The Number of Embryos Available for Transfer Predicts Successful Pregnancy Outcome in Women Over 39 Years With Normal Ovarian Hormonal Reserve Testing¹

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Purpose: The purpose was to determine whether the number of embryos available for transfer following IVF in women over age 39 predicted a successful pregnancy outcome.

Methods: Retrospective analysis of 455 consecutive IVF cycles in women ≥ 40 years of age.

Results: Few cycles were canceled (29/455, 6.4%) or produced no embryos (5/455, 1.1%). Women 40–43 years of age with normal ovarian reserve had a significantly greater delivery rate when ≥ 4 embryos were available for transfer than when < 4 embryos were available (17.8% versus 2.4%, $P = 0.002$). Subsequent IVF cycles, from women with normal FSH whose first cycle produced < 4 embryos, produced delivery rates of 13.0% when ≥ 4 embryos were available. Women with abnormal ovarian reserve or age ≥ 44 years had very low delivery rates (1.2% and 1.4% respectively).

Conclusions: The number of embryos available for transfer significantly predicts delivery from IVF–ET among reproductively older women. Many women age 40–43 with normal ovarian reserve can achieve pregnancy through IVF.

KEY WORDS: age; FSH; in vitro fertilization; ovarian reserve; pregnancy outcome.

INTRODUCTION

The probability of a liveborn pregnancy following infertility treatment declines progressively with advancing female age. Maternal age is one of the most im-

portant factors affecting infertility treatment success. Ovarian hormonal reserve (FSH levels) also correlates inversely with fecundity and directly with spontaneous abortion rates (1–4). Maternal age and ovarian hormonal reserve are at least partially independent predictors of fecundity (3,5). These two factors are among the principal information used to counsel patients regarding their predicted expectation of success from infertility treatments.

Women ≥ 40 years with robust ovarian responses to hormonal stimulation demonstrate reasonable pregnancy rates while poor responders have low fertility rates following infertility treatment (6,7). The ovarian response to gonadotropin stimulation can be quantified by, among other measures, the total number of oocytes retrieved, the number of mature oocytes retrieved, and the number of embryos formed following IVF. The number of embryos transferred represents an important factor correlating with implantation of at least one embryo (6,7).

We hypothesized that the number of embryos available for transfer, as a measure of ovarian response during an IVF treatment cycle, would help predict successful pregnancy outcome when combined with other known predictors. To test this hypothesis, we studied ovarian response, fertilization rates, and pregnancy rates among infertile couples with female partners ≥ 40 years of age who were undergoing IVF.

MATERIALS AND METHODS

Patient Selection

We retrospectively analyzed data collected from 455 consecutive IVF cycles initiated at the Genetics & IVF Institute in 296 women ≥ 40 years of age over

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a 5-year period ending in 1998. Institutional review board approval was obtained to extract data from patient medical records. During pretreatment consultation, each couple was counseled extensively regarding realistic expectations for pregnancy success, considering their individual medical circumstances and, when available, the results of ovarian hormonal reserve testing (FSH levels). Women considered to have a poor prognosis for establishing pregnancy were allowed to proceed into cycle, if they wished to do so after such counseling.

Ovarian Reserve Tests

All couples were encouraged to obtain pretreatment basal and clomiphene-stimulated FSH ovarian hormonal reserve testing. Some couples elected to forego this testing because they intended to attempt IVF regardless of their reproductive prognosis.

Serum FSH levels were measured on cycle day 2–5 and when possible on cycle day 10 after a clomiphene challenge, 100 mg/day (Days 5–9). FSH levels were obtained within 6 months of treatment. FSH tests were measured at the Genetics & IVF Institute or in several other commercial laboratories. Serum FSH measurements vary among assay kits (8), thus all FSH measurements should ideally be performed in the laboratory. Nevertheless, FSH values above 12 mIU/mL are generally associated with a low yield of eggs, poor embryo production, and low pregnancy rates (2,3). Ovarian reserve was categorized as abnormal based on a cutoff of ≥ 12 mIU/mL using the maximum basal or clomiphene-stimulated FSH level.

IVF Cycle Characteristics

Pituitary suppression during IVF cycles was accomplished with gonadotropin releasing hormone agonist (usually leuprolide acetate, TAP Pharmaceuticals, Deerfield, IL) using either a long-term (preceding luteal phase initiation) or short-term (Day 2 cycle initiation) protocol. Ovarian stimulation was accomplished with intramuscular or subcutaneous injection of FSH and rarely with human menopausal gonadotropins. In subsequent cycles in the same patient, the starting FSH dosage was based on the maximum dose used in the preceding cycle and often the gonadotropin releasing hormone agonist suppression was reduced. Oocyte retrieval was scheduled when the lead follicles were at least 18 mm in diameter. Results from conventional IVF and

intracytoplasmic sperm injection (ICSI) cycles were combined.

Cycle Cancellation

Cycle cancellation occurred either when the patient failed to respond to gonadotropin stimulation at 750 IU FSH/day or when the patient chose to terminate the cycle because of a poor ovarian response such as very few follicles. We perform oocyte retrieval for even a single follicle whenever the patient desires to do so, since future IVF cycles for such patients often do not yield larger numbers of follicles.

Embryo Transfer Categories

We recommended transfer of at least four embryos, if available, in women 40 years and older. A receiver operating curve (ROC) analysis of the mature eggs retrieved, fertilization rate, total embryos produced, and the number of embryos transferred versus the liveborn pregnancy rate is depicted in Fig. 1. The area under the ROC curve was greatest for the number of embryos transferred ($.761 \pm .036$) relative to the number of mature eggs ($.673 \pm .043$), the fertilization rate ($.569 \pm .048$), or the total number of embryos ($.720 \pm .046$). We chose a cutoff value of 4 embryos transferred, which maximized the true positive predictions while minimizing the false positive predictions. Therefore, cycle data were analyzed in two groups according to the number of embryos available for transfer, ≥ 4 embryos or 1–3 embryos. Embryo transfer was performed with ultrasound guidance using a Wallace or Frydman catheter, but occasionally using a Tomcat or Norfolk catheter. Over 90% of the transfers occurred 2 days after retrieval.

Pregnancy Outcomes

Pregnancies were classified into two groups and listed as total (at least two rising hCG levels measured at least 11 days after embryo transfer) and delivered (delivery greater than 26 weeks gestation) pregnancies.

Statistical Analysis

We excluded cycles from statistical analysis when a patient requested transfer of three embryos even though four or more were available, there was no response to ovarian stimulation, or no embryos were

Fig. 1. ROC curves of the number of embryos transferred (circles), the total number of embryos (squares), the number of mature oocytes retrieved (triangles), and the fertilization rate (diamonds).

formed from the retrieved oocytes. Spare frozen embryo transfer cycles were also excluded.

Statistical analysis was performed with SPSS statistical software (SPSS, Chicago, IL). ROC analysis, exact chi-square, analysis of variance, and multinomial logistic regression were used. P values <0.05 were considered statistically significant.

RESULTS

Patients

Two hundred ninety-six women, 40 years and older, initiated 455 IVF cycles. Two hundred eighty-two women underwent oocyte retrieval and 421 IVF em-

bryo transfers occurred. Two hundred women initiated only one cycle while 96 women initiated multiple cycles.

Cycles excluded from data analysis are listed in Table I. Sixteen cycles were excluded for transferring only three embryos despite the availability of four or more embryos but 12.5% (2/16) of these cycles resulted in a liveborn baby. Twenty-nine cycles (7.1%) were canceled in 22 women for lack of an ovarian response to high-dose gonadotropin stimulation (750 IU/day). Five cycles (1.1%) progressed to egg retrieval but no viable embryos formed. Nineteen women had some cycles excluded but also succeeded in completing at least one successful cycle that was included for statistical analysis.

Table I. Inclusion and Exclusion Summary

Age group	Patient group	Excluded cycles								Grand totals	
		Included cycles		Transferred 3 embryos when ≥ 4 available		Cycle canceled: No response		No embryos for transfer			
		Patients	Cycles	Patients	Cycles	Patients	Cycles	Patients	Cycles	Patients	Cycles
40–41	Only included cycles	135	191							135	191
	Some included cycles	9	11							9	11
	Only excluded cycles			4	5	3	4	2	1		10
42–43	Only included cycles	83	127	4	4	2	3	1	1	7	8
	Some included cycles	6	9							6	9
	Only excluded cycles			2	2	3	5	1	2		9
44+	Only included cycles	36	62	3	3	5	6	0	0	8	9
	Some included cycles	4	5							4	5
	Only excluded cycles			2	2	3	4	1	1		5
Totals		273	405	15	16	22	29	5	5		
Grand total										296	455

Ovarian Reserve Tests

In the majority of women, only basal FSH testing (198/296, 66.9%) was performed but some women completed a clomiphene challenge test (48/296, 16.2%). Evaluation of ovarian reserve identified 29% (71/246) with abnormal tests while the remaining 71% (175/246) had normal serum FSH values (<12 mIU/mL). Fifty patients (16.9%) declined FSH testing.

Women with normal FSH levels initiated 286 IVF cycles, women with abnormal FSH levels initiated 107 cycles, and women who declined FSH testing initiated 56 cycles. Table II lists the results of ovarian stimulation segregated by ovarian reserve group. Women with normal ovarian reserve produced more mature oocytes ($P < 0.0001$) and embryos ($P < 0.01$) than women with abnormal ovarian reserve.

More cycles were canceled among women with abnormal ovarian reserve (25/107, 23.4%) than among women with normal ovarian reserve (9/285, 3.2%; $P < 0.0001$). The highest FSH level associated with delivery was 17 mIU/mL. Maternal age did not significantly influence the probability of a canceled cycle after correcting for FSH levels.

Pregnancy Outcomes

Table II lists the pregnancy outcomes segregated by age, ovarian reserve (FSH level) group, and number of embryos available for transfer. As expected,

the number of delivered pregnancies was significantly greater in the youngest age group ($P = 0.011$), among women with normal ovarian reserve ($P = 0.035$), and in cycles with at least four embryos transferred ($P = 0.04$). Among all embryo transfer cycles (including some excluded cycles), women ≥ 44 years rarely produced a liveborn baby (1/69, 1.4%).

Cycles from women with normal ovarian reserve had a significantly higher delivery rate with transfer of at least four embryos (18/109, 16.5%) than from cycles with only 1–3 embryos transferred (3/158, 1.9%; $P < 0.0001$). Cycles from women with normal ovarian reserve who produced only 1–3 embryos yielded delivery rates similar to cycles from women with abnormal ovarian reserve (1.9% versus 1.2% respectively). The implantation rates were similar between women transferring four or more embryos and women transferring <4 embryos within each group.

Ninety-four couples underwent 138 additional IVF cycles after the first cycle failed to result in a liveborn infant. The number of embryos available for transfer in the first cycle was highly correlated with the number of embryos available for transfer in subsequent cycles ($P = 0.042$). If the first cycle resulted in <4 embryos for transfer then 72% (58/81) of subsequent cycles also yielded <4 embryos for transfer. When the first cycle resulted in ≥ 4 embryos transferred then 58% (33/57) of subsequent cycles also resulted in ≥ 4 embryos transferred. Women with <4 embryos in the first cycle who later produced ≥ 4 embryos had a delivery rate (13.0%) similar to high embryo producers in the first cycle.

Table II. Pregnancy Outcomes From IVF Cycles^a

Age group	Ovarian reserve test	Embryos transferred	Number of cycles	Total pregnancies	Spontaneous abortions	Delivered pregnancies	Implantation rate (%)
40–41	Normal	1–3	77	9 (11.7%)	6 (66.7%)	3 (3.8%)	4.2
		>4	64	19 (29.7%)**	8 (42.1%)	11 (16.4%)**	4.7
	Abnormal	1–3	25	1 (4.0%)	1 (100.0%)	0 (0.0%)	0.0
		>4	8	1 (12.5%)	1 (100.0%)	0 (0.0%)	1.9
	Declined	1–3	18	6 (36.3%)	4 (66.7%)	2 (13.6%)	10.9
		>4	9	5 (55.6%)	2 (40.0%)	3 (33.3%)	8.9
42–43	Normal	1–3	46	4 (8.7%)	4 (100.0%)	0 (0.0%)	0.0
		>4	37	12 (32.4%)**	5 (41.7%)	7 (18.9%)**	5.5
	Abnormal	1–3	26	8 (30.8%)	7 (87.5%)	1 (3.8%)	4.1
		>4	4	1 (25.0%)	1 (100.0%)	0 (0.0%)	0.0
	Declined	1–3	10	1 (10.0%)	1 (100.0%)	0 (0.0%)	0.0
		>4	13	3 (23.1%)	3 (100.0%)	0 (0.0%)	1.8
44+	Normal	1–3	35	2 (5.7%)	2 (100.0%)	0 (0.0%)	2.9
		>4	8	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.0
	Abnormal	1–3	15	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.0
		>4	4	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.0
	Declined	1–3	3	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.0
		>4	3	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.0

^a 2/16 cycles excluded for transfer of 1–3 embryos when ≥ 4 were available yield a delivered pregnancy (41 and 44 years).

** $P < 0.01$, ≥ 4 versus 1–3 embryos transfer.

Multiple births occurred in only three couples that delivered a baby (10%, 3/30). The three multiple births were all twins despite transferring as many as ten embryos, though rarely were more than 6–7 embryos transferred.

DISCUSSION

Our study demonstrates that women 40–43 years with normal ovarian reserve who produce at least four embryos for transfer from IVF have delivery rates of 15–20% per cycle. To achieve this level of success, we often needed to be aggressive with the number of embryos transferred. We did not compromise patient safety since only 10% of births were multiples and all three were twins. Furthermore, since the implantation rates for cycles with < 4 embryos and ≥ 4 embryos were similar, the improved pregnancy rate is not from better egg quality but rather from an increased probability that at least one of the embryos is normal. This observation supports the policy of transferring more embryos in women of advanced age without significantly increasing risk of multiple births.

Delivery rates of this magnitude make IVF an attractive option for this patient population. Unfortunately, about half of the women with normal ovarian reserve respond poorly to ovarian stimulation, producing < 4 embryos for transfer, and their delivery rates of $< 5\%$ are identical to those of women with abnormal ovarian reserve tests. Unfortunately, we cannot predict into which group an individual patient

will fall before initiating an IVF cycle. Furthermore, a good ovarian response alone is of limited value since 20% of cycles in women with an abnormal FSH also produced at least four embryos, yet none of these cycles resulted in a delivered baby.

The number of embryos produced in an index cycle significantly correlates with the number of embryos produced in subsequent follow-up cycles. Nevertheless, when the initial cycle yielded < 4 embryos, 38% of follow-up cycles yielded four or more embryos with a delivery rate of 13%. Thus, an additional cycle may be reasonably attempted after a single poor response cycle if the couple is willing.

Two previous studies showed that the number of embryos produced correlated with pregnancy rates in women ≥ 40 (6,7). Widra *et al.* (6) reported a delivery rate similar to our data of 21% when at least four embryos were transferred in women ≥ 40 . They did not find any differences in age groups presumably because of their small sample sizes and they did not group cycles according to FSH levels. Roest *et al.* (7) combined all cycles in women ≥ 40 but did not measure FSH levels routinely. They found that good ovarian response cycles yielded higher numbers of oocytes and pregnancy rates compared to poor ovarian response cycles. Our study extends these results by demonstrating that ovarian reserve test results affect the outcome even when the number of embryos produced is high.

Abnormal ovarian reserve testing uniformly predicted a poor pregnancy outcome among the women

included in this study. Several prior studies (2,3,9,10) demonstrated similar prognostic value of both basal and clomiphene-stimulated FSH levels for predicting pregnancy rates. Age alone is a significant predictor of success (3) and our study illustrates this point since only 1% of women delivered a baby if their age was ≥ 44 years.

Women with elevated FSH values demonstrate greater variability among repeated FSH levels than do women with normal FSH levels (11–13). The predictive value of a single FSH level in women over 40 years is likely to be limited to a few months (14,15). Since most women do not routinely undergo repeated FSH testing every few months, including the women in our study, some women will be categorized incorrectly into the normal ovarian reserve group. Thus, a poor ovarian response (1–3 embryos) after normal FSH testing may indicate occult abnormal ovarian reserve.

CONCLUSIONS

An important strength of the present study is the low cycle cancellation rate, which provides a less biased data set upon which to base conclusions. Our study demonstrates that women with abnormal ovarian reserve tests, maternal age >43 years, or normal ovarian reserve tests but whose IVF cycles consistently produce <4 embryos for transfer are unlikely to deliver a child. These patients should strongly consider adoption or donor egg IVF. Still, viable pregnancies occasionally occur in this group of women and for some couples the benefits, even if only psychological, from the opportunity to create their own genetic child outweigh the risks, and expense of an IVF cycle. We believe that couples, in which the female partner is 40–43 years with normal ovarian reserve, should not be discouraged or prevented from attempting IVF with their own gametes.

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