THE PREDICTORS OF CLINICAL FERTILIZATION IN IVF CYCLES

S. Esmailzadeh[1], M. Faramarzi[2] and M. Farsi[3]

1) Department of Obstetrics and Gynecology, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran
2) Department of Midwifery, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran
3) Department of Anatomy and Embryology, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran

Abstract- We examined the demographic characteristics of couples, ovarian response and sperm quality to determine the predictors of clinical fertilization in IVF cycles. A descriptive and analytic study was conducted using data and biologic specimens obtained Fatemeh-Alzahra of Babol infertility center from 2004 to 2005. Only data from 315 women who had medical indication for conventional IVF treatment were included in the analysis. Treatment using ICSI was excluded. In the univariate analysis, the following variables affected on fertilization rate: the length of infertility, the number of IVF cycle, basal LH serum on 2 days, the number of administration of hMG, the duration of ovarian stimulation, the number of follicles, the number of oocytes retrieved, the number of oocytes stage II and III, sperm count, sperm motility, sperm grading III and IV. In the multivariate analysis, the strongest predictor of positive fertilization was the mean number of oocytes retrieved. Also, the mean number of oocytes stage II and stage III were positive predictors of fertilization. The mean of basal LH serum on day 2 and the mean duration of ovarian stimulation were negative predictors of fertilization. Ovarian response to gonadotropins and the quality of oocytes were main predictors of fertilization. Although some parameters of sperm quality were significant variables of fertilization rate in univariate analysis, in multivariate analysis one's effects were negligible. This information should be used when selecting couples for IVF cycles or oocytes for fertilization to raise the rate of clinical fertilization.

Acta Medica Iranica, 46(2): 87-94; 2008

Key words: Clinical fertilization, predictor, IVF

INTRODUCTION

Most IVF cycles are performed with use of stimulation protocols with GnRH agonist for cycle control followed by controlled ovarian hyperstimulation (COH). These protocols increase the number of oocytes and embryos available and achieve the highest pregnancy rates (1). Because of the time, high drug costs, and emotional expenditure incurred by patients under going in vitro fertilization, identifying predictive factors for fertilization in IVF therapy is extremely important.

The outcome of IVF treatment is highly dependent on ovarian response to hormonal stimulation. The concept of diminished ovarian reserve has gained general acceptance in infertility medicine. The association of poor ovarian response due to diminished ovarian reserve with cycle cancellation and a significant decline in success rates

Received: 18 Apr. 2006, Revised: 2 Nov. 2006 Accepted: 19 Nov. 2006

Corresponding Author:
M. Faramarzi, Ms midwifery, PhD student of psychology of Tarbiat Modares University, Department of Midwifery, Faculty of Medicine, Babol University of Medical Sciences, Gang Afroz Street, Babol, Iran.
Tel:+09111122259
Fax:+98 0111 3264925
Email: mahbob330@yahoo.com

is well known. A variety of screening tests have been developed to provide a reliable assessment of the ovarian reserve and to predict response to gonadotropin stimulation. Aging is associated with progressive follicular depletion and diminished oocyte quality, which is accompanied by a reduction in the size or activity of the cohort of follicles available to respond to gonadotropin stimulation (2).The other factors which have been investigated as possible predictors of ovarian response include ovarian volume (3, 4), the number of antral follicles (5, 6), evaluation of ovarian stromal blood flow (7), assessment of hormonal markers such as serum FSH (8), LH (9), estradiol (E_2) and inhibin B as well as anti-mullerian hormone (10).

Numerous prognostic factors must be addressed in counseling subfertile couples who consider IVF. Important factors included the number of previous treatment cycle the number of former successful cycle and cycle cancellation. Other important determinants of pregnancy outcome in IVF are the number of aspirated oocytes, the proportion of fertilized oocytes, the number of quality of embryos, the time between oocyte aspiration and embryo transfer, and cause of infertility. Semen quality is another prognostic factor that includes sperm concentration, percent motility, quality of motility, and sperm morphology (11).

Oocytes retrieved from patients following controlled ovarian hyperstimulation show varying stages of meiotic maturity. Some studies indicated that oocyte morphology has important role on fertilization rate. Complete nuclear and cytoplasmic of oocytes is essential for the activation of oocytes at fertilization and development of embryos. An oocyte is considered to reach nuclear maturity when its meiosis is arrested again at metaphase II with the presence an extruded of the first polar body (12).

Although a large number of studies have been conducted in relation to prediction of IVF outcome, a number of methodological problems are encountered including sampling variability and clinical heterogenicity. So far trials have been conducted in single (13, 14) or combinations of a few predictive factors (15, 16). A wide range of sample size present is seen in small studies (7).

The main purpose of this study was to examine the large groups of the possible predictors of the clinical fertilization through multiple regression analysis. We considered the influencing of following variables on model regression for clinical fertilization: the characteristics of couple including women's age, men's age, length of infertility, the number of IVF cycle, the some predictive marker of ovarian reserve including; FSH serum, LH serum, the number of administration of hMG, the duration of ovarian stimulation, the number of follicles, the number of oocytes retrieved, prognostic values of semen quality including; sperm concentration, sperm motility, sperm morphology, sperm grading, and prognostic values of oocyte staging.

MATERIALS AND METHODS

A descriptive and analytic study was conducted using data and biologic specimens obtained as part of an infertility study of Fatemeh-Alzahra of Babol infertility center from 2004 to 2005. Patients registered for IVF enrolled in this study, except for those with following conditions: (I) different stimulation protocols; (II) failure to retrieved oocytes (III) poor sperm parameters (*i.e*, < 5,000,000 spermatozoa, < 50% motility, > 90% abnormal spermatozoa) (IV) fertilization using ICSI technique in a cycle with IVF failure. In this study, treatment using ICSI was excluded, indicating that patients with severe male infertility were not included. A total of 315 couples were included in this analysis.

Our analysis was approved and supported by all board members of the Fatemeh-Alzahra infertility center. We performed no therapeutic intervention on patients; thus, institutional review board approval was not needed. Identifiable information on patient was removed from the database before release to the authors.

Upon the onset of menstrual bleeding in a spontaneous cycle preceding GnRH-analogue treatment couple infertile contacted the center. They were seen on day 2-3 when the clinical history was taken and semen quality analysis, ovarian ultrasonography were performed. Also, blood samples to assays serum FSH and LH were drawn. Ovarian

ultrasonography was performed using the FukuDa (ESAOTE- AU: 350) Transvaginal probe 5 MHZ.

Specimen of semen was obtained by masturbation and collected in a clean container. In the swim-up procedure, semen sample were mixed with 10 mL Ham's F10 media containing human serum albumin (HSA) and centrifuged at 569× g (1800 r.p.m) for 10 min. We used normal values suggested by the World Health Organization (WHO) to interpret of semen quality and the grading of sperm motility (17).

All the women used the standard long protocol of pituitary suppression with gonadotropin releasing hormone (GnRH) agonist followed by administration of urinary gonadotropin for ovarian stimulation (18). The standard starting dose of urinary gonadotropins was 2-4 ampules (150-300 IU/I FSH activity)/day depending on the patient's age, basal serum FSH concentrations, and appearance of ovarian response (the total number of basal antral follicles) (19). As the patient's ovarian response is usually dependent on these three factors, we designed the following starting dose of gonadotropin for the protocol of the institute. Women who had one of this condition, age<35, or FSH < 8 IU/I, or the number of follicle antral > 10 were administered 2 ampules, and age> 35 or FSH 8-10 IU/I or the follicle antral < 10 were given 3 ampules. If women had two or three factors for poor response to ovaries, we started them on 4 ampules/day. This chosen initial daily dosage of gonadotropin was maintained until day 6. Transvaginal ultrasound was done at this time to determine follicular response. If no response had occurred by these measurements, the gonadotropin dosage was increased by 1-2 ampules/ day every 3-4 days until a response was evident on ultrasound (or until maximum dosage was reached). The maximum dosage was 8 ampules per day (20). Once an ovarian response was obtained, treatment typically was continued without a further increase in dose. Transvaginal ultrasound was performed every 2 days to evaluate follicular size, number, and quality. When the largest measured follicle reached a maximum diameter of 18-19 mm or more, 10,000 IU of hCG was administered intramuscularly.

As no reliable biochemical test of follicular fluid has been developed that can accurately and rapidly assess oocyte maturation status with microscope Nicon T300. Most classification systems rely on direct visualization of maturational status, morphology of the oocyte, and appearance of companion cumulus oophorus and cornea radiate cells. We classified oocytes according to the criteria of Table 1.

Fertilization was assessed 15-18 hours after insemination (Fig. 1). Oocytes were classed as fertilized if two pronuclei (2PN) were present and the second polar body had been extruded. Abnormally fertilized oocytes (1 PN or 3 PN) were excluded. Testing for pregnancy was performed about 15 or 16 days after hCG administration. A positive test of pregnancy was followed with an ultrasound to detect gestational sac at 5 weeks menstrual age.

Statistical analysis of the data was performed with SPSS software for windows, version 10.0. Couple's characteristics, predictors of ovarian reserve, quality of semen analysis, oocytes staging, were summarized and compared using means, standard deviation and *t* tests. To achieve primary end points of this study prediction of clinical fertilization, we performed multiple regression analysis which was carried out in a backward stepwise manner. All of the following predictive variables were entered into the model as independent variables to begin with: women's age, men's age, the number of IVF cycle, length of infertility, the number of hMG administration, the duration of ovarian stimulation, LH serum, FSH serum, the number of follicles, the count of sperm, the percent of sperm motility, the percent of normal sperm morphology, the percent of sperm grade I, II, III, IV, the percent of sperm grade III, IV after swim-up procedure, the percent of sperm motility after swim-up procedures, the count of sperm after swim-up procedure, the number of oocytes retrieved, and the number of oocytes grade I, II, IV. Significance level for all of the analysis was $P < 0.05$.

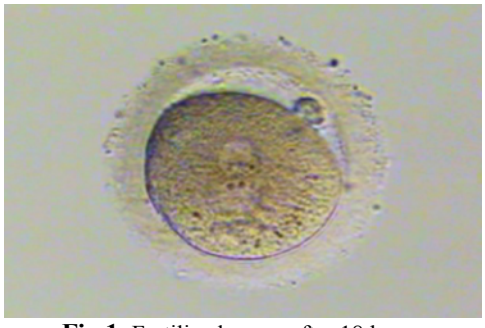

Fig 1. Fertilized ovum after 18 hours

Table 1. Maturational status of the oocyte

Maturational status	Cumulus	Corona	Germinal vesicle	Polar Body	Classification
Meiosis I, Prophase I	Compact	Compact	+	-	Immature (stage I)
Meiosis I, Metaphase I	Expand	Slightly compact	-	-	Intermediate (stage II)
Meiosis II, Metaphase II	Expand	Expand	-	+	Mature (stage III)

RESULTS

Of 315 cycles with oocyte retrieved, 194 (61.6%) resulted in embryo transfer. Cycles with no clinical fertilization were seen in 121 (38.4%) of cases.

Table 2 compares the demographic characteristics of women with positive clinical fertilization with negative clinical fertilization women. The mean length of infertility and the mean number of IVF cycles in positive fertilized women were significantly less than those of negative fertilized women. women's age and men's age were not significantly different between two groups of women with positive and negative fertilization.

Table 3 compares the ovarian response, sperm quality between women with positive clinical fertilization with negative clinical fertilization women. There was not significant difference between two groups in the mean of basal FSH on day 2, the mean number of oocytes stage I and II. The mean of basal LH serum on day 2, the mean number of administration of hMG ampules, the mean duration of ovarian stimulation in positive fertilized women was significantly less than negative fertilized women. The mean number of follicles, the mean number of oocytes retrieved, and the mean number of oocytes stage III in positive fertilized women were significantly more than negative fertilized women. There were not significant difference between two groups in sperm quality pre swim- up including the percent of normal morphology, the percent of motile sperm grade I, II. There was significant difference between two groups in the sperm quality pre swim- up including sperm counts, the percent of motile sperm III, IV. The comparison of sperm quality post swim-up in two groups showed that positive fertilized women had significantly the mean sperm count and the percent of sperm grade III and sperm grade IV more than negative fertilized women. All variables related to clinical fertilization (24 variables in tables 2 and 3) were considered for the multivariate regression model. The significant predictors of clinical fertilization in backward stepwise regression analysis are showed in table 4. On multivariate, the strongest predictor of fertilization in IVF cycles was the mean number of total oocytes retrieved. The other strong predictors of positive fertilization were: the mean number of oocytes stage II and stage III. The mean of basal LH serum on day 2, and the mean duration of ovarian stimulation were the other predictors with negative effects on fertilization rate.

Table 2. Comparison between characteristics of couples with negative and positive clinical fertilization in IVF cycles

Characteristics of couples	Fertilization				F*	P-value
	Yes (n= 194)		NO (n=121)			
	Mean	± SD	Mean	±SD		
Women's age (y)	28.2	5.5	29.8	5.1	0.64	0.42
Men's age (y)	34.3	5.9	35.4	5.7	0.7	0.95
Length of infertility (y)	6.9	4.7	8.6	5.9	4.4	0.03
No. IVF cycles	1.2	0.5	1.4	0.6	4.6	0.03

* T test is used to compare two groups. F presents the results of t test.
**P < 0.05 was considered statistically significant.

Table 3. Comparison between ovarian response and sperm quality of women with negative and positive clinical fertilization in IVF cycles

Variables	Fertilization				F	*P* value
	Yes (n = 194)		No (n = 121)			
	Mean	± SD	Mean	±SD		
Ovarian response						
Basal FSH IU/mL	5.8	2.4	5.3	2.9	0.47	0.47
Basal LH IU/mL	5.6	2.9	8.2	4.3	4.14	0.01
No of ampoules of hMG	34.8	20.2	39.5	16.9	1.30	0.03
Duration of ovarian stimulation (day)	12.3	3.3	13.2	3.2	1.62	0.01
No. of follicles	9.2	6.1	7.7	4.9	4.36	0.01
No. of oocytes retrieved	9.1	5.3	6.2	5.1	0.21	<0.0001
No. of oocytes stage I	1.4	3.3	1.2	2.1	3.47	0.68
No. of oocytes stage II	3.1	4.5	2.2	3.8	1.49	0.04
No. of oocytes stage III	3.4	4.1	1.9	3.6	2.10	0.002
Sperm quality pre swim- up						
Sperm count ($\times 10^6$m)	37.6	42.9	32.7	38.1	3.35	0.002
Total motile sperm (%)	55.0	21.3	52.1	16.8	4.93	0.005
Normal morphology (%)	61.0	10.3	59.1	18.3	1.34	0.35
Motile sperm grade I (%)	17.5	9.3	16.2	9.8	1.76	0.45
Motile sperm grade II (%)	26.4	11.0	27.9	11.8	0.25	0.49
Motile sperm grade III (%)	10.3	10.8	7.7	11.5	2.29	0.001
Motile sperm grade IV (%)	1.6	2.4	0.18	1.3	7.938	0.001
Sperm quality post swim-up						
Sperm count ($\times 10^6$ m)	44.6	27.1	32.3	26.1	1.02	0.01
Total motile sperm (%)	82.1	18.1	75.5	24.5	3.30	0.09
Motile sperm grade III (%)	30.1	16.1	22.6	16.6	1.41	0.008
Motile sperm grade IV (%)	9.4	5.4	6.5	10.5	3.34	0.002

* *t* test is used to compare two groups. $P < 0.05$ was considered statistically significant.

DISCUSSION

Results of this study indicated that of 315 started cycles, clinical fertilization rate per retrieval was 61.6%. Zalavary *et al.* analyzed 208 standard IVF treatments and reported a mean fertilization rate of 58.7± 25.3% (21). Bakkevig *et al.* in a study of 262 couples undergoing IVF treatment reported fertilization rate 61.1% in non-male smoker and 60.2% in male smoker (22). Janson *et al.* in a study with analysis of 176 IVF cycles resulted that of the total retried 1477 oocytes fertilization rate was 74.4% (12). Our study indicated that not women's age but length of infertility had negatively correlated with clinical fertilization rate. Kupka *et al.* reported that there was negative correlation between length of infertility and pregnancy rate (11). Menezo *et al.* reported that the mean of embryo transferred in

Table 4. Significant predictors of clinical fertilization in backward stepwise regression analysis

Independent variables	Unstandarazied coefficients		Standardized coefficients	*t*	Significant
	B	Std. Error	Beta		
LH	-5.21×10^{-2}	0.01	-0.37	-3.44	0.001
Duration of ovarian stimulation	-4.6×10^{-2}	0.01	-0.27	-2.51	0.01
N of oocytes stage II	4.6×10^{-2}	0.01	0.39	2.70	0.01
N of oocytes stage III	9.9×10^{-2}	0.04	0.25	2.34	0.02
No of oocytes retrieved	8.38×10^{-3}	0.01	0.73	4.92	<0.0001

* $P<0.05$ was considered statistically significant.

patient > 35 year was lower than of women with age under 30 year (23). Kupka *et al.* evaluated the effect of age for 10 age groups in logistic model and observed the best results of clinical pregnancy rate in women 27 or 28 year old (11). However, in a recent Belgian study, maternal age was as well as embryo variables, showing that the pregnancy rate after single embryo transfer was independent of maternal age in women aged < 38 years (24).

We found that there were not relationship between fertilization rate and basal FSH serum on 2-3 day. Nahum *et al.* concluded that in vitro fertilization outcome is strongly correlated with both maternal ages, basal cycle day 3 follicle stimulated hormone, and antral follicle assessment (25). Ashrafi et al reported that day –3 serum FSH was a predictor of ovarian response and estimating cancellation rate of IVF cycle (26).

Our results showed that the mean of LH serum on 2-3 day, the number of administration of hMG ampules and the duration of ovarian stimulation were higher in women with negative fertilization than of those with positive fertilization. Alike the result of this study, Penarrubi *et al.* reported that serum LH measurements during ovarian stimulation can not predict ovarian response (27). Janson *et al.* reported that the number of administration of hMG ampules was not significant variable on outcome of IVF cycles (12). On the other hand, ovarian sensitivity, assessed as number of FSH IU per oocyte retrieved, correlated with ongoing implantation (28). In addition, in a recent study the starting dose of FSH was significantly lower in cycles ongoing implantation (29).

Our results demonstrated that the number of follicles, the number of oocytes retrieved, and the number of oocytes stage III had positive relation with fertilization rate. Clear evidence suggests that the maturity of the oocytes affects the outcome of the IVF cycles in both the fertilization rate and embryo quality (12). Ebner *et al.* reported that an intact first polar body showing a smooth surface was found to be of positive prognostic value in terms of fertilization and embryo quality, as well as as implantation and pregnancy rate (30-32). In contrast, De sutter *et al*. were not able to correlate oocyte morphology with fertilization rate or embryo quality (33). Serhal reported a pregnancy rate 24% in patients with transfers derived solely from normal oocytes compared with those from oocytes with cytoplasmic abnormalities (3%) (34), and similar results were reported elsewhere (35). This negative impact on treatment outcome may be explained by a higher rate of aneuploidy found in dysmorphic oocytes (36).

Our findings suggested that count of sperm and the number of sperm grade III and IV post swim- up had relationship with clinical fertilization. Obara *et al.* demonstrated that there was no correlation between semen volume and fertilization in IVF cycle. In contrast, sperm concentration, sperm motility, progressive motility, total motile count and normal sperm morphology were significantly correlated with the fertilization rate. Also, he concluded that there was no correlation between sperm concentration, sperm motility, progressive motility and fertilization rate in post swim-up, but sperm morphology was significantly correlated with fertilization rate (37).

According to the result of multivariate regression model, the positive predictors of clinical fertilization were: the mean number of oocytes retrieved and the mean number of oocytes stage II and stage III. The mean of basal LH serum on day 2 and the mean duration of ovarian stimulation were negative predictors of fertilization rate. Balba reported that oocyte morphology does not affect fertilization rate, embryo quality and implantation rate after intracytoplasmic sperm injection (38). Thurin *et al.* in a Scandinavian study in the multivariate analysis found that the first IVF cycle, conventional IVF as fertilization method and 4-cell embryo were a statically higher ongoing implantation rate than did second IVF cycle, ICSI and non-cell embryo (39).

In conclusion, ovarian response to gonadotropins (the number of oocytes retrieved and duration of stimulation) and the quality of oocytes are main predictors of fertilization. Also this study indicated that although some parameters of sperm quality were significant variables of fertilization rate in univariate analysis, in multivariate analysis one's effects were negligible. This information should be used when selecting couples for IVF cycles or oocytes for embryo formation to reduce the rate of negative fertilization.

Acknowledgments

We thank all of the women and men who participated in this study.

Conflict of interests

We have no competing interests.

REFERENCES

1. Out HJ, Mannaerts BM, Driessen SG, Coelingh Bennink HJ. Recombinant follicle stimulating hormone (rFSH; Puregon) in assisted reproduction: more oocytes, more pregnancies. Results from five comparative studies. Hum Reprod Update. 1996 Mar-Apr; 2(2):162-171.
2. Scott RT Jr, Hofmann GE. Prognostic assessment of ovarian reserve. Fertil Steril. 1995 Jan; 63(1):1-11.
3. Lass A, Brinsden P. The role of ovarian volume in reproductive medicine. Hum Reprod Update. 1999 May-Jun;5(3):256-266.
4. Syrop CH, Dawson JD, Husman KJ, Sparks AE, Van Voorhis BJ. Ovarian volume may predict assisted reproductive outcomes better than follicle stimulating hormone concentration on day 3. Hum Reprod. 1999 Jul; 14(7):1752-1756.
5. Ng EH, Chan CC, Tang OS, Ho PC. Antral follicle count and FSH concentration after clomiphene citrate challenge test in the prediction of ovarian response during IVF treatment. Hum Reprod. 2005 Jun; 20(6):1647-1654.
6. Kupesic S, Kurjak A. Predictors of IVF outcome by three-dimensional ultrasound. Hum Reprod. 2002 Apr; 17(4):950-955.
7. Engmann L, Sladkevicius P, Agrawal R, Bekir JS, Campbell S, Tan SL. Value of ovarian stromal blood flow velocity measurement after pituitary suppression in the prediction of ovarian responsiveness and outcome of in vitro fertilization treatment. Fertil Steril. 1999 Jan; 71(1):22-29.
8. Bancsi LF, Huijs AM, den Ouden CT, Broekmans FJ, Looman CW, Blankenstein MA, te Velde ER. Basal follicle-stimulating hormone levels are of limited value in predicting ongoing pregnancy rates after in vitro fertilization. Fertil Steril. 2000 Mar; 73(3):552-557.
9. Noci I, Biagiotti R, Maggi M, Ricci F, Cinotti A, Scarselli G. Low day 3 luteinizing hormone values are predictive of reduced response to ovarian stimulation. Hum Reprod. 1998 Mar; 13(3):531-534.
10. Eldar-Geva T, Ben-Chetrit A, Spitz IM, Rabinowitz R, Markowitz E, Mimoni T, Gal M, Zylber-Haran E, Margalioth EJ. Dynamic assays of inhibin B, anti-Mullerian hormone and estradiol following FSH stimulation and ovarian ultrasonography as predictors of IVF outcome. Hum Reprod. 2005 Nov;20(11):3178-3183.
11. Kupka MS, Dorn C, Richter O, Felberbaum R, van der Ven H. Impact of reproductive history on in vitro fertilization and intracytoplasmic sperm injection outcome: evidence from the German IVF Registry. Fertil Steril. 2003 Sep; 80(3):508-516.
12. Ho JY, Chen MJ, Yi YC, Guu HF, Ho ES. The effect of preincubation period of oocytes on nuclear maturity, fertilization rate, embryo quality, and pregnancy outcome in IVF and ICSI. J Assist Reprod Genet. 2003 Sep; 20(9):358-364.
13. Frattarelli JL, Bergh PA, Drews MR, Sharara FI, Scott RT. Evaluation of basal estradiol levels in assisted reproductive technology cycles. Fertil Steril. 2000 Sep; 74(3):518-524.
14. El-Nemr A, Al-Shawaf T, Sabatini L, Wilson C, Lower AM, Grudzinskas JG. Effect of smoking on ovarian reserve and ovarian stimulation in in-vitro fertilization and embryo transfer. Hum Reprod. 1998 Aug; 13(8):2192-2198.
15. Balakier H, Bouman D, Sojecki A, Librach C, Squire JA. Morphological and cytogenetic analysis of human giant oocytes and giant embryos. Hum Reprod. 2002 Sep; 17(9):2394-2401.
16. Ng EH, Tang OS, Ho PC. The significance of the number of antral follicles prior to stimulation in predicting ovarian responses in an IVF programme. Hum Reprod. 2000 Sep; 15(9):1937-1942.
17. World Health Organization. Laboratory Manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge, England: Cambridge university press, 1992.
18. Tan SL, Kingsland C, Campbell S, Mills C, Bradfield J, Alexander N, Yovich J, Jacobs HS. The long protocol of administration of gonadotropin-releasing hormone agonist is superior to the short protocol for ovarian stimulation for in vitro fertilization. Fertil Steril. 1992 Apr; 57(4):810-814.
19. Frattarelli JL, Lauria-Costab DF, Miller BT, Bergh PA, Scott RT. Basal antral follicle number and mean ovarian diameter predict cycle cancellation and ovarian responsiveness in assisted reproductive technology cycles. Fertil Steril. 2000 Sep; 74(3):512-517.

20. Land JA, Yarmolinskaya MI, Dumoulin JC, Evers JL. High-dose human menopausal gonadotropin stimulation in poor responders does not improve in vitro fertilization outcome. Fertil Steril. 1996 May; 65(5):961-965.
21. Zalavary S, Kristina S. Computer-aided semen analysis assessment of the correlation between sperm motility parameters and fertilization, embryo development or pregnancy in standard IVF cycles. Acta Obstet Gynecol Scand. 2005; 84:510. [Abstract].
22. Bakkevig IH, Sunde A, Rein IT, Boe S, Pacey A, Hausken J. Male smoking and effect on fertilization capacity in IVF. Acta Obstet Gynecol Scand. 2005; 84:509. [Abstract].
23. Ménézo Y, Barak Y. Comparison between day-2 embryos obtained either from ICSI or resulting from short insemination IVF: influence of maternal age. Hum Reprod. 2000 Aug;15(8):1776-1780.
24. De Neubourg D, Gerris J, Mangelschots K, Van Royen E, Vercruyssen M, Elseviers M. Single top quality embryo transfer as a model for prediction of early pregnancy outcome. Hum Reprod. 2004 Jun; 19(6):1476-1479.
25. Nahum R, Shifren JL, Chang Y, Leykin L, Isaacson K, Toth TL. Antral follicle assessment as a tool for predicting outcome in IVF--is it a better predictor than age and FSH? J Assist Reprod Genet. 2001 Mar; 18(3):151-155.
26. Ashrafi M, Madani T, Tehranian AS, Malekzadeh F. Follicle stimulating hormone as a predictor of ovarian response in women undergoing controlled ovarian hyperstimulation for IVF. Int J Gynaecol Obstet. 2005 Oct; 91(1):53-57.
27. Peñarrubia J, Fábregues F, Creus M, Manau D, Casamitjana R, Guimerá M, Carmona F, Vanrell JA, Balasch J. LH serum levels during ovarian stimulation as predictors of ovarian response and assisted reproduction outcome in down-regulated women stimulated with recombinant FSH. Hum Reprod. 2003 Dec; 18(12):2689-2697.
28. Holte J, Bergh T, Tilly J, Pettersson H, Berglund L. The construction and application of a prediction model to monimize twin implantation rate at a preserved high pregnancy rate. Berlin: ESHRE; 2004. P. 394.
29. Popovic-Todorovic B, Loft A, Ziebe S, Andersen AN. Impact of recombinant FSH dose adjustments on ovarian response in the second treatment cycle with IVF or ICSI in "standard" patients treated with 150 IU/day during the first cycle. Acta Obstet Gynecol Scand. 2004 Sep; 83(9):842-849.
30. Ebner T, Yaman C, Moser M, Sommergruber M, Feichtinger O, Tews G. Prognostic value of first polar body morphology on fertilization rate and embryo quality in intracytoplasmic sperm injection. Hum Reprod. 2000 Feb; 15(2):427-430.
31. Ebner T, Yaman C, Moser M, Sommergruber M, Pölz W, Tews G. Embryo fragmentation in vitro and its impact on treatment and pregnancy outcome. Fertil Steril. 2001 Aug; 76(2):281-285.
32. Ebner T, Yaman C, Moser M, Sommergruber M, Jesacher K, Tews G. A prospective study on oocyte survival rate after ICSI: influence of injection technique and morphological features. J Assist Reprod Genet. 2001 Dec; 18(12):623-628.
33. De Sutter P, Dozortsev D, Quian C, Dhont M. Oocyte morphology dose not correlate with fertilization rate and embryo quality after intracytoplasmic sperm injection. Hum Reprod. 1996; 11: 595–597.
34. Serhal PF, Ranieri DM, Kinis A, Marchant S, Davies M, Khadum IM. Oocyte morphology predicts outcome of intracytoplasmic sperm injection. Hum Reprod. 1997 Jun; 12(6):1267-1270.
35. Loutradis D, Drakakis P, Kallianidis K, Milingos S, Dendrinos S, Michalas S. Oocyte morphology correlates with embryo quality and pregnancy rate after intracytoplasmic sperm injection. Fertil Steril. 1999 Aug; 72(2):240-244.
36. Kahraman S, Yakin K, Dönmez E, Samli H, Bahçe M, Cengiz G, Sertyel S, Samli M, Imirzalioğlu N. Relationship between granular cytoplasm of oocytes and pregnancy outcome following intracytoplasmic sperm injection. Hum Reprod. 2000 Nov; 15(11):2390-2393.
37. Obara H, Shibahara H, Tsunoda H, Taneichi A, Fujiwara H, Takamizawa S, Idei S, Sato I. Prediction of unexpectedly poor fertilization and pregnancy outcome using the strict criteria for sperm morphology before and after sperm separation in IVF-ET. Int J Androl. 2001 Apr; 24(2):102-108.
38. Balaban B, Urman B, Sertac A, et al. Oocyte morphology does not affect fertilization rate, embryo quality and implantation rate after intracytoplasmic sperm injection. Hum. Reprod 1998; 13: 3431-3433.
39. Thurin A, Hardarson T, Hausken J, Jablonowska B, Lundin K, Pinborg A, Bergh C. Predictors of ongoing implantation in IVF in a good prognosis group of patients. Hum Reprod. 2005 Jul; 20(7):1876-1880.