Human Reproduction, Vol.27, No.1 pp. 232–243, 2012
Advanced Access publication on November 10, 2011 doi:10.1093/humrep/der380

human reproduction

REVIEW *Reproductive endocrinology*

Impact of 'LH activity' supplementation on serum progesterone levels during controlled ovarian stimulation: a systematic review

J.N. Hugues*

Reproductive Medicine Unit, Jean Verdier Hospital, University Paris XIII, Avenue du 14 Juillet, Bondy 93143, France

*Correspondence address. E-mail: jean-noel.hugues@jvr.aphp.fr

Submitted on June 16, 2011; resubmitted on September 27, 2011; accepted on October 11, 2011

BACKGROUND: The influence of LH on serum progesterone rise during gonadotrophin stimulation is a matter of debate. The purpose of this analysis was to assess the impact of supplementation with 'LH activity' products on serum progesterone changes before hCG administration in GnRH analog-treated women.

METHODS: A computerized literature search was performed to identify studies comparing FSH treatment alone to those that provided supplementation with 'LH activity' using hMG, recombinant (r)LH (rLH) or hCG in GnRH analog protocols. Data regarding stimulation regimens were extracted from those that reported serum progesterone levels at the time of hCG in order to assess the specific role of LH activity products.

RESULTS: Serum progesterone determination at the time of hCG administration was performed in 34 out of 108 studies comparing the effects of FSH alone or in combination with LH activity products. In a vast majority, no significant difference in serum progesterone could be found between stimulation regimens. However, in four studies where LH activity (three hMG and one rLH) was administered from the beginning of ovarian stimulation, serum *P*-values were significantly decreased. In contrast, in two studies where LH activity (hCG) was provided during the late follicular phase, serum *P*-values were significantly increased. Analysis of confounding factors showed that the intensity of ovarian stimulation is the most important determining factor to explain serum progesterone elevation at the time of hCG administration,

CONCLUSIONS: This systematic review shows that providing LH activity supplementation in combination with FSH during ovarian stimulation does not have a consistent effect on serum progesterone concentrations at the time of hCG administration. However, these data also suggest that, in accordance with physiological concept, the timing of LH activity administration could influence the impact on serum progesterone changes.

Key words: progesterone / LH activity / controlled ovarian stimulation

Introduction

The issue of serum progesterone rise on the day of hCG administration (serum progesterone^{hCG}) during controlled ovarian stimulation (COS) is still a matter of several controversies. As recently emphasized, serum progesterone rise observed during the final stages of COS is not actually premature luteinization because it takes place in patients treated with GnRH analogs, which fully control further LH surge. Luteinization, the process by which the mature follicle is transformed into a corpus luteum, is mainly induced by an LH surge (Murphy, 2000). In contrast, the subtle and gradual increase in progesterone levels observed during the last day of ovarian stimulation reflects only the total amount of progesterone secreted by maturing follicles in the absence of any premature uncontrolled LH surge.

Many questions have been raised regarding the incidence of progesterone elevation in GnRH-analog-controlled cycles specifically on the impact on cycle IVF outcome. A meta-analysis (Venetis *et al.*, 2007) concluded that there was a lack of evidence for a negative impact of high *P*-values on implantation rate. However, this conclusion has recently been challenged (de Ziegler *et al.*, 2008; Fleming, 2008; Kolibianakis *et al.*, 2011; Kyrou *et al.*, 2011) for several reasons: results confounded by the use of different GnRH analog protocols, methodological flaws such as the unreliability of commercial non-extraction assays (Coucke *et al.*, 2007), arbitrary choice of defined threshold (0.9 ng/ml) while the use of receiver operator characteristics curve

might have been more appropriate. Using this approach, Bosch *et al.* (2010) reported a negative impact of serum progesterone increase with a threshold value of 1.5 ng/ml in a large single-center study where GnRH agonist and antagonist cycles were analyzed separately. This threshold value has been proposed in a recent study that additionally emphasizes the predictive value of progesterone/estradiol (E_2) ratio (Elgindy, 2011). Furthermore, the consequences of serum progesterone rise on IVF outcome might be drastically different depending on whether it occurs in women with a strong or weak response to COS. Indeed, a low clinical pregnancy rate was mainly observed when the response to COS was weak (Fanchin *et al.*, 1997). Therefore, confounding factors such as patients' ovarian reserve, oocyte and embryo quality need to be also considered when assessing the consequences of serum progesterone rise on cycle outcome following COS (De Ziegler *et al.*, 2008; Younis, 2011).

The potential negative impact of elevated progesterone on cycle outcome seems to be related to inadequacy of the endometrium as demonstrated in oocyte donor program (Melo *et al.*, 2006) and in studies where endometrial gene expression has been assessed (Labarta *et al.*, 2011; Van Vaerenberg *et al.*, 2011). However, it has been suggested that the endometrial receptivity is only temporally defective because blastocysts transferred on Day 5 would have an excellent potential for implantation even in the presence of high progesterone levels at the time of hCG (Papanikolaou *et al.*, 2009; Elgindy, 2011). Whether endometrial receptivity is able to recover from a detrimental impact observed when embryos are replaced on Day 3 remains to be elucidated. Nevertheless, these data emphasize the relevance of measuring serum progesterone values at the time of hCG in order to adjust the strategy for embryo transfer.

Many factors have been associated with an increased risk of progesterone rise during GnRH analog-controlled cycles. Several studies have emphasized the critical role of ovarian parameters such as the total dose of FSH administered as well as the intensity of the ovarian response assessed by serum E_2 levels on the day of hCG and the number of follicles or oocytes on the day of ovarian pick-up (Filicori *et al.*, 2002a,b; Bosch *et al.*, 2003, 2005, 2008; Andersen *et al.*, 2006; Kyrou *et al.*, 2009a,b). These data suggest that the increase in granulosa cells activity is the main driver of progesterone elevation. They also show that a multivariate analysis is absolutely required to separate confounding factors.

In this context, the role of LH is still unclear due to the complexity of the regulation of ovarian steroïdogenesis by gonadotrophins. If we have a closer look at the role of LH (Payne and Hales, 2004), it is critical to separate the effects on theca and granulosa cells (Fig. 1).

- On the one hand, LH constitutively acts on theca cell receptors to stimulate Cytochrome P450 CYP 17 enzymatic complex (17 hydroxylase and 17–20 lyase activities), responsible for the conversion of progesterone (Δ4 pathway) or pregnenolone (Δ5 pathway) to 17-hydroxylated products and androgens. The actual resulting effect is a decrease in progesterone production by theca cells. In contrast, LH acts on 3β hydroxysteroid dehydrogenase (HSD) type II to stimulate the conversion of pregnenolone to progesterone. Therefore, the overall effect of LH on theca cell progesterone production depends on the balance between these enzymatic activities.
- On the other hand, LH acts on granulosa cells when LH receptors have been induced by FSH at the later stage of follicular phase.

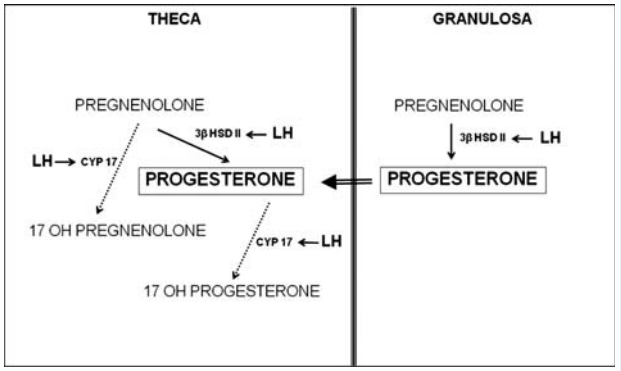

Figure 1 Effects of LH on progesterone production during the follicular phase. Left part**:** LH acting on theca cells via constitutive receptors present during the whole follicular phase has a dual effect on progesterone synthesis. Stimulation of 3β HSD type II induces a positive effect on progesterone production. Activation of CYP 17 which converts progesterone and pregnenolone to 17 OH derivates reduces progesterone synthesis. The overall effect depends on the respective activation of these two enzymatic activities Right part**:** LH acts on granulosa cells via receptors present only during the late follicular phase. LH activates 3β HSD type II enzymatic activity with a positive effect on progesterone production. 3β HSD II: 3 beta hydroxysteroid deshydrogenase type II CYP 17: Cytochrome P450 17 α-hydroxylase.

In vitro experiments have clearly demonstrated that LH has a synergistic effect with FSH on granulosa cells to stimulate progesterone production (Young *et al.*, 1992; Lindeberg *et al.*, 2007), and that LH is far more potent than FSH on granulosa cells to produce steroids as assessed by cAMP accumulation (Young *et al.*, 1994).

This complex biphasic and development-related effect of LH on progesterone production is likely to explain the controversy regarding the actual role of LH in serum progesterone elevation before hCG administration.

Few studies have been published so far on the role of endogenous LH environment. Most of them concluded for the lack of correlation between serum progesterone elevation and LH levels or the area under the curve of serum LH during the time of stimulation (Ubaldi *et al.*, 1996; Bosch *et al.*, 2003, 2005). However, a close relationship between serum LH and progesterone in GnRH agonist cycles has been recently observed in two other reports (Hugues *et al.*, 2011; Yding Andersen *et al.*, 2011). These divergent conclusions may be explained by the use of different assays that cannot reflect LH bioactivity. It has been also recently reported that serum progesterone concentrations at the time of hCG administration (serum progesterone^{hCG}) are lower following GnRH antagonist regimens when compared with GnRH agonist protocols (Bosch *et al.*, 2010). However, it is still uncertain whether this observation can be explained by the different endogenous LH environment created by each protocol or by other confounding factors such as ovarian parameters.

The purpose of this review was to evaluate the impact of exogenous 'LH activity' products on progesterone elevation before triggering of ovulation. Over the last three decades, several molecules have been

Table I Main characteristics of 21 studies comparing hMG/hMG-HP versus urinary/recombinant FSH.

Authors, review/year	Study	Intervention	Population	Protocol GnRH analog	Gonadotrophins starting dose × duration	Patients *n*
Bentick *et al.*, Fertil Steril/1988	Prosp	IVF	Previous failure	Long (Bus IN)	uFSH 150 × 7	13
	Random	Consec cycles			hMG 150 × 7	13
Djuijkers *et al.*, Hum Reprod /1993	Prosp	IVF	Unselected	Long (Bus IN)	uFSH 150 Fixed	10
	Random				hMG (3/1) 150 Fixed	10
Check *et al.*, Gynecol Obstet Invest/1995	Prosp	IVF	Unselected	Long (Leup)	uFSH 300	19
	Random				hMG 300	30
Filicori *et al.*, Fertil Steril/1996	Prosp	IUI	Unselected	Long (Deca LP)	uFSH 150 × 5	10
					hMG 150 × 5	10
Fleming *et al.*, Hum Reprod/1996	Prosp	IVF	Unselected	Long (Bus IN)	uFSH IM 225 × 7	20
	Random				uFSH Sc 225 × 7	20
					hMG 225 × 7	20
Imthurn *et al.*, Hum Reprod/1996	Prosp	IVF-ICSI	Unselected	Short (Deca)	uFSH × 5	40
	Random	Consec cycles			hMG × 5	53
Duijkers *et al.*, Int J Fertil/1997	Prosp	IVF	Unselected	Long (Bus IN)	rFSH (β) 150 × 2	6
	Random				HMG 150 × 2	7
Adonakis *et al.*, Fertil Steril/1998	Prosp	IVF	Unselected	Long (Bus IN)	uFSH-HP 300	
	Random				≥1 Foll >15 mm: uFSH 225	16
					≥1 Foll >15 mm: hMG 225	11
Teissier *et al.*, Hum Reprod/1999	Prosp	IVF	Unselected	Long (Deca)	rFSH (α) 150–225	26
					hMG 150–225	13
Gordon *et al.*, Fertil Steril/2001	Prosp	IVF	Unselected	Long (Bus IN)	uFSH 225 × 4	39
	Random				rFSH (β) 225 × 4	30
					hMG (3/1) 225 × 4	30
					hMG 225 × 4	29
Filicori *et al.*, J Clin Endocrinol Metab/2001	Prosp	IUI	Unselected	Long (Deca LP)	uFSH 150 × 14	25
	Random				hMG 150 × 14	25
Filicori *et al.*, Hum Reprod/2002b	Prosp	IUI	Unselected	Long (Deca LP)	uFSH 150 × 14	30
	Random				hMG (4/1) 150 × 14	30
					uFSH 75 + hMG 75 × 14	30
					hMG 150 × 14	30
Filicori *et al.*, Fertil Steril/2003	Prosp	IUI	Unselected	Long (Deca LP)	rFSH (α) 150 × 14	25
	Random				hMG 150 × 14	25
Balasch *et al.*, RBM Online/2003	Prosp	ICSI	Unselected	Long (Deca LP)	rFSH (α) 150 × 14	25
	Random	First cycle			hMG 150 × 14	25
Kilani *et al.*, Hum Reprod/2003	Prosp	ICSI	Unselected	Long (Deca LP)	rFSH (α) 150 × 14	50
	Random				hMG-HP 150 × 14	50
Andersen *et al.*, Hum Reprod/2006	Prosp	IVF-ICSI	Unselected	Long (Deca)	rFSH (α) 225 × 5	368
	Random				hMG-HP 225 × 5	363
Smitz *et al.*, Hum Reprod/2007	Prosp	IVF-ICSI	Unselected	Long (Deca)	rFSH (α) 225 × 5	341
	Random				hMG-HP 225 × 5	335
Bosch *et al.*, Hum Reprod/2008	Prosp	IVF-ICSI	Unselected	Anta 0.25 mg Cetrorelix	rFSH (α) 225 × 2	126
	Random				hMG-HP 225 × 2	122
Orvieto *et al.*, RBM Online/2009	Prosp	IVF Consec cycles	High FSH/LH ratio	Agonist (11) Antagonist (7)	First rFSH Second hMG	18 18
Melo *et al.*, Fertil Steril/2010	Prosp	Oocyte Donation	Unselected	Long (Leup)	rFSH (α) 225 × 5	346
	Random				rFSH 150 + hMG-HP 75 × 5	349
					hMG-HP 225 × 5	333
Requena *et al.*, Hum Fertil (Camb)/2010	Prosp	IVF-ICSI	Unselected	Anta 0.25 mg Ganirelix	rFSH (α) 225 × 3	46
	Random				rFSH 225 × 5; hMG-HP	46
					hMG-HP 225 × 3	44

Results are expressed as mean ± SEM or median (M) and interquartile range (p25 and p75).
NS, not significant; NA, not available; AUC, area under the curve; FF, follicular fluid; MII, metaphase 2, IM, intramuscular; Sc, subcutaneous; (α), Follitropin α; (β) Follitropin β.

Table II Main characteristics of 21 studies comparing hMG/hMG-HP versus urinary/recombinant FSH.

Study	Progesterone hCG (serum (ng/ml) follicular fluid (mcg/ml) AUC (ng/ml day)	E_2 hCG (serum (pg/ml) follicular fluid (ng/ml)	Size/foll (mm/n)	Oocytes (*n*)	Total GnRH dose (IU)	Duration stimulation (days)	Conclusion (difference in *P*-values according to GnRH)
Bentick *et al.* (1988)	0.3 (0.1)	1.283 (431)	Total/10.1 (0.9)	5.8 (1.4)	1.815 (187)	11.4 (0.8)	NS
	0.5 (0.1)	1.586 (278)	Total/10.2 (1.6)	8.5 (1.4)	1.822 (270)	11.1 (0.9)	
Djuijkers *et al.* (1993)	NS (NA)	1.743	≥15/6 (3–18)	8 (4–11)		9 (9–15)*	NS
		2.479*	≥15/8.5 (4–22)*	13 (4–23)*		8 (7–10)	
Check *et al.* (1995)	0.7 (0.1)	1.701 (171)	Total/20.6 (2.6)	16.6 (2)			NS
	0.5 (0.07)	1.990 (242)	Total/20 (2.7)	15.5 (1.9)			
Filicori *et al.* (1996)	0.8 (0.1)	353 (38)	>15/7.1 (0.5)		1905 (165)	12.1 (0.6)	NS
	Follicular fluid: 2.8 (1)						
	0.57 (0.03)	438 (64)	>15/6.2 (0.6)		2.227 (225)	13.1 (0.8)	
	Follicular fluid: 2.8 (0.8)						
Fleming *et al.* (1996)	1.26 (0.14)	2.508 (542)	>16/≈5.5 (0.5)	NS (NA)		13.8 (0.9)	
	1.67 (0.22)	2.076 (226)	>16/≈5.4 (0.7)	NS (NA)		14 (0.9)	NS
	1.41 (0.11)	2.235 (253)	>16/5.5 (0.5)	8.05 (0.5)		11.5 (0.7)	
Imthurn *et al.* (1996)	1.3 (0.1)	2.887 (218)		10.3 (0.7)	2.437 (135)	11 (0.2)	NS
	1.4 (0.1)	3.432 (218)		12.9 (1)	2.340 (150)	11 (0.2)	
Djuijkers *et al.* (1997)	follicular fluid 9.7 (6.3–12.3)	2.057 (1.471–3.119)	≥15/12 (10–17)	11 (9–18)		10 (9–11)	NS
	follicular fluid 12.6 (5.6–17.9)	1.662 (997–4.195)	≥15/9 (5–15)	7 (2–18)		11 (10–11)	
Adonakis *et al.* (1998)							NS
	1.56 (0.16)	2.042 (266)					
	1.82 (0.23)	3.067 (257)*					
Teissier *et al.* (1999)	0.87 (0.04)	1.115 (50)					NS
	Follicular fluid: 5.1 (0.4)						
	0.86 (0.05)	1.240 (70)					
	Follicular fluid:6.8 (0.2)						
Gordon *et al.* (2001)	1 (0.7–1.2)	828 (518–1.523)	Total/13 (9–16)	11 (8–15)	2.025 (1.575–2.700)	10 (9–11)	NS
	1.1 (0.8–1.6)	1.471 (744–1.954)	Total/13 8–17)	12 (7–15)	2.025 (1.800–2.250)	10 (9–11)	
	0.9 (0.7–1.3)	1.245 (796–1.897)	Total/14 (11–16)	9 (8–11)	2.025 (1.575–2.250)	10 (8–11)	
	1 (0.6–1.3)	1.201 (782–1744)	Total/14 (10–20)	10 (8–14)	1.800 (1.800–2.250)	9 (9–10)	
Filicori *et al.* (2001)	AUC: 5.4 (0.5)	1.133 (81)	>14/8.4 (0.8)*		2.520 (180)*	16.1 (0.8)*	NS
	AUC: 4.5 (0.5)	1.080 (53)	>14/6.3 (0.5)		1.770 (82)	12.6 (0.6)	
Filicori *et al.* (2002a,b)	AUC: 6.5 (0.6)	1.210 (79)	<10/5.6 (0.4)*		2.323 (158)*	14.4 (0.7)*	NS
	AUC: 7.5 (0.6)	1.166 (90)	<10/3.3 (0.3)		2.080 (122)	13.8 (0.6)	
	AUC: 6.7 (0.4)	1.147 (72)	<10/3.1 (0.2)		1.813 (95)	11.8 (0.5)	
	AUC: 5.7 (0.6)	1.114 (62)	<10/0.9 (0.2)		1.818 (72)	12.1 (0.4)	
Filicori *et al.* (2003)	AUC: 8.4 (0.4)*	1.210 (76)	<10/6.3 (0.9)*		1.897 (97)*	12.4 (0.5)*	P lower in hMG group
	AUC: 5.2 (0.6)	1.226 (96)	<10/2.4 (0.5)		1.627 (60)	10.8 (0.4)	
Balasch *et al.* (2003)	AUC: 13.6 (1)	1.981 (260)	>17/6.9 (0.5)*	11.8 (0.9)*	2.245 (180)*	14.7 (0.6)*	NS

Continued

Table II *Continued*

Study	Progesterone hCG (serum (ng/ml) follicular fluid (mcg/ml) AUC (ng/ml day)	E_2 hCG (serum (pg/ml) follicular fluid (ng/ml)	Size/foll (mm/n)	Oocytes (*n*)	Total GnRH dose (IU)	Duration stimulation (days)	Conclusion (difference in *P*-values according to GnRH)
	AUC: 16.7 (1.5)	2.003 (236)	>17/5.1 (0.5)	9.1 (0.9)	1.020 (75)	12.7 (0.4)	
Kilani *et al.* (2003)	2.1 (0.1)	933 (109)	>14/8.4 (0.6)	6.8 (0.6)	2.025 (112)*	12.9 (0.5)*	NS
	AUC: 13 (0.9)						
	2.1 (0.1)	1.342 (127)*	>14/8.5 (0.6)	7.9 (0.7)	1.680 (75)	11 (0.4)	
	AUC: 11.4 (0.9)						
Andersen *et al.* (2006)	1.07 (0.03)*	1.798 (47)	Total/15.9 (0.4)*	11.8 (0.3)*	2.385 (32)	10.1 (0.1)	P lower in hMG group
	0.81 (0.02)	1.961 (61)*	Total/14.8 (0.36)	10 (0.3)	2.508 (38)*	10.4 (0.1)*	
Smitz *et al.* (2007)	Follicular fluid: 10.56 (M)*	follicular fluid 930 (M)					follicular fluid P lower in hMG group
	Follicular fluid: 8.83 (M)	follicular fluid 1.060 (M)*					
Bosch *et al.* (2008)	0.99 (0.04)*	1.750 (87)		14.4 (0.7)*	2.624 (71)		P lower in hMG group
	0.73 (0.04)	2.066 (92)*		11.3 (0.5)	2.481 (90)		
Orvieto *et al.* (2009)	0.7 (0.1)	1.609 (178)		11.2 (1.4)	2.220 (131)	10.4 (0.3)	NS
	0.6 (0.1)	1.957 (168)*		11.3 (1.4)	2.467 (186)	10.6 (0.4)	
Melo *et al.* (2010)	1.01 (0.02)	2.850 (40)		19.3 (0.4)	2.500 (13)	10.4 (0.1)	NS
	0.9 (0.02)	2.740 (43)		19.5 (0.4)	2.350 (11)	10.5 (0.1)	
	0.9 (0.02)	2.710 (38)		18.7 (0.4)	2.450 (17)	10.6 (0.1)	
Requena *et al.* (2010)	0.45 (0.23–1.21)	1.154 (761–2.249)	14–17/5 (3–8)	12.2 (1)	2.111 (127)		NS
	0.20 (0.11–0.45)	1.350 (1.019–2.119)	14–17/4 (2–7)	10.2 (0.7)	2.542 (117)		
	0.34 (0.18–0.54)	1.841 (1.154–2.836)	14–17/5 (3–7)	10.2 (0.7)	2.444 (141)		

Results are expressed as mean ± SEM or median (M) and interquartile range (p25 and p75).
NS, not significant; A, not available; AUC, area under the curve; FF, follicular fluid; MII, metaphase 2; IM, intramuscular; Sc, subcutaneous; (α), Follitropin α ; (β) Follitropin β.
Significance: $^*P < 0.05$.

manufactured to provide 'LH activity'. hMG, a urinary-derived product with both FSH and LH activities, were initially used for clinical practice. The 'LH activity' was supplied by a combination of LH and hCG to provide the equivalence of 75 IU standard of LH biological activity and to comply with the regulation of the European and US Pharmacopea. Following subsequent purification (hMG-HP), the nature of LH activity was switched to hCG alone at a concentration of ~10 IU, equivalent to 75 IU LH in term of biological activity. Recombinant LH, launched in the early 2000s, gave the opportunity to use FSH and LH separately with different ratios. Finally, urinary hCG and, more recently, recombinant hCG have been also used to provide 'LH activity' in addition to FSH.

Therefore, clinical trials published in peer reviews and dealing with a comparative analysis of the effects of FSH alone or associated with 'LH activity' products on serum progesterone^{hCG} in cycles where endogenous LH surge was controlled by GnRH analog protocols were analyzed. For reasons previously mentioned, the influence of confounding factors related to the ovarian response to FSH was also considered. In contrast to meta-analysis, this systematic review was not aimed at providing statistical analysis. The main objective was to identify studies where serum progesterone^{hCG} determination was available and to assess the specific effect of the 'LH activity' supply on serum progesterone variation.

Materials and Methods

Identification of studies

A computerized literature search was performed on the bibliographic databases. The search strategy aimed at identifying both retrospective and prospective studies on the basis of the following clinical question: 'among patients treated with FSH and GnRH analogs, is the addition of products with 'LH activity' associated with significant variation in serum progesterone levels at the time of hCG administration?' The search was limited to studies in humans. Meeting proceedings were not considered

since unpublished studies cannot be adequately assessed for their design and quality. All publications released from 1980 to 2010 were reviewed.

Studies identified

Literature search yielded 108 studies that were potentially able to answer the research question. Fifty-six studies were set up to compare the effects of hMG/hMG-HP versus urinary/recombinant FSH. Twenty one of them provided data regarding serum progesterone^{hCG} and/or follicular fluid progesterone concentrations on the day of oocyte retrieval. Among the 34 studies reporting comparative data between patients treated with urinary/recombinant FSH alone or with addition of recombinant LH, six reported the values of serum progesterone^{hCG}. Finally, 18 studies were performed to compare the effects of FSH alone or with addition/substitution with hCG. In seven of them, serum progesterone^{hCG} were available.

Data extraction

The following data were recorded from each study where serum progesterone^{hCG} value was available: authorship, review and year of publication; type of studies (retrospective or prospective/randomized); interventions; characteristics of population; GnRH analog protocols; gonadotrophin protocols including FSH stimulation regimen and the protocol for 'LH activity' supply; number of enrolled patients in each group; ovarian parameters including serum estradiol (E_2) and serum progesterone^{hCG}, the number of large and medium sized follicles and/or oocytes, duration of ovarian stimulation and total FSH dose used.

Results

The results are presented according to the type of 'LH activity' added to FSH.

Studies comparing hMG or hMG-HP versus urinary or recombinant FSH

Of the 56 studies comparing hMG and FSH, 21 provided information regarding *P* values. Progesterone concentrations were measured in serum at the time of hCG administration in 17 studies, in follicular fluid at the time of oocyte retrieval in two studies and in both serum and follicular samples in 2 studies. Details of the studies are shown in Tables I and II.

The intervention was IVF or ICSI in a majority of studies (16/21), oocyte donation in one study and IUI) in four studies.

The most prescribed GnRH analog regimen was the GnRH agonist protocol (17 long and 1 short) while, in only two studies, a GnRH antagonist protocol was used. In one study, both GnRH analogs were compared.

Table III Main characteristics of six studies comparing FSH alone versus FSH and recombinant LH.

Authors, review/year	Study	Intervention	Population	Protocol GnRH analog	FSH administration starting dose × duration	FSH/LH administration time of LH administration	Patients *n*
Cédrin-Durnerin *et al.*, Hum Reprod/2004	Prosp	IVF-ICSI	Unselected	Antagonist flexible	rFSH (α) 150–300 × 5	≥1 Foll 14–16 mm: rFSH	96
	Random		(Pre-tt : OCP)	Cetro 3 mg		≥1 Foll 14–16 mm: rFSH + rLH 75	107
Sauer *et al.*, RBM Online/2004	Prosp	ICSI	Unselected	Antagonist fixed S7	rFSH (α) 225 × 5	rFSH 75–450	18
	Random		(Pre-tt : OCP)	Cetro 3 mg		S7–S10: rFSH 150 + rLH 225	20
				Long (Leup)		rFSH 75–450	18
Ferraretti *et al.*, Fertil Steril/2004	Prosp	ICSI	Hypo-response to FSH	Long (Deca LP)	rFSH (α) 150–300 × 10	S10: Increased FSH dose	50
	Random					S10: Increasing FSH dose + rLH 75–150	54
						S10: additional hMG	22
Griesinger *et al.*, Hum Reprod/2005	Prosp	IVF-ICSI	Unselected	Antagonist fixed S6	rFSH (α) 150 × 5	rFSH	54
	Random			Cetro 0.25 mg		S6: rLH 75/150	55
Kovacs *et al.*, Fertil Steril/2010	Prosp	IVF-ICSI	Unselected	Long (Bus)	rFSH (α) 150 × 5	rFSH	25
	Random					S1: rLH 75 for 4 days	25
Bosch *et al.*, Fertil Steril/2010	Prosp	IVF-ICSI	Unselected	Antagonist fixed S6	<35 years : rFSH (α) 225 × 5		172
	Random		(Pre-tt : OCP)	Cetro 0.25 mg	rFSH (α) 150 × 5	S1: rLH 75	161
					>35 years : rFSH (α) 300 × 5		142
					rFSH (α) 225 × 5	S1: rLH 150	150

Results are expressed as mean ± SEM or median (M) and interquartile range (p25 and p75).
NS, not significant; NA, not available; 2PN, 2 pronuclei; (α), Follitropin α; (β) Follitropin β.

Regarding the choice of gonadotrophins, urinary FSH preparations were compared with hMG in 10 studies. In another six studies, comparisons were performed between hMG and recombinant FSH. Finally, hMG-HP preparations were compared with recombinant FSH (follitropin alpha) in six studies.

The analysis of follicular fluid progesterone concentrations following hMG or hMG-HP administration showed the absence of significant difference in three studies, while a significant lower value than in rFSH-treated cycles was reported in one study. In that study, the number of developing follicles and retrieved oocytes was significantly lower in patients treated with hMG-HP.

In 16 of 19 studies reporting data on serum progesterone^{hCG} values, no significant difference was observed. In the three others, serum progesterone^{hCG} concentrations were significantly lower in patients treated with hMG when compared with those treated with FSH. In these studies, different GnRH analog protocols were used and the ovarian response following hMG administration was significantly lower than after FSH treatment. In addition, no correlation was observed between the duration of ovarian stimulation and the value of serum progesterone^{hCG} administration.

Therefore, this analysis—comparing the effects of hMG and FSH preparations on serum progesterone rise eventually—shows that the occurrence of a serum decrease after hMG treatment is restricted to a small proportion of patients (15.8%) and is constantly associated with a weaker ovarian response assessed by the number of follicles and oocytes.

Studies comparing FSH alone versus FSH and recombinant LH

Study details are set out in Tables III and IV. In 6 of 34 studies, serum progesterone^{hCG} values were reported. The intervention was IVF-ICSI in these six studies. The GnRH analog used was an agonist (2), an antagonist (3) or both (1). Recombinant FSH was constantly used and comparison was performed with cycles supplemented by recombinant LH (rLH) doses (range: 75–225 IU) prescribed from the beginning (2) or the middle part of the stimulation (4).

In five studies, no significant difference in serum progesterone^{hCG} was observed. In one study, serum progesterone^{hCG} was significantly lower in patients supplemented with rLH when compared with FSH alone. In that study, the administration of LH (75–150 IU) was started on Day 1 of the stimulation, and the number of follicles was significantly reduced, at least in older women (36–39 years) whose duration of ovarian stimulation was significantly longer.

Therefore, this analysis—comparing the effects of FSH alone or in addition with rhLH—cannot demonstrate any consistent effect of rLH on serum progesterone rise. The decrease in serum progesterone observed in the study where rhLH administration was started on Day 1, was associated with a diminished ovarian response to COS.

Table IV Main characteristics of six studies comparing FSH alone versus FSH and recombinant LH.

Study	Progesterone hCG (ng/ml)	E_2 hCG (pg/ml)	Size/foll (mm/*n*)	Oocytes (*n*)	FSH dose (IU)	Duration stimulation days	Conclusion difference in *P*-values
Cédrin-Durnerin *et al.* (2004)	1 (0.1)	1.012 (67)		9.8 (0.5)	2.239 (63)	11.7 (0.1)	NS
	1.1 (0.1)	1.467 (76)*		9.9 (0.5)	2.235 (71)	11.8 (0.1)	
Sauer *et al.* (2004)	1.9 (0.2)	1.540 (224)	≥14/13.7 (1.9)	(2 PN) 9.3 (1.3)	2.228 (85)	9.3 (0.2)	NS
	1.7 (0.2)	2.440 (250)	≥14/14.1 (1.9)	(2 PN) 10.1 (1.7)	2.214 (137)	9.4 (0.4)	
	1.7 (0.2)	2.931 (378)	≥14/13.2 (1.3)	(2 PN) 9.5 (1.2)	2.230 (149)	9.7 (0.4)	
Ferraretti *et al.* (2004)	0.55 (0.03)	1.020 (M)		8.2 (M)	4.320* (84)		NS
	0.51 (0.01)	1.731 (M)*		11.1 (M)*	3.560 (120)		
	05 (0.04)	1.539 (M)		10.9 (M)	3.225 (203)		
Griesinger *et al.* (2005)	0.8 (0.04)	1.488 (112)		7.7 (0.7)	1.874 (88)		NS
	0.9 (0.1)	1.925 (170)*		7.9 (0.7)	2.083 (94)		
Kovacs *et al.* (2010)	0.79 (0.05)	1.923 (263)	>14/9.4 (0.7)	9 (1.2)	1.503 (85)	11.4 (0.3)	NS
	0.88 (0.1)	1.996 (221)	>14/9.7 (0.6)	10.2 (0.9)	1.370 (51)	12 (0.3)	
Bosch *et al.* (2010)	0.85 (0.03)*	1.542 (62)		11.3 (0.5)	2.318 (48)*	9.9 (0.1)	Progesterone lower in rLH group
	0.69 (0.03)	1.625 (71)		10.9 (0.5)	1.840 (50)	10.7 (0.3)*	
	0.89 (0.04)*	1.388 (61)		10.1 (0.5)*	2.562 (61)	10.3 (0.2)	
	0.67 (0.03)	1.560 (66)		8.4 (0.4)	2.560 (62)	10.6 (0.2)	

Results are expressed as mean ± SEM or median (M) and interquartile range (p25 and p75).
NS, not significant; NA, not available; 2PN, 2 pronuclei; (α), Follitropin α; (β) Follitropin β.
Significance: *$P < 0.05$.

Studies comparing FSH alone versus FSH and hCG

Among 18 studies published from 1999 to 2010 (Tables V and VI), seven presented data on serum progesterone^{hCG} levels. The intervention was IVF-ICSI (5) or IUI (2). In four studies, a GnRH agonist protocol was prescribed, whereas a GnRH antagonist was used in two and both GnRH analogs were used in one study. Recombinant FSH was prescribed in six of seven studies, and hCG administration was started from S1 or S2 in two studies and from the mid follicular phase in the five others.

In five studies, no significant difference in serum progesterone concentrations could be observed between patients treated with FSH and hCG when compared with those treated with FSH alone. In contrast, in two studies, a significant increase in progesterone AUC or progesterone^{hCG} value was observed. In both studies, hCG administration was started when FSH was discontinued (Day 8 of ovarian stimulation or at a follicular size >12–13 mm) and the total dose of FSH was significantly lower in patients treated with hCG when compared with those treated with FSH alone. In addition, in one of these two studies, the duration of stimulation was significantly shorter in patients supplemented with hCG.

Therefore, this analysis comparing serum progesterone rise at the time of triggering of ovulation in women treated with FSH alone or a combination of FSH-hCG shows that hCG administration can be associated with a rise in serum progesterone.

Table V Main characteristics of seven studies comparing FSH alone versus FSH and hCG.

Authors, review/year	Study	Intervention	Population	Protocol GnRH analog	FSH administration starting dose × duration	FSH/hCG administration Time of hCG administration	Patients *n*
Filicori *et al.*, J Clin Endocrinol Metab/1999	Prosp Random	IUI	Unselected	Long (Deca LP)	uFSH 150 × 14 uFSH 150 × 14	 S1: hCG 50	10 10
Filicori *et al.*, J Clin Endocrinol Metab/2002a	Prosp Random	IUI	Unselected	Long (Deca LP)	rFSH (β) 150 rFSH (β) 150 × 7 discontinued rFSH (β) 150 × 7 discontinued rFSH (β) 150 × 7 discontinued	 S8: rFSH 50 + hCG 50 S8: rFSH 25 + hCG 100 S8 : hCG 200	10 7 7
Filicori *et al.*, Fertil Steril/2005	Prosp Random	ICSI	Unselected	Long (Deca LP)	rFSH (α/β)/hMG 225–300 rFSH (α/β)/hMG 225–300 discontinued	 ≥6 Foll >12 mm and E_2 >600 : hCG 200	24 24
Gomes *et al.*, Eur J Obstet Gynecol/2006	Prosp Random	ICSI	Unselected	Long (Leup)	rFSH (β) 200 rFSH (β) 200 discontinued rFSH (β) 200 discontinued	 ≥1 Foll 12–13 mm : hMG 225 ≥Foll 12–13 mm : hCG 200	17 17 17
Serafini *et al.*, Fertil Steril/2006	Prosp Random	IVF-ICSI	Unselected	Antag Cetro 0.25 flexible Antag Cetro 0.25 flexible Long (Leup)	rFSH (α) 150–300 × 5 rFSH (α) 150–300 discontinued rFSH (α) 150–300 × 5	 2 Foll 13–14 mm/S6: FSH 75 + hCG 200	96 106 98
Lossl *et al.*, Hum Reprod/2008	Prosp Random	IVF-ICSI	Unselected	Antag Cetro 0.25 flexible	D2 rFSH (α) 150 × 5 D2 Antag 3 mg; D2-D4 Anast 1 mg D5 rFSH (α) 150 × 5	 D2: hCG 1.250 single dose	50 53
Blockeel *et al.*, Hum Reprod /2009	Prosp Random	ICSI	Unselected	Antag Gani 0.25 fixed D6	rFSH (β) 200 × 6 rFSH (β) 200 discontinued	 ≥6 Foll >12 and E_2 >600: hCG 200	32 29

Results are expressed as mean ± SEM or median (M) and interquartile range (p25 and p75).
NS, not significant; NA, not available; AUC, area under the curve; OR, oocyte retrieval; MII, metaphase 2; (α), Follitropin α; (β) Follitropin β.

Table VI Main characteristics of 7 studies comparing FSH alone versus FSH and hCG.

Study	Progesterone hCG (serum (ng/ml) follicular fluid (mcg/ml) AUC (ng/ml day)	E_2 hCG (serum (pg/ml) follicular fluid (ng/ml)	Size/foll (mm/*n*)	Oocytes (*n*)	Gn dose (IU)	Duration stimulation days	Conclusion
Filicori *et al.* (1999)	AUC: 6.4 (1)	977 (27)	>14/7.9 (0.8)		2.670 (164)*	17.3 (0.7)*	NS
	AUC: 5.6 (0.7)	1.171 (151)	>14/8.7 (0.7)		1.725 (84)	12.5 (0.6)	
Filicori *et al.* (2002a,b)	AUC: 7.4 (1)	1.034 (51)	<10/8.1 (0.5)*		1.920 (146)*	13.7 (1)	Progesterone higher in hCG group (50 and 100)
	AUC: 10.7 (0.8)*	1.274 (113)	<10/3.1 (1.6)		1.325 (40)	13.4 (0.7)	
	AUC: 10.7 (0.8)*	1.223 (106)	<10/3 (1.3)		1.180 (15)	13.1 (0.6)	
	AUC: 8.1 (0.7)	1.271 (105)	<10/1.9 (0.7)		1.050 (0)	12.7 (0.6)	
Filicori *et al.* (2005)	1.1 (0.1)	2.358 (234)	>14/11.4 (1.2)	(MII) 8 (0.7)	2.779 (160)*	11.6 (0.2)	NS
	Follicular fluid: 15 (1)						
	1.1 (0.1)	3.235 (317)*	>14/12.2 (0.9)	(MII) 8.2 (0.6)	1.960 (99)	11.9 (0.1)	
	Follicular fluid: 13.5 (0.6)						
Gomes *et al.* (2007)	2 (0.3)	2.057 (252)	OR 21.6 (2)	10.2 (0.9)	1.929 (73)*	9 (7–13)	Progesterone higher in hCG group (200)
	1.5 (0.1)	2.164 (308)	OR 19.3 (3)	10.9 (1.4)	1.256 (48)	10 (7–13)	
	2.6 (0.3)*	3.238 (503)	OR 20.7 (3.2)	11.6 (1.5)	1.244 (46)	10 (8–11)	
Serafini *et al.* (2006)	1 (0.1)	2.411 (273)		11.6 (0.8)	2.198 (83)*	NA	NS
	1.3 (0.1)	4.231 (382)*		10.3 (0.5)	1.675 (59)		
	0.8 (0.1)	2.1353 (302)		10.6 (0.5)	2.157 (81)		
Lossl *et al.* (2008)	Serum: NS		≥14/6 (M)	8 (M)	1.425 (M)	8 (8–9)	NS
	Follicular fluid: 14.1 (M)	Follicular fluid: 216 (M)					
	Serum: NS		≥14/7 (M)	8 (M)	1.650 (M)*	10 (9–10)	
	Follicular fluid: 14.6 (M)	Follicular fluid: 270 (M)*					
Blockeel *et al.* (2009)	1.2 (0.1)	2.044 (213)		12.3 (1)	1.617 (50)	8.2 (0.3)	NS
	1.1 (0.2)	2.250 (322)		11.1 (1)	1.273 (48)	8.7 (0.3)	

Results are expressed as mean ± SEM or median (M) and interquartile range (p25 and p75).
NS, not significant; NA, not available; AUC, area under the curve; OR, oocyte retrieval.
MII, metaphase 2; (α), Follitropin α; (β) Follitropin β.
Significance: *$P < 0.05$.

Discussion

This review does not demonstrate any significant impact of 'LH activity' administration on serum progesterone elevation at the time of hCG administration. Indeed, this analysis of 108 studies comparing administration of FSH alone or in combination with products with LH activity (hMG, rhLH and hCG) shows that: (i) assessment of serum progesterone^{hCG} values was recorded in 34 of 108 published reports (31.5%). (ii) A significant decrease in serum progesterone^{hCG} was observed following administration of 'LH activity' products in four of them (11.8%), while a significant increase in serum progesterone was reported in two others (5.9%). (iii) In a vast majority of published reports (82.3%), the occurrence of serum progesterone elevation was not correlated to 'LH activity' supplementation.

Several questions regarding the issue on serum progesterone elevation during COS remain unanswered. Indeed, 20 years after

the first report on this issue (Schoolcraft *et al*., 1991), both the incidence and the effect on cycle outcome are still a matter of debate. As recently emphasized (Fleming, 2008), one of the most clinically relevant drawbacks is related to serum progesterone measurement usually done by the commercial kit assays with a huge variability (Coucke *et al*., 2007). In most of the studies reviewed, this methodological drawback was minimized by the use of the same assay for comparison between treatment groups. In contrast, the determination of the threshold *P*-value over which cycle outcome could be altered is clearly dependent on the choice of serum progesterone assay. This heterogeneity between progesterone assays can probably account for the divergent conclusion between studies.

The main objective of this review was to assess the specific impact of 'LH activity' products on the risk of serum progesterone^{hCG}. However, as reported in several studies, many confounding factors are involved in the process of serum progesterone rise. The most significant is actually the intensity of the ovarian response to FSH. Indeed, a clear relationship exists between serum progesterone^{hCG} values and the total FSH dose, E_2 values at the time of hCG as well as the number of recruited follicles or retrieved oocytes (Filicori *et al*., 2002a,b; Bosch *et al*., 2003, 2005, 2008; Andersen *et al*., 2006; Kyrou *et al*., 2009a,b). This relationship has been commonly explained by the large recruitment of follicles, each producing progesterone in granulosa cells under the influence of FSH stimulation, which activates 3β HSD type II enzymatic activity. This means that assessment of the role of 'LH activity' products on serum progesterone rise should take into account the intensity of the ovarian response.

It has also been recently suggested that the duration of the follicular phase could be an important determining factor in the occurrence of serum progesterone rise and, therefore, in the risk of cycle failure (Kyrou *et al*., 2011). Indeed, increasing the duration of stimulation could result in a larger number of follicles and higher E_2 levels, which could negatively impact cycle outcome throughout an earlier expression of progesterone receptors (Kolibianakis *et al*., 2002; Kyrou *et al*., 2009a,b). In accordance with this concept, it has been suggested that adjustment of the timing of hCG administration according to serum progesterone concentrations might improve cycle outcome (Harada *et al*., 1996). However, in this review, no significant correlation could be observed between the duration of ovarian stimulation with LH-like activity products and serum progesterone rise.

The most clinically relevant finding of this analysis is that serum progesterone^{hCG} values are poorly dependent on LH activity supplementation during COS. This lack of correlation can be explained by discrepancies in the design of studies.

Firstly, both materials and doses of 'LH activity' largely differed. Because biological activity of hCG is about 7-fold higher than LH, comparison between studies requires an adjusted analysis. In studies where LH supplementation was provided by rLH or hMG, the daily dose used (75–150 IU rLH or 10–20 IU hCG) was very close to the minimal effective dose (The European Recombinant Human LH Study Group, 1998). In contrast, in patients supplemented with hCG, the usual range of daily dose was 50–200 IU, equivalent to 350–1400 IU of LH. This huge difference in 'LH activity' supply could partly explain the divergent effects on serum *P*-values. These data emphasize the need to better assess the effects of lower doses of hCG.

Most importantly, the relationship between the period of LH exposure and the effects on progesterone secretion is another critical issue to be addressed. Indeed, our analysis shows that a decrease in serum progesterone was mainly observed when 'LH activity' supply was administered from the beginning of COS. In contrast, patients who displayed an increase in serum progesterone received LH activity supplementation only during the late part of COS. This differential effect of 'LH activity' support is in agreement with the concept derived from physiology that the effects of LH upon progesterone secretion are development regulated: a dual effect of LH on theca cells is observed from the early follicular phase, while LH has a strong positive effect on progesterone output by granulosa cells during the late follicular phase. Therefore, it might be assumed that adding 'LH activity' from the beginning of the ovarian stimulation can result in a decreased progesterone production, while a stimulatory effect is predominantly observed when LH supplementation is provided during the late stage of COS. These data are in good accordance with a recent report showing that supplementation with rhLH from Day 1 of FSH stimulation has a positive impact on pregnancy rate in older patients (Bosch *et al*., 2011), while it is not the case when addition of LH activity is provided on Day 6–8 of the ovarian stimulation. Further clinical trials are required to confirm this hypothesis.

In conclusion, this review assessed the effects of supplementation with different 'LH activity' products on the elevation of serum progesterone during COS. Our data indicate that providing LH supplementation does not seem to have a consistent effect on progesterone output and that the major determining factor remains the degree of the ovarian response to FSH. This analysis also suggests that the timing of LH administration could have a significant impact on the occurrence of serum progesterone rise during COS. Further studies are required to better identify the optimal dose and timing for 'LH activity' supply.

Conflict of interest

The author has participated in advisory boards for Merck Serono and Merck Sharp Dhome.

References

Adonakis G, Deshpande N, Yates R, Fleming R. Luteinizing hormone increases estradiol secretion but has no effect on progesterone concentrations in the late follicular phase of *in vitro* fertilization cycles in women treated with gonadotropin-releasing hormone agonist and follicle-stimulating hormone. *Fertil Steril* 1998;**69**:450–453.

Andersen AN, Devroey P, Arce JC. for the MERIT group. Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: a randomized assessor-blind controlled trial. *Hum Reprod* 2006;**21**:3217–3227.

Balasch J, Penarrubia J, Fabregues F, Vidal E, Casamitjana R, Manau D, Carmona F, Creus M, Vanrell JA. Ovarian responses to recombinant FSH or hMG in normogonadotropic women following pituitary desensitization by a depot GnRH agonist for assisted reproduction. *Reprod Biomed Online* 2003;**7**:35–42.

Bentick B, Shaw RW, Iffland CA, Burford G, Bernard A. A randomized comparative study of purified follicle stimulating hormone and human menopausal gonadotropin after pituitary desensitization with buserelin for superovulation and *in vitro* fertilization. *Fertil Steril* 1988;**50**:79–84.

Blockeel C, De Vos M, Verpoest W, Stoop D, Haentjens P, Devroey P. Can 200 UI of hCG replace recombinant FSH in the late follicular

phase in a GnRH-antagonist cycle? A pilot study. *Hum Reprod* 2009; **24**:2910–2916.

Bosch E, Valencia I, Escudero E, Crespo J, Simon C, Remohi J, Pellicer A. Premature luteinization during gonadotropin-releasing hormone antagonist cycles and its relationship with *in vitro* fertilization outcome. *Fertil Steril* 2003;**80**:1444–1449.

Bosch E, Escudero E, Crespo J, Simon C, Remohi J, Pellicer A. Serum luteinizing hormone in patients undergoing ovarian stimulation with gonadotropin-releasing hormone antagonists and recombinant follicle-stimulating hormone and its relationship with cycle outcome. *Fertil Steril* 2005;**84**:1529–1532.

Bosch E, Vidal C, Labarta E, Simon C, Remohi J, Pellicer A. Highly purified hMG versus recombinant FSH in ovarian hyperstimulation with GnRH antagonists-a randomized study. *Hum Reprod* 2008;**23**:2346–2351.

Bosch E, Labarta E, Crespo J, Simon C, Remohi J, Jenkins J, Pellicer A. Circulating progesterone levels and ongoing pregnancy rates in controlled ovarian stimulation cycles for *in vitro* fertilization: analysis of over 4000 cycles. *Hum Reprod* 2010;**25**:2092–2100.

Bosch E, Labarta E, Crespo J, Simon C, Remohi J, Pellicer A. Impact of luteinizing hormone administration on gonadotropin-releasing hormone antagonist cycles: an age-adjusted analysis. *Fertil Steril* 2011; **95**:1031–1036.

Cédrin-Durnerin I, Grange-Dujardin D, Laffy A, Parneix I, Massin N, Galey J, Theron L, Wolff P, Conord C, Clement P *et al*. Recombinant human LH supplementation during GnRH antagonist administration in IVF/ICSI cycles: a prospective randomized study. *Hum Reprod* 2004; **19**:1979–1984.

Check JH, O'Shaughnessy A, Nazari A, Hoover L. Comparison of efficacy of high dose pure follicle stimulating hormone versus human menopausal gonadotropins for in vitro fertilization. *Gynecol Obstet Invest* 1995;**40**:117–119.

Coucke M, Devleeschouwer N, Libeer JC, Schiettecatte J, Martin M, Smitz J. Accuracy and reproducibility of automated estradiol-17β and progesterone assys using native serum samples: results obtained in the Belgian external assessment scheme. *Hum Reprod* 2007; **22**:3204–3209.

De Ziegler D, Bijaoui G, Chaperon C. Pre-hCG elevation of plasma progesterone: good, bad or otherwise. *Hum Reprod Update* 2008;**14**:393.

Djuijkers IJM, Vemer HM, Hollanders JMG, Willemsen WNP, Bastiaans LA, Hamilton CJCM, Thomas CMG, Born GF. Different follicle stimulating hormone/luteinizing hormone ratios for ovarian stimulation. *Hum Reprod* 1993;**8**:1387–1391.

Djuijkers IJM, Willemsen WNP, Hollanders HMG, Hamilton CJCM, Thomas CMG, Vemer HM. Follicular fluid hormone concentration using gonadotropin preparations with different FSH/LH ratio. II. Comparison of hMG and recombinant FSH. *Int J Fertil* 1997;**42**:431–43.

Elgindy EA. Progesterone level and progesterone/estradiol ratio on the day of hCG administration: detrimental cutoff levels and new treatment strategy. *Fertil Steril* 2011;**95**:1639–1644.

Fanchin R, Righini C, Olivennes F, Ferreira AL, de Ziegler D, Frydman R. Consequences of premature progesterone elevation on the outcome of *in vitro* fertilization : insignts into a controversy. *Fertil Steril* 1997; **68**:799–805.

Ferraretti AP, Gianaroli L, Magli MC, D'Angelo A, Farfalli V, Montanaro N. Exogenous luteinizing hormone in controlled ovarian hyperstimulation for assisted reproduction technics. *Fertil Steril* 2004;**82**:1521–1526.

Filicori M, Flamigni C, Cognigni GE, Falbo A, Arnone R, Capelli M, Pavani A, Mandini M, Calderoni P, Brondelli L. Different gonadotropin and leuprolide ovulation induction regimens markedly affect follicular fluid hormone levels and folliculogenesis. *Fertil Steril* 1996;**65**:387–393.

Filicori M, Cognigni GE, Taraborrelli S, Spettoli D, Ciampaglia W, Tabarelli de Fatis C, Pocognoli P. Luteinizing hormone activity supplementation enhances follicle-stimulating hormone efficacy and improves ovulation induction outcome. *J Clin Endocrinol Metab* 1999;**84**:2659–2663.

Filicori M, Cognigni GE, Taraborrelli S, Spettoli D, Ciampaglia W, Tabarelli de Fatis C, Pocognoli P, Cantelli B, Boschi S. Luteinzing hormone activity in menotropins optimizes folliculogenesis and treatment in controlled ovarian stimulation. *J Clin Endocrinol Metab* 2001;**86**:337–343.

Filicori M, Cognigni GE, Tabarelli C, Pocognoli P, Taraborrelli S, Spettoli D, Ciampaglia W. Stimulation and growth of antral ovarian follicles by selective LH activity administration in women. *J Clin Endocrinol Metab* 2002a;**87**:1156–1161.

Filicori M, Cognigni GE, Pocognoli P, Tabarelli C, Spettoli D, Taraborrelli S, Ciampaglia W. Modulation of folliculogenesis and steroidogenesis in women by graded menotrophin administration. *Hum Reprod* 2002b; **17**:2009–2015.

Filicori M, Cognigni GE, Pocognoli P, Tabarelli C, Ferlini F, Perri T, Parmegiani L. Comparison of controlled ovarian stimulation with human menopausal gonadotropin or recombinant follicle-stimulating hormone. *Fertil Steril* 2003;**80**:390–397.

Filicori M, Cognigni FE, Gamberini E, Parmegiani L, Troilo E, Roset B. Efficacy of low-dose human chorionic gonadotropin alone to complete controlled ovarian stimulation. *Fertil Steril* 2005;**84**:394–401.

Fleming R. Progesterone elevation on the day of hCG: methodological issues. *Hum Reprod Update* 2008;**14**:391–392.

Fleming R, Chung CC, Yates RWS, Coutts JRT. Purified urinary follicle stimulating hormonee induces different hormone profiles compared with menotrophins, dependent upon the route of administration and endogenous luteinizing hormone activity. *Hum Reprod* 1996; **11**:1854–1858.

Gomes MKO, Vieira CS, Moura D, Luiz A, Manatta A, Leite SP, Reis RM, Ferriani RA. Controlled ovarian stimulation with exclusive FSH followed by stimulation with hCG alone, FSH alone or hMG. *Eur J Obstet Gynaecol Reprod Biol* 2007;**130**:90–106.

Gordon UD, Harrisson RF, Fawzy M, Hennelly B, Gordon AC. A randomized prospective assessor-blind evaluation of luteinizing hormone dosage and in vitro fertilization outcome. *Fertil Steril* 2001; **75**:324–331.

Griesinger G, Schultze-Mosgan A, Dafopoulos K, Schroeder A, Schroer A, von Otte S, Hornung D, Diedrich K, Felberbaum R. Recombinant luteinizing hormone supplementation to recombinant follicule-stimulating hormone induced ovarian hyperstimulation in the GnRH-antagonist multiple-dose protocol. *Hum Reprod* 2005; **20**:1200–1206.

Harada T, Katagiri C, Takao N, Toda T, Moi Y, Terakawa N. Altering the timing of human chorionic gonadotropin injection according to serum progesterone (P) concentrations improves embryo quality in cycles with subtle P rise. *Fertil Steril* 1996;**65**:594–597.

Hugues JN, Massé-Laroche E, Reboul-Marty J, Boiko O, Meynant C, Cédrin-Durnerin I. Impact of endogenous LH serum levels on progesterone elevation on the day of hCG. *Fertil Steril* 2011;**96**:600–604.

Imthurn B, Macas E, Rosselli M, Keller PJ. Nuclear maturity and oocyte morphology after stimulation with highly purified follicle stimulating hormone compared to human menopausal gonadotrophin. *Hum Reprod* 1996;**11**:2387–2391.

Kilani Z, Dakkak A, Ghunaim S, Cognigni GE, Tabarelli C, Parmegiani L, Filicori M. A prospective, randomized, controlled trial comparing highly purified hMG with recombinant FSH in women undergoing ICSI: ovarian response and clinical outcomes. *Hum Reprod* 2003; **18**:1194–1199.

Kolibianakis E, Bourgain C, Albano C, Osmanagaoglu K, Smitz J, Van Steirteghem A, Devroey P. Effect of ovarian stimulation with recombinant follicle-stimulating hormone, gonadotropin releasing hormone antagonists, and human chorionic gonadotropin on

endometrial maturation on the day of oocyte pick-up. *Fertil Steril.* 2002; **78**:1025–1029.

Kolibianakis EM, Venetis CA, Bontis J, Tarlatzis BC. Significantly lower pregnancy rates in the presence of progesterone elevation in patients treated with GnRH antagonists and gonadotrophins: a systematic review and meta-analysis. *Curr Pharm Biotechnol* 2011; June 9 (Epub ahead of print).

Kovacs P, Kovats T, Kaali S. Results with early follicular recombinant luteinizing hormone supplementation during stimulation for in vitro fertilization. *Fertil Steril* 2010;**93**:475–479.

Kyrou D, Popovic-Todorovic B, Fatemi HM, Bourgain C, Haentjens P, Van Landuyt L, Devroey P. Does the estradiol level on the day of human Chorionic Gonadotrophin administration have an impact on pregnancy rate in patients treated with rec-FSH/GnRH antagonist? *Hum Reprod* 2009a;**24**:2902–2909.

Kyrou D, Kolibianakis EM, Venetis CA, Miliaras D, Theodoridis T, Tzevelekis F, Bontis J, Tarlatzis BC. Steroid receptor expression in human endometrium during the follicular phase of stimulated cycles. *Hum Reprod* 2009b;**24**:2931–2935.

Kyrou D, Kolibianakis EM, Fatemi HM, Camus M, Tournaye H, Tarlatzis BC, Devroey P. High exposure to progesterone between the end of menstruation and the day of triggering final oocyte maturation is associated with a decreased probability of pregnancy in patients treated by in vitro fertilization and intracytoplasmic sperm injection. *Fertil Steril* 2011;**96**:884–888.

Labarta E, Martinez-Conejero JA, Alama P, Horcajadas JA, Pellicer A, Simon C, Bosch E. Endometrial receptivity is affected in women with high circulating progesterone levels at the end of the follicular phase: a functional genomics analysis. *Hum Reprod* 2011;**26**:1813–1825.

Lindeberg M, Carlstrom K, Ritvos O, Hovatta O. Gonadotrophin stimulation of non luteinized granulosa cells increases steroid production and the expression of enzymes involved in estrogen and progesterone synthesis. *Hum Reprod* 2007;**22**:401–406.

Lossl K, Yding Andersen C, Loft A, Freiesleben NLC, Bangsboll S, Andersen AN. Short-term androgen priming by use of aromatase inhibitor and hCG before controlled ovarian stimulation for IVF. A randomized controlled trial. *Hum Reprod* 2008;**23**:1820–1829.

Melo MAB, Meseguer M, Garrido N, Bosch E, Pellicer A, Remohi J. The significance of premature luteinisation in an oocyte-donation programme. *Hum Reprod* 2006;**21**:1503–1507.

Melo M, Bellver J, Garrido N, Meseguer M, Pellicer A, Remohi J. A prospective, randomized; controlled trial comparing three different gonadotroin regimens in oocyte donors: ovarian response, in vitro fertilization outcome and analysis of cost minimization. *Fertil Steril* 2010;**94**:958–964.

Murphy BD. Models of luteinisation. *Biol Reprod* 2000;**63**:2–11.

Orvieto R, Homburg R, Meltcer S, Rabinson J, Arteby E, Nahum R. HMG improves IVF outcome in patients with high basal FSH/LH ration: a preliminary study. *Reprod Biomed Online* 2009;**18**:205–208.

Papanikolaou E, Kolibianakis EM, Pozzobon C, Tank P, Tournaye H, Bourgain C, Van Steirteghem A, Devroey P. Progesterone rise on the day of human chorionic gonadotropin administration impairs pregnancy outcome in day 3 single-embryo transfer while has no effect on day 5 single blastocyst transfer. *Fertil Steril* 2009;**91**:949–952.

Payne AH, Hales D. Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. *Endocr Rev* 2004; **25**:947–970.

Requena A, Landeras J, Martinez-Navarro L, Calatayud C, Sanchez F, Maldonado V, Munoz M, Fernandez M, Gonzalez A, Lopez S *et al.* Could the addition of hp-hMG and GnRH antagonists modulate the response in IVF-ICSI cycles? *Hum Fertil (Camb)* 2010;**13**:41–51.

Sauer MV, Thornton MH II, Schoolcraft W, Frishman GN. Comparative efficacy and safety of cetrorelix with or without mid-cycle recombinant LH and leuprolide acetate for inhibition of premature LH surges in assisted reproduction. *Reprod Biomed Online* 2004; **9**:487–493.

Schoolcraft W, Sinton E, Schlenker T, Huynh D, Hamilton F, Meldrum DR. Lower pregnancy rate with premature luteinization during pituitary suppression with leuprolide acetate. *Fertil Steril* 1991; **56**:563–566.

Serafini P, Yadid I, Motta EL, Alegretti JR, Fioravanti J, Coslovsky M. Ovarian stimulation with daily late follicular phase administration of low-dose human chorionic gonadotropin for *in vitro* fertilization: a prospective, randomized trial. *Fertil Steril* 2006;**86**:830–838.

Smitz J, Andersen AN, Devroey P, Arce JC. Endocrine profile in serum and follicular fluid differs after ovarian stimulation with hp-hMG or recombinant FSH in IVF patients. *Hum Reprod* 2007;**22**:676–687.

Teissier MP, Chable H, Paulhac S, Aubard Y. Recombinant human follicle stimulating hormone versus human menoapusal gonadotrohin induction: effects in mature follicle endocrinology. *Hum Reprod* 1999; **14**:2236–2241.

The European Recombinant Human LH Study Group. Recombinant human luteinizing hormone (LH) to support recombinant human follicle-stimulating hormone (FSH)-induced follicular development in LH - and FSH—deficient anovulatory women: a dose-finding study. *J Clin Endocrinol Metab* 1998;**83**:1507–1513.

Ubaldi F, Camus M, Smitz J, Coelingh Benninck H, Van Sterteghem A, Devroey P. Premature luteinisation in *in vitro* fertilization cycles using gonadotropin-releasing hormone agonist (GnRH-a) and recombinant follicle-stimulating hormone (FSH) and GnRH-a and urinary FSH. *Fertil Steril* 1996;**66**:275–280.

Van Vaerenberg M, Blockeel C, Van Lommel L, In't Veld P, Schuit F, Kolibianakis EM, Devroey P, Bourgain C. Progesterone rise on hCG day in GnRH antagonist/FSH stimulated cycles affects endometrial gene expression. *Reprod Biomed Online* 2011;**22**:263–271.

Venetis CA, Kolibianakis EM, Papanikolaou E, Bontis J, Devroey P, Tarlatzis BC. Is progesterone elevation on the day of human chorionic gonadotrophin administration associated with the probability of pregnancy in *in vitro* fertilization? A systematic review and meta-analysis. *Hum Reprod Update* 2007;**13**:343–355.

Yding Andersen C, Bungun L, Nyboe Andersen A, Humaidan P. Preovulatory progesterone concentrations associated significantly to follicle number and LH concentrations but not to pregnancy rate. *Reprod Biomed Online* 2011;**23**:187–195.

Young EL, Baird DT, Reichert LE, Hillier SG. Hormonal regulation of the growth and steroidogenic function of human granulose cells. *Hum Reprod* 1992;**74**:842–849.

Young EL, Hillier SG, Turner M, Baird DT, Ng SC, Bongso A, Ratnam SS. Differential regulation of cholesterol side-chain cleavage (P450scc) and aromatase (P450arom) enzyme mRNA expression by gonadotrophins and cyclic AMP in human granulose cells. *J Mol Endocrinol* 1994; **12**:239–249.

Younis JS. Elevated P level on the day of hCG administration is related to FSH dose: is it whole truth? *Hum Reprod* 2011;**26**:498–499.