Controversies in IVM

Shauna Leigh Reinblatt · Weon-Young Son ·
Einat Shalom-Paz · Hananel Holzer

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Abstract

Purpose To explore four areas of controversy: the benefits of gonadotropin priming, benefits and timing of hCG trigger as well as the ideal protocols for endometrial preparation and luteal support.

Methods A literature review was performed to explore the current evidence

Results Current evidence suggests that Gonadotropin priming in combination with hCG prior to collection benefits patients with normal ovaries. In PCOS patients 10,000 IU hCG 38 h before retrieval increases the total number and rate of oocyte maturation. Gonadotropin priming may also benefit PCOS patients. The ideal timing of hCG trigger appears to be when the leading follicle is 10–12 mm. Sparse data exists regarding luteal support protocols.

Conclusions There is still a need for well-designed studies to establish ideal methods for oocyte priming, timing of retrieval, endometrial preparation and luteal support. Further studies must incorporate both clinical and basic science principles of ovarian, follicular and endometrial physiology.

Keywords In-vitro maturation · Priming · PCOS · Unstimulated

In-vitro maturation (IVM) has achieved significant advances from its initial description by Pincus [1] and Edwards [2] to

Capsule This review will explore the benefits of gonadotropin priming, benefits and timing of hCG trigger and the ideal protocols for endometrial preparation and luteal support.

S. L. Reinblatt (✉) · W.-Y. Son · E. Shalom-Paz · H. Holzer
MUHC Reproductive Center, Department of Obstetrics and
Gynecology, Royal Victoria Hospital,
Montreal, Quebec, Canada H3A 1A1
e-mail: shauna.reinblatt@muhc.mcgill.ca

its current widespread clinical applications. Despite these advances, however, there still exist many controversial issues surrounding this treatment, many of which will be covered elsewhere in this issue. This review will focus on the following issues: FSH/hMG priming, hCG priming, timing of collection, and finally, endometrial priming and luteal support.

Gonadotropin priming: results in PCOS and regularly-cycling patients

In ovulatory women, the rationale behind FSH priming is to favour more follicles to attain ovulatory potential as well as oocyte maturation [3, 4]. In some patients with polycystic ovarian syndrome (PCOS), oocytes do not develop ovulatory capacity. Others might not attain an adequately thick endometrial lining. In both scenarios, such patients may benefit from gonadotropin “priming” in the mid-to-late follicular stage [5]. Despite these different mechanisms, results from studies of gonadotropin priming in IVM have thus far been conflicting.

FSH priming in PCOS patients

Mikkelsen et al. studied the impact of FSH priming during the early follicular phase on IVM outcomes in PCOS patients and found improved maturation, implantation and pregnancy rates [5]. A benefit was also reported by Suikkari et al. in 2000 [6], although they began the FSH in the luteal phase of the previous cycle. In their retrospective study of PCO patients, Elizur et al. found more MII-stage oocytes at retrieval and higher maturation rates by 24 h post-collection in patients who received hMG

150 IU daily until their linings had reached 8 mm or a dominant follicle developed [7].

Despite these more recent findings of a benefit, Trounson et al. initially found no improvement of FSH priming on oocyte developmental competence [8]. This is in keeping with a study by Lin et al. in 2003. They randomized 60 women with PCOS to receive either recombinant FSH for 6 days prior to hCG trigger or no priming prior to hCG. All patients had documented oligo- or amenorrhea. They did not find a significant difference between the two groups in terms of maturation, fertilization or pregnancy rates. They also found similar endometrial thickness on the day of retrieval. For these reasons, they concluded that FSH priming was not beneficial in PCOS patients [9].

FSH priming in women with regular cycles

Given the reported benefits in PCOS patients, some investigators hypothesized that gonadotropin priming might also enhance maturation, fertilization and embryo development rates in women with regular cycles. In 1998, Wynne et al. showed that a 5 day course in normo-ovulatory patients yielded improved IVM rates [10]. However, there were no embryo transfers in their study. In a randomized study by Mikkelsen et al. in 1999, ten patients received 3 days of 150 IU FSH from days 3 to 5, while ten others received no exogenous FSH. There was no difference in maturation, fertilization, cleavage or embryo development between the two groups [11].

Fadini et al. then designed a prospective, randomized trial to address this issue. Patients were randomized to one of four groups; no priming, hCG alone, FSH alone or a combination of HCG and FSH priming. FSH was given from days 3 to 6 at a dose of 150 IU/day and 10,000 IU hCG was used to trigger oocyte maturation. The group that received both FSH priming and hCG trigger showed significantly improved oocyte maturation, implantation and pregnancy rates [12]. These findings suggest that FSH and hCG might work in concert to affect oocyte maturation and fertilization potential. Clearly, more randomized studies are required to determine the benefits of gonadotropin priming in various patient populations.

HCG trigger: the rationale for a beneficial effect

Given that retrieval of matured oocytes in in-vitro fertilization (IVF) takes place prior to the endogenous LH surge, hCG has been used to trigger maturation. Through its interaction with the LH receptor [13], hCG is known to trigger GV-breakdown, the resumption of meiosis and oocyte maturation [4]. This process has been demonstrated to occur even in smaller, non-dominant follicles [14].

For these reasons, the same effect was proposed for IVM cycles. hCG trigger was shown to favour dispersion of cumulus cells surrounding immature oocytes and these oocytes showed greater LH receptor expression, maturation rates and blastocyst development potential [15, 16]. Although the exact mechanism is not established, hCG promotes maturation in oocytes >10 mm and enhances smaller germinal vesicle stage oocytes to acquire maturation and developmental competence in vivo [15]. In 2010, Son et al. reported that hCG induces a dispersed cumulus pattern, faster maturation rates in-vitro, as well as better embryonic developmental potential when hCG is used [17].

In 2000, Chian et al. conducted a randomized, controlled trial in which they demonstrated significantly improved maturation rates when 10,000 IU hCG “priming” was administered 36 h prior to oocyte retrieval. They also reported earlier maturation in the primed group, but were unable to demonstrate higher pregnancy rates [18]. In 2001, Child et al. reported better clinical pregnancy rates in PCOS patients triggered with hCG [19] compared to rates reported previously by Cha et al., although that study included both intrauterine and intrafallopian embryo transfers [20].

In 2006, Son et al. found more MII-stage oocytes at the time of collection, faster maturation of immature oocytes, as well as faster blastocyst development in hCG-primed cycles [14]. In 2008, we found that MII stage oocytes retrieved at retrieval produced better quality embryos than from oocytes matured in vitro. Cycles in which MII stage oocytes were retrieved led to better pregnancy rates (40% vs 23%) and implantation rates (12% vs 8%) than in cycles where no in-vivo matured oocytes were collected [21]. These results were corroborated by other groups as well [22, 23].

On the other hand, others have reported good pregnancy rates without giving hCG. In a 2001 study, Mikkelsen et al. did not give their patients hCG. However, their patients *were* given FSH, which may have also induced oocyte maturation [5]. In Zhao et al.’s 2009 retrospective study of 118 PCOS patients, they showed a 40% clinical pregnancy rate and a 15% implantation rate in patients who did not receive hCG or gonadotropin priming. Interestingly, they only retrieved oocytes smaller than 10 mm and cancelled cycles in which a dominant follicle had reached >10 mm [24].

In women with normal ovaries and regular cycles, Soderstrom-Antilla et al. performed IVM in 191 patients without hCG priming and reported clinical pregnancy rates of almost 20% [25]. In the previously-mentioned study by Fadini et al., the group that received hCG alone had the lowest pregnancy rates of all groups in their study [12]. In summary, embryological data supports a benefit from hCG trigger in patients with PCO or PCOS, whereas those with regular cycles and normal ovaries probably also require FSH.

Timing of collection: at what follicle size?

Timing of IVM collections is generally adjusted according to the size of the leading follicle, if one has developed as well as adequate endometrial thickness. Ideally, this allows development of at least one MII-stage oocyte, but before the beginning of apoptosis in sibling oocytes. Some authors believe that sibling oocytes have good developmental competence despite the presence of a leading follicle and these smaller oocytes could therefore contribute to overall pregnancy rates. In such protocols, often referred to as “natural cycle” IVF, the oocyte collected from the leading follicle at 15–18 mm is fertilized immediately and the remaining sibling oocytes are cultured and matured in the lab [26–28]. Unfortunately, none of these studies clearly traced the immature oocytes through their development into embryos to study subsequent implantation rates.

Other authors believe that once a dominant follicle is selected, the sibling oocytes have lower developmental competence. In fact, animal studies have clearly shown that the presence of a dominant follicle has a detrimental effect on surrounding oocytes [29]. Clearly, some controversy still exists as to the optimal time to perform IVM collections.

In non-triggered cycles

In 1999, Cobo et al. reported higher blastocyst rate when oocytes were collected prior to reaching 10 mm and actually cancelled cycles in which a leading follicle was found. Unfortunately, they did not transfer the embryos and therefore an impact on pregnancy rates cannot be deduced [30]. On the other hand, Mikkelsen et al. did not find any difference in pregnancy rates when the leading follicle was >12 mm at collection as compared to cycles where all follicles were <12 mm [31]. Russell et al. reported better outcomes when oocyte collection occurred before the leading follicle measured over 13 mm [32]. Fadini et al. corroborated that the presence of a follicle >13 mm was associated with lower pregnancy rates and recommended collection above 11 mm [33]. Given these results, if hCG trigger is not used, the retrieval should be scheduled as soon as the leading follicle measures >10 mm.

In hCG-triggered cycles

We undertook a study at our centre to compare pregnancy rates in three groups: patients triggered when the leading follicle was below 10 mm, those triggered between 10 and 14 mm, and those triggered above 14 mm. Despite similar maturation, fertilization and embryo development rates in all three groups, higher clinical pregnancy and implantation

rates were seen in the group of patients triggered when their dominant follicle was between 10 and 14 mm at collection as compared to groups with follicles <10 mm or > 14 mm size. In fact, 90% of patients had MII stage oocytes collected when their largest follicle had reached 12 mm. In the group with a leading follicle >14 mm, only one pregnancy was achieved from IVM embryos [15, 21].

We now administer hCG trigger once a leading follicle of 10–12 mm has developed. Randomized studies are needed to clearly delineate the optimal timing of IVM collections and to better understand the impact of the dominant follicle on its sibling oocytes.

Interval between hCG trigger and oocyte retrieval

In terms of timing between hCG trigger and oocyte collection, early studies of IVM applied the same principles as for IVF and used 36 h. Later, it was postulated that allowing more time post-hCG might increase the number of mature oocytes. In a retrospective study of 120 IVM cycles in 113 PCOS patients, clinical outcomes were compared between waiting 35 versus 38 h prior to retrieval. The group with the prolonged interval post-hCG produced a higher number of in-vivo matured oocytes, more had expanded cumulus cells, and higher in vitro maturation rates. These factors lead to a higher number of good quality embryos available for transfer. However, the fertilization, cleavage and percentage of good quality embryos were comparable between the two groups [34]. We currently use an interval of 38 h after hCG trigger to schedule retrievals. Further studies are required to determine if the interval between hCG trigger and collection has an effect on oocyte maturation in-vivo.

Does a dose–response relationship exist for HCG priming?

Given that doses are often adjusted in conventional IVF cycles for patients at risk for OHSS [35], Guleki et al. questioned whether higher doses of hCG would improve MII-oocyte yield. They carried out a RCT whereby patients were randomized to receive the usual 10,000 IU or a higher dose of 20,000 IU. They did not find any difference in maturation, fertilization, pregnancy or implantation rates [36]. However, oocytes were immediately cultured after retrieval instead of being assessed for maturity. We currently assess oocytes prior to culture because oocytes from small follicles matured in-vivo were found to yield better pregnancy rates than those matured in-vitro [21]. Further study regarding this issue requires a randomized

trial of various doses and assessing and inseminating oocytes on the day of retrieval.

Endometrial preparation and luteal support

In IVM, estradiol levels are physiological and retrieval occurs before the lining has been fully estrogenized. Once follicles are aspirated, there is insufficient progesterone support from the corpus luteum for endometrial receptivity. This lack of synchrony might explain the lower implantation rates seen in IVM [37, 38]. Oocyte recipients from donors undergoing IVM demonstrated higher pregnancy and implantation rates than PCOS patients undergoing autologous IVM, suggesting that endometrial preparation is a significant predictive factor of successful outcomes [39].

It has also been shown that endometrial thickness is an important predictor of implantation [40], which is especially relevant to anovulatory patients with thin linings at the time of collection. IVF studies have reported better implantation rates when endometrial thickness was >7 mm [40] and this is also presumed to be the case in IVM. In fact, some authors recommend embryo cryopreservation if endometrial thickness <7 mm on day of transfer [23].

The protocol for endometrial preparation used currently is similar to that originally described by Trounson et al. [41]. Various doses of estrogen, ranging from 4 to 8 mg daily, are given prior to collection in order to thicken the lining. Progesterone is added either immediately or shortly following the retrieval. With the knowledge that endometrial thickness affects pregnancy rates and that the exact forms and doses were not well established, Elizur et al. questioned the optimal endometrial preparation method. They retrospectively compared low-dose hMG to micronized estrogen to thicken the lining in IVM cycles when lining was below 6 mm on day 6–10. The two medications produced similar thickening the lining but hMG was associated with higher numbers of in-vivo matured oocytes found at collection and higher maturation rates at 24 h. Despite a trend towards higher implantation and pregnancy rates, the difference was not statistically significant [7]. These results must therefore be corroborated through a prospective, randomized trial.

Studies of oocyte recipients have shown that at least 6 days of estrogen stimulation is required for endometrial receptivity [42]. Russell et al. showed improved maturation rates and embryo development in patients treated with estrogen beginning mid-follicular versus when estrogen was initiated early in the follicular phase [43]. Other protocols start estrogen supplementation either on the day of collection or beginning just prior to collection. This best mimics the natural estrogen rise from the dominant follicle

in natural cycles. In terms of progesterone, supplementation is generally begun on the day of collection, as this is when progesterone rises post- LH surge in natural cycles. The optimal regimen and specific doses have not yet been studied in randomized trials. In addition, it is possible that endometrial receptivity differs between PCOS and regularly ovulating women.

The McGill “flexible” IVM protocol

At our centre, patients have an ultrasound on day 2 or 3 of either a regular cycle or a Provera-induced withdrawal bleed. Once follicles are counted and measured, a repeat ultrasound is scheduled for day 6–12, depending on the patient’s cycles. Once the leading follicle reaches 10–12 mm and her lining is >6 mm, hCG trigger is given 38 h prior to oocyte retrieval.

If, on the second ultrasound, no follicle has developed above 8–9 mm or the lining does not exceed 6 mm, 150 IU hMG is prescribed for 3 days and the ultrasound is repeated. In rare cases, additional gonadotropins are given prior to reaching criteria for oocyte collection. The need for hMG is assessed on a case-by-case basis, taking into consideration each patient’s ovulatory status, previous cycle outcomes and the above criteria. For this reason, we refer to our protocol as the “flexible” IVM protocol.

We have recently reported that the addition of gonadotropins leads to significantly improved endometrial thickness (7.9 mm versus 7.1 mm in “pure” IVM cycles, $p0.008$), a trend towards increased implantation rates (20% versus 14% in “pure” IVM cases, $p0.07$) as well as significantly increased clinical pregnancy rates (66% versus 48% in “pure” IVM, $p0.05$) (Unpublished data).

Summary points

1. Giving FSH or hMG alone has not shown any benefit in patients with regular cycles but might have some benefit in those with PCOS.
2. Using hCG alone to trigger maturation is beneficial and common practice in PCOS patients. This might not be the case in normo-ovulatory patients.
3. Adding several days of gonadotropins in combination with hCG trigger is beneficial in patients with regular cycles and probably in PCOS patients as well. It improves oocyte maturation, as well as endometrial receptivity.
4. Timing the collection should proceed as follows: If hCG is used, it should be given once the leading follicle measures 12 mm and the collection should be performed before this follicle reaches 14 mm.

5. The optimal time interval between the administration of hCG trigger and oocyte retrieval has not been established in a randomized trial but it appears that 38 h maximizes the chance to retrieve a mature follicle.
6. In terms of endometrial thickness and luteal support, it is generally accepted that thicker linings lead to improved implantation rates. However, the optimal dosing regimen is still unknown as data is sparse.
7. In order to clarify these and other important controversies in IVM, further prospective studies must incorporate both clinical and basic science principles of ovarian, follicular and endometrial physiology.

Conflict of interest None

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