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ASSISTED REPRODUCTION TECHNOLOGIES

Improved pregnancy rates with luteinizing hormone supplementation in patients undergoing ovarian stimulation for IVF

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Abstract
Introduction Luteinizing hormone (LH) is believed to play a role in follicle maturation during the natural cycle. However, the need for co-treatment with recombinant LH (rLH) for controlled ovarian stimulation is controversial.
Purpose The primary objective of our study was to determine if pregnancy rates are improved when rLH is used in addition to rFSH for ovarian stimulation. Secondary outcomes were fertilization rate, implantation rate and live birth rate.
Methods A retrospective cohort study was performed of 1565 IVF or ICSI cycles. Outcomes were compared between ovarian stimulation cycles from 2007 when rLH and rFSH was used (n=765) to 2006 when rFSH only was used (n-800).
Results Improved outcomes were found for rLH + rFSH versus rFSH alone for; pregnancy rate (61% and 54% respectively, p=0.006), live birth rate (49% and 42% respectively, P=0.01), fertilization rate (74% versus 72% respectively, p=0.04 and implantation rate (41% versus 37% respectively, p=0.03).
Conclusions Our large retrospective cohort study showed an improved pregnancy rate and live birth rate with rLH supplementation. This was associated with an improved fertilization and implantation rate and therefore may reflect an improvement in oocyte quality and/or uterine receptivity.

Keywords Luteinizing hormone · In vitro fertilization · Controlled ovarian hyperstimulation · Follicle stimulating hormone · Pregnancy rate · Live birth rate

Capsule Large cohort observation demonstrated improved pregnancy outcomes with the use of lutenizing hormone in addition to follicle stimulating hormone for in-vitro fertilization.

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Introduction

Both follicle stimulation hormone (FSH) and luteinizing hormone (LH) act synergistically for follicle maturation in the natural cycle [4]. In the two cell-two gonadotropin model [7] FSH acts on granulosa cells to stimulate follicle development and estradiol production and LH acts on theca cells to produce androgens. The androgens are then aromatized in the granulosa cell to produce additional estrogen. However, in the later stages of follicle development LH has been found to also promote growth and maturation of mid sized follicles through LH receptors on the granulosa cell [3].

During ovarian stimulation for IVF the pituitary gland is initially suppressed to prevent premature LH surge and ovulation. This pituitary inhibition results in suppression of both endogenous FSH and LH. The use of recombinant LH (rLH) along with recombinant FSH (rFSH) for ovarian stimulation has been shown to be beneficial for patients with hypogonadotrophic hypogonadism [19], however LH supplementation for other patient groups or indications is still controversial.

Prior meta-analyses have not suggested any improvement in pregnancy outcomes with LH supplementation [11,16]. However, a recent study of normogonadotrophic women showed an increase in the number of oocytes collected, number of oocytes in metaphase II, fertilization rate,

implantation rate and live birth rate in those that had LH supplementation [9].

In addition, it is unclear if there may be a specific population of patients or type of stimulation protocol that have improved outcomes with LH supplementation. Specific populations that have been suggested to benefit include patients greater than 35 years of age [1,13,14,16], suboptimal responders to ovarian stimulation ([6, 12, 16]) and those undergoing specifically a GnRH antagonist cycle [2] or GnRH agonist cycles [9].

As an appropriate subgroup to benefit from LH supplementation has not yet been identified, the question still arises whether the co-administration of LH in ovarian stimulation leads to improved cycle outcomes for the general population undergoing ovarian stimulation for IVF/ICSI.

The primary objective of this study was to determine whether pregnancy rates were improved when rLH was used along with rFSH, compared to ovarian stimulation with rFSH alone in a large sample size of patients undergoing IVF/ICSI.

Secondary objectives were to determine whether other markers of successful IVF were improved with rLH supplementation (fertilization rate, implantation rate and live birth rate). In addition, we examined whether there were detrimental effects of rLH co-administration in terms of ovarian hyperstimulation syndrome.

Methods

A retrospective cohort study was performed comparing pregnancy rates from IVF/ICSI cycles completed in 2006 when only rFSH was used in ovarian stimulation protocols, versus 2007 when rLH was introduced as standard clinic protocol for ovarian stimulation in addition to rFSH. Ethical approval for the study was obtained from the University of Calgary, Office of Medical Bioethics (ID number E-23701). During the study period, other confounders were limited between these two years as there were no changes in physicians, embryologists or embryology protocols. An anonymous database from the Regional Fertility Program of all patients undergoing IVF or ICSI cycles between 2006 and 2007 was used.

All IVF or ICSI cycles that resulted in oocyte retrieval were included. Ovarian stimulation cycles that were initiated between January 1, 2006 to December 31, 2006 used rFSH alone, while ovarian stimulation cycles initiated between January 1, 2007 to December 31, 2007 used rLH and rFSH. Third party reproduction cycles such as for donor oocyte or gestational surrogacy were excluded.

Stimulation protocols used either GnRH agonist (Suprefact, Sanofi-Aventis Inc., Laval, Quebec, Canada) for pituitary down-regulation or GnRH antagonist (Cetrotide, EMD Serono Canada Inc., Mississauga, Ontario Canada) to prevent a premature surge. Following baseline ultrasound, ovarian stimulation was performed in 2006 with rFSH alone (Gonal-F, EMD Serono Canada Inc., Mississauga, Ontario, Canada) from day 1 until the day of hCG trigger unless 'coasting' with no rFSH for the last 1–2 days prior to hCG trigger was deemed necessary. In 2007 rFSH and 75 IU of rLH (Luveris, EMD Serono Inc., Mississauga, Ontario, Canada) were used from day 1 until the day of hCG trigger unless 'coasting' with no rFSH or rLH was deemed necessary prior to the trigger day. Response was monitored using serial transvaginal ultrasound and estradiol measurements and dose adjustments were made as necessary. Final oocyte maturation was triggered using human chorionic gonadotropin (hCG) (Chorionic Gonadotropin, Pharmaceutical Partners of Canada Inc., Richmond Hill, Ontario, Canada) when there were ≥2 follicles ≥18 mm in average diameter measured in 2 dimensions. Ultrasound-guided transvaginal oocyte retrieval was performed 35 hours later. Standard insemination or ICSI was used depending on semen parameters. A standard in-house embryo scoring system was used and there was no change in the scoring method during the study period of 2 years. Ultrasound-guided embryo transfer was performed either on day 3 or day 5, and the number of embryos transferred was based on institutional policies that conformed to national guidelines. Urine pregnancy test was performed 16 days later, and if positive, an ultrasound was performed at 7 weeks gestational age. Patients were given vaginal progesterone (Prometrium, Merk Frosst Canada Inc., Kirkland, Quebec, Canada) for luteal support until 10 weeks gestational age.

Statistical analysis

Patient characteristics and stimulation parameters were collected to compare the two cohorts. The primary outcome was total pregnancy rate (defined as the number of positive pregnancy tests per total number of cycles) and clinical pregnancy rate (defined by the number of pregnancies with at least one fetal heart rate on ultrasound per total number of cycles). Secondary outcomes were live birth rate (defined as at least one live infant born >20 weeks at the time of delivery per total number of cycles), fertilization rate (defined as the proportion of oocytes or metaphase II oocytes that fertilized normally per total number of oocytes insemination or injected by ICSI), percentage of usable embryos per patient (defined as the sum of embryos deemed suitable for transfer or cryopreservation per total number of fertilized embryos for each patient) and implantation rate (defined by the number of gestational sacs on ultrasound per total number of embryos transferred).

Data analysis was performed using SPSS software. Categorical data were analysed by the Chi squared test and

continuous data were analysed where appropriate by the Student's t-test or Mann-Whitney U test. Statistical significance was defined by a P value <0.05.

Results

A total of 800 cycles from 2006 (rFSH alone) and 765 cycles from 2007 (rLH + rFSH) were included in the study. Baseline patient characteristics were similar for the two cohorts as shown in Table 1. There was a higher percentage of GnRH agonist protocols performed in 2007 versus 2006, whereas there was a higher percentage of GnRH flare protocols for 2006 versus 2007 (Table 1). The ovarian stimulation cycle parameters were similar between 2006 and 2007 with the same number of oocytes retrieved, percentage of cycles that involved ICSI, percentage of cycles in which an embryo transfer occurred and number of embryos transferred (Table 2).

Our primary outcome of pregnancy rate was significantly higher in 2007 when rLH was used in addition to rFSH for ovarian stimulation versus 2006 when only rFSH was used. The rate was higher for both the total pregnancy rate (61% in 2007 compared to 54% in 2006; P=0.006) and for clinical pregnancy rate (56% in 2007 compared to 49% in 2006; P=0.005). For both outcomes the odds ratio was 1.3, with a 95% confidence interval of 1.1–1.6 (see Table 2). There was no significant difference in the percentage of heterotopic (0.1% each year) or ectopic (0.4% each year) pregnancies in the study groups.

The improved pregnancy rate in rLH supplemented cycles also translated to a higher live birth rate at 49% for 2007 versus 41% for 2006, P=0.010 (see Table 2). The multiple pregnancy rate was similar between 2006 and 2007 (triplets 0.9% and 0.8% respectively and twins 36% and 32% respectively).

The percentage of usable embryos per patient (defined as the sum of embryos deemed suitable for transfer or cryopreservation per total number of fertilized embryos for each patient) was not different between the two cohorts (Table 2), with the mean number of cryopreserved embryos also being similar between the two groups (5.3 for rFSH and 5.0 for rFSH + rLH). However, cycles supplemented with rLH demonstrated an improvement in both fertilization and implantation rates compared to cycles that used rFSH only (Table 2).

There was no significant difference in the rates of ovarian hyperstimulation syndrome (OHSS) between the two cohorts. A total of 13 patients per year (1.6% in 2006 and 1.7% in 2007) were diagnosed with severe OHSS requiring hospitalisation (P=0.9).

Table 1 Cohort characteristics

		2006 (rFSH)	2007 (rLH + rFSH)
	Age	35 yr±4 yr	35 yr±4 yr
Fertility History	Prior pregnancy	46%	46%
	Prior live birth	26%	21%
	Prior ART cycle	24%	19%
Infertility Etiology[a]	Male Infertility	40%	41%
	Unexplained Infertility	38%	36%
	Tubal Infertility	19%	19%
Stimulation Cycle Protocol	GnRH antagonist	5%	4%
	GnRH 'flare'	25%	18%
	GnRH agonist	70%	78%

[a] Etiologic factors do not amount to 100% as one or more causes may have been identified. Ovulatory dysfunction, diminished ovarian reserve and uterine factors were not collected in both years and therefore could not be compared.

Discussion

Our results showed an improvement in pregnancy rates (both total and clinical) as well as live birth rate with rLH supplementation during ovarian stimulation for IVF/ICSI. In addition, there was improved fertilization and implantation rates with rLH supplementation. Despite being limited by the retrospective design and the need for comparison between two different time periods, we were unable to identify any major confounders as the practice patterns, protocols and personnel were similar between the two years. On the other hand, the retrospective nature of the study allowed for a large sample size of patients undergoing ovarian stimulation in a single centre.

A previous meta-analysis of randomised controlled trials showed that pregnancy and live birth rates were not different between cycles using rLH supplementation versus rFSH alone [11]. However, it was cautioned in this review that the sample size available through the 7 RCTs was not adequate to determine clinical significance. In addition, only three of the RTCs (for a total n=187) used rLH supplementation in the early follicular phase as was used in our current study. Another meta-analysis [16] also did not find a significant difference in pregnancy or live birth rates but commented that the pooled pregnancy rate estimates pointed towards a beneficial effect of co-treatment with rLH, in particular with respect to decreased pregnancy-loss and improved pregnancy rates in poor-responders.

In keeping with our results, Bosch et al. [1] recently showed an improved implantation rate and a trend towards improved pregnancy rate. However, patients in the rFSH-only group were started at a higher dose of rFSH than those in the rFSH + rLH group, instead of the usual practice of tailoring an appropriate starting dose to the individual

Table 2 Ovarian stimulation outcomes

	2006 (rFSH) n=800	2007 (rLH+rFSH) n=765	p value
Number of Oocytes Retrieved	15.2±8.3	15.0±7.5	NS
ICSI Performed	512 (68%)	499 (69%)	NS
Usable Embryos	81%	78%	NS
Embryo Transfer Occurred	750 (94%)	718 (94%)	NS
Day of Embryo Transfer	3.1±0.4	3.1±0.5	NS
Number of Transferred Embryos	2.3±1.1	2.3±1.1	NS
Fertilization Rate	72%	74%	0.040
Implantation Rate	37%	41%	0.030
Total Pregnancy Rate	428 (54%)	463 (61%)	0.006
Clinical Pregnancy	394 (49%)	432 (56%)	0.005
Live Birth	337 (42%)	372 (49%)	0.010

$p<0.05$ considered significant

patient. This automatic lower starting dose of rFSH when rLH supplementation was used may have mitigated beneficial effects from the rLH supplementation. Interestingly Bosch [1] found that benefits associated with LH supplementation were only for patients greater than 35 years of age. Although our present study was not powered to find subgroup differences, a trend was seen for improved outcomes with rLH supplementation in both patients <35 years and those ≥35 years of age. When rLH + rFSH was compared to rFSH alone for patients <35 yrs of age, clinical pregnancy rate was 65% versus 58% and live birth rate 60% versus 53% respectively. Similarly, when rLH + rFSH was compared to rFSH alone for patients ≥35 yrs of age, clinical pregnancy rate was 49% versus 42% and live birth rate was 38% versus 33% respectively.

Improved outcomes have also been shown with rLH supplementation for patients who undergo GnRH agonist protocols [9]. An improvement was found in the number of oocytes retrieved, metaphase II oocytes and fertilization rate. This retrospective study also noted a trend towards higher implantation, pregnancy and live birth rates in the LH supplementation group. However, the study sample size was only 244 patients and therefore may have been underpowered to be able to show statistically significant differences. In our study GnRH agonist protocols comprised 70% (rFSH) and 78% (rLH + rFSH) of the stimulation protocols respectively, the trend for improved pregnancy rates with rLH + rFSH versus rFSH alone was still apparent (clinical pregnancy rate 57% versus 52% respectively and live birth rate 49% versus 44% respectively).

In a meta-analysis by Bosch et al. (2007) it was shown that with LH supplementation during GnRH antagonist stimulation cycles there was an improvement in the number of mature oocytes retrieved, however clinical outcomes were not statistically significant. In the present study the percentage of GnRH antagonist protocols was small (5% for rFSH and 4% for rLH + rFSH) and therefore this limits the ability to perform subgroup analysis for this specific stimulation protocol.

Studies have tried to define other subgroups that may benefit from LH supplementation. It has been suggested that rLH supplementation is effective in young, normogonadotrophic patients who demonstrate a suboptimal ovarian response to initial rFSH stimulation [6] or who had required high doses of rFSH in a previous cycle [12]. Unfortunately, we are unable to determine the number of 'suboptimal responders' within our study group to perform such a subgroup analysis. Furthermore, the definition of 'poor or suboptimal' response has been fraught with inconsistency in research studies. As this group of patients becomes better defined [8], this will facilitate more appropriate patient selection for studies and hence more meaningful analysis for clinical application.

Possible reasons for improved fertilization and implantation rates in our study with rLH supplementation include enhanced oocyte quality, embryo quality and/or uterine receptivity. This hypothesis has also been suggested in other studies. Franco et al. [9] found an increase in the number of mature oocytes, fertilization rate and implantation rate with LH supplementation that may be markers suggestive of improved quality. A role for LH in embryo development has also been suggested in bovine co-culture studies where elevated pre-ovulatory serum LH levels promoted oviduct embryo development [15]. Other studies exploring novel roles of LH have identified LH receptors in the uterine vasculature, suggesting the possibility that LH may have an important role in implantation by increasing uterine blood flow through vasodilatation and perhaps through angiogenesis and trophoblast invasion [17].

It has been hypothesised that there may be a minimal LH threshold (<0.5 IU/L) for adequate oocyte maturation [18] as well as an upper LH threshold for optimal oocyte development [10]. The optimal therapeutic range however has not yet been elucidated. Cheung et al. [5] demonstrated

comparable follicle development and successful pregnancies in subjects receiving rFSH alone compared to those receiving rFSH + rLH despite profound LH suppression as low as <0.5 IU/L. However, this was studied only in patients with polycystic ovarian syndrome, and pregnancy outcome was not the primary objective of the study as only 24 patients were examined. In our current study we did not have initial hormone profiles available to examine baseline LH levels, and therefore we are unable to identify if there was an association between outcomes and LH levels.

Conclusion

Our study suggests that pregnancy outcomes may be improved when rLH is co-administered with rFSH in ovarian stimulation for IVF/ICSI without obvious detrimental effects. Whether the mechanism of benefit is through enhanced quality of oocytes, embryos or uterine receptivity remains to be elucidated. In vitro studies are still pending to better understand the role of LH in embryo development and implantation. In addition, further clinical studies are required to identify an optimal LH therapeutic range and to clearly define the subpopulation of patients who would benefit most from LH supplementation.

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