Role of LH in controlled ovarian stimulation

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SUMMARY

Controlled ovarian stimulation has become an integral part of infertility treatment. Specific gonadotropin based protocols become the main strategies for controlled stimulation. To avoid the potentially detrimental effect of premature LH surge on oocytes and/or endometrium development, the GnRH analogs have been incorporated into controlled ovarian stimulation strategies. With the availability of recombinant gonadotropins (i.e. recombinant FSH devoided of LH activity) it is necessary to establish precise role of LH in the folliculogenesis and endometrium development. The benefit of exogenous LH may vary with the GnRH-agonists and antagonists regiment used. The optimal amount of LH or ratio FSH to LH used during therapeutically stimulated growth of follicles is still a problem that needs to be solved in the near future. Reproductive Biology 2002 2 (3): 215-227.

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INTRODUCTION

The aim of controlled ovarian stimulation is to synchronize multi-follicular development that leads to obtaining more oocytes per patient cycle and subsequently increases chances of achieving pregnancy. The fate of each follicle is controlled by an interaction between the endocrine
LH and ovarian stimulation

(pituitary gonadotropins, mainly FSH and LH, and in some species growth hormone [GH] and prolactin) and paracrine factors (intraovarian factors such as steroids, cytokines and other growth factors). In response to an increasing level of circulating FSH a cohort of antral follicles is recruited during each stimulated cycle. The growth of follicles, granulosa cell proliferation and estrogen production depend upon FSH stimulation. FSH influences estrogen synthesis through induction of granulosa cell aromatase activity that converts androgen into estrogen. An androgen of theca origin is an obligatory precursor for estradiol synthesis within the follicle and its synthesis is controlled by LH [15]. Locally produced estrogen influences the developmental ability of oocytes and also induces morphologic uterine and endometrial changes needed for embryo implantation.

The highly purified gonadotropins and recombinant gonadotropins are currently clinically available on the market; therefore, the optimal amount of LH or the ratio of FSH to LH needed in the therapeutically stimulated cycle becomes a very important issue.

Clinical aspect

After almost 15 years of use of gonadotropins to induce follicle development,there is still some controversy as to the most appropriate method of ovarian stimulation that will yield an optimum number of mature oocytes for fertilization in vitro and/or in vivo (fig. 1). Several stimulation protocols have been developed to induce multi-follicle development leading to ovulation. Currently, in all available protocols for controlled ovarian stimulation, attention is focused primarily on ovarian sensitivity to menotropins and/or gonadotropins. Stimulation is controlled by individualization of patient treatment by administering more or less gonadotropins/menotropins. To prevent premature luteinization (LH surge) the gonadotropin releasing hormone (GnRH) analogs were introduced into protocols. Typically, GnRH analogs of choice for down regulation are agonists: leuprolide acetate, commonly used in USA, and buserelin, tryptorelin and nafarelin, used in Europe. More recently, GnRH antagonists - cetrorelix and ganirelax have been introduced.

The mechanism by which GnRH–antagonists suppress pituitary gonadotropins is completely different compared to GnRH-agonists. While agonists act through down-regulation of receptors and desensitization of pituitary gonadotropic cells, antagonists bind completely to pituitary
Fig. 1. Examples of some ovarian stimulation protocols used in controlled ovarian stimulation.

ER - eggs retrieval  hCG – human chorion gonadotropin

**MICROFLARE CYCLE**

- Low dose of agonist
- Gonadotropins/menotropins

**ANTAGONIST CYCLE**

- antagonist
- Gonadotropins/menotropins

**AGONIST CYCLE**

- agonist
- Gonadotropins/menotropins

-21  1  2-3  5-7  10-12  12-14

Days of menstrual cycle
receptors and thereby prevent endogenous GnRH from exerting its stimulatory effects on pituitary cells avoiding any initial stimulatory effect typical of administration of GnRH–agonists (so-called “flare-up effect”). The antagonist mechanism of action depends on equilibrium between endogenous GnRH and the applied dose of antagonist. In contrast to agonists, the effect of antagonists is highly dose–dependent.

There are some differences in trajectory of follicular development between agonist and antagonist cycles. In antagonist cycles follicles initially grow faster and subsequent estradiol level increases more rapidly. Follicles of 18-19 mm can be present on the ovary as early as the sixth day of medication administration, because there is no pituitary suppression and endogenous LH level is between 2-8 IU/L [33]. After giving the antagonist (0.25 mg multidose), the “pituitary suppression” is fully effective within 4 to 6 hours for ganirelix and cetrorelix, respectively. The LH level is decreased about 74 %, which corresponds to a LH level <1-2 IU/L. The peak of estradiol on the day of hCG injection (hCG triggers final follicle maturation what corresponds to LH surge in the natural cycle) in the antagonist cycle is lower (2,001 pg/ml compared to the agonist group 2,768 pg/ml; [2, 5, 34]). The overall pituitary recovery from the treatment with antagonists is within 24 hours and the elimination half-life for ganirelix is within 8.5-17.1 hours and for cetrorelix within 4.1–179.3 hours. These data clearly indicate that there is a wide range in pituitary sensitivity to the antagonist and/or pituitary “escape” from suppression that probably plays an important role in appropriate stimulation of follicular growth and development of the endometrium. If the pituitary of some patients escapes very fast from the influence of the antagonist, the LH level might exceed the “ceiling level” (proposed by Gordon et al; [10]) that can adversely affect oocyte development, fertilization and possibly might initiate luteinization. But when the pituitary recovers too slowly, then profound suppression of LH might occur and more likely affect estradiol production as well as subsequent uterine lining development and implantation. This was clearly demonstrated in the ganirelix dose-finding study [33]. There were no pregnancies recorded when a 2 mg dose was used (profound suppression was present). The estradiol level in this group was lower (430 pg/ml) compared to the group with multidose 250 mg ganirelix (1160 pg/ml) where the best clinical outcome was achieved.

The importance of LH in patients with profound hypogonadotropic
hypogonadism is widely recognized. These patients require LH activity supplementation to optimize controlled ovarian stimulation and ovulation outcome [1]. This may be applicable also to normo-ovulatory women when a GnRH agonist was added to exogenous gonadotropins for controlled ovarian stimulation. The agonist can cause excessive endogenous LH deprivation at the time when ovarian follicles are becoming receptive to LH through the expression of granulosa cell LH receptors and thus, induce a functional and transitory hypogonadotropic condition in the patient.

The Westergaard et al. [36] demonstrated that the clinical outcome of IVF (In Vitro Fertilization) and ICSI (Intracytoplasmic Sperm Injection) in normogonadotropic women subjected to controlled ovarian stimulation in the long GnRH-agonist protocol was significantly correlated both to the regimen of pituitary desensitization (intranasal [IN] vs. subcutaneous administration [SC] of agonist) and to the type of gonadotropin used (human menopausal gonadotropin versus recombinant FSH). This correlation also depended on midfollicular level of circulating estradiol and LH activity. The most favorable clinical outcome was found in the group (intranasal and human menopausal gonadotropin) showing the highest midfollicular serum levels of LH activity and estradiol, whereas the least favorable outcome was seen in the group (subcutaneous and FSH) with the lowest level of these hormones. These results suggest that perhaps a subgroup of normogonadotropic women, when treated with standard long-protocol GnRH-agonist down-regulation, may benefit from supplementation with gonadotropin preparations containing LH activity (human menopausal gonadotropin or recombinant LH) during ovarian stimulation. Further study is needed to determine which patients and/or group of patients (specifically – which pituitary) is a suitable candidate for controlled stimulation with GnRH antagonist and which with agonist. Perhaps lower doses of antagonist but with increased frequency of administration would be more appropriate in therapeutically stimulated cycles in order to improve a patient’s chances of delivering a healthy baby.

**Oocytes quality**

In addition to appropriate uterine endometrial lining development, oocytes are the primary object of interest and outcome of controlled ovarian stimulation. Therefore, apart from the LH role in follicular estradiol biosynthesis,
the LH role in quality of oocytes should also be considered. Although LH receptors presence on the oocytes have not been extensively documented so far, an excessive LH level may disrupt granulosa cell communication in the cumulus oophorus, which is critical to maintain the oocyte in the late diplotene stage of meiosis until ovulation [39]. Meiotic arrest of oocytes is maintained by an oocyte maturation inhibitor, a peptide whose activity decreases with the mid-cycle LH surge in developing follicles [35]. However, early human embryo and blastocysts contain LH/hCG receptors1.

On the other hand, estrogen receptors have been identified within the oocyte [38] and estradiol-17β appears to support oocyte cellular membrane and cytoplasmic maturation [39] acting via a nongenomic Ca2+ mediated mechanism [32]. However, LH is a factor that induces final oocyte nuclear meiotic maturation, degradation of gap junctions [6] and cumulus expansion in the follicle. Eppig [6] proposed two mechanisms of LH action: 1. degradation of gap junctions between granulosa and cumulus cells results in reduction of flow of meiosis arresting substance(s); 2. the generation of a maturation–inducing signal in the granulosa introduced into oocytes via the gap junctions.

Oocytes obtained from a women with hypogonadotrophism in an IVF cycle treated with FSH showed that a LH-depleted environment yielded oocytes with a reduced fertilization rate compared with the same patient treated with combined FSH and LH [1]. No pregnancies have been reported in hypogonadotropic hypogonadal patients treated with FSH alone. Also, lower estradiol levels and a shorter length of stimulation were associated with oocyte membrane breakage characteristics (during ICSI) that yielded lower oocyte survival and fertilization rates [21]. Although fertilization and early embryonic development can occur in human oocytes that mature in an environment with a low estrogen level, the developmental potential (capability of producing a pregnancy with delivery of a baby) of these embryos appears to be questionable.

Oocytes obtained from the women who had high LH concentrations during the later stages of follicular development and in the peri-ovulatory phase were of poor quality and were associated with a reduction in the rates of fertilization and cleavage [14, 30].

Embryos obtained from such eggs were of inferior quality and displayed excessive fragmentation and asymmetric alignment of blastomeres, which suggest that oocytes were in the early stages of atresia when aspirated and fertilized. Chances of pregnancy were also low in IVF cycles associated with elevated LH concentrations, even only two days prior to HCG administration [13]. Similarly, *in vivo* conception was reduced significantly when LH concentrations were high and resulting pregnancies were more likely to end in miscarriage [12, 25]. The high level of LH appears to alter follicular theca androgen metabolism resulting in higher testosterone and lower androstendione concentrations in follicular fluid [19]. Such a shift in androgen availability may impair oocyte developmental potential and subsequently successful implantation. The other possibility would be that high LH concentration may prematurely allow resumption of meiotic maturation of oocytes [16] and resulting oocytes would then be post-mature and would likely fail to become fertilized and/or produce embryos with developmental potential. Also, premature reactivation of meiosis caused by high basal concentrations of LH may lead to karyotic abnormalities and demise of the embryo quality. Plachot et al. [23] observed an enhanced incidence of chromosome anomalies in oocytes recovered after administration of high doses of LH and FSH - 21 to 50 ampoules of human menopausal gonadotropin/cycle (41%) compared with 6-20 ampoules/cycle (23%).

**Steroidogenesis and implantation**

Follicular steroidogenesis in accordance with the two–cell, two-gonadotropins theory is primarily supported by the synergistic action of FSH and LH. The exact requirements of LH for normal steroidogenesis and follicular development and oocyte maturation are not known.

Ovarian stimulation virtually with all the currently used agonist-protocols results in reduced LH serum concentrations in the early and mid-luteal phase. LH concentrations of 1-2 IU/L [22] or <3 IU/L [10] have usually been recorded despite prolonged use of GnRH-agonist suppression. Chappel and Howles [3] demonstrated that the amount of LH required for ovarian steroidogenesis is only minimal, and residual endogenous LH secretion after suppression with agonists is sufficient to support FSH-induced follicular development in down regulated patients. Schoot et al. [29] believe that low (<1 IU/L) LH plasma concentration sufficiently facilitates estrogen
synthesis in controlled ovarian stimulation regimens with agonist, whereas Filicori et al. [7] believed that LH amounts of 1-2 IU/L would be more appropriate. Fleming et al. [8] showed that follicular fluid estradiol level, oocyte yield and fertilization were improved when LH concentrations were > 0.5-1 IU/L. It might be true that the LH requirement for maintenance of follicular steroidogenesis is likely to be low because <1% of follicular LH receptors needs to be occupied for continuation of ovarian steroidogenesis [3].

Schoolcraft et al. [28] found a significantly higher implantation rate of transferred blastocysts in patients subjected to standard long protocol GnRH-agonist down regulation and ovarian stimulation with FSH and LH supplementation than with FSH alone despite a similar percentage of blastocysts formation in these two groups. Fleming et al. [8] obtained similar results and demonstrated that severe midfollicular LH suppression did not significantly affect proportion of supernumerary embryos developing to blastocysts. On the other hand, Westergaard et al. [37] showed, that nearly half of 200 normogonadotropic women undergoing IVF and ICSI treatment in connection with long-protocol GnRH-agonist down regulation and recombinant FSH experienced midfollicular levels of LH <0.5 IU/L and significantly lower serum estradiol than did the other half of the women with LH levels >0.5 IU/L. The women with low midfollicular LH (more suppressed) had a significantly higher risk of early pregnancy loss and consequently a significantly lower chance of delivering a child than did the women with midfollicular LH >0.5 IU/L (less suppressed). These findings indicate that detrimental effects of low LH level may manifest themselves after implantation.

The important role of LH in the implantation process was also evident in the dose–finding study with GnRH antagonist [33]. When a high dosage of antagonist (2 mg of ganirelix) was administered daily, a profound LH suppression was noted and significantly reduced implantation. This was probably due to impairment of endometrial receptivity as the estradiol level on the day of hCG injection was lower (430 pg/ml) compared to the group receiving 250 µg of antagonist (1160 pg/ml). Although, the high multiple doses of ganirelix in these IVF cycles did not adversely affect the quality of embryos. Excess of these embryos was cryopreserved and replaced in subsequent thawed cycles. The ongoing pregnancy rate per freeze-thaw cycle was 25% [17]. Thus, the implantation capacity of these embryos was not impaired and provided further evidence that LH requirements for normal
steroidogenesis is minimal and that LH might play a direct important role in implantation. Gordon et al. [10] showed that a dose–related increase in implantation rate was associated with increased level of exogenous LH. The exact mechanism, how LH influences implantation events, is not known.

Gonadotropin LH/hCG receptors were detected in human endometrial tissue using immunocytochemical techniques [26]. In the endometrium, these receptors were present in glandular and luminal epithelial cells as well as in stromal cells. All cells in the secretory phase contained more receptors than the cells from the proliferative phase of the cycle. Therefore, a direct effect of LH on the endometrium during the implantation cannot be ruled out. The uterus of LH receptor knockout mice showed decreased expression of more than 250 genes as determined by complimentary DNA (cDNA) expression arrays. These decreases for the most part were not completely reversed by estradiol and progesterone replacement therapy, implying that they are under LH control [24]. Studies on endometrial effects of steroids in natural, estradiol and progesterone replacement and controlled ovarian stimulation cycles have recognized the role of ovarian hormones in priming endometrial proliferation and differentiation. There are dissimilarities in endometrial histological changes of the luteal phase of controlled ovarian stimulation and steroid replacement cycles. Garcia et al. [9] have shown an advancement of histological transformation of the endometrium in controlled ovarian stimulation cycles but glandular transformations are delayed in estradiol and progesterone replacement cycles in women with inactive and active ovaries [27, 31]. However, the endometrial morphology and implantation receptivity in the frozen embryos transferred cycles were similar whether endogenous gonadotropins were suppressed [20] or not [4]. This may suggest that gonadotropins have no direct effects on endometrium. Moreover, Li et al. [18] correlated the follicular phase LH concentrations with endometrial biopsy specimens obtained in the luteal phase. No correlation was found between follicular LH concentrations and endometrial development in the periimplantation period. However, the endometrial biopsy might not have been the best choice as a variable for correlation. It is a subjective measure with a high risk of inaccuracy and perhaps with an insufficient level of sensitivity. In spite of the fact that hypothalamus and pituitary hormones as well as the presence of their receptors on the uterus have recently captured scientific attention [11, 24], there are still a lot of unanswered questions.
CONCLUSIONS

Proper application of pure hormones or recombinants of FSH and LH in the controlled ovarian stimulation requires knowledge of basic endocrinology and physiology in clinical approaches. By introducing different analogs for suppression of pituitary function (especially when antagonists are involved), it is becoming necessary to pay close attention to pituitary and ovarian sensitivity in order to maintain appropriate speed of follicular development. The threshold value of LH that should be used to discriminate between LH concentrations considered sufficient and those considered too low or too high is still unknown. Therefore, specific dose–finding studies need to be conducted in order to evaluate the optimal dosage of LH in steroidogenesis and the implantation process. In future, emphasis should be placed on identifying patients or groups of patients that benefit the most from using agonist or antagonist for suppression of pituitary following optimal response to FSH and LH.

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