



## REVIEW ARTICLE

## What are the advantages of the DuoStim strategy?

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**Abstract** In recent years, folliculogenesis in women has been shown to occur in a wave-like pattern, with two or three waves of follicular development during one menstrual cycle. Moreover, it has been shown that follicles formed beyond the follicular phase have similar ovulation potential, opening new windows for assisted reproductive treatment (ART). Thus, the double stimulation strategy has been proposed as an attractive alternative to obtain a higher number of eggs and embryos in a shorter period of time, which would theoretically increase the cumulative live birth rate and reduce the time to pregnancy. In this protocol, controlled ovarian stimulation (COS) is performed twice in the same menstrual cycle, taking advantage of both the follicular and the luteal phase. The results obtained so far indicate that luteal phase stimulation is not inferior to follicular phase stimulation. Additionally, the double stimulation protocol has proved to be as safe as conventional COS, while improving reproductive outcomes when compared to single follicular phase stimulation. In this context, it also emerges as an interesting option for emergency fertility preservation in oncologic patients. However, the absence of randomized controlled trials and cost-effective analysis raises important concerns that need to be addressed in depth via well-designed studies.

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### PALABRAS CLAVE

Duostim;  
Doble estimulación;  
Fase lútea;  
PGT-A

### ¿Cuáles son las ventajas de la estrategia DuoStim?

**Resumen** En los últimos años, se ha demostrado que la foliculogénesis en las mujeres se produce en un patrón ondulatorio, con 2 o 3 oleadas de desarrollo folicular durante un ciclo menstrual. Además, se ha demostrado que los folículos formados más allá de la fase folicular tienen un potencial ovulatorio similar, lo que abre nuevas ventanas para el tratamiento de

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reproducción asistida. Así, la estrategia de doble estimulación se ha propuesto como una alternativa atractiva para obtener un mayor número de óvulos y embriones en un periodo más corto, lo que teóricamente aumentaría la tasa acumulada de nacidos vivos y reduciría el tiempo hasta el embarazo. En este protocolo, la estimulación ovárica controlada (COS) se realiza 2 veces en el mismo ciclo menstrual, aprovechando tanto la fase folicular como la lútea. Los resultados obtenidos hasta ahora indican que la estimulación de la fase lútea no es inferior a la estimulación de la fase folicular. Además, el protocolo de doble estimulación ha demostrado ser tan seguro como el COS convencional, al tiempo que mejora los resultados reproductivos en comparación con la estimulación en fase folicular única. En este contexto, también surge como una opción interesante para la preservación de la fertilidad de emergencia en pacientes oncológicas. Sin embargo, la ausencia de ensayos controlados aleatorizados y de análisis de rentabilidad crea importantes preocupaciones que deben ser abordadas en profundidad mediante estudios bien diseñados.

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## Introduction

Traditionally, follicular development in the ovaries was described to occur only in the early follicular phase. During ovarian stimulation (OS), antral follicles grow following the administration of exogenous gonadotropins. Recently, the presence of several waves of cyclical follicular development within the same menstrual cycle in humans has been proven (Baerwald et al., 2003a). This implies that most women have multiple waves of follicular development, although only one wave is ovulatory, due to the inhibitory effect of progesterone preventing a second LH surge and ovulation. However, it has been suggested that follicles formed during the luteal phase may have the same ovulation potential in the presence of the LH surge, thus offering new possibilities for ovarian stimulation (Kuang et al., 2014; Cardoso et al., 2017).

Improvements in vitrification techniques and the development of new exogenous gonadotropins have allowed the emergence of innovative OS protocols, such as random start or luteal phase stimulation (Liu et al., 2017), especially relevant in cases that require urgent intervention (Tsampras et al., 2017; Vaiarelli et al., 2018). After the publication of convincing data using these approaches, different researchers have described the double stimulation protocol, referred to as DuoStim, Dual Stim or Shanghai protocol (Cimadomo et al., 2018; Ubaldi et al., 2016). Double stimulation (DuoStim) consists in performing two OS within the same menstrual cycle, one in the follicular phase and the other in the luteal phase (Table 1).

## Concept of multiple waves and stimulation in the Luteal Phase

The ovulatory cycle follows a series of events (Baerwald et al., 2003b): after menstruation there is an increase in serum FSH levels above a critical threshold necessary for the recruitment of a follicle cohort. From this cohort, one or more follicles will be selected for preferential growth while the rest will suffer atresia. As the follicles grow, aromatase activity increases along with estradiol and inhibin B levels,

exerting negative “feedback” on the pituitary secretion of FSH. Similarly, the dominant follicle becomes less dependent on FSH and begins to express more LH receptors. The estradiol peak occurs one day before the LH peak, when the concentration of progesterone and inhibin A begins to rise. Ovulation takes place 24 h after the LH peak, giving rise to the oocyte and the corpus luteum (CL). If there is no fertilization, the CL suffers atresia and stops producing progesterone, starting a new cycle.

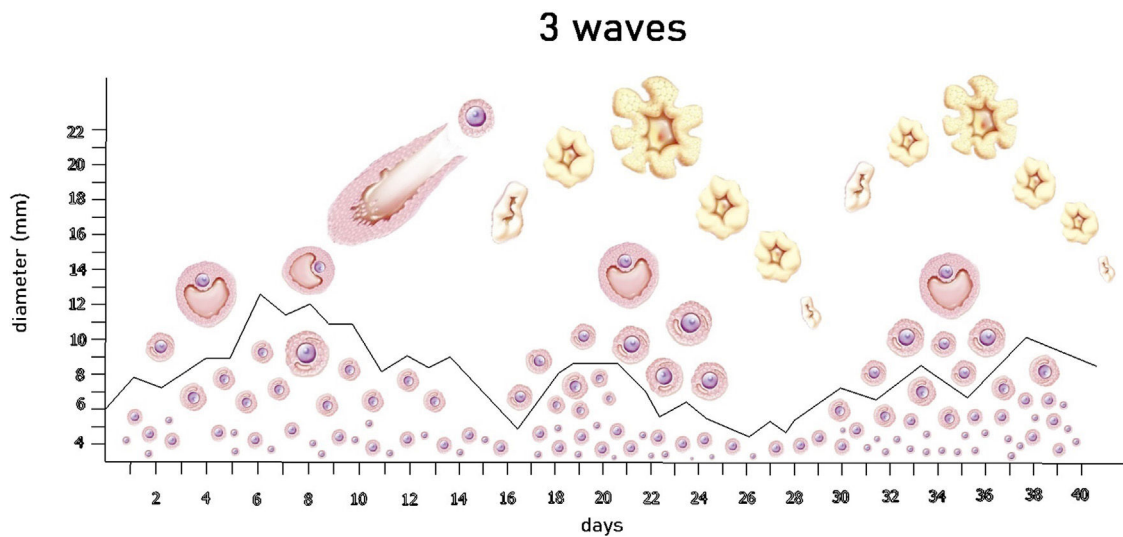
In the last decade, the existence of two or three cyclic waves of follicular growth has been proven (Baerwald et al., 2003a,b). Only one wave is ovulatory, although the development of a dominant follicle may occur in all of them. The hormone profile detected in the other waves is not followed by a peak of estradiol and LH, which prevents ovulation (Baerwald et al., 2003a,b). The dynamics of multiple waves of follicular growth are represented in Fig. 1.

Former studies had already reported the existence of healthy antral follicles in the luteal phase, but had suggested that more than 90% were atretic and the others had limited steroidogenic competence, as well as low potential for pre-ovulatory development (McNatty et al., 1983). Since the description of this wave-like model, new ovarian stimulation strategies have been proposed that could take advantage of the ovulatory potential of the follicles recruited in other waves. While some are more cumbersome and seek to synchronize the administration of exogenous gonadotropins with the follicular development waves (De Mello Bianchi et al., 2010), others are more manageable and propose starting ovarian stimulation at any time of the cycle, early or late, and either in the follicular or the luteal phase (Vaiarelli et al., 2017; Alvaro Mercadal et al., 2018).

The exact mechanisms involved in the multiple waves of follicular development are not yet known, but the possibility of performing luteal phase stimulation has gained increasing attention; thus, over the past years, it has been shown that luteal phase stimulation is similar to follicular phase stimulation with regard to the quantity and quality of oocytes and embryos and the fertilization rate, as well as implantation and pregnancy rates (Buendgen et al., 2013; Kuang et al., 2014; Wang et al., 2015). Regarding

**Table 1** Protocols for DuoStim.

	N	Trigger	Follicles unpunctured	Time interval prior to start 2nf OS cycle
Kuang et al., 2014 (7)	38	GnRH agonist	<10 mm	1 day
Ubaldi et al., 2016 (9)	43	GnRH agonist	–	5 days
Cardoso et al., 2017 (24)	13	GnRH agonist	–	5 days
Liu et al., 2017 (21)	103	hCG	≤11 mm	1–3 days
Tsampras et al., 2017 (22)	10	hCG	–	0–7 days
Vaiarelli et al., 2018 (25)	310	GnRH agonist	–	5 days
Cimadomo et al., 2018 (20)	170	GnRH agonist	–	5 days
Cecchino et al., 2020	87	GnRH agonist	<10 mm	5days


**Figure 1** 3 waves.

gestational, perinatal and neonatal outcomes, comparable results were reported in terms of miscarriage rates, multiple and ectopic pregnancies, time of birth, birthweight, and congenital anomalies (Kuang et al., 2014; Wang et al., 2015; Li et al., 2016).

Not long ago, a systematic review and meta-analysis of more than 300 luteal phase stimulations concluded that, although luteal phase cycles may require longer duration of stimulation and higher doses of gonadotropins, reproductive outcomes are similar (Boots et al., 2016). In the same year, the longest study to date was published comparing conventional stimulation with more than 700 cases of luteal phase stimulation in women with normal ovarian reserve. Once more, no significant differences were found in stimulation, reproductive and neonatal outcomes. In fact, the authors reported a higher amount of mature and good quality embryos after luteal phase stimulation compared to follicular phase stimulation (Wang et al., 2015).

### Double stimulation protocol (DuoStim)

DuoStim is based on the wave-like pattern of follicle recruitment that occurs during the ovarian cycle. It seems to be especially indicated in cases of marked follicular

asynchrony. However, this protocol has already been used in different clinical settings: low response to conventional stimulation (Kuang et al., 2014), low ovarian reserve and poor prognosis (Ubaldi et al., 2016; Cimadomo et al., 2018), advanced maternal age (Liu et al., 2017) and optimization of fertility preservation in oncological patients (Tsampras et al., 2017).

The DuoStim protocol involves a second ovarian stimulation in the luteal phase immediately after the first ovarian puncture, taking advantage of the full potential of the same menstrual cycle. The theoretical benefit of this protocol is that it shortens the time-to-pregnancy by obtaining a greater number of eggs, which would mean a greater number of embryos and higher chances of success. In fact, a very recent retrospective study including more than 14,000 in vitro fertilization (IVF) cycles has shown that the number of available eggs is directly related to the cumulative live birth rates after IVF (Polyzos et al., 2018). It must be highlighted that ovarian stimulation must be rational, safe and individualized, avoiding any type of harm to the patient.

The strategy used in the DuoStim protocol is represented in Fig. 2. First, conventional stimulation is performed in the follicular phase, followed by ovulation induction with a GnRH agonist or hCG; small follicles must be left unpunctured. Inhibition of the LH surge can be achieved with GnRH

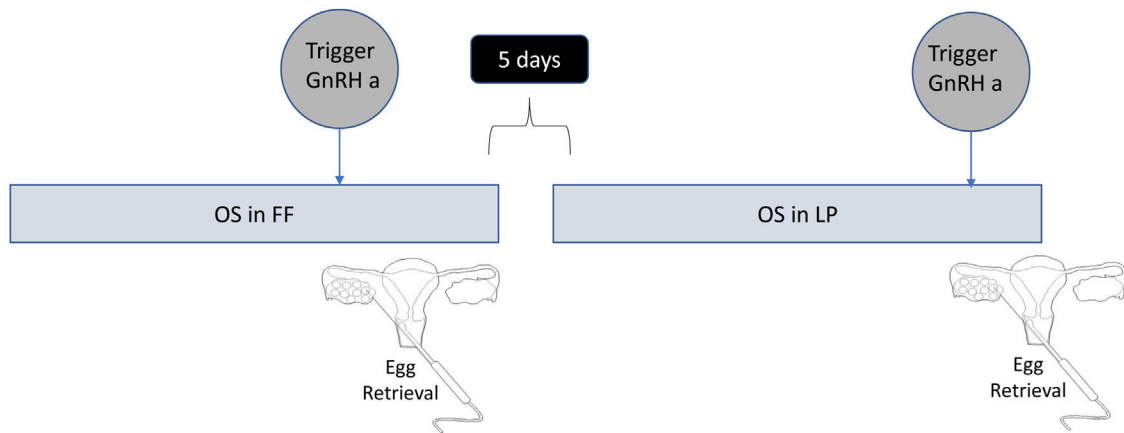


Figure 2 DuoStim protocol.

antagonist or progesterone administration. A second luteal phase stimulation is started between the first and fifth day after the first oocyte retrieval, usually with the same protocol used in the first stimulation, although various ways of stimulating in the luteal phase have been described. The theoretical advantage of inducing oocyte maturation with a GnRH agonist after the first stimulation is that it would allow better control of the second stimulation due to faster luteolysis. It is important to bear in mind that this protocol requires a deferred embryo transfer in a subsequent endometrial cycle. The different protocols used by the authors showed in [Table 1](#).

## Results of double stimulation

There is currently limited research on DuoStim for poor ovarian response (POR), so the conclusions are based on some retrospective studies published to date. In the first published study using the double stimulation protocol in low responders, [Kuang et al. \(2014\)](#) identified a greater number of retrieved oocytes and, consequently, a greater number of fertilized eggs and embryos formed in the second follicular puncture compared to the first. However, the reproductive outcomes were similar when comparing both stimulations. Similar results were described in later studies. A large case-control series of patients aged over 38 published by [Liu et al.](#) reported more eggs in the luteal phase, but comparable pregnancy rates ([Liu et al., 2017](#)). In both studies, the dose of gonadotropin required was higher in the luteal phase ([Kuang et al., 2014; Liu et al., 2017](#)).

Next, [Ubaldi et al. \(2016\)](#) published a prospective observational study in which double stimulation was used in women with low ovarian reserve undergoing Preimplantation Genetic Testing for Aneuploidy (PGT-A). They verified that the number of oocytes and embryos, the quality of the embryos, the aneuploidy rate and the reproductive results were similar when comparing follicular and luteal phase stimulations in DuoStim cycles ([Cimadomo et al., 2018](#)). Interestingly, there were no differences in the total dose of gonadotropins administered in the two phases ([Cimadomo et al., 2018](#)). Subsequently, DuoStim was compared to single conventional stimulation and it was concluded that double stimulation allowed the recovery of a greater number of

mature eggs in a single menstrual cycle ([Baerwald et al., 2012](#)).

In our series, [Cecchino et al.](#) published a retrospective analysis comparing the results of luteal versus follicular phase stimulation in 87 DuoStim cycles. Our population had a mean age of 38.4 with an AMH value of 1.03 and an antral follicle count of 6.6, all compatible with poor reproductive prognosis. Furthermore, most of the patients already had multiple IVF failures and poor oocyte quality. The number of oocytes and blastocysts obtained was greater after luteal phase stimulation ([Cecchino et al., 2020](#)).

Recently, [Glujovsky](#) published the most updated and complete systematic review ([Glujovsky et al., 2020](#)) on the results of non-conventional OS protocols, including the DuoStim strategy. In the DuoStim section, they included 4 studies (an RCT, a prospective cohort, a retrospective and a before-after study). All of these studies compared DuoStim with single conventional stimulation in poor responders that underwent IVF cycles ([Kuang et al., 2014; Ubaldi et al., 2016; Vaiarelli et al., 2020](#)). DuoStim showed a higher number of both oocytes retrieved and mature oocytes, compared to just one conventional protocol. An observational study by [Vaiarelli et al.](#) showed that more women had at least one euploid blastocyst using DuoStim compared to single conventional stimulation (33% vs 19.3%,  $p < 0.05$ ), but whether or not DuoStim increases clinical pregnancy rates remains unclear. A single small prospective cohort reported higher live birth rates with DuoStim. The most important limitation of all these studies is that none of them compared the DuoStim strategy with two consecutive conventional OS.

## Conclusions

- Double stimulation is an alternative in cases in which it is necessary to obtain a larger number of eggs in less time, for example, in patients with low ovarian reserve/response, advanced reproductive age, and oncological patients. Likewise, it could be interesting in cases of marked asynchrony at the end of the follicular phase stimulation.
- The physiology of double stimulation is based on variable recruitment during the menstrual cycle.

- The induction of final oocyte maturation can be performed using GnRH agonist or hCG; GnRH agonists, however, induce early luteolysis that could facilitate the control of the second stimulation.
- It seems that a greater number of oocytes is obtained from stimulation in the luteal phase compared to the follicular phase.
- The need for higher doses of gonadotropins in luteal phase stimulation is a controversial issue.
- DuoStim could offer our patients the possibility of performing two stimulations in less time.
- No randomized clinical trials have been performed to prove that DuoStim is better compared to two conventional stimulations.

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