Cumulative live birth rates do not increase after 4 complete cycles in women with poor ovarian response: a retrospective study of 1,825 patients

Meng Wang, M.D., Lei Jia, M.S., Xiao-Lan Li, M.D., Jia-Yi Guo, B.S., Cong Fang, Ph.D., Rui Huang, Ph.D., and Xiao-Yan Liang, Ph.D.

From the Reproductive Medicine Center, The Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, China.

Objective: To investigate whether the cumulative clinical pregnancy rates (CCPR) and cumulative live birth rates (CLBR) increase as the oocyte retrieval cycle increases in women with poor ovarian response.
Design: Retrospective cohort study.
Setting: Not applicable.
Patient(s): Women diagnosed of poor ovarian response (POR) according to the Bologna criteria and who completed in vitro fertilization or intracytoplasmic sperm injection cycles between January 2014 and December 2018.
Intervention(s): Not applicable.
Main Outcome Measure(s): The conservative and optimistic estimations of CCPR and CLBR.
Result(s): The conservative and optimistic estimates of CCPR peaked at the 6th complete cycle, reaching 36.44% and 71.61%, respectively. However, the conservative and optimistic estimates of CLBR peaked at the 4th complete cycle, reaching 20.22% and 38.31%, respectively. The live birth rate per complete cycle of mild stimulation protocol was comparable to other protocols after adjusting for the confounding factors. For patients $\leq$35 years, the live birth rate per complete cycle of progestin-primed ovarian stimulation (adjusted odds ratio = 0.51, 95% confidence interval: 0.30–0.87) and gonadotropin-releasing hormone antagonist protocol (adjusted odds ratio=0.45, 95% confidence interval: 0.24–0.81) were significantly lower than that of the mild stimulation.
Conclusion(s): It is not advisable to initiate more than four complete cycles for POR patients since CLBR do not increase after that. For POR patients $\leq$35 years, the live birth rate per complete cycle increased in women with mild stimulation protocol. (Fertil Steril Rep® 2021;2:201–8. ©2021 by American Society for Reproductive Medicine.)
Key Words: Controlled ovarian stimulation protocols, cumulative clinical pregnancy rates, cumulative live birth rates, poor ovarian response

Poor ovarian response (POR) is defined as the failure of the ovary to respond to the standard stimulation protocol, leading to an inadequate retrieval of oocytes from patients (1). Thus, POR patients have lower clinical pregnancy rates and live birth rates following in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) (2).

Clinical pregnancy rates and live birth rates were previously used as outcome parameters for IVF and ICSI, calculated as the probability of achieving pregnancy or live birth in one embryo transfer cycle. But such parameters are inadequate to evaluate the success rates of one oocyte retrieval cycle, given the fact that the embryos may have been cryopreserved and transferred to the uterus in multiple cycles. Cumulative clinical pregnancy rates (CCPR) and cumulative live birth rates (CLBR), which indicate the likelihood of achieving pregnancy and live birth per complete cycle (in which all the embryos from one retrieval cycle are transferred, or at least one live birth has been achieved), are considered as all-inclusive parameters (3). Previous studies of CLBR in women with POR undergoing IVF/ICSI have been inconsistent (4–6), varying from 14.9% to

Received September 11, 2020; revised January 12, 2021; accepted January 24, 2021.
Disclosures: M.W. has nothing to disclose. L.J. has nothing to disclose. X-L.L. has nothing to disclose. J-Y.G. has nothing to disclose. C.F. has nothing to disclose. R.H. has nothing to disclose. X-Y. L. has nothing to disclose.
Supported by the National Key Research and Development Program of China (2017YFC1001601).
Reprint requests: Xiao-Yan Liang, Ph.D., Reproductive Medicine Center, The Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou 510655, China (E-mail: liangxy2@mail.sysu.edu.cn).

Fertil Steril Rep® Vol. 2, No. 2, June 2021 2666-3341

https://doi.org/10.1016/j.xfre.2021.01.004

31.9% after 4–6 complete cycles. However, CCPR has not been investigated in POR patients.

There is little consensus on the impact of controlled ovarian stimulation protocols on the live birth rates of women with POR. A meta-analysis suggested that no one protocol was superior to the others (7). Other studies suggest that the natural cycle protocol is associated with the lowest live birth rate among all the protocols, and that the luteal phase ovarian stimulation and gonadotropin-releasing hormone antagonist (GnRH-ant) protocols are superior to the GnRH agonist (GnRHa) long protocols (4). However, few studies have examined the effects of the controlled ovarian stimulation protocols on patients of different age groups.

Based on current research, it is not clear whether increasing oocyte retrieval cycles will increase the likelihood of some patients to achieve live births. We hypothesize that after several complete cycles, additional egg retrieval will not benefit the patients due to increasing age and the potential damage brought by repeated ovarian stimulations. Furthermore, mild stimulation protocol may result in higher CLBRs by using a lower dose of gonadotropin.

Therefore, this study investigated the CLBR and CCPR in a POR cohort with different age groups. Clinical pregnancy rates per complete cycle, live birth rates per complete cycle were analyzed and their compared with live birth rates per complete cycle among different controlled ovarian stimulation protocols.

MATERIALS AND METHODS

Study Design and Participants

This was a retrospective, single-center cohort study. It was approved by the Ethics Committee of the Sixth Affiliated Hospital of Sun Yat-sen University (Approval no.: 2017ZSLYEC-0165). We included patients who were diagnosed of POR according to the Bologna criteria [8] and were admitted to the Reproductive Medicine Center of the Sixth Affiliated Hospital of Sun Yat-sen University from January 2014 to December 2018 to receive IVF/ICSI. The exclusion criteria were: (I) having some cryopreserved embryos from one oocyte retrieval left while a live birth was yet to be achieved; (II) cases involving oocyte thawing, donation, or preimplantation genetic testing. All the patients had been followed up by telephone until April 2019. Also, cycles with missing values for any of the analyzed predictors were excluded. Only the first clinical pregnancy was considered if more than one clinical pregnancy was achieved in a complete cycle. None of the participants achieved more than one live birth. Patients were grouped according to their age at the time of the first oocyte retrieval cycle: Group 1, $\leq$35 years; Group 2, 36–40 years; Group 3, 41–43 years; Group 4, $\geq$44 years. The CCPR, CLBR, and live birth rates per a complete cycle were calculated and compared among the groups. Within each group, the effects of controlled ovarian stimulation protocols on LBR were evaluated.

Controlled Ovarian Stimulation Protocols

The controlled ovarian stimulation protocols were performed as previously reported (8). For mild stimulation, an oral medication (clomiphene 50–100 mg/day and letrozole 2.5–5 mg/day) or an injectable medication (gonadotropin $\leq$150 IU/day) without an antagonist was administered from the 2th or 3rd day of menstrual cycle continuously until the trigger period. Alternatively, the drugs were administered for five consecutive days from cycle day 2–3, after which no medication was taken until the trigger period. The progestin-primed ovarian stimulation protocol (PPOS) included luteal phase ovarian stimulation (when 150–300 IU/day of gonadotropin was injected subcutaneously from the 1st-3rd day of menstrual cycle after oulation until the trigger day) and follicular phase regimen (when 150–300 IU/day of gonadotropin with 10 mg/day of medroxyprogesterone acetate or 0.1g/day of progesterone was administered between the 2nd and 3rd day of menstrual cycle until the trigger day); GnRH-ant protocol (a fixed protocol was adopted with 150–300 IU/day of gonadotropin being administered from either day 2 or day 3 of the menstrual cycle to the trigger day. Six days later, 0.25 mg/day of GnRH-ant was added until the trigger); GnRHa long protocol (GnRHa was administered on the 20th day of the menstrual cycle for about 14 days for pituitary down-regulation. Once the serum LH $<$ 5 IU/L, E2$<$50 pg/ml, and the endometrium thickness $<$ 5 mm, gonadotropin was added until the trigger day); modified long protocol (similar to the GnRHa long protocol, except that the short-acting GnRHa was stopped after 14 days), natural cycle, and others (including the modified natural cycle [no gonadotropin was administered, human chorionic gonadotropin was administered for the trigger when the dominant follicles reached 18 mm in diameter], and short protocol [0.1 mg/day short-acting GnRHa and 150–300 IU/day gonadotropin were administered simultaneously on the 2nd and 3rd day of the menstrual cycle until the trigger]).

When at least two follicles reached 18 mm in diameter, 4000–10000 IU human chorionic gonadotropin was administered for triggering. The follicles were aspirated after 36 hours. The oocytes were fertilized by IVF or ICSI, depending on the quality of the sperm cells. On day 3, the embryos were evaluated by Scott's criteria (9): grades I and II with $\geq$4 cells were defined as transferable embryos; with $\geq$6 cells being of good quality. For embryos transferred in fresh cycles, the day 3 embryos accounted for the majority and the blastocysts accounted for the minority. Surplus embryos were vitrified on day 3 or day 5-6 as determined by the quality and quantity of embryos and patients' preferences (10).

For frozen-thawed embryo transfer cycles (FET), hormone replacement treatment or natural cycle was adopted for endometrium preparation. Luteal support continued to 10 gestational weeks if clinical pregnancy was confirmed by a visible gestational sac through transvaginal ultrasound. Live birth was defined as at least a live-born infant after 22 gestational weeks. Twins or multiple births in one parturition were regarded as one live birth.

Outcome Measures

The primary outcomes were the conservative and optimistic estimates of CLBR of women with POR. The secondary outcomes were the conservative and optimistic estimates of CCPR, clinical pregnancy rates per complete cycle, live birth

TABLE 1

Characteristics of patients during the initiating cycle in the overall and separate age groups.

Characteristics	All	Group 1 ≤35 years	Group 2 36–40 years	Group 3 41–43 years	Group 4 ≥44 years	*p*
Number of cycles	2773	573	788	916	496	
Age (years)	41 (36–43)	32 (30-34)	39 (37-40) [a]	42 (41-43) [a,b]	45 (44-46) [a,b,c]	**<0.01**
BMI (kg/m^2)	22.6 (20.8-24.6)	21.5 (19.9-23.4)	22.4 (20.8-24.5) [a]	23.0 (21.3-24.8) [a,b]	23.2 (21.5-25.2) [a,b]	**<0.01**
Type of infertility			[a]	[a,b]	[a,b]	**<0.01**
Primary infertility	817 (29.4%)	356 (62.1%)	253 (32.1%)	133 (14.5%)	75 (15.1%)	
Secondary infertility	1956 (70.6%)	217 (37.9%)	535 (67.9%)	783 (85.5%)	421 (84.9%)	
Duration of infertility (years)	4 (2-7)	4 (2-6)	4 (2-8) [a]	3 (1-6) [a,b]	3 (2-6) [a,b]	**<0.01**
Infertility factors				[a,b]	[a,b,c]	**<0.01**
Male factor	101 (3.6%)–	25 (4.4%)	39 (4.9%)	25 (2.7%)	12 (2.4%)	
Diminished ovarian reserve	2443 (88.1%)	489 (85.3%)	660 (83.8%)	832 (90.8%)	462 (93.1%)	
Others	229 (8.3%)	59 (10.3%)	89 (11.3%)	59 (6.4%)	22 (4.4%)	
Baseline FSH (IU/L)	9.1 (7.0–11.7)	9.2 (6.8–11.8)	9.1 (7.2–11.6)	8.8 (7.0–11.3)	9.4 (7.0–12.0)	0.15
Baseline LH (IU/L)	4.7 (3.6–5.6)	4.5 (3.2–5.1)	4.8 (3.4–5.5)	4.6 (3.4–5.7)	4.8 (3.4–5.8)	0.08
Baseline estradiol (ng/L)	47.2 (30.2–57.6)	49.5 (30.9–58.7)	49.8 (32.3–57.1)	46.2 (29.8–58.3)	41.3 (28.2–56.3)	0.07
AMH (ng/ml)	0.6 (0.4–0.9)	0.7 (0.4–0.9)	0.6 (0.4–1.0)	0.6 (0.4–0.9)	0.6 (0.3–0.9) [b]	**<0.01**
AFC	4 (3–6)	4 (3–6)	5 (3–6) [a]	4 (3–6) [a,b]	3 (2–5) [a,b,c]	**<0.01**
Total dose of gonadotropin (IU)	1350 (900–2100)	1350 (900–2025)	1500 (900–2250) [a]	1500 (975–2250) [a]	1200 (750–2025) [b,c]	**<0.01**
Duration of gonadotropin mediation (days)	8 (6–10)	8 (6–9)	8 (6–10)	8 (6–9)	7 (5–9) [b,c]	**<0.01**
Controlled ovarian stimulation protocols				[a]	[b,c]	**<0.01**
Mild stimulation	804 (29.0%)	184 (32.1%)	237 (30.1%)	241 (26.3%)	142 (28.6%)	
PPOS	762 (27.5%)	157 (27.4%)	194 (24.6%)	267 (29.1%)	144 (29.0%)	
GnRH-ant protocol	691 (24.9%)	145 (25.3%)	192 (24.4%)	234 (25.5%)	120 (24.2%)	
Modified long protocol	172 (6.2%)	16 (2.8%)	53 (6.7%)	79 (8.6%)	24 (4.8%)	
Natural cycle	99 (3.6%)	21 (3.7%)	25 (3.2%)	22 (2.4%)	31 (6.2%)	
GnRHa long protocol	54 (1.9%)	8 (1.4%)	25 (3.2%)	17 (1.9%)	4 (0.8%)	
Others	191 (6.9%)	42 (7.3%)	62 (7.9%)	56 (6.1%)	32 (6.4%)	
Oocytes retrieved	3 (2–4)	3 (2–4)	3 (2–4)	3 (2–4)	2 (1–4) [a,b,c]	**<0.01**
Method of fertilization						0.41
IVF	2151 (77.5%)	451 (79.0%)	602 (76.4%)	702 (76.5%)	395 (79.5%)	
ICSI	622 (22.5%)	120 (21.0%)	186 (23.6%)	215 (23.4%)	102 (20.5%)	
Transferable embryos	2 (1–2)	2 (1–2)	2 (1–2)	2 (1–3)	1 (1–2) [b,c]	**<0.01**
Good quality embryos	1 (1–2)	1 (1–2)	1 (1–2)	1 (1–2)	1 (1–2) [c]	**0.03**

Bold font indicates statistical significance with a *P* value <0.05. Continuous variables are presented as median (interquartile range) and compared by Kruskal-Wallis H test. Categorical variables are presented as counts (percentage in each study group) and compared by the $\chi 2$ test. Bonferroni-adjusted *P* value of 0.0083 (6 tests in total) was set as the threshold in multiple comparisons.
BMI: body mass index; FSH: follicle-stimulating hormone; LH: luteinizing hormone; AMH: antimüllerian hormone; AFC: antral follicle count; controlled ovarian stimulation: controlled ovarian stimulation; PPOS: progestin-primed ovarian stimulation protocol; GnRH-ant: gonadotropin-releasing hormone antagonist; GnRHa: gonadotropin-releasing hormone agonist;IVF: in vitro fertilization; ICSI: intracytoplasmic sperm injection.
[a] statistically different from Group 1.
[b] statistically different from Group 2.
[c] statistically different from Group 3.

Wang. CLBR of women with poor ovarian response. Fertil Steril Rep 2021.

rates per complete cycle, and the effects on live birth rates per complete cycle of controlled ovarian stimulation protocols. The conservative estimate assumed that the patients who dropped out would not achieve clinical pregnancy or live birth if they had continued, while the optimistic estimate was based on the assumption that dropouts would have had the same clinical pregnancy or live birth rates as those who returned (11).

Statistical Analysis

Statistical analysis was performed using the computing environment R (version 3.5.1). According to Kolmogorov-Smirnov test, all continuous variables did not conform to the normal distribution. So, they were presented as median (interquartile range, IQR). Categorical variables were presented as counts (percentage). The Kruskal-Wallis H test, Chi-square test, and Fisher exact test were carried out for intergroup comparisons as appropriate. Results were considered statistically significant at $p<0.05$ and Bonferroni-adjusted *P* value was used in multiple comparisons. Kaplan-Meier method was applied to generate the live birth event curves in the subgroups, while the log-rank test was used to compare different curves. Generalized estimating equations (GEE) with exchangeable correlation structure were adopted to account for the cluster effect brought about by the repeated cycles of one patient. Variables with $P<.05$ in the univariate analysis were included in the multivariate analysis as confounding factors to illustrate the effects of controlled ovarian stimulation protocols on LBR.

RESULTS

Characteristics of Participants

Among the 2641 patients diagnosed of POR who initiated 5489 ovarian stimulation cycles, 2716 (49.5%) ovarian

TABLE 2

Clinical pregnancy rates, live birth rates per complete cycle, as well as conservative and optimistic estimates of cumulative clinical pregnancy/live birth rates per patient

				Estimations of CCPR			Estimations of CLBR	
Cycle count	patients treated (n)	clinical pregnancy (n)	CPR (95%CI)	Conservative (95%CI)	Optimistic (95%CI)	live birth (n)	LBR (95%CI)	Conservative (95%CI)	Optimistic (95%CI)
1	1825	456	24.99 (23.00–26.97)	24.99 (23.00–26.97)	24.99 (23.00–26.97)	270	14.79 (13.17–16.42)	14.79 (13.17–16.42)	14.79 (13.17–16.42)
2	665	146	21.95 (18.81–25.10)	32.99 (30.83–35.14)	41.46 (39.20–43.72)	73	10.98 (8.60–13.35)	18.79 (17.00–20.59)	24.15 (22.18–26.11)
3	206	52	25.24 (19.31–31.17)	35.84 (33.64–38.04)	56.23 (53.96–58.51)	21	10.19 (6.06–14.33)	19.95 (18.11–21.78)	31.88 (29.74–34.02)
4	53	9	16.98 (6.87–27.09)	36.33 (34.12–38.54)	63.67 (61.46–65.87)	5	9.43 (1.56–17.30)	20.22 (18.38–22.06)	38.31 (36.08–40.54)
5	16	1	6.25 (-5.61–18.11)	36.38 (34.18–38.59)	65.94 (63.76–68.11)	0	0.00 (0.00–0.00)	20.22 (18.38–22.06)	38.31 (36.08–40.54)
6	6	1	16.67 (-13.15–46.49)	36.44 (34.23–38.65)	71.61 (69.55–73.68)	0	0.00 (0.00–0.00)	20.22 (18.38–22.06)	38.31 (36.08–40.54)
7	2	0	0.00(0.00–0.00)	36.44 (34.23–38.65)	71.61 (69.55–73.68)	0	0.00(0.00–0.00)	20.22 (18.38–22.06)	38.31 (36.08–40.54)
Total	2773	665	23.98 (22.39–25.57)	-	-	369	13.31 (12.0–14.6)	-	-

CPR: clinical pregnancy rates per complete cycle; CI: confidence interval; CCPR: cumulative clinical pregnancy rates; LBR: live birth rates per complete cycle.;CLBR: cumulative live birth rates.

Wang. CLBR of women with poor ovarian response. Fertil Steril Rep 2021.

stimulation cycles of 1683 women were excluded. A total of 2067 (37.7%) ovarian stimulation cycles of 1390 women failed to get a transferrable embryo, and the embryos of 648 ovarian stimulation cycles of 552 women were not used up. Finally, this study included 2,773 fresh cycles and 1,702 following FET cycles of 1,825 POR women (Table 1). The median (interquartile) age, body mass index (BMI), baseline follicle-stimulating hormone (FSH), antimüllerian hormone, and antral follicle counts (AFC) of the participants were 41(36–43) years old, 22.6 (20.8–24.6) kg/m^2, 9.1 (7.0–11.7) IU/L, 0.6 (0.4–0.9) ng/ml and 4 (3–6), respectively. Secondary infertility accounted for the majority (70.6%) of the participants. Diminished ovary reserve (88.1%) constituted the major indicators of IVF/ICSI. Mild stimulation, PPOS, and GnRH-ant protocols each constituted nearly one-quarter of the controlled ovarian stimulation protocols. 77.5% of the cycles adopted IVF and the others underwent ICSI for fertilization. The number of oocytes retrieved as well as the high-quality embryos were low, varying from 1 to 3.

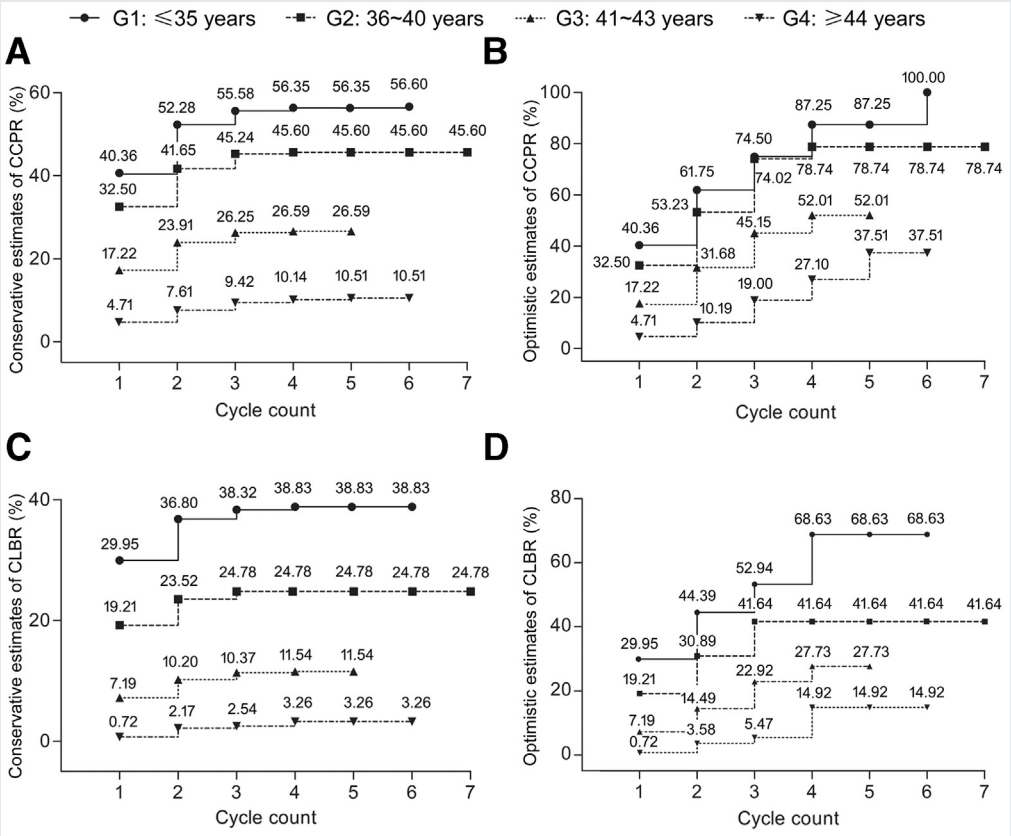
The BMI was significantly higher in the older age groups. The proportion of secondary infertility and diminished ovarian reserve increased with age. But the duration of infertility and AFC were significantly lower in the older age groups. The baseline levels of FSH, estradiol, and methods of fertilization were comparable among the groups. Women $\geq$44 years (Group 4) had the lowest number of oocytes retrieved (median 2, IQR 1–4) and transferable embryos (median 1, IQR 1–2), but the number of good quality embryos showed no difference among the different groups (P>.05).

Patients dropped out of the IVF/ICSI cycle, though they did not achieve live birth (Supplemental Figure 1). Compared with the POR patients who underwent the 2nd complete cycles, the dropout patients had significantly lower BMI (22.4 [20.7–24.4] vs 22.7 [21.0–24.7] kg/m^2), lower proportion of diminished ovarian reserve (84.8% VS 90.7%), lower basal FSH (8.9 [6.9–11.0] vs 9.1 [7.2–12.2] IU/L) and higher AMH (0.7 [0.4–1.0] vs 0.6 [0.4–0.9] ng/ml) (Supplemental Table 1). However, patients who proceeded with or quit the 3rd complete cycle had no significant difference in the baseline characteristics. This is also the case in the 4th to 7th complete cycles.

The CCPR and CLBR in the Entire Cohort

CCPR and CLBR increased with the number of complete cycles, then stabilized at subsequent complete cycles (Table 2). At the 6th complete cycle, the conservative and optimistic estimates of CCPR reached the peak values of 36.44% and 71.61%, respectively. But the counterparts of CLBR peaked at the 4th complete cycle, reaching 20.22% and 38.31%, respectively. Although one clinical pregnancy was obtained at the 5th complete cycle, no live birth was achieved. The overall CPR and LBR were 23.98% (95% confidence interval (CI), 22.39%-25.57%) and 13.31% (95% CI, 12.0%-14.6%), respectively.

FIGURE 1



The conservative and optimistic estimates of CCPR and CLBR when women with are stratified by age. A. The conservative estimations of CCPR; B. The optimistic estimations of CCPR; C. The conservative estimations of CLBR; B. The optimistic estimations of CLBR. CCPR: cumulative clinical pregnancy rates; CLBR: cumulative live birth rates; POR: poor ovarian response.

Wang. CLBR of women with poor ovarian response. Fertil Steril Rep 2021.

CCPR and CLBR in Subgroups

The CCPR and CLBR showed a decreasing trend as the age of the participants increased (Figure 1). Group 1, the youngest group, presented with the highest CCPR and CLBR. The conservative estimates of the CCPR at the last complete cycle from Group 1 to 4 (from the youngest to the oldest) were 56.60%, 45.60%, 26.59%, and 10.14%. The corresponding optimistic estimates were 100.00%, 78.74%, 52.01%, and 37.51%. As for CLBR, the conservative estimates from Group 1 to 4 were 38.83%, 24.78%, 11.54%, and 3.26%; and the optimistic estimates were 68.63%, 41.64%, 27.71%, and 14.92%. The clinical pregnancy rates and live birth rates per complete cycle exhibited the same trend (Supplemental Table 2).

As shown in the entire cohort, CCPR and CLBR were initially elevated in the subgroups as the number of complete cycles increased. After a certain cut-off point, the curve stabilized, which appeared earlier in CLBR than in CCPR. The CCPR of Group 1 to 4 stabilized at the 6th, 4th, 4th, and 5th complete cycles, respectively. On the other hand, CLBR stopped increasing at the 4th, 3rd, 4th, and 4th complete cycles.

Impacts of Controlled Stimulation Protocols on the Live Birth Rates per Complete Cycle

According to the univariate analysis, the following factors had an effect on the live birth rates per complete cycle of the cohort, age, BMI, type of infertility, infertility factors, baseline FSH, LH, AMH, AFC, the dose of gonadotropin administered, duration of gonadotropin medication, complete cycle counts, and oocytes retrieved (supplemental Table 2). After adjusting for these confounding factors with the GEE model, th results showed that mild stimulation was not inferior to the other controlled ovarian stimulation protocols in terms of live birth rates per complete cycle (Table 3). Only age (adjusted odds ratio (aOR) = 0.86, 95% CI: 0.84–0.88), BMI (aOR = 0.94, 95% CI: 0.90–0.98), oocytes retrieved (aOR = 1.15, 95% CI: 1.09–1.22), secondary infertility (aOR = 1.41, 95% CI: 1.05–1.90), and diminished ovarian reserve (aOR = 0.52, 95% CI: 0.31–0.88) were statistically significant.

However, the subgroup analysis suggested that controlled ovarian stimulation protocols had varied associations with live birth rates per complete cycle. In Group 1, PPOS (aOR=0.51, 95% CI: 0.30–0.87) and GnRH-ant protocols (aOR=0.45, 95% CI: 0.24–0.81) adversely affected the live

TABLE 3

The multivariable analysis of live birth rates per complete cycle in different age groups.

	All		Group 1: ≤35years		Group 2: 36–40 years		Group 3: 41–43 years		Group 4: ≥44 years	
	aOR	*p*	aOR	*p*	aOR	*p*	aOR	*p*	aOR	*p*
controlled ovarian stimulation protocols										
Mild stimulation	Reference		Reference		Reference		Reference		Reference	
PPOS	0.72 (0.51–1.02)	0.06	0.51 (0.30–0.87)	**0.01**	1.05 (0.57–1.93)	0.89	1.23 (0.59–2.58)	0.59	1.25(0.13–11.63)	0.85
GnRH-ant protocol	0.81 (0.56–1.15)	0.24	0.45 (0.24–0.81)	**0.01**	1.74 (0.97–3.14)	0.07	0.95 (0.42–2.16)	0.90	1.79 (0.15–21.01)	0.65
Modified long protocol	0.78(0.40–1.51)	0.47	0.35 (0.09–1.14)	0.14	1.34 (0.46–3.91)	0.59	1.56 (0.40–5.93)	0.53	2.53 (0.04–159.24)	0.66
Natural cycle	1.19 (0.54–2.62)	0.67	0.74 (0.19–2.94)	0.67	2.41 (0.72–8.07)	0.16	0.80 (0.14–3.72)	0.70	4.36×10^{-12} (0–0)	**<0.01**
GnRHa long protocol	0.73 (0.30–1.77)	0.48	1.580×10^{-18}(0–0)	**<0.01**	1.60 (0.48–5.38)	0.45	2.31 (0.47–11.40)	0.30	2.67×10^{-11} (0–0)	**<0.01**
Others	0.75 (0.45–1.25)	0.27	0.39 (0.17–0.90)	**0.03**	1.68 (0.77–3.68)	0.20	0.55 (0.13–2.34)	0.42	9.52×10^{-12} (0–0)	**<0.01**
Age	0.86 (0.84–0.88)	**<0.01**	0.98 (0.90–1.05)	0.52	0.89 (0.77–1.02)	0.10	0.50 (0.35–0.70)	**<0.01**	0.35 (0.17–0.74)	**<0.01**
BMI	0.94 (0.90–0.98)	**<0.01**	0.95 (0.88–1.02)	0.16	0.96 (0.89–1.03)	0.26	0.87 (0.78–0.97)	0.01	0.98 (0.74–1.28)	0.85
Type of infertility										
Primary infertility	Reference		Reference		Reference		Reference		Reference	
Secondary infertility	1.41 (1.05–1.90)	**0.02**	1.22 (0.80–1.85)	0.36	1.76 (1.10–2.82)	**0.02**	0.77 (0.39–1.52)	0.44	2.25 (0.12–6.90)	0.59
Infertility factors										
Male factor	Reference		Reference		Reference		Reference		Reference	
Diminished ovarian reserve	0.52 (0.31–0.88)	**0.01**	0.71 (0.27–1.85)	0.48	0.46 (0.22–0.98)	**0.04**	0.52 (0.17–1.58)	0.25	0.07 (0.01–0.85)	**0.04**
Others	0.63 (0.33–1.20)	0.16	0.92 (0.28–3.00)	0.88	0.49 (0.19–1.26)	0.14	0.50 (0.17–1.58)	0.35	5.62×10^{-13} (0–0)	**<0.01**
Basal FSH	0.98 (0.96–1.01)	0.13	1.01 (0.98–1.05)	0.54	0.96 (0.92–0.996)	0.03	0.97 (0.89–1.04)	0.36	0.94 (0.77–1.54)	0.56
Basal LH	0.98 (0.94–1.02)	0.33	1.00 (0.97–1.03)	0.95	0.97 (0.89–1.06)	0.52	0.91 (0.78–1.06)	0.21	0.79 (0.41–1.51)	0.47
AMH	0.97 (0.78–1.21)	0.76	0.89 (0.57–1.40)	0.62	1.04 (0.69–1.58)	0.84	1.04 (0.79–1.37)	0.76	2.76 (0.98–7.75)	0.06
AFC	1.05 (0.997–1.11)	0.06	1.02 (0.93–1.13)	0.63	1.02 (0.94–1.11)	0.59	1.11 (1.00–1.23)	**0.04**	0.88 (0.59–1.30)	0.21
Total dose of gonadotropin (IU)	1.00 (1.00–1.00)	0.83	1.00 (1.00–1.00)	0.50	1.00 (1.00–1.00)	0.15	1.00 (1.00–1.00)	0.98	1.00 (1.00–1.00)	0.85
Duration of gonadotropin mediation (days)	1.03 (0.96–1.11)	0.44	1.02 (0.89–1.15)	0.82	1.10 (0.98–1.24)	0.11	0.90 (0.74–1.09)	0.28	0.86 (0.53–1.40)	0.54
Oocytes retrieved	1.15 (1.09–1.22)	**<0.01**	1.18 (1.07–1.32)	**<0.01**	1.10 (1.00–1.21)	0.06	1.18 (1.01–1.36)	**0.03**	1.08 (0.78–1.49)	0.54

PPOS progestin-primed ovarian stimulation protocol; *GnRH-ant* gonadotropin-releasing hormone antagonist; *GnRHa* GnRH agonist; *BMI* body mass index; *FSH* follicle-stimulating hormone; *LH* luteinizing hormone; *AMH* antimüllerian hormone; *AFC* antral follicle count; *aOR* adjusted odds ratio; $p<0.05$ was statistically significant.

Wang. CLBR of women with poor ovarian response. Fertil Steril Rep 2021.

birth rates per complete cycle compared with the mild stimulation protocol. The modified GnRHa long protocol (aOR=0.35, 95% CI: 0.09–1.14, $P = .14$) and natural cycle (aOR=0.74, 95% CI: 0.19–2.94, $P = .67$) also showed adverse influences but the differences were not statistically significant. Controlled ovarian stimulation protocols exhibited no significant association with the live birth rates in Group 2 to 4.

DISCUSSION

In this study, we retrospectively analyzed 2,773 complete cycles of 1,825 women with POR at a single center and compared CCPR and CLBR in the entire cohort as well as the different age groups. We concluded that CLBR and CCPR initially increased with the number of complete cycles, then stabilized after the 4th complete cycle. The actual CCPR may lie between the conservative and optimistic estimates (36.44% - 71.61%), as the CLBR (20.22% - 38.31%). The CCPR, CLBR, clinical pregnancy rates per complete cycle, and live birth rates per complete cycle decreased with age. Mild stimulation protocol contributed to the higher live birth rates per complete cycle for POR patients younger than 35 years compared with the PPOS and the GnRH-ant protocols.

The CCPR and its relationship to CLBR have not been previous investigated. Our data showed that CLBR stopped increasing after the 4th complete cycle, while CCPR stopped increasing after the 6th complete cycle, either in the entire cohort or age subgroups. That means after four complete cycles, women with POR achieved no live birth, even if some of them had become pregnant. Therefore, it is not advisable that specialists initiate more than four complete cycles for women with POR.

One possible explanation lies in the increase in age during the treatment period, perhaps, because the quantity and quality of oocytes usually decrease with age (12). In this vein, the mitochondria, the main organelles that determine the capacity of normal fertilization, show increasing instability of their deoxyribonucleic acid with increasing age. Mitochondrial biogenesis in oocytes and the surrounding granulosa cells are severely impeded by aging (13). Furthermore, a higher apoptosis rate of mural granulosa cells is correlated with older age, which may impair the quality of oocytes (14). The aneuploidy rate of embryos also increased with age (15). As a result, the clinical pregnancy rates per complete cycle, and live birth rates per complete cycle decrease accordingly. The CLBR observed in our study differed from those of previous studies. Yang (5) reported the conservative estimates of CLBR in 401 women with POR to be 31.9%. Specifically, the CLBR was 48.0% for <35 years, 30.1% for 35–39 years, and 16.9% for ≥ 40 years. Xu (4) found the conservative estimates of CLBR to be 14.9% for >3000 POR patients, 22% for those ≤ 30 years old, 18.3% for 31–34 years, 17.2% for 35–37 years, 13.5% for 38–40 years, 10.5% for 41–43 years, and 4.4% for ≥ 44 years. Our results lie between these two studies. Several factors might account for the differences. First, compared with the Yang study, the patients we recruited were more advanced in age, had higher baseline FSH and lower AFC. Although the participants in Xu's study were younger than ours, the proportion of diminished ovarian reserve was higher. Second, Xu recruited patients who received treatment between 2002 and 2016, while our patients were treated between 2014 and 2018. Over the past decade, optimization of treatment protocols, embryo culture procedures, vitrification and thawing methods, and luteal support may have contributed to the increase in CLBR (16). Finally, Xu's study did not exclude patients with genetic or chromosomal abnormalities associated with infertility, which might lead to lower CLBR.

Consistent with many other studies (4, 5, 17), our study showed that age was inversely correlated with CCPR and CLBR, indicating that oocyte quality and ovarian reserve in older patients were reduced. Despite the poor response to gonadotropin stimulation, younger POR patients might have more residual follicles. Furthermore, according to the logistic regression analysis of the data of 1730 biopsied blastocysts, for every 1-year increase in the female age, the probability of aneuploid embryos increases by 10% (OR=1.1, 95% CI: 1.1–1.2) (15). Older women were likely to have no euploid embryos (26–37 years old: 2%-6%, 42 years old: 33%, 44 years old: 53%) (18). The decreased probability of aneuploidy brought higher success rate for the younger patient. From this perspective, clinical trials of POR that require homogeneity among participants should take age into consideration. The POSEIDON criteria (19), a new classification of low responders developed by the Poseidon Group in 2016, may be a superior option. The POSEIDON criteria stratify women with low predicted prognosis by age and ovarian reserve. Patients in the same group of POSEIDON classification might have similar live birth rates.

The suitable controlled ovarian stimulation protocols for POR have been discussed in many studies, but no consensus has been reached. After adjusting for the confounding factors, the live birth rates per complete cycle of POR patients using mild stimulation was not inferior to those who adopted other controlled ovarian stimulation protocols. Furthermore, for POR women ≤ 35 years, the live birth rates per complete cycle of mild stimulation were higher. The effectiveness of mild stimulation in women ≤ 35 years may be due to avoiding disturbances on oocyte quality by high gonadotropin dose (20). Previous studies have reported that the live birth rates for POR is independent of controlled ovarian stimulation protocols (21), and that mild protocol leads to live birth rates in POR patients that are comparable to conventional protocols (22–24). But the live birth rates in these studies were results of a single embryo transfer cycle, instead of a complete cycle. Also, they were not discussed separately in different age groups. Further prospective and randomized studies are needed to confirm the benefit of mild stimulation on POR patients younger than 35 years old.

This study is not without limitations. First, its retrospective design has an inherent defect such that we could not identify the reasons for patients dropping out. Besides, the cycles without live births but with remaining embryos were excluded, which might have led to a selection bias, such that those embryos were discarded due to poor quality. Furthermore, not every patient insisted on receiving seven complete cycles. Only a small number of patients received more than four complete cycles. Therefore, the result might

have deviated from the actual value. Despite the limitations above, the strengths of this study should not be missed. Our study provided a unique insight into the relationship between the CCPR and CLBR and explored the controlled ovarian stimulation protocols suitable for different age groups. Our large patient cohort was a better representation of the current state of patients with POR. The insights of this study could be useful in the clinical evaluation and treatment of women with POR. However, further research is necessary to explore the underlying reasons for the poor responses in order to develop clinical interventions to overcome such challenges.

CONCLUSION

The results of this study suggest that younger women with POR have a higher CCPR and CLBR. Repeated cycles may help improve CLBR in women with POR in the first four complete cycles. Therefore, it is not advisable to initiate more than four complete cycles in such women. Mild stimulation was not inferior to the conventional ovarian stimulation protocols in terms of live birth rates per complete cycle. For young ($\leq$35 years) women with POR, mild stimulation may be beneficial to live birth rates per complete cycle compared with the use of other protocols.

REFERENCES

1. Pandian Z, McTavish AR, Aucott L, Hamilton MP, Bhattacharya S. Interventions for 'poor responders' to controlled ovarian hyper stimulation (COH) in in-vitro fertilisation (IVF). Cochrane Database Syst Rev 2010:CD004379.
2. Law YJ, Zhang N, Venetis CA, Chambers GM, Harris K. The number of oocytes associated with maximum cumulative live birth rates per aspiration depends on female age: a population study of 221 221 treatment cycles. Hum Reprod 2019;34:1778–87.
3. Maheshwari A, McLernon D, Bhattacharya S. Cumulative live birth rate: time for a consensus? Hum Reprod 2015;30:2703–7.
4. Xu B, Chen Y, Geerts D, Yue J, Li Z, Zhu G, et al. Cumulative live birth rates in more than 3,000 patients with poor ovarian response: a 15-year survey of final in vitro fertilization outcome. Fertil Steril 2018;109:1051–9.
5. Yang Y, Sun X, Cui L, Sheng Y, Tang R, Wei D, et al. Younger poor ovarian response women achieved better pregnancy results in the first three IVF cycles. Reprod Biomed Online 2016;32:532–7.
6. Yin H, Jiang H, He R, Wang C, Zhu J, Cao Z. Cumulative live birth rate of advanced-age women more than 40 with or without poor ovarian response. Taiwan J Obstet Gynecol 2019;58:201–5.
7. Jeve YB, Bhandari HM. Effective treatment protocol for poor ovarian response: A systematic review and meta-analysis. J Hum Reprod Sci 2016;9:70–81.
8. Chen P, Li T, Jia L, Fang C, Liang X. Should all embryos be cultured to blastocyst for advanced maternal age women with low ovarian reserve: a single center retrospective study. Gynecol Endocrinol 2018;34:761–5.
9. Scott LA, Smith S. The successful use of pronuclear embryo transfers the day following oocyte retrieval. Human reproduction (Oxford, England) 1998;13:1003–13.
10. Fang C, Huang R, Wei LN, Jia L. Frozen-thawed day 5 blastocyst transfer is associated with a lower risk of ectopic pregnancy than day 3 transfer and fresh transfer. Fertil Steril 2015;103:655–61.e3.
11. Raz N, Shalev A, Horowitz E, Weissman A, Mizrachi Y, Ganer Herman H, et al. Cumulative pregnancy and live birth rates through assisted reproduction in women 44-45 years of age: is there any hope? J Assist Reprod Genet 2018;35:441–7.
12. Vollenhoven B, Hunt S. Ovarian ageing and the impact on female fertility. F1000Res 2018;7.
13. May-Panloup P, Boucret L, Chao de la Barca JM, Desquiret-Dumas V, Ferré-L'Hotellier V, Morinière C, et al. Ovarian ageing: the role of mitochondria in oocytes and follicles. Hum Reprod Update 2016;22:725–43.
14. Fan Y, Chang Y, Wei L, Chen J, Li J, Goldsmith S, et al. Apoptosis of mural granulosa cells is increased in women with diminished ovarian reserve. J Assist Reprod Genet 2019;36:1225–35.
15. Minasi MG, Colasante A, Riccio T, Ruberti A, Casciani V, Scarselli F, et al. Correlation between aneuploidy, standard morphology evaluation and morphokinetic development in 1730 biopsied blastocysts: a consecutive case series study. Hum Reprod 2016;31:2245–54.
16. Lee J, Kim EJ, Kong HS, Youm HW, Kim SK, Lee JR, et al. Establishment of an improved vitrification protocol by combinations of vitrification medium for isolated mouse ovarian follicles. Theriogenology 2018;121:97–103.
17. Leijdekkers JA, Eijkemans MJC, van Tilborg TC, Oudshoorn SC, van Golde RJT, Hoek A, et al. Cumulative live birth rates in low-prognosis women. Hum Reprod 2019;34:1030–41.
18. Franasiak JM, Forman EJ, Hong KH, Werner MD, Upham KM, Treff NR, et al. The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. Fertil Steril 2014;101:656–63.e1.
19. Poseidon G, Alviggi C, Andersen CY, Buehler K, Conforti A, De Placido G, et al. A new more detailed stratification of low responders to ovarian stimulation: from a poor ovarian response to a low prognosis concept. Fertil Steril 2016;105:1452–3.
20. Lu C-L, Yan Z-Q, Song X-L, Xu Y-Y, Zheng X-Y, Li R, et al. Effect of exogenous gonadotropin on the transcriptome of human granulosa cells and follicular fluid hormone profiles. Reproductive biology and endocrinology: RB&E 2019;17:49.
21. Polyzos NP, Nwoye M, Corona R, Blockeel C, Stoop D, Haentjens P, et al. Live birth rates in Bologna poor responders treated with ovarian stimulation for IVF/ICSI. Reprod Biomed Online 2014;28:469–74.
22. Song D, Shi Y, Zhong Y, Meng Q, Hou S, Li H. Efficiency of mild ovarian stimulation with clomiphene on poor ovarian responders during IVF⁄ICSI procedures: a meta-analysis. Eur J Obstet Gynecol Reprod Biol 2016;204:36–43.
23. Youssef MA, van Wely M, Al-Inany H, Madani T, Jahangiri N, Khodabakhshi S, et al. A mild ovarian stimulation strategy in women with poor ovarian reserve undergoing IVF: a multicenter randomized non-inferiority trial. Hum Reprod 2017;32:112–8.
24. Medicine PCotASfR. Comparison of pregnancy rates for poor responders using IVF with mild ovarian stimulation versus conventional IVF: a guideline. Fertil Steril 2018;109:993–9.