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COMMENTARY

Ovarian manipulation in ART: going beyond physiological standards to provide best clinical outcomes

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Abstract

Current knowledge on ovarian physiology has challenged the traditional concept of folliculogenesis, creating the basis for novel ovarian stimulation protocols in assisted reproduction technology. The purpose of this review was to evaluate the efficacy of novel clinical interventions that could aid clinicians in individualizing their protocols to patients' characteristics and personal situations. We conducted a literature review of the available evidence on new approaches for ovarian stimulation from both retrospective and prospective studies in the PubMed database. Here, we present some of the most important interventions, including follicle growth in the gonadotropin-independent and dependent stage, manipulation of estradiol production throughout ovarian stimulation, control of mid-cycle gonadotropin surges, and luteal phase support after different stimulation protocols and trigger agents. The latest research on IVF has moved physicians away from the classical physiology, allowing the development of new strategies to decouple organ functions from the female reproductive system and challenging the traditional concept of IVF.

Keywords Double stimulation · Dual triggering · Fertility preservation · Luteal phase ovarian stimulation · Poor responders · Random-start ovarian stimulation

Introduction

Throughout much of recent medical history, illness and treatment have been considered in terms of a profoundly simple model in which physiological knowledge should be applied and either medical treatment or surgical repair performed when a vital organ begins to lose some function. When reproductive organs begin to function abnormally, in vitro fertilization (IVF) has classically been proposed as a clinical approach to help infertile couples achieve a pregnancy. Since the birth of the first "test-tube baby," ovarian stimulation (OS) protocols have been based on the administration of exogenous gonadotropins to induce the growth of multiple follicles during the follicular phase of the menstrual cycle, increasing the number of mature oocytes fertilized by a sperm sample. However, the latest research in IVF has moved physicians away from the classical physiology, allowing the development of new strategies to decouple organ functions from the female reproductive system in an attempt to achieve the best clinical outcomes for patients, and challenging the traditional concept of IVF.

The "freeze-all" strategy based on vitrification represents one of the biggest breakthroughs in reproductive medicine, allowing embryos to be transferred in a subsequent cycle and emerging as an alternative to fresh embryo transfer during IVF. Compared to slow freezing, current oocyte and embryo vitrification protocols yield excellent survival rates, up to 97% in young women [10, 43], and implantation and pregnancy rates for oocytes derived from vitrification/warming cycles are not different from those of fresh oocytes [11]. Establishment of the vitrification technique has dramatically changed routine clinical practice in reproductive centers; contrary to previous belief, ovarian function does not necessarily go hand in hand with the uterus, enabling a decoupling of both organs in assisted reproductive technologies (ARTs).

Such a dissociation of the uterus and ovarian function may occur in several clinical scenarios. First, the egg donation program allows a pregnancy to be established after manipulation

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of the recipient's uterus with proper hormonal replacement therapy using donor oocytes. Second, the ovary may be stimulated in a progesterone-enriched milieu, raising the possibility of starting OS in a phase other than the follicular phase. A report published in 1987 described multiple follicular development after IVF in the presence of a viable intrauterine pregnancy [16], challenging the previously held notion that a single cohort of antral follicles grows only during the follicular phase of the menstrual cycle. Similarly, recent studies on oncology patients and low responders have demonstrated that OS can be initiated in the luteal phase of the menstrual cycle with optimal reproductive outcomes [62, 84].

Taken together, these findings show that we, as physicians, have found several ways to manipulate ovarian function, shifting from the classical model to novel approaches aimed to provide the best standard of care to all patients. Such an improvement leads to the customization of fertility treatments in a broad range of clinical situations, such as fertility preservation in oncology patients or low responders, enabling the medical treatment to be synchronized with the patient's lifestyle. The present review summarizes recent modifications in ovarian physiology over the past few decades in the setting of clinical ART, showing how modern medicine is able to manipulate each step of OS (Fig. 1). This review describes not only the clinical interventions that can be pursued at different points of OS but also their clinical applications in current IVF treatments, focusing on oncology patients undergoing fertility preservation, high and low responders, and menopausal women.

Methods

We attempted to clarify the main aspects of ovarian manipulation and its clinical applications by synthesizing the results of primary studies. A broad PubMed and Medline database search was performed for all case reports, retrospective, and prospective studies. The following search terms were used, both alone and combined: GnRH analogues, clomiphene citrate, in vitro activation, mild stimulation, kisspeptin, letrozole, luteal phase OS, fertility preservation, random-start OS, double stimulation, oocyte and embryo cryopreservation, tamoxifen. From the total number of publications associated with the topic, selection criteria were applied based on the relevance of the topic, outcomes of interest, and potential clinical application. Only English full-text publications were reviewed. The same terms were used to search for ongoing clinical trials in the US NIH database ClinicalTrials.gov and among institutional guidelines and protocols.

Ovarian physiology

Over the past few decades, reproductive medicine has been based on initial primate studies published in parallel with the development of ARTs in the 1980s, aiming to better understand the mechanisms underlying follicular growth and atresia, corpus luteum function, and ovum maturation. A landmark paper describing the recruitment and selection of the dominant follicle, the intrafollicular milieu, and ovarian regulation of pituitary function represented the biological basis and standard for future research in ovarian physiology [27].

The primate ovary is characterized by the development of a cohort of follicles from which only one follicle commonly reaches the pre-ovulatory stage, resulting in a single ovulation each month [35], as most follicles fail to complete the maturation process and undergo atresia. In humans, primordial follicles undergo initial recruitment to enter the growing pool of primary follicles, requiring more than 120 days to reach the secondary follicle stage, whereas 85 days are needed to grow into the early antral stage. According to classical studies, the basal growth of follicles from the pre-antral to the selectable stage occurs in a gonadotropin-independent fashion due to the low proliferative activity of granulosa cells, low expression of steroidogenic enzymes, and downregulation of FSH receptors. After reaching a size of 2 mm, follicles become more dependent on FSH. Increased circulating levels of FSH and multiple locally produced intrafollicular factors allow a cohort of antral follicles (2–5 mm in diameter) to escape apoptotic demise [25]. Therefore, follicle development may be divided into gonadotropin-responsive (pre-antral follicle stage) and gonadotropin-dependent (beyond early antral stage) stages. Although development to the antral stage is not dependent on FSH, pre-antral follicles are responsive to FSH treatment and regulated by several local intra-ovarian factors (e.g., granulosa cell-derived C-type natriuretic factor [CNP]) and signaling pathways (e.g., AKT and mTOR), allowing the development of new therapeutic approaches [28].

The development of novel OS protocols has challenged the traditional concept of ovarian physiology, leading to modern clinical interventions that have dramatically changed clinical care in endocrine-infertility treatments. In the present review, some of the most important interventions are addressed, including follicle growth in the gonadotropin-independent stage, random-start OS protocols, manipulation of estradiol production throughout OS, control of mid-cycle gonadotropin surges, and progesterone secretion.

Interventions for gonadotropin-independent follicular growth

Hippo signaling pathway—patient with primary ovarian insufficiency

The development of technologies to grow oocytes from the most abundant primordial follicles to pre-antral follicles has challenged the concept that follicle growth may not be

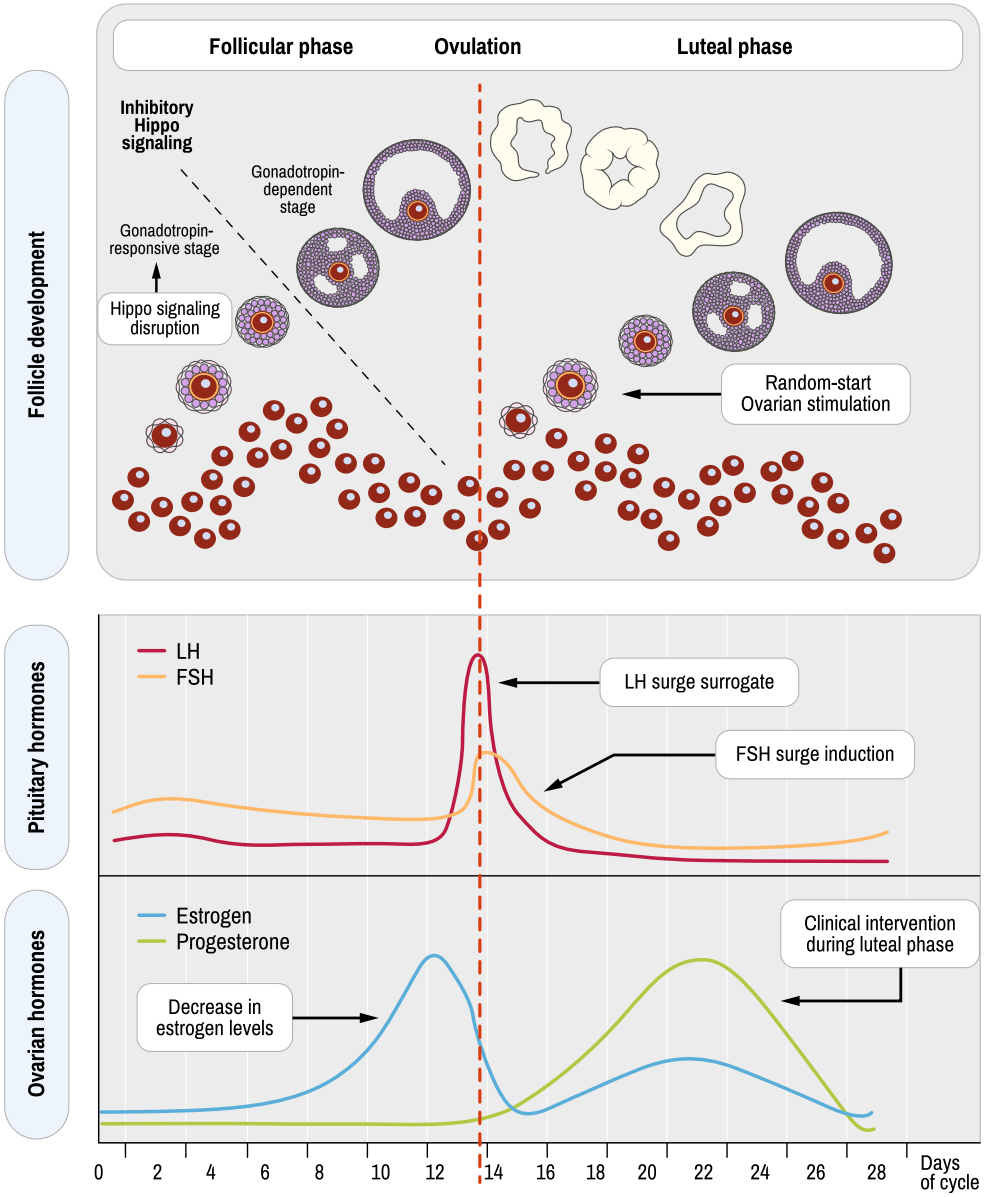

Fig. 1 Diagram of various events during the menstrual cycle and potential clinical interventions. From top to bottom: emergence and regression of follicular waves in women with two follicular waves, pituitary and ovarian hormone fluctuations. Adapted from De Mello Bianchi et al. [14]

activated in the gonadotropin-independent phase. The Hippo-signaling pathway is involved in the control of organ homeostasis and size in animals. The pathway consists of several negative regulators acting in a kinase cascade, phosphorylating and inactivating key Hippo signaling effectors that play a crucial role in restraining cell proliferation and promoting apoptosis. The development of pre-antral and antral follicles has been shown to be restrained by inhibitory Hippo signaling effectors, such as Yes-associated protein (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ) (Fig. 1). After Hippo signaling is disrupted, high YAP levels increase downstream CCN growth factors and inhibitors of apoptosis, leading to cell growth and proliferation. In mammalian ovaries, most ovarian follicles are constrained to grow under physiological conditions due to the local inhibitory effect of Hippo signaling.

A murine model has demonstrated that ovarian fragmentation disrupts the Hippo signaling pathway and promotes actin polymerization, leading to increased follicle growth and the generation of mature oocytes [34]. Previous studies using phosphatase and tensin homolog deleted from chromosome 10 (PTEN) knockout mice indicated that the Akt signaling pathway plays a prominent role in the activation and development of dormant primordial follicles [54]. Treating ovarian fragments containing secondary follicles with Akt stimulators induces an increase in follicle growth. The same group combined these two methods in an in vitro activation (IVA) method that may be relevant in women with primary ovarian insufficiency (POI), as it may induce the growth of residual follicles and increase the number of mature oocytes after IVF treatment.

Kawamura et al. [34] reported the first birth after ovarian vitrification and IVA/grafting to promote follicle growth followed by auto-transplantation. Briefly, ovaries from 27 infertile patients were removed via laparoscopic surgery, cut into strips, and vitrified. After histological analysis, frozen ovarian strips were thawed, fragmented into approximately 100 1–2 mm^2 cubes, and treated with Akt stimulating drugs for 2 days. After grafting ovarian tissue back into the patients beneath the Fallopian tube serosa, follicle growth was monitored during IVF [34]. After adding 10 new patients to this cohort, they found that ovaries from 20 of the 37 patients contained residual follicles, 9 of which exhibited follicle growth in auto-grafts, with 24 oocytes retrieved from 6 patients. After IVF and embryo transfer in four patients, one miscarriage and two successful deliveries were achieved [75]. In addition to these two healthy babies delivered after IVA at St. Mariana University Hospital [34, 75], two more healthy babies were born after cryopreservation-free IVA in China and Spain [33].

Interestingly, histological analysis of ovarian cortices and the time from POI diagnosis to ovariectomy are the main prognostic factors for IVA outcome [93]. Such a procedure represents a systematic activation of residual follicles, leading to follicle growth and the production of mature oocytes. The present approach may be relevant not only for treating infertile POI women, but also for fertility preservation in cancer patients undergoing sterilizing treatment and premenopausal women with a diminished ovarian reserve. Despite providing more mature oocytes for embryonic development in women of advanced maternal age, this approach does not overcome the age-related defects in oocytes.

Interventions for gonadotropin-dependent follicular growth

Random start—oncological patients

In assisted conception cycles, gonadotropin administration is initiated from the early follicular phase onward to extend the "window of recruitment," allowing a larger cohort of follicles to escape atresia. Over the past decade, there has been growing interest in developing new protocols based on the concept of alternative timing for starting OS for fertility preservation. However, the dogma suggesting that synchronized follicular development may only be achieved in early follicular phase-start OS, based on the theory of single-wave follicular development and the strong belief in local inhibitory effects of the corpus luteum and progesterone in the luteal phase, has limited the development of new protocols.

The "wave theory" of follicular recruitment initially emerged form veterinary medicine's characterization of follicular wave dynamics in domestic farm animals [20, 69]. Two patterns of follicular waves are known: the major wave pattern, in which a single leading follicle becomes dominant after a few days and is finally ovulated while the remaining follicles become atretic, and the minor wave pattern, in which follicular dominance does not occur and all follicles undergo atresia. In humans, follicular wave dynamics characterized in women during the natural menstrual cycle indicate that the day of follicular recruitment/wave emergence varies depending on the number of waves developed throughout the cycle. Sixty-eight percent of women exhibit two waves of follicle development, and 32% exhibit three waves during the interovulatory interval (IOI). In both two- and three-wave cycles, the final wave emerging in the early follicular phase of the cycle is ovulatory, whereas the preceding waves developed in the luteal phase are anovulatory [5]. The wave theory of follicle recruitment challenges the classical concept of folliculogenesis and is the basis for current luteal phase OS protocols.

Oocyte vitrification is the treatment of choice for fertility preservation in both oncology and non-oncology patients because it yields excellent survival rates after thawing and optimal clinical results (Practice Committees of American Society for Reproductive Medicine and Society for Assisted Reproductive Technology, [61]). In oncology patients, OS based on a GnRH antagonist protocol is the treatment of choice. Madrigano et al. [46] demonstrated that the average time interval from the first evaluation to oocyte retrieval in a subset of oncology patients that underwent OS before the onset of adjuvant therapies was 33.3 days. This aspect is relevant in oncology patients, as there is a narrow window of opportunity for egg harvesting due to the limited time frame until the initiation of chemo and/or radiotherapy, and sometimes, the patients do not have enough time to undergo OS.

To overcome this issue, a novel strategy for emergency fertility preservation has been developed in the last few years based on the possibility of starting OS at any time of the cycle. However, there are limited data in the literature regarding random-start OS and emergency fertility preservation. The initiation of OS during the luteal phase of the cycle in cancer patients was first introduced by von Wolff et al. [84], who compared a study group treated with simultaneous administration of GnRH antagonist and recombinant FSH in the luteal phase to a control group based on standard follicular phase stimulation [84]. No significant differences were found in either the dosage or duration of gonadotropin administration between the two groups. Therefore, although the number of oocytes was slightly higher in the follicular phase group (13.1 vs. 10.0), the difference was not significant. Similarly, the maturation (83.7 vs. 80.4%) and fertilization rates (61.0 vs. 75.6%) were comparable between both groups. These findings were confirmed by subsequent studies and case series with a small number of patients [7, 73].

Recently, Von Wolff et al. [83] carried out the largest study evaluating random-start stimulation as part of fertility preservation therapies to date. The study included 684 women who underwent OS prior to gonadotoxic therapy and were subsequently divided into three groups according to the day of stimulation initiation: group A, early proliferative phase (day 1–5 of the cycle); group B, mid-late proliferative phase (day 6–14); and group C, luteal phase (after day 14). The total gonadotropin dosage was significantly higher in group C than groups A and B due to a slightly prolonged stimulation time in group C compared to the others. However, the number of oocytes obtained was significantly higher in groups B and C compared to group A. This study confirms the results of previous smaller studies, indicating that such time-optimized stimulations can be started at any point in the cycle before gonadotoxic treatment, including the luteal phase [83].

Double stimulation in poor ovarian response

Despite establishing a variety of protocols and adjuvant therapies to improve the poor ovarian response (POR), these patients still present with compromised reproductive outcomes, making the management of POR a challenge for reproductive specialists. The luteo-follicular recruitment of follicles in poor responders was first proposed by Rombauts et al. [62], who evaluated the possibility of increasing the number of oocytes after OS by commencing OS in the luteal phase of the previous cycle in patients who previously had a suboptimal OS cycle [62]. A total of 40 patients downregulated by GnRH agonist received 150 IU of recombinant FSH therapy and were randomized into two groups: the experimental group, which started gonadotropin administration on day 25 of their previous cycle, and the control group, which started it on day 3 of the treatment cycle. No significant difference was reported in the number of oocytes collected and the authors concluded that initiating prolonged administration of exogenous gonadotropin in the luteal phase does not prevent more recruitable follicles from undergoing atresia.

In light of these findings, some authors hypothesized that continuous OS with two oocyte retrievals within the follicular and luteal phases of the same menstrual cycle (double stimulation) may represent a clinically valuable alternative to poor responders when no oocytes are retrieved following IVF with follicular phase OS [87]. Kuang et al. carried out a pilot study to evaluate the efficacy of double stimulation during the follicular and luteal phases in women with POR undergoing mild OS IVF (Shanghai protocol). During stage one of the treatment protocol, letrozole was added to clomiphene citrate and 150 IU HMG to stimulate follicular development. Subsequently, stage two was initiated that day or the day after the first oocyte retrieval with letrozole and higher doses of gonadotropins (225 IU HMG). Between the two stages, no significant differences were found in the mature oocyte, fertilization, and cleavage rates. Similarly, the number of top-quality embryos and cryopreserved embryos was similar between the first and second oocyte retrievals. [41]. Interestingly, a subsequent study reported a similar number of euploid blastocysts per injected MII oocyte after follicular-phase stimulation and LPS were performed during the same menstrual cycle in low responders [81]. Therefore, double stimulation in this subset of patients may augment the final number of transferable blastocysts, increasing the chances of having euploid embryos for transfer and improving reproductive outcomes.

Interventions for estradiol secretion in the follicular phase

Ovarian hyperstimulation syndrome and oncological patients

In a normal menstrual cycle, the development of a single dominant follicle during the follicular phase is associated with increased estradiol production, mediated by aromatase activity in granulosa cells [24]. Similarly, in the setting of IVF, a greater number of growing follicles leads to increased serum estradiol levels, the magnitude of which depends on gonadotropin dose and patient characteristics. Moreover, the concomitant use of LH-containing drugs in OS protocols has been shown to increase estradiol production, as it dose-dependently increases androgen production at the follicular level for later aromatization to estrogens [8].

Oocyte freezing to avoid the potential adverse effects of high estradiol on OHSS and implantation-placentation

The impact of elevated estradiol levels on the day of oocyte triggering on reproductive outcomes has been a matter of debate over the past few decades. Some investigators have demonstrated that elevated estradiol levels after OS have no effect on IVF reproductive outcomes [86, 90], whereas others have shown that they may impair endometrial receptivity and, thus, IVF outcome [2]. Moreover, estrogens have been shown to regulate vascular endothelial growth factor-A (VEGF-A) expression through an estrogen receptor (ER)-alpha-dependent pathway, which may be involved in the development of ovarian hyperstimulation syndrome (OHSS) and in the pathophysiology of breast cancer [82].

Oncofertility protocols with aromatase suppression

Regardless of the potential negative effect of supraphysiological estradiol levels on clinical outcomes, modulation of estradiol production should be considered in patients with estrogen-sensitive malignancy, such as endometrial or ER-positive breast

cancer. In this scenario, 2.5 or 5 mg/day letrozole (depending on the patient's ovarian reserve) should be administered simultaneously with OS to keep estradiol at physiological levels and continued until the trigger day [72]. In clinical trials, the use of letrozole in patients with estrogen-sensitive cancers has resulted in significantly lower maximum serum estradiol levels in both random- and conventional-start cycles [9]. By releasing the hypothalamic-pituitary axis from negative estrogenic feedback due to blockade of the aromatization of androgens into estrogens, letrozole may increase follicular growth secondary to an increase in intra-ovarian androstenedione and testosterone concentrations [42]. In addition, letrozole does not adversely affect reproductive outcomes, yielding a similar number of total and mature oocytes and similar maturation and fertilization rates, regardless of its use in random- or conventional-start protocols [9, 55]. Interestingly, the concurrent use of letrozole throughout OS has been shown to not affect the relapse-free survival rate after a median follow-up of approximately 2 years [3]. On the basis of these data, OS with simultaneous administration of letrozole and gonadotropins seems to be a safe and effective approach in oncology patients, at least in the short-term.

Other drugs have also been co-administered during conventional OS protocols for a fertility preservation IVF cycle in breast cancer patients. Tamoxifen citrate, a selective estrogen receptor modulator (SERM), competes with estrogen for binding sites in the ER in target tissues, such as the breast. Tamoxifen has been used for more than 30 years, with significant efficacy in reducing breast cancer recurrence and improving patient survival as the treatment of choice in young premenopausal breast cancer patients [31] despite potential elevated estrogen levels during treatment [12]. There are several reasons for using tamoxifen rather than letrozole. First, unlike letrozole, tamoxifen does not require special approval for clinical use and has been used in fertility treatment for several decades. Second, a recent study demonstrated that co-administration of tamoxifen during stimulation is safe and efficient with favorable fertility preservation despite higher estradiol serum levels [52]. However, there is no evidence for the potential use of tamoxifen in LPS protocols.

Interventions for mid-cycle gonadotropin surge control

In the IVF setting, a major cause of cycle cancelation during OS is the occurrence of premature LH surges. Thus, conventional protocols for ovarian induction involve the challenge of controlling ovulation inhibition [80]. In the early days, all efforts were focused on detecting the pre-ovulatory increase in LH in women undergoing OS with gonadotropins for IVF, measuring either the urine or serum LH concentration [18]. However, up to 30% of the patients have a premature LH surge resulting in cancelation of the cycle, despite close monitoring throughout OS [71] .

Since the first days of IVF, hCG has been the gold standard for inducing final oocyte maturation, as it closely resembles the main structural and biological characteristics of LH. Although both molecules activate the LH/hCG receptor, there is an important difference regarding their half-lives (60 min for LH vs. > 24 h for hCG) [13, 88]. Such a difference may have relevant implications, as the prolonged half-life of hCG may induce sustained luteotropic activity associated with the occurrence of undesired side effects due to the release of some vasoactive molecules, such as VEGF. This molecule plays a prominent role in the development of OHSS, the most life-threatening complication associated with ARTs [82].

Over the past decade, several IVF strategies have been developed to avoid the premature LH surge and to trigger oocyte maturation, improving oocyte quality and avoiding the risk of OHSS.

Avoiding the premature LH surge

1. GnRH analogs

The introduction of GnRH agonist into IVF protocols in the 1980s, in either the early follicular phase or luteal phase of the previous menstrual cycle (short and long protocols, respectively), was an effective approach for preventing a premature increase in LH due to downregulation, gaining widespread popularity in clinical medicine until present day [58, 76]. Furthermore, the use of the GnRH agonist protocol in combination with gonadotropins was associated with major benefits, including enhanced follicular recruitment, allowing the recovery of a larger number of oocytes, and improvements in the routine patient treatment schedule [46, 96]. In contrast, the use of GnRH agonists in the long protocol was associated with some shortcomings, including a long treatment period until the occurrence of desensitization, increased risk of OHSS, and more frequent occurrence of side effects due to hypo-estrogenemia [79].

Against this background, the introduction of GnRH antagonists in ARTs to prevent the premature LH surge seemed to overcome some major disadvantages associated with GnRH agonists, opening the door to a more "friendly" IVF [17]. Immediate and profound suppression of the hypothalamic-pituitary axis to prevent the endogenous LH surge resulted in a shorter duration of stimulation and fewer exogenous gonadotropins required for stimulation, reducing the side effects caused by profound hypo-estrogenemia [56]. These characteristics, which were associated with lower OHSS rates and excellent overall IVF outcomes, improved the patient's experience throughout IVF treatment compared to GnRH agonist and became the cycle protocol of choice in most IVF units [37, 79].

2. Clomiphene citrate

Clomiphene citrate (CC) is an anti-estrogen agent approved for the treatment of ovulatory dysfunction. CC acts in the hypothalamus, leading to an increase in endogenous FSH and the LH pulse frequency, resulting in a moderate gonadotropin stimulus to the ovary and subsequent ovulation [89]. In conventional regimens, CC at doses of 50 to 250 mg/day was started in the early follicular phase and discontinued after 5 days of treatment to induce ovulation [67]. However, continuous administration of CC throughout OS in mild stimulation protocols exerts an opposite effect. Kato et al. [77] developed a pioneering protocol characterized by oral CC administration (50 to 100 mg/day) with an extended regimen from cycle day 3 until the day before the GnRH agonist was given to trigger oocyte maturation. Premature onset of LH surge occurred in less than 3.5% of cases [77]. This effect has also been shown in donors with good antral follicle count, as longer stimulation was achieved with CC and the premature LH surge prevented by the anti-estrogen effect of CC on the pituitary in the presence of increased estrogen levels [68]. Therefore, continuous administration of CC may be a valid method for blocking pituitary function, representing the third method to prevent premature LH surge after conventional OS with GnRH agonist and antagonist.

3. Progestins

There is increasing evidence that progestins may also be a valid method of preventing the premature LH surge during OS. The LH-suppressing effects of progestins have been widely utilized in the past in research of oral contraceptive pills [23, 59]. Specifically, medroxyprogesterone acetate (MPA) has a moderate to strong progestin action and does not interfere with the measurement of endogenous progesterone production [70]. Administration of 10 mg/day MPA from day 3 of the menstrual cycle with human menopausal gonadotropin (hMG; 150–225 IU/day) has been shown to be an effective and feasible strategy for inhibiting a premature increase in LH in patients undergoing OS for IVF without impairing pregnancy outcomes [85]. In addition, despite the use of a greater amount of gonadotropins due to less sensitivity of the follicles to gonadotropin administration in the high progesterone environment, the MPA-based protocol is associated with a reduced risk of developing OHSS in both the follicular and luteal phases [40, 42, 85]. Recently, other progestins have been shown to be an effective oral alternative for preventing premature LH surges during OS in normal ovulatory women undergoing IVF, with optimal reproductive outcomes in frozen-thawed embryo transfer [94, 95].

Oocyte maturation triggering to improve quality

1. Dual triggering

Over the past decade, the combination of a single dose of GnRH agonist with a reduced or standard dose of hCG trigger for final oocyte maturation in IVF cycles has been suggested as a clinical approach to improve the oocyte maturation rate. Fabris et al. [22] demonstrated that dual trigger in patients with more than 50% immature oocytes in a previous IVF cycle with hCG yielded a better number of mature oocytes and reproductive outcomes [22]. Compared to GnRH-a alone, the “dual trigger” strategy has been shown to significantly improve the live-birth rate (52.9 vs. 30.9%) in high responders without increasing the risk of significant OHSS [26]. In normal responders, dual trigger also improves clinical outcomes compared to the standard dosage of hCG trigger in GnRH antagonist IVF cycles [45]. The dual trigger concept has also been used in poor responders, yielding better oocyte retrieval rates than GnRH-a alone [32, 47]. Furthermore, the dual trigger may be clinically relevant in cases of empty follicle syndrome and a history of a high number of immature oocytes retrieved on the ovum pickup day [32].

2. FSH surge

In most mammalian species, spontaneous ovulation is preceded by surges in both LH and FSH due to the combination of accumulated pituitary GnRH receptors and increased GnRH secretion. The FSH surge has been shown to play an important role in the acquisition of oocyte competence in vitro, inducing oocyte maturation and ovulation [74, 91]. Classically, standard IVF treatment requires hCG administration to stimulate the LH surge 36 h later and induce final oocyte maturation. However, FSH levels may be decreased at this crucial step due to pituitary downregulation in current stimulation protocols and because the last gonadotropin dose may be administered up to 2 days before oocyte retrieval.

Given this background, Lamb et al. [44] carried out a randomized clinical trial to determine whether an additional FSH bolus administered at the time of the hCG trigger improves the developmental competence of the oocyte. Although fertilization was significantly improved in the treatment arm, no significant differences were observed in clinical pregnancy or live-birth rates. Despite these promising initial findings, larger studies are needed to further investigate whether such an approach has any potential benefit in terms of clinical outcomes in patients undergoing IVF [44].

Oocyte maturation triggering to avoid OHSS

1. GnRH agonists

With the introduction of GhRH antagonists in OS protocols during the 1990s, the use of a single bolus of GnRH agonist to trigger an endogenous LH surge and, thus, final oocyte maturation and ovulation was proposed as an alternative to hCG [53]. The administration of GnRH had some advantages, including a reversible effect, rapid action, and shorter duration of the endogenous LH surge, which is associated with a reduced risk of OHSS development [36]. Furthermore, unlike hCG use, the use of GnRH agonist to trigger final oocyte maturation also induces an FSH surge, which may act synergistically with LH to promote oocyte nuclear maturation and cumulus expansion [92]. On the other hand, one of the main shortcomings of using GnRH agonist for triggering ovulation is decreased clinical pregnancy rates due to luteal phase insufficiency [39], despite supplementation with progesterone and estradiol. In the last few years, several protocols have been suggested to overcome luteal phase insufficiency when using GnRH agonist to trigger ovulation.

2. Kisspeptins

Kisspeptins (KPs) involve a group of peptide hormones encoded by the KISS1 gene in humans that play a crucial role in human neuroendocrine regulation [64]. The location of KP neurons differs between animal species. In humans, KP neurons are located in the hypothalamus and seem to play a central role in the generation of GnRH pulses in mammalian species [63]. Both central and systemic administration of KP have been shown to stimulate GnRH secretion, which then stimulates the secretion of both LH and FSH from the anterior pituitary [50, 57]. However, this KP-induced stimulatory effect can be blocked by GnRH antagonists [65]. Although the majority of studies have been carried out in animals, the stimulatory effect of KP has also been demonstrated in humans. Recently, Abbara et al. [1] showed that KP-54 is a promising approach for effectively and safely triggering oocyte maturation, successfully achieving live births in women at high risk of developing OHSS who were undergoing IVF treatment [1]. However, larger randomized studies are needed to compare the efficacy and safety of KP with currently used triggers before it can be commercialized and introduced into routine clinical practice.

Interventions for luteal phase support

Although the present review does not focus on luteal phase support, it may be crucial in the IVF setting for the development of the early pregnancy. Current evidence suggests a luteal phase deficiency in IVF cycles, regardless of the use of GnRH agonist or antagonist protocols. Moreover, GnRH agonist-induced decreases in progesterone levels when used as an ovulation trigger leads to impaired clinical outcomes and lower chances of pregnancy [66]. Given this background, several strategies have been proposed for luteal phase support in women at high risk of developing OHSS who receive GnRHa as an ovulation trigger to facilitate fresh embryo transfer. Kol et al. [38] demonstrated that luteal phase support with a total of two boluses of 1500 IU hCG on the day of oocyte retrieval and 4 days later reverts luteolysis after GnRHa trigger [38]. In addition, GnRHa trigger combined with intensive luteal steroid support (exogenous progesterone administration and oral estradiol) can facilitate fresh embryo transfer, though the occurrence of late-onset OHSS cannot be ruled out completely [30]. Although these approaches have been shown to be effective in achieving optimal pregnancy rates [29], the "freeze-all" protocol after GnRHa trigger and deferred embryo transfer has become the gold standard in patients at high risk of OHSS.

In the case of insufficient secretion of progesterone and estrogens by the lutean cells of the corpus luteum, recent studies have suggested a potential beneficial effect of GnRH agonist when administered as luteal phase support, supporting the corpus luteum by stimulating the secretion of LH by gonadotroph cells or by acting directly on the endometrium through locally expressed GnRH receptors [60]. A previous study evaluated the effects of GnRH agonist on clinical outcomes when administered in a single dose 6 days after ICSI, concluding that luteal-phase GnRH agonist administration enhances ICSI reproductive outcomes in both GnRH agonist- and GnRH antagonist-treated OS cycles [78]. Following this line of reasoning, a recent study demonstrated that intranasal GnRHa is effective in achieving luteal-phase support in high-responder patients triggered with GnRHa, avoiding OHSS [6]. However, a recent meta-analysis of 2776 women reported that there is low-quality evidence that adding GnRH agonist during the luteal phase improves the likelihood of ongoing pregnancy, concluding that this intervention requires further research before being integrated into clinical practice [49].

In the case of corpus luteal insufficiency, progesterone-based luteal phase support may be used. Although the preferred route of administration for progesterone is still an area of active research, both intramuscular and vaginal progesterone have become the standard of care for luteal-phase support. A recent comparison between different routes of progesterone administration demonstrated a lack of clinically significant differences between subcutaneous and vaginal progesterone for luteal-phase support in patients undergoing IVF [19]. However, a recent interim analysis of a three-arm, randomized, controlled, non-inferiority trial of 645 cycles demonstrated significantly poorer ongoing pregnancy rates following vitrified-warmed blastocyst transfer when vaginal-only

Table 1 Potential clinical interventions and applications of ovarian manipulation in ART derived from current knowledge in women undergoing IVF treatment. *DA* dopamine, *IVA* in vitro activation, *IVF* in vitro fertilization, *KP* kisspeptin, *OHSS* ovarian hyperstimulation syndrome, *POI* primary ovarian insufficiency, *WOI* window of implantation

	IVA	Random start	Dual stimulation	Letrozole/ Tamoxifen	Trigger with GnRHa/KP	Oocyte/embryo freezing	DA agonist/GnRH ant
Oncological patient		X		X	X	X	
High responder					X	X	X
Poor responder			X	X	X	X	
Menopausal patient	X						

progesterone replacement was administered [15]. In light of this finding, randomization of patients to the vaginal progesterone-only arm was stopped. Although intramuscular progesterone replacement for vitrified-warmed blastocyst transfer may be recommended based on these data, further studies are needed to evaluate whether other scenarios of assisted reproduction could benefit from this approach.

Future technique/advance

There is some evidence for the possibility of an unexpected return of ovarian function and fertility after bone marrow transplantation in both animal models and humans [4]. Over the past decade, interest has increased in alternatives to generating autologous germ cells in vitro that may be relevant in menopausal women who want to produce their own genetic offspring. The derivation of human germ cells from human embryonic stem cells and skin-derived stem cells has been reported [21], confirming that stem cells possess the potential to form germ cells, which is meaningful for the treatment of infertility. A recent paper also demonstrated the conversion of human germ cells from human somatic cells using key gene regulators [51]. Although promising, studies focusing on genetic and epigenetic regulation of the whole cycle of germ cell fate are needed before the introduction of this technique into the clinical setting.

Limitations

One of the main limitations of the present review is the limited number of randomized studies focused on LPS, as most of the studies found in the literature are case reports or retrospective studies with heterogeneous inclusion criteria, bias in the study design, and a limited number of participants. Therefore, all of the findings should be interpreted with caution. A larger number of well-designed, large-scale, randomized, controlled trials with live-birth rates are needed to better assess the efficacy of luteal-phase OS in IVF patients and elucidate the optimal stimulation regimen for this challenging group of patients.

Conclusions

Current knowledge of ovarian physiology has challenged the traditional concept of folliculogenesis, creating the basis for novel OS protocols in ARTs and leading to the development of new clinical applications (Table 1). Luteal-phase initiation of OS appears to be a safe and effective method for yielding competent oocytes with comparable reproductive and perinatal outcomes compared to conventional protocols. Thus, random-start COH provides a significant advantage in oncology patients eligible for fertility preservation, allowing OS to be initiated at any point in the menstrual cycle and decreasing the total time for an IVF cycle. Furthermore, double stimulation and subsequent cryopreserved embryo transfer is a promising approach for poor responders with previous failure of conventional IVF regimens. Menopausal women would benefit from the IVA method, as it may induce the growth of residual follicles and yield a decent number of oocytes after IVF treatment. However, there is an urgent need for large long-term cohort studies/randomized clinical trials to better elucidate the role of these new approaches in IVF before they are integrated into routine clinical practice.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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