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Original Article

Maternal age and serum β-hCG concentrations at 14-16 days after embryo transfer are predictive factors for spontaneous abortion in IVF/ICSI cycles in Northeast China

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Abstract: *Purpose:* The objective of this current study was to find the most possible risk factors determining spontaneous abortion and predicting ongoing pregnancy outcomes in IVF/ICSI cycles to improve patient counselling by giving reliable predictive information. *Methods:* A retrospective data analysis was carried out on 426 IVF/ICSI cycles with clinical pregnancies performed from May 29, 2011 to December 31, 2015. Related factors between spontaneous abortion (SA group) and ongoing pregnancy (OP group) were analyzed. *Results:* A total of 363 patients had ongoing pregnancies and while 63 patients had spontaneous abortions. Concerning related factors, maternal age and serum β-hCG concentrations at 14-16 days after embryo transfer were found to be significant in predicting pregnancy outcomes. Significantly higher maternal ages ($p < 0.001$) and lower β-hCG values ($p < 0.001$) were obtained in the SA group compared with OP group. Cut-off values of maternal age, β-hCG value, and their logistic model in predicting ongoing pregnancy were 33 years (sensitivity 77.69% and specificity 47.62%), 502.4 mIU/mL (sensitivity 61.16% and specificity 76.19%), and 0.83393 (sensitivity 70.52% and specificity 71.43%), respectively. *Conclusion:* Maternal age, serum β-hCG concentrations at 14-16 days after embryo transfer, and logistic models are useful tools in predicting pregnancy outcomes following IVF-ET.

Keywords: Spontaneous abortion, *in vitro* fertilization embryo transfer, intracytoplasmic sperm injection, predictive factors

Introduction

With the development of *in vitro* fertilization (IVF), embryo transfer (ET), and intracytoplasmic sperm injection (ICSI), more and more people suffering from infertility have been cured by assisted reproductive technology (ART). The average clinical pregnancy rate of ART is 30%-40% [1]. However, because of the influence of many factors, the live birth rate of ART is only 20%-30% [2]. High incidence of miscarriage after IVF/ICSI is one of the most important reasons. It has been reported that women undergoing ART have an increased risk of spontaneous miscarriage compared to those who conceive spontaneously [3, 4]. The average incidence of clinically apparent miscarriage varies between 10% and 15%. Miscarriage rates after infertility vary between 18% and 30% [3, 5-7]. Mechanisms underlying this increased risk remain unclear [8].

Due to many uncertain factors, couples undergoing IVF are often plagued by anxiety and stress related to their infertility, subsequent treatment, and pregnancy outcome. Yong et al. showed that patients undergoing IVF experience the highest level of psychological stress at the time of the pregnancy test, as waiting for results can be very stressful [9]. This emotional change may have adverse effects on outcomes in IVF [10]. Therefore, prediction of pregnancy outcomes is of great importance to both patients and clinicians. If ART outcomes can be

predicted reliably, patients will reduce anxiety and other unnecessary costs, while clinicians monitor and manage pregnancies in advance.

Many studies have been implemented to predict the success of IVF pregnancies. Some serum markers have been reported to be predictive, including human chorionic gonadotrophin (hCG), progesterone and oestradiol, activing, inhibin, and CA-125. Jee Hyun Kim et al. suggested that for prediction of intrauterine and ongoing pregnancies, hCG was better than progesterone and inhibin A. The predictive performance of progesterone and inhibin A was similar. A single measurement of serum hCG levels is sufficient to predict pregnancy outcomes in IVF-ET patients [11]. Many other authors have also demonstrated that hCG is the most predictive serum marker [12-18].

In addition, other factors such as body mass index (BMI) and endometrial thickness have been reported to be predictors for pregnancy outcomes [19, 20]. Xi Y et al. showed that endometrial thickness is an independent variable predictive of clinical pregnancies, spontaneous abortions, and live births [20]. In summary, there are many factors influencing pregnancy outcomes. However, the most useful factors in discriminating between spontaneous abortions and ongoing pregnancies have not been determined.

Therefore, the objective of this current study was to find the most possible factors determining spontaneous abortions in IVF/ICSI cycles. This study used these data to design a model to predict spontaneous abortions and ongoing pregnancy outcomes to improve patient counselling by giving reliable predictive information.

Materials and methods

Researchers reviewed records of all IVF/ICSI patients treated between May 29, 2011 and December 31, 2015. All patients meeting the following inclusion criteria were included: fresh IVF or ICSI treatment cycle, embryo transfer on day 3 after oocyte retrieval, positive serum pregnancy test on day 14-16 after embryo transfer, and presence of one or more intrauterine gestational sac(s) on ultrasound on day 28. Only one treatment cycle per patient was included. If patients had more cycles resulting in pregnancies within the study period, the first cycle was included. Pregnancies after ectopic pregnancies and artificially terminated pregnancies were excluded.

Abortions were registered as having occurred on the day that an empty gestational sac or fetal demise was recorded by transvaginal ultrasound, regardless of time of expulsion or evacuation by curettage. Multiple gestations that partially ended before and partially after 28 weeks were regarded as ongoing pregnancies. An ongoing pregnancy was defined as a viable intrauterine pregnancy of at least 28 weeks gestation.

The following information was collected for analysis: maternal age, paternal age, infertility duration, female body mass index, baseline hormone levels (FSH, LH, E_2), hormone levels on the day of hCG administration (LH, E_2, P), endometrial thickness on the day of embryo transfer, number of embryos transferred, number of high-quality embryos transferred, number of retrieved oocytes, number and rate of MII oocytes, number and rate of 2PN, serum β-hCG levels 14-16 days after embryo transfer, type of infertility, causes of infertility, female factor, endometrial type on the day of embryo transfer, insemination method, sperm origin, and ovarian response.

The significance of each of these factors was determined by statistical analyses. Logistic regression analysis was performed utilizing significant factors. Receiver operating characteristic (ROC) curve was obtained to assess the predictive value of this model and individual indicators.

Statistical analysis

Statistical analyses were performed using *t*-tests, Chi-squared tests, and binary logistic regression analysis. *P*-values < 0.05 were considered statistically significant. Analyses were performed using Statistical Package for Social Sciences (SPSS) 17.0 (SPSS Inc., Chicago, IL, USA). Variables were primarily tested with univariate or multivariate analyses, for relation with SA. Variables with *P*-values < 0.05 were selected for binary logistic regression analysis. Crude odds ratios (OR) and 95% confidence intervals (CI) were calculated as SA risks. Receiver operating characteristic (ROC) curve was used to determine the model and individu-

Table 1. Comparison of patient characteristics and related variables between SA and OP group

	SA group	OP group	*P*-value
Number of cycles	63	363	
Maternal age (years)	32.68 ± 4.48	30.19 ± 3.92	< 0.001*
Paternal age (years)	34.81 ± 5.70	31.87 ± 5.04	< 0.001*
Infertility duration (years)	4.83 ± 3.36	4.05 ± 3.08	0.061
Female BMI (kg/m^2)	22.92 ± 3.73	22.16 ± 3.29	0.141
Baseline hormone levels			
FSH (mIU/ml)	7.33 ± 1.95	7.06 ± 2.46	0.245
LH (mIU/ml)	6.16 ± 5.90	6.78 ± 26.53	0.231
E_2 (pg/ml)	50.21 ± 27.47	56.51 ± 61.88	0.542
Hormone levels on the day of hCG administration			
LH (mIU/ml)	2.97 ± 3.71	2.35 ± 2.61	0.186
E_2 (pg/ml)	3327.52 ± 2006.79	3429.44 ± 1974.93	0.490
P (ng/ml)	1.00 ± 0.52	1.03 ± 0.47	0.421
Endometrial thickness on the day of embryo transfer (mm)	11.01 ± 2.40	11.39 ± 2.15	0.234
Number of embryos transferred	2.02 ± 0.42	2.05 ± 0.30	0.474
Number of high-quality embryos[a] transferred	1.97 ± 0.18	1.98 ± 0.16	0.748
Number of retrieved oocytes	9.73 ± 4.8	10.60 ± 4.48	0.122
Number of MII oocytes	8.75 ± 4.11	9.50 ± 4.12	0.190
Number of 2PN	6.71 ± 3.48	6.95 ± 3.49	0.668
MII oocytes rate (%)	92.17 ± 11.96	90.05 ± 12.19	0.093
2PN rate (%)	71.76 ± 21.78	66.80 ± 21.00	0.087
Serum β-hCG levels 14-16 days after embryo transfer (mIU/mL)	454.25 ± 411.52	757.93 ± 664.53	< 0.001*
Type of infertility			0.302
Primary	40 (63.5%)	255 (70.2%)	
Secondary	23 (36.5%)	108 (29.8%)	
Causes of infertility			0.229
Male factor	21 (33.3%)	138 (38.0%)	
Female factor	31 (49.2%)	131 (36.1%)	
Both factors	9 (14.3%)	79 (21.8%)	
Unexplained infertility	2 (3.2%)	15 (4.1%)	
Female factor			0.346
No history	23 (36.5%)	151 (41.6%)	
Uterine disease	3 (4.8%)	14 (3.9%)	
Ovarian disease	7 (11.1%)	29 (8.0%)	
Tubal factor	12 (19.0%)	98 (27.0%)	
Complic factor	18 (28.6%)	71 (19.6%)	
Endometrial type on the day of embryo transfer			0.701
Type A	1 (1.6%)	13 (3.6%)	
Type B	48 (76.2%)	267 (73.6%)	
Type C	14 (22.2%)	83 (22.9%)	
Insemination method			0.464
IVF	31 (49.2%)	181 (49.9%)	
ICSI	23 (36.5%)	148 (40.8%)	
Early rescue ICSI	9 (14.3%)	34 (9.4%)	
Sperm origin			0.087
Fresh ejaculate sperm	60 (95.2%)	316 (87.1%)	
Testicular sperm	3 (4.8%)	47 (12.9%)	
Ovarian response			0.011*
Normal ovarian response	40 (63.5%)	263 (72.5%)	
Poor ovarian response	5 (7.9%)	6 (1.7%)	
High ovarian response	18 (28.6)	94 (25.9%)	

BMI: Body mass index, *PN:* pronucleus, *MII:* metaphase II oocyte, *IVF:* in vitro fertilization, *ICSI:* intracytoplasmic sperm injection. [a]high-quality embryos have at least six cells, less than 20% fragmentation with even blastomeres or minor unevenness between blastomeres. *$p < 0.05$.

Table 2. Risk factors analysis for SA by univariately logistic regression analysis

	P-value	Odds ratio	95% confidence interval
Maternal age	0.022*	0.895	0.813-0.984
Paternal age	0.148	0.950	0.886-1.018
Ovarian response	0.133	0.783	0.568-1.077
Serum β-hCG levels 14-16 days after embryo transfer	< 0.001*	1.002	1.001-1.002

*$p < 0.05$.

Table 3. Multivariate logistic regression analysis of indicators used to predict SA

Parameters	β	OR (CI)	*P*-value
Maternal age	-0.151	0.860 (0.800-0.924)	< 0.001*
Serum β-hCG levels 14-16 days After embryo transfer	0.002	1.002 (1.001-1.002)	< 0.001*
Constant	5.613	273.865	< 0.001*

*$p < 0.05$.

al indicator cutoff levels that best discriminated between SA and OP. An area of 1.0 under the ROC curve denoted a perfect test while an area of 0.5 was no better than chance.

Results

Between May 29, 2011 and December 31, 2015, a total of 426 treatment cycles with clinical pregnancies remained for analysis. There were 63 cycles (14.79%) in the SA group and 363 cycles (85.21%) in the OP group.

Univariate analysis between the SA group and OP group showed higher maternal age and lower serum β-hCG levels at 14-16 days after embryo transfer. Both were statistically significant between the two groups (**Table 1**). Average maternal age of the the SA group was 32.68 ± 4.48 years while that of the OP group was 30.19 ± 3.92 years. Average paternal age of the SA group was 34.81 ± 5.70 years while that of the OP group was 31.87 ± 5.04 years. Both maternal ($p < 0.001$) and paternal ($p < 0.001$) patients were significantly older in the SA group. Serum β-hCG concentration at 14-16 days after embryo transfer in the SA group was 454.25 ± 411.52 mIU/mL while that of the OP group was 757.93 ± 664.53 mIU/mL. Maternal and paternal age and serum β-hCG concentration 14-16 days after embryo transfer showed a significant relationship to SA in the univariate model ($p < 0.001$). All other variables were not significantly different.

Chi-squared tests for types of infertility, causes of infertility, female factors, endometrial types, insemination methods, sperm origin, and ovarian response between the SA group and OP group revealed that ovarian response was associated with SA ($p < 0.05$). All other variables were not significantly associated to SA (**Table 1**).

Logistic regression analysis showed that OR for SA associated with maternal age of 0.0895 (95% confidence interval 0.813-0.984; $p = 0.022$) and OR for SA associated with serum β-hCG levels at 14-16 days after embryo transfer of 1.002 (95% confidence interval = 1.001-1.002; $p < 0.001$). The two variables were independently positively associated with SA. The other two variables were not significantly different (**Table 2**).

A logistic regression model was established to assess the effects of maternal age and serum β-hCG levels (**Table 3**). According to this logistic model, the probability of SA was equal to the following: exp(z)/(1 + exp(z)), where z = 5.613 + (-0.151 × maternal age) + (0.002 × β-hCG value).

Using ROC curves, optimal cutoff, maternal age, and β-hCG values were calculated, respectively. Area under the curve (AUC) of the ROC for the logistic model was 0.734 ($p < 0.001$) and cutoff for this model was 0.83393 (sensitivity 70.52% and specificity 71.43%). AUC of the ROC for maternal age was 0.657 ($p < 0.001$) and the cutoff for maternal age was 33 years (sensitivity 77.69% and specificity 47.62%). AUC of the ROC for β-hCG was 0.694 ($p < 0.0001$) and the cutoff for β-hCG was 502.4

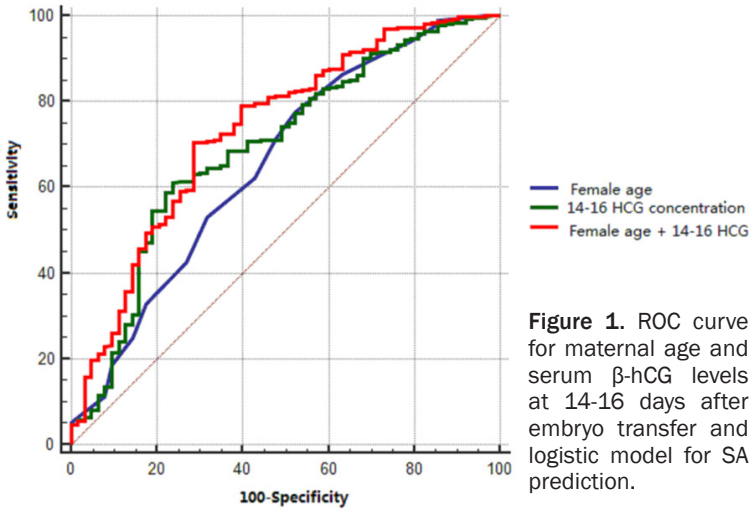

Figure 1. ROC curve for maternal age and serum β-hCG levels at 14-16 days after embryo transfer and logistic model for SA prediction.

mIU/mL (sensitivity 61.16% and specificity 76.19%) (**Figure 1**).

Discussion

The incidence of miscarriage after IVF/ICSI treatment is caused by several factors. It is widely believed that different levels of embryonic chromosomal abnormalities are the leading cause of early pregnancy loss [21]. In a sense, spontaneous abortion after IVF-ET is a process of optimization of human beings. However, clinicians should continue to attach great importance to finding risk factors related to SA after IVF/ICSI, improving live birth rates as much as possible.

Many studies have been carried out to find possible factors to predict the success of IVF pregnancies. Some serum markers have been reported to be predictive, including human chorionic gonadotrophin (hCG), progesterone and oestradiol, activin and inhibin, and CA-125. In addition, other factors such as body mass index (BMI) and endometrial thickness have been reported to as predictors for pregnancy outcomes [19, 20].

In this current study, the following related information was collected for analysis: maternal age, paternal age, infertility duration, female body mass index, baseline hormone levels (FSH, LH, E_2), hormone levels on the day of hCG administration (LH, E_2, P), endometrial thickness on the day of embryo transfer, number of embryos transferred, number of high-quality embryos transferred, number of retrieved oocytes, number and rate of MII oocytes, number and rate of 2PN, serum β-hCG levels 14-16 days after embryo transfer, type of infertility, causes of infertility, female factor, endometrial type on the day of embryo transfer, insemination method, sperm origin, and ovarian response. Finally, maternal age and serum β-hCG levels at 14-16 days after embryo transfer were found to be strong predictors of SA. Differences in age between pregnancies that were SA and those that were ongoing were strongly significant ($p < 0.001$), as were differences within serum β-hCG levels at 14-16 days after embryo transfer ($p < 0.001$). This significance indicates that these variables may be predictive of SA or the implantation success.

Therefore, this present study established a model to predict SA in infertility patients after IVF/ICSI. This model includes maternal age and serum β-hCG levels at 14-16 days after embryo transfer. According to ROC curve analysis, AUC of this logistic model (AUC = 0.734) was greater than that of other two individual parameters.

Data from this study showed that mean maternal age of the SA group was 32.68 ± 4.48 years while that of the OP group was 30.19 ± 3.92 years. Mean maternal ages in the two groups were significantly different. Therefore, incidence of SA increased with age. Many studies have reported that advanced maternal age is associated with poor pregnancy outcomes after IVF/ICSI. Nybo Andersen et al. investigated overall abortion incidence by reviewing a total of 634,272 women and 1,221,546 pregnancy outcomes. Risk of spontaneous abortion was lower (8.9%) in women aged 20-24 years and higher (74.7%) in those aged 45 years or more. High maternal age was a significant risk factor for spontaneous abortion regardless of number of previous miscarriages, calendar period, or parity [22]. Philippe Tummers et al. collected 1,597 clinical IVF/ICSI cases with known pregnancy outcomes. They tabulated the number of miscarriages or fetal demise per intervals of 2 weeks and compared outcomes

in terms of fetal survival of 1,200 singleton pregnancies with that of 397 twin pregnancies. According to maternal age, the investigator divided the pregnancies into two groups. The first group contained maternal age < 35 years and in the second group patients were > 35 years. A significant difference was found when miscarriage of women < 35 years of age was compared with miscarriage rates of women > 35 years. This significant difference was found in both singleton and twin pregnancy groups. In both singleton and twin pregnancies, incidence of abortion increased with age [23]. This present study is consistent with these studies in that incidence of spontaneous abortion increased with age. This result may be attributed to deterioration of the oocyte and to endocrine variations that occur with aging [21]. Therefore, this variable was selected for inclusion in multivariate logistic regression analysis.

β-hCG, originally found in the urine of pregnant women in 1927, is secreted and produced by syncytial trophoblasts. Its serum levels represent trophoblastic mass and function. β-hCG can be detected in the maternal serum as early as 8-11 days following oocyte retrieval, roughly doubling every 2 days in early pregnancy [17]. Many previous studies have analyzed the value of serum β-hCG levels for predicting clinical pregnancy outcomes in IVF/ICSI treatment cycles. Zhang Q et al. analyzed serum hCG levels 17 days after oocyte retrieval and clinical pregnancy outcomes in patients with positive serum hCG. They showed that, compared with normal clinical pregnancies (live births), hCG levels of spontaneous miscarriages were markedly lower (357.15 IU/L vs. 596.80 IU/L, $p < 0.001$) [17]. Wu GX et al. also showed the conclusion that average β-hCG values of miscarriage were lower than that of live-birth pregnancies on day 12 after ET ($p < 0.001$) [18]. Both studies demonstrated the same conclusion that β-hCG could be used as a marker to predict outcomes of pregnancies after IVF. It has been reported as the most reliable predictive biochemical marker in assessment of pregnancy outcomes.

Some investigations have analyzed β-hCG concentrations for cutoff values to differentiate an ongoing pregnancy from a non-ongoing pregnancy [4, 11] or a viable pregnancy from a nonviable pregnancy [24]. Bjercke et al. found a single β-hCG measurement of 55 IU/l on day 12 after embryo transfer to be reliable in predicting occurrence of early pregnancy loss. In another study performed by Gengxiang Wu et al., AUC of the ROC for miscarriage was 0.720 ($p < 0.001$). This present study showed that AUC of the ROC for miscarriage was 0.694 ($p < 0.0001$). In this study, ongoing pregnancy included pregnancies that did not result in a live birth. Mean β-hCG concentration at 14-16 days after embryo transfer was statistically different between the SA group (454.25 ± 411.52) and OP group (757.93 ± 664.53) ($p < 0.001$). Therefore, this variable was selected for inclusion in multivariate logistic regression analysis.

Other researchers have found serum concentrations of progesterone, estradiol, and hCG, measured 12 to 13 days post-ET, to be higher in ongoing pregnancies than in abortions [25]. However, there were no significant differences regarding progesterone and estradiol in the current study. One deficiency of this present study was that fewer samples were included in the SA group than the OP group. Larger samples are necessary for more accurate determination of predictive value.

In summary, it can be concluded that maternal age and serum β-hCG levels measured at 14-16 days after embryo transfer in IVF/ICSI treatment cycles are helpful in predicting pregnancy outcomes. This study provides a logistic model to predict SA. Cutoff values determined by ROC curve analysis are useful for discriminating between spontaneous abortions and ongoing pregnancies. Higher maternal age and lower β-hCG values indicate greater need for repeated measurement and closer follow up of IVF/ICSI patients at high risk for spontaneous abortion.

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Disclosure of conflict of interest

None.

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