REVIEW ARTICLE

# Pharmacological Options to Trigger Final Oocyte Maturation in *In Vitro* Fertilization

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## Abstract

Human chorionic gonadotropin (hCG) has been the gold standard in the induction of final oocyte maturation since the pioneer days of *in vitro* fertilization (IVF). But owing to its long half-life, it leads to increased risk of ovarian hyperstimulation syndrome (OHSS). Trigger of GnRH agonist is a more physiological trigger, effective, and safe, as it significantly reduces or eliminates risk of OHSS. Newer options of dual trigger and double trigger are discussed in this review, which can be used as modified luteal phase support in patients with expected suboptimal oocyte maturation. Newer pharmaceutical options such as Kissepeptins, key in central regulation of neuroendocrine system, and GnRH release need more studies before being implemented in general practice. This review includes details of various trigger options for final oocyte maturation and about combination of trigger options aiming safe and effective outcomes.

**Keywords:** Dual trigger, GnRH agonist, Human chorionic gonadotropin trigger, Kisspeptins, Ovarian hyperstimulation syndrome.

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## Introduction

One in six couples are affected by infertility, and it has been recognized as the fifth serious global disability by the World Health Organization (WHO).[1] *In vitro* fertilization (IVF) is the process in which controlled ovarian stimulation is done with gonadotropins in their pharmacological dose, while premature luteal hormone (LH) surge is prevented by either GnRH agonist or antagonist protocol. Since many years, human chorionic gonadotropin (hCG) has been used to induce final maturation and ovulation as surrogate to endogenous LH.[2] Ovum pickup to retrieve oocytes is timed after adequate LH exposure but prior to ovulation. After adequate LH exposure, there occurs initiation of resumption of meiosis oocyte maturation and attainment of fertilization competence. Under LH exposure, corpus luteum is formed by the remaining part of the follicle, which releases sex steroids, mainly progesterone, pivotal in preparing endometrium for implantation.[3] Various triggers available for inducing oocyte maturation are hCG, GnRH agonist, recombinant LH, and recently Kissepeptins.

Human chorionic gonadotropin trigger, owing to its long half-life and its luteotropic action, increases the risk of ovarian hyper stimulation syndrome (OHSS). GnRH agonist, although a physiological trigger causing surge of both LH and follicle-stimulating hormone (FSH) and safe in polycystic ovarian syndrome (PCOS) patients due to short LH surge, has inadequate luteal phase due to luteolysis of follicles, resulting in decrease in pregnancy rates. GnRH agonist trigger being safe, effective, and physiologic has shifted the IVF practice. Agent for oocyte maturation should be safe and efficacious in terms in pregnancy rates. Thus, the recently concept of dual trigger and double trigger has been introduced combining hCG and GnRH agonist. Individualization of choosing single agent or combination trigger will optimize safety and results of IVF.

### Physiology of Oocyte Maturation

- Natural cycle LH surge leads to meiotic maturation, disruption of cumulus oocyte complex and ovulation, luteinization of granulosa cells, and formation of corpus luteum that releases sex steroids for luteal phase support.
- Human oocytes are arrested at the prophase of the first meiotic division until the preovulatory LH surge, and oocyte maturation is a critical process to the success of IVF treatment, during which the oocyte gains competence for fertilization
- Oocyte maturation is initiated by LH-like exposure that can be provided by hCG, GnRH agonist, recombinant LH, or kisspeptin.
- Oocyte maturation is combination of nuclear and cytoplasmic maturation.
- Nuclear maturation (chromosomal segregation) is maturation of oocyte from metaphase I (immature stage) to metaphase II (mature stage), along with extrusion of first polar body, due to fall in intra-oocyte cyclic adenosine monophosphate (cAMP) concentration under LH exposure.[3] The diploid cell transitions

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to haploid gamete and attains competence for fertilization by the haploid spermatozoa.
- Cytoplasmic maturation is associated with changes in dynamics of cytoskeleton, organelle redistribution, calcium release activity, and storage of mRNA, transcription factor and proteins. This creates asymmetry enabling polar body extrusion with minimal loss of cytoplasm.[4]
- The resumption of meiosis is signaled by germinal vesicle breakdown (GVBD), and the interval between LH receptor activation and first stage of meiosis is 18 hours[5]
- Total duration of nuclear maturation including time to GVBD is estimated to be 20 to 22 hours[6]

## Options Available to Trigger Final Oocyte Maturation

Options are shown in Flowchart 1 below.

### *Description of Various Trigger Options*

*hCG trigger:* It has been pioneer for inducing final oocyte maturation, luteinization of granulosa cells, resumption of meiosis since decades, and as surrogate for natural midcycle LH surge.

- hCG binds to same receptor due to biological and structural similarity to natural LH (share same alpha subunit and 85% of amino acid structure of beta subunit)[7] and can activate the same receptor to mimic action of natural LH surge.
- It allows progesterone secretion by promoting maintenance of corpus luteum.
- It may be linked in peri-trophoblastic immune tolerance development, thus facilitating trophoblastic invasion, expediting fetal development in the endometrium.[8]

### *Difference between hCG and LH*

The main difference is the half-life, approximately 60 minutes[9] for LH, while hCG has long half-life >24 hours.[10] Human chorionic gonadotropin can lead to life-threatening condition such as OHSS. Activation of LH receptor by hCG and LH is not equivalent due to difference in intracellular signaling and kinetics. LH has more effect on antiapoptotic extracellular proliferative signaling, related kinase ½, and AKT, while hCG has more cAMP and steriodogenic action.[11]

### *Dose of hCG Trigger*

5,000–10,000 units of urinary hCG (intramuscular) or recombinant hCG 250–500 µg (subcutaneously).

Duration of hCG surge is dose dependent[12] (0 hours for 100 IU, 24 hours for 300 IU, and 48 hours for 1000 IU), although lower doses can induce oocyte maturation, granulosa cell luteinization but are insufficient in ensuring optimal cytoplasmic maturation and adequate corpus luteum function. Thus, higher dose of hCG can influence duration at which hCG levels can be maintained above threshold and amplitude of hCG level can be attained.

5,000 IU as the minimum effective dose of u-hCG and oocyte yield [(number of oocytes divided by number of follicles 14 mm)] did not increase in doses >5,000 IU.[13]

Dose less than 2,000 IU of urinary hCG has less probability of retrieving oocytes and has increased chance of cycle cancelation, failed or low fertilization, or no embryos available to transfer.[14] Higher doses of hCG can be beneficial in patients with higher body mass index (BMI) for efficacious triggering.[15]

Systematic review concluded that the clinical outcomes were similar between women receiving 5,000 or 10,000 IU of u-hCG. The incidence of OHSS was not reduced in the high-risk population even with lower dose of u-hCG. The dose of u-hCG for final oocyte maturation in women referred for IVF needs to be individualized.[16]

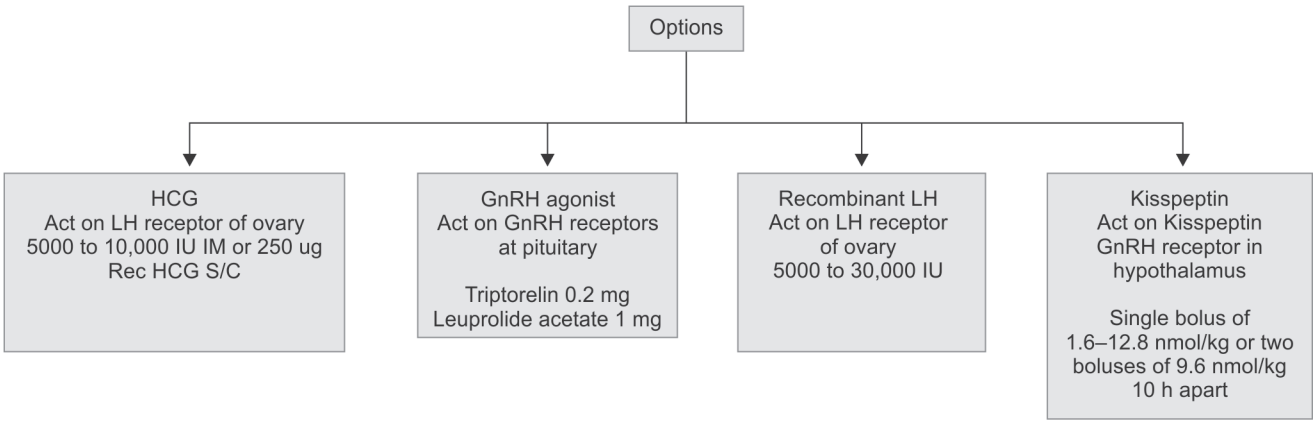
### *Difference between uhCG and rhCG*

250 µg of rec hCG is equivalent to 10,000 IU of hCG

- Urinary hCG has more impurities, batch to batch variability, and risk of leading to immunological reaction.
- Rec hCG is filled by mass, offer better dose precision and low batch to batch variability, contamination free, allows subcutaneous administration, and is better tolerated by patients.

Driscoll et al. in phase III double-blind randomized trial found no difference in number of oocytes retrieved: >10 mm per follicle on day of trigger, number of mature oocytes, number of cleaved embryos in rhCG verses uhCG groups.[17] A larger randomized controlled study done on 297 patients found no difference in number of oocytes following 10,000 of uhCG, 250 µg of rhCG, or 500 µg rhCG. The authors found two more zygotes/cleaved embryos in the 500-µg rhCG group than the 250-µg rhCG with increased risk of OHSS (9% vs 3%] in the former. The authors thus recommended 250 µg rhCG for clinical use due to convenience and lower risk of OHSS.[18] Cochrane review which included 18 studies in 2,952 women undergoing IVF or intracytoplasmic sperm injection found no difference between rhCG and uhCG or between RhLH and uhCG in rates of live birth/ongoing pregnancy or OHSS.[19]

**Flowchart 1:** Various options to trigger oocyte maturation



OHSS risk is high with hCG trigger due to:

- Long half-life
- Luteotropic action
- Due to its direct action on stimulated follicles, it causes release of vasoactive compounds mainly vascular endothelial growth factor (VEGF). Angiongenic factors lead to third space accumulation and depletion of intravascular volume. Fluid shift to interstitial spaces causes pleural effusion, ascites, hypotension, oliguria, thromboembolism. and organ failure[20]

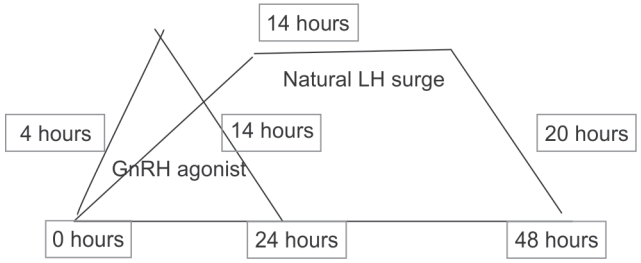
*GnRH agonist trigger:* Nakano et al. first described it as option to induce final maturation.[21] With introduction of GnRH antagonist protocols, GnRH agonist was considered an option due to fact that antagonist has short duration and rapid irreversible action, thus allowing receptor to be active to GnRH agonist single bolus action

*GnRH surge vs natural LH surge:* GnRH agonist surge lasts for 24–36 hours and comprises only two phases of short ascending limb (lasting 4 hours) and long descending limb (lasting 20 hours)[22] in contrast to natural midcycle LH surge which lasts for 48 hours and consists three phases.[23] This difference has striking role in reduction of risk of OHSS, owing to shorter duration of endogenous LH surge[24] (Flowchart 2).

### *Advantages of GnRH Agonist Trigger*

- GnRH agonist is considered to be a physiological trigger, as it induces release of both FSH and LH after displacing antagonist and activating the receptor. This flare effect is comparable to natural midcycle surge of gonadotropins.[21]
- Simultaneous FSH surge promotes LH receptor formation on granulosa cells, cumulus expansion, and nuclear maturation, and synergist action with LH promotes final oocyte maturation and provides optimal environment.[25]
- It eliminates severe OHSS in combination owing to rapid and reversible luteolysis and inhibits secretion of vasoactive compounds mainly VEGF from the corpus luteum. Largest randomized trial in population at risk of OHSS (follicles 15–25) found no case of OHSS despite low-dose hCG rescue, followed by doing fresh embryo transfer and showed comparable reproductive outcome to hCG.[26]
- It is preferred in PCOS patients (follicles >25) to eliminate risk of OHSS: Segmented IVF (cryopreservation of embryos and transferring on later date).
- Fertility preservation: There is need to avoid high estradiol levels in these patients, and thus GnRH agonist with antagonist protocol is preferred. GnRH eliminates OHSS, gives more metaphase II oocytes, and thus more embryos. It can be used in random start protocols reducing the waiting period, as pituitary is capable to respond sufficiently in luteal phase.
- It is preferred in oocyte donors for final oocyte maturation and results in retrieval of oocytes of similar quality to hCG and hence gives similar results in recipient, eliminating the risk of severe OHSS.[27] It simplifies egg donor program for clinician, as it eliminates the need for estradiol monitoring during stimulation, and post-OPU decreases the burden of follow-up owing to short luteal phase (4–6 days), reduced ovarian volume, and much diminished abdominal distension in donors.

**Flowchart 2:** Difference between GnRH and natural LH surge




### *Dose for Final Oocyte Maturation*

Inj Triptorelin 0.2 mg, Inj leuprolide acetate 1 mg, Buserelin nasal spray 500 µg.

Parneix et al., in 231 women, compared different ovulation induction agents, including triptorelin, nafarelin, leuprolide, or hCG and concluded that all regimes lead to ovulation with no superiority of one analog to other in terms and gave similar pregnancy rates between test groups and the control hCG group. There is insufficient evidence in the literature to support any preference of one GnRH agonist agent over another, although the dosing may vary.[28]

*Luteal phase after GnRH agonist trigger:* Through pituitary downregulation, GnRH agonist reduces the levels of LH, which is inadequate to support ongoing function of corpus luteum, thus leading to fall in serum progesterone and suboptimal implantation. Thus, duration of LH surge after agonist trigger is sufficient to induce oocyte maturation but not sufficient to maintain adequate corpus luteum.[29] Thus, despite supplementation of adequate luteal phase support, there is reduction in pregnancy rates and increase in pregnancy loss due to luteal phase insufficiency.

Modified luteal phase support options with GnRH agonist trigger are:

- Low-dose hCG at time of oocyte retrieval: Single bolus of 1,500 IU of hCG along with standard luteal support.[30]
- Dual trigger: Low-dose hCG with GnRH agonist trigger, which rescues corpora lutea by giving additional signal required for luteinization.
- Very low doses of hCG: Recombinant hCG (125 IU) given daily starting during the stimulation cycle.[31]
- Intensive luteal support: To start with progesterone injection 50 mg daily with three 0.1 mg transdermal E2 patches, replaced every other day from day of oocyte retrieval. Serum levels closely monitored and dose adjustment done to maintain serum levels of progesterone >20 ng/mL and serum estradiol >200 pg/mL.[32]
- Recombinant LH: Given as 300 IU, in six alternate doses starting from day of OPU along with 600 mg daily vaginal progesterone.[33]
- Fixed low-dose hCG: Three fixed doses of 500 IU administered every third day along with progesterone supplementation starting form day of OPU.[34]

### *Recombinant LH*

Given in dose of 5,000 to 30,000 IU subcutaneously.

It is as effective as 5,000 IU hCG and more safe, as risk of OHSS is nil. Multicentric double-blind trial showed single dose of rh-LH is effective in inducing final follicular maturation and early luteinization in IVF and embryo transfer patients and is comparable to 5,000 IU u-hCG, with significant reduction in OHSS. The dose

of rh-LH giving the highest efficacy to safety ratio was between 15,000 and 30,000 IU.[35]

It is not routinely used as it is expensive and cumbersome in administration. Data on rise of LH in first 24 hours are not clear, and thus timing of oocyte retrieval may not be optimal and offers no additional advantage over hCG.

*Kisspeptins:* They are recently discovered peptide hormones that play a role in regulation of neuro-endocrinology of human reproduction. Kisspeptins are potent stimulators of the hypothalamic–pituitary–gonadal axis.[36] They signal directly to GnRH neurons, leading to release of GnRH in the portal circulation, which in turn leads secretion of both FSH and LH from the anterior pituitary.[37]

Preliminary studies are in favor of using Kisspeptins in practice for final oocyte maturation. Abbara at al. concluded that kisspeptins can be used as natural pharmacologic option for induction of ovulation due its effectiveness in eliciting LH surge for final oocyte maturation, with successful live births and importantly eliminating risk of OHSS.[38]

*Dosing:* Kisspeptin-54 in single bolus of 1.6 to 12.8 nmol/kg or two boluses of 9.6 nmol/kg 10 hour apart (16–18).[39]

### *Criteria to be Fulfilled for Giving Ovulation Trigger*

Trigger is usually given when two or three follicles are >17 mm on ultrasound monitoring and/or serum estradiol in range of 1,500 to 3,000 pg/mL or 100 to 200 pg/mL/follicle

Follicles in range from 16 to 22 mm give maximum oocyte yield.[40] The size of follicles at time of trigger influences likelihood of getting mature oocytes as diameter of the leading follicle provides representation of other follicles when they are growing in a tight cohort.

### *Timing of Ovum Pickup in Terms of Trigger*

Ovum pickup is timed 35 hours after hCG trigger, as in some women ovulation was seen at 36 hours following administration of intramuscular hCG.[41]

A total of 533 patients undergoing IVF with long protocol, with interval ranging from 33 to 41 hours between uhCG and ovum pickup, were studied. No significant differences were observed in the ratio of oocytes retrieved divided by the number of follicles punctured in the interval groups (63.8%: 33–<36 hours, 60.9%: 36–<38 hours, 62.5%: 38–<41 hours). The author did not find significant differences in the number of oocytes retrieved, fertilization rates, or clinical pregnancy rates.[42] In stimulated cycles, there is evidence to suggest that extending the interval between hCG administration and oocyte retrieval beyond the standard 41 hours is unlikely to lead to the frequent occurrence of premature ovulation;[42] however, there is insufficient evidence to suggest if outcome will improve with longer intervals between ovum pickup and trigger.[43] Option of double trigger explained subsequently highlights on flexibility of time between ovum pickup and trigger.

### *Empty Follicle Syndrome*

This is frustrating to patients and clinicians and is characterized by lack of oocytes retrieved despite adequate ovarian response on ovarian stimulation and meticulous aspiration of follicles. It is classified as genuine and false empty follicle syndrome. Etiology can be uncertain in most cases after hCG could be due to human error in administration or pharmaceutical error. Although in one study incidence of EFS after hCG and GnRH agonist trigger (3.1% vs 3.5%, respectively) was reported to be similar, but there is difference in pathology due to difference in their site of action.[44]

Cases of EFS after hCG trigger in antagonist cycles are solved by changing to GnRH agonist, which being more physiological causes release of both FSH and LH promoting adequate follicular maturation and thus preventing EFS.[45]

### *Suboptimal Response to GnRH Agonist Trigger*

In small subset of patients, there can be suboptimal response with inadequate oocyte yield following GnRH agonist trigger. This suboptimal response can range from much fewer oocytes than expected to empty follicle syndrome.

In PCOS patients, suboptimal response can be seen due to self-priming of gonadotroph cells in pituitary by high E2 and suboptimal delivery of gonadotropins due to inadequate neovascularization owing to more number to intermediate follicles. Thus, post-GnRH agonist trigger, the resultant endogenous FSH and LH could be inadequate. Higher number of mature oocytes and higher pregnancy rates are seen after a repeat dose of GnRHa trigger 12-hour following the first dose due to maintenance of sustained level of gonadotropins.[46]

Poor outcome or canceled retrieval was seen in patients with serum LH <15 IU, 12 hours post-agonist trigger. Overall reported incidence of suboptimal response to GnRH-a trigger was 5.2%.[47] Various risk factors found were baseline LH <0.1, serum LH on day of trigger <0.5, long-term use of oral contraceptives, and low BMI hypothalamic dysfunction. In all, 25% chance of suboptimal response was seen when serum LH was undetectable on the day of trigger. It is recommended to individualized final oocyte maturation in patients with risk factors for having suboptimal response to GnRH a trigger.[47] Role of dual trigger and double trigger that provide more sustained support of corpus luteum is explained further in the review. Human chorionic gonadotropin component in combination trigger acts as second rescue in such event of suboptimal response to GnRH agonist trigger.

### *Dual Trigger*

Dual trigger was first defined as the concept of a combination of GnRH agonist and a low-dose hCG (1,000 to 2,500 IU) in triggering final oocyte maturation[48] in high responder patients. There was absence of OHSS and acceptable fertilization, implantation, clinical and ongoing pregnancy rates, and early pregnancy loss.[48]Role and application of dual trigger were further explored in various studies. Ding et al. conducted a meta-analysis including 527 women from four eligible RCTs to investigate the efficacy of the dual trigger in comparison to hCG alone (Schachter et al.[49] in 2008, Decleer et al.[50] in 2014, Kim et al.[51] in 2014, Mahajan et al.[52] in 2016). The analysis did not demonstrate any difference in the number of oocytes, mature oocytes, zygotes, or implantation rate, although it did find an increase in the pregnancy rate in the GnRHa-supplemented group when compared to hCG alone (relative risk, 1.55; 95% CI, 1.17–2.06).[53] In summary, additional FSH exposure is suggested to enhance oocyte maturation, although LH/hCG plays a dominant role and the additional impact of FSH is likely to be small.

Advantages of dual trigger:

- GnRH component is more physiological and causes release of both endogenous FSH and LH and increases the number of mature oocytes retrieved, resulting in optimization of live births.

- hCG component supports corpus luteum function and supports implantation.
- Potential enhancement of endometrial receptivity by the GnRH-a component.
- Dual combined trigger leads to higher levels amphiregulin and epiregulin (ligands of epidermal growth factor), which plays important role in cumulus expansion,[54] oocytes maturation,[55] and thus resumption of meiosis.

Large prospective trials are needed to exactly evaluate the role of dual trigger before routine implementation in practice in different groups of patients undergoing IVF.

*Double Trigger*

The concept of the "double trigger" represents a combination of a GnRH agonist and a standard hCG, when used 40 and 34 hours prior to ovum pickup, respectively.[56]

Double trigger prolongs the time between ovulation trigger and ovum pickup, aiming to overcome any existing granulosa cell dysfunction, facilitating cumulus cell expansion and meiotic maturation and thus results in retrieving more mature oocytes and plays role in patients with abnormal follicular maturation despite adequate response to COH.

In patients with <50% number of oocytes retrieved per number of dominant follicles on day of trigger, double trigger has led to significant increase in number of oocytes retrieved and number of high-quality embryos when compared to hCG alone.[57]Double trigger has been offered in the treatment of patients with immature oocyte syndrome where less than 25% of the expected oocytes are retrieved or with poor/low oocytes yield and empty follicle syndrome.[58]

*Indications of Dual Trigger and Double Trigger*

- Previous history of >25% immature oocytes retrieved
- Empty follicle syndrome
- To prevent OHSS in PCOS patients and get adequate luteal phase support in PCOS patients
- Poor responders

### Individualization of Combination Trigger

- Normal responder: Combination of GnRH agonist with standard hCG bolus (5,000–10,000 IU) administered together and OPU done at 35–37 hours: significant increase in mature oocytes, number of good-quality embryos for transfer, and cryopreservation.[59] It is not recommended if fresh embryo transfer is planned.
- Poor responder: Combination of GnRH agonist with standard hCG bolus (5,000–10,000 IU) administered together and OPU done at 34 hours[59] (improves oocyte yield).
- High responders: Increases mature oocyte yield, rescues corpus luteum improving pregnancy rates, and reduces OHSS rates.
    - 15 to 25 follicles: Dual trigger in form of GnRH agonist and low-dose hCG 1,500 IU one hour post-oocyte retrieval with modified luteal phase support[26]
    - Follicles <14: Concomitant GnRH agonist with 1,500 IU hCG for final oocyte maturation.

## Conclusion

In this review article, we have reviewed different pharmacological options for triggering of final oocyte maturation in ART. Time has come in terms of individualization of choice of ovulation trigger for final maturation in ART. The risk of OHSS has been reduced from the time GnRHa trigger has been used for final oocyte maturation although hCG has been the gold standard for decades. The advantage of GnRHa trigger is segmentation of IVF in patients with high risk of OHSS, where a freeze all policy can be performed followed by frozen embryo transfer in the subsequent cycle to minimize the risk of OHSS and have a higher cumulative pregnancy rate. Also, depending on the number of oocytes retrieved, GnRHa trigger opens the possibility to tailor the luteal phase support.

Kisspeptins for final follicular maturation are in the horizon. The promising results of a preliminary study need to be further explored in large clinical trials.

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