



Review Article

Dual Stimulation

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ABSTRACT

The traditional concept of starting controlled ovarian stimulation (COS) exclusively in the early follicular phase has been challenged by the recent evidence on the multiple wave theory of follicular recruitment within a menstrual cycle. This understanding has led to the development of a new ovarian stimulation protocol for in vitro fertilisation (IVF) known as Dual stimulation or DuoStim, especially for poor responders. Dual stimulation implies follicular phase stimulation (FPS) followed by luteal phase stimulation (LPS) within the same menstrual cycle. The main advantage of this protocol includes an increase in the number of oocytes and embryos obtained per menstrual cycle, thus improving the chance of live birth per DuoStim cycle compared to single conventional stimulation. Dual stimulation appears to be beneficial for poor responders and advanced maternal age women, especially to rescue poor blastocyst yield after one conventional COS and avoid further maternal ageing between IVF attempts. This strategy appears to have led to a significant reduction in patient dropout rates in these women. It is also a relevant approach for patients undergoing the accumulation of embryos for pre-implantation genetic testing, where one aims to maximise the number of oocytes/embryos in a limited period. The majority of evidence has shown better performance of LPS in comparison to FPS in the context of the number of oocytes and embryos obtained in DuoStim cycles. However, mandatory freeze all, high cycle cancellation and lengthening of stimulation days in LPS are a few drawbacks. However, presently, multicycle counselling using DuoStim or similar protocols appears to be a relevant change needed in IVF to increase oocyte/embryo number in a short time frame and thus reduce attrition.

Keywords: Double stimulation, Dual stimulation, DuoStim, IVF, Poor responders

INTRODUCTION

Dual stimulation (DuoStim), as the name implies, means two controlled ovarian stimulation (COS) cycles for in vitro fertilisation (IVF) within one menstrual cycle with the purpose of maximising oocyte/embryo collection in a shorter period. It is used in cases undergoing COS for IVF where we anticipate that we may not be able to obtain an adequate number of oocytes by one stimulation cycle to achieve a live birth. Recent evidence on the physiology of follicular recruitment suggests that follicular recruitment by the 'multiple wave' theory within every menstrual cycle. It implies that two to three cohorts of antral follicles are recruited and available for stimulation in a single menstrual cycle, both in the follicular and luteal phases. This concept is contradictory to the previous understanding that follicular growth occurs only during the follicular phase of the menstrual cycle under the single wave theory of follicular recruitment.^[1]

DuoStim is a newer ovarian stimulation protocol used for COS in IVF first introduced by Kuang *et al.* in 2014 (Shanghai protocol), to collect embryos for poor prognosis women entering the IVF program.^[2] This method combines consecutive stimulations in the follicular and luteal phases of a single ovarian cycle to maximise the number of oocytes, hence embryos, formed within a cycle. It also needs to be understood that all embryos will have to be cryopreserved in the DuoStim protocol, ruling out the option of fresh transfer. This protocol could only be established after the development and standardisation of oocyte or embryo vitrification in contrast to the slow freezing programme used earlier. All embryos generated in these stimulation cycles are transferred in subsequent frozen thaw embryo transfer cycles only. This review aims to summarise the current evidence regarding the utility of DuoStim since it was first used by Kuang *et al.* in 2014 in poor responder women undergoing IVF cycles.^[2]

WHOM SHOULD BE CONSIDERED FOR DUOSTIM?

Poor responders are the ones for whom DuoStim was initially developed by Kuang *et al.* in 2014,^[2] and it is still the most common indication for this protocol. Almost 40% of women entering an IVF programme are poor responders belonging to any of the four Poseidon groups.^[3] They either have advanced maternal age, lower ovarian reserve or an unexpected poor response to stimulation. Another reason for undertaking the DuoStim protocol is when, despite an adequate number of oocytes obtained in the COS cycle, embryo conversion is poor, resulting in an insufficient number of embryos required to achieve a live birth. With increasing experience of using DuoStim in IVE, it has also become the preferred method of COS for women undergoing IVF for pre-implantation genetic testing (PGT) to collect more embryos in a limited period for the purpose of genetic testing for monogenic disorders or structural rearrangement. It can also be used for advanced maternal age women undergoing IVF for infertility to allow PGT for aneuploidy, since only 20% of embryos are euploid in women over 40 years of age.^[4] Fertility preservation is another common area for the use of DuoStim; it has been used for fertility preservation in cancer patients awaiting surgery or chemo/radiotherapy, where they have at least a month to undergo two stimulation cycles. However, in these patients, due to paucity of time, a random start protocol is more commonly undertaken, which can be started any time in a menstrual cycle to obtain oocytes/embryos for cryopreservation, not delaying the onset of their cancer treatment. Here the concept of multiple wave theory is applicable, like in the DuoStim protocol, enabling us to start stimulation during the mid-follicular, luteal, or mid-luteal phase also.

PROTOCOLS

Various regimes have been described in studies published on DuoStim since its first use in 2014 by Kwang's group, with differences in stimulation protocols, methods of luteinising hormone (LH) suppression, type of trigger, and gap days between two stimulations.

Stimulation Protocols

Both mild and conventional stimulation have been used in studies on DuoStim. Kuang *et al.* in 2014 were the first to describe double stimulation (Shanghai protocol) using mild stimulation in the follicular phase.^[2] Following this, many other studies used mild stimulation protocols in follicular phase stimulation (FPS) and/or luteal phase stimulation (LPS) [Table 1].^[5-7] Ubaldi *et al.* in 2016 were the first to describe conventional stimulation in DuoStim.^[8] In most subsequent studies to date, the conventional stimulation protocol has been used [Table 2]. Although mild stimulation using clomiphene citrate and/or letrozole has been described for poor responders, evidence does not support their use due to the high risk of cycle cancellation and a smaller number of oocytes obtained.^[9]

Methods of LH Suppression

Different strategies have been adopted to prevent premature LH surge during FPS and LPS. These include the use of either a GnRH antagonist (GnRH-ant), progesterone, clomiphene citrate or ibuprofen [Tables 1 and 2]. Use of progesterone during LPS not only prevents premature LH surge but also avoids menstruation during oocyte retrieval (OCR), reducing the possible risk of infection. Progesterone-primed ovarian stimulation (PPOS) was introduced by Kuang *et al.* in 2015 for suppressing the LH surge in COS freeze-all cycles.^[10] It is now being extensively used in cases where fresh transfer is not planned, such as donor cycles, oocyte/embryo freezing for cancer patients, social reasons or cycles requiring PGT. However, the use of PPOS in DuoStim protocols to suppress LH surge in the luteal phase has been scarce as of now. Ours was one of the few studies to use medroxyprogesterone acetate (MPA) 10 mg per day in LPS to prevent LH surge [Figure 1].^[11,12] This also precludes the use of antagonists to control the LH surge, reducing the number of injections in LPS, thereby improving patient compliance and decreasing treatment cost.

Type of Trigger

GnRH agonist is the most used trigger in FPS of DuoStim to promote early luteolysis so that the corpus lutea does not interfere with transvaginal ultrasound follicle assessment in LPS. In our study, human chorionic gonadotropin (hCG) was

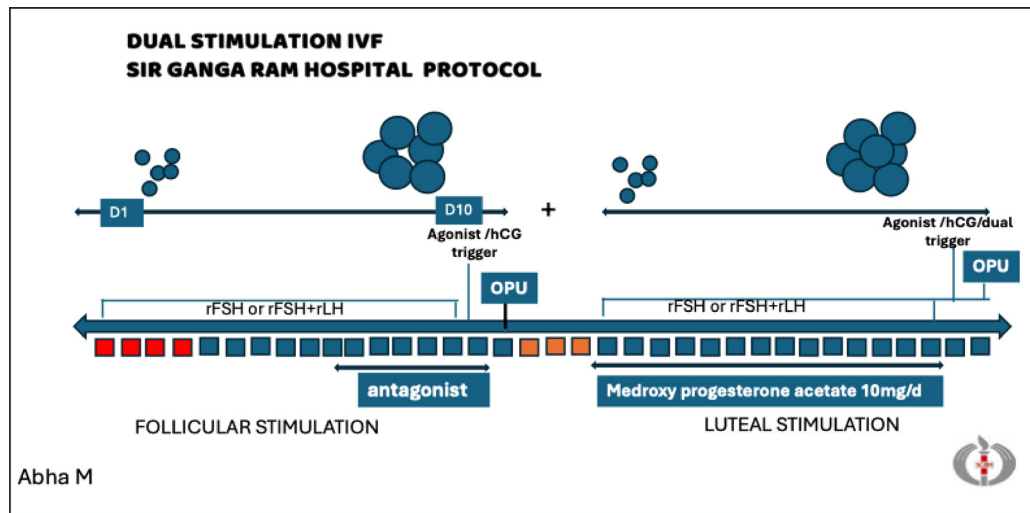


Figure 1: DuoStim protocol used at our center.

also often used as a trigger in FPS to allow the option of fresh transfer in cases where DuoStim was started in a previous poor responder with adequate reserve to allow the option of fresh transfer without compromising the luteal phase in case she made an adequate number of transferable or usable embryos. However, we found that this trigger did not interfere with follicle monitoring in LPS, as the first ultrasound was done directly after 11 days of FPS OCR (after 8 days of stimulation post 3 days of OCR).^[12] In LPS, either hCG or a GnRH agonist or both have been equally used in studies.

Gap Days Between FPS and LPS

The number of gap days between the first OCR and the start of the next stimulation differs in most studies. There have been studies which start luteal stimulation on the next day of OCR, and these are generally studies using mild stimulation protocols.^[2,5-7] Our study started luteal stimulation after 3 days, while Ubaldi *et al.*, Alsberg *et al.* and Vairelli *et al.* started it after 5 days.^[8,13,14] The long 5-day gap between the two stimulations is beneficial in two ways: the number of usable blastocysts is known before starting the luteal stimulation; hence, one could decide the need for DuoStim. Apart from this, even though most studies show a longer luteal stimulation in DuoStim protocols, the number of stimulation days of LPS was approximately 1–2 days shorter in a 5-day gap of LPS start compared to the early start of luteal stimulation after the first OCR.

MERITS

DuoStim can increase the probability of attaining a live birth by significantly increasing the number of oocytes and usable embryos per menstrual cycle in comparison to conventional

follicular stimulation alone, particularly in women with low ovarian reserve or poor responders. Regarding the performance of LPS vs. FPS in the majority of studies, LPS performed better than FPS in terms of the number of oocytes and usable embryos, including our study.^[5,12-14] A possible explanation could be the recruitment of a more synchronised antral follicle cohort in LPS due to high oestradiol levels during FPS and constant progesterone exposure post OCR.^[15] Gonadotropin stimulation and high follicular oestradiol levels may increase FSH receptor sensitivity in granulosa cells of pre-antral and small antral follicles, leading to a greater response in LPS.^[16]

One of the main benefits observed in most studies was reduced patient dropout rates, including our study. From a psychological perspective, the greater the couple's expectations invested in their first attempt, the greater their disappointment in case of failure, and the higher the risk of treatment dropout. In our study, the majority of women with poor ovarian reserve or response and previous IVF failures agreed to undergo DuoStim after counselling, especially when a financial benefit was added to such cycles.^[12] Hence, in poor responders, dropout rates tend to be higher after failure of a single conventional IVF cycle, particularly when no oocytes or embryos are obtained. Our study found that LPS rescued 70% of cycles where no embryos were formed in FPS.^[12]

All studies have shown comparable oocyte and embryo competence obtained after FPS and LPS. Use of PPOS in the luteal phase makes DuoStim more patient friendly and cost effective by alleviating the need for antagonist injections and reducing frequent monitoring in LPS. In addition, it also averts menstruation during LPS, reducing the possible risk of infection during OCR.^[12,13]

Table 1: DuoStim studies using mild stimulation.

Authors	Study design	Study population	No. of patients	FPS protocol	FPS trigger	LPS protocol	LPS trigger	Conclusion
Kuang <i>et al.</i> ^[2]	Pilot	At least two of these criteria: (a) Age > 40 years (b) ≤3 oocytes with conventional COS (c) AFC < 5 (d) FSH > 10 IU (e) Prior ovarian surgery	38	CC 25 mg/day (from D3 to day prior to trigger), Letroz 2.5 mg/day (from D3 for 4 days), hMG 150 IU (from D6 alternate day) LH suppression: CC 25 mg/day till the day prior to trigger, Ibuprofen 0.6 g on trigger day and the day after trigger	GnRH _a	Letroz: 2.5 mg/day + (from day of OPU until at least 1 follicle ≥ 12 mm) + hMG 225 IU/day (from day of OPU) LH suppression: MPA 10 mg/day, Ibuprofen 0.6 g on trigger day and the day after trigger	GnRH _a	Significantly higher number of oocytes retrieved in LPS vs. FPS.
Zhang <i>et al.</i> ^[5]	Retrospective	At least two of these criteria (Bologna criteria): (a) Age ≥ 40 years or any risk factor for POR (b) ≤3 oocytes with conventional COS (c) AFC < 5–6 or AMH < 0.5–1.1 ng/ml	153	CC 50 mg/day (from D3 until trigger day), FSH 150 IU/day (if follicle growth <1 mm/day) LH suppression: CC 50 mg/day till trigger day, Ibuprofen 0.3 g/6 hours from the trigger to OPU day	GnRH _a	hp-FSH 150–225 IU/day next day after OPU LH suppression: none	hCG	Significantly higher number of oocytes, embryos and implantation rate in LPS vs. FPS.
Rashtian and Zhang ^[6]	Retrospective	Day 3 FSH >15 IU/ml and AFC < 8 with at least one failed conventional IVF	69	Oral contraceptive pill for 1 week before starting FPS, CC 50 mg/day until trigger LE 2.5 mg/day (for 5 days), FSH 75 IU/day LH suppression: GnRH-ant	GnRH _a	CC 50 mg/day, Letroz 2.5 mg/day, FSH 75 IU/day (from the day after OPU) LH suppression: none	hCG	Similar numbers of oocytes in FPS and LPS; DuoStim doubles the number of oocytes when compared to FPS only.
Madani <i>et al.</i> ^[7]	Prospective	Bologna criteria	121	CC 25 mg/day (from D3 to 1 day before trigger), LE 2.5 mg/day (from D3 for 5 days), hMG 150 IU (from D6 every alternate day) LH suppression: CC 25 mg/day till the day prior to trigger, Ibuprofen 0.6 g on trigger day and the day after trigger	GnRH _a	LE 2.5 mg/day (from the day after OPU until at least 1 follicle ≥ 14 mm), hMG 225 IU/day (from the day after OPU) LH suppression: Ibuprofen 0.6 g on the trigger day and the day after trigger	GnRH _a	Fertilisation rate and number of embryos are higher in FPS vs. LPS; DuoStim is a time-saving and patient-friendly regimen.

COS: Controlled ovarian stimulation, CC: Clomiphene citrate, LE: Letrozole, OPU: Oocyte pick up, POR: Poor ovarian responder, hMG: Human menopausal gonadotropin, GnRH_a: GnRH agonist, GnRH-ant: GnRH antagonist. FSH: Follicle stimulating hormone, IU: International unit, FPS: Follicular phase stimulation, LPS: Luteal phase stimulation, LH: Luteinising hormone, IVF: In vitro fertilization.

Table 2: DuoStim studies using conventional stimulation.

Authors	Study design	Study population	No. of patients	FPS protocol	FPS trigger	LPS protocol	LPS trigger	Conclusion
Ubaldi <i>et al.</i> ^[8]	Prospective	AFC \leq 6, AMH \leq 1.5 ng/ml and/or \leq 5 oocytes retrieved in previous cycle	51	rFSH 300 IU/day and rLH 75 IU/day LH suppression: GnRH-ant	GnRHa	Same as FPS started 5 days after OPU	GnRHa	Number of oocytes, fertilisation, blastulation and euploidy rate comparable in FPS and LPS. Higher chance of obtaining > 1 euploid blastocyst with DuoStim.
Alsbjerg <i>et al.</i> ^[13]	Retrospective	Bologna criteria	54	Single coriofolliotropin alfa 150 mg on D2/3, rFSH 300–375 IU/day (from stimulation D6; rLH supplemented (if female age \geq 35 years) LH suppression: GnRH-ant	GnRHa	Same as FPS started 5 days after OPU	GnRHa or HCG	Significantly higher oocyte number but similar number of embryos in LPS vs. FPS. DuoStim is a valid option to FPS alone due to the decrease in risk of cycle cancellation.
Luo <i>et al.</i> ^[11]	Retrospective	Bologna criteria	304	rFSH or HP FSH and hMG (150–300 IU/day) LH suppression: GnRH-ant	hCG or GnRHa	hMG 225 IU/day 1 day after OPU LH suppression: MPA 10 mg/day	GnRHa	Similar numbers of oocytes in FPS and LPS; performing DuoStim doubles the number of oocytes when compared to FPS alone.
Bourdon <i>et al.</i> ^[17]	Observational	Poor prognosis patients fulfilling Poseidon criteria (groups 1–4) and \leq 42 years	53	300 IU/day FSH or hMG LH suppression: GnRH-ant	GnRHa	Same as FPS after 1–6 days of OPU	GnRHa	Significantly less number of oocytes with LPS in comparison to FPS.
Vaiarelli <i>et al.</i> ^[14]	Multicenter observational study	At least two of these criteria: AMH \leq 1.5 ng/ml, AFC \leq 6, \leq 5 oocytes retrieved in a previous cycle, \geq 35 years old	827	Pre-treatment with luteal oestradiol 4 mg/day, rFSH 300 IU/day, and rLH 150 IU/day (from D2/3) LH suppression: GnRH-ant	GnRHa	Same as FPS after 5 days of OPU LH suppression: GnRH-ant		Significantly higher number of mature oocytes with LPS. Blastulation and euploidy rate are comparable between FPS and LPS. Patients with at least one euploid blastocyst increased from 42.3% to 65.5% with DuoStim when compared with FPS alone.
Majumdar <i>et al.</i> ^[12]	Retrospective	Low prognosis group according to Poseidon or poor response in FPS	118	rFSH 300 IU/day. LH suppression: GnRH-ant	hCG or GnRHa	Same as FPS after 3 days of OPU LH suppression: MPA 10 mg/day after 48 hours of OPU	hCG or GnRHa	Significantly higher number of oocytes in LPS vs. FPS. 70% of cycles were rescued by LPS where no embryos formed in FPS.

r: Recombinant, HP: Highly purified, OPU: Oocyte pick up, hMG: Human menopausal gonadotropin, GnRHa: GnRH agonist, GnRH-ant: GnRH antagonist, MPA: Medroxy progesterone acetate, AFC: Antral follicle count, AMH: Anti mullerian hormone, FSH: Follicle stimulating hormone, LH: Luteinising hormone, IU: International unit, FPS: Follicular phase stimulation, LPS: Luteal phase stimulation.

DEMERITS

Studies have shown an increase in the number of days of stimulation required in LPS compared to FPS by 1–3 days. Researchers have tried to reduce it by increasing the time gap between the OCR day in FPS to as much as 5 days before starting luteal stimulation.^[8,12–14] The addition of letrozole at the beginning of luteal stimulation has also been proposed to help reduce days of stimulation by a few studies.^[2,7] There are also higher cycle cancellation rates in LPS compared to FPS, due to no response (22% in our study).^[11,12,14,17] Another demerit of LPS is the occurrence of menstrual bleeding during stimulation and/or during the subsequent OCR. This, apart from posing a risk of infection, may be a cause of apprehension to patients. However, it can be overcome by using PPOS in LPS instead of the antagonist protocol.^[12,13] The DuoStim protocol does not allow the option of fresh transfer; hence, mandatory freeze-all is necessary. However, in our study, the use of the hCG trigger in FPS allowed patients to opt out of the DuoStim protocol and undergo a fresh transfer in case of good embryo conversion.^[12] Currently, there is a lack of well-designed studies or a large, robust randomised controlled trial (RCT) analysing the effectiveness of DuoStim. We also need studies on the cost-effectiveness analysis of this protocol.

COMPARISON WITH OTHER NEWER STIMULATION PROTOCOLS FOR POOR RESPONDERS

BISTIM Protocol

An alternate method for collecting more oocytes by two stimulation cycles has been proposed as the bistimulation (BISTIM) protocol, where two follicular stimulations are done in two consecutive cycles. Massin *et al.* in 2023 conducted an RCT to compare this protocol to DuoStim and found comparable results in the number of oocytes and embryos obtained.^[18] However, many of the study findings have been challenged by Ubaldi *et al.* in a subsequent publication.^[19] The BISTIM protocol defeats the main purpose of DuoStim, which is to shorten the time of oocyte collection and embryo generation. Though BISTIM allows the option of fresh transfer, the dropout rates in BISTIM are significantly higher because often, if one good-quality embryo is formed in the first stimulation, couples may be tempted to go through a fresh transfer and opt out of BISTIM. In addition, if pregnancy occurs and fails to reach live birth, the second stimulation is delayed by 6–12 months, which becomes a major drawback for poor responders with advanced age and/or low ovarian reserve.

DuoStim Fresh

Another method of double stimulation in low-prognosis women less than 40 years old has been proposed recently. In

this protocol, which is called ‘DuoStim fresh’, first stimulation is started in the luteal phase for oocyte collection and embryo vitrification, followed by a second stimulation after 5 days in the subsequent follicular phase and fresh embryo transfer of the best embryo formed so far. This method of stimulation was compared with a single conventional follicular stimulation, and there was no difference in oocyte numbers in the three stimulation cycles.^[20] In this protocol, the time taken for embryo collection is the same as in the standard DuoStim protocol. The only advantage of the ‘DuoStim fresh’ protocol is that the option of fresh transfer is available. However, the probability of obtaining a higher number of oocytes and embryos in the luteal phase, which was seen in studies using the conventional DuoStim protocol, is not seen in this study. The explanation for this is that probably the standard DuoStim, where FPS precedes LPS, stimulates the smaller follicles to an extent that they outnumber and grow better in the luteal phase compared to stimulation started directly in the luteal phase.

CONCLUSION

Of all women undergoing IVF, protocols for normal and high responders have been very well delineated with the aim of reducing complications and optimising results. However, in poor prognosis women, the pursuit for an ideal protocol to optimise results and achieve successful live birth continues. This group mainly consists of women with advanced age or low ovarian reserve who, on one hand, have less time and on the other hand suffer extreme disappointment after one failed embryo transfer due to no surplus embryos. As a result, the burden of going through another stimulation cycle leads to a huge dropout rate in these women. Two stimulations within one menstrual cycle are proving to be a good tool to accumulate embryos in the shortest possible time, also reducing dropout rates significantly. Till we find a method to improve oocyte and embryo competence in ageing women undergoing IVF, such as mitochondrial transfer or a similar procedure, multicycle counselling using DuoStim or a similar protocol appears to be a relevant change needed in IVF. In women undergoing PGT for genetic disorder/aneuploidy or fertility preservation for cancers, this protocol appears suitable for embryo pooling. In most studies on DuoStim, LPS found a higher number of usable embryos compared to FPS. This advantage was not observed in reverse stimulations such as luteal followed by follicular (DuoStim fresh) or two subsequent follicular stimulations (BISTIM protocol). However, we need more robust RCTs to substantiate the effectiveness of the DuoStim protocol as an ideal protocol for this group of women undergoing IVF. The practical utility of offering such treatments must be to make such protocols affordable to support the couple’s chance of achieving a live birth to fulfil their dream of parenthood.

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