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Synchronization of endogenous and exogenous FSH stimuli in controlled ovarian hyperstimulation (COH)*

Dominique de Ziegler[1,2,5], Anna-Stina Jääskeläinen[1], Pierre-Alain Brioschi[1], Renato Fanchin[3] and Carlo Bulletti[4]

1Department of OB/GYN, Hospital of Nyon, Switzerland, [2]Columbia Laboratories, 19, Rue du Général Foy, 75008 Paris, [3]Department of OB/GYN, H™pital Antoine Béclère, Clamart, France, and [4]Department of OB/GYN, University of Bologna, Italy

[5]To whom correspondence should be addressed

We have previously observed that exogenous oestradiol can delay the intercycle increase in plasma follicle stimulating hormone (FSH). The increase in plasma FSH that follows discontinuation of exogenous oestradiol peaks after 3 days. We have now studied the possibility of using exogenous oestradiol to synchronize the increase in endogenous FSH with the onset of human menopausal gonadotrophin (HMG) treatment in controlled ovarian hyperstimulation (COH). A total of 30 women aged 35.1 ± 6.3 years (mean ± SD) undergoing ovarian stimulation received 2 mg of oestradiol valerate twice daily starting on day 25 of the previous menstrual cycle until the first Tuesday following menses. Ovarian stimulation was initiated 3 days later. On the last day of oestradiol treatment, plasma oestradiol, FSH and luteinizing hormone (LH) (mean ± SEM) were 566 ± 53 (pmol/l), 3.8 ± 0.4 (IU/l) and 5.5 ± 0.8 (IU/l) respectively. After 3 days, the FSH and LH (mean ± SEM) had increased to 6.7 ± 0.7 and 6.9 ± 0.7 (IU/l) respectively while oestradiol decreased to 251 ± 29 (pmol/l). The mean number (± SEM) of HMG ampoules used was 25.1 ± 2.7 and treatment lasted 11.3 ± 0.9 days. Five women became pregnant for a pregnancy rate (ongoing) of 19 (15)%. If all women aged >40 years (six women who did not become pregnant) were excluded from analysis the pregnancy rate (ongoing) was 24 (19%). These results indicate that exogenous oestradiol can safely be used for the synchronization of endogenous and exogenous FSH stimuli in COH. This approach provides the practical advantage of permitting an advanced timing of the onset of COH treatments when gonadotrophin-releasing hormone (GnRH) agonists are not used, which improves treatment convenience for patients and team members alike. Further development of this model may enable control of the onset of natural cycles which may find practical applications for timing assisted reproductive techniques (intrauterine insemination or in-vitro fertilization) in the natural cycle.

Key words: controlled timing/FSH/oestradiol/ovarian stimulation

Introduction

The ultimate phase of follicular growth is initiated by the slight increase in plasma follicle stimulating hormone (FSH) that occurs in the intercycle interval. This increase in FSH stimulates the pool of recruitable follicles and ultimately leads to single follicular dominance and ovulation ~14 days later while the other follicles undergo atresia. In controlled ovarian hyperstimulation (COH), exogenous gonadotrophins are administered to amplify and sustain the gonadotrophic stimulus in order to prevent single follicular dominance by rescuing the rest of the cohort of follicles from atresia and hence achieve multiple ovulation.

In cycling individuals whose endogenous gonadotrophins are not suppressed, exogenous gonadotrophin treatments [human menopausal gonadotrophin (HMG), purified or recombinant FSH] are arbitrarily initiated on cycle day 2 or 3. This represents the best practical compromise for an approximate synchronization between the onset of exogenous gonadotrophins and the normal acme of the intercycle increase in FSH. The onset of HMG treatment, however, remains arbitrary because of the inherent variability in the point at which the intercycle FSH elevation occurs. Moreover, it is impossible to identify the actual intercycle FSH elevation by a single blood sample because of the small amplitude of the increase which precludes the possibility of adjusting the onset of HMG treatment individually. Hence, in real life the intercycle FSH elevation can occur before, concomitantly or after the onset of HMG treatment without clinical means of controlling for this variable. Plasma FSH elevation before the onset of HMG treatment is likely to occur in women whose cycles are short. Hence, it is possible that HMG is administered after mechanisms leading to follicular dominance have already taken place, which is likely to hamper the efficacy of COH. Conversely, if the elevation in endogenous FSH has not yet taken place when HMG treatment is initiated, exogenous gonadotrophins become the sole source of ovarian stimulation which may increase treatment needs (number of HMG ampoules).

The nature of the mechanisms that control the intercycle FSH elevation remains a subject for debate. Roseff *et al.* (1989) have proposed that in the menstrual cycle it is the decrease in inhibin occurring following the demise of the corpus luteum (inhibin A) that represents the triggering signal for the early follicular phase FSH elevation. In previous work

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however (Le Nestour *et al.*, 1993), we have challenged this view, postulating rather that it is the end luteal phase decrease in circulating oestradiol concentrations that plays the prime stimulating role for the intercycle FSH rise, a hypothesis concordant with findings made in non-human primates by Zeleznik *et al.* (1985). In this earlier study we extended artificially by 3 days the luteal phase levels of oestradiol with exogenous oestradiol treatment and observed a proportional delay in the FSH rise without alterations in the end luteal phase decrease in inhibin levels. The maximum of the FSH rise was observed 3 days after withdrawal of the oestradiol treatment (Le Nestour *et al.*, 1993). In a preliminary attempt to take clinical advantage of the possibility of inhibiting the intercycle FSH rise with physiological amounts of exogenous oestradiol we tested the possibility of priming endometrial receptivity with a gonadotrophin-releasing hormone (GnRH) agonist in women using cryopreserved embryos and whose ovaries were activated with oestradiol and progesterone only without suppressing gonadotrophins (de Ziegler *et al.*, 1991; Lelaidier *et al.*, 1992, 1995). Our results showed that exogenous oestradiol effectively and reliably (>90%) prevented follicular recruitment for up to 2 weeks (de Ziegler *et al.*, 1991) and offered a simple mean to prime endometrial receptivity and determine in advance the date for transferring cryopreserved embryos (Lelaidier *et al.*, 1992, 1995).

In the present study we examined a new distinct practical application of the control exerted by oestradiol over the intercycle elevation in FSH. Because of inherent variability in the timing of the intercycle FSH elevation, we attempted to control the endogenous FSH elevation with exogenous oestradiol in order to achieve an optimal synchronization with the onset of exogenous FSH treatment in COH. Specifically, we followed the two following objectives: (i) to provide a more physiological approach to multiple follicular stimulation and possibly improve the quality of COH and its outcome while diminishing the overall need for HMG or recombinant FSH; (ii) to improve the scheduling of treatments for patients and team members when GnRH agonists are not used by synchronizing endogenous FSH rises that initiate new menstrual cycles with the onset of exogenous FSH treatment (HMG or recombinant FSH) for COH.

Material and methods

Population characteristics

A total of 30 women 35.1 ± 6.3 years of age (mean ± SD) who were candidates for COH for ovulatory disorders (n = 5), male factor (n = 22) and/or unexplained/insufficiently explained infertility (n = 17) were invited to participate in our synchronized endogenous–exogenous gonadotrophin stimulation protocol. Six women were > 40 years of age (range 41–46).

Treatment and monitoring

Starting on day 25 (or ~3 days before the anticipated menses) women received oestradiol valerate (Progynova®; Schering Pharmaceuticals AG, Berlin, Germany) 2 mg twice daily until the first Tuesday that followed the onset of menses, defined as functional day (FD) 0, i.e. 1–8 days after the onset of menses. On the Friday that followed the discontinuation of oestradiol valerate treatment, defined as FD 3, an ultrasound scan was performed to confirm the absence of follicular recruitment (no follicle ⩾11 mm) and ovarian cyst. Women were then started on HMG using a predetermined dose (ranging from 150–300 IU/day) for the first 6 days of treatment. The initial dose of HMG (first 6 days) was established according to past experience (prior COH cycle), clinical symptoms, age, menstrual cycle length, etc. From the sixth day of treatment onward, HMG treatment was adjusted as per ultrasound and plasma oestradiol data.

Blood sampling

Women were sampled on the last day of oestradiol treatment or FD 0 and on the third day after discontinuation of oestradiol or FD 3 for FSH, luteinizing hormone (LH) and oestradiol.

Results

Oestradiol treatment was started 7.1 ± 3.3 days (mean ± SD) before the onset of menses and continued for 5 ± 2 days (mean ± SD) thereafter. Three patients were cancelled before receiving HCG due to excessive (n = 2) or insufficient ovarian response (n = 1). Results were analysed for the 27 women in whom ovulation was triggered by HCG.

Plasma FSH, LH and FSH/LH ratios on the last day of oestradiol treatment (FD 0) and the first day of HMG treatment (FD 3) are illustrated in Table I. No cyst or maturing follicle ⩾10 mm was found at baseline (FD 0). The mean increase in plasma FSH in response to oestradiol withdrawal was of 2.7 ± 0.7 mIU/ml. There was a strong correlation between the plasma FSH/LH ratios on FD 0 and FD 3 ($P < 0.001$).

COH required 25.1 ± 2.7 ampoules of HMG (mean ± SEM) and lasted 11.3 ± 0.9 days on average. Five women became pregnant, giving an pregnancy rate of 19% (ongoing pregnancy rate = 15%). When excluding six women aged >40 years who did not conceive were excluded, the pregnancy rate becomes 24% (19% ongoing). The hormonal parameters of pregnant women were not different from those of the entire group.

Discussion

Our results confirm that it is feasible, practical and not counter-productive to use exogenous oestradiol for controlling the timing of the early follicular phase increase in FSH and to then adjust the onset of HMG treatment accordingly. While prior work had suggested that exogenous oestradiol could delay the intercycle FSH elevation, the clinical usefulness of this principle and its application for synchronizing endogenous and exogenous FSH stimuli had never been tested and therefore needed to be verified by a pilot study. The small number of patients treated with this original protocol do not permit us to assess the possible superiority of this novel COH approach over conventional treatments. Our positive results do, however, warrant the initiation of full-scale randomized clinical trials to verify the theoretical value of this paradigm. Our results confirm our previous observation that progesterone levels alone trigger menses irrespective of actual oestradiol levels (de Ziegler *et al.*, 1987).

The improvement to the scheduling of HMG treatments

Table I. Plasma gonadotrophins and oestradiol concentrations on functional day (FD) 0 and FD 3

	FD 0			FSH/LH	FD 3			FSH/LH
	Oestradiol pmol/l	FSH mIU/l	LH mIU/m		Oestradiol pmol/l	FSH mIU/ml	LH mIU/ml	
Mean	566	3.8	5.5	1.0	251	6.7	6.9	1.2
SEM (n = 27)	53	0.4	0.8	0.2	29	0.7	0.78	0.2

FSH = follicle stimulating hormone; LH = luteinizing hormone.

(starting on a pre-set day of the week) provided by controlling of the intercycle increase in FSH, and consequently the true functional onset of new menstrual cycles, offers genuine clinical advantages for patients and team members alike. Previously, advanced programming of the onset of COH treatment cycles was limited to complex approaches using temporary gonadotrophin suppression with GnRH agonists or non-physiological molecules, including oral contraceptives (Frydman *et al*., 1986, 1987). Our present protocol in which the timing of the intercycle increase in FSH is controlled with physiological amounts of oestradiol (amounts of oestradiol that keep plasma oestradiol concentrations within the luteal phase range) provides the same practical advantages without the complexity, the cost and the increased risk of ovarian hyperstimulation inherent to the use of GnRH agonists. COH cycles programmed with oestradiol, however, do not provide protection against premature ovulation. In COH cycles programmed with oral contraceptive pill, the added treatment had to be taken for at least a full month before COH (Frydman *et al*., 1986, 1987; Kemeter and Feichtinger, 1989; Rainhorn *et al*., 1987). Furthermore, the use of synthetic molecules may have persistent and possibly deleterious effects notably on the endometrium and ovarian response to HMG. Moreover, these regimens also withheld the menstrual period until the desired time, while only the increase in FSH needs to be delayed.

The possibility of controlling the timing of the intercycle FSH elevation with physiological amounts of oestradiol offers the prospect of developing new and original therapies for improving the quality of the ovulatory process. As recently emphasized by Edwards *et al.* (1996), the trend over the years has been for the successive methods of ovarian stimulation used to increase the number of follicles recruited per patient. As stated by these authors 'high-order ovarian stimulation could be injurious to women's health' in view of the prevailing fears that multiplying the number of ovulatory scars may favour the onset of ovarian cancer. Gaining control of the triggering mechanism for the intercycle FSH elevation may represent a first step toward 'revolutionizing ovarian stimulation'. Indeed, controlling the timing of the intercycle FSH elevation may lead toward the use of milder doses of HMG or recombinant FSH to only slightly increase the signal's amplitude and/or duration and hence obtain two or three follicle ovarian stimulations. Moreover, our new approach for timing the onset of COH would be also a logical complement to the emerging timely use of GnRH antagonists during the end follicular phase in order to prevent premature ovulation (Frydman *et al.*, 1991; Ubaldi *et al*., 1996).

Controlling the functional onset of the menstrual cycle, i.e. the intercycle FSH elevation, may also provide completely new perspectives for studies on the unstimulated menstrual cycle while avoiding tedious efforts to identify the LH surge. Finally, the use of physiological amount of oestradiol starting on about day 25 of the previous cycle, is harmless in the event of an undiagnosed pregnancy as this treatment is commonly prescribed to egg donation recipients (Lelaidier *et al.*, 1995).

In conclusion, our results confirm that it is possible to control the timing of the early follicular phase increase in FSH with physiological amounts of exogenous oestradiol and to synchronize endogenous and exogenous FSH stimuli in COH. The present data are encouraging and prove our hypothesis that oestradiol concentrations control the intercycle FSH elevation. Further work however, must evaluate the true possible advantages of this original approach particularly in women with short menstrual cycles in whom the spontaneous intercycle FSH elevation is likely to occur too early. Finally, the possibility of controlling the functional onset of the menstrual cycle, i.e. the intercycle increase in plasma FSH, with physiological amounts of oestradiol offers fascinating possibilities for original approaches for improving the quality of the ovulatory process.

References

Edwards, R.G., Lobo, R. and Bouchard, P. (1996) Time to revolutionize ovarian stimulation. *Hum. Reprod.*, **11,** 917–919.

de Ziegler, D., Cedars, M.I., Randle, D. *et al.* (1987) Suppression of the ovary using a GnRH agonist prior to stimulation for oocyte retrieval. *Fertil. Steril.*, **48,** 807–810.

de Ziegler, D., Cornel, C., Bergeron, C. *et al.* (1991) Controlled preparation of the endometrium with exogenous estradiol and progesterone in women having functioning ovaries. *Ferti. Steril.*, **56,** 851–855.

Frydman, R., Forman, R., Rainhorn, J.D. *et al.* (1986) A new approach to follicular stimulation for *in vitro* fertilization: programmed oocyte retrieval. *Fertil. Steril.*, **46,** 657–662.

Frydman, R., Rainhorn, J.D., Forman, R. *et al.* (1987) Pregnancies following fixed schedule ovulation induction and embryo cryopreservation during diagnostic infertility laparoscopy. In Feichtinger W. and Kemeter P. (eds), *Future Aspects In Human In Vitro Fertilization.* Springer, Berlin, Heidelberg, New York, pp. 63–70.

Frydman, R., Cornel, C., de Ziegler, D. *et al.* (1991) Prevention of premature LH and progesterone rise with a gonadotropin releasing hormone antagonist (Nal–Glu), in controlled ovarian hyperstimulation. *Fertil. Steril.*, **56,** 923–927.

Kemeter, P. and Feichtinger, W. (1989) A fixed stimulation protocol for *in vitro* fertilization (IVF) without determinations of hormones in blood. *Acta Europ. Fertil.*, **20,** 63–70.

Lelaidier, C., de Ziegler, D., Gaetano, J. *et al.* (1992) Controlled preparation of the endometrium with exogenous oestradiol and progesterone: a novel regimen not using a gonadotrophin-releasing hormone agonist. *Hum. Reprod.*, **7,** 1353–1356.

Lelaidier, C., de Ziegler, D., Freitas, S. *et al.* (1995) Endometrium preparation with exogenous estradiol and progesterone for the transfer of cryopreserved blastocysts. *Fertil. Steril.*, **63,** 919–921.

Le Nestour, E., Marraqui, J., Lahlou, N. *et al.* (1993) Role of estradiol in the rise in follicle-stimulating hormone levels during the luteal-follicular transition. *J. Clin. Endocrinol. Metab.*, **77,** 439–442.

Rainhorn, J.D., Forman, R.G., Belaisch-Allart, J. *et al.* (1987) One year's experience with programmed oocyte retrieval for IVF. *Hum. Reprod.*, **2,** 491–494.

Roseff, S.J., Bangah, M.L., Kettel, L.M. *et al.* (1989) Dynamic changes in circulating inhibin levels during the luteal-follicular transition of the human menstrual cycle. *J. Clin. Endocrinol. Metab.*, **69,** 1033–1039.

Ubaldi, F., Albano, C., Peukert, M. *et al.* (1996) Subtle progesterone rise after the administration of the gonadotrophin-releasing hormone antagonist Cetrorelix in intracytoplasmic sperm injection cycles. *Hum. Reprod.*, **11,** 1405–1407.

Zeleznik, A.J., Hutchison, J.S., Schuler, H.M. (1985) Interference with the gonadotropin-suppressing actions of estradiol in macaques overrides in selection of a single preovulatory follicle. *Endocrinology*, **117,** 991–999.

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