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Original Article

Impact of the number of retrieved oocytes on pregnancy outcome in *in vitro* fertilization

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ABSTRACT

Objective: To evaluate the impact of the number of retrieved oocytes on pregnancy outcome.
Materials and Methods: We retrospectively examined the cycles of 2491 women undergoing *in vitro* fertilization therapy at Taipei Medical University (Taipei, Taiwan) from August 1995 to March 2009. We divided them into three groups based on their response rate (where H = high, M = middle, and L = low). We conducted this study to evaluate and compare pregnancy outcome in these three groups.
Results: The total number of retrieved oocytes had a significantly positive correlation with peak E2 levels, and the number of fertilized oocytes, good quality embryos, and available frozen embryos. The number of retrieved oocytes had a positive correlation with pregnancy rates and a negative correlation with fertilization rates. The implantation and abortion rates among the three groups were essentially the same. Compared to the middle and higher responders, the pregnancy rates for lower responders were significantly lower. The pregnancy rates for middle responders and higher responders were not significantly different.
Conclusion: The benefits of more retrieved oocytes between the lower and the middle responders were obvious. However, the benefits and risks for retrieving more oocytes for the middle and the higher responders remained controversial.

Introduction

Ovarian response during controlled ovarian hyperstimulation (COH) is the most important factor for evaluating the pregnancy outcome in assisted reproductive technique. Age and ovarian reserve are both major predictive factors for *in vitro* fertilization (IVF) outcome [1]. The higher responders produced more oocytes with a smaller dose of exogenous gonadotropins. The lower responders required a higher dose of gonadotropins to produce a smaller number of oocytes [2–4]. Furthermore, the higher and lower responders usually registered higher and lower serum E2 levels, respectively [2,4]. The oocytes obtained from various responders should be under different endocrine milieu [5]. We question whether there are any differences between the quality and developmental potential of oocytes obtained from the various responders.

Numerous studies about the number of retrieved oocytes and pregnancy outcome have been published with controversial results [5–10]. While remaining within the margins of safety required to prevent ovarian hyperstimulation syndrome, some authors suggested that the more oocytes obtained, the higher the chance of conception [6,7]. Others proposed that mild ovarian stimulation with a modest number of retrieved oocytes would optimize implantation rates [9].

Understanding the relationship between the number of retrieved oocytes and the quality of oocytes/embryos is critically important when the ovarian stimulation protocol is determined. We conducted this study to determine the number of retrieved oocytes during COH and to evaluate their impact on oocyte/embryo development potential and pregnancy outcome.

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Materials and methods

This study was approved by the Institutional Review Board of Taipei Medical University Hospital, Taipei, Taiwan (101IVF-TMU 01).

We performed a retrospective analysis of IVF-embryo transfer cycles at Taipei Medical University from August 1995 to March 2009. All women were chosen by computer to meet the following criteria: (1) women who were between 20 years old and 42 years old; (2) their IVF cycles had undergone downregulation with leuprolide acetate, followed by ovarian stimulation with exogenous gonadotropins; (3) their IVF cycles had at least one embryo transfer; (4) only the data from first cycles were used; (5) their IVF cycles used fresh nondonor eggs and embryos.

To rank ovarian response, the cycles of a total of 2491 women were arbitrarily classified according to the number of oocytes harvested as middle (M), higher (H), and lower (L) responders. Group M consisted of the middle responders (retrieved oocytes: 5–11, $n = 1298$); group H comprised the higher responders (retrieved oocytes: >11, $n = 579$); and group L consisted of the lower responders (retrieved oocytes: <5, $n = 614$). Oocytes were evaluated by their fertilization rates. Embryos were evaluated by their morphological appearance just prior to the transfer (about 66–72 hours after insemination). Oocyte quality was judged by the implantation rate. In this study, we defined the implantation rate as the number of gestational sacs seen with ultrasound at 6–7 weeks of pregnancy, divided by the number of embryos replaced per transfer [11,12].

IVF protocol

The cycles for all of the women were suppressed with the gonadotropin-releasing hormone agonist. Ovarian stimulation was performed using the gonadotropins available at the time of treatment. The stimulation protocol and starting dose were chosen according to the woman's age and an assessment of her ovarian function. Each woman's cycle was monitored by vaginal ultrasound 6 days after ovarian stimulation. The dosage of follicle-stimulating hormone was adjusted individually (usually in a step-down manner) 7 days after ovarian stimulation for all women. HCG (Profasi; Serono Laboratories, Randolph, MA, USA) 10,000 IU [intramuscularly (i.m.)] was administered when three or more follicles were >16 mm at their largest diameters. Transvaginal follicular aspiration was performed 35–36 hours later. Progesterone supplementation (50 mg/d, i.m.) and/or utrogesterone (micronized progesterone; 100 mg) (Laboratoire Piette International S.A., Beinheim, France) was begun on the day of transfer and was continued until a pregnancy test was performed. If the test was positive, progesterone supplementation was given until Week 10 of the pregnancy. A clinical pregnancy was defined as the presence of a gestational sac by ultrasound at 6–7 weeks. All oocytes were assessed at aspiration for corona/oocyte morphology (corona density, ooplasm color, oocyte shape, and nuclear morphology) and fertilization outcome.

Transfer of the embryos into the uterus was performed approximately 70–72 hours postsperm injection or insemination (Day 3 transfers). Embryos were transferred in a transfer medium consisting of HTF Medium (Irvine Scientific, Santa Ana, CA, USA), supplemented with 10% Plasmanate (Talecris Biotherapeutics, Inc., Research Triangle Park, NC, USA), and loaded into a TDT SET (Laboratoire CCD, Paris, France). The number of embryos to be transferred was determined immediately prior to the transfer based on the quality of the embryo and women's age. The excess embryos were cryopreserved (cleaving stage). Only the results of "fresh" transfers were considered in this study.

Embryo quality grading

The morphological condition (grading) of cleaving embryos was assessed immediately prior to the transfer following the criteria outlined by Veeck [13]. The grading system is as follows, with Grade 1 representing the best morphological condition: Grade 1—preembryo with blastomeres of equal size, no cytoplasmic fragments; Grade 2—preembryo with blastomeres of equal size, minor cytoplasmic fragments or blebs; Grade 3—preembryo with blastomeres of distinctly unequal size, few or no cytoplasmic fragments; Grade 4—preembryo with blastomeres of equal or unequal size, significant cytoplasmic fragmentation; Grade 5—preembryo with few blastomeres of any size, and severe or complete fragmentation. Good embryos are defined as Grade 1 or Grade 2 embryos [12].

Statistical analysis

Statistical analysis was performed using SPSS 13.0 for Windows (SPSS, Inc., Chicago, IL, USA). Data are presented as mean ± standard error. More than two groups' means were compared using one-way analysis of variance *post hoc* range (Dunnett's) tests, with equal variances not assumed (Table 1; Figures 1 and 2). Correlations were performed using Pearson's correlation coefficient. Data are presented as mean ± standard error. A p level < 0.05 was considered statistically significant.

Results

The cycles of 2491 women (average age, 34.4 ± 4.2 years; range, 20–40 years) resulting in pregnancy rates with 30.4% were analyzed. Table 1 shows the causes of infertility and their pregnancy outcome among the 2491 women. Causes of infertility included: endometriosis (34.6%, 863/2491); male infertility (23.23%, 579/2491); tubal factor infertility (19.4%, 483/2491); polycystic ovary syndrome (PCOS) (2.5%, 63/2491); combined multiple infertility causes (11.9%, 296/2491); and, others (8.3%, 207/2491). Women with PCOS had the best pregnancy outcome in this study. Compared to the other causes of infertility, the women with PCOS had significantly higher peak E2 levels (2213 ± 1663 vs. 1311 ± 1049, $p < 0.001$), more retrieved oocytes (15.0 ± 8.5 vs. 8.3 ± 5.1, $p < 0.001$), more good embryos (2.8 ± 2.0 vs. 2.2 ± 1.7, $p < 0.001$), more frozen embryos (4.4 ± 5.5 vs. 0.9 ± 2.4, $p < 0.001$), higher implantation rates (21.5 ± 26.4 vs. 11.6 ± 20.3, $p < 0.001$), and higher pregnancy rates (50.8 ± 50.4 vs. 29.9 ± 45.8, $p = 0.003$). However, the women with PCOS had a significantly lower percentage of good embryos in fertilized eggs (35.6 ± 24.1 vs. 47.2 ± 33.1, $p = 0.006$), and a higher percentage of severe ovarian hyperstimulation syndrome [4.8% (3/63) vs. 0.8% (20/2428), $p = 0.001$].

In the analysis of correlations for all 2491 cycles, the total number of retrieved oocytes had a significantly positive correlation with the peak estradiol level ($\gamma = 0.559$, $p < 0.001$), as well as the number of fertilized oocytes ($\gamma = 0.834$, $p < 0.001$), good embryos ($\gamma = 0.329$, $p < 0.001$), and available frozen embryos ($\gamma = 0.617$, $p < 0.001$). The number of retrieved oocytes had a positive correlation with pregnancy rates ($\gamma = 0.120$, $p < 0.001$), and a significantly negative correlation with fertilization rates ($\gamma = -0.236$, $p < 0.001$). The number of retrieved oocytes also had a positive correlation with implantation ($\gamma = 0.076$, $p < 0.001$), and a significantly negative correlation with the percentage of good embryos ($\gamma = -0.269$, $p < 0.001$).

Table 2 shows the ovulation and pregnancy outcome according to the ovarian responses of the women. There was no significant difference in average age among the higher (H), middle (M), and lower (L) responders. The parameters for comparison of the groups

Table 1
Pregnancy outcome classified by indications of infertility.

	Total	Endometriosis	Male	Tubal	PCOS	Combined	Others	*p*
Cases	2491	863	579	483	63	296	207	
Age (y)	34.4 ± 0.08	34.5 ± 0.14	34.1 ± 0.17	34.4 ± 0.20	34.4 ± 0.53	34.3 ± 0.24	34.7 ± 0.29	0.547
Range of oocyte number	2–40	2–31	2–34	2–35	3–38	2–40	2–34	<0.001
Duration of infertility	3.8 ± 0.06	3.9 ± 0.10	3.6 ± 0.11	3.7 ± 0.12	3.2 ± 0.26	3.8 ± 0.16	3.8 ± 0.20	0.265
Oocytes no.	8.5 ± 0.11	7.5 ± 0.15	8.6 ± 0.21	9.3 ± 0.25	15.0 ± 1.07	8.9 ± 0.34	8.0 ± 0.37	<0.001
Peak estradiol	1334.2 ± 22.0	1166.3 ± 31.0	1420.2 ± 49.3	1412.7 ± 51.0	2212.5 ± 216.6	1406.6 ± 69.7	1270.0 ± 70.6	<0.001
Fertilization rates (%)	66.6	68.1	61.3	70.9	64.7	63.1	70.5	<0.001
Implantation rates (%)	11.9	12.9	10.1	11.1	21.5	14.0	8.7	<0.001
Pregnancy rates (%)	30.4	32.7	25.9	29.6	50.8	35.8	21.7	<0.001
Abortion rates (%)	23.0	19.9	30.0	20.3	18.8	25.6	28.9	0.183
Live birth rates (%)	23.4	26.2	18.1	23.6	41.3	27.4	15.5	<0.001
No. of embryo transfer	3.2 ± 0.02	3.1 ± 0.04	3.2 ± 0.05	3.2 ± 0.05	3.5 ± 0.13	3.2 ± 0.07	3.0 ± 0.08	0.011
No. of fertilized egg	5.4 ± 0.07	4.9 ± 0.10	5.0 ± 0.13	6.3 ± 0.17	9.5 ± 0.76	5.4 ± 0.22	5.3 ± 0.02	<0.001
No. of good embryo	2.2 ± 0.04	2.0 ± 0.05	2.1 ± 0.08	2.3 ± 0.25	2.8 ± 0.11	2.4 ± 0.12	2.4 ± 0.12	0.079
% of good embryo in fertilized egg	46.9	47.3	46.9	42.0	35.6	51.7	53.3	0.088
No. of frozen embryo	1.0 ± 0.07	0.7 ± 0.09	0.7 ± 0.14	1.6 ± 0.70	4.4 ± 0.16	1.0 ± 0.19	1.1 ± 0.19	<0.001

Data are presented as mean ± standard error.
PCOS = polycystic ovary syndrome.

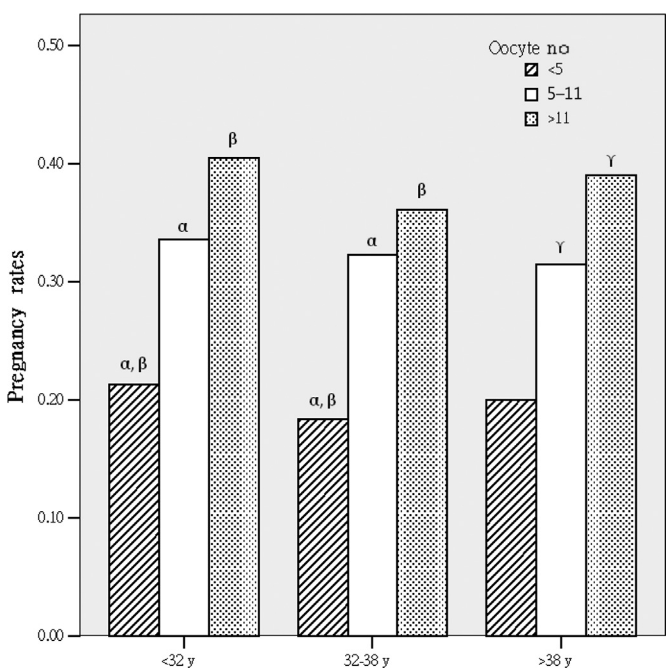

Figure 1. Pregnancy rates in high, middle, and low responders classified by young (<32 years), middle-aged (32–38 years) and older (≥39 years) women. α = L vs. M <0.05; β = L vs. H <0.05; ϒ = M vs, H<0.05.

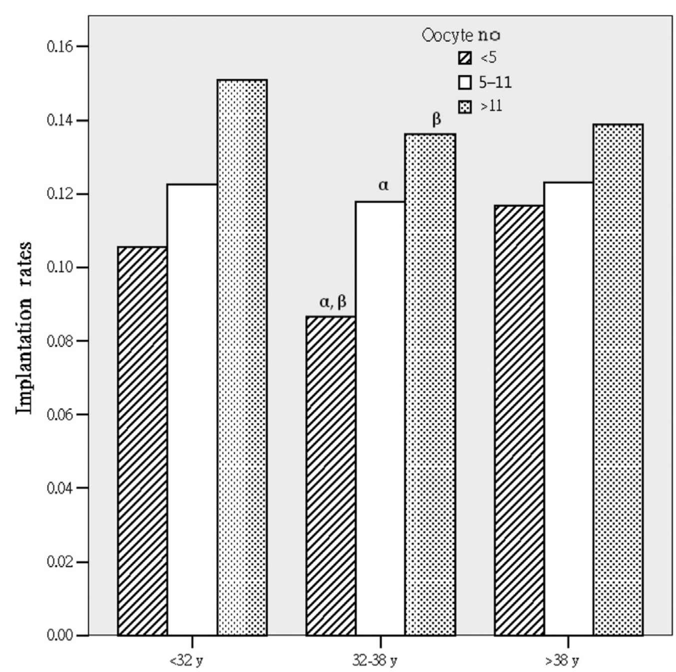

Figure 2. Implantation rates in high, middle, and low responders classified by young (<32 years), middle (32–38 years) and older (≥39 years) women. α = L vs. M < 0.05; β = L vs. H < 0.05.

included peak estradiol level, number of fertilized eggs, number of good embryos, number of frozen embryos, fertilization rates, percentage of good embryos, and percentage of fertilized eggs transferred, which show a strong relationship as (H vs. M), (H vs. L), and (M vs. L) are all significantly different. Lower responders had significantly lower pregnancy rates than middle or higher responders. However, the implantation rates and abortion rates were not significantly different among the three groups.

An important aspect of this study was evaluating the results from aging oocytes, so the ovarian responders were subgrouped according to age. The younger subgroup (<32 years), the middle-aged subgroup (32–38 years), and the older subgroup (≥39 years) were analyzed in each group. Figure 1 shows the mean of pregnancy rates among the lower, middle, and higher responders in each different age group. The higher responders had significantly higher pregnancy rates than the lower responders among the younger women (<32 years, 40.5 ± 49.3 vs. 21.3 ± 41.1, $p = 0.001$), middle-aged women (32–38 years, 36.1 ± 48.1 vs. 18.4.3 ± 38.8, $p < 0.001$), and older women (>38 years, 39.0 ± 49.0 vs. 20.0 ± 40.2, $p = 0.005$). Middle responders also had significantly higher pregnancy rates than the lower responders among the younger women (<32 years, 33.5 ± 47.3 vs. 21.3 ± 41.1, $p = 0.011$) and middle-aged women (32–38 years, 32.3 ± 46.8 vs. 18.4.3 ± 38.8, $p < 0.001$).

As for the implantation rates, Figure 2 shows that the only significant difference was found in middle-aged women (32–38 years). The lower responders among the middle-aged women had significantly lower implantation rates than the middle responders (8.7 ± 20.0 vs. 11.8 ± 19.6, $p = 0.045$) and the higher responders (8.7 ± 20.0 vs. 13.6 ± 20.9, $p = 0.005$). Although the implantation rates among the lower, middle, and higher responders were not

Table 2
Pregnancy outcome in lower (L), middle (M), and higher (H) responders.

	Total	Lower responders	Middle responders	Higher responders
Group		L	M	H
Cases	2491	614	1298	579
Age (y)	34.4 ± 0.1	34.3 ± 0.2	34.3 ± 0.1	34.7 ± 0.2
Range of oocyte number	2–40	2–4	5–11	12–40
Duration of infertility	3.8 ± 0.1	3.9 ± 0.1	3.7 ± 0.1	3.8 ± 0.1
Oocytes no.	8.5 ± 0.1	3.1 ± 0.1[a,b]	7.7 ± 0.1[a,c]	16.1 ± 0.1[b,c]
Peak estradiol	1334.2 ± 22.0	685.2 ± 18.8[a,b]	1243.1 ± 23.3[a,c]	2218.3 ± 57.9[b,c]
Fertilization rates (%)	66.6 ± 0.4	76.5 ± 1.0[a,b]	64.9 ± 0.6[a,c]	59.8 ± 0.8[b,c]
Implantation rates (%)	11.9 ± 0.4	9.7 ± 0.9	12.0 ± 0.6	14.0 ± 0.9
Pregnancy rates (%)	30.4 ± 0.1	19.4 ± 1.6[a,b]	32.4 ± 1.3[a]	37.7 ± 2.0[b]
Abortion rates (%)	23.0 ± 1.5	23.5 ± 3.9	21.6 ± 2.0	25.2 ± 2.9
Live birth rates (%)	23.4 ± 8.5	14.8 ± 1.4[a,b]	25.4 ± 1.2[a]	28.2 ± 1.9[b]
No. of embryo transfer	3.2 ± 0.02	2.3 ± 0.04[a,b]	3.5 ± 0.03[a]	3.5 ± 0.04[b]
No. of fertilized egg	5.4 ± 0.08	2.3 ± 0.04[a,b]	4.9 ± 0.05[a,c]	9.6 ± 0.17[b,c]
No. of good embryo	2.2 ± 0.04	1.3 ± 0.04[a,b]	2.2 ± 0.04[a,c]	3.1 ± 0.10[b,c]
% of good embryo in fertilized egg	46.9 ± 6.6	57.3 ± 1.6[a,b]	47.3 ± 8.4[a,c]	34.9 ± 1.1[b,c]
% of fertilized egg transferred	74.1 ± 5.7	97.0 ± 0.4[a,b]	76.7 ± 0.7[a,c]	44.1 ± 1.0[b,c]
No. of frozen embryo	1.0 ± 0.05	0.0 ± 0.01[a,b]	0.5 ± 0.04[a,c]	3.4 ± 0.18[b,c]
Ovarian hyperstimulation case and %	23 (0.9%)	2 (0.3%)	4 (0.3%)[c]	17 (2.9%)[c]

Data are presented as mean ± standard error.
[a] L versus M < 0.05.
[b] L versus H < 0.05.
[c] M versus H < 0.05.

significantly different among the younger and the older women, the trend of increasing implantation rates from lower to middle to higher responders was very obvious.

Discussion

Increasing pregnancy rates and decreasing complication rates are the primary goals for controlled ovarian stimulation. Enhanced oocyte recovery resulted from improvements in the technique of COH. Our study shows that the total number of retrieved oocytes has a significantly positive correlation with the peak estradiol level, along with the number of fertilized oocytes, good embryos, and available frozen embryos. The number of retrieved oocytes has a positive correlation with pregnancy rates and implantation rates. The results supported the idea of the pregnancy rate increasing with an increase in the number of retrieved oocytes [6,7]. Although higher counts of oocytes obtained from ovarian stimulation were accompanied by lower fertilization rates, there still were more fertilized oocytes, good embryos, and frozen embryos available among the higher responders. Our results confirmed those of Kok et al [14], who reported that although the oocytes from high responders contain a greater fraction of immature oocytes, the pregnancy outcome is not impaired. Actually, our results showed that higher responders had a trend of better implantation rates in all age groups, which may be effected by the number of available embryo among various responders. According to their ovarian responses, the higher responders produced significantly more embryos available for selection and transfer. The lower responders transferred almost all of their available cleavage embryos without selection. Only about 44% of the cleavage embryos were transferred among the higher responders compared with 97% of the cleavage embryos being transferred among the lower responders in our study. It is not surprising, therefore, that the embryo transfers in the higher response group had a tendency toward higher implantation rates. Our results confirmed those Yih et al [6], who reported that higher clinical pregnancy rates were seen in those women who had more oocytes retrieved, regardless of age and ovarian reserve. The age-related decline in the success of IVF is largely attributable to a progressive decline in ovarian oocyte quality and quantity [15]. The women in our study presented with similar average ages in the lower, middle, and higher responders, proving that ovarian response, in an age-independent fashion, is highly predictive of IVF outcome [6].

The lower responders had significantly lower pregnancy and implantation rates, confirming that lower response is a valid predictor of poor outcome [8]. Retrieving more oocytes provides a clear benefit to the middle responders in contrast to the lower responders. Without significant risk, the middle responders recorded significantly higher pregnancy rates and more available embryos. Although some low responses may have resulted from decreasing the ovarian pool of growing follicles [16], the problem continues to be finding ways to recruit all of the low responder's available oocytes. Modified stimulation protocol or the use of high-dose exogenous gonadotropins may prove helpful to some women [17,18]. However, this study could not determine whether, for an initially lower response woman, increasing the gonadotropin dose would improve the ovarian response and lead to a better pregnancy outcome [8–10]. Compared to the middle responders, the higher responders had a significantly higher risk for severe ovarian hyperstimulation syndrome without significantly improved pregnancy rates. However, the higher responders had more frozen embryos that could be transferred in the future, thereby increasing their cumulative pregnancy rates.

While investigating the impact of high estrogen levels on embryo implantation rates, some studies have suggested that COH leads to reduced implantation rates [5,19,20]. Others have suggested that exposure of the developing endometrium to COH during IVF cycles does not inhibit embryo implantation or affect the pregnancy, delivery [4,21], or even the high implantation rates [22]. Our study demonstrated the positive correlation between the number of retrieved oocytes, the estradiol levels, and the implantation rates. We did not find that the quality of oocytes obtained from the high responders was lower than the oocytes obtained from the middle or lower responders. Although we could suspect an alteration in uterine receptivity in the high responders [20], the implantation rates were not significantly different in the lower, middle, and higher responders.

As for the fertilization rates in the groups with higher numbers of retrieved oocytes, our results confirmed the findings of previous reports that the fertilization rates in higher responders were lower than those of normal responders [14,22,23]. However, the implantation rates of the higher responders were not impaired. The low fertilization rates in the high responders should result from a high count of immature oocytes, but not from the quality of the mature oocytes. This finding can be explained by Tarín and Pellicer's [24] suggestion that lower fertilization in the high oocytes retrieved group is the consequence of higher cytoplasmic immaturity. Our results confirmed previous studies that reported that live birth rate increased steadily with the number of retrieved oocytes up to about 15 oocytes; however, the benefit and risk of retrieval of more than 15 oocytes should be questioned [25,26].

Age is one of the important independent factors affecting the ability of embryos to implant [11,12]. Aging of the ovary and low responders coincided in many situations [16]. However, our study showed a tendency toward higher pregnancy and implantation rates for women who retrieved more oocytes regardless of whether they were in the younger, middle, or older age group.

Generally speaking, our results showed that the more oocytes retrieved, the better the pregnancy outcome. Without increased risk, the middle responders especially accrued benefits from more retrieved oocytes in comparison to the lower responders. However, controversy continues to exist for the middle responders and the higher responders regarding the risks and benefits of retrieving higher numbers of oocytes.

These findings highlight the importance of not relying solely on age when presenting and discussing IVF outcome data. They also provide useful information for helping patients interpret their IVF cycle response [6]. To predict pregnancy outcome, as discussed above, the more oocytes retrieved, the more cleavage embryos are available for use, and the higher the cumulative pregnancy rate. However, the aim of this study is to evaluate the correlation between the number of oocyte retrieved and pregnancy outcome. This study did not consider the patient's underlying disease and stimulation protocol. It should be the weakness of this study.

To summarize, we suggest the following: (1) the smaller the number of retrieved oocytes during COH, the poorer the pregnancy outcome; (2) the implantation rates of embryos obtained from high or low responders are comparable to the oocytes of middle responders; and (3) middle responders especially received more benefits from retrieving more oocytes than the lower responders. However, the benefits of retrieving more oocytes from the middle and higher responders remain controversial.

Conflicts of interest

The authors have no conflicts of interest relevant to this article..

Author contributions

Ming-I Hsu participated in the study conception and design, and analysis and interpretation of data, Chii-Ruey Tzeng participated in the conception and design of the study, and Chia-Woei Wang and Chi-Huang Chen were involved in the acquisition of data.

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