Full Length Research Paper

Common predictive parameters anticipating pregnancy among poor and normoresponder patients in ICSI

Bahar Sarıibrahim

Zeynep Kamil Obstetrics and Gynecology Research and Education Hospital IVF Unit, Istanbul/Turkey
E-mail: baharsariibrahim@hotmail.com; Tel:05058942924; Fax:02622334641

Multiple parameters are being considered in the field of IVF/ICSI success. It is difficult to estimate patient's response to ovarian stimulation in poor responder IVF/ICSI patient group. The objective of this study is to find the common predictive parameters anticipating positive pregnancy results among poor responder and normoresponder IVF/ICSI patients. It was a retrospective study, examining medical records of 96 poor responder, 117 normoresponder patients between the ages 23-39 years who performed ICSI between June 2013-March 2015 at Zeynep Kamil Obstetrics and Gynecology Research and Education Hospital IVF Unit. Study (poor responder) and control (normoresponder) groups were evaluated in regard of positive pregnancy results. According to the results of AUCs and ROC curves five significant variables; the number of retrieved oocytes, the number of M2 oocytes, the number of fertilized oocytes, the day of embryo transfer and normoresponder group could be considered as diagnostic markers in the study and control groups and clinically these markers might be accepted as predictive parameters. The number of retrieved oocytes, the number of M2 oocytes, the number of fertilized oocytes, the day of embryo transfer and being in normoresponder group could be accepted as predictive parameters anticipating positive pregnancy results among poor and normoresponder IVF patients. These parameters can also help couples deciding on subsequent IVF treatments or counseling for donor egg selection option.

Keywords: Poor responder, ART outcome predictor

INTRODUCTION

All of the efforts in assisted reproduction is for achieving healthy pregnancies and babies. In IVF/ICSI treatment pregnancy success is almost dependent on both quantity and quality of oocytes and embryos. Besides this, patient's age, ovarian reserve, preceding controlled ovarian hyperstimulation results, transferred embryo number, the day of embryo transfer, endometrial receptivity all are related factors for IVF/ICSI success.

Ovarian reserve which could be said as predictor of fertility potential of women declines with age. The estimation of ovarian reserve has critical importance for the fertility potential of women. Various methods for evaluating the ovarian reserve have been used in clinical practice (Fauser et al., 2008). Among them, the measurement of baseline follicle stimulating hormone (FSH) and estradiol (E2) levels, evaluating the number of antral follicles by ultrasonography and the estimation of antimullerian hormone (AMH) levels are the more widely used worldwide.

The quality of retrieved oocytes is strongly related with ICSI success. The oocytes having germinal vesicle and not having first polar body at perivitellin space can be said as immature. It's made ICSI only to the oocytes reached M2 stage and threw the first polar body.

The optimal number of retrieved oocytes is a contentious issue in assisted reproduction. In one study regarding the impact of oocyte yield groups; (1-3 group A), (4-9 group B), (10-15 group C), (>15group D) on fresh LBR and cumulative LBR after utilization of all cryo preserved embryos, it was reported that cumulative LBR significantly increased with the number of oocytes retrieved (Drakopoulos et al., 2016). High responders demonstrated significantly higher LBR than women with normal, suboptimal and poor ovarian response. However in another study, Blais et al. reported that live birth rates were highest when the number of retrieved oocytes was 11-15 and retrieval of more than 15 oocytes was not associated with significant increases in achieving conception and birth.

With our best knowledge we couldn't find any literature in the subject of predictive parameters anticipating positive pregnancy results among poor and normoresponder groups. In this current study, it was investigated the predictive parameters between the groups; poor responder and normoresponder groups, regarding cycle outcome measure positive pregnancy rate.

MATERIALS AND METHODS

This retrospective study was performed at Zeynep Kamil Obstetrics and Gynecology Research and Education Hospital IVF Unit. We examined medical records of 96 poor responder, 117 normoresponder patients between the ages 23-39 years who had IVF treatment between June 2013-March 2015. Poor response was defined as less or equal to five oocytes retrieved at ovum pickup, normoresponse was defined as six to fifteen oocytes retrieved at ovum pickup. 96 poor responder patients was in study group, 117 normoresponder patients was in control group.

The patients with male factor infertility (TPMSS<5.000.000), endometriosis, systemic disease, chromosomal abnormalities in either male or female and the patients with cancelled cycles due to poor response were excluded from the study. Approval was obtained from Bioethics Committee of Zeynep Kamil Education and Research Hospital.

After getting written informed consent from patients, long agonist, antagonist or microdose flare-up protocols were administered to patients. During the long agonist protocol, subcutaneous (sc) daily 0,5 mg leuprolide acetate (lucrin) injections applied on day 21 of the preceding cycle and decreased to 0,25 mg upon ovarian suppression confirmed by serum E2 levels of<50 pg/ml and serum LH levels of 5 IU/ml, endometrial thickness under 5 mm measured by ultrasonography and fallowed by utilization of hMG (human menopausal gonadotropin, Menogon; Ferring, İstanbul, Turkey) and recombinant FSH (GonalF; Serono, Istanbul, Turkey). During the antagonist protocol, patients received hMG and recombinant FSH starting on day 2or 3 and 0,25 mg cetrorelix (Cetrotide; Asta Medica, Frankfurt, Germany) was administered daily when leading follicle reached 13-14 mm in diameter. Recombinant hCG (250 microgram sc, Ovitrelle, Serono, Istanbul, Turkey) was applied when at least one leading follicle reached a mean diameter of 18 mm and the serum E2 concentration was >500 pg/ml. Thirty six hour after hCG injection ultrasound-guided oocyte retrieval was performed.

Embryo transfers were performed on day 2,3,4 or 5 under ultrasound guidance. The best quality embryos using standard embryological criteria and grading systems were transferred. In patients <35 year old 1 embryo was transferred in the first and second embryo transfer, 2 embryos were transferred in the third embryo transfer and in patients > 35 year old 2 embryos were transferred according to the Turkish law.

For luteal support after embryo transfer, vaginal progesterone (Crinone % 8 gel, Serono, İstanbul, Turkey) supplementation twice a day until menstruation or until 12 week gestation in case of clinical pregnancy occurrence.

In this study we investigated the predictive parameters between the groups poor and normoresponders in regard of positive pregnancy results.

Statistical analysis

Mean and standard deviation (SD) were calculated for continuous variables. The normality of the variables was analyzed by Kolmogorov–Smirnov test, and the associations between the categorical variables were evaluated with the Chi-square test with Yates Correction. The mean values of two groups and two percentages were compared by using Student's t test for two independent groups. Receiver Operating Characteristics (ROC) analysis of modern multivariate statistical methods was employed to find and Area Under Curves (AUC) values for diagnostic markers of the study variables. The ROC curve is a plot expressing the relationship between the true positive rate (sensitivity) and the false positive rate (1-specificity). The area under the ROC curve represents the ability of the screening instrument to discriminate between the study and control.

Two-sided p values were considered statistically significant at P≤0.05. Statistical analyses were carried out by using the statistical packages for SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Table 1. The mean and standard deviation and the results of analyses

	Pregnancy (-)		Pregnancy (+)		p
	$\bar{x}$	±SD	$\bar{x}$	±SD	
age	30.98	4.00	30.82	4.61	0.793
duration of infertility	8.898	1.66	8.67	1.62	0.370
fsh	6.22	3.22	6.03	3.13	0.689
afc	10.92	5.75	12.14	5.82	0.158
The initial dose of gonadotropine	298.9	109.2	278.4	98.4	0.195
Total dose of gonadotropin	2671	900.2	2461	912.2	0.236
Maximum estradiol	1689	755.2	1914	765.3	0.147
The number of retrieved oocyte	7.22	5.29	9.46	5.26	0.005
The number of methaphase 2 (M2) oocyte	4*		9*		0.008
The number of fertilized ocyte	3*		5*		0.017
ICSI rate(%)	78.85**		80.02**		0.455
The day of embryo transfer	2.88	0.87	3.29	0.99	0.003
Fertilization rate (%)	67.87**		66.03**		0.978
Implantation rate(%)	.	.	78.73**		-

*Median values; two groups were analysed by Mann whithney U test, the mean and ratios of two different groups were analysed by Student's t test.

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Table 2. Comparison of "Groups", "The number of embryos transferred" between the study group and control.

		Pregnancy (-)	Pregnancy (+)	χ^2	p
Group	Control (%)	70 (59.8)	47 (40.2)	11.41	0.001
	Study (%)	78 (81.2)	18 (18.8)		
The number of	1	128 (73.1)	47 (26.9)	6.195	0.013
embryos transferred	2	20 (52.6)	18 (47.4)		

The relation between "group" and "Study,control" were found significant (p=0.001), and also "The number of embryos transferred" were found significant (p=0.013).

RESULTS

Descriptive statistics values as the mean, standard deviation ($\bar{x} \pm SD$), median and ratio values of the variables according to two different groups were calculated and analyzed by Student's t test. Results are presented by Table 1.

According to the results of Table 1 the number of retrieved oocyte, the number of M2 oocyte, the number of fertilized oocyte, the day of embryo transfer were found significantly different for two different groups (p<0.05).

Cross tabulation of "Groups", "The number of embryos transferred" and "Study, control groups" were analysed by Chi-Squre test. The results were presented by Table 2.

Variables were found significant in the differences between study and control groups were tested by ROC analysis can be considered as a diagnostic markers. Figure 1 shows that the areas under curves (AUC) on ROC curves for the variables.

The areas under the ROC curves, Standard errors, significant (p), and Confidence intervals (95%CI) for the number of retrieved oocyte, the number of M2 oocyte, the number of fertilized oocyte, the day of embryo transfer and normoresponder group were presented by Table 3.

The area under the ROC curve represents the ability of the screening instrument to discriminate between the study and controls.
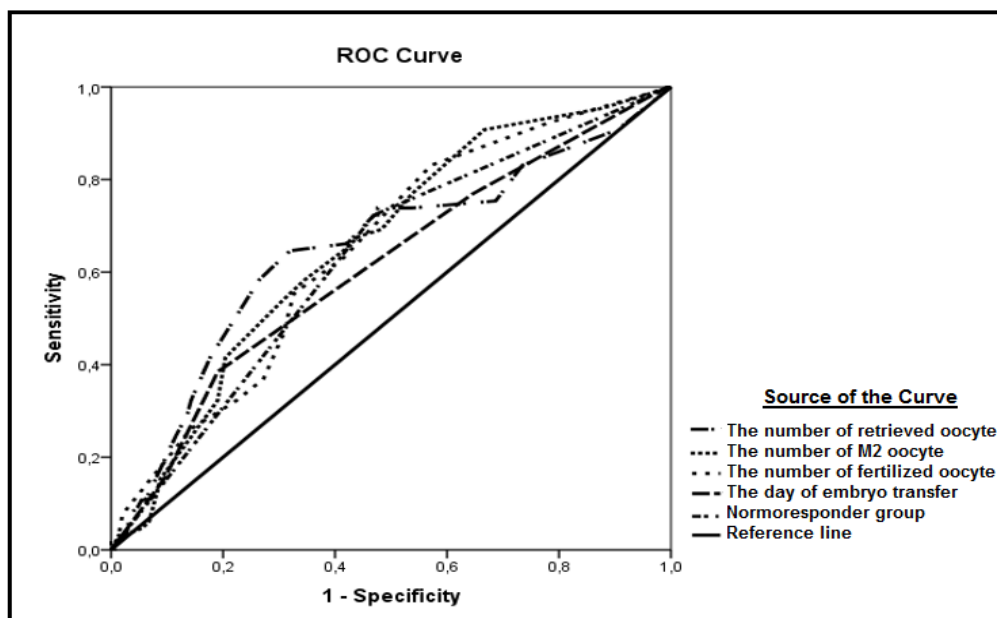
Figure 1. The areas under curves (AUC) on ROC curves for the variables.

Table 3. The areas under the ROC curves and significant values

Test Result Variable(s)	Area	Std. Error	P	Asymtotic 95% Confidence interval	
				Lower Bound	Upper Bound
Toplanan_oosit	,649	,043	,001	,564	,734
M2oosit_sayi	,658	,039	,000	,581	,734
Fertilize_oosit_sayi	,647	,040	,001	,569	,725
Transfer_gunu	,610	,042	,011	,526	,693
Grup	,627	,041	,003	,547	,707

AUC: Area Under Curve
SE: Standerd Error of AUC value
P: Significant Value
95%CI: Confidence Interval with 95%

The AUCs and 95%CI values of variables were as follows; "The number of retrieved oocyte"; 64.90% (95%CI: 56.4-73.4), p=0.001, the number of M2 oocyte: 65.8% (95%CI: 58.1-73.4), p<0.001, the number of fertilized oocyte: 64.7% (95%CI: 56.9-72.5), p=0.001, the day of embryo transfer 61.0% (95%CI: 52.6-69.3), p=0.011) and normoresponder group 62.7% (95%CI: 54.7-70.7), p=0.003

According to the results of AUCs and ROC curves five significant variables could be considered as diagnostic markers in the study and control.

DISCUSSION

It's almost impossible to predict pregnancy in natural conception and also in IVF patients. Poor ovarian response during ovulation induction seems to be a major problem for infertility specialists. Predicting pregnancy in poor responder patients is another problematic condition in infertility patients.

In this study we have found according to the results of AUCs and ROC curves five significant variables that could be considered as diagnostic markers in poor and

normoresponder groups. In clinical practice significant variables found to be as diagnostic markers might be accepted as predictive parameters for pregnancy. These are retrieved oocyte number, M2 oocyte number, fertilized oocyte number, the day of embryo transfer and normoresponder group.

The number of retrieved oocyte is almost dependent on patient's age, ovarian reserve and ovarian hyperstimulation protocol. Optimal number of retrieved oocyte is a contentious issue. Previous studies evaluating the relationship between the number of retrieved oocytes and pregnancy rates have reported inconsistent results in showing that pregnancy rates increased with an increasing number of oocytes (Meniru, 1997), best pregnancy rates being obtained with number of oocytes of 10-15 (KablyAmbe et al., 2008), or 7-16 (Molina Hita Ma del et al., 2008). Besides Sunkara et al. (Sunkara et al., 2011) reported in their research analyzing 400135 IVF cycles, live birth rate rose with an increasing number of eggs up to 15, plateaued between 15 and 20 eggs and steadily declined beyond 20 eggs. In our study we have found predictive parameters anticipating positive pregnancy rates as retrieved oocyte number (AUC-64.9%) and normoresponder group (AUC-62,7%). This means pregnancy chance increases with the increasing number of retrieved oocyte in poor responder group and being in normoresponder group that means having six to fifteen retrieved oocytes either increases pregnancy chance.

Zhang et al. evaluated cumulative live birth rate according to the rate of use of metaphase 2(M2) oocytes in conventional ovarian stimulation protocol for IVF/ICSI and reported no significant difference between the M2 oocyte yield groups; (low: 1-2; intermediate: 3-6; high; 7 and over) in terms of cumulative live birth rate (Zhang et al., 2011). However McAvey et al. (McAvey et al., 2011), reported in their research that there was an advantage obtaining six or more M2 oocytes compared to obtaining five and less oocytes and there was not an advantage obtaining 10 or more or 15 or more oocytes compared to obtaining 6-9 oocytes. In our study M2 oocyte number is a predictive parameter anticipating positive pregnancy results in poor responder and normoresponder groups (AUC %65.8). This means pregnancy chance increases with increasing M2 oocyte number in poor and normoresponder patient groups.

Ciepiela et al. (Ciepiela et al., 2015) evaluated the impact of limiting fertilized oocyte number to 6 and 10 to ICSI results with good-prognosis patients in their research. They found no significant difference in the implantation rate and ongoing pregnancy rate between patients who electively limited fertilized oocyte number to 6 and 10.Only fewer embryos available for ET, lower number of frozen embryos per cycle, lower rates of

cycles with embryo cryopreservation were found in fertilized oocyte number 6 group. However in our study we have found fertilized oocyte number as a predictive parameter anticipating positive pregnancy results in poor and normoresponder groups (AUC-%64.7). This means with the increasing number of fertilized oocyte, the chance of positive pregnancy results increases in poor and normoresponder patients.

Also we have found the day of embryo transfer as a predictive parameter in poor and normoresponder groups (AUC-%61). This means oncoming the day of embryo transfer has the chance of getting better pregnancy results. Similar to our findings Kaur et al. (Kaur et al., 2014), Van der Auwera et al. (Auwera et al., 2002), Mangalraj et al. (Mangalraj et al., 2009) reported in their researches better clinical pregnancy and implantation rates of blastocyst stage transfer than cleavage stage transfer.

There are several limitations to the present research. First one is its retrospective design. Secondly we couldn't use basal AMH predicting ovarian reserve that is used widely all over the world. And lastly we couldn't compare our result with high responder patients.

In summary, we report that retrieved oocyte number, M2 oocyte number, fertilized oocyte number, the day of embryo transfer and normoresponder group could be accepted as predictive parameters anticipating positive pregnancy results among poor and normoresponder groups. These markers may help guide poor responder patients as they might have the same chance getting positive pregnancy results with normoresponder patients. These parameters can also help couples deciding on subsequent IVF treatments or counseling for donor egg selection option. Further study is needed to identify predictive parameters anticipating positive pregnancy results in poor, normo and high responder IVF patients.

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