RBMO

REVIEW

Use of progestins to inhibit spontaneous ovulation during ovarian stimulation: the beginning of a new era?

BIOGRAPHY

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KEY MESSAGE

Ovulation inhibition by exogenous progestins in ovarian stimulation cycles is safe, effective and economical. Pregnancy outcomes are promising and risk for OHSS seems lower than for conventional stimulation protocols.

ABSTRACT

Advances in oocyte and embryo cryopreservation for assisted reproduction prompted new approaches to ovarian stimulation. Attention has been paid to progesterone and its derivatives to block the LH surge, as oocyte vitrification removes possible harmful effects of progestins on endometrial receptivity. This review summarizes the current status of progestin use to inhibit ovulation during ovarian stimulation compared with conventional ovarian stimulation. Progestin-primed ovarian stimulation is shown to effectively inhibit spontaneous ovulation, without affecting the number of retrieved oocytes and embryo quality. Reproductive outcomes from ovarian stimulation with progestins appear similar to those from conventional ovarian stimulation, although large trials are needed to confirm this. Use of progestins allows better control of LH concentrations, lower costs and easier (oral) administration. Therefore, progestin-primed ovarian stimulation could be the first choice for ovarian stimulation in fertility preservation, oocyte donation and preimplantation genetic testing cycles. So-called 'non-conventional' ovarian stimulation protocols (luteal and random-start, double ovarian stimulation), which always require oocyte or embryo cryopreservation, may also use progestins to inhibit the endogenous LH surge. Since the 'freeze-all' strategy with delayed transfer is mandatory, high responders undergoing IVF could benefit more from this approach. Economic advantage remains to be demonstrated, as do pregnancy and neonatal outcomes.

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KEYWORDS

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Ovarian stimulation
Ovulation inhibition
Progestin-primed ovarian stimulation
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INTRODUCTION

IVF has been increasingly employed over the last few decades. The retrieval of oocytes in adequate numbers is based on the principles of ovarian stimulation. In addition to the use of gonadotrophins to recruit multiple follicles, it is mandatory to use a drug for preventing the untimely outbreak of the recruited follicles. The first substances used to obtain ovulation inhibition were gonadotrophin-releasing hormone (GnRH) agonists, followed by GnRH antagonists. However, GnRH analogues have multiple disadvantages. These include cost, poor manageability (the drug must be accurately prepared and needs subcutaneous injection) and various side effects. This has prompted interest in medical alternatives.

After the publication of two studies on luteal phase ovarian stimulation (*Li et al., 2016; Wang et al., 2016a*) demonstrating consistent LH suppression with no spontaneous surge, investigation has been carried out into whether exogenous progesterone could be applied in ovarian stimulation cycles with the aim of inhibiting ovulation. The obvious assumption, in both luteal phase ovarian stimulation and stimulation in the follicular phase with exogenous progestins, is the need to cryopreserve the entire cohort of embryos derived from retrieved oocytes (*Massin, 2017*). In previous decades, progesterone could not be considered for use during ovarian stimulation because it was known to have a negative impact on endometrial receptivity. Since newly advanced vitrification techniques have made possible superior quality cryopreserved embryo and reliable warming, it has become feasible to break through the standard sequence of ovarian stimulation–retrieval–transfer (FIGURE 1). Progesterone's inhibition of ovulation and the efficacy of the 'freeze-all' protocol suggest that progesterone may be used as an alternative to a GnRH analogue for suppressing premature LH surges during ovarian stimulation in IVF cycles using the freeze-all strategy. This review proposes to elucidate the state of the art of progestin-primed ovarian stimulation (PPOS), with its advantages and limitations.

MATERIALS AND METHODS

A literature search was conducted using PubMed until March 2019. The following keywords were used to generate the list of citations: ovarian stimulation, ovulation inhibition, GnRH analogues, progestins, PPOS. A review of English-language publications was conducted. Articles and their references were examined to identify other potential studies. All the articles considered of interest between those identified are reported in this review.

CONVENTIONAL PROTOCOLS FOR OVARIAN STIMULATION AND THE ROLE OF GNRH ANALOGUES

The advent of IVF saw oocyte retrieval from a single follicle in a natural cycle. However, the disadvantages of having only one oocyte to work with led to the introduction of ovarian stimulation for IVF. Ovarian stimulation is employed aiming to stimulate the growth of several follicles and, consequently, to obtain as many high-quality oocytes as possible (*Cavagna et al., 2011*). More oocytes means more embryos, which offers the possibility of embryo selection; this in turn helps to improve pregnancy rates (*Mahajan, 2013*).

In conventional ovarian stimulation protocols, the administration of exogenous gonadotrophins maintains FSH and LH concentrations above a critical threshold needed to stimulate the development of many follicles, thus allowing the retrieval of multiple oocytes in a single cycle (*Alper and Fauser, 2017*). The early rise in oestradiol concentrations, due to the development of multiple follicles at the same time, may promote an extemporaneous LH surge, leading to spontaneous ovulation and to the consequent premature end of the respective cycle. In order to avoid such an effect, pituitary suppression has,

Organization of a conventional ovarian stimulation (COS) protocol with a GnRH antagonist, versus a progestin primed ovarian stimulation (PPOS) protocol.

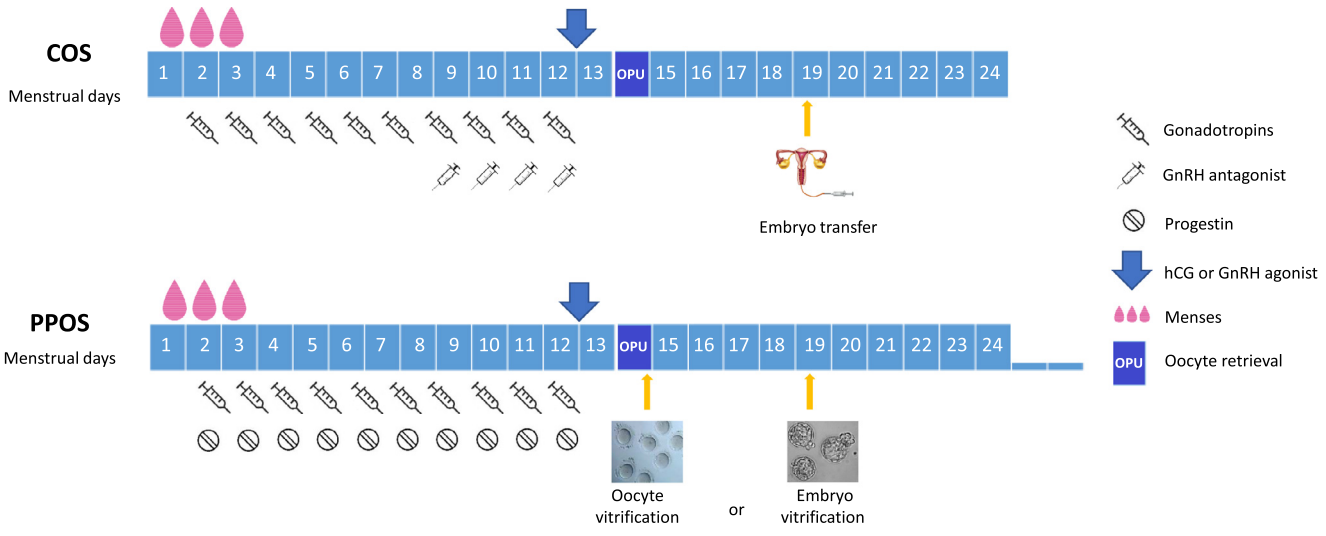

FIGURE 1 Organization of a conventional ovarian stimulation (OS) protocol with gonadotrophin-releasing hormone (GnRH) antagonist versus a progestin-primed ovarian stimulation (PPOS) protocol. HCG, human chorionic gonadotrophin.

over the last 30 years, been employed using GnRH and its analogues. Pituitary suppression was initially attempted using GnRH agonists, but GnRH antagonists have more recently been introduced. Final maturation of the oocyte and ovulation are then typically triggered using a bolus of GnRH agonist, human chorionic gonadotrophin (HCG; a hormone that is biologically similar to LH but with a longer half-life) or both (*Cavagna et al., 2011*).

GnRH agonists are more potent and have a longer half-life than native GnRH, from which they are derived. Developed in the 1980s, they bind to pituitary receptors in the hypophysis and induce the release of large amounts of FSH and LH (a flare-up effect), and an increase in the number of GnRH receptors (up-regulation) (*Kumar and Sharma, 2014*). However, after prolonged use, internalization of the GnRH agonist–receptor complex occurs, which results in a decrease in the number of GnRH receptors (down-regulation). As a result, the pituitary becomes refractory to stimulation by GnRH, leading to a decrease in circulating gonadotrophins and thus preventing a premature LH surge.

Acting differently, GnRH antagonists promptly suppress pituitary gonadotrophin by GnRH–receptor competition (*Kumar and Sharma, 2014*). Gonadotrophin secretion is decreased within hours of antagonist administration and no flare-up effect occurs. Moreover, the discontinuation of GnRH antagonist treatment results in rapid, predictable recovery of the pituitary–gonadal axis as the pituitary receptor system remains intact. GnRH antagonists have provided clinicians with flexibility in terms of administration, offering patients a friendlier method of ovarian stimulation (*Tarlatzis and Kolibianakis, 2007*).

The use of GnRH analogues may, however, lead to a series of side effects. Despite their long time in clinical use, GnRH agonists are still associated with the complexity of achieving consistent down-regulation and an increased risk of ovarian hyperstimulation syndrome (OHSS) from an HCG trigger (*Zhu et al., 2015*). Moreover, the administration of GnRH analogues leads to their accumulation and subsequent concentration in the peripheral circulation, with consequent possible extrapituitary effects. As GnRH receptors have been described in the ovary and the presence of specific GnRH agonistic and antagonistic binding has been demonstrated in human luteinized granulosa cells, these compounds, particularly GnRH agonists, can induce the formation of functional follicular cysts (*Mehta and Anand Kumar, 2000*), probably as a result of their flare-up effects. The incidence of functional cysts has been reported to be in the range 2–40%, the frequency being higher in older women, when the administration of the agonist begins in the follicular phase rather than in mid-luteal phase, and in women with increased concentrations of basal FSH (*Fiszbajn et al., 2000*). Other disadvantages of GnRH agonists include hypo-oestrogenaemia and the requirement for a prolonged period of down-regulation, with subsequent high costs. This extensive treatment period before desensitization implies not only an increased cost of treatment, but also a prolonged hormonal exposure, associated with menopausal symptoms, induced by gonadotrophin suppression.

Protocols with GnRH antagonists have fewer complications and are more convenient for patients because of the shorter treatment time and lower number of injections. However, the effectiveness of antagonists is still under debate. According to multiple studies comparing a GnRH agonist protocol and GnRH antagonist protocol, effectiveness, the number of oocytes retrieved and the number of embryos obtained are significantly lower when the antagonist is used (*Wang et al., 2017*). Furthermore, the cycle cancellation rate seems higher in women who received GnRH antagonist protocols compared with GnRH agonist protocols (*Kahyaoğlu et al., 2017*). A varied proportion (0.34–38%) of patients using a GnRH antagonist has been demonstrated to experience a premature LH surge, especially seen in older women and women with diminished ovarian reserve (*Bosch et al., 2003; Reichman et al., 2014*). Indeed, GnRH antagonists, at the doses currently used in IVF programmes, have been demonstrated to be unable to block the stimulating effect of exogenous oestrogen on the LH surge in women with unstimulated ovaries, suggesting that their clinical efficacy in IVF cycles is determined by the ovarian stimulation process (*Messinis et al., 2005, 2010*). Moreover, it is important to note that, as the development of an antagonist with an acceptable pharmacokinetic and safety profile has been more difficult than for agonists, some experience on the part of clinicians is still needed in their use.

These disadvantages have prompted interest in exploring convenient alternatives to prevent premature LH surges in ovarian stimulation, and research is still ongoing.

THE PHYSIOLOGICAL ROLE OF ENDOGENOUS PROGESTERONE IN THE PITUITARY LH SURGE

Extensive research has been undertaken to elucidate the exact signals responsible for inducing the pituitary surge in LH secretion that lies at the basis of ovulation. The major regulatory factors of the gonadotrophin surge have been identified as hypothalamic GnRH, ovarian steroids such as oestradiol and progesterone, and various other regulatory factors such as cytokines, leukotrienes and glucocorticoid, adrenergic and dopaminergic stimuli.

GnRH is released in a pulsatile manner by neurons that have their origin in the arcuate nucleus; it is released in the median eminence in the perivascular space and then enters the capillaries of the hypophyseal portal system (*Chabbert-Buffeta et al., 2000*). It has to be discharged with a frequency and amplitude within a critical range for normal gonadotrophin secretion to occur. Gonadotrophins are synthesized on rough endoplasmic reticulum in the gonadotrophic cells, packaged into secretory granules and stored. Actual secretion depends on migration and activation of the mature secretory granules at the cell membrane (*Shoham et al., 1995*).

The major control element regulating gonadotrophin concentrations is ovarian steroid feedback on the anterior pituitary. As it has been observed that GnRH neurons do not possess classic steroid receptors, the surge-inducing signals seem to be transmitted to the GnRH neurosecretory system through a series of one or more interneurons (*Harris et al., 1999*).

Oestradiol plays a central role in the secretion of LH by the pituitary gland. Produced by the granulosa cells of the developing follicle, it exerts a negative feedback on LH production in the early follicular phase of the ovarian cycle.

During the follicular phase, however, once oestrogen concentrations have reached a critical level as oocytes mature within the ovary in preparation for ovulation, they begin to exert positive feedback on LH production, leading to the LH surge. The LH surge increases intrafollicular proteolytic enzymes, weakening the wall of the ovary and allowing the mature follicle to pass through (*Holesh and Lord, 2017*). This change from a negative to a positive feedback on LH secretion happens via both the pituitary gland and the hypothalamus. In the pituitary region, it is caused by an increase in sensitivity to GnRH (due to an increase in GnRH receptors on gonadotrophic cells), a possible enhancement of the availability of GnRH in the pituitary via an inhibition of GnRH metabolism, and a lowering of the GnRH concentration needed for the secretion of LH. At the hypothalamic level, the effect is direct, via the neuropeptide kisspeptin: steroid-sensitive kisspeptin neurons are located in the anterior ventral periventricular nucleus and neighbouring periventricular nucleus, and co-express oestrogen and progesterone receptors (*Messinis et al., 2014; Stephens et al., 2015*).

Progesterone is also a key signal in the complicated mid-cycle dynamics. Progesterone is a steroid hormone that is responsible for preparing the endometrium for uterine implantation of the fertilized egg. If a fertilized egg implants, the corpus luteum secretes progesterone in early pregnancy until the placenta develops and takes over progesterone production for the rest of the pregnancy (*Holesh and Lord, 2017*).

The neuroendocrine effects of progesterone are mediated by the classic progesterone nuclear receptors. They exist in different isoforms and are up-regulated by oestrogen, while progesterone down-regulates its own receptors (*Chabbert-Buffeta et al., 2000*). Despite the importance of progesterone in the control of GnRH surge generation, the neural mechanisms through which progesterone interacts with oestradiol to regulate the gonadotrophin surge are not completely understood. The actions of progesterone action may be synergistic with, or antagonistic to, the actions of oestradiol, depending on hormone ratios and the timing of exposure (*Custodia-Lora and Callard, 2002*). Progesterone seems to have a permissive role in the preovulatory LH peak: experiments have shown a rise in progesterone preceding the LH surge by several hours in the pre-ovulatory period (*Hoff et al., 1983*); in various studies, exogenous progesterone has been shown to induce a peak in LH if administered to oestrogen-primed women (*Liu and Yen, 1983*). On the other hand, progesterone is known to have an inhibitory effect on ovulation. Studies originally focused on contraception have shown that progesterone is able to block the LH surge (*Evans et al., 2002; Heikinheimo et al., 1996*). Its inhibitory effect on follicular growth has been at the base of the design of progestin-only contraceptives, which suppress follicular growth and thus inhibit ovulation after a sustained administration.

The timing of administration of progesterone has been shown to be critical in determining its effect upon the pre-ovulatory LH surge, whether it is stimulating or inhibiting, in different animal studies. Various analyses have suggested that the generation of the LH surge is characterized by an oestradiol-dependent period, during which the oestradiol signal is read, and an oestradiol-independent period, during which the signal is transmitted through a cascade of neuronal events to the GnRH neurosecretory system, with the release of a surge of GnRH. Progesterone has been demonstrated to block the oestradiol-induced signal soon after its transmission (immediately after oestradiol removal), in the early part of oestradiol-independent period of surge generation, probably via an inhibition of transmission of the stimulatory signal through the intraneuronal system than links the oestradiol-receptive neurons with the GnRH neurons (*Harris et al., 1999*). Changes in progesterone nonetheless cause dramatic modifications in GnRH pulse frequency: its removal induces an acceleration of the pulse generator, while its administration slows the pulse frequency, with LH secretion being consequently modified (*Chabbert-Buffeta et al., 2000*). Progesterone priming seems in fact 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 (*Soules et al., 1984*).

EXOGENOUS PROGESTINS AND PITUITARY INHIBITION

Progestins are hormones that produce numerous physiological actions. In women, these include developmental effects, neuroendocrine actions involved in the control of ovulation, the cyclical preparation of the reproductive tract for fertilization and implantation, and major actions on mineral, carbohydrate, protein and lipid metabolism. Progesterone represents the only natural progestin (*Brunton et al., 2011*).

The therapeutic use of progestins largely reflects an extension of their physiological activities. The two most frequent uses of progestins are for contraception, either alone or with an oestrogen, and in combination with oestrogen for hormone therapy in postmenopausal women. Progesterone has also been historically used largely in the prevention of threatened abortion for its quality of inhibition of uterine contractility, even if this treatment is of questionable benefit. It is also used worldwide for the prevention of preterm birth, for endometrioisis and for uterine fibroids. Progesterone-receptor antagonists are also available. The main use of antiprogestins has been for medical abortion and for uterine fibroids, but other uses are theoretically possible.

Besides natural progesterone, produced and secreted normally in the human female by the corpus luteum, the placenta and in small quantities the adrenal cortex, there is a broad spectrum of steroids with progesterone-like actions, derived from different parent compounds. Close to natural progesterone is retroprogesterone, followed by the 17-hydroxyprogesterone (i.e. medroxyprogesterone acetate [MPA]) and the 19-norprogesterone derivatives. A clinically important group and the basis of the success of hormonal contraception are the 19-nortestosterone derivatives, subdivided into estranes (i.e. norethindrone, norethisterone acetate) and gonanes (i.e. norgestrel, levonorgestrel). Spirolactone derivatives (i.e. drospirenone) have also been developed for clinical use.

Two isoforms of the progesterone receptor exist, A and B, encoded by a single gene. The biological activities of progesterone receptors A and B are distinct: in most cells, progesterone

TABLE 1 PROGESTOGENIC EFFECT AT THE ENDOMETRIUM LEVEL AND ANTI-GONADOTROPHIC EFFECT OF VARIOUS PROGESTINS

Progestin	Transformation dose (mg/cycle)	Transformation dose (mg/day)	Ovulation inhibition dose (mg/day)
Progesterone	4200	200–300	300
Dydrogesterone	140	10–20	>30
Medrogestone	60	10	10
Medroxyprogesterone acetate	80	5–10	10
Cyproterone acetate	20	1.0	1
Norethisterone acetate	30–60	–	0.5
Levonorgestrel	6.0	0.15	0.05
Dienogest	6.0	–	1.0
Nomegestrol acetate	100	5.0	5.0
Drospirenone	50	–	2.0

Adapted from *Schindler et al., 2003*.

receptor B mediates the stimulatory activities of progesterone; progesterone receptor A strongly inhibits this action and is also a transcriptional inhibitor of other steroid receptors. Current data, from a study in rats, suggest that co-activators and co-repressors interact differentially with the two receptors, and this may account, at least in part, for the differential activities of the two isoforms (*Gordon et al., 2015*) . One of the essential requirements of any compound with progesterone-like activity is that it is able to bind to the progesterone receptors.

All progestins have in common the so-called progestogenic effect, which is the induction of a characteristic change in the oestrogen-primed endometrium (*Schindler et al., 2003*). The final progestogenic activity of any substance also depends on the route and timing of administration. This is often expressed as the difference in the dose required for endometrial transformation in a woman, called the transformation dose, and this varies widely between different progestins. There are large variations between progestins in the multitude of other biological effects elicited, the capacity to inhibit ovulation being one of those. Progestins have been selected for clinical use based on differences in the dose necessary for inhibition of ovulation: for example, MPA may interfere with ovulation at a dose of 10 mg/day, whereas 300 mg/day of progesterone is required to obtain the same effect. This implies that, over the years, progestins have been used in clinical practice much more broadly than progesterone itself, and as a lower dose is required to obtain the same effect, lower costs are involved (TABLE 1).

THE USE OF PROGESTINS TO PREVENT THE LH SURGE IN OVARIAN STIMULATION CYCLES

As progestins have been demonstrated to inhibit ovulation, it has been asked whether exogenous progesterone could replace the use of GnRH agonists or antagonists in ovarian stimulation protocols (*Massin, 2017*). Different studies have demonstrated consistent LH suppression during ovarian stimulation in the luteal phase, with no spontaneous LH surge (*Kuang et al., 2014; Wang et al., 2016a*). It has been presumed that those pituitary glands secretions could have been transiently suppressed by high doses of progesterone during luteal phase ovarian stimulation. This supposition is in agreement with Letterie's study (*Letterie, 2000*) showing that a combination of ethinyl oestradiol and norethindrone administered for 5 days beginning on day 6 or 8 of the menstrual cycle permitted folliculogenesis, but inhibited the mid-cycle LH surge and consequently ovulation during ovarian stimulation. However, the administration of progestins would lead to the impossibility of fresh embryo transfer because of a severe impairment in endometrial receptivity. The modern technology of vitrification allows safe cryopreservation of oocytes and embryos with a post-warming survival rate very close to 99%. Hence the transfer of fresh embryos to a uterus that has been newly subjected to hormonal stimulation is no longer required. The transfer of cryopreserved–thawed/warmed embryos in the freeze-all embryo protocol has nonetheless been reported in some studies to result in improved pregnancy and delivery outcomes (*Devroey et al., 2011; Doody, 2014; Wong et al., 2014*). The inhibition of the LH surge with exogenous progesterone associated with the efficacy of the so-called freeze-all protocol clearly indicates that progestins could be an alternative to GnRH agonists and antagonists for suppressing the LH surge during ovarian stimulation in IVF/ intracytoplasmic sperm injection ICSI cycles.

Studies available in the literature so far, involving more than 2600 patients (*Begueria et al., 2019; Chen et al., 2017; Dong et al., 2017; Kuang et al., 2015; Wang et al., 2016b; Yu et al.,2018; Zhu et al., 2015, 2016, 2017a, 2017b*), are summarized in TABLE 2. The first study on the use of a progestin during ovarian stimulation was published by Kuang and colleagues in 2015 (*Kuang et al., 2015*). It aimed to investigate the use of MPA to prevent the LH surge, and compared cycle characteristics and pregnancy outcomes in subsequently frozen-thawed embryo-transfer cycles, using a short protocol as a control. MPA was used as an alternative to progesterone for its advantages: it is progestative and slightly androgenic, and does not interfere with the measurement of endogenous progesterone production. In the study group, human menopausal gonadotrophin (HMG, at a dose of 150 U/day in patients with an antral follicle count higher than 20 or a slightly elevated FSH basal value, and at a dose of 225 U/day for all other patients) and MPA (10 mg/day) were simultaneously administered beginning on day 3 of the menstrual cycle. From day 7–8 of the cycle, every 2–4 days, ultrasound follicular monitoring was performed and the serum concentrations of LH, FSH, oestradiol and progesterone were measured. As in the other studies mentioned, the progestin was administered until the day ovulation was triggered. Ovulation was triggered with triptorelin (0.1 mg), a GnRH agonist, or co-triggered by triptorelin and HCG (1000 U) when at least three dominant follicles reached 18 mm in diameter. A short protocol was used in the control group, with the administration of triptorelin (0.1 mg/day) beginning on day 2 of the menstrual cycle and HMG (150–225 U/day, with the same administration dose criteria used in the study group) beginning on day 3. Aspirated oocytes were then fertilized *in vitro*, and viable embryos were cryopreserved for later

TABLE 2 SUMMARY OF THE STUDIES PUBLISHED ON PROGESTIN-PRIMED OVARIAN STIMULATION CYCLES

	ProgestinPopula-tionStudy design	Group	Number of patients	Number of oocytes	Number of embryos	Implantation rate(%)	Pregnancy or Live birth rate (%)
Kuang et al., 2015	MPA Normal responders Prospective controlled study	Study group (10 mg MPA)	150	9.9 ± 6.7	7.0 ± 5.3	31.9	42.6 (LBR)
		Control group (Short agonist)	150	9.0 ± 6.0	6.4 ± 4.4	27.7	35.5 (LBR)
Wang et al., 2016b	MPA PCOS patients Prospective RCT	Study group (10 mg MPA)	60	15.2 ± 7.8	10.6 ± 5.9	48.6	65.3 (CPR)
		Control group (Short agonist)	60	15.8 ± 8.4	9.7 ± 5.2	42.6	53.5 (CPR)
Dong et al., 2017	MPA Normal responders Prospective RCT	Study group (4 mg MPA)	150	9.6 ± 5.9	3.7 ± 3.0	30.1	42 (LBR)
		Control group (10 mg MPA)	150	9.8 ± 6.3	4.2 ± 2.6	30.9	48.7 (LBR)
Chen et al., 2017	MPA Poor responders Prospective controlled study	Study group (10 mg MPA)	102	1.0	1.1	21.4	11.8 (CPR)
		Control group (Natural cycle)	102	0.7 $P < 0.05$	0.8 $P < 0.05$	15.3	5.9 (CPR)
Zhu et al., 2015	Utrogestan Normal responders Retrospective study	Study group (200 mg Utrogestan)	187	10.9 ± 5.7	4.99 ± 2.51	33.5	54.2 (CPR)
		Control group (Short agonist)	187	10.6 ± 6.2	4.45 ± 2.46 $P < 0.05$	34.0	51.6 (CPR)
Zhu et al., 2016	Utrogestan PCOS patients Retrospective study	Study group (200 mg Utrogestan)	123	13.2 ± 7.4	9.0 ± 5.2	46.6	64.6 (CPR)
		Control group (Short agonist)	77	13.1 ± 7.9	7.8 ± 4.7	31.3 $P < 0.05$	51.6 (CPR) $P < 0.05$
Zhu et al., 2017a	Utrogestan Normal responders Prospective RCT	Study group (100 mg Utrogestan)	150	9.8 ± 5.7	6.5 ± 4.0	38.6	50 (CPR)
		Control group (200 mg Utrogestan)	150	10.2 ± 5.4	6.7 ± 4.0	36.	51.3 (CPR)
Yu et al.,2018	Dydrogesterone Normal responders Prospective RCT	Study group (20 mg DYG)	260	10.8 ± 6.3	6.9 ± 4.4	40.0	57.6 (CPR)
		Control group (10 mg MPA)	256	11.1 ± 5.8	7.0 ± 4.5	45.9	62.3 CPR)
Zhu et al., 2017b	DYG Normal responders Prospective controlled study	Study group (20 mg DYG)	125	8.22 ± 5.46	2.23 ± 2	38.68	53 (CPR)
		Control group (100 mg Utrogestan)	125	8.8 ± 5.62	2.69 ± 2.38	35.71	51.7 (CPR)
Begueria et al., 2019	MPA Oocyte donors Prospective RCT	Study group (10 mg MPA)	86	15.1 ± 8.3			22 (LBR)
		Control group (GnRH antagonist)	87	14.6 ± 7			31 (LBR)

Number of oocytes and number of embryos are given as means ± SD.

CPR, clinical pregnancy rate; DYG, dydrogesterone; LBR, live birth rate; MPA, medroxyprogesterone acetate; PCOS, polycystic ovary syndrome; RCT, randomized controlled trial.

transfer in a subsequent cycle in both protocols, after adequate preparation of the endometrium. The number of oocytes retrieved in the study group was slightly higher than in the short protocol, although the difference did not reach significance, and the mean duration of stimulation and HMG dose were significantly higher than in the control group ($P < 0.05$). No significant differences were found in oocyte maturation rate, fertilization rate or cleavage rate between the two groups. In addition, the number of good-quality embryos and cryopreserved embryos showed was not significantly different between the two groups. No patient experienced moderate or severe OHSS during the study. No significant difference was found in the incidence of premature LH surge in the study group compared with the control group (0.7% versus 0%). Similarly, no statistically significant differences were found in clinical pregnancy rates, implantation rates and live birth rates in the study group compared with the controls. It is important to consider that no congenital malformations were found in any of the live-born babies. The results of the study provided first-time evidence that MPA is an effective oral alternative for the prevention of premature LH surges in women undergoing ovarian stimulation, and the pregnancy outcomes from frozen-thawed embryo transfer cycles indicated that the embryos originating from this regimen had a similar development potential to those from the control group.

This same protocol has been applied among patients with polycystic ovary syndrome (PCOS) in a prospective controlled study comparing the MPA protocol with a short protocol (*Wang et al., 2016b*). Women with PCOS planning to have an IVF represent a therapeutic challenge: they are predisposed to poor oocyte quality, low fertilization rates and high miscarriage rates. Moreover, they are at high risk of developing OHSS when stimulation is used. There is therefore an unsatisfied interest in alternative ways of ovarian stimulation that would be associated with improved efficacy and decreased incidence of OHSS in those patients. The fertilization rate and the ongoing pregnancy rate per transfer in the study group were higher than those in the control group (77.69 ± 16.59% versus 70.54 ± 19.23%, $P < 0.05$; 58.67% versus 42.86%, $P < 0.05$). Two cases of OHSS were reported in the short protocol group, while none was seen in the MPA group (although this result was not significantly different); a possible reduction in the incidence of moderate to severe OHSS using MPA should, however, be viewed with caution due to the small numbers included.

As it is desirable to be able to identify a minimum dose of MPA, another randomized prospective controlled trial was conducted comparing PPOS protocols using 4 mg versus 10 mg of MPA (*Dong et al., 2017*). Previous studies of contraception had indicated that 10 mg MPA could be used to inhibit ovulation, whereas 5 mg MPA failed to do so (*Wikström et al., 1984*); this study did not, however, show any premature LH surge in the group receiving 4 mg/day of MPA. The number of oocytes retrieved and viable embryos were similar in the two groups. The administration of 4 mg/day of MPA was then demonstrated to be sufficient to prevent un untimely LH rise in ovarian stimulation cycles.

The follicular phase dynamics of progestin-primed minimal stimulation and natural-cycle IVF have also been prospectively compared in poor responders (*Chen et al., 2017*). As these patients cannot benefit from increasing gonadotrophin doses, natural-cycle IVF with minimal ovarian stimulation is a patient-friendly option. In order to avoid untimely ovulation in a natural cycle, the use of MPA to block the premature LH surge was explored. The incidence of a spontaneous LH surge and premature ovulation were significantly lower in the MPA group (1.0% versus 50%, $P < 0.05$; 2% versus 10.8%, $P < 0.05$) and the number of oocytes retrieved and viable embryos was higher (both $P < 0.05$). Progesterone priming is therefore a promising approach to overcome premature ovulation during minimal stimulation for poor responders.

Some inconsistencies have been reported regarding reproductive outcomes with the use of MPA. A recent trial (*Begueria et al., 2019*) aimed to evaluate the non-inferiority of MPA compared with a GnRH antagonist on the number of mature oocytes retrieved in oocyte donation cycles. This large trial was conducted on 252 oocyte donors, of whom 173 reached oocyte retrieval, and reported no differences in length of ovarian stimulation, dose of gonadotrophin needed, endogenous hormonal profile or number of mature oocytes between women treated with MPA or a GnRH antagonist. All oocytes were inseminated by ICSI, and the fertilization rate, morphological score of the resulting embryos and proportion of top quality blastocysts were similar between the two groups. Regarding reproductive outcomes in oocyte recipients, worse pregnancy rates have been reported in recipients of oocytes from the MPA group. The biochemical pregnancy rate was 44% versus 57% ($P = 0.023$), the clinical pregnancy rate 31% versus 46% ($P = 0.006$) and the ongoing pregnancy rate 27% versus 40% ($P = 0.015$) for MPA and GnRH antagonists, respectively. The live birth rate was not significantly different when progestin was used instead of GnRH antagonist (22% versus 31%, respectively) Given that this was a non-inferiority study with number of retrieved oocytes as the primary outcome, further investigations specifically aiming to assess live birth as a main outcome are needed.

Progestins other than MPA have also been explored in PPOS protocols (*Zhu et al., 2015*). Conducted under the same conditions as for MPA, a retrospective study compared Utrogestan taken orally in the form of soft capsules (200 mg/day) with HMG with a short protocol. Despite the higher amount of HMG (1884.22 ± 439.47 IU versus 1446.26 ± 550.48 IU, $P < 0.05$), the number of mature oocytes was not significantly different in these groups of normal responders. In contrast, the number of viable embryos was significantly higher in the Utrogestan group compared with the short protocol ($P < 0.05$), although there was no significant difference in the ongoing pregnancy rate.

Utrogestan protocols were also demonstrated to be feasible in improving oocyte quality in a retrospective study conducted on PCOS patients (*Zhu et al., 2016*). PCOS patients were administered Utrogestan (200 mg/day) and HMG (150–225 U/day) from day 3 of the menstrual cycle and compared with PCOS patients undergoing a short protocol. No difference was shown in the number of instances of OHSS in each group. The fertilization rate, viable embryo rate per oocyte retrieved, implantation rate and clinical pregnancy

rate were significantly higher in the study group than the control group (all $P < 0.05$), showing a possible new choice for PCOS patients undergoing ovarian stimulation in combination with embryo cryopreservation.

The Utrogestan and HMG protocol used in the previous study was also applied in a randomized controlled study in normal responders aiming to demonstrate the efficacy of a lower dosage of Utrogestan (*Zhu et al., 2017a*). A dose of Utrogestan of 100 mg/day was used in the study group, while in the control group 200 mg/day was employed. Pituitary LH concentrations were suppressed after 6 days of Utrogestan treatment at 100 mg/day, no premature LH surge was observed, and there were no significant differences between the study and control groups throughout the ovarian stimulation. The safely of Utrogestan has been investigated in a recent retrospective cohort study by Wang and colleagues (*Wang et al., 2018*); neonatal outcomes and live birth defects after Utrogestan-primed ovarian stimulation were compared with those of the infants conceived after conventional ovarian stimulation. No treatment-related difference was shown between the two groups, the congenital malformations being only related to the multiple births.

MPA is generally preferred over Utrogestan because the administration of a natural exogenous progesterone such as the latter can interfere with measurement of serum progesterone, leading to the possible neglect of potential premature luteinization (*Yu et al., 2018*). However, MPA may lead to stronger pituitary suppression and thus require a higher dosage of gonadotrophin and a longer duration of ovarian stimulation than with the conventional ovarian stimulation protocol (*Kuang et al., 2015*). In an attempt to test new synthetic progestins that represent the most suitable option for PPOS, dydrogesterone (DYG) has recently been compared with MPA as part of a PPOS protocol (*Yu et al., 2018*). The results of this prospective randomized controlled trial showed comparable oocyte retrieval and viable embryo numbers in the two groups, with similar pregnancy outcomes. Moreover, DYG was able to effectively suppress the premature LH surge, while not interfering with the measurement of endogenous progesterone (*Yu et al., 2018*). Similar results have been obtained comparing DYG and Utrogestan: in a prospective controlled study published in 2017 (*Zhu et al., 2017b*) in which DYG was shown to be similar to Utrogestan in preventing premature LH surges and in terms of clinical outcomes. The extensive worldwide use of DYG for the treatment of threatened and recurrent miscarriage, as well as for the luteal phase support in infertile patients, suggests also its long-term safety (*Yu et al., 2018*)

ADVANTAGES AND DISADVANTAGES OF PROGESTINS IN OVARIAN STIMULATION

The above-mentioned studies show that the use of progesterone during ovarian stimulation is effective in blocking the LH surge, whether endogenous or exogenous, and does not affect the number of oocytes collected or the quality of the embryos obtained. Total freezing of the oocytes (or embryos) obtained and delayed transfer are, however, mandatory. In particular, the data shown are very reassuring regarding fertility preservation and oocyte donation, as these situations do not require consequent embryo transfer (*Massin, 2017*). The potential harmful effect of the hormonal environment on endometrial receptivity are therefore avoided.

Some studies have reported concerns about prolonged exposure of the developing follicles to progesterone. Although previous studies and a meta-analysis (*Melo et al., 2006; Shapiro et al., 2010; Venetis et al., 2013*) have shown that progesterone elevation in the late follicular phase has no adverse effect on oocyte and embryo quality, suggesting that elevated exposure of the developing follicles to progestins is safe, several recent publications have challenged this concept. Elevated progesterone concentrations on the day of oocyte maturation induction have in fact been said to significantly reduce the formation rate of top-quality blastocysts (*Huang et al., 2016; Vanni et al., 2017*). Progesterone elevation on the day of HCG administration has been said also to adversely affect the cumulative live birth rate per oocyte retrieval cycle (*Bu et al., 2014*), even if this result seems more dependent on the detrimental effect of progestin on the endometrium.

Other than in women seeking fertility preservation, PPOS may be proposed as a first-choice protocol in all conditions where ovarian stimulation and oocyte retrieval are not followed by a fresh embryo transfer (**TABLE 3**). Ovarian stimulation in donors and in preimplanation genetic testing (PGT) cycles may be obviously based on progestin instead of GnRH analogue administration. All 'non-conventional' ovarian stimulation protocols (luteal and random start, double ovarian stimulation) may be associated with the use of PPOS as they are always associated to the "freeze-all" and segmentation of the cycle. Other patients who can benefit from progesterone-blocking protocols are those at risk of OHSS since for these patients there is very frequently the application of the "freeze-all" strategy. One important advantage of the association between a progestin and FSH/HMG in high responders is that the triggering may be exerted by the GnRH agonist, which helps to avoid early-onset OHSS. In addition, cryopreservation of all embryos with delayed transfer can diminish the risk of late-onset OHSS. Other advantages over the use of a progestin in preventing the LH surge are oral administration, easier access and more control over LH concentrations (Wang et al., 2016). This programme is also more patient-friendly as fewer injections are required and it is much cheaper.

The potential economic advantage of the application of protocols involving progesterone is easily understood by calculating the expense of one of these protocols compared with protocols involving GnRH analogues. Although in some of the studies mentioned above the duration of stimulation with FSH/HMG was higher in PPOS protocols than in those using analogues, the difference was not significant in most studies. The cost related to gonadotrophin use is therefore not significantly different between the two protocols. The substantial economic difference becomes evident by comparing the cost of GnRH analogues with that of progestins. The total expense for the GnRH analogue in a GnRH antagonist cycle may vary from €190 to €320. The economic burden is drastically reduced by using progestins. With MPA, a total outlay of €10–15 is sufficient to inhibit ovulation in the IVF cycle. The cost-effectiveness of progestins compared with GnRH antagonists has also been highlighted in a recent article

TABLE 3 WHEN PROGESTIN-PRIMED OVARIAN STIMULATION MAY BE PROPOSED AS THE FIRST-CHOICE PROTOCOL

Donor stimulation
Fertility preservation
PGT-A and PGT-M cycles
Double ovarian stimulation (and non-conventional protocols)
IVF in women at risk of ovarian hyperstimulation syndrome

PGT-A, preimplantation genetic testing for aneuploidies; PGT-M, preimplantation genetic testing for monogenic/single gene defects.

by Evans and colleagues (*Evans et al., 2019*), limited to planned freeze-only cycles and to high-responder patients for whom a 'freeze only' is likely and the risk of OHSS is high.

Despite these advantages, progesterone-blocking strategies associated with delayed embryo transfer may have some weaknesses. The total dose of gonadotrophins used in those protocols could be higher in comparison with common protocols; nonetheless, patients need to return and be rescheduled for cryopreserved embryos transfer. Data on subsequent cryopreserved embryo transfer are still limited. Those protocols furthermore require a change in the practice of current IVF programmes, a good cryopreservation programme, and further evaluation on medical and economical aspects, as many of the conclusions are based on retrospective studies with limited number of patients.

CONCLUSIONS

The application of progestins for inhibit ovulation in ovarian stimulation cycles for IVF has been shown to be effective and safe, with good results reported in terms of the number and quality of the oocytes and embryos obtained, and the low risk of OHSS. The large-scale application of PPOS could be revolutionary for several reasons. With the growing use of IVF, it is preferable to make the treatment as convenient as possible for the patients, possibly converting the route of administration from subcutaneous injections to oral intake. The cost of progestin compared with GnRH analogues also seems extremely beneficial. However, further studies are needed, especially on reproductive, obstetric and long-term neonatal outcomes, before this protocol can be introduced on a wider scale.

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