were similar in high and low implantation groups (Table 2).

Morphological characteristics of oocytes were compared considering oocyte grades. Mean number of grade (V) oocytes was statistically greater in high implantation group (4.05±0.644 vs. 2.51± 0.407) (p=0.035). Our results were also compared based on age and PCO diagnosis. Women younger than 35 years old in high implantation group had higher number of grade(V) oocytes than low implantation group (4.08±0.697 vs. 2.7±0.445) (p=0.038).

Assessing our results based on PCO diagnosis, we found that the number of oocytes grade (IV) were significantly higher in high implantation group with non-PCOs diagnosis (4.82±2.87 vs. 4.25±3.6) (p=0.043). Non-PCOs women in low implantation group had also greater number of grade II oocytes compared to the other group (0.69±0.204 vs. 0.15± 0.086) (p= 0.017) (Table 2).

High implantation group also showed higher mean size of follicle (19.32 ± 2.17) than the other group (18.07 ± 1.76) (p=0.014) (Table 2). This difference was statistically seen in the stratum of women under 35 years of age (19.4± 2.27 vs. 18.14±1.78) (p=0.032) and non-PCO diagnosis (19.17± 2.25 vs. 17.84± 1.79) (p=0.028).

Table 1: Comparison of patient's characteristics between high and low implantation groups

	High implantation	Low implantation	p-value
Age(yr)			
(Mean±SD)	29.8±4.008	29.8±4.66	0.926
BMI			
(Mean±SD)	28.11±8.13	27.12±8.07	0.551
Type of infertility			
First	97.6%	100%	0.376
Second	2.4%	0%	
PCO idagnosis			
Yes	16.7%	100%	0.248
No	83.3%	72.1%	
ET day			
Day 2	54.8%	69.1%	0.155
Day 3	45.2%	30.9%	
Stimulation day			
(Mean±SD)	11.12±2.89	10.15±2.43	0.061

Table 2: Comparison of oocyte related factors between high and low implantation groups

	High implantation	Low implantation	p-value
Number of Oocyte retrieved	10.17±4.41	8.96±5.61	0.239
Foollicle size	19.32±0.37	18.07±0.32	0.014*
Oocyte grades			
Grade I	0	0.06±0.385	0.064
Grade II	0.34±1.063	0.81±1.743	<0.0001*
Grade III	1.37±2.19	0.72±1.84	0.063
Grade IV	4.24±2.98	4.42±3.72	0.921
Grade V	4.05±0.644	2.51±0.407	0.035*
Grade VI	0.1±0.37	0.45±1.105	0.019*

* Significant difference

Discussion

Some previous studies have shown that embryo's factors such as number of embryos transferred are significant predictors for implantation and multiple pregnancy rates (10-12). In this retrospective study, we only measured the ovarian factors which may have potential impact on implantation. Different stimulation protocols have variable effects on oocyte number and oocyte qualities. Some studies demonstrated that pregnancy rates increased when more oocytes were retrieved (13); however we found no significant difference about oocyte numbers between high and low implantation groups. Similarly, Glitcher et al. (8) found no association between total numbers of follicles with high order multiple pregnancies. Hendriks et al. (14) in their meta-analysis found the performance of antral follicle count is clearly poor for predicting pregnancy. They believed that this test merely represents the quantitative aspect of ovarian reserve and the occurrence of pregnancy in IVF is largely dependent on oocyte quality.

Morphological characteristic of oocytes are the other aspect which could be evaluated during induction ovulation programs. In our study the number of top quality oocytes (grade V) was significantly higher in high implantation group than the other group. In other words, oocyte quality had a more important role than oocyte number in implantation. Although Wittemer et al. (13) found no association between morphological characteristic of oocyte with pregnancy rate, others have reported that better fertilization and embryo development rates occurred with "good quality" oocytes (2, 3). Good oocyte quality has reported to be directly related to an increase in intrafollicular oxygenation (15). Other investigations have further demonstrated that follicular blood flow and the content of dissolved oxygen were positively related to the ability of the oocyte-embryo to develop successfully, compared with avascular follicles which did not result in any pregnancies (16).

Our results showed that there was no significant difference about oocyte grade (V) in high and low implantation group who were older than 35 years old and had PCO diagnosis. In young women, ovulation stimulation is usually associated with an increased ovarian response (17) and this might lead to more oocytes number with better qualities that is an important factor in high implantation and multiple pregnancy rate. On the other hand, there is an expected decrease in ovarian response with increasing age of women (7). Because implantation rates decrease dramatically after the age of 38 years (18), it may explain the lack of difference in number of good oocyte quality between high and low implantation groups in older women. Previous results also showed that the percentage of high-quality oocytes and the fertilization rate were significantly lower in patients with polycystic ovaries (PCO) (19, 20).

Our result demonstrated that follicle size was significantly higher in high-implantation group (19.32±0.37 vs. 18.07±0.32) (p=0.014). It is believed that larger follicles contain the most mature oocytes, which have the greatest potential of pregnancy (8). Follicle size was merely as a positive factor for high implantation in women younger than 35 years old having non-PCO diagnosis. In other words, for PCOS and older women this variable could not have an effective role on implantation. Richmond et

al. (21) showed that in fecund cycles, follicles with a diameter of 15 mm have an 8% chance of producing a viable implantation, and that 14 mm follicles show a much reduced incidence (4%).

Conclusion

Follicle size, oocyte quality had positive effect on high implantation potential. From a clinical perspective, further consideration should be given to investigating treatment protocols improving oocyte quality rather than maximizing the number of oocyte obtained.

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