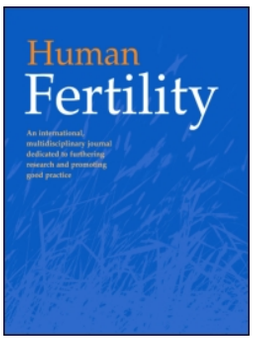

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ORIGINAL ARTICLE

Concomitant use of FSH and low-dose recombinant hCG during the late follicular phase versus conventional controlled ovarian stimulation for intracytoplasmic sperm injection cycles

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ABSTRACT

The objective of this study was to investigate the effects of low-dose hCG supplementation on ICSI outcomes and controlled ovarian stimulation (COS) cost. Three hundred and thirty patients undergoing ICSI were split into groups according to the COS protocol: (i) control group ($n = 178$), including patients undergoing conventional COS treatment; and (ii) low-dose hCG group ($n = 152$), including patients undergoing COS with low-dose hCG supplementation. Lower mean total doses of FSH administered and higher mean oestradiol level and mature oocyte rates were observed in the low-dose hCG group. A significantly higher fertilization rate, high-quality embryo rate and blastocyst formation rate were observed in the low-dose hCG group as compared to the control group. The miscarriage rate was significantly higher in the control group compared to the low-dose hCG group. A significantly lower incidence of OHSS was observed in the low-dose hCG group. There was also a significantly lower gonadotropin cost in the low-dose hCG group as compared to the control group ($1235.0 ± 239.0 × $1763.0 ± 405.3, $p < 0.001$). The concomitant use of low-dose hCG and FSH results in a lower abortion rate and increased number of mature oocytes retrieved, as well as improved oocyte quality, embryo quality and blastocyst formation and reduced FSH requirements.

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ICSI; infertility; ovarian stimulation

Introduction

Controlled ovarian stimulation (COS) is an essential step of assisted reproductive techniques (ART) that induces the recruitment and development of multiple follicles, thus enabling the retrieval of many oocytes in order to improve the chance of pregnancy, through conventional *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) (Fauser & Devroey, 2003).

The most important hormone in the stimulation of ovarian folliculogenesis is follicle-stimulating hormone (FSH) (Bentov, Kenigsberg, & Casper, 2012), and thus, the main agents used in COS are gonadotropin preparations. These preparations are administered in association with gonadotropin-releasing hormone (GnRH) agonists or antagonists to prevent a premature luteinizing hormone (LH) surge (Macklon, Stouffer, Giudice, & Fauser, 2006).

Gonadotropin preparations are fairly expensive (Verberg et al., 2009). A less-costly ART treatment can be obtained through a reduction in the amount of FSH administered. Sullivan, Stewart-Akers, Krasnow, Berga, and Zeleznik (1999) described an innovative COS protocol, based on sequential FSH administration during early follicle development followed by the addition of human chorionic gonadotropin (hCG) or LH in late folliculogenesis. Because granulosa cells begin to express LH receptors after the initial FSH-induced follicular growth (Shima, Kitayama, & Nakano, 1987; Takao et al., 1997), LH or hCG could replace FSH in late folliculogenesis to complete COS.

The application of low-dose hCG or LH has been investigated in both GNRH agonist (Aflatoonian, Yousefnejad, Eftekhar, & Mohammadian, 2012; Filicori et al., 1999b, 2002a 2002b, 2005) and GnRH antagonist protocols (Blockeel et al., 2009; Koichi, Yukiko, Shima, & Sachiko, 2006). Several studies have demonstrated that adding LH activity through the administration of low-dose hCG (50–200 IU) in the late stages of COS supports full follicle development (Fauser & Van

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Heusden, 1997; Filicori & Cognigni, 2001; Filicori et al., 1999a, 1999b, 2001, 2002a, 2002b, 2005; Sullivan et al., 1999; Thompson et al., 1995) and successful pregnancies (Koichi et al., 2006; Serafini et al., 2006). Additionally, it shortens the stimulation interval and significantly reduces FSH requirements and treatment costs (Filicori et al., 2002a; Maldonado, Franco, Setti, Iaconelli, & Borges, 2013).

Although there is some evidence that LH activity may be useful to sustain ART cycle outcomes at reduced cost, it is unknown whether supplementation with low-dose hCG in the late stages of COS is associated with improved oocyte quality and embryo development. The objective of this study was to investigate the effects of low-dose hCG supplementation on: (i) oocyte quality, (ii) embryo quality; (iii) ICSI cycle outcome; and (iv) treatment costs in patients undergoing ART.

Materials and methods

Study design

This retrospective cohort trial included patients undergoing their first ICSI cycle at a private university-affiliated IVF centre between May 2007 and May 2012. In our centre, we have an IVF assistance programme for low-income infertile couples. For those patients, ovarian stimulation is achieved by the administration of recombinant FSH and low-dose hCG. In patients undergoing IVF at our private clinic, ovarian stimulation is achieved by the administration of recombinant FSH only. Therefore, the 330 enrolled patients were split into groups according to the COS protocol: (i) the control group ($n = 178$), which included patients undergoing conventional COS; and (ii) the low-dose hCG group ($n = 152$), which included patients undergoing COS with low-dose hCG supplementation. Groups were compared regarding laboratory and clinical outcomes and the cost of treatment.

To be selected for the present study, patients had to meet the inclusion criteria as follows: (i) age <36 years old; (ii) body mass index (BMI) ≥ 20 and <30 kg/m^2; (iii) regular menstrual cycles of 25–35 days; (iv) the presence of both ovaries and an intact uterus; (v) basal FSH <10 mIU/mL; (vi) normal karyotype for both male and female partners; (vii) the absence of endometriosis III and IV; (viii) the absence of hydrosalpinx; (ix) the absence of previous oncological treatments; (x) the absence of important systemic disease; and (xi) negative result in a screening for sexually transmitted diseases. All cases of severe spermatogenic alteration, including frozen and surgically retrieved sperm, were also excluded from the study.

Written informed consent was obtained in which patients agreed to share the outcomes of their own cycles for research purposes, and the study was approved by the Research Ethics Committee from the Faculdade de Medicina de Jundiaí (reference number: 410/2012).

Controlled ovarian stimulation protocols

Controlled ovarian stimulation was achieved by pituitary blockage using a GnRH antagonist (Cetrotide, Serono, Geneva, Switzerland), and ovarian stimulation was performed using either exclusively recombinant FSH (Gonal-F; Serono, Geneva, Switzerland) or recombinant FSH along with recombinant hCG (Ovidrel®, Serono, Geneva, Switzerland).

For patients in the control group, COS was performed as follows: on day three of the cycle, ovarian stimulation commenced with 225 IU of recombinant FSH on a daily basis until the day of ovulation trigger. When at least two follicles $\geq$14 mm were visualized by ultrasound, patients received 0.25 mg cetrorelix acetate as a GnRH antagonist subcutaneously (SC), until the day of ovulation trigger (Figure 1(a)).

Patients in the low-dose hCG group received 225 IU of recombinant FSH on day three on a daily basis (day 1 of ovarian stimulation = S1), for three days. On S4, the recombinant FSH dose was reduced to 150 IU until the visualization of at least two follicles $\geq$14 mm, at which time we began the administration of cetrorelix acetate 0.25 mg subcutaneously (SC). The day after starting antagonist therapy, the recombinant FSH dose was reduced to 75 IU and the concomitant SC administration of recombinant low-dose hCG (7.7 μg, equivalent to 200 IU hCG), which was obtained via the dilution of one ampoule of 250 μg of r-hCG (Ovidrel®; EMD Serono, Inc., Rockland, MA), was initiated and continued for 2 days. After that, recombinant hCG and the GnRH antagonist were administered until the day of ovulation trigger (Figure 1(b)). Both ovarian stimulation protocols were based on our previously published data (Cavagna et al., 2010; Maldonado et al., 2013).

The subsequent steps of the treatment were the same for both treatment regimens. When three or more follicles attained a mean diameter of $\geq$17 mm, hCG 250 μg was administered SC. Oocyte retrieval was performed 36 hours later, through transvaginal ultrasonography.

Preparation of oocytes

Retrieved oocytes were maintained in culture medium (Global® for fertilization, LifeGlobal, CT) supplemented

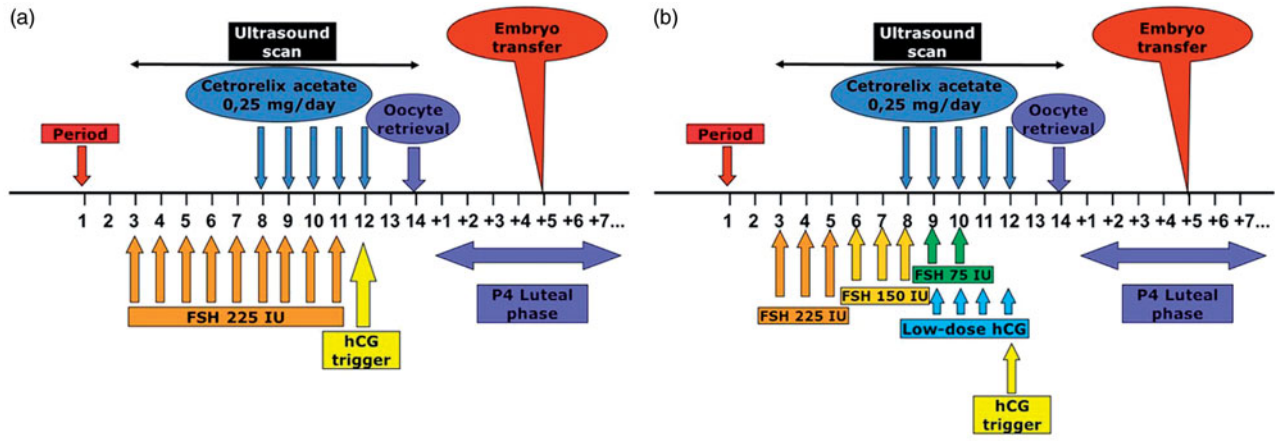

Figure 1. Controlled ovarian stimulation protocol using (a) recombinant FSH; and (b) recombinant FSH concomitantly with recombinant low-dose hCG.

with 10% protein supplement (LGPS, LifeGlobal, CT) and covered with paraffin oil (Paraffin oil P.G., LifeGlobal, CT) for two to three hours before the removal of cumulus cells. The surrounding cumulus cells were removed after exposure to a HEPES-buffered medium containing hyaluronidase (80 IU/mL, LifeGlobal, CT) and any remaining cumulus cells were mechanically removed by gently pipetting with a hand-drawn Pasteur pipette (Humagen Fertility Diagnostics, VA).

Oocyte morphology was assessed just before sperm injection (4 h after retrieval) using an inverted Nikon Diaphot microscope (Eclipse TE 300; Nikon®, Tokyo, Japan) with a Hoffmann modulation contrast system under 400X magnification. The following oocyte dimorphisms were recorded: (i) cytoplasmic granularity; (ii) vacuoles in the ooplasm; (iii) smooth endoplasmic reticulum clusters (sERC) in the ooplasm; (iv) large perivitelline space (PVS); (v) PVS granularity; (vi) fragmented polar body (PB); (vii) zona pellucida (ZP) abnormalities; and (viii) oocyte shape abnormalities.

Oocytes that had released the first polar body were considered mature and were used for ICSI.

Intracytoplasmic sperm injection

Intracytoplasmic sperm injection was performed in a microinjection dish prepared with 4 μL droplets of buffered medium (Global® w/HEPES, LifeGlobal, CT) and covered with paraffin oil on the heated stage of an inverted microscope (37.0 ± 0.5 °C). Approximately, 16 h after ICSI, fertilization was confirmed by the presence of two pronuclei and the extrusion of the second polar body. Embryos were maintained in a 50 μL drop of culture medium (Global®, LifeGlobal, CT) supplemented with 10% protein supplement and covered with paraffin oil in a humidified atmosphere under 6% CO_2 at 37 °C for three days.

Embryo morphology evaluation and embryo transfer

Embryo morphology was assessed at the zygote stage (16–18-h post-ICSI) and on the mornings of days two, three and five of embryo development using an inverted Nikon Diaphot microscope (Eclipse TE 300; Nikon, Tokyo, Japan) with a Hoffmann modulation contrast system under 400X magnification. Immediately before embryo transfer, the embryo morphology was also assessed.

To evaluate the cleavage-stage morphology, the following parameters were recorded: (i) the number of blastomeres; (ii) the percentage of fragmentation; (iii) the variation in blastomere symmetry; (iv) the presence of multinucleation; and (v) defects in the zona pellucida and cytoplasm. High-quality cleavage-stage embryos were defined as those with all of the following characteristics: (i) four cells on day two or 8 – 10 cells on day three; (ii) < 15% fragmentation; (iii) symmetric blastomeres; (iv) the absence of multinucleation; (v) colourless cytoplasm with moderate granulation and no inclusions; (vi) the absence of perivitelline space granularity; and (vii) the absence of zona pellucida dimorphisms. Embryos lacking any of these characteristics were considered to be of low quality.

To evaluate the blastocyst, embryos were given a numerical score from one to six based on their degree of expansion and hatching status, as follows: (i) an early blastocyst with a blastocoel less than half the volume of the embryo; (ii) a blastocyst with a blastocoel greater than half the volume of the embryo; (iii) a full blastocyst with a blastocoel that completely filled

the embryo; (iv) an expanded blastocyst; (v) a hatching blastocyst; and (vi) a hatched blastocyst. Full blastocysts, expanded blastocysts, hatching blastocysts and hatched blastocysts were considered as those that had reached the blastocyst stage.

All embryo transfers were performed by the same gynaecologist, at the blastocyst stage, using a soft catheter with transabdominal ultrasound guidance. Up to three embryos were transferred per patient.

Luteal-phase support

Luteal-phase support was provided by vaginal administration of 200 mg of micronized progesterone (Utrogestan®; Farmoquímica, Rio de Janeiro, Brazil), three times a day starting one day after oocyte retrieval, and continued until 12 weeks of gestation in the presence of a positive hCG test.

Clinical follow-up

A pregnancy test was performed 10 days after embryo transfer. All women with a positive test had a transvaginal ultrasound scan two weeks after the positive test. A clinical pregnancy was diagnosed when the fetal heartbeat was detected. Pregnancy rates were calculated per transfer. Implantation rates were calculated by dividing the number of gestational sacs by the number of transferred embryos. Miscarriage was defined as pregnancy loss before 20 weeks. Miscarriage rates were calculated per clinical pregnancy.

OHSS was clinically diagnosed by the presence of symptoms such as abdominal distension and pain, nausea, vomiting, diarrhoea, shortness of breath, accumulation of fluid in the abdominal/pelvic cavity, increased ovarian volume, serum oestradiol >5000 pg/dl, and the presence of 30 or more follicles on the day of hCG trigger. In these cases, all embryos were cryopreserved and transferred in a subsequent cycle, after endometrial preparation.

Statistical analysis

Sample size calculations were based on the assumption that a 15% difference in implantation rate would mean a clinically significant difference. Consequently, to achieve this difference, at least 68 cycles would be needed in each treatment group (with a significance level of 5% and power of 85%).

Data are expressed as mean ± standard deviation for continuous variables; percentages were used for categorical variables. Mean values were compared by Student's *t* parametric test or Mann–Whitney non-parametric test. Percentages were compared by the Chi-squared or Fisher exact test, only when the expected frequency was five or fewer.

Logistic regression analysis was performed to evaluate the influence of the COS protocol on oocyte dimorphisms, embryo quality and blastocyst formation chance. The regression analyses were adjusted for maternal age and type of infertility, as these variables are considered potential confounders in the association between the COS protocol and response variables. Results were expressed as odds ratios (OR), 95% confidence intervals (CI) and *p* values.

Results were considered to be significant at the 5% critical level ($p < 0.05$). Data analysis was conducted using Minitab 16 Software (Minitab Inc., PA).

Results

Patient characteristics

This study included 330 patients that were split into two groups according to the COS protocol: (i) 152 were allocated to the low-dose hCG group; and (ii) 178 to the control group. Patient demographic variables are shown in Table 1. The overall ($n = 330$) mean age was 29.1 ± 6.9 years and BMI was 23.9 ± 3.6 kg/m^2. The two treatment groups were similar with respect to female age and BMI: the mean female age and BMI in the low-dose hCG group were 29.1 ± 5.7 years and

Table 1. Patient demographic characteristics and stimulation outcomes in the low-dose hCG and control groups.

Variables	Low-dose hCG group ($n = 152$)	Control group ($n = 178$)	*p* value
Female age (years)	29.1 ± 5.7	29.2 ± 7.8	0.916
BMI (kg/m^2)	24.3 ± 3.4	23.6 ± 3.6	0.990
Total dose of FSH (IU)	1434 ± 277	2051 ± 472	<0.001
Causes of infertility			
Male factor	94/152 (61.8)	108/178 (60.7)	0.828
Male and female factor	20/152 (13.2)	23/178 (12.9)	0.949
Tubal factor	22/152 (14.5)	26/178 (14.6)	0.973
Unexplained infertility	16/152 (10.5)	21/178 (11.8)	0.715
Oestradiol levels on the day of hCG trigger (pg/ml)	2767 ± 1830	1310 ± 1017	<0.001
Number of aspirated follicles	20.7 ± 11.9	20.7 ± 14.2	0.980
Number of retrieved oocytes	13.5 ± 7.9	15.3 ± 9.8	0.078
MII oocyte rate (%)	78.9	72.8	0.004

BMI: Body mass index; IU: International unit; MII: metaphase II.
Note: values are mean ± SD, unless otherwise noted.

Table 2. ICSI outcome in the low-dose hCG and control groups.

Variables	Low-dose hCG group ($n = 152$)	Control group ($n = 178$)	p value
Fertilization rate (%)	79.1	70.9	<0.001
High quality embryos rate on D3 (%)	78.8	58.2	0.007
Blastocyst formation rate (%)	32.7	23.6	<0.001
Number of transferred embryos	2.0 ± 0.8	1.9 ± 0.9	0.180
Implantation rate (%)	26.9	26.7	0.963
Pregnancy rate (per transfer) (%)	44.4	46.7	0.702
Miscarriage rate (%)	4.7	17.1	0.022

Note: values are mean ± SD, unless otherwise noted.

Table 3. Influence of the low-dose hCG protocol on oocyte morphology.

Oocyte dysmorphisms	OR	95% CI	p value
Intracytoplasmic granulation	0.14	0.05–0.39	<0.001
Intracytoplasmic sERC	0.45	0.22–0.92	0.021
Intracytoplasmic vacuole	1.21	0.96–1.52	0.103
PVS granularity	1.04	0.80–1.36	0.757
PVS size	1.03	0.70–1.51	0.892
ZP abnormalities	0.92	0.70–1.20	0.526
Shape abnormalities	0.98	0.55–1.75	0.949
Fragmented PB	1.25	0.87–2.16	0.119

CI: confidence interval; OR: odds ratio; PB: polar body; PVS: perivitelline space; sERC: smooth endoplasmic reticulum cluster; ZP: zona pellucida.

24.3 ± 3.4 kg/m^2, respectively, whereas in the control group, these values were 29.2 ± 7.8 years and 23.6 ± 3.6 kg/m^2, respectively. No relevant differences were found between the low-dose hCG group and the control group for the causes of infertility, which were male factor (61.8% and 60.7%, respectively), male and female factor (13.2% and 12.9%, respectively), tubal factor (14.5% and 14.6%, respectively) and unexplained infertility (10.5% and 11.8%, respectively) (Table 1).

Stimulation and ICSI outcomes

A lower mean total dose of FSH administered (1434 IU ±277 vs. 2051 IU ±472, $p < 0.001$) and a higher mean oestradiol level on the day of ovulation trigger (2767 pg/ml ±1830 vs. 1310 pg/ml ±1017, $p < 0.001$) were observed in the low-dose hCG group as compared to the control group. The number of follicles and retrieved oocytes as well as the oocyte yield were similar between the groups; however, a significantly higher mature oocyte rate (78.9% vs. 72.8%, $p = 0.004$) was observed in the low-dose hCG group (Table 1).

A significantly higher fertilization rate (79.1% vs. 70.9%, $p < 0.001$), high-quality embryo rate (78.8% vs. 58.2%, $p = 0.007$) and blastocyst formation rate (32.7% vs. 23.6%, $p < 0.001$, OR: 1.57, CI: 1.23–2.01, $p < 0.001$) were observed in the low-dose hCG group as compared to the control group. The mean number of embryos transferred, implantation and pregnancy rates were not significantly different between the groups; however, the miscarriage rate was significantly higher in the control group as compared to the low-dose hCG group (17.1% vs. 4.7%, $p = 0.022$, OR: 0.24, CI: 0.06–0.89, $p = 0.018$) (Table 2). Additionally, a significantly lower incidence of OHSS was observed in the low-dose hCG group (1.3% vs. 6.7%, $p = 0.025$).

Regarding oocyte quality, a significantly lower rate of oocytes presenting intracytoplasmic dimorphisms was observed in the low-dose hCG group (0.9% vs. 5.3%, $p < 0.001$, OR: 0.17, CI: 0.07–0.43, $p < 0.001$), especially the presence of sERC (OR: 0.45, CI: 0.22–0.92, $p = 0.021$) and intracytoplasmic granularity (OR: 0.14, CI: 0.05–0.39, $p < 0.001$) (Table 3).

Finally, we observed a significantly lower gonadotropin cost in the low-dose hCG group as compared to the control group ($1235.0 ± 239.0 × $1763.0 ± 405.3, $p < 0.001$).

Discussion

The results from this study suggest that the concomitant use of low-dose hCG and FSH does not reduce the chance of implantation and pregnancy and results in a reduced rate of abortion. In addition, it increased the number of mature oocytes retrieved and the oocyte quality, expending less FSH. It also resulted in a higher fertilization rate, an increased high-quality cleavage-stage embryo rate and increased blastocyst formation rate, with a lower incidence of OHSS.

Our results are in accordance with those obtained by Filicori et al. (2005), who demonstrated that low-dose hCG administration is associated with a more oestrogenized intrafollicular environment, fewer small preovulatory follicles, higher fertilization rates, and no signs of premature luteinization. Additionally, Lee, Miller, Elkinnd-Hirsch, and Scott (2004) showed that advantages of this protocol also include lower consumption of FSH and COS cost, and a follicle pattern that may predispose patients to a reduced risk of OHSS.

Previous reports demonstrated that profound suppression of LH through the use of GnRH agonists (Coppola et al., 2003; Fleming et al., 1998; Westergaard, Laursen, & Andersen, 2000) or antagonists (Al-Inany et al., 2011) may lead to undesirable results. This suggests that supplementation with recombinant LH or hCG could benefit patients undergoing COS. Previous studies have shown encouraging outcomes when hCG supplementation was performed in GnRH-agonist (Beretsos et al., 2009; Berkkanoglu, Isikoglu, Aydin, & Ozgur, 2007; De Placido et al., 2005; Drakakis et al., 2009; Filicori et al., 2002a, 2002b, 2005;

Gomes et al., 2007) and GnRH-antagonist cycles (Koichi et al., 2006; Serafini et al., 2006; Van Horne, Bates, Robinson, Arthur, & Propst, 2007), when compared to conventional COS cycles. A recently published Cochrane Review (Martins, Vieira, Figueiredo, & Nastri, 2013) suggested that the use of low-dose hCG does not decrease the chance of pregnancy when compared with the conventional COS protocol.

In the present study, low-dose hCG supplementation resulted in superior oocyte and embryo quality but not a higher rate of pregnancy. Embryo implantation depends not only on the proper embryo development, but also on the acquisition of a receptive endometrium, and the proper dialogue between (Dominguez, Pellicer, & Simon, 2003). In addition, it has been suggested that pregnancy rate is inversely related to serum progesterone levels on the day of hCG administration (Bosch et al., 2010; Hamdine et al., 2014; Wu et al., 2012; Xu et al., 2012), regardless of oocyte quality (Xu et al., 2012). Raised concentration of progesterone in the late follicular phase is likely to influence the secretory changes of the endometrium leading to an asynchrony between embryo and endometrial dialogue, which may result in reduced implantation (Bourgain & Devroey, 2003).

We also observed a decreased incidence of oocyte defects, such as sERC and intracytoplasmic granularity, was observed when low-dose hCG was used. Indeed, the impact of oocyte morphology on embryo development has been suggested by many authors (Ebner et al., 2000; Suppinyopong, Choavaratana, & Karavakul, 2000; Wilding et al., 2007; Xia, 1997). There is growing evidence to support the hypothesis that elevated FSH is an underlying cause of low oocyte quality. Recently, the concept that 'more may not be better' has started to emerge (Inge, Brinsden, & Elder, 2005) and led to the acceptance of 'minimal-stimulation' and 'patient-friendly' protocols (Revelli, Casano, Salvagno, & Delle Piane, 2011), in which individualized approaches allow for the optimization of oocyte yield while avoiding the risk of OHSS (Kovacs, Sajgo, Kaali, & Pal, 2012).

The aforementioned hypothesis may also explain the lower miscarriage rate observed among patients in the low-dose hCG group. Previous reports showed that women with low LH concentrations had a significantly increased risk of early pregnancy loss (Westergaard et al., 2000).

An important implication of the present study is the fact that the introduction of low-dose hCG during COS led to a higher number of oocytes retrieved while expending less FSH, and because this hormone is a relatively expensive drug, a less costly treatment was achieved. Therefore, this protocol may be a suitable option for developing countries, where ART treatments do not qualify for reimbursement and for couples with limited financial resources.

Our study has some limitations. As mentioned earlier, serum progesterone and LH levels were not obtained. In addition, the evaluation of cases was retrospective, and there was unavoidable selection bias. These limitations could be overcome by conducting a large-scale prospective study in the near future.

In conclusion, our evidence suggests that hCG supplementation can be used to reduce the FSH dose required in COS protocols, resulting in treatment that is significantly less costly. Moreover, supplementation with low-dose hCG may increase oocyte quality, fertilization rate, embryo quality and blastocyst formation. However, its effect on implantation and pregnancy remains controversial.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

Aflatoonian, A., Yousefnejad, F., Eftekhar, M., & Mohammadian, F. (2012). Efficacy of low-dose hCG in late follicular phase in controlled ovarian stimulation using GnRH agonist protocol. *Archives of Gynecology and Obstetrics*, *286*, 771–775. doi: 10.1007/s00404-012-2337-z.

Al-Inany, H.G., Youssef, M.A., Aboulghar, M., Broekmans, F., Sterrenburg, M., Smit, J., & Abou-Setta, A.M. (2011). Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. *Cochrane Database of Systematic Reviews*, 2011, CD001750. doi: 10.1002/14651858.CD001750.pub3.

Bentov, Y., Kenigsberg, S., & Casper, R.F. (2012). A novel luteinizing hormone/chorionic gonadotropin receptor mutation associated with amenorrhea, low oocyte yield, and recurrent pregnancy loss. *Fertility and Sterility*, *97*, 1165–1168. doi: 10.1016/j.fertnstert.2012.02.002.

Beretsos, P., Partsinevelos, G.A., Arabatzi, E., Drakakis, P., Mavrogianni, D., Anagnostou, E., … Loutradis, D. (2009). "hCG priming" effect in controlled ovarian stimulation through a long protocol. *Reproductive Biology and Endocrinology*, *7*, 91. doi: 10.1186/1477-7827-7-91.

Berkkanoglu, M., Isikoglu, M., Aydin, D., & Ozgur, K. (2007). Clinical effects of ovulation induction with recombinant follicle-stimulating hormone supplemented with recombinant luteinizing hormone or low-dose recombinant human chorionic gonadotropin in the midfollicular phase in microdose cycles in poor responders. *Fertility and Sterility*, *88*, 665–669. doi:10.1016/j.fertnstert.2006.11.150.

Blockeel, C., De Vos, M., Verpoest, W., Stoop, D., Haentjen, P., & Devroey, P. (2009). Can 200 IU of hCG replace recombinant FSH in the late follicular phase in a GnRH-antagonist cycle? A pilot study. *Human Reproduction*, *24*, 2910–2916. doi: 10.1093/humrep/dep253.

Bosch, E., Labarta, E., Crespo, J., Simón, C., Remohí, J., Jenkins, J., & Pellicer, A. (2010). Circulating progesterone levels and ongoing pregnancy rates in controlled ovarian stimulation cycles for in vitro fertilization: analysis of over 4000 cycles. *Human Reproduction*, *25*, 2092–2100. doi: 10.1093/humrep/deq125.

Bourgain, C., & Devroey, P. (2003). The endometrium in stimulated cycles for IVF. *Human Reproduction Update*, *9*, 515–522. doi: 10.1093/humupd/dmg045.

Cavagna, M., Maldonado, L.G., de Souza Bonetti, T.C., de Almeida Ferreira Braga, D.P., Iaconelli, Jr., A., Borges., & Jr, E. (2010). Supplementation with a recombinant human chorionic gonadotropin microdose leads to similar outcomes in ovarian stimulation with recombinant follicle-stimulating hormone using either a gonadotropin-releasing hormone agonist or antagonist for pituitary suppression. *Fertility and Sterility*, *94*, 167–172. doi: 10.1016/j.fertnstert.2009.02.075.

Coppola, F., Poti, E.R., Barusi, L., Ferrari, B., Salvarani, M.C., & Vadora, E. (2003). Profound luteinizing hormone suppression induces a deleterious follicular environment during assisted reproduction technology. *Fertility and Sterility*, *79*, 459–460. doi: 10.1016/S0015-0282(02)04671-X.

De Placido, G., Alviggi, C., Perino, A., Strina, I., Lisi, F., Fasolino, A., … Mollo, A. Italian Collaborative Group on Recombinant Human Luteinizing Hormone. (2005). Recombinant human LH supplementation versus recombinant human FSH (rFSH) step-up protocol during controlled ovarian stimulation in normogonadotrophic women with initial inadequate ovarian response to rFSH. A multicentre, prospective, randomized controlled trial. *Human Reproduction*, *20*, 390–396. doi: 10.1093/humrep/deh625.

Dominguez, F., Pellicer, A., & Simon, C. (2003). The chemokine connection: hormonal and embryonic regulation at the human maternal-embryonic interface–a review. *Placenta*, *24*, S48–S55. doi: 10.1016/S0143-4004(03)00134-6.

Drakakis, P., Loutradis, D., Beloukas, A., Sypsa, V., Anastasiadou, V., Kalofolias, G., … Antsaklis, A. (2009). Early hCG addition to rFSH for ovarian stimulation in IVF provides better results and the cDNA copies of the hCG receptor may be an indicator of successful stimulation. *Reproductive Biology and Endocrinology*, *7*, 110. doi: 10.1186/1477-7827-7-110.

Ebner, T., Yaman, C., Moser, M., Sommergruber, M., Feichtinger, O., & Tews, G. (2000). Prognostic value of first polar body morphology on fertilization rate and embryo quality in intracytoplasmic sperm injection. *Human Reproduction*, *15*, 427–430. doi: 10.1093/humrep/15.2.427.

Fauser, B.C., & Devroey, P. (2003). Reproductive biology and IVF: ovarian stimulation and luteal phase consequences. *Trends in Endocrinology and Metabolism*, *14*, 236–242. doi: 10.1016/S1043-2760(03)00075-4.

Fauser, B.C., & Van Heusden, A.M. (1997). Manipulation of human ovarian function: physiological concepts and clinical consequences. *Endocrine Reviews*, *18*, 71–106. doi: 10.1210/edrv.18.1.0290.

Filicori, M., & Cognigni, G.E. (2001). Clinical review 126: Roles and novel regimens of luteinizing hormone and follicle-stimulating hormone in ovulation induction. *Journal of Clinical Endocrinology and Metabolism*, *86*, 1437–1441. doi: 10.1210/jcem.86.4.7385.

Filicori, M., Cognigni, G.E., Gamberini, E., Parmegiani, L., Troilo, E., & Roset, B. (2005). Efficacy of low-dose human chorionic gonadotropin alone to complete controlled ovarian stimulation. *Fertility and Sterility*, *84*, 394–401. doi: 10.1016/j.fertnstert.2005.02.036.

Filicori, M., Cognigni, G.E., Tabarelli, C., Pocognoli, P., Taraborrelli, S., Spettoli, D., & Ciampaglia, W. (2002a). Stimulation and growth of antral ovarian follicles by selective LH activity administration in women. *Journal of Clinical Endocrinology and Metabolism*, *87*, 1156–1161. doi: 10.1210/jcem.87.3.8322.

Filicori, M., Cognigni, G.E., Taraborrelli, S., Parmegiani, L., Bernardi, S., & Ciampaglia, W. (2002b). Intracytoplasmic sperm injection pregnancy after low-dose human chorionic gonadotropin alone to support ovarian folliculogenesis. *Fertility and Sterility*, *78*, 414–416. doi: 10.1016/S0015-0282(02)03243-0.

Filicori, M., Cognigni, G.E., Taraborrelli, S., Spettoli, D., Ciampaglia, W., & de Fatis, C.T. (1999a). Low-dose human chorionic gonadotropin therapy can improve sensitivity to exogenous follicle-stimulating hormone in patients with secondary amenorrhea. *Fertility and Sterility*, *72*, 1118–1120. doi: 10.1016/S0015-0282(99)00426-4.

Filicori, M., Cognigni, G.E., Taraborrelli, S., Spettoli, D., Ciampaglia, W., de Fatis, C.T., & Pocognoli, P. (1999b). Luteinizing hormone activity supplementation enhances follicle-stimulating hormone efficacy and improves ovulation induction outcome. *Journal of Clinical Endocrinology and Metabolism*, *84*, 2659–2663. doi: 10.1210/jcem.84.8.5884.

Filicori, M., Cognigni, G.E., Taraborrelli, S., Spettoli, D., Ciampaglia, W., Tabarelli, C., … Boschi, S. (2001). Luteinzing hormone activity in menotropins optimizes folliculogenesis and treatment in controlled ovarian stimulation. *Journal of Clinical Endocrinology and Metabolism*, *86*, 337–343. doi: 10.1210/jcem.86.1.7108.

Fleming, R., Lloyd, F., Herbert, M., Fenwick, J., Griffiths, T., & Murdoch, A. (1998). Effects of profound suppression of luteinizing hormone during ovarian stimulation on follicular activity, oocyte and embryo function in cycles stimulated with purified follicle stimulating hormone. *Human Reproduction*, *13*, 1788–1792. doi: 10.1093/humrep/13.7.1788.

Gomes, M.K., Vieira, C.S., Moura, M.D., Manetta, L.A., Leite, S.P., Reis, R.M., & Ferriani, R.A. (2007). Controlled ovarian stimulation with exclusive FSH followed by stimulation with hCG alone, FSH alone or hMG. *European Journal of Obstetrics, Gynecology and Reproductive Biology*, *130*, 99–106. doi: 10.1016/j.ejogrb.2006.05.025.

Hamdine, O., Macklon, N.S., Eijkemans, M.J., Laven, J.S., Cohlen, B.J., Verhoeff, A., … Broekmans, F.J. CETRO trial study group. (2014). Elevated early follicular progesterone levels and in vitro fertilization outcomes: a prospective intervention study and meta-analysis. *Fertility and Sterility*, *102*, 448–454.e1. doi: 10.1016/j.fertnstert.2014.05.002.

Inge, G.B., Brinsden, P.R., & Elder, K.T. (2005). Oocyte number per live birth in IVF: were Steptoe and Edwards less wasteful?. *Human. Reproduction*, *20*, 588–592. doi: 10.1093/humrep/deh655.

Koichi, K., Yukiko, N., Shima, K., & Sachiko, S. (2006). Efficacy of low-dose human chorionic gonadotropin (hCG) in a GnRH antagonist protocol. *Journal of Assisted Reproduction and Genetics*, *23*, 223–228. doi: 10.1007/s10815-006-9036-2.

Kovacs, P., Sajgo, A., Kaali, S.G., & Pal, L. (2012). Detrimental effects of high-dose gonadotropin on outcome of IVF: making a case for gentle ovarian stimulation strategies. *Reproductive Sciences*, *19*, 718–724. doi: 10.1177/1933719111432859.

Lee, A., Miller, K., Elkinnd-Hirsch, K., & Scott, R. (2004). The "un-coast"— use of low-dose hCG alone to complete ovarian folliculogenesis in a high responder. *Fertility and Sterility*, *82*, S120. doi: 10.1016/j.fertnstert.2004.07.304.

Macklon, N.S., Stouffer, R.L., Giudice, L.C., & Fauser, B.C. (2006). The science behind 25 years of ovarian stimulation for in vitro fertilization. *Endocrine Reviews*, *27*, 170–207. doi: 10.1210/er.2005-0015.

Maldonado, L.G., Franco, J.G., Jr., Setti, A.S., Iaconelli, A., Jr., & Borges, E. Jr., (2013). Cost-effectiveness comparison between pituitary down-regulation with a gonadotropin-releasing hormone agonist short regimen on alternate days and an antagonist protocol for assisted fertilization treatments. *Fertility and Sterility*, *99*, 1615–1622. doi: 10.1016/j.fertnstert.2013.01.095.

Martins, W.P., Vieira, A.D., Figueiredo, J.B., & Nastri, C.O. (2013). FSH replaced by low-dose hCG in the late follicular phase versus continued FSH for assisted reproductive techniques. *Cochrane Database of Systematic Reviews*, *2013*, CD010042. doi: 10.1002/14651858.CD010042.pub2. .

Revelli, A., Casano, S., Salvagno, F., & Delle Piane, L. (2011). Milder is better? Advantages and disadvantages of "mild" ovarian stimulation for human in vitro fertilization. *Reproductive Biology and Endocrinology*, *9*, 25. doi: 10.1186/1477-7827-9-25.

Serafini, P., Yadid, I., Motta, E.L., Alegretti, J.R., Fioravanti, J., & Coslovsky, M. (2006). Ovarian stimulation with daily late follicular phase administration of low-dose human chorionic gonadotropin for in vitro fertilization: a prospective, randomized trial. *Fertility and Sterility*, *86*, 830–838. doi: 10.1016/j.fertnstert.2006.02.110.

Shima, K., Kitayama, S., & Nakano, R. (1987). Gonadotropin binding sites in human ovarian follicles and corpora lutea during the menstrual cycle. *Obstetrics and Gynecology*, *69*, 800–806. Retrieved from: http://journals.lww.com/greenjournal/Abstract/1987/05000/Gonadotropin_Binding_Sites_in_Human_Ovarian.24.aspx.

Sullivan, M.W., Stewart-Akers, A., Krasnow, J.S., Berga, S.L., & Zeleznik, A.J. (1999). Ovarian responses in women to recombinant follicle-stimulating hormone and luteinizing hormone (LH): a role for LH in the final stages of follicular maturation. *Journal of Clinical Endocrinology and Metabolism*, *84*, 228–232. doi: 10.1210/jcem.84.1.5389.

Suppinyopong, S., Choavaratana, R., & Karavakul, C. (2000). Correlation of oocyte morphology with fertilization rate and embryo quality after intracytoplasmic sperm injection. *Journal of the Medical Association of Thailand*, *83*, 627–632. Retrieved from: http://www.jmatonline.com/index.php/jmat/article/view/4163.

Takao, Y., Honda, T., Ueda, M., Hattori, N., Yamada, S., Maeda, M., … Wimalasena, J. (1997). Immunohistochemical localization of the LH/HCG receptor in human ovary: HCG enhances cell surface expression of LH/HCG receptor on luteinizing granulosa cells in vitro. *Molecular Human Reproduction*, *3*, 569–578. doi: 10.1093/molehr/3.7.569.

Thompson, K.A., LaPolt, P.S., River, J., Henderson, G., Dahl, K.D., & Meldrum, D.R. (1995). Gonadotropin requirements of the developing follicle. *Fertility and Sterility*, *63*, 273–276. doi: 10.1016/S0015-0282(16)57354-3.

Van Horne, A.K., Bates, G.W., Jr., Robinson, R.D., Arthur, N.J., & Propst, A.M. (2007). Recombinant follicle-stimulating hormone (rFSH) supplemented with low-dose human chorionic gonadotropin compared with rFSH alone for ovarian stimulation for in vitro fertilization. *Fertility and Sterility*, *88*, 1010–1013. doi: 10.1016/j.fertnstert.2006.12.051.

Verberg, M.F., Macklon, N.S., Nargund, G., Frydman, R., Devroey, P., Broekmans, F.J., & Fauser, B.C. (2009). Mild ovarian stimulation for IVF. *Human Reproduction Update*, *15*, 13–29. doi: 10.1093/humupd/dmn056.

Westergaard, L.G., Laursen, S.B., & Andersen, C.Y. (2000). Increased risk of early pregnancy loss by profound suppression of luteinizing hormone during ovarian stimulation in normogonadotrophic women undergoing assisted reproduction. *Human Reproduction*, *15*, 1003–1008. doi: 10.1093/humrep/15.5.1003.

Wilding, M., Di Matteo, L., D'andretti, S., Montanaro, N., Capobianco, C., & Dale, B. (2007). An oocyte score for use in assisted reproduction. *Journal of Assisted Reproduction and Genetics*, *24*, 350–358. doi: 10.1007/s10815-007-9143-8.

Wu, Z., Li, R., Ma, Y., Deng, B., Zhang, X., Meng, Y., … Qiao, J. (2012). Effect of HCG-day serum progesterone and oestradiol concentrations on pregnancy outcomes in GnRH agonist cycles. *Reproductive Biomedicine Online*, *24*, 511–520. doi: 10.1016/j.rbmo.2012.02.003.

Xia, P. (1997). Intracytoplasmic sperm injection: correlation of oocyte grade based on polar body, perivitelline space and cytoplasmic inclusions with fertilization rate and embryo quality. *Human Reproduction*, *12*, 1750–1755. doi: 10.1093/humrep/12.8.1750.

Xu, B., Li, Z., Zhang, H., Jin, L., Li, Y., Ai, J., & Zhu, G. (2012). Serum progesterone level effects on the outcome of in vitro fertilization in patients with different ovarian response: an analysis of more than 10,000 cycles. *Fertility and Sterility*, *97*, 1321–1327.e4. doi: 10.1016/j.fertnstert.2012.03.014.