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Double or dual stimulation in poor ovarian responders: where do we stand?

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***Abstract*:** Recent advances in our recognition of two to three follicular waves of development in a single menstrual cycle has challenged the dogmatic approach of ovarian stimulation for *in vitro* fertilization starting in the early follicular phase. First shown in veterinary medicine and thereafter in women, luteal phase stimulation–derived oocytes are at least as competent as those retrieved following follicular phase stimulation. Poor ovarian responders still remain a challenge for many decades simply because they do not respond to ovarian stimulation. Performing follicular phase stimulation and luteal phase stimulation in the same menstrual cycle, named as double stimulation/dual stimulation, clearly increases the number of oocytes, which is a robust surrogate marker of live birth rate in *in vitro* fertilization across all female ages. Of interest, apart from one study, the bulk of evidence reports significantly higher number of oocytes following luteal phase stimulation when compared with follicular phase stimulation; hence, performing double stimulation/dual stimulation doubles the number of oocytes leading to a marked decrease in patient drop-out rate which is one of the major factors limiting cumulative live birth rates in such poor prognosis patients. The limited data with double stimulation/dual stimulation-derived embryos is reassuring for obstetric and neonatal outcome. The mandatory requirement of freeze-all and lack of cost-effectiveness data are limitations of this novel approach. Double stimulation/dual stimulation is an effective strategy when the need to obtain oocytes is urgent, including patients with malignant diseases undergoing oocyte cryopreservation and patients of advanced maternal age or with reduced ovarian reserve.

Keywords: double stimulation, dual stimulation, number of oocytes, poor ovarian response

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Introduction

Despite the remarkable progress in assisted reproductive technologies, management of poor ovarian responders (POR) still remains a challenge, simply because they do not respond to treatment. The prevalence of POR varies from 5.6% to 35.1%,[1–5] depending on differences in the definition of poor response. Although many strategies have been proposed to treat such poor prognosis patients, there is still no clear superiority of one treatment *versus* another to enhance reproductive outcome.

Concise definition and stratification of subgroups of POR patients is essential for inter-study comparison of various interventions. The Bologna consensus criteria was first described in 2011 under the auspices of the European Society of Human Reproduction and Embryology (ESHRE) and has been a great achievement for classifying such patients.[6] Before this criteria, it is of interest that more than 40 different definitions for poor ovarian response have been used among 47 randomized trials and no more than 3 trials used the same definition, whereas even trials from the same research groups used different definitions across different trials.[7] As expected, a huge heterogeneity in study populations of the available studies and meta-analyses was seen, resulting in adoption of interventions of ambiguous value. Although the Bologna criteria was an important step, there is still marked heterogeneity of various subgroups regarding live birth rates (LBR).[8–10] In our study of 821 POR patients fulfilling Bologna

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criteria, prognosis, in general, was poor with less than 10% of LBR.[8] However, the LBRs were not homogeneous and 'young proven' PORs had the most favorable pregnancy outcome.

Overall, the pregnancy rates attained with *in vitro* fertilization (IVF) in POR patients are low being less than 8%.[9–12] Polyzos and colleagues[11] in a cohort of 485 PORs reported an LBR per cycle of 7.1% in patients <40 years and 5.2% in women $\geqslant 40$ years old; in this study, the only independent variable related to the LBR was the number of oocytes. Indeed, the number of eggs is a robust surrogate outcome for LBR in IVF across all female age groups;[13] in women aged 35–37, the estimated effect of collecting three oocytes compared with two oocytes was a relative increase in the observed LBR by 28%.[13] Thus, retrieving even one more oocyte in this patient population makes a huge difference in prognosis and any attempt that would increase the number of eggs would be a very important step to enhance reproductive outcome.

High drop-out rate is one of the major factors limiting the cumulative LBRs in POR patients[14] Although the etiologic factors for drop-out may differ from one population to another,[15] poor prognosis per se is an important contributory factor, especially in POR patients.[14] Pooling of oocytes[16] and embryos[17] have been reported to decrease the drop-out rate and hence increase the cumulative LBRs.[16]

Establishment of efficient vitrification techniques at every stage of preimplantation embryo development[18,19] along with our enhanced understanding of the physiologic, biochemical, and molecular mechanisms underlying antral follicular wave dynamics[20–22] have permitted the first description of double stimulation (DS) in 2013[23] and a modified version of DS couples with preimplantation genetic testing for aneuploidy (PGT-A), named dual stimulation (DuoStim) in 2016,[24] followed by several studies from different centers.[25–35] The goal of this mini-review article is to cover the available evidence of DS/DuoStim in POR patients.

Search procedure

Criteria for inclusion were established before literature search. Inclusion was limited to studies that were published of randomized controlled trials (RCTs), prospective/retrospective cohort studies and case series reports comparing the ovarian stimulation (OS) characteristics, embryological data, and pregnancy outcome between follicular phase stimulation (FPS) and luteal phase stimulation (LPS) in the same ovarian cycle. A thorough search of PubMed database was performed using combinations of the following keywords: 'IVF', 'In vitro fertilization', 'Intracytoplasmic Sperm Injection', 'ICSI', 'Assisted Reproductive Techniques', 'Assisted Reproductive Technologies', 'ART', 'Follicular Wave', 'DuoStim', 'Luteal phase stimulation', 'Luteal phase ovarian stimulation', 'Dual stimulation', 'Double stimulation', 'Ovarian stimulation', 'Fertility preservation'. After screening from the title and abstract, we excluded the data published as abstract, meeting proceeding, book chapter, review articles, and articles published in languages other than English. Finally, we included 12 studies comparing FPS and LPS in the same ovarian cycle (Table 1).

Physiologic basis of DS/DuoStim: theories of follicular development

The physiologic mechanisms underlying recruitment and selection of antral follicles in women are not fully elucidated. Three distinct theories of follicular recruitment have been proposed, including continuous recruitment (theory 1), single recruitment episode (theory 2), and follicular waves (theory 3).[21] Single recruitment episode and follicular waves theories are, indeed, part of the cyclic recruitment concept. According to the continuous recruitment theory (theory 1), small antral follicles $\leqslant$4–6 mm are recruited to grow continuously, at all stages of reproductive life, independent of gonadotropins.[36–38] The follicle destined to ovulate is selected, by chance, from the continuous supply of antral follicles, by being at the right stage of maturity to respond to the rise in follicle-stimulating hormone (FSH) that occurs following luteal regression.[38–40] According to the single recruitment episode theory (theory 2), a cohort of 2–5 mm follicles is recruited from a continuous supply of antral follicles once during each menstrual cycle.[41–44] There is, however, increasing evidence to suggest that multiple cohorts (also referred to as 'waves') of antral follicles are recruited during the menstrual cycle (theory 3).[20,21] Follicular waves have been described in veterinary medicine, although, some species-specific differences appear to exist. In a large population of healthy women, emergence of

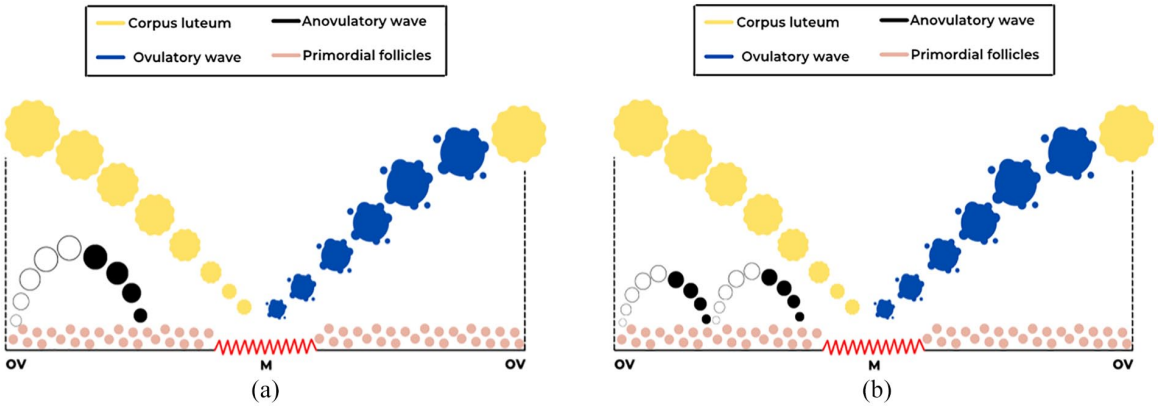

Figure 1. Schematic illustrations of the 2 (a) and 3 (b) follicular waves during the menstrual cycle.
M, menstruation; OV, ovulation.

a wave of 4–14 follicles ⩾4–5 mm was detected either two or three times during the interovulatory interval;[22] 68% of women exhibited two waves of follicle recruitment during the interovulatory interval, while the remaining 32% exhibited three waves (Figure 1(a) and (b)). In women with two follicular waves, an anovulatory wave emerged at the time of ovulation (i.e. early luteal phase) followed by emergence of the ovulatory wave during the early follicular phase. In women with three waves, an anovulatory wave emerged at the time of ovulation, a second anovulatory wave emerged during the mid to late luteal phase, and a third wave (the ovulatory wave) emerged in the early to mid-follicular phase.[22] The wave theory challenges the classical concept of folliculogenesis and is the basis for DS/DuoStim.

How to perform double/dual stimulation?

DS/DuoStim, with the intention to increase the number of retrieved eggs, is performed in a single menstrual cycle and composed of FPS and LPS. Although POR patients are the primary target, fertility preservation cases, in whom time is an important issue, may also benefit from this approach.

The recent available evidence suggests that, in POR patients, mild OS regimens (low-dose gonadotropins with/without oral agents), when compared with traditional OS protocols, offer comparable reproductive outcome albeit lower cost.[45,46] Across all the available studies on DS/DuoStim, different OS protocols have been described for FPS and LPS. Regarding FPS, luteal estrogen priming may be used to promote synchronization and coordination of follicular growth.[47,48] Either mild or conventional OS regimens can be employed for FPS and LPS. For mild OS, clomiphene citrate (CC), letrozole (LE) with/without low-dose exogenous gonadotropins can be used. Conventional OS using a 225–450 IU daily dose of exogenous FSH with/without luteinizing hormone (LH)/LH-like activity can also be used for FPS and LPS.

Different strategies can be employed to avoid premature LH surge during FPS and LPS, including GnRH-antagonist (GnRH-ant) use, exogenous progestins and/or Ibuprofen. A flexible GnRH-ant scheme is the most commonly employed strategy; GnRH-ant is started when the leading follicle attains a mean diameter of 12–14 mm and is continued until and including the day of triggering. Exogenous progestins may also be used for this purpose, especially during LPS, not only to avoid premature LH surge but also to avoid menses during oocyte retrieval[49] to decrease the risk of infection.[25,29] Although Ibuprofen is used in some studies, the precise role to avoid premature ovulation in this patient population needs to be proven in further studies.[50,51]

GnRH-agonist (GnRH-a) is most commonly used to trigger final oocyte maturation for both FPS and LPS. Dual/double triggering has been recently suggested to increase the number of eggs retrieved and enhance reproductive outcome in POR patient undergoing IVF.[52–54] In a retrospective study of 384 cycles fulfilling Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number (POSEIDON) Group 4 patients, the dual triggering was associated with significantly

Table 1. The available studies with their design, follicular/luteal phase stimulation protocols, modes of trigger and conclusions.

Author	Design	Inclusion criteria	*N*	FPS Protocol	FPS Trigger	LPS Protocol	LPS Trigger	Conclusions
Kuang and colleagues[25]	Pilot study	At least two of the following criteria: (1) >40 years old, (2) history of ovarian surgery, (3) history of poor response, ⩽3 oocytes with conventional stimulation, (4) AFC < 5, day 2–3 FSH 10–19 IU/L	38	CC: 25 mg/day (from D_3 to trigger-1 day) + LE: 2.5 mg/day (from D_3 for 4 days) + hMG: 150 IU (from D_6, every other day) + Ibuprofen 0.6 g on the trigger day and trigger + 1 day	GnRH-a	LE: 2.5 mg/day + (from OPU/OPU + 1 day until at least one follicle reaches 12 mm) + hMG 225 IU/day (from OPU/OPU + 1 day) + MPA 10 mg/day + Ibuprofen 0.6 g on the trigger day and trigger + 1 day	GnRH-a	Significantly more oocytes after LPS
Ubaldi and colleagues[24]	Prospective noninferiority	AFC ⩽ 6 + AMH ⩽ 1.5 ng/ml and/or ⩽ 5 oocytes retrieved in previous cycle	51	rec-FSH 300 IU/day + rec-LH 75 IU/day (from D_2) + GnRH-ant (when the leading follicle reaches 13–14 mm)	GnRH-a	rec-FSH 300 IU/day + rec-LH 75 IU/day (from OPU + 5 day) + GnRH-ant (when the leading follicle reaches 13–14 mm)	GnrH-a	Number of oocytes, M-2 oocytes, fertilization, blastulation and euploidy rates comparable with FPS and LPS. Increased yield of >1 euploid blastocyst with DuoStim.
Wei and colleagues[28]	Retrospective	At least two of the following criteria: (1) >40 years old, (2) history of poor response, ⩽3 oocytes with conventional stimulation (3) AFC < 6	23	CC: 50 mg or LE: 2.5 mg/day (from $D_{2\text{-}3}$ for 5 days) + hMG:75-150 IU/day (following CC or LE from $D_{7\text{-}8}$) Medication to avoid LH surge is NA	GnRH-a	CC: 50 mg/day or LE: 2.5 mg/day (from OPU + 1 day to 1 follicle reaches 12 mm) + hMG:225 IU/day + MPA 10 mg/day	10,000 IU hCG	Significantly more oocytes collected after LPS
Zhang and colleagues[26]	Retrospective	At least two of the following criteria: (1) ⩾40 years old or any other risk for POR, (2) history of poor response, ⩽3 oocytes with conventional stimulation (3) AFC < 5–6 or AMH < 0.5–1.1 ng/ml	153	CC 50 mg/day (from D_3 until triggering day) + hp-FSH 150 IU/day (if the follicles grow < 1 mm/ day) + Ibuprofen 0.3 g/every 6 h from the trigger to OPU day	GnRH-a	hp-FSH 150–225 IU/day	10,000 IU hCG	Significantly more oocytes and embryos following LPS. Significantly higher implantation rate with LPS-derived embryos
Rashtian and Zhang[27]	Retrospective	Day 3 FSH > 15 IU/ml + AFC < 8 and at least one failed conventional IVF cycle	69	Oral contraceptive pill for at least 1 week before starting FPS, CC: 50 mg day (until the trigger day) + LE: 2.5 mg/day (for 5 days) + FSH: 75 IU/day + GnRH-ant (when the estradiol level > 500 pg/ml or the follicle reaches > 14 mm)	GnRH-a	CC:50 mg day + LE: 2.5 mg/day + FSH: 75 IU/day (from OPU + 1 day) Medication to avoid LH surge is NA	hCG	Similar number of oocytes at FPS and LPS; performing DS doubles the number of oocytes when compared with FPS-only. a
Zhang and colleagues[29]	Retrospective	At least 2 of the following criteria: 1) ⩾40 year old or any other risk factor for POR, 2) history of poor response, ⩽3 oocytes with a conventional stimulation, 3) AFC < 5-7 or AMH < 0.5-1.1 ng/ml	61	CC 50–100 mg/day (from D_3 for 4 days) + hMG 75–150 IU/day (from D_8) Medication to avoid LH surge is NA	250 µg rec-hCG	CC 50–100 mg/day + hMG 75 IU-150/day (from OPU + 2–7 days) + Dydrogesterone 20 mg/day	NA	Significantly more oocytes albeit lower M-2 rate with LPS. Similar CPR and LBR with FPS- and LPS-derived embryos.

(Continued)

Table 1. (Continued)

Author	Design	Inclusion criteria	*N*	FPS Protocol	FPS Trigger	LPS Protocol	LPS Trigger	Conclusions
Jin and colleagues[30]	Retrospective	At least two following criteria: (1) ⩾40 years old, (2) history of poor response, ⩽3 oocytes with conventional stimulation, (3) FSH ⩾ 12 mIU/ml, AMH < 1.2 ng/ml or AFC ⩽ 6	76	CC 50–100 mg/day or LE: 5 mg/day (from D_3 for 5 days) + hMG:150–300 IU/day (from D_{6-7}) + GnRH-ant: 0.125 mg/day (when the follicle reaches 12 mm or LH > 8 IU/L)	GnRH-a or (5000–10,000 IU hCG)	CC 50–100 mg/day + hMG 150–300 IU/day (from OPU + 3 days) Medication to avoid LH surge is NA	5000–10,000 IU hCG	Significantly higher number of oocytes and embryos with LPS when compared with FPS. Similar pregnancy outcome with FPS- and LPS-derived embryos.
Madani and colleagues[31]	Prospective	⩽3 oocytes with conventional stimulation + at least one of the following criteria: (1) ⩾40 years old or other risk factors for POR (2) AFC < 5–7 or AMH < 0.5–1.1 ng/ml	121	CC 25 mg/day (from D_3 to trigger -1 day) + LE: 2.5 mg/day (from D_3 for 5 days) + hMG: 150 IU (from D_6, every other day) + Ibuprofen 0.6 g on the trigger day and trigger + 1 day	GnRH-a	LE: 2.5 mg/day (from OPU + 1 day until at least one follicle reaches 14 mm) hMG: 225 IU/day (from OPU + 1 day) + Ibuprofen 0.6 g on the trigger day and trigger + 1 day	GnRH-a	DS is time efficient and patient friendly
Alsberg and colleagues[32]	Retrospective	Bologna criteria	54	Single-dose Coriofollitropin alfa 150 mg (D_{2-3}) + rec-FSH 300–375 IU/day (from stimulation D_6; rec-LH was supplemented if female age ⩾ 35 years old) + GnrH-ant (from stimulation D_5)	GnRH-a	Single-dose Coriofollitropin alfa 150 mg (OPU + 4 day) + rec-FSH 300–375 IU/day (from stimulation D_6; rec-LH was supplemented if female age ⩾ 35 years old) + GnrH-ant (from stimulation D_5)	GnRH-a or 10,000 IU hCG (if there was no oocyte in the FPS)	Significantly higher number of oocytes but similar number of embryos with LPS. DuoStim is a valid alternative to FPS-only decreasing the risk of cycle cancelation.
Luo and colleagues[33]	Retrospective	At least two of the following criteria: (1) ⩾40 years old, (2) history of poor response, ⩽3 oocytes with conventional stimulation (3) AMH < 1.1 ng/ml or AFC < 7	304	rec-FSH or hp-FSH and hMG (150–300 IU/day) + GnRH-ant	rec-hCG, urinary hCG or GnRH-a	hMG 225 IU/day + MPA 10 mg/day (OPU + 1 day)	GnRH-a	Significantly higher number of oocytes and cryopreserved embryos with LPS. rec-FSH or GnRH-a performs better than urinary hCG for triggering LPS.
Bourdon and colleagues[34]	Observational study	Poor prognosis patients fulfilling POSEIDON criteria (Groups 1–4) and ⩽42 years old	53	300 IU/day FSH or hMG + GnRH-ant (from stimulation D_6)	GnRH-a	300 IU/day FSH or hMG + GnRH-ant (from stimulation D_6)	GnRH-a	The only study reporting significantly less number of oocytes with LPS.
Vaiarelli and colleagues[35]	Multicentre observational study	At least two of the following criteria: AMH ⩽ 1.5 ng/ml, AFC ⩽ 6, ⩽ 5 oocytes retrieved in a previous cycle, ⩾35 years old	827	Pre-treatment with luteal estradiol priming 4 mg/day + rec-FSH 300 IU/day + rec-LH: 150 IU/day (from D_{2-3}) + GnRH-ant (at least one follicle reaches 13–14 mm)	GnRH-a	rec-FSH 300 IU/day + rec-LH: 150 IU/day (from OPU + 5 days) + GnRH-ant (at least one follicle reaches 13–14 mm)	GnRH-a	Significantly higher number of M-2 oocytes with LPS. Blastulation and euploidy rates comparable following FPS and LPS. The rate of patients with at least one euploid blastocyst increases from 42.3% to 65.5% with DuoStim when compared with FPS-only.

AFC, antral follicle count; AMH, anti-mullerian hormone; CC, clomiphene citrate; CPR, clinical pregnancy rate; DS, double stimulation; DuoStim, dual stimulation; FPS, follicular phase stimulation; FSH, follicle-stimulating hormone; GnRH-a, GnRH agonist trigger; GnRH-ant, GnRH antagonist; hCG, human chorionic gonadotrophin; hMG, human menopausal gonadotrophin; hp-FSH, highly purified FSH; hp-hMG, highly purified hMG; LBR, live birth rate; LE, letrozole; LPS, luteal phase stimulation; MPA, medroxyprogesterone acetate; M-2 oocyte, metaphase-2 oocyte; M-2 rate, metaphase-2 oocyte rate; NA, not available; OPU, oocyte pick up; POR, poor ovarian responders; POSEIDON, Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number; rec-FSH, recombinant FSH; rec-hCG, recombinant hCG; rec-LH, recombinant LH.

higher number of retrieved oocytes, metaphase II oocytes, fertilized oocytes, day-3 embryos, and top-quality day-3 embryos.[52] To our knowledge, no study has employed dual/double triggering for either FPS or LPS during DS/DuoStim. Although urinary and recombinant human chorionic gonadotropin (hCG) use is associated with similar number of oocytes and clinical pregnancy rates (CPR) in IVF,[55,56] a recent study suggested that the use of GnRH-a or recombinant hCG (rec-hCG) performed better than urinary hCG (u-hCG) in both the FPS and LPS.[33]

Current available evidence comparing FPS and LPS in POR patients

Concomitant FPS and LPS was first reported in a 41-year-old POR woman by Xu and Li in 2013.[23] For FPS, they used 50–100 mg CC coupled with a daily dose of 150 IU FSH; despite two leading follicles of 16 and 18.5 mm in mean diameter in the right ovary on the day of triggering with GnRH-a, no oocyte could be retrieved. Although the patient wanted to drop-out at this stage, she was persuaded to undergo LPS, since she had had two antral follicles in the left ovary. Following LPS with 100 mg CC and a daily dose of 150 IU FSH, triggering was accomplished 10,000 IU u-hCG and one oocyte was retrieved; a cleavage stage embryo was cryopreserved. Of interest, the egg retrieval was performed 21 and 25 h after triggering in FPS and LPS, respectively. Unfortunately, the patient did not conceive with frozen embryo transfer (FET) of the available embryo.

Since this initial case report, several studies with different design, different OS regimens during FPS and LPS, and number of patients have been reported in PORs.[24–35] Although DS has also been employed for fertility preservation,[34,57] the available studies for this purpose have been excluded in this mini-review.

Kuang and colleagues,[25] in a pilot study, reported the so-called 'Shanghai protocol', in 38 POR patients fulfilling Bologna criteria. For FPS, CC (25 mg/day) was started on cycle day 3 and continued until the day before triggering; in addition, LE at a dose of 2.5 mg/day was used during cycle days 3–6 along with 150 IU human menopausal gonadotropins (hMG) every other day starting from cycle day 6. Ibuprofen 0.6 g on the trigger day and the following day was prescribed. One day after egg retrieval, if the patient had at least two antral follicles 2–8 mm in diameter, LPS was performed, using LE (2.5 mg/day) and 225 IU daily hMG. Medroxy-progesterone acetate (MPA) and ibuprofen were used to avoid premature ovulation. GnRH-a triggering was employed for triggering for both FPS and LPS. Following FPS, the mean number of oocytes retrieved was 1.7 ± 1.0; this figure was 3.5 ± 3.2 following LPS ($p = 0.001$). Of the 38 patients, 26 (68.4%) succeeded in producing 1–6 cleavage stage cryopreserved embryos; 21 patients underwent 23 FET, resulting in 11 ongoing pregnancies (47.8%).

In 2016, Wei and colleagues[28] confirmed the initial results of Kuang and colleagues, with the same protocol adopted in 23 POR patients fulfilling Bologna criteria; the number of oocytes was significantly higher with LPS when compared with FPS (3.5 ± 3.4 *versus* 1.6 ± 1.1, $p = 0.01$). In the same year, Ubaldi and colleagues,[24] using a prospective paired noninferiority observational study design, performed the so-called DuoStim in 51 POR patients [anti-mullerian hormone (AMH) $\leqslant 1.5$ ng/ml, antral follicle count (AFC) $\leqslant 6$ follicles, and $\leqslant 5$ oocytes retrieved in previous IVF cycles]. There were two distinctions from the previous two studies; first, a GnRH-ant protocol with a fixed recombinant FSH (rec-FSH) 300 IU/day dose combined with recombinant LH (rec-LH) 75 IU/day were used in both FPS and LPS. Second, PGT-A was performed. A GnRH-a was used for triggering final oocyte maturation for both FPS and LPS. In this study, the number of metaphase-2 (M-2) oocytes, fertilization rate, number of biopsied blastocysts, and euploidy rates were comparable following FPS and LPS. The authors concluded that DuoStim increased the final euploid blastocyst yield per ovarian cycle when compared with FPS-only.[24]

In 2017, Zhang and colleagues,[26] in a retrospective study of 153 POR patients fulfilling Bologna criteria, in line with the previous studies, reported that LPS resulted in significantly more oocytes, M-2 oocytes, and zygotes when compared with FPS. Of interest, in this study, embryos obtained following LPS yielded higher implantation rates (7.84 *versus* 27.69, $p = 0.014$).

In 2018, three retrospective studies were reported on DS.[27,29,30] Jin and colleagues,[30] in 260 POR patients fulfilling Bologna criteria, compared DS (Group A, $n = 76$) to LPS-only with conventional

OS (Group B; $n=52$) and FPS-only with mild OS (Group C; $n=132$). In Group A, although the number of oocytes and embryos available in the FPS were significantly less compared with LPS, performing DS increased both the number of oocytes and available embryos when compared with those of Group B and Group C. The reproductive outcome was comparable following FET in Groups A, B, and C. Rashtian and Zhang compared FPS and LPS in 69 POR patients; the definition of POR was day 3 FSH >15 IU/L, AFC <8 and at least one failed conventional IVF.[27] The mean age was 42 years. A GnRH-ant protocol with rec-FSH, LE, and CC were used for both FPS and LPS. The ovulation was triggered with a GnRH-a in FPS and with hCG in LPS. The number of oocytes retrieved was comparable between the FPS and LPS; hence, performing DS in a single menstrual cycle doubled the number of oocytes when compared with FPS-only. Zhang and colleagues,[29] in 61 patients fulfilling Bologna criteria, reported that the number of oocytes retrieved in LPS was significantly higher compared to FPS, although LPS yielded a lower rate of M-2 oocytes. However, CPR and LBR attained were not statistically different.

In 2019, Madani and colleagues[31] published a prospective clinical study of 121 patients fulfilling the Bologna criteria. Of the 121 eligible patients, 104 completed both FPS and LPS. DS was performed by LE, CC, hMG, and Ibuprofen and triggering was accomplished by a GnRH agonist. The authors concluded that 'this protocol can be considered a time-efficient and patient friendly regimen'. Alsbjerg and colleagues[32] reported a case series of 54 PORs classified according to the Bologna criteria; the mean age was 37 years. FPS was performed with corifollitropin-alfa; from the sixth day of OS, a daily bolus of 300 IU rec-FSH was added. Patients $\geqslant 35$ years old were treated with gonadotropins containing LH activity and younger patients were treated with rec-FSH only. Fixed GnRH-ant protocol was used starting on the fifth day of OS and GnRH-a triggering was employed for both FPS and LPS. The mean number of oocytes retrieved was significantly higher in LPS compared with FPS (3.7 ± 2.6 *versus* 2.4 ± 2.1, $p=0.002$) despite a significantly higher gonadotropin consumption and duration of stimulation during LPS. However, the mean number of embryos vitrified was comparable. The authors concluded that DuoStim using corifollitropin alfa and a subsequent individualized FSH dose appear to be a valid alternative to conventional follicular stimulation, decreasing the risk of cycle cancelation.

In 2020, three studies evaluated the performance of DS/DuoStim. Luo and colleagues,[33] using a retrospective study design, performed DuoStim in 304 patients fulfilling Bologna criteria.[33] For FPS, exogenous gonadotropin at a daily dose of 150–300 IU and a GnRH-ant was used. Triggering final oocyte maturation was accomplished with u-hCG (10,000 IU), rec-hCG (250 µg), or a GnRH-a. If $\geqslant 2$ follicles 5–10 mm were noted one day after egg retrieval, LPS was carried out using hMG at a daily dose of 225 IU along with 10 mg/day daily dose of MPA. Consistent with the previous studies,[26,28,29,32,35] the authors reported that LPS resulted in a significantly higher number of oocytes retrieved, normally fertilized oocytes, cleaved embryos, cryopreserved embryos, and good quality embryos when compared with those counterparts during FPS. The three different agents used for triggering at the end of FPS resulted in comparable embryological outcome. However, of interest, the rates of cryopreserved embryos and good quality embryos were significantly higher following LPS in those patients who were triggered by rec-HCG or GnRH-a when compared with u-hCG at the end of FPS or LPS. This unexpected finding is in contrast with previous studies reporting comparable oocyte yield and CPR following u-hCG and rec-hCG trigger in patients undergoing conventional IVF with fresh embryo transfer using the long GnRH-agonist protocol.[55,56]

In a recent French observational cohort study,[34] 77 patients underwent DS; of those 77 patients, 53 were poor prognosis patients fulfilling POSEIDON criteria (Group I, $n=12$; Group II, $n=23$; Group III, $n=5$; Group IV, $n=13$) and the remaining 24 underwent DS for fertility preservation. In contrast to the previous studies, the number of oocytes was significantly higher following FPS compared with LPS (4.83 ± 3.26 *versus* 3.64 ± 3.18, $p=0.019$, respectively). Of note, the total FSH dose and duration of stimulation during FPS were significantly less when compared with LPS. Differences in patient population might contribute to the discrepant results with the previous studies since in this study not only patients with diminished ovarian reserve (POSEIDON Groups III and IV) were included but also those with hypo-response despite adequate ovarian reserve (POSEIDON Group I and II).

Ubaldi and colleagues[24] have contributed several manuscripts on DuoStim[14,35,58–60] following their initial noninferiority study in 2016. As mentioned previously, distinct from the previous studies, PGT-A and single euploid vitrified–warmed blastocyst transfer is their policy. DuoStim protocol involved a pretreatment with luteal oestradiol priming (4 mg/day of oestradiol valerate) on day 21 of the previous menstrual cycle to promote the synchronization of the follicular growth. FPS was started with a fixed dose of rec-FSH 300 IU/day plus rec-LH 150 IU/day for 4 days. A flexible GnRH-ant is administered daily following identification of a leading follicle of 12–14 mm in diameter both during FPS and LPS until the day of ovulation trigger. The final maturation of oocytes is triggered with a subcutaneous bolus of GnRH-a. Five days after the first retrieval, LPS is started with the same protocol and daily dose regardless of the number of visible antral follicles. A total of 310 patients fulfilling at least two of the following parameters, AMH $\leqslant$ 1.5 ng/ml, AFC $\leqslant$ 6, previous oocytes retrieved $\leqslant$ 5, and maternal age $\geqslant$ 35 years, underwent DuoStim.[58] The mean number of M-2 oocytes was significantly higher following LPS compared with FPS (4.7 ± 3.0 *versus* 4.0 ± 2.5, $p < 0.01$). The fertilization, blastulation, and euploidy rates were comparable. Importantly, the rate of patients obtaining one euoploid blastocyst increased from 42.3% (131/310) after FPS to 65.5% (203/310) with the contribution of LPS. In a larger series of 827 women undergoing DuoStim, the same group recently reported similar clinical, obstetric, and neonatal outcome following transfer of LPS-derived euploid blastocysts when compared with FPS-derived ones.[35]

Comparison of DS/DuoStim ve FPS-only

In line with the above given data, and as expected, the available four studies[14,30,61,62] comparing DS/DuoStim with conventional OS (single FPS) in POR patients report significantly less cycle cancelation rates[30,61] and significantly higher number of oocytes[30,61,62] M-2 oocytes,[61,62] blastocysts,[14] and cryopreserved/available embryos[30,61] with DS/DuoStim.

Critics of the available data

It is clearly evident that DS/DuoStim increases the number of oocytes when compared with FPS-only. It is of interest that, apart from one study,[34] the number of oocytes retrieved at LPS is either the same[24,27,31] or higher[25,26,28–30,32,33,35] when compared with FPS (Table 2). The reason for this is not clear but may be related to more synchronous follicular development due to high estrogen and progesterone levels during LPS.[59] Moreover, such *in vivo* milieu at the LPS stage may lead to an increase in angiogenic factors, thereby promoting the sensitivity of granulocytes to FSH.[63] Another hypothesis is a possible flare-up effect derived from the GnRH agonist trigger in the FPS, which might induce a down-regulation in the expression of AMH in the follicles from the anovulatory wave, thereby increasing the number of follicles with a 3–4 mm diameter recruited in the LPS.[64] However, all these speculations need to be confirmed, as well as the role of endocrine and paracrine factors better unveiled, to understand the mechanisms modulating the recruitment of follicles growing in the anovulatory wave of the ovarian cycle. However, one should also keep in mind that different OS regimens with higher gonadotropin consumption[25,26,31–34] and duration of stimulation[26,33–35] may also contribute to higher number of oocytes at LPS.

LPS-derived oocytes show similar competence as FPS-derived ones, including fertilization, blastulation, and euploidy rates.[24,35,58] A recent study reported no significant differences in the miRNA signature of the follicular fluid during FPS and LPS stages,[65] complementing embryological and chromosomal equivalence between these two stages.

A recent DELPHI consensus reported that 'we recommend that it should only be used when the need to obtain oocytes is urgent, including patients with malignant diseases undergoing oocyte cryopreservation and patients of advanced maternal age or with reduced ovarian reserve'.[66] The recent ESHRE Ovarian Stimulation Guideline for IVF/ICSI states that 'Due to absence of RCT, comparing a double stimulation within a same cycle with mandatory postponed transfer and two conventional stimulations, we cannot recommend the double stimulation in poor responder patients'.[67] Future randomized controlled trials comparing two consecutive conventional OS (two FPS-only) with DS/DuoStim are warranted to delineate the role of this strategy in PORs. Moreover, in the personalized medicine era, other large-scale studies are warranted to delineate the features, beyond classification as of

Table 2. The laboratory data and reproductive performance of follicular and luteal phase stimulation of the available studies.

Author	No. of oocytes		Fertilization rate		No./rate of cryopreserved embryos		Clinical pregnancy rate		Miscarriage/early pregnancy loss		Ongoing pregnancy/live birth rate	
	FPS	LPS	FPS	LPS	FPS	LPS	FPS	LPS	FPS	LPS	FPS	LPS
Kuang and colleagues[25]	1.7 ± 1.0[a]	3.5 ± 3.2[a]	69.8%	75.6 %	0.9 ± 1	1.3 ± 1.4 (Cleavage + Blastocyst)	61.5 (8/13)	71.4% (5/7)	12.5 (1/8)	20% (1/5)	53.8% (7/13)	57.1% (4/7)
Ubaldi and colleagues[24]	5.1 ± 3.4	5.7 ± 3.3	69.7%	78.6%	0.6 ± 0.8	0.7 ± 0.8*	85.7% (6/7)	75% (6/8)	16.7% (1/6)	16.7% (1/6)	71.4% (5/7)	62.5% (5/8)
Wei and colleagues[28]	1.6 ± 1.1[b]	3.5 ± 3.4[b]	100%	100%**	NA	NA	50% (1/2)	33% (4/12)	0% (0/2)	0% (0/12)	NA	NA
Zhang and colleagues[26]	2.2 ± 1.6[c]	3.3 ± 2.6[c]	75.9%	73.1%	NA	NA	10.71% (3/28)[d]	38.89% (14/36)[d]	0% (0/3)	14.2% (2/14)	10.7% (3/28)	27.8% (10/36)
Rashtian and Zhang[27]	1.6 ± 0.2	1.9 ± 0.2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Zhang and colleagues[29]	1.3 ± 0.9[e]	1.8 ± 1.1[e]	78.5%	86.9%	NA	NA	21.4% (3/14)	13.8% (4/29)	0% (0/3)	25% (1/4)	14.3% (2/14)	10.2% (3/29)
Jin and colleagues[30]	1 (1–2)[f]	2 (1–4)[f]	96.0%	95.7%	NA	NA	35% (7/20)	37.5% (12/32)	0% (0/7)	8.1% (1/12)	NA	NA
Madani and colleagues[31]	1.52 ± 1.16	1.50 ± 1.98	NA	NA	1.75 ± 0.99[g]	0.85 ± 1.22[g] (Cleavage)	NA	NA	NA	NA	NA	NA
Alsberg and colleagues[32]	2.4 ± 2.1[h]	3.7 ± 2.6[h]	NA	NA	1.5 ± 0.9	1.8 ± 1.8 (Cleavage + Blastocyst)	NA	NA	NA	NA	NA	NA
Luo and colleagues[33]	1.71 ± 1.30[i]	3.58 ± 4.54[i]	62.43%	62.50%	0.9 ± 0.78[j]	1.82 ± 2.54[j] (Cleavage + Blastocyst)	NA	NA	42.85% (6/14)	21.15%(11/52)	NA	NA
Bourdon and colleagues[34]	5.25 ± 3.38[k]	3.83 ± 3.14[k]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Vaiarelli and colleagues[35]	4.8 ± 2.1[l]	6.6 ± 6.6[l]***	NA	NA	1.5 ± 0.8	1.8 ± 1.1*	NA	NA	15% (14/94)	14% (16/118)	44% (80/182)	49% (102/207)

FPS, follicular phase stimulation; ICSI, intracytoplasmic sperm injection; LPS, luteal phase stimulation; NA, not applicable.
[a]$p = 0.001$.
[b]$p = 0.01$.
[c]$p < 0.001$.
[d]$p = 0.040$.
[e]$p = 0.035$.
[f]p level not available.
[g]$p = 0.03$.
[h]$p = 0.002$.
[i]$p = 0.000$.
[j]$p = 0.000$.
[k]$p = 0.001$.
[l]$p < 0.01$.
*No. of euploid blastocysts.
**Fertilization rate with ICSI.
***No. of M_2 oocytes.

a poor prognosis, to predict which couples might benefit the most from a DS/DuoStim protocol.

The mandatory freeze-all and lack of cost-effectiveness data are the weaknesses of DS/DuoStim. Although initial findings of comparable obstetric and neonatal outcome of FPS- and LPS-derived embryos are reassuring,[35,68] further large-scale studies are warranted for the long-term safety of this approach.

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