Kasum M., Franulić D., Čehić E., Orešković S., Lila A., Ejubović E. (2017) ***Kisspeptin as a promising oocyte maturation trigger for in vitro fertilisation in humans.*** **Gynecological Endocrinology, 33 (8). pp. 583-7. ISSN 0951-3590**

http://www.tandfonline.com/loi/igye20

http://dx.doi.org/10.1080/09513590.2017.1309019

Review article

Kisspeptin as a promising oocyte maturation trigger for in vitro fertilisation in humans

Running title: Kisspeptin – oocyte maturation trigger in IVF

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Abstract

The aim of this review is to analyse the effectiveness of exogenous kisspeptin administration as a novel alternative of triggering oocyte maturation, instead of currently used triggers such as human chorionic gonadotrophin (hCG) or gonadotrophin releasing hormone (GnRH) agonist, in women undergoing in vitro fertilisation (IVF) treatment. Kisspeptin has been considered a master regulator of two modes of GnRH and hence gonadotrophin secretion, pulses and surges. Administration of kisspeptin-10 and kisspeptin-54 induces the luteinizing hormone (LH) surge required for egg maturation and ovulation in animal investigations and LH release during the preovulatory phase of the menstrual cycle and hypothalamic amenorrhoea in humans. Exogenous kisspeptin-54 has been successfully administered as a promising method of triggering oocyte maturation, following ovarian stimulation with gonadotrophins and GnRH antagonists in women undergoing IVF, due to its efficacy considering achieved pregnancy rates compared to hCG and GnRH agonists. Also, its safety in patients at high risk of developing ovarian hyperstimulation syndrome is noteworthy. Nevertheless, further studies would be desirable to establish the optimal trigger of egg maturation and to improve the reproductive outcome for women undergoing IVF treatment.

Keywords: kisspeptin, physiological mechanisms, exogenous administration, oocyte maturation trigger, IVF

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The role of kisspeptins in reproduction

The aim of the paper is to analyse the role of kisspeptins in reproduction as well as current modalities of treatment infertility. Kisspeptin is a polypeptide hormone, a product of the kisspeptin gene and its receptor, which plays a crucial role in the regulation of reproduction. Despite the fact that gonadotrophin releasing hormone (GnRH) has been considered to play a pivotal role in controlling reproductive functions, a hypothalamic neuropeptide kisspeptin, has recently emerged as a key regulator of the hypothalamo-pituitary-gonadal (HPG) axis, which plays a major role in the regulation of GnRH neurons. Kisspeptin has been considered a master regulator of two modes of GnRH and hence gonadotrophin secretion, pulses and surges. Although direct actions of kisspeptins on GnRH neurons are dominant, current data argue against a unimodal mechanism of action, because such primary effects of kisspeptins appear to be insufficient to attain fertility. Owing to more recent investigations it appears that GnRH pulse and surge activity is a product of the integration of multiple signals, because kisspeptin pathways may also be mediated indirectly by other central regulators of GnRH neurons including neurokinin B, dynorphin A, supstance P, cocaine, amphetamine, gamma-aminobutyric acid -glutamate and nitric oxide. In contrast to the intra-hypothalamic roles of kisspeptin as a master regulator of reproductive functions generating GnRH pulses and surges, less is known about the physiological significance of kisspeptins in other tissues such as, the human placenta, pancreatic islet cells, aorta and coronary vessels, umbilical vessels, and a number of brain cells types. Although kisspeptin primarily operates centrally to regulate the HPG axis, peripheral administration of different isoforms (kisspeptin-10 and kisspeptin-54) has been shown to stimulate GnRH and gonadotrophin release. Since recently potential therapeutic applications of kisspeptins justified its use in clinical parctice for treatment of infertility such as novel oocyte maturation triggers in in vitro fertilisation (IVF), in prevention of ovarian hyperstimulation syndrome and prediction of ovulation. Exogenous kisspeptin-54

has been successfully administered as a promising method of triggering oocyte maturation, following ovarian stimulation with gonadotrophins and GnRH antagonists in women undergoing IVF, due to its efficacy considering achieved pregnancy rates compared to human chorionic gonadotrophin (hCG) and GnRH agonists. Also, its safety in patients at high risk of developing ovarian hyperstimulation syndrome is noteworthy. Since kisspeptin levels are positively correlated to estradiol levels, increase kisspeptin surge in serum and urine may be used as a marker for dominant follicle development and pre-ovulation. Nevertheless, further research with larger studies would be desirable in the future to improve the reproductive outcome.

Introduction

Since the early days of *in vitro* fertilisation (IVF), human chorionic gonadotrophin (hCG) has been used as the gold standard for triggering final oocyte maturation instead of the natural midcycle luteinizing hormone (LH) surge [1]. During the 1990's it became possible to trigger oocyte maturation with a single bolus of a gonadotrophin-releasing hormone (GnRH) agonist as an alternative to hCG, when the third generation GnRH antagonist was introduced in ovarian stimulation protocols. The induction of final follicular maturation using GnRH agonists offers potential advantages over hCG, because its triggering elicits a surge of gonadotrophins resembling the natural midcycle gonadotrophin surge, resulting in the retrieval of more mature oocytes and a significant reduction in ovarian hyperstimulation syndrome (OHSS) cases [2].

Despite the fact that GnRH plays a pivotal role in controlling reproductive functions, a hypothalamic neuropeptide kisspeptin, has recently emerged as a key regulator of the hypothalamo-pituitary-gonadal (HPG) axis, which plays a major role in the regulation of GnRH neurons. However, GnRH pulsatility is a product of the integration of multiple signals from other central regulators. Two other neuropeptides, neurokinin B and dynorphin A, which have come under the spotlight recently for their role in the regulation of GnRH pulse generation, are thought to be co-secreted with kisspeptin [3]. Following paracrine stimulatory and inhibitory inputs from neurokinin B and dynorphin A, kisspeptin directly signals GnRH neurons to release GnRH, which in turn stimulates the secretion of LH and follicle stimulating hormone (FSH). In addition, kisspeptin mediates the negative and positive gonadal steroid feedback loop as well as adult fertility, controls the onset of puberty, relays information regarding the body's energy stores and serves as a vital link between the reproduction and energy homeostasis of the body [4]. In contrast to the intra-hypothalamic roles of kisspeptin

as a master regulator of reproductive functions generating GnRH pulses and surges, less is known about the physiological significance of kisspeptins in other tissues such as, the human placenta, pancreatic islet cells, aorta and coronary vessels, umbilical vessels, and a number of brain cells types [5]. Furthermore, compelling evidence accumulated in the last few years, has revealed that kisspeptins have emerged as important gatekeepers of key aspects of reproductive maturation and function, from sexual differentiation of the brain and puberty onset to adult regulation of gonadotrophin secretion and the metabolic control of fertility [6].

Although kisspeptin primarily operates centrally to regulate the HPG axis, peripheral administration of different isoforms (kisspeptin-10 and kisspeptin-54) has been shown to stimulate GnRH and gonadotrophin release and to activate the LH surge required for egg maturation and ovulation in animal studies [7,8]. These findings have been subsequently demonstrated in females with normal menstrual cycles and hypothalamic amenorrhoea, with increments in plasma LH and FSH in the preovulatory phase [9-13]. Since data from investigations of animal and human studies has suggested that the peripheral administration of kisspeptin-10 and kisspeptin-54 is involved in the generation of the LH surge, it was hypothesized that exogenous kisspeptin could be used to trigger egg maturation in women with subfertility undergoing IVF treatment. According to the initial results from a recently published study, a subcutaneous bolus of kisspeptin-54 has been used to trigger oocyte maturation effectively in place of hCG in a FSH/GnRH antagonist IVF protocol. It seems that kisspeptin offers a novel alternative to trigger oocyte maturation in IVF treatment, evidenced by high rates of oocyte maturation in women with normal ovarian reserve [14]. Furthermore, kisspeptin-54 may be used as a promising approach to effectively and safely trigger oocyte maturation in women undergoing IVF treatment at high risk of developing OHSS [15]. The aim of this review is to analyse the effectiveness of exogenous kissppetin as

a new alternative for triggering oocyte maturation following ovarian stimulation for IVF treatment.

Kisspeptin actions in the regulation of GnRH activity

KISS1, the gene encoding kisspeptins, was originally identified in 1996 as a novel human malignant melanoma metastasis-supressor gene, whose expression might supress malignant melanoma cells [16]. The kisspeptin receptor, a member of the rhodopsin family of G-protein coupled receptors, was discovered in 1999, originally designated GPR54 and later termed KISS1R, has been demonstrated in various tissues, including the placenta, brain, pituitary, gonads, liver, pancreas, intestines, aorta, coronary artery and umbilical vein [17,18]. Three surrogate agonist peptides were isolated later from the placenta extracts in 2001, which were originally called metastin (a 54 amino acid protein), for its ability to inhibit cancer metastasis or kisspeptins (54, 14, and 13 amino acid peptides). The initial human kisspeptin precursor (prepro-kisspeptin), a 145-amino-acid protein, is cleaved to a 54 amino acid protein (the most abundant kisspeptin in human circulation with a half-life of 28 minutes) and to several other smaller peptide fragments, including 14, 13, and 10 amino acid peptides, which are collectively named kisspeptins. Two of them, kisspeptin-10 and kissppetin-54 isoforms, have been used by exogenous administration to probe the potential clinical applications of kisspeptin. The kisspeptins numbers correspond to the number of amino acids. Kisspeptin-10 shares the common C-terminal decapeptid sequence, which leads to the strong binding with their receptors. The C-terminal part of the peptides is responsible for the high affinity binding and the activation of the kisspeptin receptor, because kisspeptin-54, -14, and -13 as well as kisspeptin-10 have the same affinity, efficacy and biological activity at the level of KISS1R. There is a consensus that kisspeptin is a direct trigger of GnRH secretion in

mammals, which plays a major role in regulating GnRH neurons by activation of its native KISS1R expressed on the cell body/proximal dendrites of most GnRH neurons [3,19-22]. The hypothesis that kisspeptin neurons regulate GnRH secretion through the activation of the KISS1R on the plasma membrane of GnRH neurons has been confirmed experimentally, because selective elimination of the receptor from GnRH cells induces a hypogonadal phenotype [23]. The localisation of the majority of kisspeptin cell bodies in humans has been demonstrated in the infundibular (arcuate) nucleus in close apposition with GnRH neurons in the hypothalamus, and a second dense population of kisspeptin cells in the preoptic (anteroventral periventricular) area. Kisspeptin is produced by the axons which forming dense pericapillary plexuses in the infundibular stalk, the site of GnRH neurosecretion, where kisspeptin and GnRH neuronal networks are in close proximity via axo-somatic, axo-dendritic and axo-axonal contacts [24].

Although the mechanisms by which kisspeptins directly regulate GnRH secretion are not absolutely clear, it has been demonstrated in various species that the translocation of kisspeptin along the phospholipid bilayer toward its receptor can evoke very potent depolarisation in GnRH neurons with increases in intracellular calcium and induction of the release of GnRH. The main signaling pathway involves Gq proteins and the activation of phospholipase C, MAP kinase phosphorylation via protein kinase C and mobilisation of calcium in the endoplasmic reticulum via phosphatidylinositol-3-kinase. In addition, the adenylate cyclase protein kinase A signaling pathway involving Gs proteins can also be activated, leading to a rise in extracellular calcium influx. Kisspeptin depolarises and excites GnRH neurons primarily through the activation of transient canonical receptor potential channels and the inhibition of K+ channels. [6,25]. The activation of the KISS1R results in a biphasic release of intracellular calcium, a rapid increase followed by a more sustained second-phase calcium response. The slower phase is maintained by kisspeptin receptor

trafficking involving internalisation, recycling and recruitment from an intracellular pool, to prevent desensitisation following an initial acute phase [26]. Although direct actions of kisspeptins on GnRH neurons are dominant, current data argue against a unimodal mechanism of action, because such primary effects of kisspeptins appear to be insufficient to attain fertility. Moreover, GnRH pulsatility is a product of the integration of multiple signals, because kisspeptin pathways may also be mediated indirectly by other central regulators of GnRH neurons. Regulators that appear to be of particular importance include members of the tachykinin peptide family, neurokinin B, substance P and neuropeptide A [27,28].

Recently neurokinin B and the endogenous opioid dynorphin A have been demonstrated across a range of species from rodents to humans in the infundibular/arcuate nucleus due to frequent co-localisation of kisspeptin and neurokinin B neurons. It is becoming increasingly apparent that kisspeptin, co-secreted with neurokinin B and dynorphin (KNDy neuropeptides), regulates the GnRH pulse generation, as key hypothalamic regulators (KNDy hypothesis) in response to dynamic changes in steroid hormone concentrations, because most kisspeptin neurons express the ERα. [3,29]. According to the evidence for KNDy model in the ewe, it is widely accepted that KNDy neurons may represent the long-sought GnRH pulse generator responsible for driving synchronous release of GnRH and gonadotrophins. An increase in endogenous neurokinin B initiates a positive feedback loop and each GnRH pulse by the activation of the neurokinin-3 receptor (NK3R) within KNDy neurons, to release kisspeptin onto GnRH neurons. However, dynorphin A, stimulated from the same neurons by neurokinin B, inhibits GnRH pulse frequency and terminates GnRH pulses acting directly on KNDy neurons. Thus, neurokinin B and dynorphin A control a the synchronised activity of KNDy neurons and kisspeptin solely activates GnRH neurons to regulate pulsatile GnRH secretion (Fig.1) [30]. Nevertheless, the role of neurokinin B in GnRH pulse regulation remains controversial because its effects have been more variable, with some studies in

rodents showing stimulatory or inhibitory effects on gonadotrophins. It is now clear that sex steroid milieu, pubertal status, and gender are important factors governing the effects of neurokinin B on gonadotrophin release [31]. Moreover, co-localisation of the NK3R in neurokinin neurons of the arcuate nucleus and the lack of the NK3R in GnRH neurons suggest that the actions of neurokinin B on GnRH neurosecretory activity in the ewe may be mediated indirectly via other neurones and neuropeptides [32]. Aiming to investigate the KNDy hypothesis in humans by assessing for the first time the effects of coadministration of kisspeptin-54, neurokinin B, and an opioid receptor antagonist, naltrexone, the results confirmed significant interactions between the KNDy neuropeptides on LH pulsatility and gonadotrophin release [33]. However, KNDy neurons may serve as a major target in the positive feedback actions of estradiol and might also be involved in the preovulatory GnRH/LH surge generation, in addition to the mode of the GnRH pulse generator under the negative feedback action of oestrogen [29]. Despite the widely reported role of kisspeptin neurons in the preoptic area mediating the positive feedback action of oestrogen as a trigger of the preovulatory GnRH/LH surge, a recent study reports that both anteroventral periventricular and arcuate kisspeptins may be important in the generation of GnRH/LH surges in rats [5, 34]. It appears that anteroventral periventricular/periventricular preoptic nucleus (AVPV/PeN) kisspeptin neurons play a critical role in induction of GnRH/LH surge in female mice, because 17β-Estradiol increases the persistent sodium current and excitability of the kisspeptin neurons, dramatically altering their firing activity. Consequently. AVPV/PeN kisspeptin neurons generate spontaneous and repetitive burst firing, which is required for the high-frequency-stimulated release of kisspeptin for exciting GnRH neurons and potentially generating the GnRH surge [35].

In search of additional neuropeptides in kisspeptin and neurokinin B neurons in the infundibulum of postmenopausal women, co-localisation experiments have provided evidence

of the presence of the anorectic hypothalamic peptide cocaine- and amphetamine-regulated transcript in contacts with other peptidergic cells, including GnRH-IR neurons [36]. Under some circumstances, kisspeptin may also exert indirect actions on GnRH neurons with both gamma-aminobutyric acid (GABA)-glutamate and nitric oxide (NO) signalling being modified in the vicinity of the GnRH neuron cell bodies. Selective activation of GABA(A) receptors decreased kisspeptin-induced gonadotrophin secretion, whereas their blockade elicited robust LH and FSH bursts and protracted responses to kisspeptin-10 when combined with GABA(B) receptor inhibition. It seems that NO is also involved in the control of GnRH release because LH responses to kisspeptin-10 were protracted after inhibition of NO synthesis [37]. Furthermore, the gonadal steroid 17β-estradiol which conveys vital feedback information, has recently been demonstrated to modulate the excitability of kisspeptin as well as GnRH neurons by altering the expression and/or function of channel transcripts that orchestrate the downstream signalling of kisspeptin in GnRH neurons [38]. Since kisspeptin cells and their KISS1R have been demonstrated throughout the brain in addition to the GnRH neurons, it may be that kisspeptin is involved in regulating the activity of multiple neuronal circuits in other brain regions. Therefore, it is becoming increasingly likely that kisspeptin acts as a neuromodulator through the KISS1R within multiple different neuronal networks in the brain, but also exhibits neuromodulatory actions typical of other neuropeptides through other RF amide receptors such as the neuropeptide FF receptors [39].

Exogenous kisspeptin – a novel ovulation trigger

Although kisspeptin primarily operates at central levels as a master regulator of GnRH pulse generation and secretion of gonadotrophins [3-5], the initial use of kisspeptins by peripheral administration in animal experiments demonstrated that metastatin and kisspeptin-10

successfully induce the release of gonadotrophins and ovulation via activation of the hypothalamic GnRH neurons [7,8]. Furthermore, investigations involving exogenous administration of the natural, unmodified peptide sequence of kisspeptin-10 and kisspeptin-54 have subsequently been performed in females with normal menstrual cycles [11] and hypothalamic amenorrhoea [13], using various doses and routes of delivery with no adverse effects having been reported. According to these findings, kisspeptin most potently induces gonadotrophin release during the preovulatory phase of the menstrual cycle, followed by the luteal phase, and then the follicular phase [9,10]. An elevation of plasma kisspeptin with consequent LH release has been suggested as a novel mechanism for manipulation of the HPG axis in women. Kisspeptin has been identified as a new therapeutic agent who may show potential for the treatment of reproductive disordes in the future [9-13]. Although a single subcutaneous bolus injection of kisspeptin-54 temporarily increases the number of LH pulses in healthy women, further studies would be advisable to investigate its therapeutic potential to restore LH pulsatility in cases with impaired GnRH secretion [12]. The mechanism by which kisspeptin stimulates the LH surge predominantly during the preovulatory phase may be explained by a potential role of estradiol-positive feedback on gonadotrophin secretion, because the plasma concentration of estradiol is greater in investigations with an LH surge compared to those without LH surges [40]. Since women with hypothalamic amenorrhoea are more sensitive to kisspeptin than healthy women, kisspeptin is more likely to cause tachyphylaxis at higher doses. Nevertheless, kisspeptin can persistently stimulate gonadotrophin secretion in women with hypothalamic amenorrhoea if the correct doses are chosen. During intravenous infusion of kisspeptin-54, the mean peak number of pulses was 3-fold higher and the mean peak LH pulse secretory mass was 6-fold higher when compared to vehicle. Therefore, the determination of the dose range which kisspeptin-54 treatment

increases basal and pulsatile LH secretion in would represent a basis for studying the potential of kisspeptin-based therapies to treat women with hypothalamic amenorrhoea [13].

Based on the data that exogenous kisspeptin may activate the LH surge required for egg maturation and ovulation in animal investigations [7,8] stimulate LH release in the preovulatory phase of the cycle [9,10] and hypothalamic amenorrhoea [13] in females, recent studies investigated the potential for kisspeptin as a novel method of triggering oocyte maturation in women undergoing IVF [14,15]. In a recent pilot study of 53 women undergoing a recombinant FSH plus GnRH antagonist IVF protocol a single subcutaneous injection of kisspeptin-54 was administered 24hrs after the last GnRH antagonist injection. Fertilisation occurred in 92% (49/53) of cases, with biochemical and clinical pregnancy rates of 40% (21/53) and 23% (12/53), respectively. In the 12 women achieving clinical pregnancies, eight women had singleton pregnancies and two women had twin pregnancies i.e. 12 babies, two further women having had miscarriages. The findings of the study suggest for the first time that kisspeptin-54 sufficiently triggers egg maturation to result in fertilisation, embryo implantation, and successful live birth in women with subfertility undergoing IVF therapy. Nevertheless, further studies would be required to estimate the clinical utility of kisspeptin-54 during IVF therapy when compared to established pharmacological triggers of egg maturation [14]. According to data from a later study, kisspeptin-54 may be a promising approach which can be used efficiently and safely to trigger egg maturation in women at high risk of developing OHSS while undergoing IVF treatment. In a randomised clinical trial of 60 women following a standard recombinant FSH/GnRH antagonist protocol, patients received a single injection of kisspeptin-54 to trigger oocyte maturation. The achieved pregnancy rates compared favourably with currently used pharmacological triggers of oocyte maturation. Oocyte maturation occurred in 95% of cases with biochemical pregnancy, clinical pregnancy, and live birth rates per transfer (n = 51) of

63, 53, and 45%, respectively, with no cases of clinically significant OHSS and adverse events associated with the kisspeptin-54 injection. Despite attractive results with the use of kisspeptin-54 to trigger oocyte maturation in women at high risk of OHSS undergoing IVF treatment, additional large randomised studies would be desirable to compare the efficacy and safety of kisspeptin-54 vs. currently used triggers to verify the optimal trigger of oocyte maturation [15]. In order to overcome the drawback of kisspeptin's short half-life, a kisspeptin-10 analogue (compound 6, C6) has recently been synthesised, capable of triggering ovulation in ewes after a single intramuscular injection, although a longer half-life increases the chance of tachyphylaxis unless the doses are not appropriately reduced. Nevertheless, these results imply that kisspeptin-10 analogues opens up new possibilities for the treatment of reproductive disorders in humans and may find application in the management of livestock reproduction [41].

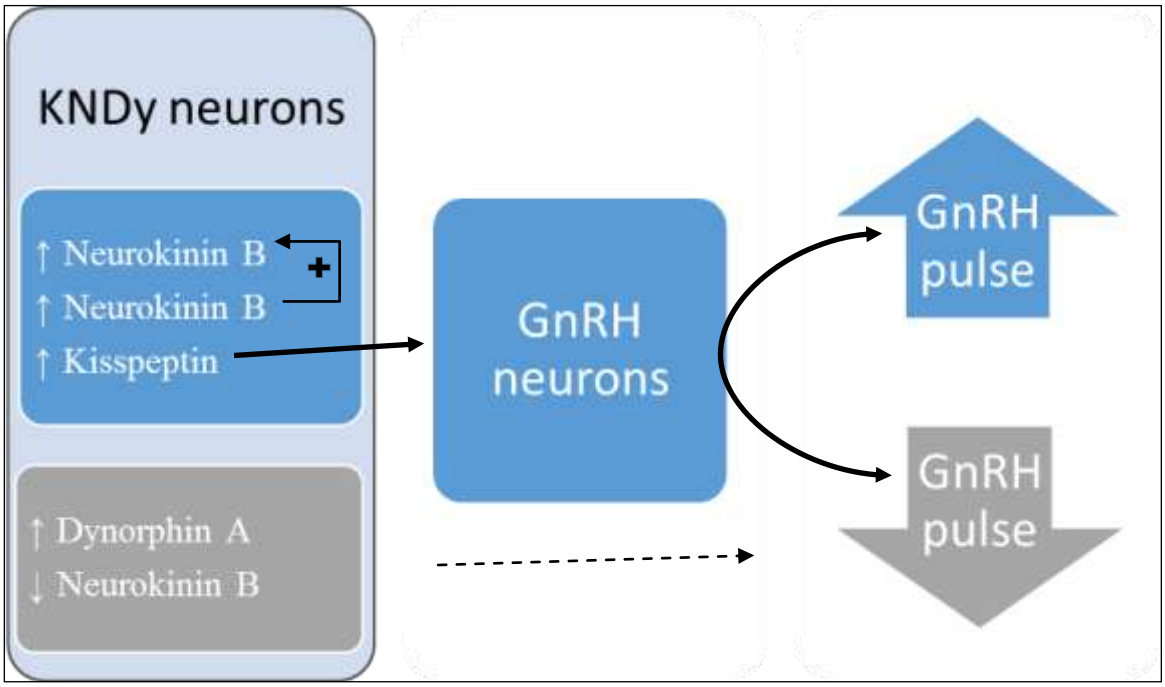
Conclusions

Peripheral kisspeptin administration could be used as a novel and promising method of triggering oocyte maturation in women undergoing IVF therapy due to its efficacy estimated through pregnancy rates when compared to pharmacological triggers currently in use. Furthermore, it is noteworthy that kisspeptin may also be administered effectively and safely to trigger oocyte maturation in patients at high risk of developing OHSS. Nevertheless, further research with larger studies will be required in the future to determine the clinical utility of kisspeptin, establish the optimal trigger of egg maturation, and to improve the reproductive outcome for women undergoing IVF treatment.

Declaration of Interest

The authors report no declarations of interest.

Fig.1. Schematic illustration of the GnRH pulse generator



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