Comparison of the C Umulative Live Birth Rates After One ART Cycle Including All Subsequent Frozen–thaw Cycles in Women Undergoing IVF Using Progestin Primed Ovarian Stimulation Versus Long GnRH Agonist Protocol

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Abstract

Background: The efficacy and reproductive outcomes of progestin primed ovarian stimulation protocol (PPOS) were previously compared to rarely used ovarian stimulation protocol and also the live birth rate were reported by per embryo transfer rather than cumulative live birth rates (CLBRs). Does the use of PPOS improve the cumulative live birth rates (CLBRs) and shorten time to live birth when compared to long GnRH agonist protocol in women with normal ovarian reserve?

Methods: A retrospective cohort study was designed to include women aged<40 with normal ovarian reserve (regular menstrual cycles, FSH <10 IU/L, antral follicle count >5) undergoing IVF from January 2017 to December 2019. The primary outcome was cumulative live birth rates (CLBRs) within 18 months from the day of ovarian stimulation.

Results: A total of 995 patients were analyzed. They used either PPOS (n=509) or long GnRH agonist (n=486) protocol at the discretion of the attending physicians. Both groups had almost comparable demographic and cycle stimulation characteristics except for duration of infertility which was shorter in the PPOS group. In the GnRH agonist group 372 cases (77%) completed fresh embryo transfer, resulting into 218 clinical pregnancies and 179 live birth. The clinical pregnancy rate, ongoing pregnancy, and live birth per transfer were 58.6%, 54.0%, 53.0% respectively. In the PPOS, no fresh transfer was carried out. During the study period, the total number of initiated FET cycles with thawed embryos was 665 in the PPOS group and 259 in the long agonist group. Of all FET cycles, a total of 206/662 (31.1%) cycles resulted in a live birth in the PPOS group versus 110/257 (42.8%) in the long agonist group (OR: 0.727; 95% CI: 0.607–0.871; p<0.001) .The implantation rate of total FET cycles was also lower in the PPOS group compared with that in the agonist group 293/1004 (29.2%) and 157/455 (34.5%) (OR: 0.846; 95% CI: 0.721–0.992; p= 0.041). Cumulative live birth rates after one complete IVF cycle including fresh and subsequent frozen embryo cycles within 18 months follow up were significantly lower in the PPOS group compared that in the long agonist group 206/509 (40.5%) and 307/486 (63.2%), respectively (OR: 0.641; 95% CI: 0.565-0.726). The average time from ovarian stimulation to pregnancy and live birth was significantly shorter in the long agonist group compared to the PPOS group (p<0.01) In Kaplan-Meier analysis, the cumulative incidence of ongoing pregnancy leading to live birth was significantly higher in the long agonist compared in the PPOS group(Log rank test, p<0.001). Cox regression analysis revealed stimulation protocol adopted was strongly associated with the cumulative live birth rate after adjusting other confounding factors (OR =1.917 (1.152-3.190), p=0.012) .

Conclusion: Progestin primed ovarian stimulation was associated with a lower cumulative live birth rates and a longer time to pregnancy / live birth than the long agonist protocol in women with a normal ovarian reserve.

Background

Gonadotropin releasing hormone (GnRH) analogues are essential in IVF to prevent a premature LH surge [1–4]. Inadequate suppression can cause early ovulation and affect oocyte quality and embryo development resulting in a low pregnancy rate [5, 6]. Despite their overall effectiveness, GnRH analogues are associated with insufficient ovarian response and cycle cancellation in 5–20% of all IVF cycles [7, 8]. Furthermore, GnRH analogues have been criticized as increasing IVF protocol complexity, resulting in increased costs and the need for an HCG trigger in GnRH agonist cycles, which increases the risk of ovarian hyperstimulation syndrome [9].

Concerning the adverse attributes of GnRH analogues, Kuang et al proposed the need for pituitary suppression methods that are more convenient, less costly and safer for patients. When given as cotreatment with exogenous gonadotropins for IVF, medroxyprogesterone acetate (MPA) was used in place of GnRH analogues to block the LH

surge [10]. Prior studies indicate that compared with GnRH analogues, the use of MPA results in effective pituitary suppression with similar outcomes such as cycle cancellation rates, oocyte number and quality, fertilization rate, cleavage rate, blastocyst quality and pregnancy [11]. Because of the adverse effects of premature progesterone exposure on the endometrium, however, progestin cycles require a freeze-all IVF cycle with subsequent frozen embryo transfer (FET). Additionally, progestin cycles have been shown to require more gonadotropins compared with short GnRH agonist cycles. Several investigators have claimed that progestin cycles are more patient friendly and cost-effective [11–17].

Progestins seem to provide higher pregnancy rates than the short GnRH agonist protocol following cryopreserved embryo transfers [10, 11, 15]. However, in most trials, the efficacy and reproductive outcomes of PPOS regimen were compared to short GnRH agonist protocol, which is now rarely used in many assisted reproduction programs and also the live birth rate were reported by per embryo transfer rather than cumulative live birth rates (CLBRs) which can reflect the real efficacy of ovarian stimulation in ART [18–21]. Since many women with normal ovarian reserve are suitable for fresh embryo transfer in long agonist protocols, whether this would be the case compared with the more common long GnRH agonist protocol in which fresh transfer can be accomplished in the majority cases.

Therefore, the aim of the present study was to compare cumulative live birth rates and time to live birth in women with normal ovarian reserve following progestin primed ovarian stimulation protocol with long GnRH agonist protocol.

Methods

Study design and participants

Patients

A retrospective study of infertile women with normal ovarian reserve attending the Assisted Reproduction clinic, Shanghai First Maternity and Infant Hospital for IVF from January 2017 to December 2019 was undertaken, and each patient was followed for 18 months from the day of the ovarian stimulation. Ethical approval was not required for the retrospective analysis.

Women were included if they fulfilled the following inclusion criteria: (i) less than 40 years of age; (ii) having indications for IVF; (iii) regular menstrual cycles over the previous 3-month period (25 - 35 days in duration); (iv) antral follicle count (AFC) of more than 5 on menstrual cycle day 2 - 3, and basal serum FSH concentration of no more than 10 IU/L. Women were excluded if they had: (i) diagnosis of polycystic ovarian syndrome, (ii) an abnormal uterine cavity shown on hysterosalpingogram or hysteroscopy, (iii) moderate or severe endometriosis, (iv) use of donor eggs/sperm, (v) preimplantation genetic testing, (vi) rescue intracytoplasmic sperm injection (ICSI) or half ICSI, (vii) still having cryopreserved embryos but continuing to the next fresh IVF cycle.

Women were offered either progestin-primed ovarian stimulation protocol (PPOS group) or agonist long protocol (agonist group) at the discretion of the attending physicians or subject to the wishes of the couple.

Ovarian stimulation

Women started their IVF with ovarian stimulation using either PPOS or long agonist protocols. For the long agonist protocol, gonadotropin-releasing hormone analogue (GnRHa) (1.88mg Triptorelin acetate, Diphereline, Ipsen Pharma Biotech, France) was given for pituitary desensitisation from the mid-luteal phase in the previous cycle. On Day 2–3

of the menstrual cycle, they underwent transvaginal ultrasound examination and serum oestradiol measurement. Human menopausal gonadotrophin (hMG) (Lebaode, Lizhu, china) or recombinant FSH (Puregon, Organon, Dublin, Ireland or Gonal F, Merck Serono S.p.A, Modugno, Italy) was given at 150–225 IU per day based on the antral follicle count (AFC), age of women and previous ovarian response, according to the standard operation procedures of the centre. For the PPOS protocol, Medroxyprogesterone MPA (MPA, 10 mg/d, Shanghai Xinyi Pharmaceutical Co., China) was also given from day of the ovarian stimulation until the day of ovulation trigger. Ovarian response was monitored by serial transvaginal scanning with or without hormonal monitoring. Further dosage adjustments were based on the ovarian response at the discretion of the clinicians in charge.

When three leading follicles reached ≥18 mm in diameter, Ovidrel 250 microgram (Merck Serono S.p.A., Modugno, Italy) or triptorelin (0.1 mg; Decapeptyl, Ferring Pharmaceuticals, Netherlands) and hCG (2000 IU; Lizhu Pharmaceutical Trading Co., China) were given to trigger final maturation of oocytes. Oocyte retrieval was performed around 36 hours later.

Fertilization and embryo evaluation

Semen samples were prepared by the swim-up procedure. About 2 hours after oocyte retrieval, each oocyte was inseminated with approximately 20,000–30,000 motile spermatozoa. If the total number of motile sperm was $<10^5$ after washing or normal morphology was <1%, intracytoplasmic sperm injection (ICSI) was performed. Oocytes were decoronated and checked for the presence of two pronuclei to confirm fertilization. Embryos were graded on day 3 after retrieval as grade one to grade six according to the evenness of each blastomere and the percentage of fragmentation. Embryos of 6-8 cells and of grade one or two were regarded as top quality embryos. Some non-top-quality embryos were placed in extended culture until they reached the blastocyst stage.

Fresh embryo transfer

In the long agonist protocol, a maximum of two embryos was replaced on Day 3 after retrieval under transabdominal ultrasound guidance. Luteal phase support was given by vaginal or intramuscular progesterone at the discretion of the attending physicians. A pregnancy test was carried out 2 weeks after the transfer. All who had a positive pregnancy test had a transvaginal ultrasound scan 2 weeks after the positive pregnancy test (4 weeks after embryo transfer) to identify the presence of a gestation sac with a foetal heart signifying an ongoing pregnancy. All pregnant women were contacted or traced for the pregnancy outcomes after delivery or miscarriage.

Cryopreservation and frozen embryo transfer (FET)

Surplus embryos of day 3 top quality embryos or good-morphology Day 5 or 6 blastocysts in the long agonist group and all the viable embryos/blastocysts in the PPOS group were cryopreserved using vitrification. Those who did not get pregnant in the stimulated IVF cycle and those who postponed embryo transfer would undergo frozen embryo transfer (FET) at least 2 months after the stimulated cycle if they had at least one frozen embryo.

Vitrification was performed with MediCult Vitrifification Cooling (Origio, Denmark) using ethylene glycol, propylene glycol, sucrose as cryoprotectant. Embryos were vitrified one by one at room temperature. For the warming procedure following vitrification, the straw was cut and the capillary was pulled from the straw out of the liquid nitrogen, and immediately warmed one by one using MediCult Vitrification Warming (Origio, Denmark). After warming, embryos were transferred to a culture dish for evaluation and further embryo development. Only embryos with more than 50% of blastomeres present after thawing were transferred in FET cycles.

FETs were carried out in natural cycles for ovulatory women and in clomiphene induced or hormonal cycles for anovulatory women. Up to two embryos or blastocysts were transferred in FET cycles.

Outcomes measures

The primary outcome measure was the cumulative live birth rate within 18 months from the first day of ovarian stimulation. LBR which was calculated by including the first live birth generated during the one complete IVF cycle including fresh and all subsequent FET cycles.

Secondary outcome measures included incidence of premature LH surge (LH ≥10 IU/l), fertilization rate, clinical pregnancy, ongoing pregnancy, live birth rate, miscarriage, multiple pregnancy, and implantation rates in both fresh and FET cycles. Number of cycle cancellations, number of oocytes retrieved, number of obtained oocytes, number of embryos available for transfer, number of cryopreserved embryos, number of FET cycles started, moderate and severe ovarian hyperstimulation syndrome (OHSS), time to ongoing pregnancy were also compared. A baby born alive after 22 weeks gestation was classified as a live birth. Clinical pregnancy was defined as the presence of at least one gestational sac on ultrasound at 6 weeks. Ongoing pregnancy was the presence of at least one foetus with heart pulsation on ultrasound beyond 10 weeks. Miscarriage rate was defined as the number of miscarriages before 22 weeks divided by the number of women with clinical pregnancy. Multiple pregnancy was a pregnancy with more than one gestational sac detected on ultrasound at 6 weeks. Fertilization rate was the percentage of zygotes with two visible pronuclei among inseminated oocytes. Implantation rate was calculated as the number of gestational sacs seen on scanning divided by the number of embryos replaced. Time to ongoing pregnancy leading to live birth as the time from day of ovarian stimulation to an ongoing pregnancy that led to a live birth.

We analyzed all cycles finished before 18 months after the first day of starting ovarian stimulation - whether cancelled, pregnant, or non - pregnant. To ensure validation of complete cycles, all enrolled subjects agreed to use all frozen embryos before proceeding with a new fresh IVF/ICSI cycle.

Statistical analyses

One sample of the Kolmogorov - Smirnov test was used to test the normal distribution of continuous variables. Continuous variables were given as mean ± SD if normally distributed, and as median (interquartile range) if not normally distributed. Statistical comparison was carried out by Student's t-test, Mann - Whitney U-test for continuous variables and chi-square test for categorical variables, where appropriate.

Cox proportional hazard model was used to evaluate the relative prognostic significance of female age, BMI, the number of retrieved oocytes and the primary diagnosis of infertility in relation to CLBR.

Generalized estimated equation regression analyses(GEE) were made for the individual treatment groups in all FET cycles with transfer to evaluate the impact of independent variables on the total LBRs from FET (n = 919).

All pregnancies within 18 months from ovarian stimulation were analyzed, whether achieved by fresh or frozen IVF cycle. The Kaplan-Meier method was used to calculate the cumulative proportion of ongoing pregnancies leading to live births, and time to pregnancy was graphically depicted by cumulative incidence curves. The log-rank test was used to measure whether significant differences existed in the cumulative incidence curves. Patients who did not reach the primary outcome (live birth) including those achieved a continuing pregnancy that did not lead to live birth were censored. Statistical analysis was performed using the Statistical Program for Social Sciences (SPSS Inc., Version 24.0, Chicago, USA). The two-tailed value of $P < 0.05$ was considered statistically significant.

Results

Out of 995 women who met the selection criteria, 509 women used the PPOS protocol while 486 women used the long agonist protocol. One woman in the PPOS group had premature ovulation before oocyte retrieval. No transferable embryos were available in 61women in the PPOS group and 19 women in the long agonist group resulting in cycle cancellation, the cancellation rate was significantly higher in the PPOS group than in the agonist group (12.0% versus 3.9%, p < 0.001). Within 18 month follow up, 76 (14.9%)women in the PPOS group and 55 (11.3%) women in the agonist group who did not achieve live birth but still have cryopreserved embryos were also included for analysis (Figure1).

Demographic and the index stimulation cycle characteristics

Baseline characteristics of two groups are presented in Table I. No significant differences were found with regard to age of women, basal AFC, basal FSH level, number of previous IVF cycles, body mass index, cause of infertility, proportion of primary infertility and insemination methods between the two groups except for duration of infertility, which was significantly shorter in the PPOS group compared to that in the long agonist group.

The starting dose of FSH was higher (225 IU versus 150 IU, P < 0.001), days of stimulation is shorter (8 days versus 11 days, P < 0.001) and total FSH dose was lower (1800 IU versus 2025 IU, P < 0.001) in the PPOS group compared to those in agonist group. Serum estradiol levels (2740 pg / ml versus 2496 pg / ml, P < 0.05) and LH level on HCG day (2.6 IU/ml versus 0.7 IU/ml, P < 0.001) was higher in the PPOS groups than those in the long agonist group. However, there was no significant difference in the serum progesterone level on the hCG day between the two groups. One women in the PPOS groups experienced premature LH surge while none was seen in the long agonist group. No patient experienced OHSS in the PPOS groups, while 4 patients (0.8%) in the long agonist group were administered into hospital due to moderate or severe OHSS. (Table 1)

Average number of oocytes obtained (9 versus 12, P < 0.001), number of oocytes fertilized (7 versus 8, P < 0.001), number of cleaving embryos (6 versus 8, P < 0.001) and number of transferable embryos (3 versus 4, P < 0.001) was lower in the PPOS group as compared to that in the long agonist group. However, no differences were found in fertilization rate, cleavage rate, number of blastocyst formation and number of good quality embryos between the two groups (Table I) .

Fresh embryo transfer

In the GnRH agonist group 372 cases (77%) completed fresh embryo transfer, resulting into 218 clinical pregnancies and 197 live birth. The clinical pregnancy rate, ongoing pregnancy, and live birth per transfer were 58.6%, 54.0%, 53.0% respectively. Seventeen (4.6%) and four (1.1%) women miscarried <12 weeks and >12 weeks of gestation respectively. Fresh transfer was canceled in 114 women due to elevated serum progesterone level on the trigger day, risk of OHSS, suboptimal endometrial thickness or having no transferable embryos. In the PPOS, no fresh transfer was carried out.

Frozen embryo cycles

Of all allocated patients, the total number of initiated FET cycles with thawed embryos was 665 in the PPOS group and 259 in the long agonist group. In the PPOS group, 662/665 (99.5%) had one frozen embryo transfer compared to 257/259 (99.2%) in the long agonist group. In the majority of FET cycles Day-3 embryos were thawed and transferred. Presence of top quality of embryos after thawing and endometrial thickness were similar between the two groups.

More women had double embryo transfer in the frozen embryo cycles in the agonist group (75.5%) than in the PPOS group (50.8%). Hormonal cycles used for endometrium preparation were used in more FET cycles in the PPOS group 482/662 (72.8%) compared to 104/257 (40.5%) in the long agonist group (P < 0.001) (Table III).

Women in the PPOS group were less likely to have a live birth following their first FET cycle 139/433 (32.1%) compared to those in the long agonist group 85/192 (44.3%) (OR: 1.721; 95% CI: 0.588–0.884; P = 0.003). However, this difference disappeared after inclusion of additional FET cycles. Of all FET cycles, a total of 206/662 (31.1%) cycles resulted in a live birth in the PPOS group versus 110/257 (42.8%) in the long agonist group (OR: 0.727; 95% CI: 0.607–0.871; P <0.001) .The implantation rate of total FET cycles was also lower in the PPOS group compared with that in the agonist group 293/1004 (29.2%) and 157/455 (34.5%) (OR: 0.846; 95% CI: 0.721–0.992; P = 0.041) (Table IV).

Generalized estimated equation regression analyses (GEE) regression by the women's age, BMI, AFC, duration of infertility, stimulation protocol (PPOS / agonist), type and cause of infertility, cycle number, insemination method, endometrium preparation, embryo transfer day, number of embryos replaced, endometrial thickness revealed that female age (OR: 0.938; 95% CI: 0.894-0.983), type of infertility (OR: 0.643; 95% CI: 0.459-0.901), endometrial thickness (OR: 1.162; 95% CI: 1.068-1.265), treatment protocol (OR:1.585; 95% CI: 1.031-2.437), and number of embryos replaced (OR:1.585; 95% CI: 1.031-2.437) were associated with live birth per FET cycle of women treated with PPOS and GnRH agonist protocol (Table V).

Cumulative pregnancy and LBRs

Cumulative pregnancy and live birth rates are listed in Table II. The CLBR after one complete IVF cycle including fresh and subsequent frozen embryo cycles within 18 months follow up were significantly lower in the PPOS group compared that in the long agonist group 206/509 (40.5%) and 307/486 (63.2%), respectively (odds ratio (OR): 0.641; 95% CI: 0.565-0.726). The average time from ovarian stimulation to pregnancy and live birth was significantly shorter in the long agonist group compared to the PPOS group (P < 0.001) (Table II). In Kaplan-Meier analysis, the cumulative incidence of ongoing pregnancy leading to live birth was significantly higher in the long agonist compared in the PPOS group.(Log rank test, P < 0.001) (Fig. 2)

Cox proportional hazard model using the stepwise method by the women's age, stimulation protocol (PPOS/agonist), body mass index, duration of infertility, total FSH dosage, number of retrieved oocytes, causes of infertility, starting dose of FSH, days of stimulation, oestradiol and LH level on HCG day, only stimulation protocol and starting dose of FSH was entered in this model and revealed stimulation protocol adopted was strongly associated with the cumulative live birth rate after adjusting other confounding factors.(OR =1.917 (1.152-3.190), P = 0.012) (Table VI).

Discussion

The main finding of this study was that the CLBR in women with normal ovarian reserve after the one oocyte retrieval including fresh and all subsequent frozen embryo cycles were significant lower in the PPOS group compared with that in long agonist group, 40.5% versus 63.2% respectively. Moreover, the time to pregnancy and live birth was significantly shorter in the long agonist group compared with that in the PPOS group.

The results of the study indicated that progestins were capable of effectively preventing premature ovulation in IVF cycles. No significant difference was found in the incidence of premature LH surge and premature ovulation between the PPOS group and the long agonist group, although serum LH levels on HCG day were significantly lower in the long agonist group. The inhibitory effect of progestin on ovulation has been the basis of the design of progestin-only

contraceptives, which suppress follicular growth and thus inhibit ovulation after a sustained administration. Progestin priming seems to slow the LH pulse frequency, augments the pulse amplitude and reduces the mean plasma LH concentrations compared with those in untreated women in some studies [22, 23] .

Progestin cycles have been shown to require more gonadotropins compared with short GnRH agonist cycles [11–17]. However, in the present study we found total gonadotropin dose was lower and the day of stimulation was shorter in the PPOS group compared to that in long GnRH agonist group. This may be due to prolonged pituitary suppression in the long agonist protocol which was started from the mid-luteal phase of the previous cycle, and prolonged pituitary down-regulation by GnRHa might contribute to improved endometrial receptivity [24].

In this study we found number of oocytes obtained, number of oocytes fertilized, number of cleaving embryos and number of transferable embryos was lower in the PPOS group as compared to that in the long agonist group. The results are in contrast with previous studies which showed comparable embryological characteristics in progestin and short GnRH agonist cycles [11–17]. Studies with FET cycles provide an opportunity to estimate two different protocols on oocyte quality and subsequent embryo development potential. In the first and total FET cycles, we found significantly lower clinical pregnancy and live birth rate per frozen embryo transfer as well as implantation rate in PPOS group compared to those in long agonist group. Furthermore, if we combined data from fresh and FFT cycles, the total implantation rate and pregnancy rate per transfer was still significantly lower in the PPOS group indicating the embryos originating from the PPOS protocol may have a reduced development potential to those from the long agonist group. While some researches indicate that elevated progesterone levels do not have a negative impact on the FET results of stimulated cycles using PPOS [10, 16, 25], in most trials, the efficacy and reproductive outcomes of PPOS regimen were compared to short GnRH agonist protocol, which is now rarely used and is recommended to be replaced by the long agonist or the antagonist protocol [26, 27]. One randomized trial [28] compared use of medroxyprogesterone versus a GnRH antagonist on the number of mature oocytes retrieved in oocyte donation cycles. Though no difference was found in the number of mature oocytes between the two groups, the clinical pregnancy rate was 31% versus 46% (P = 0.006) and the ongoing pregnancy rate 27% versus 40% (P = 0.015) for medroxyprogesterone and GnRH antagonists, respectively. This suggests a possible impairment of oocyte quality when medroxyprogesterone was used in ovarian stimulation.

It is difficult to directly compare our results with previous studies as none of the available study evaluated the effect of PPOS on cumulative live birth rates nor assessed time to ongoing pregnancy. In this study we report cumulative live birth rates in one complete cycle, which is the outcome of interest for infertile couples. Not only just single fresh or FET cycle live birth, but also results from one IVF cycle including all subsequent frozen embryo cycles performed within an 18-month period were evaluated, thereby giving the actual efficacy of these two strategies in the daily practice can be compared. Other strengths include none of the patients lost to follow-up in the study, leading to an increased reliability of our outcomes. Furthermore, we performed a Kaplan-Meier analysis to compare cumulative success rate in each group, as it assumed that women who did not return for subsequent FET cycles had the same chance of a pregnancy resulting in a live birth as those who returned for treatment [19]. Time to pregnancy was much shorter in the long agonist group which is also an important factor to evaluate the efficacy of IVF treatment [29] and further strengthen the overall result as PPOS is not beneficial with respect to the cumulative outcomes in two groups.

Safety profile such as ectopic pregnancy rate, miscarriage rate was similar in progestin and GnRH agonist cycles. No patient experienced moderate or severe OHSS in the PPOS group owning to it is applicable for the use of a GnRHa for ovulation trigger and freezing all embryos [30]. In contrast, though not reaching significant difference, there were four cases of severe OHSS in the long agonist group in which HCG trigger was used and fresh embryo transfer was

undertaken in the stimulated cycle. Therefore, PPOS may be more suitable for high responders but not for normal responders in whom a freeze all is likely and OHSS risk is high [31, 32].

A cost-effectiveness study comparing PPOS with the short GnRH agonist and GnRH antagonist protocols suggested that PPOS was associated with significantly higher cost per live birth when conventional protocols using GnRH analogues were completed with a fresh transfer [33]. According to data shown in this study, we do not think that PPOS combined with an elective freeze all approach is currently justified for all IVF cycles, because avoiding a fresh transfer does not seem beneficial in the absence of a medical indication when a fresh embryo transfer is not intended [34, 35].

Our study is limited by its retrospective design. Although we did not calculate the sample size, around 500 cases in each group had enough power to distinguish the 20% difference of the cumulative live birth between the two groups. Cox regression analysis was carried out for controlling the basis possibly produced by imbalanced characteristics between the two groups. Further randomized trials with adequate sample size would be needed to confirm these findings.

Conclusion

In conclusion, in women with a normal ovarian reserve, progestin primed ovarian stimulation was associated with a lower cumulative live birth rates and a long time to pregnancy /live birth than the long agonist protocol.

Declarations

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Authors' contributions

Zhiqin Chen, Xiaoming Teng and Hong Chen designed the study. Zili Sun, Zheng Wang, Di Yao and Kunming Li searched the literature and collected the data. Hong Chen performed statistical analyses. Zhiqin Chen, Hong Chen and Ernest Hung Yu Ng wrote the manuscript. All authors read and approved the manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

Ethics approval and consent to participate

Ethical approval: All protocols and data collections and storage performed in this study involving human participants were approved by the Institutional Research Ethics Committee of Shanghai First Maternity and Infant Hospital (NU IREC: KS21302) and were in accordance with the 1964 Helsinkideclaration and its later amendments.

Consent for publication

All authors read and approved the fnal manuscript.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table I. Demographic and cycle stimulation characteristics

	PPOS group (n=509)	Agonist group (n=486)	P value
Female age (years)	31.4±3.8	31.2±3.7	0.451
Infertility duration (years)	3 (2-4)	3 (2-5)	0.015
Primary infertility, n (%)	263 (51.67%)	281 (57.82%)	0.051
Cycle number, n (%)			0.110
First cycle	306 (60.1%)	316 (65.0%)	
Basal antral follicle count	14 (10-20)	16 (12.75-17)	0.989
Basal FSH level (IU/L)	6.3 (4.8-7.3)	6.06 (5.1-7.0)	0.521
Body mass index (kg/m^2)	22.7±7.5	21.9±3.3	0.355
Cause of subfertility, n (%)			0.911
Tubal	298 (58.5%)	271 (55.8%)	
Male factor	77 (15.1%)	87 (18.0%)	
Unexplained	42 (8.3%)	40 (8.2%)	
Anovulatory	11(2.2%)	10 (2.1%)	
Endometriosis	4 (0.8%)	4 (0.8%)	
Mixed factors	77 (15.1%)	74 (15.1%)	
Insemination method, n (%)			0.381
IVF	303 (59.5%)	276 (56.8%)	
ICSI	206 (40.5%)	210 (43.2%)	
Starting dose of FSH (IU)	225 (225-225)	150 (150-150)	<0.001
Days of stimulation	8 (8-9)	11 (10-12)	<0.001
Total FSH dosage (IU)	1800 (1575-2025)	2025 (1650-2475)	<0.001
Oestradiol level on HCG day (pg/ml)	2740 (1670-4205)	2496(1758-3098)	0.019
LH level on HCG day (IU/ml)	2.6 (1.5-4.3)	0.7 (0.4-1.0)	<0.001
Progesterone level on the hCG day (ng/L)	0.8 (0.6-1.2)	0.9 (0.7-1.2)	0.189
Cancellation rate, n (%)	61 (12.0%)	19 (3.9%)	<0.001
Incidence of premature LH surge, n (%)	5 (1.0%)	0 (0%)	0.062
Incidence of premature ovulation, n (%)	1 (0.2%)	0 (0%)	1.000
No. of moderate or severe OHSS	0 (0%)	4 (0.8%)	0.057
No. of oocytes obtained	9 (5-14)	12 (8-16)	<0.001

No. of oocytes fertilized	7 (3.5-10.5)	8 (5-12)	<0.001
Fertilization rate %	85.7 (68.4-100)	83.3 (66.7-93.8)	0.097
No. of cleaving embryos	6 (3-10)	8 (5-12)	<0.001
Cleavage rate (%)	100(100-100)	100(100-100)	0.815
No. of top quality embryos	2 (1-4)	2(0-4)	0.680
No. of embryos frozen	3 (2-5)	2 (1-4)	<0.001
No. of transferrable embryos	3 (2-5)	4 (2-6)	<0.001
No. of blastocyst formation	1 (0-2)	1 (0-2)	0.198

PPOS: progestin-primed ovarian stimulation.

BMI: body mass index.

FSH: follicle-stimulating hormone.

Values are presented as mean ± SD or median (25th and 75th percentile) or number (%). Mann–Whitney U test or Pearson test was carried out according to the data distribution and statistical principles.

Table II Cumulative reproductive outcomes from one complete cycle including fresh and frozen embryo transfer for 18 months follow up

	PPOS group (n=509)	Agonist group （n=486）	P-value	95% CI for the relative risks (RR)
No. of cycles				
Fresh ET	0	372		
Frozen ET	662	257		
Cumulative reproductive outcomes				
Positive β-hCG (≥10 IU/L), n (%)	265 (52.1%)	344 (70.8%)	<0.001	0.736(0.665-0.814)
Clinical pregnancy, n (%)	240(47.2%)	330 (67.9%)	<0.001	0.694(0.622-0.775)
Ongoing pregnancy, n (%)	215 (42.2%)	313 (64.4%)	<0.001	0.656(0.581-0.740)
Implantation rate, n (%)	293/1004 (29.2%)	452/1137 (39.8%)	<0.001	0.734(0.651-0.828)
Miscarriage rate#, n (%)	44/250 (17.6%)	34/341 (10.0%)	0.007	1.765(1.164-2.678)
Ectopic pregnancy rate, n(%)	8/296 (2.70%)	6/374 (1.6%)	0.324	1.685(0.591-4.802)
Live birth, n (%)	206 (40.5%)	307 (63.2%)	<0.001	0.641(0.565-0.726)
After fresh embryo transfer, n (%)	0/206 (0%)	197/307 (64.2%)	<0.001	
After FET embryo transfer, n (%)	206/206 (100%)	110/307 (35.8%)	<0.001	2.791(2.403-3.242)
Time to pregnancy from starting ovulation induction				
Time to first positive hCG (days), median (Interquartile Range)	137(94-191)	30(28-120.5)	<0.001	
Time to first clinical pregnancy (days), median (Interquartile Range)	153(108.75-206.5)	44(42-131.5)	<0.001	
Time to first ongoing pregnancy (days), median (Interquartile Range)	184(136.5-235.5)	74(72-155.75)	<0.001	
Time to first live birth (days), median (Interquartile Range)	380(336-440)	277(263-346)	<0.001	

PPOS: progestin-primed ovarian stimulation.

Data are median (25th and 75th percentile) or number (%). Mann–Whitney U-test for continuous variables and chi-square test for categorical variables.# 10 patents in PPOS group and 7 patents in agonist group were miscarriaged >12 weeks of gestation.

Table III Characteristics of subsequent frozen embryo transfer (FET) cycles in the PPOS and GnRH agonist groups.

FET treatments	PPOS group (n=509)	Agonist group (n=486)	P value	95% CI for the relative risks (RR)
Patients with frozen embryos after fresh cycle of all allocated, n/n (%)	448/509 (88.0%)	212/486 (43.6%)	<0.001	2.018(1.815-2.243)
Number of patients who continued with FET cycles, n/n (%)	435/509 (85.5%)	189/486 (38.9%)	<0.001	2.198(1.955-2.471)
Total number of FET cycles where embryos where thawed, n	665	259		
Total number of FET cycles with transfer, n (%)#	662 (99.5%)	257(99.22%)	0.623	1.003(0.991-1.015)
Day 2/3 embryo transfer, n (%)	520 (78.6%)	232(90.27%)	<0.001	0.870(0.822-0.921)
Blastocyst transfer, n (%)	142(21.5%)	25(9.73%)	<0.001	2.205(1.478-3.289)
No. of blastocysts transferred in FET cycles	1 (1-1)	1(1-2)	<0.001	
No. of Day-2 or -3 embryos transferred in FET cycles	2 (1-2)	2(2-2)	<0.001	
DET, n (%)	336 (50.8%)	194(75.49%)	<0.001	0.672(0.607-0.745)
SET, n (%)	326(49.2%)	63(24.51%)	<0.001	2.009(1.599-2.523)
Endometrial preparation, n (%)		<0.001	13.615(2.204-5.931)	
Natural cycle	31 (4.7%)	137 (53.3%)		
Mild stimulation	149 (22.5%)	16 (6.2%)		
Hormonal cycles	482 (72.8%)	104(40.5%)		
Presence of top quality of embryos after thawing	0 (0-1)	0 (0-1)	0.054	
Endometrial thickness	10 (9-11.2)	10 (9-11)	0.199	

PPOS: progestin-primed ovarian stimulation, FET: frozen embryo transfer.

Data are median (25th and 75th percentile) or number (%). Mann–Whitney U-test for continuous variables and chi-square test for categorical variables. DET, double embryo transfer; SET, single embryo transfer. # 3 patients in PPOS and 2 in agonist group with no embryos available to transfer after thawing.

Table IV Reproductive outcome of fresh and subsequent FET cycles

	Reproductive outcome	PPOS group (n=509)	Agonist group (n=486)	P-value	95% CI for the relative risks (RR)
Fresh ET	No. of cycles	0	372		
	Positive p-hCG, n (%)	0 (0%)	233 (62.6%)		
	Clinical pregnancy, n (%)	0 (0%)	218 (58.6%)		
	Ongoing pregnancy, n (%)	0 (0%)	201 (54.0%)		
	Abortion <12 weeks/>12 weeks of gestation, n (%)/n (%)	0 (0%)	17 (4.6%)/4 (1.1%)		
	Live birth, n (%)	0 (0%)	197 (53.0%)		
FET1	No. of cycles	433	192		
	Positive p-hCG, n (%)	194 (44.8%)	112 (58.3%)	0.002	0.768(0.655-0.900)
	Clinical pregnancy, n (%)	167 (38.6%)	96 (50.0%)	0.008	0.771(0.641-0.928)
	Ongoing pregnancy, n (%)	143 (33.0%)	88 (45.8%)	0.002	0.721(0.588-0.884)
	Abortion <12 weeks/>12 weeks of gestation, n (%)/n (%)	24 (5.5%) /4 (0.9%)	8 (4.2%)/3 (1.6%)	0.471/0.682	1.330(0.609-2.907)/0.591(0.134-2.616)
	Live birth, n (%)	139 (32.1%)	85(44.3%)	0.003	0.725(0.588-0.894)
FET2	No. of cycles	170	51		
	Positive p-hCG, n (%)	76 (44.7%)	23 (45.1%)	0.961	0.991(0.701-1.401)
	Clinical pregnancy, n (%)	61(35.9%)	21 (41.2%)	0.492	0.871(0.593-1.280)
	Ongoing pregnancy, n (%)	53 (31.2%)	20 (39.2%)	0.284	0.795(0.529-1.196)
	Abortion <12 weeks/>12 weeks of gestation, n (%)/n (%)	8 (4.7%)/3 (1.8%)	1 (2.0%)/ 0(0%)	0.688/1.000	2.400(0.307-18.740)/NS
	Live birth, n (%)	50 (29.4%)	20 39.22%)	0.187	0.750(0.496-1.134)
≥FET3	No. of cycles	59	14		
	Positive p-hCG, n (%)	26 (44.1%)	6(42.86%)	0.935	1.028(0.526-2.009)
	Clinical pregnancy, n (%)	22 (37.3%)	6(42.86%)	0.700	0.870(0.437-1.734)
	Ongoing pregnancy, n (%)	20 (33.9%)	5(35.71%)	1.000	0.949(0.432-2.087)
	Abortion <12 weeks/>12 weeks of gestation, n (%)/n (%)	2 (3.4%)/3 (5.1%)	1(7.14%)/0(0%)	0.477/1.000	0.475(0.046-4.871)/NS

	Live birth, n (%)	17 (28.8%)	5 (35.7%)	0.747	0.807(0.359-1.812)
Total FET cycles	No. of cycles	662	257		
	Positive p-hCG, n (%)	296 (44.7%)	141 (54.9%)	0.006	0.815(0.709-0.937)
	Implantation rate, n (%)	293/1004 (29.2%)	157/455 (34.5%)	0.041	0.846(0.721-0.992)
	Clinical pregnancy, n (%)	250 (37.8%)	123 (47.9%)	0.005	0.789(0.672-0.927)
	Ongoing pregnancy, n (%)	216 (32.6%)	113 (44.0%)	<0.001	0.742(0.622-0.885)
	Abortion <12 weeks/>12 weeks of gestation, n (%)/n (%)	34 (5.1%)/10 (1.5%)	10 (3.9%)/3(1.2%)	0.428/1.000	1.320(0.662-2.632)/1.294(0.359-4.664)
	Live birth, n (%)	206 (31.1%)	110 (42.8%)	<0.001	0.727(0.607-0.871)

PPOS: progestin-primed ovarian stimulation, FET: frozen embryo transfer.

P-value, RR and 95% CI correspond to tests for difference between the two groups using chi-square tests.

Table V Regression analysis of factors predicting live birth per FET cycle of women treated with PPOS and GnRH agonist protocol

	Crude model				Adjusted model			
Variables		**Beta**	**OR(95%CI)**	**P-value**		**Beta**	**OR(95%CI)**	**P-value**
Age of women		-0.070	0.933(0.898-0.968)	<0.001*		-0.064	0.938(0.894-0.983)	0.008*
Body mass index		-0.034	0.966(0.923-1.012)	0.149		-0.020	0.98(0.93-1.034)	0.466
AFC		0.019	1.019(1.002-1.036)	0.024		0.012	1.012(0.993-1.031)	0.231
Infertility duration		0.035	1.035(0.975-1.099)	0.255		0.021	1.022(0.949-1.1)	0.570
Type of infertility								
Primary	Reference		1		Reference		1	
Secondary		-0.507	0.602(0.454-0.798)	<0.001*		-0.441	0.643(0.459-0.901)	0.010*
Causes of infertility								
Tubal	Reference		1		Reference		1	
Anovulatory		-0.008	0.992(0.676-1.457)	0.968		0.294	1.342(0.391-4.603)	0.640
Endometriosis		0.481	1.618(0.605-4.327)	0.338		-0.770	0.463(0.073-2.933)	0.414
Unexplained		0.260	1.296(0.741-2.268)	0.363		0.311	1.365(0.732-2.547)	0.328
Male		-0.800	0.449(0.088-2.294)	0.336		-0.006	0.994(0.561-1.76)	0.983
Mixed factors		0.252	1.287(0.847-1.956)	0.237		0.401	1.493(0.895-2.491)	0.125
Cycle number								
First cycle	Reference		1		Reference		1	
Repeated cycles		-0.026	0.975(0.722-1.315)	0.867		-0.051	0.95(0.666-1.355)	0.778
Treatment protocol								
PPOS	Reference		1		Reference		1	
GnRH agonist		0.505	1.656(1.217-2.255)	0.001*		0.461	1.585(1.031-2.437)	0.036*
Insemination method								

IVF	Reference		1		Reference		1	
ICSI		-0.009	0.991(0.744-1.321)	0.951		-0.190	0.827(0.525-1.302)	0.411
Endometrial preparation								
Natural cycle	Reference		1		Reference		1	
Mild stimulation		-0.177	0.838(0.536-1.308)	0.437		0.315	1.37(0.761-2.468)	0.294
Hormonal cycles		-0.199	0.820(0.579-1.160)	0.262		0.307	1.36(0.842-2.197)	0.209
Endometrial thickness		0.129	1.137(1.050-1.232)	0.002*		0.150	1.162(1.068-1.265)	0.001
No. of embryo transfered		0.816	2.261(1.675-3.051)	0.000*		0.740	2.096(1.435-3.062)	<0.001
Blastocyst transfer								
No	Reference		1		Reference		1	
Yes		-0.527	0.590(0.408-0.853)	0.005*		-0.026	0.974(0.621-1.528)	0.910

Note: Crude and adjusted odds ratios (ORs) were presented , adjusted for all covariates in the table with the use of log-binomial regression models with generalized estimating equations for pregnancy outcomes in repeated FET cycles in the same woman..

*Statistically signifificant, with P < 0.05.

Table VI Crude and HR for cumulative live birth rate (CLBR) of women treated with PPOS and GnRH agonist protocol(n = 995)

Independent covariates	Covariate strata	Coefficient (B)	Hazard ratio (95% CI)	Wald ()	P-value
Crude model					
Treatment protocol	PPOS		1		
	GnRH agonist	1.095	2.989(2.509-3.561)	150.15	<0.001
Age of women		-0.033	0.968(0.945- 0.990)	7.834	0.005
Body mass index		-0.019	0.981(0.954- 1.008)	1.961	0.161
Duration of infertility		0.019	1.019(0.982-1.057)	0.969	0.325
Type of infertility	Primary		1		
	Secondary	-0.194	0.824(0.692-0.980)	4.800	0.028
Causes of infertility				4.937	0.424
	Tubal		1		
	Anovulatory	0.170	1.185(0.948-1.482)	2.216	0.137
	Endometriosis	0.021	1.021(0.728-1.432)	0.015	0.903
	Unexplained	0.134	1.143(0.669-1.956)	0.239	0.625
	Male	-1.095	0.335(0.083-1.346)	2.377	0.123
	Mixed factors	0.055	1.057(0.818-1.366)	0.178	0.673
Starting dose of FSH		-0.011	0.990(0.988-0.992)	106.916	<0.001
Days of stimulation		0.160	1.173(1.131-1.217)	73.225	<0.001
Total FSH dosage (IU)		0.000	1(1-1)	2.389	0.122
Oestradiol level on HCG day (pg/ml)		0.000	1(1-1)	0.410	0.522
LH level on HCG day (IU/ml)		-0.092	0.912(0.851-0.978)	6.658	0.010
Number of retrieved oocytes		0.001	1.001(0.989-1.014)	0.048	0.826
Adjusted model*					
Treatment protocol	PPOS		1		
	GnRH agonist	0.651	1.917(1.152-3.190)	6.266	0.012
Starting dose of FSH		-0.005	0.995(0.989-1.001)	3.203	0.074

P-values correspond to tests for difference in a cox regression analysis with cumulative LBR as the outcome (dependent) variable. *Adjusted for the following covariates: age of women, body mass index, duration of infertility, total FSH dosage, number of retrieved oocytes and causes of infertility, starting dose of FSH, days of stimulation, oestradiol and LH level on HCG day.

Figures

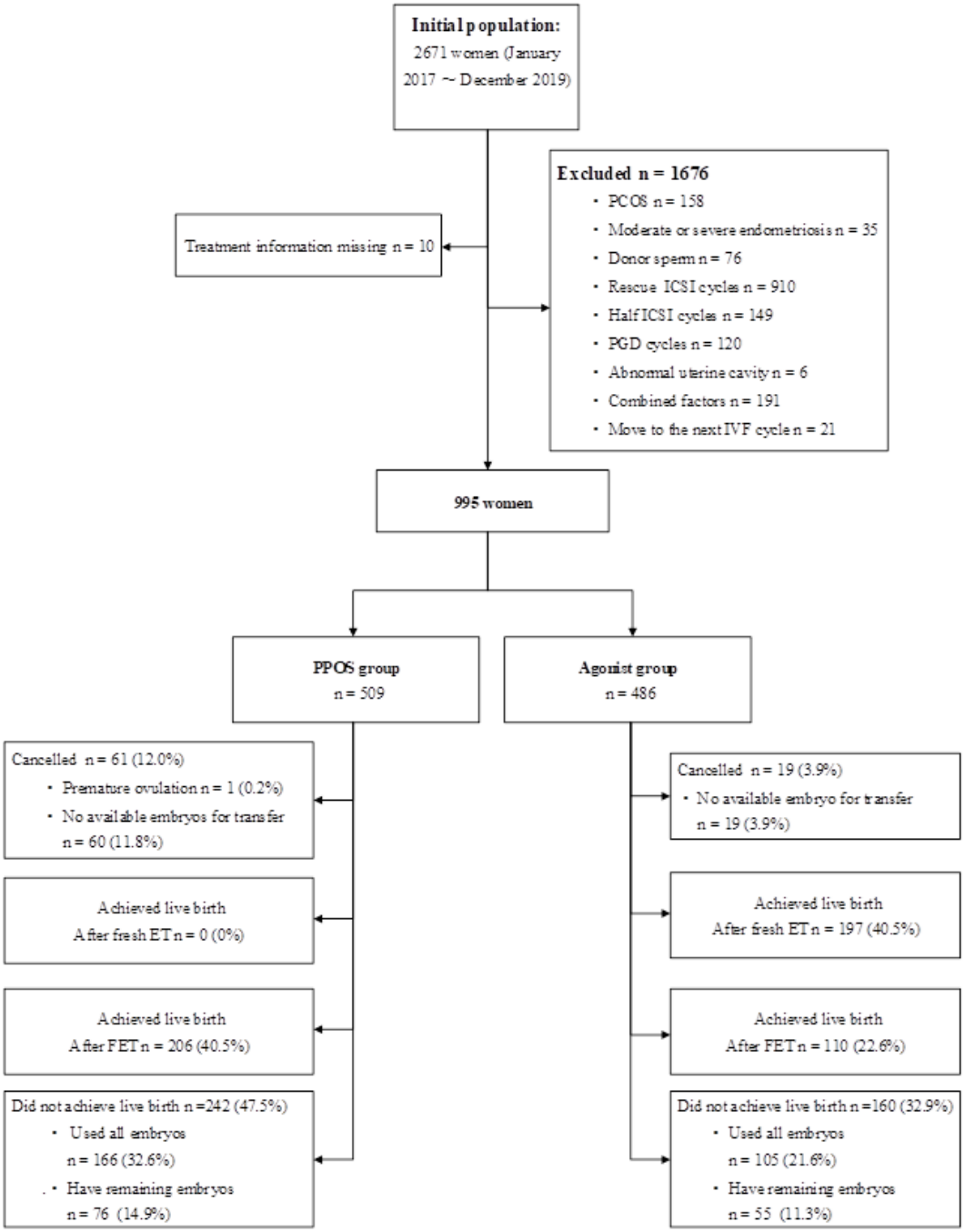
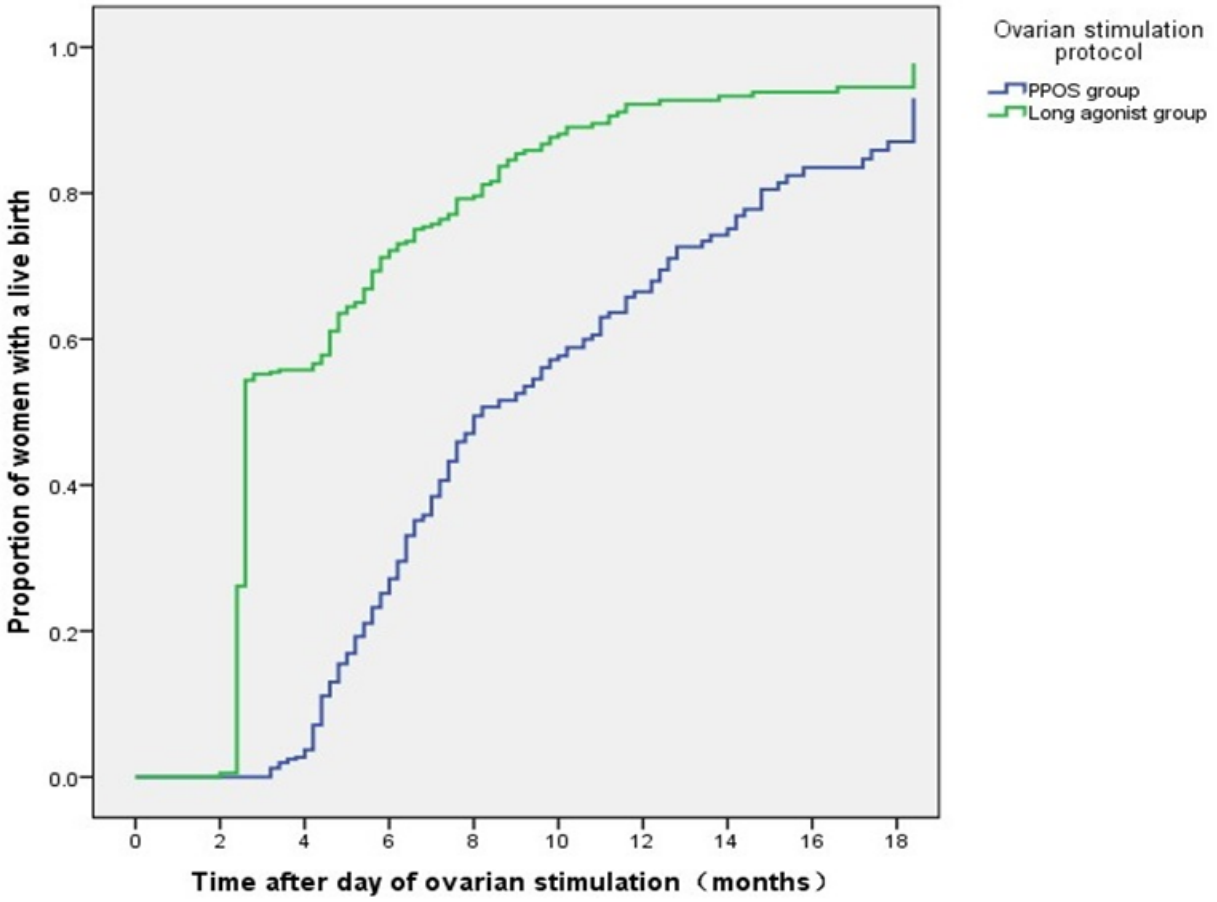


Figure 1 Patient flowchart

Figure 1

See image above for figure legend



Time after ovarian stimulation (months)	0	2	4	6	8	10	12	14	16	18
Number at risk (%); PPOS	509(100)	442(96)	374(73)	217(51)	125(43)	76(34)	46(25)	28(17)	15(13)	11(7)
Number at risk (%); Long agonist protocol	486(100)	368(44)	152(28)	89(21)	53(12)	26(8)	15(7)	12(6)	9(6)	7(2)

Figure 2

The cumulative incidence of ongoing pregnancy leading to live birth within 18 months followed-up in PPOS and long agonist group. Log-rank test($\chi 2 = 177.815$, $P < 0.001$).