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CLINICAL ARTICLE

Reproductive potential of mature oocytes after conventional ovarian hyperstimulation for in vitro fertilization

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ABSTRACT

Objective: To compare cumulative live birth rate according to the rate of use of metaphase II (MII) oocytes in conventional ovarian stimulation protocols for in vitro fertilization (IVF) or intracytoplasmic sperm injection. *Methods:* In a cohort study, patients aged 18–38 years undergoing their first IVF treatment at one US center were enrolled between February 1, 2009, and August 31, 2013. Ovarian response was categorized by the yield of MII oocytes (low: 1–2; intermediate: 3–6; high: ≥7). The main outcome measure was cumulative live birth rate over a 6-month period. *Results:* Among 250 participants, 3240 oocytes (mean ± SEM 12.96 ± 0.50) were retrieved and there were 152 (60.8%) live births. Overall, 172 (68.8%) participants had a high oocyte yield, 61 (24.4%) an intermediate yield, and 17 (6.8%) a low yield. The cumulative live birth rate was 58.8% (10/17) in the low-yield group, 55.7% (34/61) in the intermediate-yield group, and 62.8% (108/172) in the high-yield group ($P = 0.35$). *Conclusion:* In conventional ovarian stimulation, live birth rate is not affected by the ovarian response. Whether oocytes produced from a low ovarian response are biologically more effective than oocytes obtained from a high ovarian response remains to be determined.

1. Introduction

Conventional in vitro fertilization (IVF) using high doses of gonadotropins has several advantages [1,2], including the production of a high number of oocytes that can be retrieved to allow the formation of more embryos, potentially leading to high rates of pregnancy [2–4]. Outcome measures for IVF treatment commonly include live birth rate per IVF cycle and live birth rate per embryo transfer; however, live birth rate per number of retrieved mature oocytes can provide additional useful information, particularly because use of oocyte cryopreservation is increasingly widespread [5–8].

Oocyte cryopreservation allows women to electively prolong their reproductive life for family planning and provides fertility preservation for patients with cancer [7]. Outcomes after oocyte cryopreservation are good; as a result, the number of centers performing oocyte cryopreservation is growing. Thus, it would be valuable to assess the reproductive efficiency of human oocytes fertilized in vitro on the basis of oocyte yield [9]. In one study of women aged 23–43 years [9], it was reported that the live birth rate per mature oocyte retrieved—the oocyte utilization rate (OUR)—was approximately 5% for women aged 37 years or younger, 3.8% for women aged 38 years, and 0.8% for women aged 43 years.

The aim of the present study was to determine the metaphase II (MII) oocyte efficiency, as represented by cumulative live birth rate, among fairly young women with normal ovarian reserve who underwent conventional ovarian hyperstimulation for IVF with single or double embryo transfer.

2. Materials and methods

In a cohort study, women aged 18–38 years with normal menstrual cycles who underwent a first conventional IVF treatment at the New Hope Fertility Center, New York, NY, USA, between February 1, 2009, and August 31, 2013, were enrolled. The inclusion criteria were infertility due to unexplained, male, and tubal factors. Women with pre-existing medical conditions and those with a body mass index (BMI; calculated as weight in kilograms divided by the square of height in meters) of less than 18.5 or more than 32 were excluded. In addition, participants who had a baseline cycle day-3 follicle-stimulating hormone (FSH) level of 13 mIU/mL or higher were excluded because of potentially diminished ovarian reserve. The study was approved by the Institutional Review Board of New York Downtown Hospital (IRB approval reference no.: JZ-09-08) and the Biomedical Research Alliance of New York (BRANY). Informed consent was obtained from each participant before enrollment.

Conventional ovarian hyperstimulation was performed by the long gonadotropin-releasing hormone (GnRH) agonist protocol using leuprolide acetate (Teva, Sellersville, PA, USA), which was started at

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the mid-luteal phase to achieve ovulation suppression. Ovarian stimulation was achieved with injections of high-dose gonadotropins, including Bravelle and/or Menopur (Ferring, Parsippany, NJ, USA), Follistim (Merck, White House Station, NJ, USA), or Gonal F (EMD Serono, Rockland, MA, USA), at a starting dose of 150–300 IU daily depending on the age and ovarian reserve of the participant. The GnRH agonist was administered daily, along with the gonadotropin injections. During ovarian stimulation, participants were closely monitored by transvaginal ultrasonography and measurements of serum estradiol, progesterone, and luteinizing hormone.

When at least two follicles reached a diameter of 18 mm or greater, oocyte maturation was induced with human chorionic gonadotropins, such as Novarel (Ferring), Pregnyl (Merck), or Ovidrel (EMD Serono). The retrieved oocytes were fertilized by either conventional IVF or intracytoplasmic sperm injection [10]. All embryos were subsequently cultured until the blastocyst stage, and one or two blastocysts were transferred in a fresh cycle. The remaining surplus blastocysts were frozen by vitrification. The frozen embryos were then thawed and subsequently transferred in a natural or artificial cycle with oral Estrace (Actavis Pharma, Parsippany, NJ, USA) within 6 months of oocyte retrieval.

The participants were subdivided into three subgroups according to the yield of mature MII oocytes after oocyte retrieval: "low" represented a yield of 1–2 oocytes, "intermediate" a yield of 3–6 oocytes, and "high" a yield of 7 or more oocytes. Patients who had no oocytes retrieved were excluded from the data analysis.

The main outcome measure was cumulative live birth rate over a 6-month period, which was calculated as the number of births divided by the total number of participants in each subgroup. The likelihood of live birth was expressed as an odds ratio (OR) with 95% confidence intervals (CIs).

Secondary outcomes were OUR, implantation rate, clinical pregnancy rate, number of blastocysts formed, total dose of gonadotropins used per cycle, number of fertilized oocytes, and number of days of stimulation (i.e. number of days during which the participants received gonadotropins). The implantation rate was defined as the number of gestational sacs observed on ultrasonography at 6 weeks of pregnancy divided by the number of blastocysts transferred. A clinical pregnancy was defined as at least one intrauterine sac at 6 weeks of gestation, and live birth was defined as a neonate born after 22 weeks of gestation or weighing at least 500 g. The cumulative clinical pregnancy rate over a 6-month period was calculated as the number of pregnancies divided by the total number of participants in each subgroup. The OUR was calculated as the number of live births over a 6-month period divided by number of MII oocytes produced after only one cycle of ovarian stimulation and oocyte retrieval.

Prism software (GraphPad, San Diego, CA, USA) was used to perform all data analyses. Power analysis indicated that 73 participants in each group would be needed to detect a 20% difference in live birth rate with 80% power and a one-tailed α value of 0.05.

Continuous variables were expressed as mean ± SEM and compared by *t* test or analysis of variance (ANOVA) as appropriate. Categorical variables were expressed as number (percentage) and compared by χ^2 test. Multivariate logistic regression analyses were used to evaluate low, intermediate, and high ovarian responses as independent correlates of a live birth. To estimate the predictive ability of the number of MII oocytes and age (the strongest predictor of a subsequent live birth), receiver operating characteristic (ROC) curves were generated and the area under the ROC curve (AUC) was determined. $P < 0.05$ was considered statistically significant.

3. Results

During the study period, 250 participants enrolled in the study. The demographic and clinical characteristics of the participants are summarized in Table 1. Among all participants, 3240 total oocytes were retrieved (mean ± SEM 12.96 ± 0.50; range 1–43), of which 2525 were MII oocytes (10.1 ± 0.42; range 1–38), yielding a maturity rate of 77.9%. Over a 6-month period, 152 live births occurred, giving a live birth rate of 60.8%. Three patients had no oocytes retrieved.

Table 1
Demographic and baseline characteristics (n = 250).[a]

Characteristic	Value
Age, y	32.02 ± 0.26 (22–38)
Body mass index [b]	24.34 ± 0.19 (18.6–30.7)
Baseline FSH, mIU/mL	8.37 ± 0.15 (3–12)
Primary infertility	122 (48.8)
Nulliparous	200 (80.0)
Ethnic origin	
White	116 (46.4)
Black	58 (23.2)
Hispanic	44 (17.6)
Asian	24 (9.6)
Mixed/other	8 (3.2)
Infertility diagnosis	
Tubal	93 (37.2)
Unexplained	60 (24.0)
Male	43 (17.2)
Mixed male/female	23 (9.2)
Other	31 (12.4)

Abbreviation: FSH, follicle-stimulating hormone.
[a] Values are expressed as mean ± SEM (range) or number (percentage).
[b] Calculated as weight in kilograms divided by the square of height in meters.

Most participants (172/250 [68.8%]) had a yield of seven MII oocytes or more. Only 17 (6.8%) participants had a low ovarian response, and 61 (24.4%) had an intermediate ovarian response. Women who had a high ovarian response produced significantly more fertilized oocytes and more blastocysts than did women who had an intermediate ovarian response ($P < 0.001$) (Table 2). In turn, women who had an intermediate ovarian response produced significantly more fertilized oocytes and more blastocysts than did women who had a low ovarian response ($P < 0.001$) (Table 2).

The implantation rate was highest for women with a low MII oocyte yield, followed by those with an intermediate MII oocyte yield, and then a high MII oocyte yield ($P < 0.001$) (Table 2, Fig. 1). The number of blastocysts transferred was significantly higher among participants with a high ovarian response than among those with an intermediate ovarian response ($P < 0.001$), and significantly higher among participants with an intermediate ovarian response than among those with a low ovarian response ($P < 0.001$) (Table 2). However, the rates of clinical pregnancy and live birth did not differ among the three groups (Table 2). The MII OUR was inversely related to ovarian response: it was 38.5% in the group with low ovarian yield, 12.2% in the intermediate-yield group, and 4.9% in the high-yield group ($P < 0.05$) (Fig. 1).

Among all participants, logistic regression showed that age was a negative predictor for achieving a live birth ($P = 0.018$). In multivariate logistic regression, after adjusting for day-3 FSH, day-3 estradiol, number of days of stimulation, and total dose of gonadotropins used per cycle, the number of MII oocytes retrieved (i.e. low, intermediate, or high) did not affect the likelihood of achieving a live birth (OR 0.99, 95% CI 0.96–1.04; $P = 0.088$) (Table 3).

In ROC curve analysis, the AUC for number of MII oocytes was 0.46 ± 0.02 (95% CI 0.97–1.05; $P = 0.77$), and that for age was 0.61 ± 0.31 (95% CI 0.86–0.98; $P = 0.01$) (Fig. 2). A threshold of eight or more mature oocytes produced the highest likelihood of achieving a live birth (positive likelihood ratio 1.15), but with poor sensitivity (61.5%) and specificity (46.5%).

4. Discussion

The present study has reported the efficiency of MII oocyte yield as reflected by cumulative live birth rate over a 6-month period among fairly young women with normal ovarian reserve who underwent

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Table 2
Clinical data by oocyte yield (n = 250).[a]

Clinical characteristics	1–2 oocytes (n = 17)	3–6 oocytes (n = 61)	≥7 oocytes (n = 172)	*P* value[b]
Total no. of mature oocytes retrieved	26	278	2221	
Age, y	30.65 ± 0.95	32.21 ± 0.48	32.09 ± 0.32	0.31
Day-3 FSH, mIU/mL	8.88 ± 0.49	8.85 ± 0.26	8.15 ± 0.18	0.087
Day-3 estradiol, pg/mL	60.59 ± 4.48	47.90 ± 2.48	52.08 ± 1.67	0.083
Length of stimulation, d	9.94 ± 0.39	10.46 ± 0.23	10.45 ± 0.53	0.060
Total dose of gonadotropins per cycle	2132 ± 104	2217 ± 49[c]	2020 ± 14[c]	<0.001
Fertilized oocytes	1.50 ± 0.12[d,e]	3.77 ± 0.18[c,d]	10.79 ± 0.41[c,e]	<0.001
Blastocysts formed per patient	1.12 ± 0.23[e]	3.19 ± 0.22[c]	7.14 ± 0.34[c,e]	<0.001
Blastocysts transferred per patient	1.01 ± 0.14[d,e]	1.57 ± 0.07[c,d]	1.84 ± 0.028[c,e]	<0.001
Total blastocysts transferred per group	13[d,e]	102[c,d]	429[c,e]	0.001
Implantation	11/13 (84.6)[d,e]	58/102 (56.9)[c,d]	181/429 (42.2)[c,e]	<0.001
Spontaneous abortion	1	23	61	0.17
Cumulative clinical pregnancy[f]	10 (58.8)	35 (57.4)	120 (69.8)	0.17
Cumulative live birth[f,g]	10 (58.8)	34 (55.7)	108 (62.8)	0.35

Abbreviation: FSH, follicle-stimulating hormone.
[a] Values are given as number, mean ± SEM, number (percentage), or number/total blastocysts transferred per group (percentage), unless indicated otherwise.
[b] Analysis of variance was used for continuous variables; χ^2 test for categorical data.
[c] Significant difference for 3–6 oocytes vs ≥7 oocytes.
[d] Significant difference for 1–2 oocytes vs 3–6 oocytes.
[e] Significant difference for 1–2 oocytes vs ≥7 oocytes.
[f] Cumulative rate over a 6-month period after one cycle of oocyte retrieval.
[g] One pending pregnancy in the intermediate group, 10 in the high group.

only one cycle of oocyte retrieval after conventional controlled ovarian hyperstimulation for IVF. For all participants, fresh single or double embryo transfer was carried out, followed by frozen embryo transfer of the remaining cryopreserved supernumerary blastocysts when necessary. The live birth rate was similar between the women who had a high number of MII oocytes and embryos transferred and those who had fewer MII oocytes and fewer embryos transferred. Notably, a yield of eight or more mature oocytes was determined to predict a live birth over a 6-month period, but with poor sensitivity and specificity.

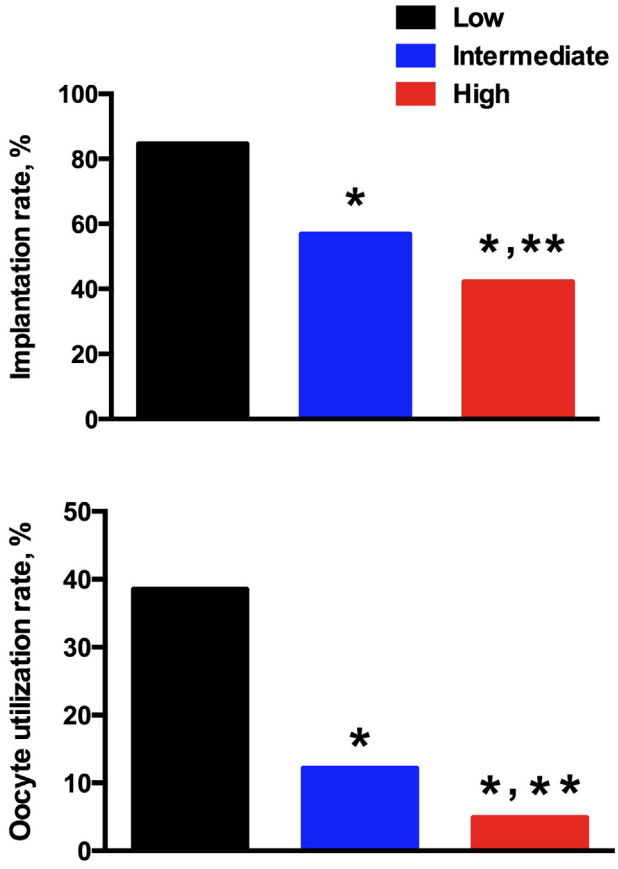

Fig. 1. Implantation rate and oocyte utilization rate during conventional ovarian stimulation for in vitro fertilization, by ovarian response. The single asterisk indicates a significant difference from the low-yield group ($P < 0.05$). The double asterisk indicates a significant difference from the intermediate-yield group ($P < 0.05$).

The present results are consistent with other reports [2,9,11], one of which [9] assessed the efficiency of oocyte utilization among women younger than 38 years according to an oocyte yield of low, intermediate, or high. Similar to the present findings, live birth rates were equivalent across the three groups [9]. Additionally, an apparent difference in the numbers of oocytes required for each live birth was reported: women in the low-yield group used 9.6 oocytes for each live birth, as compared with 25.1 and 51.5 oocytes, respectively, in the intermediate- and high-yield groups. Taken together, these data support the hypothesis that oocyte wastage occurs in cases with a high oocyte yield because women with high oocyte numbers did not have significantly higher live birth rate.

One possible explanation for the inverse relationship between oocyte efficiency and ovarian response might be the negative impact of supraphysiological estrogen levels on the embryo and/or endometrium during fresh embryo transfers [12–15]. This might, in part, explain why IVF centers are increasingly using the "freeze-all" embryo strategy rather than fresh embryo transfer. Several recent studies have shown that the success rates of frozen–thawed embryo transfer are similar to, if not better than, the success rates of fresh embryo transfer [16,17]. Indeed, data collected by the US Centers for Disease Control and Prevention [18] on assisted reproductive technology procedures in all US fertility clinics from 1997 to 2011 showed that the success rates of both fresh and frozen–thawed embryo transfer cycles (donor eggs not included) have increased over the past 14 years for women of all ages. Notably, the increase in success rates seems to be greater for frozen–thawed embryos than for fresh embryos [18]. So far, the etiology of these differences is unknown, although suboptimal endometrial

Table 3
Likelihood of live birth according to the yield of MII oocytes.

MII yield per cycle	OR (95% CI)	*P* value[a]	Live birth rate (%)
Low (1–2 oocytes)	2.0 (0.3–14.2)	0.48	58.8
Intermediate (3–6 oocytes)	0.9 (0.6–1.4)	0.60	55.7
High (≥7 oocytes)	1.0 (0.9–1.1)	0.63	62.8

Abbreviations: MII, metaphase II; OR, odds ratio; CI, confidence interval.
[a] Significance did not change after adjusting for the number of blastocysts transferred.

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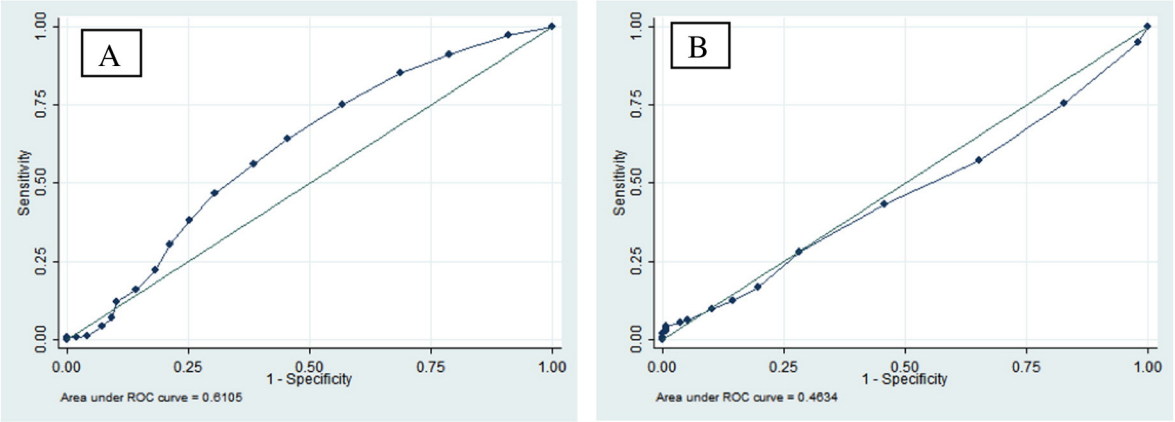

Fig. 2. ROC curves predicting cumulative live birth for (A) age and (B) number of metaphase II oocytes. The area under curve for age was significant ($P = 0.01$), whereas that for number of metaphase II oocytes was not ($P = 0.77$). Abbreviation: ROC, receiver operating characteristic.

development, as mentioned above, has been suggested to be a risk factor for the adverse outcomes of assisted reproductive technology [19].

A meta-analysis has suggested that the number of oocytes needed to achieve ideal implantation rates is dependent on the controlled ovarian hyperstimulation protocol that is used [20]. A yield of five retrieved oocytes was previously associated with a high ongoing pregnancy rate per embryo transferred among patients undergoing mild ovarian stimulation using the GnRH antagonist protocol, whereas a yield of 10 retrieved oocytes was optimal after conventional ovarian hyperstimulation [20]. In agreement, the present findings indicated that a yield of eight or more mature oocytes predicted a live birth over a 6-month period. This provides an additional dimension to the argument that more gonadotropins is not always better, especially when it pertains to conventional ovarian stimulation for IVF [21,22].

There are several limitations to the present study. First, serum anti-Müllerian hormone level and/or antral follicle count were not used to exclude women with diminished ovarian reserve. Instead, the study relied on age and day-3 FSH. Second, women with a wide age range (18–38 years) were included, which might have caused variability in the results; a study sample with a narrower age range would have been ideal. Third, the participants underwent the long GnRH agonist protocol, which several large centers are now abandoning owing to the availability and use of patient-friendly GnRH antagonist protocols, making the data a little less generalizable. Last, the findings cannot be generalized to women with diminished ovarian reserve.

In conclusion, the present study has extended previous findings on conventional IVF by indicating that a low yield of MII oocytes led to a higher OUR without affecting the live birth rate over a 6-month period. Aggressive ovarian stimulation and retrieval of large cohorts of oocytes do not necessarily benefit the patient clinically. Whether gentle ovarian stimulation might offset the oocyte wastage observed in conventional IVF needs to be evaluated in future studies.

Conflict of interest

The authors have no conflicts of interest.

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