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COMMENTARY

Why more is less and less is more when it comes to ovarian stimulation

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Abstract
Purpose The purpose of the present study is to describe the possible mechanisms which may explain the apparent paradox of "less is more." Mild ovarian stimulation for in vitro fertilization (IVF) minimizes ovarian hyperstimulation syndrome (OHSS) and multiple gestations without compromising the pregnancy rate (PR).
Methods The pertinent English literature (PubMed) addressing mild stimulation for IVF/assisted reproductive technology (ART) and publications addressing "mild" or "soft" controlled ovarian stimulation (COS) vs conventional COS for IVF, OHSS, natural cycle IVF, and IVF outcome in association with COS was searched.
Results Four possible mechanisms can be put forward to explain the apparent paradox of "less is more." (1) In the natural or mild stimulation cycles, the healthiest follicles are selected by the principle of "quality for quantity"; (2) high estradiol (E_2) in the late follicular phase significantly correlated with higher rates of small for gestational age (SGA) and low-birth-weight (LBW) neonates; (3) anti-Mullerian hormone (AMH), LH, testosterone, and E_2 are significantly higher in natural cycle (NC)-IVF than in stimulated IVF follicles, suggesting an alteration of the follicular metabolism in stimulated cycles; and (4) supraphysiological E_2 may increase the growth hormone-binding protein (GH-BP) bio-neutralizing GH and diminishing the resultant insulin-like growth factor (IGF) levels, necessary for optimal synergism with follicle-stimulating hormone (FSH).
Conclusions It is suggested to aim at the retrieval of around eight to ten eggs. Mild stimulation should be the common practice for IVF. In cases where more than ten ova are retrieved or high E_2 levels are reached, either intentionally or unintentionally, "freeze-all policy" should be considered and embryo transfer (ET) done in a subsequent natural cycle.

Keywords Minimal ovarian stimulation · Mild controlled ovarian stimulation (COS) · In vitro fertilization (IVF) · Ovarian hyperstimulation syndrome (OHSS) · Growth hormone-binding protein (GH-BP)

Capsule Four possible mechanisms are discussed that shed light on the question of why less gonadotropin during COH may in fact be better than more:
1. In the natural or mild stimulation cycles, the healthiest follicles are selected by the principle of quality for quantity.
2. High E_2 significantly correlated with SGA and LBW neonates.
3. Better intrafollicular hormonal milieu in minimal stimulation.
4. Supraphysiological E_2 may increase the GH-BP bio-neutralizing GH and diminishing the resultant IGF levels, necessary for optimal synergism with FSH.

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Introduction

Since the delivery of the first in vitro fertilization (IVF) generated neonate in 1978, the clinical practice of assisted reproductive technology (ART)/IVF has undergone many changes and variations. Indeed, whereas the successful pregnancy of Louise Brown has been achieved in a natural cycle, the pendulum of clinical practice has soon after swung over to controlled ovarian stimulation (COS) with the rationale that retrieval of many ova may increase the clinical pregnancy rate (PR) [1]. However, in the last decade, the clinical practice pendulum has turned back toward mild (the so-called "soft")

ovarian stimulation for the retrieval of a lower number of oocytes for IVF [2, 3]. Low-dose stimulation regimens for IVF have many synonyms: "mild," "light," soft, "mini," "minimal," "low cost," "modified natural cycle," and "low dose." Milder ovarian stimulation protocols for IVF were developed for minimizing the adverse effects of the more aggressive COS, mainly ovarian hyperstimulation syndrome (OHSS) and multiple gestations. Furthermore, it has been observed that the mild ovarian stimulation generating a relatively modest number of oocytes is associated with better implantation rates and PR. Therefore, the concern of reducing the number of retrieved oocytes following mild COS appears more clinically appealing and "patient friendly" [2, 3]. What is the rationale and possible explanation to the improved outcome of the soft COS or why *less is more*? At least four possible mechanisms can be put forward to explain this apparent paradox:

1. Natural selection: "quality for quantity"
2. Early-gestation high estradiol (E_2) effect on fetal growth
3. Better intrafollicular hormonal milieu
4. The GH/insulin-like growth factor (IGF)/growth hormone-binding protein (GH-BP) system

Materials and methods

The English literature (PubMed) of the last 10 years has been searched for publications addressing mild or soft COS vs conventional COS for IVF, OHSS, natural cycle IVF, and IVF outcome in association with COS.

Results

Natural selection: quality for quantity

In the natural cycle of spontaneous folliculogenesis, the best and healthiest follicle, which will ultimately ovulate, is selected using the selection principle of quality for quantity. Out of 700–1000 primordial follicles, which start the long journey of folliculogenesis, lasting somewhere between 4 and 9 months, only one, usually, reaches the stage of dominant follicle and ovulates [4, 5]. Thus, nature eliminates the less than ideal follicles with aneuploidy or other suboptimal genetic, hormonal, or growth factor stimulation, enabling for continuation of species by the best and healthiest ova. Indeed, Baart et al. [6, 7] have shown that milder ovarian stimulation for IVF reduces aneuploidy in the human preimplantation embryos. These investigators have shown in a convincing preimplantation genetic screening (PGS) experiment that the mild COS generating a lower oocyte yield, compared to the conventional COS, was associated with a decrease in the proportion of aneuploid embryos [6]. The number of euploid embryos was identical regardless of whether eight embryos were generated, after conventional COS, or only four embryos, after mild COS [6]. Hohmann et al. [8] have also shown that mild COS generated high-quality embryos and PR comparable to those following conventional ovarian stimulation. Different from conventional COS where four or less generated ova did not generate pregnancies, most gestations obtained following mild COS occurred in women where four or less oocytes were retrieved [6, 7]. Fauser et al. [9] have summarized the studies performed to develop the concept of mild stimulation aiming to obtain fewer than eight oocytes. They have defined mild COS as administration of low doses of gonadotropins in the gonadotropin-releasing hormone (GnRH) antagonist protocol and/or oral compounds (such as clomiphene citrate or similar anti-estrogenic compounds or aromatase inhibitors) for IVF, aiming at limiting the number of retrieved oocytes to less than eight. They [9] summarized the balance between IVF success and patient discomfort vs. complications and cost, challenging the conventional practice to attempt generation of a large number of oocytes as an integral part of a successful IVF program, and the possible implication of simpler COS protocols aiming to retrieve fewer oocytes. In support of the recommended mild COS, they [9] cited a randomized controlled trial (RCT) [10], whereby the term live birth rate, but not the PR/cycle, after mild COS+ single embryo transfer (ET), was similar to that of conventional IVF. However, only few authors and publications [11, 12] have supported this concept of mild COS emphasizing the benefits regarding cost-effectiveness, equity of access, minimal risk for mother and offspring, and minimal burden for patients.

Many others do not agree with this attitude; the analysis by Sunkara et al. [13] suggests that the optimal number of oocytes retrieved to secure a good live birth rate is between 8 and 15 and that this number predicts the chance of a live birth in all age groups. They [13] have found a significant association between the number of retrieved ova and live birth rate (LBR); the LBR rose with an increasing number of eggs up to ~15, plateaued between 15 and 20, and declined beyond 20 ova. Moreover, others [14] found that increasing the number of oocytes did not increase the number of aneuploid embryos, but it did increase the chance of having at least one euploid embryo for transfer. Using array comparative genomic hybridization (CGH) analysis, Ata et al. [14] have demonstrated that aneuploidy did not correlate with the number of generated embryos. Whereas the detrimental effect of COS on egg quality is doubtful, it is unequivocally accepted that implantation is impaired in high responders, most probably due to a diminished endometrial receptivity, induced by high estrogen levels [15, 16]. However, the latter publication [16] concluded that high E_2 levels are deleterious to embryo adhesion in vitro, mainly because they have a direct toxic effect on the embryo

that may occur at the cleavage stage. Therefore, besides the endometrial effect, an additional detrimental effect of supraphysiological estrogen concentrations on the embryo itself is possible. Similarly, high progesterone levels in the late follicular phase in conventional IVF COS protocols, associated with high ovarian response, have also been found to be detrimental to PR [17–20]. In contradiction to Valbuena et al. [16] who concluded that high E_2 levels are deleterious to embryo adhesion, due to a direct toxic effect on the embryo itself at the cleavage stage, Fatemi et al. [21] claim that the potential implantation of these embryos is preserved, demonstrated by the fact that the cumulative PRs in high responders are higher than in normal responders. Also, embryo aneuploidies were not increased after moderate ovarian stimulation with respect to non-stimulated cycles in the same patient [22], and higher responses provided more euploid blastocysts [14, 23]. Therefore, the whole concept of mild stimulation has not obtained ubiquitous worldwide acceptance by the majority of reproductive endocrinologists and ART practitioners, and after the introduction of GnRH agonist (GnRHa) trigger to minimize OHSS, the idea seems to have lost its momentum. A Cochrane database systematic review [24] has concluded that GnRHa as a final oocyte maturation trigger in fresh autologous cycles is associated with lower LBR, lower ongoing PR (>12 weeks), and higher early miscarriage rate (<12 weeks). However, GnRHa as an oocyte maturation trigger could be useful for women who choose to avoid fresh transfers (for whatever reason), women who donate oocytes to recipients, or women who wish to freeze their eggs for later use in the context of fertility preservation [24].

Most recently, Arce et al. [25] have demonstrated a significant positive relationship between the dose of recombinant follicle-stimulating hormone (FSH) administration in COS for IVF and the number of retrieved oocytes, both in high-anti-Mullerian hormone (AMH) and low-AMH patients. As expected, the women in the high AMH stratum had significantly more blastocysts than those in the low AMH stratum, but in neither stratum did the increased oocyte yield at higher gonadotropin doses result in a similar increase in the numbers of total blastocysts or high-quality blastocysts [25]. These recent findings are in keeping with the older, and previously cited, findings of Baart et al. [6, 7]. Arce et al. [25] have postulated that there may exist a threshold level for the starting gonadotropin dose, related to the AMH level, above which more intense stimulation has only a limited effect on increasing the number of competent oocytes.

Similarly, Evans et al. [26] have recently shown that laboratory-based studies demonstrate morphological and molecular changes in the endometrium and reduced responsiveness of the endometrium to human chorionic gonadotropin, resulting from conventional COS. The published data suggest reduced endometrial receptivity in conventional COS cycles and support the clinical observations that ET of frozen-thawed embryos in natural or minimally stimulated IVF cycles not only reduces the risk of OHSS but also improves outcomes for both the infertile patient and her neonate [26].

A logical possible compromise, between the two attitudes, regarding cost-effectiveness, is to aim at the retrieval of around eight to ten eggs since this number is close to the number claimed to have the advantages of mild stimulation (up to eight ova) and, at the same time, within the number range of maximal PR and LBR (8–15 ova) according to the conventional stimulation policy. This suggested target may optimize success without significantly compromising safety, cost, and patients' comfort.

Early-gestation high E_2 detrimental effect on fetal growth

It has been postulated that a disrupted endocrine environment may disturb the growth of the fetus and induce chronic adult diseases in later life [27, 28]. Animal experiments in baboons have shown that high E_2 concentrations in the first trimester of pregnancy could impair blood flow to the placenta and lead to fetal growth restriction [29]. More recently, Hu et al. [30] have shown that high maternal E_2 environment in the first trimester is correlated with increased risks of low-birth-weight (LBW) and small for gestational age (SGA) neonates. High concentrations of E_2 in the late follicular phase of IVF cycles correlated with high E_2 levels in the generated gestations at 4 and 8 weeks of gestation and significantly correlated with higher rates of SGA and LBW neonates vs. spontaneous pregnancies or those generated by ET of thawed embryos, associated with much lower, physiological E_2 levels. This study [30] suggested that conventional COS could induce an increase in E_2 levels not only before and during implantation but also afterward, and the high, supraphysiological E_2 concentrations on the day of hCG administration can serve as an effective marker for the E_2 milieu before, during, and after implantation and in early gestation. E_2 may crucially affect the process of implantation and spiral artery invasion and remodeling and influence various aspects of placental function and fetal growth [31, 32]. Low, physiological levels of estrogens are necessary at early conception to ensure normal extravillous cytotrophoblast spiral artery invasion [30–32].

Other suggested mechanisms, possibly explaining the association of high E_2 levels and LBW, are thyroid dysfunction and disturbed plasma levels of long-chain polyunsaturated fatty acids [30, 33].

More recently, Xu et al. [34] have assessed the cardiovascular functions of children born to mothers with OHSS, compared to children of mothers with non-OHSS IVF pregnancies, and spontaneously conceived children. They [34] have found that children of OHSS mothers showed a significantly decreased ratio of early-to-late mitral peak velocities, reduced systolic and diastolic diameters of common carotid arteries, and impaired flow-mediated dilation compared with non-OHSS IVF and spontaneously conceived children. They concluded that children born to ovarian-hyperstimulated women

displayed cardiovascular dysfunctions, suggesting supraphysiological E_2 and progesterone levels as underlying mechanisms [34].

Therefore, supraphysiologic levels of E_2 should be avoided, preferring mild COS over high or conventional COS. In cycles where high E_2 levels are inadvertently reached, one may consider a "freeze-all" policy, whereby no ET is performed in that cycle but postponed to next cycle, in a natural or minimal endometrial stimulation cycle and thawed ET. However, a possible drawback of this policy is an increased risk of delivering large for gestational age (LGA), macrosomic neonates [35].

In addition to the negative effects of high E_2 levels on the neonates, there is increasing evidence that ART is frequently associated with preeclampsia and other pregnancy-associated complications that have impacts on the cardiovascular health of both the mother and child [36, 37]. As an example, high estrogen levels, produced during IVF cycles by high numbers of corpora lutea, are associated with greater odds of developing preeclampsia [36].

Physiologic intrafollicular hormonal milieu

Is the steroid hormone profile of the follicular fluid (FF) different in the naturally matured follicles (natural cycle (NC)-IVF), from the conventional gonadotropin COS-IVF?

Von Wolff et al. [38] have compared the intrafollicular hormonal milieu between NC-IVF and stimulated COS. Their working hypothesis was that FF from NC-IVF follicles could be considered ideal since evolution has perfected folliculogenesis, whereas pharmacologic endocrine manipulations are likely to demonstrate an adverse disruption of the endocrine milieu [38]. This working hypothesis is also supported by the higher implantation rate in NC-IVF compared to conventional COS-IVF [12, 38]. Indeed, these investigators [38] have shown that AMH, LH, testosterone, E_2, and androstenedione are significantly higher, in NC-IVF than in COS-IVF follicles, suggesting an alteration of the follicular metabolism in stimulated IVF as a possible mechanism of suboptimal outcome. The significantly higher AMH concentration in the FF from NC-IVF is in keeping with higher implantation rates vs COS-IVF, since AMH has been shown to be a marker of high PR and better implantation potential [39–41]. Furthermore, the significantly higher androgen concentrations in the FF of NC-IVF vs COS cycle are in keeping with the recently hypothesized augmenting effect of androgens on early folliculogenesis [38]. It is therefore conceivable and understandable why COS with supraphysiological plasma levels of sex hormones may be detrimental to the intrafollicular hormonal milieu, physiological folliculogenesis, and oocyte maturation.

The GH/IGFs/GH-BP system

FSH stimulates normal folliculogenesis synergistically with IGFs. IGF-I and IGF-II stimulate folliculogenesis in vivo and in vitro, granulosa cell (GC) proliferation, and steroidogenesis and inhibit apoptosis [42–47] being important promoters not only of follicular growth, but also of follicular selection.

Furthermore, IGFs may augment the expression of gonadotropin receptors and response of the ovarian cells and oocytes to gonadotropins considered the main local mediators of gonadotropins' action in the ovary [48–50]. Gonadotropins and IGFs synergistically activate ovarian follicular functions. IGF-I is a mediator of GH [42–44], oxytocin, and leptin action on ovarian cells [44]. The ovarian effect of GH is to increase the IGF levels and augment folliculogenesis. The GH is bound in plasma to GH-BP which is increased by E_2 [51–55]. The GH-BP is identical to the extramembranal residue of the GH receptor and is believed to be the product of proteolytic cleavage of the extracellular domain of the GH receptor [51–55]. The GH-BP binds GH in plasma similarly to the binding of the GH ligand by its receptor. It has been suggested that supraphysiologic levels of E_2 (>6000 pmol/L) may increase GH-BP to very high levels which may bio-neutralize the restricted GH and prevent the increase in the generated IGF levels, necessary for optimal synergism with FSH [51–55]. Therefore, mild COS and moderately increased plasma E_2 concentrations generate higher GH-BP and higher IGF levels resulting in enhanced folliculogenesis due to FSH-IGF synergism. On the other hand, high, supraphysiological E_2 concentrations increase the GH-BP to such high levels that may compete with the GH receptor on the restricted GH ligand causing bio-neutralization and resulting in lower generated IGF levels and suboptimal FSH-IGF synergism [51–55]. Therefore, high estrogen levels and conventional or aggressive COS may be inferior to mild COS, especially in cases of borderline or limited GH levels.

Most recently, Revelli et al. [56] have compared mild vs. "long" protocol COS in ovarian poor responders in IVF in a large prospective randomized trial. A total of 695 IVF patients with low ovarian reserve and a poor response to COS were randomly assigned to receive the clomiphene citrate/gonadotropins/GnRH-antagonist mild protocol (mild group, n=355) or the long protocol with high-dose gonadotropins (long group, n=340). Although the long protocol was associated with less cancelled cycles, higher number of overall and mature oocytes retrieved, and more generated embryos, the outcome was similar [56]. The implantation rate, clinical PR, and ongoing PR at 12 weeks were comparable [56]. In addition, the stimulation was shorter and the number of used gonadotropin units was lower in the mild COS.

Late follicular "triggering" with GnRHa instead of hCG

The last-decade popularity of GnRH antagonist protocol+ GnRHa triggering, instead of hCG, has decreased the prevalence of severe OHSS [57]. Some investigators claimed that an OHSS-free clinic could be practiced [58]. However, it has also induced an erroneous sense that the OHSS syndrome has been eliminated by this practice and the awareness and careful monitoring of the ovarian response to COS are not always stringently kept. Indeed, OHSS is substantially underreported [57]. Furthermore, severe OHSS following GnRHa trigger with the addition of 1500 units of hCG has been reported in 26 % [59]. Therefore, it has been suggested to abstain from hCG addition. Nevertheless, several others [60–62] have described severe OHSS after GnRHa trigger alone, without hCG, and freeze-all strategy. These publications support the concept that GnRHa trigger minimizes OHSS but does not completely eliminate it.

Discussion

The implication of all the presented studies is that supraphysiologic, pharmacologically increased levels of E_2 should be avoided, preferring mild COS over aggressive or conventional COS. In cycles where high E_2 levels were inadvertently reached, one should consider a freeze-all policy, whereby no ET should be performed in that cycle but postponed to next cycle in a natural or minimal endometrial stimulation and thawed ET.

In both natural cycle and mild IVF, the best follicles seem to be selected and conventional high-dose ovarian stimulation does not carry any advantages.

Compliance with ethical standards

Conflict of interest The author has nothing to disclose.

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