Understanding reproducibility of human IVF traits to predict next IVF cycle outcome

Bin Wu · Juanzi Shi · Wanqiu Zhao · Suzhen Lu ·
Marta Silva · Timothy J. Gelety

Received: 4 March 2014 / Accepted: 29 June 2014 / Published online: 15 August 2014
© Springer Science+Business Media New York 2014

Abstract

Purpose Evaluating the failed IVF cycle often provides useful prognostic information. Before undergoing another attempt, patients experiencing an unsuccessful IVF cycle frequently request information about the probability of future success. Here, we introduced the concept of reproducibility and formulae to predict the next IVF cycle outcome.

Methods The experimental design was based on the retrospective review of IVF cycle data from 2006 to 2013 in two different IVF centers and statistical analysis. The reproducibility coefficients (r) of IVF traits including number of oocytes retrieved, oocyte maturity, fertilization, embryo quality and pregnancy were estimated using the interclass correlation coefficient between the repeated IVF cycle measurements for the same patient by variance component analysis. The formulae were designed to predict next IVF cycle outcome.

Results The number of oocytes retrieved from patients and their fertilization rate had the highest reproducibility coefficients ($r=0.81\sim0.84$), which indicated a very close correlation between the first retrieval cycle and subsequent IVF cycles. Oocyte maturity and number of top quality embryos had middle level reproducibility ($r=0.38\sim0.76$) and pregnancy rate had a relative lower reproducibility ($r=0.23\sim0.27$). Based on these parameters, the next outcome for these IVF traits might be accurately predicted by the designed formulae.

Capsule The IVF traits including number of oocytes retrieved, maturity, fertilization and top quality embryos had high or middle reproducibility. Base on these parameters, the outcomes of these traits could be accurately predicted in next IVF cycle.

B. Wu (✉) · S. Lu · M. Silva · T. J. Gelety
Arizona Center for Reproductive Endocrinology and Infertility,
Tucson, AZ 85712, USA
e-mail: wubin5190@gmail.com

J. Shi · W. Zhao
Shaanxi Hospital for Women and Children Health Care, Xi'an,
Shaanxi Province, P. R. China

Conclusions The introduction of the concept of reproducibility to our human IVF program allows us to predict future IVF cycle outcomes. The traits of oocyte numbers retrieved, oocyte maturity, fertilization, and top quality embryos had higher or middle reproducibility, which provides a basis for accurate prediction of future IVF outcomes. Based on this prediction, physicians may counsel their patients or change patient's stimulation plans, and laboratory embryologists may improve their IVF techniques accordingly.

Keywords IVF traits · Reproducibility · Correlation coefficient · Prediction · IVF outcome

Introduction

Assisted reproductive technologies including in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) as well as the more recently developed pre-implantation genetics diagnosis (PGD) or screening (PGS) have now widely been used for the treatment of infertility. Since 1985, the Society for Assisted Reproduction Technology (SART) has been actively involved in the collection of data related to IVF cycle outcomes from clinics performing assisted reproductive technologies (ART) in the United States and published annual national success rates and outcomes. Based on the 2012 SART report, the national success rate of total 93,062 fresh IVF cycles resulted in live births ranges from 3.9 to 40.7 % for all age groups [www.sart.com]. Averaging data from 49 countries, the world collaborative report estimated a pregnancy rate of 27 % and a delivery rate of 19 % [1]. The possibility of a live-birth for any couple undergoing treatment depends not only on the technique and success rate of individual clinics, but also on patient age, embryo quality and the uterine environment. Therefore, more than 60 % of infertile couples will not achieve a live-birth with the first IVF cycle

treatment and many may consider subsequent IVF treatments. Before undergoing another attempt, patients experiencing an unsuccessful IVF cycle frequently request information about the probability of future success.

Evaluating the failed IVF cycle often provides useful prognostic information. Information about the probability of pregnancy after successive treatments with IVF is important for candidate patients and for the physicians who are counseling them. Thus, developing better method for predicting IVF outcomes is an area of considerable current interest in the field of assisted reproductive technology. To date, many prediction models have been developed in reproductive medicine to help assess the chances of an IVF success [2, 3]. However, none of the various predictors of human fertility is ideal and there is no single simple solution for success in assisted reproduction [4]. The objective of this study was to estimate reproducibility coefficients for key IVF traits including number of oocytes retrieved, oocyte maturity, fertilization rate, top quality embryos and pregnancy rates, and to use these parameters to develop formulae for predicting of subsequent IVF cycle outcomes.

Materials and methods

Data source

This retrospective review utilized IVF cycle data from the Arizona IVF center to perform statistical analysis and estimate reproducibility coefficients for IVF traits. Chinese IVF center data was used to test the estimation accuracy. Patient data with at least two IVF cycles within three consecutive years were collected from the Arizona Center for Reproductive Endocrinology and Infertility in USA (Arizona Center) between 2006 and 2011 and the Shaanxi Province Women and Child Hospital in China (Shaanxi Center) between 2009 and 2013. At the Arizona Center, all patient data included the first IVF cycles with pregnancy and without pregnancy and again underwent another 2 to 7 cycles. At Shaanxi Center, because of the Chinese one child birth policy for each couple, only those patients with the first IVF cycle failure may undergo a second or third IVF cycle. Thus, all these data containing 2–4 IVF cycles only included the first failed IVF cycle patients. Also, the expected values for patients with future IVF cycles at the Arizona Center during 2012 to 2013 were calculated by our formulae in order to confirm prediction accuracy.

Ovarian stimulation procedure and IVF laboratory embryo culture

In this study, two IVF centers applied different IVF procedures and embryo culture methods. The patients at different centers were treated by individual center standard stimulation

protocols before oocyte retrieval. At the Arizona Center, all patients underwent controlled ovarian stimulation following mid-luteal GnRHa down-regulation with hMG (300 to 450 IU) daily. Pelvic ultrasound and E₂ assays were performed to assess ovarian and endometrial response to ovarian stimulation. Injection of 10,000 IU hCG was administered with leading follicles measuring 18–20 mm. Oocyte retrieval was performed 36 h later by transvaginal ultrasound-guided follicular aspiration under conscious sedation. At Shaanxi Center, beginning at one week after ovulation, patients were administrated with 0.05 to 0.1 mg Decapeptyl (triptorelin) per day for 14 days. Then, ovarian stimulation was done with daily administration of 150–300 IU of human recombinant follicle-stimulating hormone (Gonal-f) and continued until the day of hCG injection. Ovarian response was monitored by serial ultrasound examination and hormone measurement. When three leading follicles were larger than 18 mm in diameter, injections of 10,000 IU hCG were administered to trigger ovulation. Oocytes were retrieved 36 h later by transvaginal ultrasound-guided follicular aspiration.

At the Arizona Center, the retrieved oocytes were cultured in the P1 medium (Irvine Scientific, Inc, California, USA) with 3 % human serum albumin for 4–6 h. Then, oocytes were routinely inseminated (in vitro fertilization, IVF) or by intracytoplasmic sperm injection (ICSI) depending on sperm quality (approximately 35 % of cycles required ICSI). Fertilization was assessed on the following day, 18 to 20 h after insemination or ICSI. If two distinct pronuclei (2PN) were observed, fertilization was confirmed. Oocyte maturity was assessed by observing the existence of oocyte polar body after cumulus cells were removed. The zygotes were cultured in 50 µl micro-drops of global medium (IVFonline, Inc, Canada) for another two days. On the third day after oocyte retrieval, embryo quality was graded based on our laboratory evaluation criteria [5]. Top quality embryos were defined as regular, spherical blastomeres with less 10 % extracellular fragmentation and had 6 to 10 blastomeres on Day 3. Embryos with more than 10 % fragmentation and irregular and less 6 blastomeres were defined as low quality. 1 to 4 embryos were selected for abdominal ultrasound-guided transfer according to patient age (patient age range 23–46). Clinical pregnancies and live births were recorded to determine the pregnancy rate. At the Shaanxi Center, the retrieved oocytes were cultured in the G-IVF medium (Vitrolife, Inc, Sweden) with 10 % human serum albumin for 3–6 h. Then, oocytes were routinely inseminated for 5 h. Hereafter, the release of the second polar body was observed. If the second polar body was not released, this oocyte was identified as unfertilized and ICSI was performed immediately. The total ICSI rate was approximately 45 % of all IVF cycles. After 18 to 20 h, the zygotes were cultured in 15 µl micro-drops of G1 (Vitrolife, Inc., Sweden) for another 2 days. On the third day after retrieval, embryo quality was graded based

on Shaanxi Center laboratory criteria [6]. Top quality embryos were defined as 6–8 even blastomeres with less than 20 % extracellular fragmentation. 1 to 3 embryos were selected for transfer according to patient age (Age 23–46). Clinical pregnancy and live-births were recorded to determine pregnant rates.

Data statistical analysis and reproducibility estimation

All data collected based on patient's age, IVF cycle times, retrieved oocyte numbers, maturity, fertilization (2PN), number of top quality embryos and pregnancy were statistically analyzed and classified to determine their percentage, average and standard deviation (mean±SD). The analysis of variance method (ANOVA) and Student's *t*-test were used for statistical evaluation and differences were considered statistically significant at $P<0.05$. The method of variance analysis was used to determine IVF trait reproducibility coefficient [7]. Here, a trait reproducibility coefficient was designed using the inter-class correlation coefficient of repeated measurements on the same individual. That it is, to determine the correlation among various IVF cycles in an infertile patient. This correlation coefficient was calculated using mean variance components that were estimated by analysis variance [8]. Based on our IVF data, the reproducibility coefficient for each IVF trait was calculated by the formula:

$$r = (\text{MSb} - \text{MSw}) / [\text{MSb} + \text{MSw}(k_0 - 1)]$$

Where

- r reproducibility coefficient for IVF traits
- MSb the mean variance among patients
- MSw the mean variance of the different IVF cycle times within a patient
- K_0 the weighted IVF cycle times. Because some patients have two IVF cycles and some patients have more than three IVF cycles. Thus, it needs to weight different patient IVF cycle times and K_0 is adjusted and calculated by following formula:

$$K_0 = (\sum K - \sum K^2 / \sum K) / n - 1$$

Here, K is IVF cycle times per patient and n indicates numbers of IVF patients.

Because patient pregnancy is a “Yes” or “No” threshold trait and does not have a contiguous number, we defined pregnancy as 1 and no pregnancy as 0 to determine the reproducibility coefficient of each IVF pregnancy trait. After estimation of reproducibility coefficient, the significant test

was conducted by student's *t*-test. $P<0.05$ was considered statistically significant.

Prediction formula of IVF trait outcome

In order to predict various trait outcomes of next IVF cycle, the following logistic regression model was designed as the prediction formula:

$$P_x = k r / [1 + (k-1)r] \times (P_k - \mu) + \mu$$

here

- P_x patient next prediction value (for example, oocyte number, maturation and fertilization rate and embryo quality etc. for next IVF cycle)
- P_k patient average of different IVF cycle times
- μ mean level of all patient IVF cycles
- k and r the average IVF cycle times of the patient and reproducibility coefficient, respectively.

In order to verify the accuracy of the prediction, 40 patients were randomly chosen from the IVF patient records in 2012–2013 at the Arizona Center. Based on patient's first cycle record on number of oocytes retrieved, maturation, fertilization and top quality embryos, the patient's second values of IVF cycle were predicted by above formula, respectively. Then, the difference between actual data and expected value was examined by χ^2 test. $P<0.05$ was considered statistically significant.

Results

Distribution of oocyte numbers by patient age

In order to show the demographics of the patient population by age and ovarian reserve, the distribution of oocyte number retrieved according to age from 23 to 46 years old was calculated based on the data of 419 IVF cycles of 163 patients at Arizona Center and 376 IVF cycles of 179 patients at Shaanxi Center (Fig. 1). As the figure shows, number of oocytes retrieved displayed a declining trend after age 36. *F* test indicated that there was a very significant difference on oocyte numbers among various ages ($P<0.01$). According to patient age and number of oocyte retrieved, the data was divided into three groups: age 23 and 35, age 36 and 39 and age 40 and 46. There was no significant difference within each group ($P>0.05$) in both IVF centers. However, there was a significant difference among three groups ($P<0.05$). The oocyte numbers in age 36 to 39 were significantly fewer than age 23 to 36 group ($P<0.01$), and the oocyte numbers at age 40 to

Fig. 1 The distribution of number of oocytes retrieved based on various ages in two different race populations. Three groups were divided from age 23 and 35, age 36 and 39 and age 40 and 46 and oocyte numbers each group were 14.3 ± 2.93 , 8.6 ± 1.19 and 4.5 ± 1.21 at Arizona Center, and 11.6 ± 3.34 , 7.2 ± 1.27 and 3.1 ± 1.3 at Shaanxi Center, respectively. There was no significant difference within each group ($P > 0.05$), but there was a

significant difference among three groups ($P < 0.05$). The numbers of oocytes in age 36 to 39 group were significantly fewer than that at age 23 to 35 group ($P < 0.01$), and significant more than that at age 40 to 46 group ($P < 0.05$). Different letters, a,b,c indicate significant difference ($P < 0.05$) and the same letters show no significant difference ($P > 0.05$)

46 were significant fewer than age 36 to 39 group ($P < 0.05$). Particularly, we noticed no significant difference among patients over 40 years old ($P > 0.05$).

The ontogenetic change at the various IVF cycles

The mean $\pm$ SD of data from a total of 419 IVF cycles of 163 patients at Arizona Center and a total of 293 IVF cycles of 139 patients at Shaanxi Center were calculated based on patient IVF cycle times (Table 1). At the Arizona Center, average retrieval cycles were 2.58 (range 2–7 cycles) per patient and average numbers of oocytes retrieved were 10.1 ± 8.6 . There were no significant changes in mean values of oocyte numbers and rates of maturity, fertilization and top quality embryos between the initial and subsequent IVF cycles ($P > 0.05$). However, the pregnancy rates showed a significant difference between the initial and subsequent IVF cycles ($P < 0.05$). The first three IVF cycles had relatively high pregnancy chance, but in the fourth and fifth cycles, the opportunity for pregnancy fell to 16 to 22 %. After five cycles, the successive IVF retrieval was no longer valid for pregnancy.

At the Shaanxi Center, the average retrieval cycles were 2.1 (range 2–4 cycles) per patient and average numbers of oocytes retrieved were 12.1 ± 8.5 . There were no significant changes in

mean values of oocyte numbers, maturity, fertilization and top quality between the initial and subsequent IVF cycles ($P > 0.05$). However, pregnancy rates in the fourth IVF cycle were significant lower than the second or third cycles ($P < 0.05$). In the Shaanxi Center data, there were no patients with more than four IVF cycles and all patients experienced a first IVF cycle failure.

Reproducibility coefficients of IVF traits

All five IVF traits observed in both IVF centers demonstrated statistically significant estimation of reproducibility coefficients (Table 2). The numbers of oocytes retrieved from patients and their fertilization rate had the highest reproducibility coefficient ($r = 0.81 \sim 0.84$). Oocyte maturation rate and top quality embryos had relative middle level reproducibility ($r = 0.38 \sim 0.76$). Pregnancy rate had lower reproducibility ($r = 0.23 \sim 0.27$).

Prediction of IVF outcome

Based on these reproducibility coefficients and patient's previous IVF records, the predictive formulae for the subsequent IVF cycle are shown in Table 3. During 2012 and 2013, forty

Table 1 Mean±SD of various IVF traits in the different IVF cycles of Arizona Center (upper) and Shaanxi Center (below)

IVF Cycle Number								
IVF Traits	1	2	3	4	5	6	7	F test
Arizona Center								
Patient No.	163	163	55	25	7	4	2	
Oocyte Number	10.7±8.6	10.5±8.0	8.9±7.6	10.1±7.5	7.4±5.5	9.0±5.7	7.0±4.6	<i>P</i> >0.05
Patient No.	160	160	53	23	7	4	2	
MII rate (%)	76.7±22.5	77.0±21.3	76.9±21.2	79.6±21.0	75.2±20.9	66.3±30.3	75.0±27.6	<i>P</i> >0.05
Patient No.	153	153	49	18	7	4	2	
2 PN rate (%)	61.1±24.0	64.6±21.8	66.3±23.0	68.6±21.6	58.0±19.2	57.0±25.6	71.5±21.3	<i>P</i> >0.05
Patient No.	103	103	33	11	3	2	2	
Top embryo (%)	52.2±30.6	55.2±31.4	52.9±30.9	65.4±22.6	40.0±40.0	65.0±21.2	42.5±21.5	<i>P</i> >0.05
Patient No.	145	145	50	18	6	4	2	
Pregnancy (%)	28.3±45.2 ^a	42.8±49.6 ^a	32.0±47.1 ^a	22.0±42.8 ^b	16.7±40.8 ^b	0 ^c	0 ^c	<i>P</i> <0.05
Shaanxi Center								
Patient No.	139	139	12	3				
Oocyte Number	12.1±8.5	12.6±6.4	13.2±6.0	16.3±7.1				<i>P</i> >0.05
Patient No.	139	139	12	3				
MII rate (%)	87.0±15.4	88.1±16.0	90.1±10.0	75.5±19.5				<i>P</i> >0.05
Patient No.	139	139	12	3				
2 PN rate (%)	62.5±20.8	69.7±25.5	62.2±26.3	42.3±13.3				<i>P</i> >0.05
Patient No.	139	139	12	3				
Top embryo (%)	30.9±18.1	36.2±26.6	30.5±23.6	48.0±3.5				<i>P</i> >0.05
Patient No.	139	139	9	3				
Pregnancy (%)	0	48.7 ^a	44.0 ^a	33.3 ^c				<i>P</i> <0.05

Note: At Arizona center, 7 IVF cycle data were collected and some patients had pregnancy in the first cycles. At Shaanxi center, patients had only four IVF cycles and all patients had no pregnancy in the first IVF cycles

Table 2 Population mean and repeatability coefficient of various IVF traits

IVF traits	Population mean±SD		Reproducibility coefficient (r)*	
	Arizona Center	Shaanxi Center	Arizona Center	Shaanxi Center
Oocyte Number	10.1±7.7	12.3±7.6	0.84	0.82
Maturation rate (%)	76.6±21.0	86.3±13.8	0.52	0.68
Fertilization rate (%)	63.8±22.8	65.7±23.7	0.82	0.81
Top quality embryo (%)	54.0±30.6	34.9±22.8	0.76	0.38
Pregnancy (%)	33.4±25.2	42.0±7.9	0.27	0.23

*All coefficients showed statistically significant values ($P<0.05$)

patients were randomly selected to predict their second or third retrieval oocyte numbers, oocyte maturation and fertilization rate, and their top quality embryo rate. χ^2 test (Table 4) indicated that there was no significant difference between predicted values and actual observed values on the number of oocytes, maturation, fertilization and top quality embryos ($P>0.05$).

Discussion

It is important for patients who are considering the next treatment with in-vitro fertilization to know the probability of success after one or more failed IVF treatments. Also, it is very important for physicians and embryologists to understand IVF trait repeatability in order to improve their IVF treatment procedures and embryo culture systems. Our findings provide this important information. First, we determined that two IVF traits including number of oocytes retrieved and

fertilization rate had very high repeatability in both IVF centers, which indicates a very close correlation between the first retrieval cycle and subsequent IVF cycles. Secondly, we used these reproducibility coefficients to form a formula to predict subsequent IVF cycle values. For example, if one patient had 5 oocytes collected in the first retrieval cycle, she would be predicted to have 5.8 oocytes in the subsequent IVF cycles. Thirdly, our result showed that the maturation rate of oocytes retrieved and top quality embryos had middle level reproducibility coefficients ($r=0.38\sim0.76$). We also may use these coefficients to predict next IVF cycle outcomes, but the accuracy may be lower than the prediction of oocyte numbers and fertilization rate. These results suggest that different ovarian stimulations plans at each IVF center may result in different degrees of oocyte maturation, and individual IVF laboratory embryologists may employ different criterion in embryo grading. Finally, the pregnancy rate had lower repeatability with $r=0.23$ at the Shaanxi Center and $r=0.27$ at the Arizona Center, which indicates that over increasing subsequent attempts, IVF pregnancy is more difficult to predict. Loughman [9] interpreted that a repeatability coefficient of less than 0.33 was very likely to be due to measurement error and using this coefficient often is both misleading and inaccurate. Thus, over the last few decades, many previously developed prediction models in reproductive medicine have not accurately predicted IVF pregnancy rates [4, 10].

Based on our clinical observation, when a patient had three IVF cycles without clinical pregnancy or live birth, the probability of her achieving a subsequent IVF cycle pregnancy would become very low (approximately 8.0 %, 2 of 25 patients). According to statistics reported in 2011 from Australia and New Zealand (2009–11) from 35 fertility centers, Sullivan's study [11] indicated that the overall results, covering all age groups, showed that the chance of delivering a liveborn baby was 21.1 % after the first cycle, increasing to 31.1 % after two cycles, 36 % after three cycles, 38.6 % after four cycles, and 40 % after five cycles. The cumulative live delivery rate did not increase markedly with additional treatments in the sixth cycle and beyond. The sixth cycle only afforded an extra 0.7 % chance of pregnancy and the seventh cycle, 0.4 %. Our results showed that there was no successful

Table 3 Prediction formulae of various IVF traits in next cycle

IVF traits	Prediction formula
Arizona Center	
Oocyte Number	$P_x = 0.84 \text{ k} / [1 + 0.84(k-1)] \times (P_k - 10) + 10$
Maturation rate (%)	$P_x = 0.52 \text{ k} / [1 + 0.52(k-1)] \times (P_k - 77) + 77$
Fertilization rate (%)	$P_x = 0.82 \text{ k} / [1 + 0.82(k-1)] \times (P_k - 64) + 64$
Top quality embryos (%)	$P_x = 0.76 \text{ k} / [1 + 0.76(k-1)] \times (P_k - 54) + 54$
Pregnancy (%)	$P_x = 0.27 \text{ k} / [1 + 0.27(k-1)] \times (P_k - 33) + 33$
Shaanxi Center	
Oocyte Number	$P_x = 0.82 \text{ k} / [1 + 0.82(k-1)] \times (P_k - 12) + 12$
Maturation rate (%)	$P_x = 0.68 \text{ k} / [1 + 0.68(k-1)] \times (P_k - 86) + 86$
Fertilization rate (%)	$P_x = 0.81 \text{ k} / [1 + 0.81(k-1)] \times (P_k - 66) + 66$
Top quality embryos (%)	$P_x = 0.38 \text{ k} / [1 + 0.38(k-1)] \times (P_k - 35) + 35$
Pregnancy (%)	$P_x = 0.23 \text{ k} / [1 + 0.23(k-1)] \times (P_k - 42) + 42$

P_x indicates predicted value for next IVF cycle; P_k indicates patient former several IVF mean; k indicates patient former IVF times

Table 4 Verification of predicted and observed values on IVF cycles in 2012–2013

Patient No.	Oocyte No.		Maturation rate (%)		Fertilization rate (%)		Embryo quality (%)	
	Expect	Observe	Expect	Observe	Expect	Observe	Expect	Observe
1	10.0	12	68.2	66.7	60.7	61.5	76.0	66.7
2	5.8	10	68.2	60.0	44.3	30.0	89.0	100.0
3	5.8	5	80.1	80.0	60.7	80.0	58.6	50.0
4	7.5	5	59.3	40.0	48.8	40.0	51.0	50.0
5	6.6	10	71.6	80.0	66.2	60.0	89.0	83.3
6	13.4	15	74.1	66.7	52.5	53.3	73.0	70.0
7	4.5	4	75.6	75.0	73.9	75.0	65.0	66.7
8	10	15	83.8	81.3	77.1	73.3	32.0	41.7
9	5.8	5	89.0	100	93.5	100	89.0	100
10	5.6	9	68.2	77.8	60.7	55.6	38.1	40.0
...	...	...	...	...	...	...	...	...
40	17.6	20	86.2	95.0	84.4	89.5	41.5	55.6
χ^2 test								
	$\chi^2=34.74$		$\chi^2=50.52$		$\chi^2=29.14$		$\chi^2=40.50$	
	$\chi^2_{0.05}=55.76$		$\chi^2_{0.05}=55.76$		$\chi^2_{0.05}=55.76$		$\chi^2_{0.05}=55.76$	
	$P>0.05$		$P>0.05$		$P>0.05$		$P>0.05$	

$P>0.05$ indicates no significant difference between expected and observed values

pregnancy after patients underwent five IVF cycles. Therefore, we strongly suggest that these patients consider using donor oocytes or donor sperm to realize their pregnancy after 4 or 5 IVF cycles.

From the point of view of genetics, the reproducibility of a trait consists of its inheritable component and permanent environmental affected component. Therefore, different patient populations under different environmental conditions may have different reproducibility coefficients. In the present study, we estimated the reproducibility coefficients of two patient populations; one in the North America (mainly White, Hispanic and African American patients) and in China (Chinese patients). Although they were two completely different patient populations, the estimated reproducibility coefficients were consistently high for oocyte number and fertilization rate, and middle for maturity and embryo quality. The pregnancy reproducibility was low in both IVF centers. These results support the observation that the number of oocytes retrieved among different patient populations really had a high reproducibility. Due to the determination of inheritance and permanent environment, an individual patient's oocyte numbers have been fixed and will decline throughout life. Human ovarian follicle number is predictable [12]. Animal research also indicates that follicle numbers are highly repeatable within individual animals [13]. These researches indicated that oocyte numbers in an individual are relatively stable with respect to age. Therefore, we may use the number of oocytes retrieved in the first or second cycle to accurately predict subsequent IVF cycle results.

To verify the prediction accuracy, 40 patients were randomly selected from the Arizona Center in 2011 to 2013 to predict their subsequent IVF cycle outcomes for the traits studied. Our results indicate that there was no significant difference between predicted values and actual observed values for these IVF traits. This further supports that these predictions are both accurate and practical. Before oocyte retrieval, the development of the ovarian follicles is monitored by vaginal ultrasound scanning of the ovaries and measurement of serum estradiol [6]. The follicular size, number, and the level of serum estradiol are used routinely by IVF physicians to predict the oocyte yield from patient's ovaries. However, the collected oocyte numbers often differ significantly from the expected values based on follicular numbers and serum estradiol level. For instance, a total of 5 oocytes were collected in the first IVF cycle from a patient in our IVF center. When this patient underwent a second IVF cycle, her ovaries displayed about 22 follicles by ultrasound monitor and her E_2 was more than 2,000 pg/ml estradiol. This patient was expected that about 20 oocytes should be collected. However, based on our reproducibility coefficient, the most probable oocyte numbers should be 5.8 for the second retrieval. In fact, only 6 oocytes were obtained for this patient. This example indicated that the prediction based on reproducibility is more accurate than dependent on the measurement of estradiol and follicle numbers.

It is possible that deviation may occur in our prediction because the reproducibility estimation is based on the standard stimulation protocol for IVF treatment in each IVF center. Thus, the reproducibility coefficients of various IVF centers

may be slightly different based on the individual stimulation protocol. Each IVF center must determine itself reproducibility coefficient of IVF traits before prediction. Also, if the protocol for gonadotrophin administration used for patient treatment has been changed, this reproducibility coefficient cannot be used accurately to estimate the subsequent IVF outcomes. For example, for a patient with few oocytes retrieved or few mature oocytes in the first cycle using the standard gonadotrophin administration, if the amount of gonadotrophin was increased or treatment time was lengthened, it is possible to obtain more matured oocytes in the second IVF cycle. Clearly, oocyte maturity has only middle level reproducibility which is easily affected by drug treatment. Changes of laboratory procedures also affect IVF trait reproducibility estimation and its accuracy. Also, if the failure of the first IVF fertilization was changed to using ICSI for the second cycle, oocyte fertilization for the same patient may show obvious differences during subsequent cycles. Our Shaanxi center data indicated that the average fertilization rate for the first IVF cycles without ICSI was 49.6 %, but on the second cycles with ICSI, the average fertilization became 65 %. It is clear that this data would not be suitable to estimate repeatability coefficients and to predict next IVF cycle outcome.

In conclusion, the introduction of the concept of reproducibility coefficients to our human IVF program allows us to predict the future IVF cycle outcomes. The traits of number of oocytes retrieved and their fertilization rate had high reproducibility, oocyte maturity and top quality embryos had middle level reproducibility, and pregnancy rate had relatively low reproducibility. Therefore, number of oocytes retrieved, maturity and fertilization rate as well as top quality embryo rates may be predicted based on former IVF cycle data, but the prediction of pregnancy rates demonstrated large variation. Based on this study, physicians may consult patients and change patient's stimulation plans for next IVF cycle and laboratory embryologists may further improve their IVF techniques accordingly.

References

1. Adamson GD, de Mouzon J, Lancaster P, Nygren KG, Sullivan E, Zegers-Hochschild F. World collaborative report on in vitro fertilization, 2000. *Fertil Steril*. 2006;85:1586–622.
2. Robertson DM. Predictors of IVF outcome: markers and protocols. *Women's Health*. 2006;2:385–8.
3. Maheshwari A, Bhattacharya S, Johnson NP. Predicting fertility. *Human Fertility*. 2008;11:109–17.
4. Leushuis E, van der Steeg JW, Steures P, Bossuyt PMM, Eijkemans MJC, van der Veen F, et al. Prediction models in reproductive medicine: a critical appraisal. *Human Reproduction Update*. 2009;15(5):537–52.
5. Fisch J, Sher G, Adamowicz M, Keskinetepe L. The graduated embryo score predicts the outcome of assisted reproductive technologies better than a single day 3 evaluation and achieves results associated with blastocyst transfer from day 3 embryo transfer. *Fertil Steril*. 2003;80:1352–2358.
6. Trounson AO, Gardner DK. *Handbook of In Vitro Fertilization* -2nd ed. CRC Press, LLC. New York. 2000; pp 68; p173–174.
7. Carstensen B. Repeatability, Reproducibility and coefficient of variation, in comparing clinical measurement methods: A practical guide, John Wiley & Sons, Ltd, Chichester. UK. 2010. doi:10.1002/9780470683019.ch9.
8. Roman RM, Wilcox CJ, Martin FG. Estimates of repeatability and heritability of productive and reproductive traits in a herd of Jersey cattle. *Genetics and Mol Biol*. 2000;23:113–9.
9. Loughman J. The statistical interpretation of the coefficient of repeatability. *Br J Ophthalmol*. 2010;94:753–6.
10. Soullier N, Bouyer J, Pouly JL, Guibert J, de La Rochebrochard E. Estimating the success of an in vitro fertilization programme using multiple imputation. *Human Reproduction*. 2008;23:187–92.
11. Sullivan E. Single embryo transfer reduces the risk of perinatal mortality in IVF. *Medical Press* 2012; <http://medicalxpress.com/news/2012-07-world-ivf-icsi-babies-total.html>.
12. Westhoff C, Murphy P, Heller D. Predictors of ovarian follicle number. *Fertil Steril*. 2000;74:624–8.
13. Ireland JJ, Ward F, Jimenez-Krassel F, Ireland JLH, Smith GW, Lonergan P, et al. Follicle numbers are highly repeatable within individual animals but are inversely correlated with FSH concentrations and the proportion of good-quality embryos after ovarian stimulation in cattle. *Human Reproduction*. 2007;22:1–9.