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CASE REPORT *Reproductive endocrinology*

Successful pregnancy after ovulation induction with human chorionic gonadotropin in a woman with selective luteinising hormone deficiency

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Abstract: Selective LH deficiency has been described in several men, but only in two women who presented normal pubertal development but secondary amenorrhoea due to anovulation. Despite its rarity, this condition represents a valuable model for studying the processes regulated by FSH or LH during late folliculogenesis and ovulation in humans. A woman previously diagnosed with selective LH deficiency due to a homozygous germline splice site mutation in *LHB* (IVS2 + 1G→C mutation) was submitted to an individualised ovarian induction protocol, first with recombinant LH and then with highly purified urinary hCG. Ovarian follicle growth and ovulation were achieved, and a healthy baby was born after an uneventful term pregnancy. The treatment described herein demonstrates that the clinical actions of exogenous LH or hCG in inducing late-stage follicular development in women with deficient LH production or performance might be interchangeable or inevitable, once FSH-dependent early follicular growth is assured.

Key word: LH deficiency / ovulation induction / infertility / hCG induction / LH / FSH / hCG

Introduction

LH belongs to the family of glycoprotein hormones that is characterised by a heterodimeric structure comprising a specific beta subunit, which conveys specificity of action, and a non-covalently linked common alpha subunit. LH plays an essential role in normal human sexual development, and its deficiency is associated with impaired reproductive function in both males and females (Latronico *et al.*, 1996; Themmen and Huhtaniemi, 2000).

Gene mutations leading to primary disorders of gonadotropic secretion or action can partially or totally prevent sexual development in mammals. Selective LH deficiency due to inactivating mutations of its beta-subunit gene (*LHB*) was initially linked to the absence of pubertal development, hypogonadism and infertility in males (Weiss *et al.*, 1992; Valdes-Socin *et al.*, 2004). Subsequently, Lofrano-Porto *et al.* (2007) described the phenotype of a woman with selective LH deficiency in a Brazilian family due to a germline splice site mutation in the *LHB* (IVS2+1G→C). This mutation induced a gross abnormality in the processing of *LHB* mRNA, leading to complete impairment of LH secretion into the circulation and consequently undetectable LH serum levels in the homozygous carrier. The phenotypes of two male siblings were similar to those of previously described rare cases. In contrast, the female sibling had a unique and peculiar phenotype, which was characterised by normal pubertal development but chronic anovulation

that evolved into secondary amenorrhoea and infertility (Lofrano-Porto et al., 2007).

Here, we report the occurrence of a full-term pregnancy in a previously reported LH-deficient woman after ovulation induction using purified urinary hCG alone, after an unsuccessful attempt with recombinant LH.

Case report

The patient and her two male siblings had been previously diagnosed with selective LH deficiency due to a germline splice site mutation in *LHB* gene in the homozygous state (IVS2+1G→C mutation), which had been shown to result in consistently undetectable serum LH levels (Lofrano-Porto et al., 2007). After undergoing normal pubertal development and menarche at the age of 13 years, the patient reported sporadic menses during the following 14 years, which finally evolved into secondary amenorrhoea. At the age of 32 years, she was referred to specialised infertility care owing to anovulatory amenorrhoea, which was proven by repeatedly low progesterone serum levels and the lack of a corpus luteum at serial ultrasound exams. She presented a typical female phenotype with a normal height and body mass index. Laboratory tests showed normal blood glucose and thyroid function and a normal 46 XX karyotype. Pelvic ultrasonography revealed a normal uterus and ovaries, with multiple antral follicles distributed throughout the ovarian tissue. Hysterosalpingography revealed normal tubal anatomy and patency, and partner semen parameters were within the normal limits of World Health Organization's reference values (Cooper et al., 2010).

This study was approved by the Research Ethics Committee of the Faculty of Medicine, University of Brasília, Brazil (CEP-FM 062/2004), and written informed consent was obtained from the patient.

Rationale

Uncommon cases of genetically caused hypogonadotropic hypogonadism represent valuable models for studying the processes regulated by pituitary gonadotropins; furthermore, women harbouring inactivating mutations in LH beta-subunit (LHB) or LH receptor (LHCGR) genes are rare in comparison to other genetic and non-genetic causes of hypogonadism. In this case, the desire for pregnancy in a woman with an *LHB* mutation and total impairment of LH secretion imposed the challenge of testing cost-effective ovarian stimulation protocols that mimic the role of LH in late folliculogenesis and ovulation. Therefore, individualised ovarian induction protocols were performed, initially with recombinant LH and then with highly purified urinary hCG.

Ovarian stimulation protocol design and results

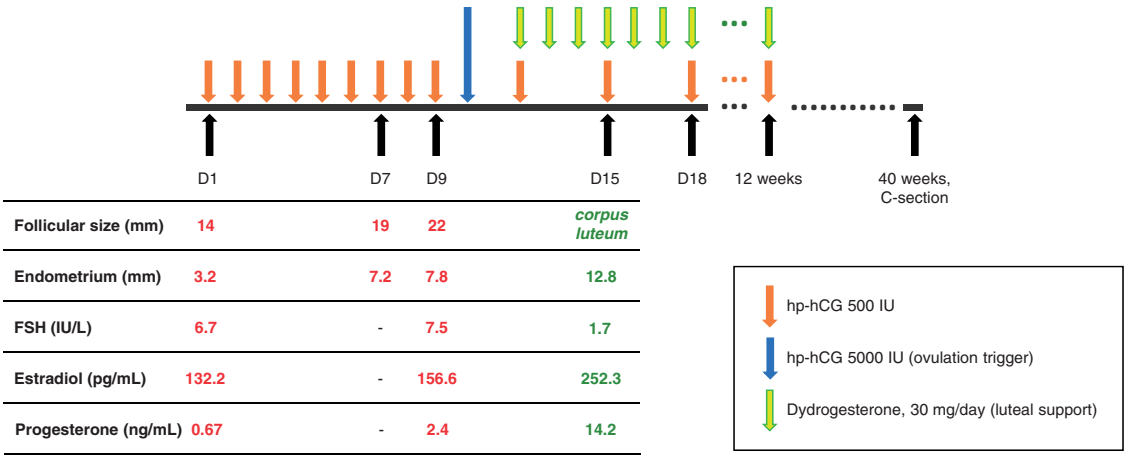
During the first appointment, 12 antral follicles and a single 13-mm follicle were identified in the sum of both ovaries, using the standardised antral follicle count technique proposed by Broekmans et al. (2010). Therefore, recombinant alfalutropin (rLH) (Luveris ®75 IU/ml, Merck S/A) was immediately started. As no follicular growth was achieved after seven days of daily 75 IU of rLH, the cycle was cancelled. Three months later, menstruation induction with oral progesterone was performed; a new cycle of ovulation induction was started with a daily dose of 150 IU rLH, but it was cancelled again after 13 days owing to a lack of response. After 5 months, menstruation was reinduced with oral progesterone. A new ovulation induction protocol consisting of increasing rLH doses was initiated, beginning with 75 IU/day and adjusted progressively, according to the following scheme: 75 IU/day from day 2 to day 3; 150 IU/day from day 4 to day 9; 300 IU/day from day 10 to day 20, until dominant follicles reached mean diameter >17 mm (20 and 19 mm). Fifty-eight ampoules of 75 IU of rLH were used in this third cycle for a total of 19 days until preovulatory follicles were formed. Thereafter, an ampoule of recombinant hCG (Ovidrel®250 µg/0.5 ml, Merck S/A) was used to induce complete follicular maturation, and ovulation was confirmed by ultrasound. Timed intercourse was recommended, and luteal phase replacement was performed with oral dydrogesterone (Duphaston® 10 mg, Abbott Biologicals B.V. Olst, Netherlands) at a daily dose of 20 mg. Unfortunately, the patient failed to achieve pregnancy.

Owing to the high amount of recombinant alfalutropin ampoules required and the high initial cost of the protocol (Verberg et al., 2009), after 14 months of the previous cycle, the researchers proposed a second ovarian stimulation cycle using highly purified hCG (hp-hCG; Brevactid® 5000 IU/ml, Ferring Arzneimittel GmbH, Berlin, Germany). Initially, a dose of 200 IU/day of hCG was used as described previously (Filicori et al., 2005; Koichi et al., 2006; Serafini et al., 2006), but no follicular growth was observed. Considering the exceptionality of this case, wherein the woman had a proven lifelong lack of endogenous LH secretion, an increased dose at 500 IU/day of hp-hCG was administered, and ovarian follicle growth and endometrial thickening were closely monitored. Nine days after the daily administration of hp-hCG 500 IU, a 22-mm follicle was obtained. Ovulation was triggered with a single hp-hCG 5,000 IU dose (Brevactid® 5000 IU/ml), and the patient was instructed to have intercourse every 2 days. Pregnancy was confirmed after 14 days. From the second day after trigger until the end of the 12th week of pregnancy, luteal phase support was provided with oral dydrogesterone at 30 mg/day, combined with subcutaneous hp-hCG 500 IU every 3 days (Fig. 1). After an uneventful 40-week pregnancy, a healthy infant was born by elective caesarean section when the mother was 35 years old.

Discussion

We report the reproductive success of an ovulation induction protocol consisting of progressive doses of r-LH and hp-hCG, which aimed to mimic the LH effect in the late follicular phase in a woman with genetically proven complete endogenous LH deficiency. To our knowledge, this is the first report of pregnancy in a woman with chronic anovulation due to selective LH deficiency.

LHB-inactivating mutations have been described in only two women. Beyond the case presented herein (Lofrano-Porto et al., 2007), the second report was of a female sibling from a Moroccan family who had a homozygous 9-base deletion in *LHB* exon 2, leading to the deletion of three amino acids at positions 30, 31 and 32 in the mutant LHB protein (Achard et al., 2009). Despite limited phenotypic data of the Moroccan female sibling, the clinical presentation of these two unrelated LH-deficient women was similar and was mainly characterised by normal pubertal development, secondary amenorrhoea and infertility. However, the phenotypes of their affected male siblings were



Follicular size (mm)	14	19	22	*corpus luteum*
Endometrium (mm)	3.2	7.2	7.8	12.8
FSH (IU/L)	6.7	-	7.5	1.7
Estradiol (pg/mL)	132.2	-	156.6	252.3
Progesterone (ng/mL)	0.67	-	2.4	14.2
β-hCG (mIU/mL)	< 0.6	-	25.4	36.6
LH (mIU/mL)	< 0.07	-	< 0.07	< 0.07

Figure 1. Schematic representation of the hp-hCG-based treatment protocol performed in a woman with selective LH deficiency. Ovarian and endometrium ultrasonographic features and hormonal levels represented in the induced follicular phase (red) and after trigger (green). C-section, caesarean section; D, day of treatment; hp-hCG, highly purified urinary human chorionic gonadotropin.

different, suggesting a complete LH deficiency in the Brazilian patient associated with the homozygous IVS2+1G→C LHB mutation, and a partial deficiency in the Moroccan case due to the p. His30_Ile32del mutation (Achard *et al.*, 2009). The partial activity of the Moroccan mutant was clinically evidenced by preserved spermatogenesis in the male affected patient. It was corroborated by a series of *in vitro* studies showing that the latter mutant protein retains heterodimerisation potential and low levels of secretion and activity. Indeed, the IVS2+1G→C mutation in our case is predicted to disrupt the LHB tertiary structure severely, thus preventing dimerisation with the alpha subunit and consequently leading to the absence of LH secretion and action (Lofrano-Porto *et al.*, 2007).

Despite the lifelong absence of LH secretion and action in the present case, oestradiol production was sufficient for adequate development of the internal genitalia throughout the pubertal and initial adult years, probably driven by FSH-responsive early ovarian follicles. Subsequent amenorrhoea and repeatedly low progesterone serum levels together with the lack of a corpus luteum confirm the essential physiological role of LH in regulating late follicular growth and triggering ovulation. (Richards *et al.*, 2002). At the time of publication, the 'experiment of nature' described by Lofrano-Porto *et al.* (2007) was innovative in supporting the concept that, unlike that in males, female pubertal development may occur in an LH-deficient state, even though the granulosa cells fail to produce sustained high levels of oestradiol associated with larger and preovulatory follicle function, probably owing to limited LH-dependent secretion of androgenic precursors by the theca cells (Themmen and Huhtaniemi, 2000; Lofrano-Porto *et al.*, 2007).

The reproductive phenotype of women who harbour homozygous inactivating mutations in *LHB* gene is phenocopied by *lhb*-knockout female mice (Ma *et al.*, 2004; Arnhold *et al.*, 2009). In these mice, despite the absence of preovulatory follicles, the LH receptor and BMP4 (bone morphogenetic protein-4) were identified in multiple antral follicles at different stages of development. However, the expression of steroidogenic enzymes and cyclooxygenase 2 was suppressed, which mirrors the impaired late follicular development observed in LH-deficient women. In animal experiments, exogenous hCG administration rescued the expression of steroidogenic and ovulatory markers in the ovaries of *lhb*-null mice and elicited a normal response to exogenous gonadotropin induction compared to wild-type controls (Ma *et al.*, 2004).

Traditionally, to improve follicular responses in assisted reproduction cycles, ovulation induction protocols employ exogenous FSH or hMG plus hCG or GnRH analogues to trigger final oocyte maturation, thereby simulating an LH surge (Ovarian Stimulation TEGGO *et al.*, 2020).

In clinical practice, specific subgroups of women suspected of impaired LH function, such as women of an older age or the potential hypo-responders or those with an unexpected low response due to common genetic variants, may benefit from optimised LH supplementation during ovarian stimulation (Alviggi *et al*, 2011; Papaleo *et al*, 2014). In a recent systematic review and meta-analysis, clinical pregnancy and implantation rates and the number of oocytes retrieved were higher for hypo-responders who received recombinant LH supplementation than for those who underwent FSH monotherapy (Conforti *et al*, 2019). Another meta-analysis that compiled different protocols of gonadotropins to support follicular growth also showed that LH addition improves oocyte quality and reduces the amount of FSH needed in antagonists GnRH protocols (Santi *et al.*, 2017). However, other studies remain inconclusive regarding the advantage of recombinant LH or any LH supplementation on live birth rates after medically assisted reproduction (Fábregues *et al.*, 2006; Nyboe

Andersen et al., 2008; Fábregues et al., 2011; Alviggi et al., 2018). A Cochrane systematic review including 36 randomised controlled studies (8125 women) found no difference between rLH combined with rFSH and rFSH alone with respect to the live birth rate (Mochtar et al., 2017). The peculiar case of selective LH deficiency presented here supports the idea that the clinical actions of LH and exogenous hCG might be interchangeable or inevitable in terms of controlled ovulation stimulation in women with deficiencies in LH production or performance.

However, protocols with LH supplementation requires complex, long and expensive treatment. Alternatively, low doses of hp-hCG starting at the mid-follicular phase through the day of ovulation trigger have been proposed, based on the physiological roles of progressively increasing LH action on late follicular maturation and the acquired LH responsiveness of granulosa cells in dominant follicles (Hillier et al., 1994; Filicori et al., 2002; Verberg et al., 2009; Martins et al., 2013).

LH and hCG are heterodimeric glycoprotein hormones, acting on the same receptor (LHCGR) but with specific intracellular-mediated signalling (Santi et al., 2017). *In vitro* models of human granulosa cells demonstrated that hCG is more potent than LH in inducing cAMP production. At the same time, LH leads to preferential activation of the ERK1/2 and AKT pathways (Casarini et al., 2012). Thus, although LH and hCG activate different pathways, whether and how they differently influence the *in vivo* response remains unclear (Santi et al., 2017).

Although unusual, hCG protocols have been proposed to increase the ovulatory response in selected cases of poor responders in previous assisted reproduction technology or women of advanced age (Casarini et al., 2018). Some studies have also indicated a potential risk of ovarian hyperstimulation syndrome based on the longer half-life of hCG and its primarily sustained luteotropic activity (Dinopoulou et al., 2016; Casarini et al., 2018). However, a direct comparison of efficacy among protocols that used LH or hCG was limited by the low number and high heterogeneity of the studies. Thus far, in the absence of scientific evidence to support the choice between LH and hCG for ovarian stimulation, the significantly lower cost of hCG should be considered in the cost evaluation of a specific ART procedure, especially in different specific conditions such as in cases where patients have a suspected LH deficiency and when several cycles may be necessary. Future studies using adequate methods comparing the use of LH and hCG would thus be of great value.

In the present case, we observed that all steps mediated by activation of the common G-protein coupled receptor (LHCGR) proceeded normally after hCG-induced late follicular growth in a healthy ovarian environment with FSH sufficiency, culminating in a normal pregnancy. This finding is noteworthy because our patient had an inactivating mutation leading to a severely defective LHB subunit, whereas LHCGR proteins were structurally and functionally preserved.

In summary, the LH/hCG-driven ovulation induction protocol described herein in a rare case of infertility due to selective LH deficiency provides a proof-of-concept for the efficacy and requirement of exogenous LH or hCG in inducing late-stage follicular development in patients with LH deficiency, once FSH-dependent early follicular growth has been assured. Moreover, it represents a valuable human model of assisted reproduction under controlled gonadotropin administration.

Data availability

The data underlying this article will be shared on request to the corresponding author.

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Authors' roles

Drafting and finalisation of the manuscript: L.M.P.S., B.R.C., A.M.Z., A.L.P.

Critical review and insightful contributions: all authors.

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Conflicts of interest

None.

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