

Ovarian stimulation for preimplantation genetic testing

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Abstract

A narrative review of the management of controlled ovarian stimulation in patients undergoing preimplantation genetic testing is presented. An electronic search was performed to identify research publications that addressed ovarian stimulation and preimplantation genetic testing published until December 2017. Studies were classified in decreasing categories: randomized controlled trials, prospective controlled trials, prospective non-controlled trials, retrospective studies and experimental studies. The aim of controlled ovarian stimulation has shifted from obtaining embryos available for transfer to yielding the maximum embryos available for biopsy to increase the odds of achieving one euploid embryo available for transfer, without the distress of inducing ovarian hyperstimulation syndrome or inadequate endometrium receptivity as vitrification and deferred embryo transfer usually will be planned. The present narrative review summarizes all treatment-related variables as well as stimulation strategies after controlled ovarian stimulation that could help patients undergoing an *in vitro* fertilization cycle coupled with preimplantation genetic testing, including the number of oocytes needed to achieve one healthy live birth, oral contraceptive pill usage, the role of mild ovarian stimulation or random-start stimulation, the stimulation protocol and type of gonadotropin of choice, the novel progesterone protocols, agonist or dual trigger as a final oocyte maturation trigger, the accumulation of oocytes/embryos and the optimal interval before proceeding with a subsequent controlled ovarian stimulation or the optimal medication to link stimulation cycles. The discussion is being presented according to how questions are posed in clinical practice. The aim of ovarian stimulation has shifted from obtaining embryos available for transfer to yielding the maximum embryos available for biopsy to increase the odds of achieving one euploid embryo available for transfer.

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Introduction

Previous years have shown great progress in the field of human assisted reproductive technology (ART), largely thanks to the ability of embryologists and reproductive physicians to consider new strategies for improving the practice of *in vitro* fertilization (IVF). Notably, one of these breakthroughs has been the implementation of preimplantation genetic testing (PGT) of embryos, a technology introduced by [Handyside et al. \(1989\)](#) that allows controlling, before transfer, if all 24 chromosomes are normal within an embryo. Several improvements have been made to the technology, such as biopsy day ([Scott et al. 2013a](#)), amplification method ([Sermon et al. 2016](#)) or platform upgrades to analyze the amplified DNA ([Sermon et al. 2016](#)) that have enhanced its validity and propelled its adoption. Even though PGT has become a common tool to filter embryos before transfer, scarce information is available regarding the optimal controlled ovarian stimulation (COS) strategy in patients undergoing IVF with the intention to genetically evaluate their embryos.

Traditionally, the goal of COS with exogenous gonadotropins has been to maximize oocyte yield in an effort to overcome the high rate of gamete and

embryo attrition ([Pellestor et al. 2006](#), [Rodriguez-Purata et al. 2015](#)). Nevertheless, the objective of COS in patients undergoing PGT has been inherently adapted. Fundamentally, COS has changed from purely obtaining one suitable embryo available for transfer to obtaining the maximum number of embryos available for biopsy in order to increase the odds of achieving one euploid embryo available for transfer.

Furthermore, in the same manner that COS is tailored according to patient demographic variables such as antimullerian hormone (AMH) ([Lensen et al. 2018](#)), antral follicle count (AFC) ([Lensen et al. 2018](#)) or basal follicle-stimulating hormone (FSH) ([Lensen et al. 2018](#)), the concept of fitting COS in order to complement a patient's infertility diagnosis has also been utilized, for example in patients who experience poor ovarian response (POR) ([Polyzos & Devroey 2011](#)) or in patients undergoing fertility preservation (FP) for medical ([Dahhan et al. 2017](#)) and non-medical reasons ([Stoop et al. 2014](#)). In this regard, specific subsets of patients have particular requirements during their treatment, and endpoints in mind should be thought with the final aim that these patients seek.

The question being, in the era of modern ART, is there an optimal stimulation strategy for patients or couples

undergoing an IVF cycle with the aim of sustaining PGT that maximizes reproductive potential? For the purpose of this review, studies related to ovarian stimulation reporting live birth (LB) in PGT cycles or the clinically relevant outcome will be selected as a surrogate outcome of embryo euploidy.

Objective

The review surveys the current literature presented on critical clinical decisions regarding the management of COS for women undergoing IVF coupled with PGT. Additionally, the review is intended to offer professionals in the field of reproductive medicine a comprehensive clinical summary of the optimal stimulation strategy when incorporating PGT in IVF programs.

Materials and methods

Search

The current narrative review was designed to take a broad overview of the topic and attempted to integrate all available clinical and scientific evidence correlating the acquisition of euploid embryos through COS. The flow discussion will be presented according to how questions are posed in clinical practice. First, decisions before COS is started, followed by decisions presented during COS and, lastly, decisions after COS is finished:

- (1) How many oocytes do we need?
 - a. Number of oocytes needed to obtain one embryo.
 - b. Number of blastocysts needed to obtain one euploid embryo.
 - c. Number of euploid embryos needed to achieve a LB.
- (2) Contraceptive pill programming.
- (3) Stimulation strategy.
 - a. Conventional vs mild stimulation.
 - b. Conventional vs random start.
- (4) Stimulation protocol.
 - a. GnRH-antagonist vs agonist protocol.
 - b. New models of LH suppression: progesterone use.
- (5) Stimulation medication.
 - a. Type of gonadotropins.
 - b. LH supplementation.
 - c. Oocyte maturation trigger.
 - d. Recombinant human chorionic gonadotropin (hCG) (r-hCG) vs GnRH-agonist trigger.
 - e. Dual trigger.
- (6) Stimulation strategies after COS.
 - a. Fresh vs frozen embryo transfer.
 - b. Embryo accumulation.
 - c. Interval between stimulations.
 - d. Medication used to link stimulations.
 - e. Interval to first frozen embryo transfer.
- (7) Association of ovarian stimulation and aneuploidy.

For this narrative review, original studies were classified into five categories according to decreasing supporting evidence: randomized controlled trials (RCTs), prospective controlled trials, prospective non-controlled trials, retrospective studies and experimental studies. Studies included in lower categories were only used if enough evidence was not provided by high-quality studies. Reports in the form of abstracts were not considered for this review. The authors performed a systematic search of the literature for studies appropriate to the clinical questions on Medical Literature Analysis and Retrieval System Online (MEDLINE), PubMed, Embase, Cumulative Index to Nursing and Allied Health Literature and Cochrane Library (<http://www.cochranelibrary.com>). The authors additionally checked citations on the Web of Science and manually searched the references of the articles. Only articles in English were included. Searches were coordinated by an expert librarian and a statistician in November 2017 and December 2017. Search updates were conducted in April 2018. All records were screened for eligibility by two independent reviewers.

Quality assessment

Interpretation of the grades of recommendations (Vermeulen *et al.* 2017) is shown in Table 1. The grades of the recommendations are only based on the strength of the supporting evidence.

Results

How many oocytes do we need?

The goal of COS is to allow the retrieval of many oocytes during a cycle of IVF (Trounson *et al.* 1981). COS compensates for inefficiencies in the following stages of the cycle such as oocyte maturation and insemination, embryo culture and transfer, and implantation (Fauser *et al.* 1999). Even though the basis of COS involves the administration of gonadotropins (Macklon *et al.* 2006), it is now very clear that a 'one-size-fits-all' approach does not exist and an individualized treatment approach can cater to a patient's unique characteristics and maximizes success, eliminates iatrogenic risks and minimizes the risk of cycle cancellation (Scott *et al.* 2013a,b, La Marca & Sunkara 2014, Oudshoorn *et al.* 2017, van Tilborg *et al.* 2017a,b).

On the other hand, PGT describes the procedure of removing one or more nuclei from embryos, blastomeres or trophectoderm cells, to test for mutations in gene sequence or aneuploidy before transfer selection (Practice Committee of Society for Assisted Reproductive Technology & Practice Committee of American Society for Reproductive Medicine (2008)). When performing COS for PGT, the goal is to obtain a maximum ovarian response, without distress of inducing ovarian hyperstimulation syndrome (OHSS) (Steward *et al.* 2014) or inadequate endometrium receptivity (Evans *et al.* 2014) at transfer.

The reduction in natural conception and moderate IVF success rates with age is attributed to both the decline

Table 1 Interpretation on the grades of recommendations (Vermeulen *et al.* 2017).

Grades of recommendations	Supporting evidence
A	Meta-analysis, systematic review or multiple randomized controlled trials (RCTs) (high quality)
B	Meta-analysis, systematic review or multiple RCTs (moderate quality)
C	Single RCT, large non-randomized trial, case-control or cohort studies (high quality)
D	Single RCT, large non-randomized trial, case-control or cohort studies (moderate quality)
GPP	Non-analytical studies, case reports or case series (high or moderate quality)
	Expert opinion

of the ovarian reserve (Broekmans *et al.* 2009) and the increase in embryonic aneuploidy rate (Pellestor *et al.* 2006, Hassold & Hunt 2009, Frasiak *et al.* 2014). As a result, an increase in the practice of PGT during IVF has been observed (De Rycke *et al.* 2015). By permitting the selection of a euploid embryo before transfer, PGT has enhanced the efficiency of ART outcomes, particularly in patients with advanced maternal age, couples in which a severe male factor has been identified or in patients suffering repeated miscarriages or implantation failure (Munné *et al.* 2002, Harper & SenGupta 2012, Hodes-Wertz *et al.* 2012).

In essence, patients/couples looking to undergo PGT are not just expecting the identification of a transferable embryo, but the accumulation of as many embryos as possible in the minimal time attainable with the least plausible associated side effects.

Traditionally, a successful outcome when undergoing ART treatment denotes the ability to achieve a healthy singleton LB. Having this in mind, several studies reported that the most important factor that increases the odds of achieving the desired outcome is the number of oocytes retrieved, with 10–15 oocytes the considered optimal range (Meniru & Craft 1997, Kably *et al.* 2001, Letterie *et al.* 2005, van der Gaast *et al.* 2006, Edgar & Gook 2007, Patrizio & Sakkas 2009, Sunkara *et al.* 2011, Stoop *et al.* 2012, Fatemi *et al.* 2013, Ji *et al.* 2013, Steward *et al.* 2014, Baker *et al.* 2015, Drakopoulos *et al.* 2016, Vaughan *et al.* 2017).

Initially, studies conveyed there was a threshold number of oocytes after which, above and below, outcomes started to compromise (Meniru & Craft 1997, van der Gaast *et al.* 2006, Patrizio & Sakkas 2009, Sunkara *et al.* 2011). However, since this information was based only on fresh cycle outcomes, later studies incorporated the concept of cumulative LB rates (LBRs) (Fatemi *et al.* 2013, Drakopoulos *et al.* 2016, Toftager *et al.* 2017), largely because of the improvements and optimization made in cryopreservation methods (Cobo & Diaz 2011, Cobo *et al.* 2012) and the increasing use of the ‘freeze-only’ policy (Roque *et al.* 2013). Before the understanding that the quality of the frozen embryos and their reproductive potential were at least similar to those observed with fresh embryos (Roque *et al.* 2013, Rodriguez-Purata *et al.* 2016a), the accepted concept was that there was a maximum probability of LB in a fresh cycle, as, when everything is taken into consideration, only a certain number of embryos were going to be

transferred in the fresh cycle, regardless of how many embryos were available for subsequent frozen embryo transfer (FET) cycles.

To date, there are no studies evaluating the optimal number of oocytes needed to achieve one healthy LB through PGT. Since in most cases PGT involves the vitrification of all biopsied embryos as the turnaround time before genetic results are received is highly variable depending on the technology used (i.e. ploidy status, single-gene disorders, specific balanced translocations), success of COS can, therefore, be segmented into the optimal number of oocytes needed to obtain one blastocyst, then into the optimal number of blastocysts needed to obtain one euploid embryo, and, lastly, into the optimal number of euploid embryos needed to achieve a LB. Included studies are summarized in Table 2.

Number of oocytes needed to obtain one embryo

Sunkara *et al.* reported a strong correlation between the number of oocytes and LBR, which rose with increasing number of oocytes up to 15, plateaued between 15 and 20 and steadily declined beyond 20 oocytes (Sunkara *et al.* 2011). It is worth mentioning that the study only analyzed fresh IVF cycle outcomes and did not take into account the impact of FET cycles on the cumulative LBR (Sunkara *et al.* 2011).

Stoop *et al.* referred that, overall, 3.83% of all retrieved mature oocytes result in a LB (Stoop *et al.* 2012). The authors reported that oocyte utilization rate (number of LBs per mature oocyte) depends largely on ovarian response between the ages of 23 and 37, and to a much lesser extent on age. But from the age of 38, the oocyte utilization rate depended largely on age, and to a lesser extent on ovarian response (Stoop *et al.* 2012). The oocyte utilization rate between the age of 23 and 37 remained constant with an average of 4.5%. But from ≥ 38 years, a significantly lower oocyte utilization rate was noted, declining from 3.8% at 38 years to 0.8% at 43 years ($P < 0.001$) (Stoop *et al.* 2012).

Fatemi *et al.* (2013) also showed how the average number of good-quality embryos increased with ovarian response: from 1.1 with 0–5 oocytes retrieved to 8.0 with >18 oocytes retrieved (recombinant FSH group). Accordingly, the number of cryopreserved embryos also increased with ovarian response: from 0.2 with 0–5 oocytes to 4.2 with >18 oocytes (Fatemi *et al.* 2013).

Table 2 Studies evaluating the optimal number of oocytes, blastocysts and euploid embryos needed to achieve one healthy LB through PGT.

Study	Variable	Metric
Number of oocytes needed to obtain one embryo		
Sunkara et al. (2011)	Retrospective study	Oocytes to LB
Stoop et al. (2012)	Retrospective study	MII to LB
Fatemi et al. (2013)	RCT double-blind	Good-quality embryos
Fatemi et al. (2013)	RCT double-blind	Cryopreserved embryos
Patrizio and Sakkas (2009)	Retrospective study	Usable embryos
Ji et al. (2013)	Retrospective study	Usable embryos
Vaughan et al. (2017)	Retrospective study	Usable embryos
Number of blastocysts needed to obtain one euploid embryo		
Fransiak et al. (2014)	Retrospective study	Aneuploidy
Fransiak et al. (2014)	Retrospective study	No-euploid embryo rate
Labarta et al. (2012)	Prospective cohort study	Euploid embryo rate
Labarta et al. (2017)	Prospective cohort study	Oocytes to euploid embryo
Ata et al. (2012)	Retrospective study	Proportion of patients with one euploid embryo
Number of euploid embryos needed to achieve a live birth		
Yang et al. (2012)	RCT, fresh	SET
Scott et al. (2013a,b)	RCT, fresh	1.86 embryos transferred
Forman et al. (2013)	RCT, fresh and frozen	SET

IR, implantation rate; LB, live birth; LBR live birth rate; MII, metaphase II oocytes; PGT, preimplantation genetic testing; PR, pregnancy rate; RCT, randomized controlled trial; SET, single embryo transfer.

Given that some published studies do not report biopsiable embryos as an outcome, transferable or usable embryos were considered as a surrogate marker for biopsiable embryos with the objective of determining how many embryos would have been available if a PGT cycle would have been carried out. First, Patrizio and Sakkas published that 31.2% of the total oocytes collected develop into usable embryos (Patrizio & Sakkas 2009). Then, Ji *et al.*

showed that the number of transferable embryos increased from 2 when 0–5 oocytes were retrieved to 9 when >16 oocytes were retrieved (Ji *et al.* 2013). Lastly, Vaughan *et al.* reported that the average usable blastocysts increased as the number of oocytes retrieved increased: 0.1 with 1–3 oocytes, 1.0 with 4–9 oocytes, 2.5 with 10–14 oocytes, 4.1 with 15–25 oocytes and 6.7 with >25 oocytes were retrieved (Vaughan *et al.* 2017).

Number of blastocysts needed to obtain one euploid embryo

The aneuploidy rate and the no-euploid embryo rate (risk of having no-euploid embryos within a single cohort) rises in a predictable way with age (Franasiak *et al.* 2014). It was found that aneuploidy rates reached >40% at age 25, reached the lowest level between the ages of 26 and 30 at around 25%, with a predictable and steady rise through age 43, at which point the rate plateaus at approximately 85% (Franasiak *et al.* 2014). The no-euploid embryo rate was nearly 30% at age 22, reached the lowest in women aged 26–37, then 33% at age 42 and was 53% at age 44. Interestingly, both the aneuploidy rate and the no-euploid embryo rate in patients younger than 26 years were higher than in patients older than 26 years. Additionally, Labarta *et al.* also published a 60% euploid embryo rate in patients <35 years (25.4 ± 4.0) (Labarta *et al.* 2012).

More recently it has been published that a total of 3.4 metaphase II (MII) oocytes were needed to obtain one euploid embryo in patients <35 years (Labarta *et al.* 2017), and, considering the median value as a reference point, the authors observed that the mean of euploid embryos was 5.0 ± 2.4 (median 5.0) when the number of oocytes obtained was \geq median (17 oocytes), and it was 2.6 ± 1.4 (median 2.5) when ovarian response was below it ($P < 0.001$). Furthermore, when the number of euploid embryos was segregated by ovarian response, the authors observed ~2 euploid embryos with 1–12 oocytes, ~3 euploid embryos with 13–16 oocytes, ~5 euploid embryos with 17–23 oocytes and ~6 euploid embryos with >23 oocytes.

Lastly, according to Ata *et al.*, when 1–4 blastocysts are obtained, 92.3% of patients <35 years will have one euploid embryo, 78.6% when 35–39 years, 60.7% when 40–42 and 37.5% when ≥ 43 years. Now, when 5–7 blastocysts are identified, 100% of patients <35 years will have one euploid embryo, 97.2% when 35–39 years, 81.3% when 40–42 and 66.7% when ≥ 43 years. Then, when 8–10 or >10 blastocysts are biopsied, essentially 100% of all patients will have at least one euploid embryo (Ata *et al.* 2012). This depicts that the more blastocysts biopsied, the higher the probability of obtaining at least one euploid embryo available for transfer, although at the same time, that probability decreases with increasing age.

Number of euploid embryos needed to achieve a LB

Three RCTs were identified reporting clinical pregnancy rates (PRs), ongoing PRs or LBRs (Yang *et al.* 2012, Forman *et al.* 2013, Scott *et al.* 2013b). PGT is correlated with higher implantation rates (IR) and higher ongoing PRs when the same number of embryos are transferred in non-PGT cycles. By enhancing embryo selection with aneuploidy screening, a single euploid embryo with higher reproductive potential can be selected for

transfer. Using this approach, elective single embryo transfer (SET) can be performed without compromising LBRs and improving the chance of having a healthy, term singleton delivery after IVF (Forman *et al.* 2014).

Yang *et al.* reported a clinical PR with PGT of 70.9% and an ongoing PR of 69.1% when a fresh euploid SET (Yang *et al.* 2012). Scott *et al.* reported sustained IRs (probability that an embryo will implant and progress to delivery) of 66.4% (with 1.86 embryos transferred) and a LBR of 84.7% (Scott *et al.* 2013b). Lastly, Forman *et al.* reported a SET's ongoing PR of 60.7% (Forman *et al.* 2013). Of note, this is the only RCT in which fresh and frozen ET cycles were accounted, and IRs were significantly improved with both strategies (60.7% after euploid SET vs 65.1% after untested two-blastocyst transfer; relative risk (RR): 0.9; 95% confidence interval (CI): 0.7–1.2) (Forman *et al.* 2013).

Treatment-related variables in PGT cycles

In conventional IVF, retrieving less than four oocytes is considered a POR, and the risk of POR increases when there is a low ovarian reserve (Ferraretti *et al.* 2011), whereas for PGT, the retrieval of <10 oocytes had been previously considered insufficient (Vandervorst *et al.* 1998). The cumulative probability of achieving a LB does not plateau at any level of ovarian response, reaching in the extreme high response (>30 oocytes) values above 75% and that the number of euploid embryos increases as the number of retrieved oocytes does (Ata *et al.* 2012, Labarta *et al.* 2017). Therefore, in PGT cycles, the preferred strategy is a stimulation protocol aiming to exploit the ovarian response as much as possible without incurring in adverse effects to the patient.

Contraceptive pill programming

Pretreatment with oral contraceptive pills (OCP) withholds natural hormone production and might help homogenize the development of the follicular cohort and help scheduling IVF cycles (Cédric-Durnerin *et al.* 2007). In the most recent systematic review addressing the influence of hormone pretreatment in IVF cycles (Farquhar *et al.* 2017) multiple subgroups were used to evaluate OCPs combined with different GnRH-analog protocols (antagonist/agonist). Treatments were compared head-to-head or versus no treatment or placebo. Various of the comparisons included only one study with limited number of participants and methodological weaknesses, so many findings from this review should be considered with caution (Farquhar *et al.* 2017). With antagonist cycles in both groups, the LBR or ongoing PR was lower in the pretreatment group (OR: 0.74, 95% CI: 0.58–0.95; 6 RCTs; 1335 women; $I^2 = 0\%$; moderate-quality evidence). There was insufficient evidence to determine whether the groups differed in rates of pregnancy loss (OR: 1.36, 95% CI:

0.82–2.26; 5 RCTs; 868 women; $I^2=0\%$; moderate-quality evidence), multiple pregnancy (OR: 2.21, 95% CI: 0.53–9.26; 2 RCTs; 125 women; $I^2=0\%$; low-quality evidence), OHSS (OR: 0.98, 95% CI: 0.28–3.40; 2 RCTs; 642 women; $I^2=0\%$, low-quality evidence), or ovarian cyst formation (OR: 0.47, 95% CI: 0.08–2.75; 1 RCT; 64 women; very low-quality evidence) (Farquhar *et al.* 2017). These results are consistent with previous reports (Griesinger *et al.* 2010, 2015).

Given that comparable outcomes are obtained, it can be concluded that the benefits of cycle scheduling with OCPs (Garcia-Velasco *et al.* 2011) (equal distribution of workload in large busy units and staff distribution, avoiding weekend retrievals in small units, synchronization of follicular cohort, avoiding excessive incubator openings) outweigh the theoretical drawbacks (i.e. higher FSH consumption and longer duration of the stimulation). Additionally, it is particularly useful in PGT programs to try to avoid biopsy procedures over the weekend.

Stimulation strategy

Conventional vs mild stimulation With the mild ovarian stimulation (MOS) approach, clinicians focus on optimizing patient care rather than obtaining the highest yield of oocytes, by minimizing treatment burden and risk of complications. MOS is associated with a greater safety profile in terms of the incidence of OHSS and of venous thromboembolism (Nargund *et al.* 2017). It is also found to be better tolerated by patients (Verberg *et al.* 2008) and less expensive (Nargund *et al.* 2017). Given that two meta-analyses of RCTs in normal responders demonstrated a lower number of retrieved oocytes in MOS (Verberg *et al.* 2009a,b, Matsaseng *et al.* 2013), it is not a strategy recommended for patients undergoing a PGT cycle.

Random-luteal phase stimulation Largely, COS is usually started at the early follicular phase (day 2–3 of the cycle) for ~8–10 days, and the retrieval of mature follicles takes place around day ~13–15 of the cycle. Beginning the COS at any time of the cycle ('random start') has been a routine in FP (Martinez 2017) without observing any change in oocytes retrieved, regardless of cycle start day (Martínez *et al.* 2014, Ubaldi *et al.* 2016). This approach is based upon the recognition of the presence of multiple waves of follicle recruitment within a single inter-ovulatory period (Baerwald *et al.* 2012).

Ubaldi *et al.* (2016) compared the euploid blastocyst formation rates obtained after follicular vs random start with an identical COS protocol in patients with POR, performed in the same menstrual cycle. Stimulations were started on day 2 of a menstrual cycle and 5 days after the first vaginal oocyte retrieval, respectively. No significant differences were found in the number of MII (3.4±1.9 vs 4.1±2.5), or biopsied blastocysts per stimulated cycle (1.2±1.2 vs 1.4±1.7). Similarly, no differences were

observed in the euploid blastocyst rate calculated either per biopsied blastocyst (46.9% vs 44.8%) or inseminated MIIs (16.2% vs 15.0%). The authors concluded that luteal phase stimulation significantly contributes to the final transferable blastocyst yield thus increasing the number of patients undergoing transfer per menstrual cycle (Ubaldi *et al.* 2016).

Notably, even though time is an important factor for all patients, it is crucial for those with a foreseeable rapid loss or decrease of fertility, including a malignancy that requires gonadotoxic treatment, removal of gonads or in poor-prognosis patients. Therefore, we think this strategy may be applied in situations where obtaining competent oocytes is an urgent task.

Stimulation protocol

Agonist vs antagonist protocol To acquire multiple oocytes during an IVF cycle, exogenous gonadotropins are administered so that multiple competent follicles can mature (Macklon *et al.* 2006). A GnRH-agonist or antagonist is given concomitantly to prevent a premature endogenous LH peak that would otherwise induce ovulation too early. GnRH-agonists may be prescribed using a long or a short protocol (Siristatidis *et al.* 2015). In all cases, an oocyte maturation triggering agent with a bolus of r-hCG or a GnRH-agonist, or both together (Bosch *et al.* 2016) is required.

A recent Cochrane review included 73 RCTs and 12,212 participants, comparing GnRH-antagonist to long GnRH-agonist protocols (Al-Inany *et al.* 2016). There was no evidence of a difference in LBR between GnRH-antagonist and long GnRH-agonist (OR: 1.02, 95% CI: 0.85–1.23; 12 RCTs, $n=2303$, $I^2=27\%$, moderate-quality evidence). The evidence suggested that if the chance of LB following GnRH-agonist is assumed to be 29%, the chance following GnRH-antagonist would be between 25% and 33%. At the same time, GnRH-antagonist was associated with a 39% lower incidence of any grade of OHSS than GnRH-agonist (OR: 0.61, 95% CI: 0.51–0.72; 36 RCTs, $n=7944$). Likewise, GnRH-antagonist was associated with a 53% lower incidence of cycle cancellation due to high risk of OHSS (OR: 0.47, 95% CI: 0.32–0.69; 19 RCTs, $n=4256$) but also 1.3 times higher risk of cancellation due to poor response than those who were treated with GnRH-agonist (OR: 1.32, 95% CI: 1.06–1.65; 25 RCTs, $n=5230$).

More recently, a systematic review and meta-analysis evaluated the effect of the type of GnRH analog used to prevent the endogenous LH surge, with special attention to differences in subset of patients and interventions (Lambalk *et al.* 2017). Fifty studies were included, of which 34 studies reported on general IVF patients, 10 studies reported on polycystic ovary syndrome (PCOS) patients and 6 studies reported on POR (Lambalk *et al.* 2017). In general IVF patients, ongoing PR was significantly lower in patients using an antagonist (RR:

0.89, 95% CI: 0.82–0.96). In women with PCOS or with POR, there was no evidence of a difference in ongoing PR between groups (RR: 0.97, 95% CI: 0.84–1.11 and RR: 0.87, 95% CI: 0.65–1.17, respectively). Subgroup analyses for various antagonist strategies compared to the long GnRH-agonist protocol showed a significantly lower ongoing PR when OCP pretreatment was combined with a flexible antagonist protocol (RR: 0.74, 95% CI: 0.59–0.91) while non-OCP, the RR was 0.84, 95% CI: 0.71–1.0. Subgroup analysis for the fixed antagonist protocol demonstrated no evidence with or without OCP (RR: 0.94, 95% CI: 0.79–1.12 and RR: 0.94, 95% CI: 0.83–1.05, respectively). Lastly, antagonist cycles resulted in statistically lower OHSS rates both in the overall IVF patients and in patients with PCOS (RR: 0.63, 95% CI: 0.50–0.81 and RR: 0.53, 95% CI: 0.30–0.95, respectively), with no data reported on patients with POR (Lambalk *et al.* 2017).

This meta-analysis showed that in relation to the odds of a sustained pregnancy in overall IVF patients and when evident ovarian dysfunction such as PCOS or POR are excluded, there is an absolute risk reduction of 3.6% in ongoing PR when a GnRH-antagonist was used (23.8% compared to 27.4% after the use of agonists). The number needed to harm was 28, that is for every 28 women treated with antagonist one less ongoing pregnancy would occur. On the other hand, use of antagonist instead of an agonist reduced the absolute rate of OHSS by 2.5%, with the number of women needed to prevent one case of OHSS being 40.

Given the moderate-quality evidence that the use of GnRH-antagonist compared with long-course GnRH-agonist protocols is associated with a substantial reduction in OHSS without reducing the likelihood of achieving LB (Al-Inany *et al.* 2016, Lambalk *et al.* 2017), the antagonist protocol is considered the recommended protocol in patients undergoing PGT. Additionally, it fosters the utilization of a GnRH-agonist for late oocyte maturation (alone or in combination with r-hCG), a strategy associated with decreasing OHSS risk (Griesinger *et al.* 2006, Shapiro *et al.* 2008, Youssef *et al.* 2014, Engmann *et al.* 2016, Eftekhar *et al.* 2017).

New models of suppression of endogenous LH surge During luteal phase stimulation, endogenous progesterone secretion from the corpus luteum inhibits a spontaneous LH surge. Administering exogenous progesterone during the follicular phase has produced a similar effect and, consequently, a newer strategy for controlling the endogenous LH peak has been recently utilized: the so-called progesterone protocols (Massin 2017). Researchers have reported successful clinical outcomes with the use of medroxyprogesterone acetate (Kuang *et al.* 2015), micronized progesterone (Zhu *et al.* 2015, 2017a) or dydrogesterone (Zhu *et al.* 2017b) during COS without affecting the total oocytes collected or the quality of the embryos obtained. There are no

previous studies evaluating this strategy in patients undergoing PGT. Given that an essential requisite would be that a fresh transfer is not programmed, theoretically, these protocols could be used in the PGT population. Further studies are necessary to confirm these judgments.

Stimulation medication

Type of gonadotropins A recent Cochrane review included 42 trials with a total of 9606 couples comparing urinary (hMG, human menopausal gonadotropin) or recombinant gonadotropins (rFSH, recombinant FSH) (van Wely *et al.* 2011). Comparing rFSH to all other gonadotropins combined, irrespective of the GnRH analog used, did not result in any evidence of a statistically significant difference in LBRs (28 trials, 7339 couples, OR: 0.97, 95% CI: 0.87–1.08). There was similarly no evidence of a difference in the OHSS rate (32 trials, 7740 couples, OR: 1.18, 95% CI: 0.86–1.61). There was no evidence of a difference in LBs when rFSH was compared with purified urinary FSH (5 trials, $n=1430$, OR: 1.26, 95% CI: 0.96–1.64) or when rFSH was compared with highly purified urinary FSH (13 trials, $n=2712$; OR: 1.03, 95% CI: 0.86–1.22). Given this results, clinical choice of gonadotropin should depend solely on availability, convenience and costs (van Wely *et al.* 2011).

When comparing rFSH isoforms, two commercially available rFSH preparations are follitropin alfa (Gonal-F, Merck Serono, Geneva, Switzerland) and follitropin beta (Puregon, Organon, Oss, The Netherlands) are the most studied. Although both preparations are synthesized by the same recombinant technology resulting in identical dimeric FSH-alfa and FSH-beta subunits, they differ in the glycosylation and purification procedures. To our knowledge, there is only one recent study compared recombinant preparations. Orvieto *et al.* compared 198 in patients using follitropin alfa, and 68 in patients using follitropin beta (Orvieto *et al.* 2009). Although both groups achieved a comparable number of retrieved oocytes, the use of follitropin beta was associated with a tendency toward a lower clinical PR, and with significantly higher estradiol levels despite the use of significantly lower total gonadotropin dose. Unfortunately, there is no information on ongoing embryos, usable embryos nor LBR. With the recent addition of new molecules into the market (Elonva; Merck Sharp & Dohme de España, Spain; or Rekovelle; Ferring Pharmaceuticals, Madrid, Spain) further large studies are required to assess the effect of the different rFSH on COS variables and IVF outcome.

LH supplementation As described in the classic ‘two cell-two gonadotropins’ theory, LH is needed to provide the granulosa cells with androgen precursors for estradiol biosynthesis by FSH (Short 1962). Thenceforth, when GnRH analogues prevent premature LH, surges and ovulation most likely deprive the growing follicles of LH. The question arises as to whether

supplementation with LH, either recombinant (rLH) or as hMG, would have beneficial effects for growing follicles and may lead to better pregnancy outcomes.

Taking LBR as a surrogate marker of embryo euploidy, in a Cochrane review of 36 RCTs (8125 women) there was insufficient evidence to determine whether there was a difference between rLH combined with rFSH vs rFSH alone in LBR (OR: 1.32, 95% CI: 0.85–2.06; $n=499$; studies=4; $I^2=63\%$, very low-quality evidence) (Mochtar *et al.* 2017). The most recent systematic review assessing the role of rLH supplementation in COS for ART in specific subgroups of patients was published by Alviggi *et al.* (2018). Six populations were investigated: (1) women with a hypo-response to rFSH monotherapy; (2) women with advanced age; (3) women co-treated with a GnRH-antagonist; (4) women with profoundly suppressed LH levels after an GnRH-agonist use; (5) normo-responder women to prevent OHSS; and (6) women with a POR to COS. rLH supplementation appears to be beneficial in two subgroups: (1) women with adequate pre-stimulation ovarian reserve parameters and an unexpected hypo-response to rFSH monotherapy and (2) women 36–39 years of age (Alviggi *et al.* 2018). Indeed, there is no evidence that rLH is beneficial in young (<35) normo-responders co-treated with a GnRH-antagonist. This is consistent with a previous study asserting better IRs in patients aged 36–39 years, compared to patients <35 years, when LH administration was added during an antagonist protocol (Bosch *et al.* 2011).

The use of rLH supplementation in women with suppressed endogenous LH levels caused by GnRH analogues and in POR remains controversial, whereas the use of rLH supplementation to prevent the development of OHSS warrants further investigation. Given that the type of patient that is more likely to undergo a PGT cycle, further RCTs in patients undergoing PGT on the effectiveness of rLH combined with rFSH in women with POR and in women of advanced age are required.

Oocyte maturation trigger

r-hCG vs GnRH-agonist trigger In antagonist protocols, an oocyte maturation triggering medication is used, and due to the intact pituitary responsiveness, this agent can be r-hCG, a GnRH-agonist or both together (Bosch *et al.* 2016). Induction of final oocyte maturation with a bolus of GnRH-agonist instead of r-hCG, in PGT cycles would have several advantages: a significant reduction or avoidance of the risk of OHSS, a more physiologic stimulus for oocyte maturation since it causes both FSH and LH release, and a luteolytic effect since endometrial receptivity and luteal function are no longer important (Gonen *et al.* 1990, Humaidan *et al.* 2011).

A recent Cochrane review included 17 RCT ($n=1847$) comparing the clinical outcomes of GnRH-agonist trigger vs r-hCG in women undergoing a GnRH-antagonist cycle (13 studies assessed fresh

autologous cycles and four studies assessed donor-recipient cycles) (Youssef *et al.* 2014). Since the donor-recipient model could be inferred as COS in PGT (stimulation in one menstrual cycle and embryo transfer in another), no evidence suggested a difference between groups in LBR (OR: 0.92, 95% CI: 0.53–1.61; one RCT, 212 women) or ongoing PR (OR: 0.88, 95% CI: 0.58–1.32; three RCTs, 372 women, $I^2=0\%$) in this subpopulation. Importantly, the authors found evidence of a lower incidence of OHSS in the GnRH-agonist triggered group than in the r-hCG group (OR: 0.05, 95% CI: 0.01–0.28; three RCTs, 374 women, $I^2=0\%$) (Youssef *et al.* 2014).

Because of all this, GnRH-agonist as a final oocyte maturation trigger could be considered a useful option for patients who choose to avoid fresh transfers. Of note, appropriate selection of patients is of utmost importance, and those with hypothalamic dysfunction are not candidates for GnRH-agonist trigger as they may not reliably respond to GnRH-agonist administration (Engmann *et al.* 2016).

Dual trigger Additionally, a dual trigger, the co-administration of GnRH-agonist and r-hCG, has been proposed to improve the oocyte yield while reducing OHSS risk (Shapiro *et al.* 2008). A recent RCT including 192 normal-responder IVF patients comparing triggering with 6500IU r-hCG alone vs 6500IU r-hCG plus 0.2mg of triptorelin, found statistically higher mean number of retrieved oocytes, mature MII oocytes and ongoing embryos in the dual trigger group compared with r-hCG (Eftekhari *et al.* 2017). Therefore, in patients not at risk of OHSS, dual trigger could be considered a valuable option.

Strategies after ovarian stimulation

Fresh vs FET

FET cycles have traditionally been associated with the utilization of ‘leftover’ embryos, as the morphologically superior embryos are selected for fresh ET. This inherent bias may have affected former analyses and could have diminished early study’s FET cycles’ PRs. In this nature, it would be unfavorable to compare the quality of ‘second best’ embryos to their morphologically ‘superior’ siblings. Although there is an increasing number of studies supporting improved clinical outcomes after FETs (Roque *et al.* 2013, Evans *et al.* 2014), fresh ET protocols are typically more affordable, require little to no additional medications and allow the patients immediate transfer. However, in the context of PGT cycles, a successful fresh day 6 transfer approach obliges not only that expanded blastocysts be available on the morning of day 5 (Rodriguez-Purata *et al.* 2016b), but also that at least one of these biopsied embryos is euploid, thereby also reducing the probability for a transfer in that cycle.

The most recent meta-analysis evaluating the effectiveness and safety of the freeze-only strategy compared to the conventional IVF strategy included four RCTs analyzing a total of 1892 women (Wong *et al.* 2017). The evidence was of moderate to low quality due to serious risk of bias and, for some outcomes, serious imprecision (Wong *et al.* 2017). Risk of bias was associated with unclear blinding of investigators for preliminary outcomes of the study, unit of analysis error and absence of adequate study termination rules (Wong *et al.* 2017). There was no evidence of a difference in cumulative LBR among freeze-only and a conventional IVF strategy (OR: 1.09, 95% CI: 0.91–1.31; 4 trials; 1892 women; $I^2=0\%$; moderate-quality evidence) (Wong *et al.* 2017). This suggests that if the cumulative LBR is 58% following a fresh transfer, LBR following a freeze-only strategy would be between 56% and 65%. The authors concluded that, with moderate-quality evidence, one strategy is not superior to the other in terms of cumulative LBRs (Wong *et al.* 2017). In terms of OHSS risk, the prevalence was statistically lower after freezing all embryos when compared to the conventional IVF strategy (OR: 0.24, 95% CI: 0.15–0.38; 2 trials; 1633 women; $I^2=0\%$; low-quality evidence) (Wong *et al.* 2017). This suggests that if the OHSS rate is 7% following a conventional IVF, the rate following a freeze-only strategy would be between 1% and 3% (Wong *et al.* 2017).

Two RCTs published recently not included in the meta-analysis (Shi *et al.* 2018, Vuong *et al.* 2018) also support these findings. Shi *et al.* randomly assigned 2157 women who were undergoing their first IVF cycle to fresh ET or embryo cryopreservation and FET in normo-ovulatory women, and the LBR did not differ significantly (50.2% and 48.7%, respectively; RR: 0.97; 95% CI: 0.89–1.06; $P=0.50$) (Shi *et al.* 2018). Vuong *et al.* (2018) randomly assigned 782 infertile women without PCOS who were undergoing a first or second IVF cycle to receive either an FET or a fresh embryo, and the transfer of frozen embryos did not result in significantly higher LBRs than the transfer of fresh embryos (33.8% and 31.5%, respectively (risk ratio: 1.07; 95% CI: 0.88–1.31)) (Vuong *et al.* 2018).

Pertaining only to the transfer of euploid embryos, only one RCT (Coates *et al.* 2017) and one retrospective study (Rodriguez-Purata *et al.* 2016a) have compared the two strategies. Coates *et al.* (2017) randomized 179 patients to either a fresh or a frozen ET. The intention-to-treat analysis considered all randomized patients in their original group of allocation regardless of achieving an ET. The IR was higher in the FET group (75%) compared with the fresh group (67%), but this difference was not significant ($P=0.3$) (Coates *et al.* 2017). The ongoing PRs and LBRs were significantly higher for the FET group compared with the fresh group: ongoing PRs of 80% in FET vs 61% in fresh, $P=0.03$; LBRs of 77% in FET vs 59% in fresh, $P=0.04$ (Coates *et al.* 2017). Despite a

strong trend toward improved LBRs with FETs, the ET strategy did not have a statistically significant effect on the probability of achieving a LB when adjusted for age and oocytes retrieved in a logistic regression model (OR: 2.1; 95% CI: 0.95–4.68; $P=0.68$) (Coates *et al.* 2017). Rodriguez-Purata *et al.* retrospectively segregated patients into fresh-only (patients who transfer their best embryo in the fresh cycle), FET-only (patients who underwent a freeze-only cycle) and a FET in which a fresh transfer was previously performed (patients who did not achieved a pregnancy in the fresh cycle and were utilizing the second-best embryo). FET-only patients were associated with a higher LBR, even when a morphologically superior embryo was already used in a previous fresh ET (FET-only group 57.6%, FET with a previous fresh ET 47.7% and fresh-only 46.5%). FET-only patients were reported to have 1.6 times higher probability of achieving a LB when compared to patients that underwent a fresh ET (OR: 1.6 (95% CI: 1.14–2.13), $P<0.05$) (Rodriguez-Purata *et al.* 2016a).

In summary, either strategy can be an equitable option for patients. Nevertheless, freezing all embryos allows for the inclusion of all blastocysts in the cohort of embryos available for analysis, which potentially culminates in a higher proportion of patients reaching transfer. This approach also reduces uncertainty in scheduling procedures and prevents the unavoidable disappointment that patients undergo when informed that there are no suitable embryos for transfer. Lastly, as mentioned before, not transferring in the fresh cycle decreases the overall risk of OHSS. Consequently, FET is the recommended strategy when undergoing PGT.

Embryo accumulation/banking

For patients with a fair response for conventional IVF but insufficient for PGT (low number of embryos available for biopsy), performing repeated stimulation cycles in order to accumulate oocytes or embryos could be a suitable strategy to increase their odds of success, turning the patient into a 'normal responder' and render a pregnancy rate per patient and per ET comparable to those of PGT cycles with on stimulation cycle. Moreover, planning the stimulation and accumulation strategy before starting the procedure may avoid the sensation of failure in patients when the recommended number of oocytes or embryos is not achieved after one attempt.

In 2013, embryo banking was utilized in 27,564 out of 121,351 (22.7%) fresh non-donor ART cycles in the United States (Kushnir *et al.* 2016). Embryo banking cycles were more frequently performed with advancing female age, increasing from 15.5% in women <35 years to 56.5% in women >44 years old (Kushnir *et al.* 2016). In a study by Martinez *et al.*, patients underwent one, two or three COS cycles until reaching ≥ 10 MII. There were no differences between groups in PRs per patient (36.8, 34.9 and 31.0%) or per ET (59.6, 56.8 and 60%,

respectively) (Martínez *et al.* 2016). Patients with a more unfavorable ovarian reserve (lower AMH and AFC) who need three stimulation cycles can reach final outcomes comparable to patients who obtain the required number of oocytes or embryos after two stimulation cycles (Martínez *et al.* 2016).

Interval between stimulations

There are no studies published evaluating if there is an optimal time interval before proceeding with a subsequent COS.

In the animal model, repeated COS leads to modifications in associated somatic cells, size and transcriptional status of germinal vesicle-stage oocytes, reductions in *in vitro* meiotic competence, with no changes in *in vivo* maturation, and significant variations in oocyte adenosine triphosphate content according to oocyte types and stimulation cycles (Combelles & Albertini 2003). Nevertheless, comparable implantation/resorption rates and fetal counts were observed following the mating of animals subjected to repeated COS (Combelles & Albertini 2003). Collectively, these observations suggest that, in a mouse model, while repeated COS has specific effects on follicular oocyte quality, following ovulation *in vivo*, oocytes can be rescued from consequences of repeated COS (Combelles & Albertini 2003).

Medication used to link stimulations

No meta-analyses, RCTs or prospective studies were identified in neither conventional IVF nor IVF with PGT. There is one retrospective study performed to establish which medication is correlated with better outcomes in two subsequent stimulation cycles in patients undergoing embryo banking. The natural option would be to start a new cycle with a spontaneous menses (SM). A constrain associated with SM is its spontaneity, without possible previous scheduling. Furthermore, in many cases, the final number of euploid embryos is not yet known and counseling of that specific couple could be inappropriate (Rodriguez-Purata *et al.* 2018). Other options to defer menses until a suboptimal number of biopsied embryos are confirmed could be the use of vaginal micronized progesterone (VMP) or OCPs (Rodriguez-Purata *et al.* 2018). After ovulation, the corpus luteum produces high levels of progesterone to maintain endometrial receptivity after fertilization has occurred. In the absence of pregnancy, the corpus luteum degenerates, causing a decrease in circulating progesterone (Jabbour *et al.* 2006) that results sloughing of the upper two-thirds of the endometrium (Maybin & Critchley 2015). Therefore, it is theorized that if high progesterone levels are continued, menses could be deferred until the complete information about the total number of biopsied embryos is obtained. Also OCPs

could be used; by prescribing combined OCPs during the luteal phase, LH and, consequently, GnRH, pulse frequency slows (Nippoldt *et al.* 1989), delaying the FSH-based follicle recruitment and consequently the start of a new cycle.

Rodriguez-Purata *et al.* (2018) compared whether SM, VMP or OCPs were associated with improved results when used to undergo a subsequent cycle. The difference in oocytes collected between first and second cycles was -0.9 in SM, -1.5 in VMP and $+0.4$ in OCPs. Although not statistically significant, more oocytes were retrieved in the second cycle when OCPs were used (9.0 ± 3.7 vs 9.4 ± 4.1), while fewer oocytes were retrieved when SM (9.4 ± 3.9 vs 8.5 ± 4.0) or VMP (9.8 ± 5.7 vs 8.2 ± 4.4) were utilized (Rodriguez-Purata *et al.* 2018). After adjusting for age, total gonadotropins used, total days of stimulation (second cycle) and treatment group in an ANCOVA model, no strategy was correlated with higher oocytes collected (power: 14.9%) or a higher difference of oocytes collected (power: 22.3%). Because of its practicality, OCP could be a more feasible option to link two cycles.

Interval to first FET

No meta-analyses, RCTs or prospective studies were identified in neither conventional IVF or IVF with PGT. Two retrospective studies have evaluated if the interval between the end of stimulation to transfer following a freeze-only cycle had an influence on clinical outcomes. Santos-Ribeiro *et al.* compared 333 FET cycles, segregated as either immediate (following the GnRH-agonist withdrawal bleeding) or delayed (by at least one menstrual cycle) transfer. Clinical PR was slightly statistically higher in the immediate FET group in the raw analysis (52.9% after immediate FET vs 41.6% after delayed-FET, $P=0.046$) (Santos-Ribeiro *et al.* 2016). After a mixed-effects multivariable regression analysis, timing of FET no longer was correlated to clinical PR (adjusted OR: 0.62, 95% CI: 0.4–1.0; predicted clinical PR of 52.5% for immediate FET vs 41.8% for delayed-FET) (Santos-Ribeiro *et al.* 2016). More recently, Ozgur *et al.* published a similar analysis but with LB as the main outcome measure. Cycles were investigated in oocyte retrieval-to-FET interval groups of 32–46, 47–61, 62–76, 77–91 and ≥ 92 days, with the 47–61-day group used as the reference group. There were no significant differences in LB rates between the groups in the overall analysis (59.7%; 57.8%, OR: 0.9 (95% CI: 0.7–1.3), $P=0.6$; 63.6%, OR: 1.2 (95% CI: 0.6–2.5), $P=0.7$; 45.7%, OR: 0.6 (95% CI: 0.3–1.2), $P=0.1$; 54.9%, OR: 0.8 (95% CI: 0.5–1.4), $P=0.5$; respectively), as well as, in sub-analyses investigating LB in terms of single blastocyst transfer, trigger-type medication, oocyte number and patient's age (Ozgur *et al.* 2018).

Table 3 Management of controlled ovarian stimulation for IVF with PGT: draft recommendations presented for consideration based on our review of evidence.

Topic	Recommendation	Grade (1)
Pretreatment with OCPs	LBR or ongoing PR is observed lower in the OCP pretreatment group The benefits of cycle scheduling with the pill outweigh the theoretical drawbacks	B
Conventional vs mild stimulation	Mild ovarian stimulation is not a strategy recommended in patients undergoing an PGT cycle	A
Conventional vs random start	Random start may be applied in situations where obtaining competent oocytes is an urgent task	C
Agonist vs antagonist	Antagonist protocol is considered the recommended protocol in patients undergoing PGT	A
Type of gonadotropin	There are no studies published in patients undergoing PGT Clinical choice of gonadotropin should depend on availability, convenience and costs	B
LH supplementation	There is insufficient evidence to make a recommendation for or against LH supplementation during COS Beneficial in two subgroups of patients: women with adequate pre-stimulation ovarian reserve parameters and an unexpected hypo-response to rFSH monotherapy women 36–39 years Studies are required in women with poor ovarian response or with advanced age	C
LH suppression by progesterone	There are no studies published in patients undergoing PGT Given that an essential requisite would be that a fresh transfer is not programmed, theoretically these protocols could be used in PGT	C
hCG vs GnRH-agonist trigger	GnRH-agonist could be useful for patients undergoing PGT Patients with hypothalamic dysfunction are not candidates for GnRH-agonist trigger	A
Dual trigger	In patients not at risk of OHSS, dual trigger could be considered a valuable option	GPP
Fresh vs FET	FET is the recommended strategy when undergoing PGT Cryopreserving all embryos allows for inclusion of all blastocysts in the cohort of embryos available for analysis	A
Embryo accumulation	Patients with more unfavorable ovarian reserve can reach final outcomes comparable to patients who obtain the required number of oocytes or embryos	GPP
Interval between stimulations	There are no studies published in patients undergoing PGT	–
Medication used to link stimulations	There are no studies published in patients undergoing PGT	–
Interval to first FET	There are no studies published in patients undergoing PGT FETs done immediately after a freeze-all protocol appear to result in pregnancy rates comparable to deferred to a later time	C
COS and aneuploidy	There is insufficient evidence to make a recommendation The absolute number of euploid embryos is more relevant than the proportion of euploid/aneuploid embryos	GPP

Vermeulen *et al.* (2017), available from: https://www.eshre.eu/~media/sitecore-files/Guidelines/Guidelines/Manual-for-guideline-development_update2017_final.pdf?la=en.

COS, controlled ovarian stimulation; FET, frozen embryo transfer; FP, fertility preservation; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; IVF, *in vitro* fertilization; LBR, live birth rate; LH, luteinizing hormone; OCP, oral contraceptive pills; PGT, preimplantation genetic testing; OHSS, ovarian hyperstimulation syndrome.

Association of ovarian stimulation and aneuploidy

COS has been proposed to influence oocyte maturation and the completion of meiosis, potentially mediating chromosomal aneuploidy and mosaicism (Verberg *et al.* 2009a). Possible mechanisms for this effect include FSH-induced chromosome dysfunction in oocytes or recruitment of poor-quality oocytes when the process of naturally selecting a dominant follicle is overridden with ovarian stimulation.

Nine studies analyzing the impact of COS on euploidy rates have been published (Baart *et al.* 2007, Verpoest *et al.* 2008, Rubio *et al.* 2010, Massie *et al.* 2011, Ata *et al.* 2012, Labarta *et al.* 2012, 2017, Barash *et al.* 2017, Sekhon *et al.* 2017). Labarta *et al.* recently maintained that aneuploidy rate did not increase with ovarian response or gonadotropin dosage (Labarta *et al.* 2017). Also, the authors observed that the number of euploid embryos was inversely related to the ovarian sensitivity index (Huber *et al.* 2013),

which correlates the amount of gonadotropins needed per oocyte obtained (Labarta *et al.* 2017). Several non-randomized studies have also determined that aneuploidy rate was not associated with the number of embryos generated (Ata *et al.* 2012, Barash *et al.* 2017, Sekhon *et al.* 2017).

Due to the fact that the number of euploid embryos available for transfer increases as the number of oocytes obtained does (it is fair to think that the number of euploid and aneuploid embryos increases in the same way as number of oocytes does), it is recommended to have in mind that the absolute number of euploid embryos at the end of a cycle seems more relevant for the prognosis of a patient than the proportion of euploid/aneuploid embryos (i.e. 33% of aneuploidy rate of a six-embryo cohort for a total of two euploid embryos would be less favorable than 40% of aneuploidy rate of a ten-embryo cohort for a total of four euploid embryos), especially considering cumulative pregnancy outcome as the optimal outcome.

Summary of findings

Up-to-date concepts decoupling ovarian and endometrial physiology have helped clinicians and embryologists in the individualization of treatment strategies in patients undergoing PGT, which has consequently developed particular characteristics (Table 3). First, it aims at obtaining a maximized number of oocytes and, consequentially, embryo development through more robust stimulation dosages. Second, it incorporates vitrification of all biopsied embryos due to the turnaround time before genetic results are received, which prevents endometrial receptivity displacement (Horcajadas *et al.* 2008). Third, it circumvents the risk of OHSS, as it exploits the use of antagonist protocol concomitant with a gonadotropin-releasing hormone (GnRH) agonist (GnRHa) trigger. And fourth, it fosters cycle segmentation by deferring embryo transfer to a subsequent menstrual cycle, which further decreases late OHSS risk, achieving an optimal embryo-endometrial synchrony. The resulting spectrum enables and promotes a COS that would otherwise be considered forceful.

To date, there are no studies evaluating the optimal number of oocytes needed to achieve one healthy LB through PGT. Hormone pretreatment with OCP facilitates IVF cycle scheduling. MOS is not a strategy recommended for patients undergoing a PGT cycle. Random-start stimulation may be applied in situations where obtaining oocytes is an urgent task. GnRH-antagonist is considered the protocol of choice in patients undergoing PGT. Clinical choice of gonadotropin should depend on availability, convenience and costs. Progesterone protocols could be used in the PGT population. GnRH-agonist as a final oocyte maturation trigger could be useful for women who for any given reason choose to avoid fresh transfers. In patients not at risk of OHSS, dual trigger could be considered a valuable option. The accumulation of oocytes/embryos could be a suitable strategy to increase the odds of success, turning low responder patients into a 'normal responder'. There are no studies published evaluating if there is an optimal interval before proceeding with a subsequent COS or the optimal medication to link stimulation cycles. The total euploid embryo cohort size is an unequivocal prognosis factor in IVF treatments, and the number of euploid embryos has to be taken into account, more importantly than the proportion of euploid/aneuploid embryos.

Declaration of interest

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