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human reproduction **OPINION**

A fresh look at the freeze-all protocol: a SWOT analysis

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ABSTRACT: The 'freeze-all' strategy with the segmentation of IVF treatment, namely with the use of a GnRH antagonist protocol, GnRH agonist triggering, the elective cryopreservation of all embryos by vitrification and a frozen-thawed embryo transfer in a subsequent cycle, has become more popular. However, the approach still encounters drawbacks. In this opinion paper, a SWOT (strengths, weaknesses, opportunities and threats) analysis sheds light on the different aspects of this strategy.

Key words: IVF / ovarian stimulation / GnRH antagonist / GnRH agonist trigger / freeze-all / vitrification / embryo cryopreservation

Introduction

GnRH antagonist protocols have increasingly become the mainstay in clinical IVF practice, especially when dealing with (predicted) high responder patients. The main reason for this progressive shift in medical practice has been the need to minimize the occurrence of the largest enemy in reproductive medicine: ovarian hyperstimulation syndrome (OHSS) (Mathur *et al.*, 2000; Griesinger *et al.*, 2006; Papanikolaou *et al.*, 2006). Besides the already significant reduction of the risk of OHSS just by the using a GnRH antagonist by itself (Al-Inany *et al.*, 2011; Youssef *et al.*, 2011), these downregulation protocols allow the implementation of additional measures to further reduce the risk of OHSS, the most notable of which is the replacement of hCG for final oocyte maturation induction by a GnRH agonist (Kolibianakis *et al.*, 2005a, 2005b; Griesinger *et al.*, 2006; Youssef *et al.*, 2010). Moreover, the addition of GnRH agonist for ovulation triggering seems to manage to practically eliminate the risk of OHSS without hindering the efficacy of the oocyte retrieval procedure when compared with hCG triggering, as shown by the similar yields in terms of oocyte maturation and embryonic development, namely in oocyte donation cycles (Acevedo *et al.*, 2006; Shapiro *et al.*, 2007; Galindo *et al.*, 2009; Hernandez *et al.*, 2009). However, the drastic luteolysis following GnRH agonist triggering is associated with an important luteal phase defect, presumably because of excessive negative steroid feedback resulting in suppressed pituitary LH release (Beckers *et al.*, 2003; Casper, 2015; Kol *et al.*, 2015). Nevertheless, the introduction of oocyte/embryo vitrification as a method of cryopreservation has increased the post-thawing survival rates significantly, providing an adequate solution to circumvent this issue. Specifically, the use of an antagonist protocol followed by a 'freeze-all' strategy and transfer of the embryo(s) in a subsequent frozen-thawed cycle seems to be a promising option with high cumulative live birth rates, mainly in patients with a high risk for OHSS (Eldar-Geva *et al.*, 2007; Griesinger *et al.*, 2007, 2010a, 2010b).

This approach resulted in the genesis of the so-called 'freeze-all' strategy with the segmentation of ovarian stimulation (using a GnRH antagonist protocol), ovulation triggering (with a GnRH agonist), the elective cryopreservation of all embryos (by vitrification) and a frozen-thawed embryo transfer in a subsequent natural or artificial cycle (Devroey *et al.*, 2011) (Fig. 1).

Cryopreservation has become an increasingly intricate part of IVF treatment and is no longer viewed as a mere supplement to fresh embryo transfer, as in the past (Doody, 2014). Although the most common reasons for cryopreservation and delayed embryo transfer are the presence of risk factors for OHSS, the need for pre-implantation genetic diagnosis or screening (PGD/PGS) or the presence of embryo/endometrial asynchrony, currently there is an accelerating trend toward the elective cryopreservation of all embryos following IVF with transfer of a thawed embryo in one or several subsequent cycles (Devroey *et al.*, 2011; Roque *et al.*, 2015). The rationale behind this hypothesis is that the transfer of an embryo into a more 'physiologic environment' would result not only in higher pregnancy rates but potentially a decrease in both maternal and perinatal morbidity, when compared with a fresh embryo transfer (Evans *et al.*, 2014). However, the debate regarding the risks and benefits of the cryopreservation of all embryos with subsequent replacement is not without controversy. First, a distinction needs to be made between the elective freezing of all embryos in all IVF cycles (universal approach) and the cases in which GnRH agonist triggering was performed in order to prevent OHSS (patient risk-based approach).

Besides, there is a lack of sufficiently robust (Grade A) evidence on the real outcome of interest, i.e. live birth rate and, thus, superiority of the freeze-all strategy cannot (yet) be advocated, and thus additional arguments should be taken into consideration, namely health economics, patients' convenience and logistic aspects/concerns of the IVF centers. Second, a paucity of data is available in terms of financial burden: the costs of both strategies have yet to be compared, as current indications are different and, therefore, these populations cannot be considered equal. In this regard, one can delineate the following SWOT analysis in order to shed some light on this relatively new freeze-all strategy that might become the gold standard for IVF stimulation in the near future (Fig. 2).

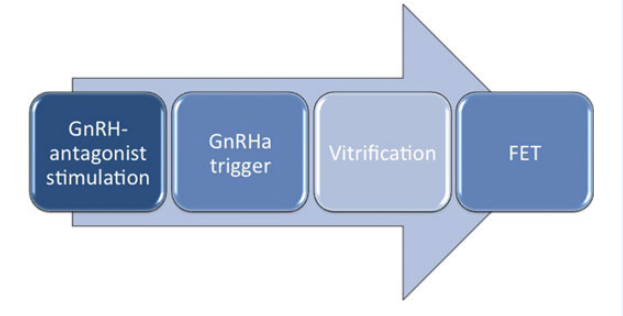

Figure 1 The 'freeze-all' protocol. GnRHa, GnRH agonist; FET, frozen embryo transfer.

Strengths

The modern treatment goal for the infertile patient is to achieve high-term, singleton live birth rates per IVF treatment started, while improving the patient's comfort. The major complication observed in today's IVF is the occurrence of OHSS, a potentially life-threatening condition (Kawwass *et al.*, 2015). When GnRH antagonist protocols were introduced for the prevention of a premature LH surge (Albano *et al.*, 1997; Itskovitz-Eldor *et al.*, 2000; European, Middle East Orgalutran Study Group, 2001), it became once more possible to trigger ovulation with a bolus of a GnRH agonist as an alternative to hCG. In the first RCTs comparing GnRH agonist triggering with hCG administration, the ongoing pregnancy rates were significantly decreased in the agonist-triggered arm (Humaidan *et al.*, 2005; Kolibianakis *et al.*, 2005a, 2005b). These poor outcomes were attributed to a potential luteal phase defect, and researchers suggested an alternative approach of cryopreserving the embryos and transferring them in consecutive frozen cycles. The first results were promising (Griesinger *et al.*, 2007), and over the years, frozen embryo transfer (FET) cycles became increasing popular in IVF practice. Therefore, the comparison with fresh cycles became inevitable.

The results of the first meta-analysis comparing fresh and FET cycles suggested a significantly higher implantation, clinical and ongoing pregnancy rate by performing FET (Roque *et al.*, 2013). These results can probably be explained by the improved embryo-endometrium synchrony, a negative consequence of ovarian stimulation on endometrial receptivity, which has been largely studied before (Kolibianakis *et al.*, 2002; Bourgain and Devroey, 2003).

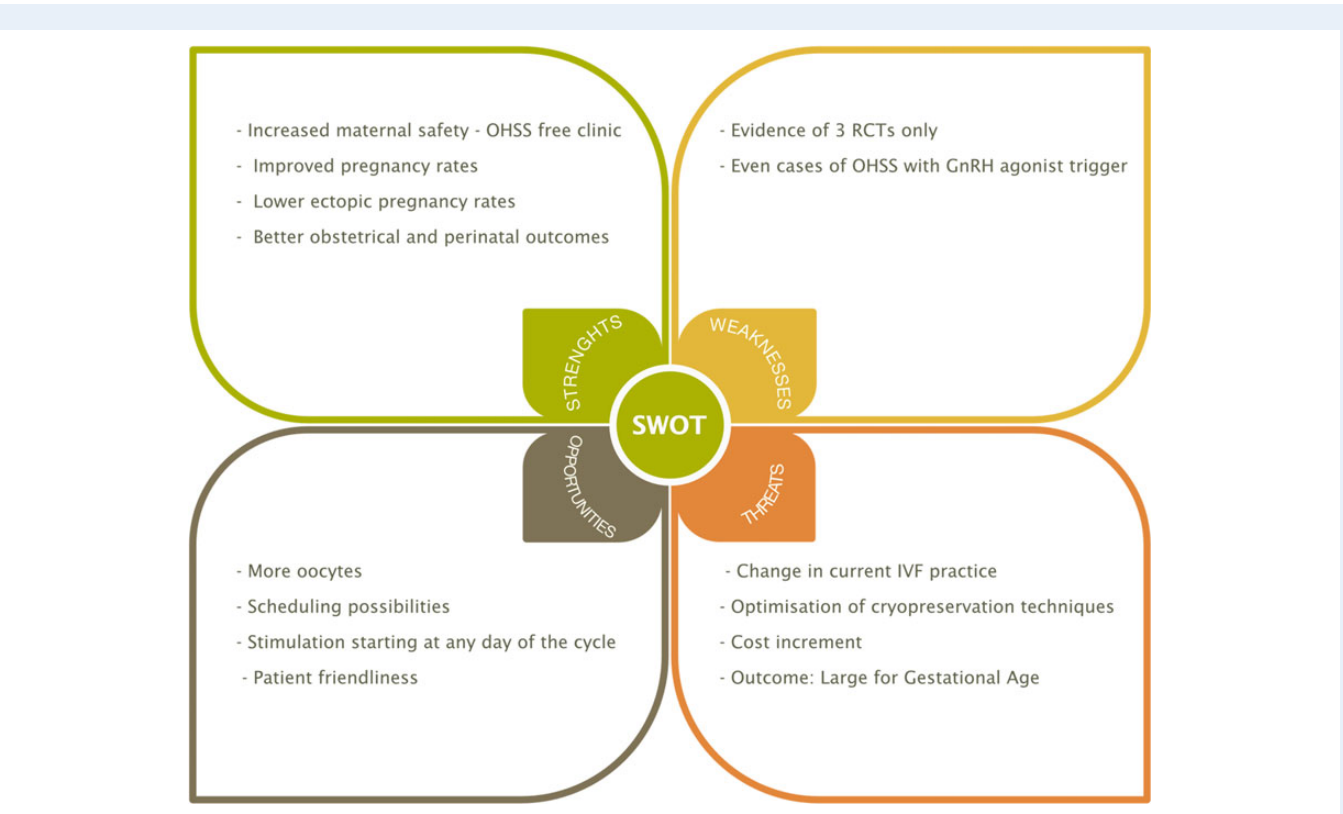

Figure 2 SWOT analysis of a freeze-all strategy. OHSS, ovarian hyperstimulation syndrome.

In addition, compared with embryo transfers following ovarian stimulation, large retrospective cohort studies (Ishihara *et al.*, 2011; Shapiro *et al.*, 2012; Huang *et al.*, 2014; Londra *et al.*, 2015) have shown that frozen-thawed embryo transfers, both at cleavage and blastocyst stages, significantly reduce the rate of ectopic pregnancy, suggesting a negative effect of ovarian stimulation on endometrial receptivity.

Previous researchers have shown that children born after assisted reproductive techniques (ART) have poorer outcomes in terms of preterm birth, low birthweight (LBW), small for gestational age and perinatal mortality when compared with newborns of natural conceptions (Helmerhorst *et al.*, 2004; Henningsen *et al.*, 2011; Bloise *et al.*, 2014). Interestingly enough, among those born from ART, the live births following embryos transferred during frozen cycles seem to have better obstetrical and neonatal outcomes when compared with children born from embryo transfers performed immediately after ovarian stimulation (Pelkonen *et al.*, 2010; Sazonova *et al.*, 2012; Wennerholm *et al.*, 2013; Ishihara *et al.*, 2014). Specifically, while the incidences of LBW and preterm birth of singleton FET pregnancies are similar to natural conceptions (Pinborg *et al.*, 2013), LBW occurs more frequently after fresh embryo transfers among women who conceived after both fresh and frozen cycles (Kalra *et al.*, 2011). Taken together, these observations provide reassuring evidence that the abnormal hormonal milieu and the suboptimal endometrial development observed in conventional ovarian stimulation cycles may be the main risk factor for at least some of these adverse outcomes. Furthermore, the physiological intrauterine conditions of FET may have a positive impact not only on endometrial receptivity and early implantation, but also on placentation and fetal growth (Pinborg, 2012).

Weaknesses

Despite the potential advantages of a freeze-all policy, the application of this technique has been limited to a safety measure to minimize the development of OHSS. One of the main reasons for this restricted use is that the benefit of the elective cryopreservation of all embryos in terms of pregnancy outcomes has only been verified in a few small and heterogeneous RCTs (Aflatoonian *et al.*, 2010; Shapiro *et al.*, 2011a, 2011b) restricted mostly to high responders. Furthermore, in the studies performed by Shapiro *et al.* (2011a, 2011b), all the available embryos were frozen at a pronuclear stage of development, a decision no longer common in most IVF centers. Such limitations are also inherent to the meta-analysis (Roque *et al.*, 2013) published later, which while confirming that FET cycles seem to be associated with better ongoing and clinical pregnancy rates, was based on only a few events deriving from heterogeneous studies. In this regard, high-quality RCTs are urgently needed, and currently registered RCTs aiming to test the above-mentioned hypothesis of the so-called 'freeze-all' strategy are ongoing (NCT00823121, NCT02471573, NCT01841528, NCT02148393, NTR3187 and ACTRN 12612000422820).

Finally, OHSS is not completely avoided even when applying a GnRH agonist as a trigger for final oocyte maturation, since cases of severe OHSS following GnRH agonist triggering without any luteal supplementation have been reported (Fatemi *et al.*, 2014; Gurbuz *et al.*, 2014; Ling *et al.*, 2014; Santos-Ribeiro *et al.*, 2015). Such case reports highlight that it still seems too early to safely apply a 'wild' ovarian stimulation approach, using higher doses of FSH stimulation.

Opportunities

Over recent years, live birth rates following the replacement of vitrified-thawed embryos have increased substantially and the success rates of these cycles have even reached those of fresh embryo transfers (Groenewoud *et al.*, 2012; Groenewoud *et al.*, 2013; Wong *et al.*, 2014). Given the fact that vitrification results in increased pregnancy rates when compared with both slow and ultra-rapid freezing (AbdelHafez *et al.*, 2010), it is likely that developments in embryo freezing systems over the past decade have finally bridged the gap, in terms of live births, between fresh and frozen-thawed cycles. Furthermore, other factors, such as the application of better morphologic embryo selection criteria or altered embryo transferred policies (e.g. elective single embryo transfer), may have also contributed to the improved pregnancy rates per frozen cycle.

Nonetheless, increased pregnancy and live birth rates are not the only factors to be taken into consideration. Specifically, for an IVF clinic, the scheduling of oocyte retrievals becomes easier, since the triggering of final oocyte maturation can be delayed instead of using the pre-specified other arbitrary cutoffs (e.g. presence of 3 follicles of 17 mm diameter), which might enhance the number of recruited mature oocytes (Kolibianakis *et al.*, 2004; Tremellen and Lane, 2010; Mochtar *et al.*, 2011; Vandekerckhove *et al.*, 2014). The endocrine profile and, mainly, high progesterone levels (>1.5 ng/ml) at the end of the follicular phase also become much less important, since the potential histological advancement of the endometrium is no longer of relevance (Bosch *et al.*, 2010). Interestingly, hormonal cycle monitoring becomes less crucial altogether, and the avoidance of oocyte retrievals during weekend days can be accomplished without the need of either oral contraceptive or estradiol valerate pretreatment (Griesinger *et al.*, 2010a, 2010b; Blockeel *et al.*, 2012; Cedrin-Durnerin *et al.*, 2012; Garcia-Velasco and Fatemi, 2015).

Another option that appears more likely with the widespread application of a freeze-all strategy is the possibility to initiate ovarian stimulation on any given day of the menstrual cycle, which is currently otherwise regarded as a last-resort treatment, until now mostly applied only in onco-fertility patients (Sonmezer *et al.*, 2011; Ozkaya *et al.*, 2012). Recently, published evidence did not find any difference in multiple perinatal outcomes when stimulation was initiated in the luteal phase (Chen *et al.*, 2015). This means that ovarian stimulation can begin on any day of the menstrual cycle, giving more room for logistical treatment changes to accommodate both the scheduling restrictions of physicians and IVF lab and the patient, who could then begin their treatment either as soon as possible or at their own convenience.

Furthermore, when speaking of flexibility for scheduling of the embryo transfer, frozen cycles with natural or artificial preparation have been shown to be equally effective, regardless of the use of GnRH downregulation (Ghobara and Vandekerckhove, 2008; Glujovsky *et al.*, 2010). However, the debate on which is the best FET cycle regimen is still open. While natural cycles are, at first look, more patient-friendly and less expensive, artificial cycles are the only option to treat women with cycle irregularities and may also contribute toward the avoidance of embryo transfers during weekends or holidays. An interesting study by El-Toukhy *et al.* (2004), employing a simplified protocol using ultrasound as the sole monitoring tool of medicated frozen cycle preparation, concluded that both the suppression of ovarian activity and hormonal monitoring are essential in FET cycles and that clinicians should not schedule

FET cycles based solely on endometrial thickness. These findings, however, are in contrast with a large cohort study of 1129 cryopreserved embryo transfer cycles, which revealed that, so long as an adequate endocrine monitoring is carried out, cryopreserved embryos can be transferred successfully in an artificial endometrium priming cycle without the use of a GnRH agonist (van de Vijver *et al.*, 2014). In this regard, further studies are needed in order to assess which FET regimen can simultaneously be as simple as possible for the patients, reduce medical visits and offer logistical and financial relief for IVF centers.

Finally, in terms of patient friendliness, the freeze-all protocol could also allow for a different approach to prevent premature LH surges, namely the use of oral medroxyprogesterone acetate (MPA) instead of injectable GnRH analogs. If an injection could be replaced by an oral medication, this would mean an enormous improvement in the quality of life for women undergoing IVF and a significant revolution in reproductive medicine (Kuang *et al.*, 2015).

Threats

Considering that the available evidence that supports higher pregnancy rates in FET compared with fresh cycles is mainly based on studies including high responders, extrapolating these data to the general population should be done with caution. In addition, a 'fresh' look at a new approach should always be able to foresee potential future hazards.

First, although most obstetrical and perinatal outcomes seem to be better following a FET, other studies have reported that it may, on the other hand, also be associated with an increased incidence of large for gestational age (LGA) in singletons (Pelkonen *et al.*, 2010; Sazonova *et al.*, 2012; Wennerholm *et al.*, 2013) even after accounting for maternal age and birth order (Pinborg *et al.*, 2014). In contrast, a previous large Japanese cohort study by Kato *et al.* (2012), which included only vitrified embryos, did not find any significant difference in LGA between children born after fresh or frozen cycles after adjusting for known confounding factors. Given that LGA and macrosomia are related to adverse obstetrical outcomes, such as stillbirth, asphyxia, shoulder dystocia and hypoglycemia (Opati *et al.*, 2015), these results may be a subject for concern and warrant confirmation by larger registry analyses that account for known paternal confounding factors. Whether the potential risk of LGA in FET singletons compared with singletons born after fresh embryo transfer is related to the freezing/thawing procedure *per se* remains unknown, and efforts should be made to evaluate causal pathways between freezing and thawing of embryos and growth potential. Most importantly, data regarding these perinatal outcomes are derived from observational studies, which may imply that the data might not be free from selection bias. Specifically, it is reasonable to assume that many of the live births that occurred following a FET took place in parous women who have already delivered in the preceding fresh cycle and are, therefore, at lower risk of adverse perinatal events.

Second, the applicability of elective vitrification of all embryos to the whole IVF population can only be a fact whenever good evidence from sufficiently powered studies becomes available, and when laboratories acquire optimal vitrification systems. However, a consensus is currently lacking in this aspect and, as a result, ART centers have developed their own freezing strategies based on their personal experiences and choices. This is an important drawback that limits our ability to effectively compare the different protocols available in order to evaluate the optimal timing for cryopreservation, the best selection criteria for embryo cryopreservation and the ideal methods for both embryo thawing and endometrial preparation for frozen-thawed embryo transfers (Groenewoud *et al.*, 2013). A cost-effectiveness analysis is also necessary to assess if the potential effects of a freeze-all policy on perinatal outcomes justify the additional cost and extra workload of elective cryopreservation.

As for the patient perception, a freeze-all policy can be met with considerable resistance. On one hand, couples are naturally more prone to opt for treatment solutions that minimize their time to pregnancy. Furthermore, although there is accumulating evidence to the contrary, patients frequently perceive FET as being inferior in terms of efficacy. On the other hand, physicians are known to be influenced in their decision-making process by a number of factors including previous experience and habits, rather than by evidence (Lode *et al.*, 2007). Thus, for the freeze-all strategy to thrive in the near future, physicians cannot disregard the importance of their own role as patient counselors and should adequately inform couples of the potential disadvantages of the temptation to always seek the instant gratification of a quick positive pregnancy test instead of opting for interventions associated with both safer and better long-term outcomes.

Conclusion

Although the major advantage of a freeze-all strategy is the potential for eliminating OHSS, several other factors also support a move toward this approach in ART. Enhanced cycle scheduling and improved organization of the IVF unit are elements that should not be overlooked. Taken together, these developments may lead to a new era in modern ART. Nevertheless, confirmation of the clinical benefits of a freeze-all strategy through well-designed clinical trials is mandatory prior to shifting our current ART practice.

Authors' roles

C.B. is responsible for the concept and drafted the manuscript. P.D. and S.R. participated in the writing of the manuscript. N.P.P. and H.T. contributed to the interpretation editing of the manuscript.

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Conflict of interest

None declared.

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