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The endometrium during and after ovarian hyperstimulation and the role of segmentation of infertility treatment

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Controlled ovarian hyperstimulation (COH) is a crucial part of assisted reproductive technologies (ART) that resulted in a substantial increase in pregnancy rates from in vitro fertilization (IVF). However, in spite of the apparent benefit of COH on an increase in the number of follicles and the number of oocytes retrieved, allowing for extended embryo culture and enabling the selection of the best quality embryo for transfer, several reports has shown that the supraphysiologic hormonal levels may indeed have a detrimental effect at the endometrial level. The current article revises the pathophysiological mechanisms through which ovarian stimulation may negatively affect endometrial receptivity. Also, the evidence is analyzed explaining how segmentation of IVF treatment may allow us to overcome the deleterious effects of hyperstimulation on endometrial receptivity. Deferred embryo transfer may be performed in a more physiologic uterine environment in a subsequent cycle, improve endometrial receptivity,

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decrease uterine contractility, diminishes the impact of premature luteinization and allow individualized stimulation according to the level of response.

Introduction

Despite significant advances in ART, many couples experience infertility as a result of failed implantation of the fertilized embryos into the uterus [1]. Implantation and live birth rates (IRs, LBRs) in ART remain low, even with high quality embryos, pointing to the importance of endometrium deficiency during implantation as a major cause of pregnancy failure [2]. In some cases, this uterine inefficiency is thought to be caused by controlled ovarian hyperstimulation (COH) per se during treatment. Therefore, it is crucial to gain a clear understanding of the mechanisms underlying embryo implantation and the role of the endometrium during and after COH in order to better address new clinical approaches to infertility.

Implantation is a regulated process that involves complex cellular communication pathways (i.e., endocrine, paracrine, autocrine, and juxtracrine) and that comprises different types of cell interactions (i.e., cell-to-cell, and cell-to-extracellular matrix). The exact course of the molecular interactions involved has not yet been determined. Two-Way intercommunication between the blastocyst and maternal uterine luminal epithelium launches the process of implantation, a sequence of events by which blood vessels of the embryo are brought into contact with the maternal circulation leading to the establishment of a functional placenta and pregnancy. Understanding the mechanism of embryonic development during preimplantation and, the implantation in the uterus has been a challenge to reproductive and developmental biologists: the ultimate goal is alleviating the problems of human infertility and favor the birth of offspring.

The endometrium, before and during implantation

The human endometrium is crucial for reproduction and is one of the most complex tissues in the human body. It derives from the mucosal lining after the fusion of the Müllerian ducts during embryonic development, and it has an exceptional regenerative capacity, as it can grow from 0.5 to 1 mm following menstruation to its maximum thickness during the secretory phase of the menstrual cycle [3].

The endometrium is organized in a *functionalis* layer and a *basalis* layer: the functionalis corresponds to the upper two-thirds and the basalis, to the lower one-third of the endometrium. Since the functional layer is a complex tissue with the ability to proliferate, secrete hormones and degenerate, it is ideal for blastocyst implantation to occur. On the contrary, the basalis layer renews the endometrium following the loss of the functionalis layer after the menstruation [4]. On the one hand, the circulating estrogen promotes the growth of the functionalis layer after every endometrial cycle during the proliferative phase [5]. On the other, the rise of progesterone levels across the secretory phase and therefore the blockage of mitosis in the epithelial cells favors cell differentiation [3]. Other phenomena begin simultaneously including the pre-decidualization around the blood vessels that further expands to the stroma below the surface epithelium [4], and there is also a decrease in stromal proliferation [5]. In the case that there is no implantation, the functional layer degenerates allowing the start of the menses and giving rise to a new menstrual cycle. Conversely, if implantation takes place, endometrial stromal cells complete decidualization and differentiation [3].

During the mid-luteal part of the menstrual cycle, following the implantation of the embryo, the endometrial stroma is characterized by edema. This event takes place thanks to the rise in prostaglandins and vascular endothelial growth factor, mediated by the progesterone and estrogen, and favoring an increase in capillary permeability. Blood vessels within the endometrium, and specifically within the muscular walls, contain receptors for sex steroids. Moreover, both the muscular

walls and the endothelium of the endometrial arterioles have the capability of synthesizing prostaglandins. Also, in the presence of estrogen and progesterone, and response to those sex steroids, prostaglandins, and other paracrine and autocrine factors, coiling of the spiral vessels can be observed led by vascular proliferation. During the same phase, around the day 22 of the cycle, the first mitoses appear.

Effect of controlled ovarian stimulation on the endometrium

The window of implantation

The endometrium undergoes a histological transformation during the natural menstrual cycle [6]. Despite methodological flaws by previous studies examining endometrial biopsies for the assessment of endometrial receptivity [7,8], biopsy of the endometrium is a reliable method to assess endometrial development.

During normal ovulatory and stimulated cycles, variation in ovarian steroid hormone levels affects the endometrium during a specific period during which it becomes receptive to embryonic implantation. This period is defined as the window of implantation (WOI) [9]. In humans, in unstimulated cycles, blastocysts begin to implant around day LH+6 and implantation process is completed by day LH+10 [10]. Previous reports have shown that in women who received ovarian stimulation for IVF, the gonadotropin treatment in relation to the supraphysiologic levels of estradiol on the day of final oocyte maturation, may have a deleterious effect on embryo implantation most owing to a potential displacement of the WOI [11].

The theory of a potential effect of ovarian stimulation on endometrial receptivity has been supported by studies demonstrating reduced implantation rates in IVF cycles as compared to natural cycles [12]. Early studies with ovarian stimulation with clomiphene citrate or gonadotrophins for IVF have shown a profound effect of ovarian stimulation on endometrial advancement [13–15], which appeared to affect pregnancy rates. According to previous reports, the conception rate in IVF cycles was 40.5% as compared to 0% conceptions in cycles where ETs occurred after cycle day 20 [16]. Subsequent studies in GnRH agonist downregulated cycles, attempted to date the endometrium either according to Noyes' criteria or by morphometric assessment in relation to unstimulated cycles. According to these studies, the day of oocyte retrieval was considered to be equivalent to day 14 in a natural cycle lasting 28 days, whereas the ovulatory stimulus was considered equivalent to the natural cycle LH surge [17–20]. Results from these studies varied according to the timing of the endometrial biopsy. Finally, the only study conducted that performed endometrial biopsies in the pre-ovulatory phase had demonstrated proliferative aspects and early secretory changes in the endometrium, even before any serum progesterone rise was observed [21].

The specific endometrial development in IVF cycles is most likely due to several factors as described in Table 1.

Effect of ovarian stimulation on progesterone levels is a frequent event in stimulated cycles. This exposure of human endometrium on high progesterone levels in stimulated may be associated with early secretory transformation [22] and subsequent mid-luteal glandular maturation arrest [23]. Similarly, the increase in serum estradiol levels following gonadotropin stimulation has also been associated with more frequent glandular-stromal dis-synchrony [24] and defective induction of progesterone receptors [25]; this has been reported to negatively affect the number of cytosolic progesterone receptors as compared to normal cycles [26]. On the other hand, hCG injection for final oocyte maturation can also be responsible for disrupted endometrial luteal phase morphology, taking into account a direct effect of hCG on endometrial maturation advancement and transformation of the endometrium during the luteal phase as described in both in vitro experiments [27] as well as in hormone replacement cycles [28]. Finally, GnRH agonists have been reported to an anti-proliferative effect on the endometrium [29,30]. The hypothesis a multi-factorial regulation of endometrial development in IVF cycles is further supported by the finding of a profound variability of endometrial patterns for similar hormone values [17,31] and the absence of a clear association between individual serum hormone levels and endometrial dating [32].

Table 1
Mechanisms influencing endometrial development in IVF cycles.

Progesterone Exposure	Increased exposure to progesterone may explain early secretory transformation (1) and subsequent mid-luteal glandular maturation arrest (2)	1. Fanchin et al., 1995 [22] 2. Ezra et al., 1994 [23]
Estradiol exposure	Elevated estradiol is associated with glandular-stromal dis-synchrony (3) and defective induction of progesterone receptors (4), with reduction of cytosolic progesterone receptors (5)	3. Basir et al., 2001 [24] 4. Gorodeski et al., 1987 [25] 5. Molina et al., 1989 [26]
hCG exposure	hCG injection is a further possible cause for disrupted endometrial luteal phase morphology. A direct effect of hCG concerning advanced endometrial maturation and acquisition of a luteal phase phenotype has been documented in in vitro experiments (6) and hormone replacement cycles (7)	6. Tang and Gurpide, 1993 [27] 7. Fanchin et al., 2001b [28]
GnRH agonist exposure	GnRH agonists have anti-proliferative effects on the endometrium (8).	8. Kim et al., 1999 [29]; Meresman et al., 2003 [30]
Summary	Everything is sustained by the finding of the large variability of endometrial patterns for similar hormone values (9) and the absence of a clear correlation between individual serum hormone measurements and endometrial dating (10)	9. Bourgain et al., 1994 [31]; Seppälä and Tiitinen, 1995 [17] 10. Ubaldi et al., 1997 [32]

Endometrial steroid receptors in stimulated cycles

Although endometrial receptors have been measured in previous studies, the use of homogenized endometrial samples in order to evaluate the endometrial steroid receptor content has not permitted the differentiation of the receptors in glandular and stromal cells. In comparison to natural unstimulated cycles, ovarian stimulation negatively affected the expression of luteal cytosolic receptors when assessed with a dextran charcoal assay [26,33]. Other studies demonstrated that ovarian stimulation followed by high ovarian response resulted in a reduction of estrogen (ER) but not progesterone receptors (PR) [34], whereas studies using enzyme immunoassays, failed to identify any differences in the receptors between unstimulated and stimulated cycles [35]. Recently, endometrial receptor status has been evaluated more precisely by the use of immunohistochemical techniques, allowing for differentiation between receptor expression in the glandular and stromal cells, while development of monoclonal antibodies against the different receptor isoforms has further allowed proper dating of the endometrium according to receptor status [36,37]. Still, published evidence should be interpreted with caution, firstly because different monoclonal antibodies and tissue processing methods may result in variable immunohistochemical staining patterns [37], while secondly, variation regarding the methods reporting the staining intensity across the studies is extensive. Still, evidence appears to be in the same line suggesting that in stimulated cycles, both glandular and stromal PRs are found to be reduced in the peri-ovulatory and luteal phase, whereas data on endometrial ERs in stimulated cycles are less clear.

Ovarian stimulation and endometrial development

Two landmark studies have performed endometrial biopsies on the day of oocyte retrieval within the actual embryo transfers, in an attempt to correlate endometrial development with pregnancy outcome [32,38]. According to the results, all endometria on the day of oocyte retrieval in both stimulation cycles showed the advancement of >2 days as compared to a natural cycle endometrium on the day of ovulation. This advancement was more evident in cycles with premature progesterone elevation on or before the day of hCG trigger; still, no correlation could be shown between endometrial secretory development and absolute progesterone values or number of days of premature progesterone elevation, for an individual patient [32]. The degree of endometrial advancement in GnRH antagonist cycles has been associated by high LH concentration at the initiation of rFSH stimulation and the length of stimulation before antagonist inhibition [38]; this correlation was not present in GnRH agonist cycles, where serum LH concentrations were lower following pituitary desensitization.

More recently, endometrial gene expression analysis has been introduced as a very promising tool in order to evaluate the impact of ovarian stimulation on the endometrium. Endometrial gene expression profile has been analyzed throughout the entire menstrual cycle [39,40], allowing classification of endometria precisely, in relation to their transcriptional profile, irrespective of the morphological facade. Still, the more recent reports have focused on the assessment of endometrial receptivity as defined by gene expression profile of the tissue during the WOI [41–45]. One of the most critical genes consistently up-regulated in all five studies was osteopontin, while several other genes have also been expressed and considered necessary for the implantation process [46,47]. Following initial studies performed in unstimulated cycles, future reports focused on identifying the genomic impact of ovarian stimulation protocols on the human endometrium during an IVF cycle. Horcajadas et al. [48] evaluated the effect of ovarian stimulation on endometrial gene expression profiles during the WOI by comparing the profiles at day HCG+7 of ovarian stimulation versus day LH+7 of a previous natural cycle in the same women [48]. According to the results, 558 genes were differentially expressed by more than two-fold when stimulated, and unstimulated cycles were compared at HCG+7 versus LH+7. Further analysis of these genes, has shown a remarkably high number of genes which were involved in endometrial receptivity (WOI genes) to be aberrantly expressed in endometria following ovarian stimulation (342 genes), demonstrating an expression pattern more similar to those in a non-receptive endometrium.

All these clearly show that endometrial development may be negatively affected and delayed under ovarian stimulation for IVF/ICSI.

Segmentation of IVF treatment: is it the key for a “healthy” endometrium?

Segmentation of IVF treatment

Recently, in an attempt to reduce the risk of ovarian hyperstimulation syndrome (OHSS), the traditional concept of IVF with ovarian stimulation, oocyte retrieval, embryo development and transfer in the same cycle, has been seriously challenged [49]. The ‘freeze-all’ strategy, with a segmentation of the IVF cycle, and the cryopreservation of the entire cohort of embryos followed by a transfer in a subsequent cycle without ovarian stimulation has been the key towards an OHSS-free clinic [50]. Still, although this appeared to be a crucial choice towards a safer stimulation and the reduction of the risk of OHSS [51], we cannot overlook that with such a strategy we may be able to overcome the deleterious effects of hyperstimulation on endometrial receptivity, as embryo transfer may be performed in a more physiologic uterine environment in a later cycle [52].

The role of vitrification in the segmentation “era”

Commensurate with the deepening in molecular knowledge in human endometrial physiology, the optimization of the vitrification method, initially described by Rall and Fahy [53], has revolutionized the cryopreservation, successfully placing vitrification, and thus the embryo cryopreservation, as a routine procedure of the IVF laboratories around the world.

Although slow freezing, a technique which enables adequate cellular dehydration through freezing at an appropriately slow rate, minimizing intracellular ice formation [54], has been the technique of choice for embryo cryopreservation for the past two decades, the optimized vitrification method has become universally used in the last years. Vitrification permits the solidification of the cells and the extracellular milieu without the formation of ice crystals in the interior of the embryo [55,56], significantly improving the efficiency of cryopreservation.

Available evidence from published RCTs demonstrate that vitrification (and warming) technique is superior to the slow-freezing method concerning clinical outcomes. Moderate-quality studies clearly show better cryo-survival rates for oocytes, cleavage-stage embryos and blastocysts [57], whereas blastocyst survival rates are significantly increase in embryo cryo-survival following vitrification when compared with slow-freezing (RR = 1.59, 95% CI 1.30–1.93; $p < 0.001$; $I^2 = 93\%$), as shown form pooled data of 3615 embryos (pooled from seven RCTs) [58–64,57]. Furthermore, clinical outcomes were significantly better with cryopreservation of cleavage-stage embryos as

compared with slow-freezing, with a higher clinical PR per cycle, (RR = 1.89, 95% CI 1.00–3.59; p = 0.05; I^2 = 71.9%) [58,60,64] and a higher LBR per cycle, as published by Debrock et al., (RR = 2.28; 95% CI 1.17–4.44; p = 0.016; 216 cycles) [64,57].

In this context, although at first, clinicians were reluctant to adopt the freeze-all strategy, the increased efficiency, reliability, and the fact that vitrification is now documented as a safe technique, has significantly strengthened our confidence, with accumulating evidence demonstrating that freezing and posterior rewarming embryos may result in comparable outcomes when contrasted with transfers in fresh cycles [65,66]. This may be even more evident if we consider that frozen embryo transfer (FET) cycles were routinely utilized as a second option, as the better-scored and morphologically superior embryos were selected first for fresh embryo transfer (ET) [67]. Therefore, it is essential to consider that this could have been a possible source of bias in previous studies, underestimating the reproductive outcomes and diminishing early study's FET cycles' PRs.

The role of segmentation in modern IVF

There is an increasing number of studies supporting improved clinical outcomes [68,69] when selecting the freeze-all strategy over the fresh ET. Most of these observed differences emerged from the impact on COH and thus endometrial receptivity. However, it also seems that this cryopreservation strategy may decrease the risks of OHSS, and besides obstetric and perinatal morbidity and mortality.

COH and endometrial receptivity

Data proves that COH can impair endometrial receptivity, through morphologic and endocrinological modifications favored by the supraphysiologic increase in the levels of progesterone and estradiol. Such reduced endometrial receptivity affects the synchronicity between the endometrium and the embryo, reducing, in turn, the implantation rates during ART treatments [38,70,71]. In the same way, those increased estradiol level may promote an altered endometrial maturation and, hence, implantation [72]. Also, the rise in progesterone, during the late follicular phase, may explain the microscopic changes in the morphology of the endometrium and the observed imaging variations in its echogenicity [73,74]. This subtle rise in the progesterone levels appears to have a negative influence on embryo implantation [75,76]. As exposed by other authors, it seems that when this endometrial advancement occurs for longer than three days, PRs decrease when a fresh ET is performed [38,77]. As mentioned above, these modifications in the endometrial ultrastructure may end in altering the embryo-endometrium synchrony, consequently, decreasing implantation rates during IVF [68]. To date, the evidence does not support a specific threshold in which progesterone levels may be considered increased and capable of leading to alterations in endometrial receptivity [78].

Despite the different hormonal variations discussed before, these do not seem to have a direct impact on embryo quality and development [70]. Thanks to the publication of studies in oocytes donors not undergoing embryo transfer, It has been possible to prove that the increase in progesterone levels probably influence the endometrium, but, do not damage oocyte quality and implantation rates in recipients and do not impact embryo quality [79,80].

In this context, segmentation of IVF may be the key for an increased endometrial receptivity. This is further reinforced if we consider the publication of recent RCTs demonstrating that, in high responders, LBRs are significantly higher with a freeze-all policy as compared with fresh ET [81], whereas lower quality evidence from cohort studies suggest that freeze-all policy may even improve outcomes in patients with implantation failure [82].

COH and uterine contractility

Accumulating evidence suggests that supraphysiologic hormonal levels may increase uterine contractility (UC) [83]. Similarly, recent studies demonstrated that uterine peristaltic wave frequency at oocyte retrieval (2.7 ± 0.7 vs. 3.6 ± 0.8, $p < 0.01$) and two days later (2.0 ± 0.9 vs. 2.4 ± 0.7, $p < 0.01$), were significantly higher in stimulated cycles when compared to natural cycles [84]. Thus, taking into account that UC at the time of embryo transfer adversely affects IVF outcomes [85], it can be

hypothesized that COH may adversely affect PRs via an effect on UC. Although no studies are comparing COH cycles and cycles with FETs, indirect evidence suggests a potentially higher ectopic PR as a result of increased UC in fresh as compared with FET [86]. A freeze-all policy could potentially be of value in an attempt to reduce UC, and therefore future research pathways could focus on the relationship of transfer strategy, uterine contractions, and hormonal levels.

COH and elevated progesterone

Progesterone elevation (PE) on the final day of oocyte maturation has been a matter of debate in the last decade. Despite conflicting initial evidence regarding its detrimental or not effect on PRs in fresh IVF transfer, recent large scale meta-analysis support that PE on the day of hCG administration is associated with severely compromised clinical outcomes [87]. Current reports suggest that a threshold of 1.5 should always be considered, beyond which PRs significantly decline further. Research is needed to confirm whether this is the correct threshold with the new progesterone assays in the market and secondly whether this affects, in the same manner, all ovarian response categories.

The effect of PE on pregnancy rates in IVF cycles has been traditionally attributed to an adverse effect of PE on the day of final oocyte maturation on the endometrium. Previous studies have shown that PE can alter the gene expression profile on the endometrium [77,88] and this can be associated with the lower pregnancy rates. This finding, in addition with lack of association between PE in the fresh IVF cycle and the probability of pregnancy in a subsequent FET cycle [87] and the lack of association of PE in the donor stimulation cycles with the probability of pregnancy in the recipient cycle, in women undergoing third-party reproduction with donor oocytes [79,80,87], clearly suggest that PE affects mainly the endometrium. Of interest, a recent retrospective cohort study, demonstrated that PE was associated with a decrease in embryo utilization rate [89] and a decrease in CLBRs was regardless of the number of oocytes retrieved [89]. Although this may imply that PE may affect oocyte quality, this should be interpreted with great caution, especially given the lack of a clear pathophysiological mechanism supporting such an effect.

In this context, it can be hypothesized that segmentation of IVF treatment might be of value in cases of PE during ovarian stimulation for IVF/ICSI since it may allow us to overcome the adverse effect of PE on the endometrium. A recent study has shown that frozen embryo transfer may ameliorate the effect of elevated progesterone seen in fresh transfer cycles, given that elevated P levels on the day of trigger during the initial fresh cycle were negatively associated with live birth only in the fresh transfer but not in subsequent FET cycles [90]. Even though the exact threshold beyond which progesterone affects pregnancy rates is still a matter of discussion, segmentation of IVF treatment could be of value for these patients in the future. Whether a freeze-all strategy is sufficient to resolve the problem of diminished endometrial receptivity or if it also affects embryo utilization rate needs to be clarified, and further studies as needed.

Segmentation according to the level of ovarian response

Table 2 summarizes the available published evidence regarding the use of segmentation of IVF treatment vs. standard IVF and fresh ET according to the level of ovarian response.

The high responder patient

Women with a high ovarian response following COH may undeniably be the group which is likely to experience the most significant benefits from the segmentation of IVF treatment. OHSS is still one of the most critical complications confronted during COH in IVF. Especially because it is potentially lethal and also because it is almost exclusively iatrogenic [91]. Numerous precautionary measures have been developed during the past few years to prevent its onset, yet, up to now, the most effective strategy is to replace the hCG by applying a GnRH agonist as the trigger for the final oocyte maturation and also by utilizing the freeze-all strategy [49]. These policies altogether have revolutionized the field of IVF, and are now known as the concept of an OHSS-free clinic. It consists of the use of an antagonist protocol, including all the measures as mentioned above [49]. The principal aim is to eliminate the onset of early and late OHSS [92]. It is important to know that two recent publications reported three cases of OHSS in

Table 2
Studies evaluating segmentation according to the level of ovarian response.

	Design	Comments	Number of patients	Primary Outcome (FET VS. Fresh)	OHSS (FET VS. Fresh)	Quality
High Responder						
(Shapiro et al., 2011a) [95]	RCT		122	Ongoing PR:77.6% vs. 65.4%, p = 0.194	Not Reported	Moderate-High
(Wu et al., 2014) [117]	Retrospective	Day 5 vs. FET	1041	Clinical PR: 59.1% vs. 43.2%	Not Reported	Low
(Chen et al., 2016) [81]	RCT	In PCOS patients	1508	LBR: 49.3% vs. 42.0%. Rate ratio 1.17 (95% CI 1.05–1.31, p = 0.004)	7.1% vs. 1.3%. Rate ratio 0.19 (95% CI 0.10–0.37, p < 0.001)	High
(Aflatoonian et al., 2018) [115]	RCT	Fresh cycle with agonist trigger	240	LBR: 30.3% vs. 29.9%, p = 0.953	35.6% vs. 42.9%, p = 0.337	Low
Normal Responder						
(Shapiro et al., 2011b) [116]	RCT	High responders were discarded	103	Ongoing PR: 78.0% vs. 50.9%, p = 0.007	20.0% vs. 35.8% (p = 0.084)	Moderate-High
(Shi et al., 2018) [97]	RCT	Ovulatory women	2157	LBR: 48.7% vs. 50.2%. Relative Risk 0.97 (95% CI 0.89–1.06, p = 0.50)	0.6% vs. 2.0%; relative risk, 0.32; 95% CI 0.14–0.74; p = 0.005	High
(Vuong et al., 2018) [98]	RCT	No PCOS	782	LBR: 31.5% vs. 33.8%. Risk ratio 1.07; 95% CI 0.88–1.31, p = 0.54	0.8 vs. 1.0 Risk Ratio 0.75; 95% CI 0.17–3.33, p = 0.99	High
Low Responder						
(Çelik et al., 2015) [101]	Retrospective	Bologna Criteria	355	LBR: 21.5% vs. 16.7%, p = 0.34	–	Low
(Berkkanoglu et al., 2017) [102]	Retrospective	<4 oocytes retrieved, day 5 vs. FET	234	LBR: 48.2% vs. 30.5%, p < 0.001	–	Low
(Roque et al., 2017) [103]	Retrospective	Bologna Criteria	433	Ongoing PR 9.6% vs. 10.1%; Relative Risk: 0.95, 95% CI: 0.52–1.73	–	Low

FET: frozen embryo transfer; Fresh: Fresh embryo transfer; RCT: randomized controlled trial; PR: pregnancy rate; LBR: live birth rate; CI: confidence interval.

patients triggered with GnRH agonist in antagonist GnRH cycles and following the adoption of a freeze-all strategy [93,94].

High responders have the great potential to have a safe and effective COH, while recent evidence suggests that efficacy of treatment may be higher following ovarian stimulation and deferred embryo transfer. A recent RCT has clearly shown that segmentation of IVF in anovulatory women with PCOS resulted in a higher proportion of LB after the first transfer when compared to fresh ET (49.3% vs. 42.0%), for a rate ratio of 1.17 (95% CI, 1.1–1.3; P = 0.004). Additionally, patients who underwent FET also had a lower proportion of miscarriage (22.0% vs. 32.7%), for a rate ratio of 0.67 (95% CI, 0.5–0.8; P < 0.001), and of OHSS (1.3% vs. 7.1%), for a rate ratio of 0.19 (95% CI, 0.10–0.37; P < 0.001) [81]. Consequently, widespread adoption of a freeze-all policy in case of high ovarian response should be reinforced.

The normal responder patient

Although the role of segmentation is evident in high responder patients, in normally responding ovulatory patients, it is still uncertain whether this strategy should be the proposed way forward. Despite the promising early reports from small RCTs [95] and meta-analysis comparing the effectiveness and safety of the freeze-all strategy vs. conventional IVF strategy [96] the quality of these early studies is low to moderate.

Therefore, despite the pathophysiological mechanism supporting the use of freeze-all as an attempt to overcome the negative effect of COH on endometrial receptivity and endometrial contractility, two recent RCTs failed to identify any superiority of freeze-all versus fresh ET in normo-ovulatory women with normal ovarian response [97,98]. First, Shi et al. [97] randomly assigned 2157 ovulatory patients undergoing their first IVF cycle to either fresh ET or embryo cryopreservation followed by FET. The LBR did not differ significantly between the FET group and the fresh ET group (48.7% and 50.2%, respectively; RR 0.97; 95% CI, 0.89–1.06; P = 0.50). There were also no significant between-group differences in IR, clinical PR, pregnancy loss and ongoing PR. FETs did result in a significantly lower OHSS risk (0.6% vs. 2.0%; RR 0.32; 95% CI, 0.14–0.74; P = 0.005). Similarly, Vuong et al. [98] randomly assigned 782 infertile women without PCOS who were undergoing a first or second IVF cycle. After the first completed cycle, ongoing pregnancy occurred in 36.3% in the FET group and 34.5% in the fresh ET group (risk ratio in the FET group, 1.05; 95% CI, 0.87–1.27; P = 0.65). LBRs after the first transfer were 33.8% and 31.5%, respectively (RR, 1.07; 95% CI, 0.88–1.31, p = 0.54).

Evident advantages with segmentation of IVF in normal responders could be the opportunity to stimulate more intensively without the risk for severe side effects and retrieve more oocytes which could lead in higher cumulative LBRs [99]. Scheduling and avoidance of the weekends and patient friendliness should also not be overlooked [100], with several however threats such as an increment in the costs and the generation of numerous supernumerary embryos which might likely never be used. All these, given the lack of superiority regarding LBR, do question an immediate swift in clinical practice and suggest that segmentation in this group can be of value in some instances given that results in similar LBR as compared with fresh ET.

The low responder patient

In women with poor ovarian response (POR), the evidence is rather weak to suggest the use of segmentation as a measure to increase outcomes in these women. Three retrospective cohort studies have been published up to date, analyzing the effect of freeze-all in these subsets of patients [101–103]. One study investigated the use of the freeze-all strategy in poor ovarian responders fulfilling the Bologna criteria, finding no differences in the outcomes when comparing fresh and elective FET (LBR 21% vs. 17%, p > 0.42) in this specific population [101]. Berkkanoglu et al. observed an improvement in the reproductive outcomes after FET (vs. fresh embryo transfer) in POR. The authors found an IR of 24.1% (fresh cycles) and 47% (FET cycles) (P = 0.026) after the evaluation of day-5 transfers. The LBR per ET was 30.5% and 48.2% (P = 0.049), respectively [102]. Nevertheless, the most recent study revealed no benefit in performing the freeze-all policy contrasted with fresh ET in POR patients (GnRH antagonist protocol). Ongoing PRs were similar between the freeze-all and fresh groups (9.6% versus 10.1%, respectively) (Relative Risk [RR]: 0.95; 95% CI: 0.52–1.73) [103].

Overall, owing to these reports and the limited number of oocytes retrieved in this patients' category, segmentation should not be routinely recommended unless other practical or clinical reasons point toward this direction, i.e., progesterone elevation during COH.

Segmentation in women of advanced age

In women of advanced age, in whom the risk of aneuploidy is increased [104], the use of comprehensive chromosome screening (CCS) is every day more frequently recommended [105]. Furthermore, CCS in the blastocyst stage with elective single embryo transfer (eSET) has been proven to be as effective as a two-embryo unscreened transfer and drastically reduces the risk of a twin pregnancy [106]. Although it not clear whether this is the way forward in women above 38 or 40 years old, segmentation of IVF treatment is essential when preimplantation genetic screening for aneuploidy (PGT-A) is considered. In the context of PGT-A cycles, a successful fresh day-six transfer approach presupposes, not only that there will be available expanded blastocysts on the morning of biopsy day [107], but also, that there will be at least one euploid blastocyst from the biopsied embryos, also decreasing the possibility for a transfer in that cycle.

A recent study has shown that segmentation is associated with improved results in women undergoing ovarian stimulation for PGT-A. Coates et al. [108] randomized 179 patients to either a fresh or a frozen ET and performed an intention-to-treat analysis. They found no differences in the IR between the FET group (75%) and the fresh group (67%) ($p = 0.3$). However, the ongoing PRs and LBR were significantly higher in the FET group when contrasted with the fresh group (ongoing PRs 80% in FET vs. 61% in fresh, $p = 0.03$; LBRs of 77% in FET vs. 59% in fresh, $p = 0.04$) [108]. In this setting, segmentation of IVF treatment should be considered in PGT-A cycles.

Segmentation of IVF: implications for obstetric and perinatal outcomes

The effect of segmentation of IVF on obstetric and perinatal outcomes is also of great interest for future planning of policymakers. In a systematic review and meta-analysis published by Pandey et al., the risk of obstetric and perinatal complications was higher following IVF/ICSI (singleton pregnancies) as compared with natural conception [109]. Some observational studies comparing fresh and FET, have found better obstetric and perinatal outcomes from FET pregnancies when compared to fresh ET, suggesting a potential advantage of a freeze-all approach as instead of a fresh ET. The supraphysiologic hormonal levels from the COS may be responsible for altered placentation, and thus an increased the risk of small size for gestational age (SGA), low birth weight (LBW), pre-eclampsia, prematurity, antepartum hemorrhage, and stillbirth [110–112]. This, may substantially favor the freeze-all approach, especially given the accumulating evidence supporting a similar risk of congenital anomalies between children conceived after fresh ET and FET [113]. Although, an increased risk of macrosomia in singletons born after FET as compared with those born following fresh ET [114], the most recent meta-analysis [111] demonstrated that FET results in a lower risk of preterm delivery (RR 0.90; 95% CI 0.84–0.97), LBW (RR 0.72; 95% CI 0.67–0.77), and SGA (RR 0.61; 95% CI 0.56–0.67), as compared to those conceived from fresh ETs. On the contrary FET was associated an increased risk of hypertensive disorders of pregnancy (RR 1.29; 95% CI 1.07–1.56), large for gestational age (LGA) (RR 1.54; 95% CI 1.48–1.61), and high birth weight (RR 1.85; 95% CI 1.46–2.33) [111].

Three RCTs, not included in previous meta-analyses, have been published recently. For instance, Shi et al. reported, in ovulatory women, that the incidence of obstetrical and perinatal complications, congenital anomalies, and neonatal death was similar between the two groups [97]. Additionally, Chen et al. studied the two strategies in women with PCOS and reported a higher frequency of preeclampsia (4.4% vs. 1.4%) in FET cycles with a risk ratio of 3.12 (95% CI, 1.26 to 7.73; $p = 0.009$), and no other significant differences in other pregnancy or neonatal complications; still, five neonatal deaths in the FET group (none in the fresh ET) were reported [81]. Lastly, including all other infertility diagnoses except for PCOS, Vuong et al. reported that the proportion of singleton babies, below the 10th percentile for birth weight, was significantly lower in the FET group than in the fresh-ET group ($p = 0.01$) [98].

Conclusions

Despite the apparent benefit of COH on multifollicular development, extended embryo culture and increased pregnancy rates (PRs), it is evident that it may have a detrimental effect on endometrial receptivity.

The 'freeze-all' strategy, with a segmentation of the IVF cycle, and the cryopreservation of the entire cohort of embryos followed by a transfer in a subsequent cycle without ovarian stimulation has been the key towards an OHSS-free clinic. Still, although this appeared to be a crucial choice towards a safer stimulation and the reduction of the risk of OHSS, we cannot overlook that with such a strategy we may be able to overcome the deleterious effects of hyperstimulation on endometrial receptivity, as embryo transfer may be performed in a more physiologic uterine environment in a subsequent cycle.

More importantly, specific scenarios and subset of patients specifically benefit from segmenting their IVF cycle by improving endometrial receptivity, decreasing uterine contractility, diminishing the impact of premature luteinization or individualizing stimulation according to the level of response to stimulation. Moreover, more significant than the implantation rates is the safety of the ART. It is clear that the freeze-all policy decreases the odds of OHSS development, and may beneficially affect obstetric and perinatal morbidity and mortality.

Practice points

- The freeze-all' strategy, cryopreservation of the entire cohort of embryos and transfer in a subsequent synthetic cycle has been the key towards an OHSS-free clinic
- There is evidence that the supraphysiologic levels of estradiol and progesterone during COH could lead to morphologic and biochemical modifications, and consequently impair endometrial receptivity
- Recent studies have demonstrated that uterine peristaltic wave frequency at oocyte retrieval and two days later were significantly higher in stimulated cycles when compared to natural cycles.
- High responder patients are the group of patients that benefit the most from a freeze-all strategy.
- It is still unclear if normo-responding patients benefit regarding pregnancy outcome from a freeze-all strategy as compared to traditional IVF.
- Segmentation should not be routinely recommended in low responding patients unless other practical or clinical reasons point toward this direction, i.e., progesterone elevation during COH.

Research agenda

- Studies on the impact of hormonal changes during ovarian stimulation on the genetic composition of an embryo.
- Gene expression profiles on the impact of vitrification.
- Cost-effectiveness of elective FET, when compared to fresh ET based on the recent RCTs in different ovarian response categories, could elucidate the value of segmentation for policy decision making.

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