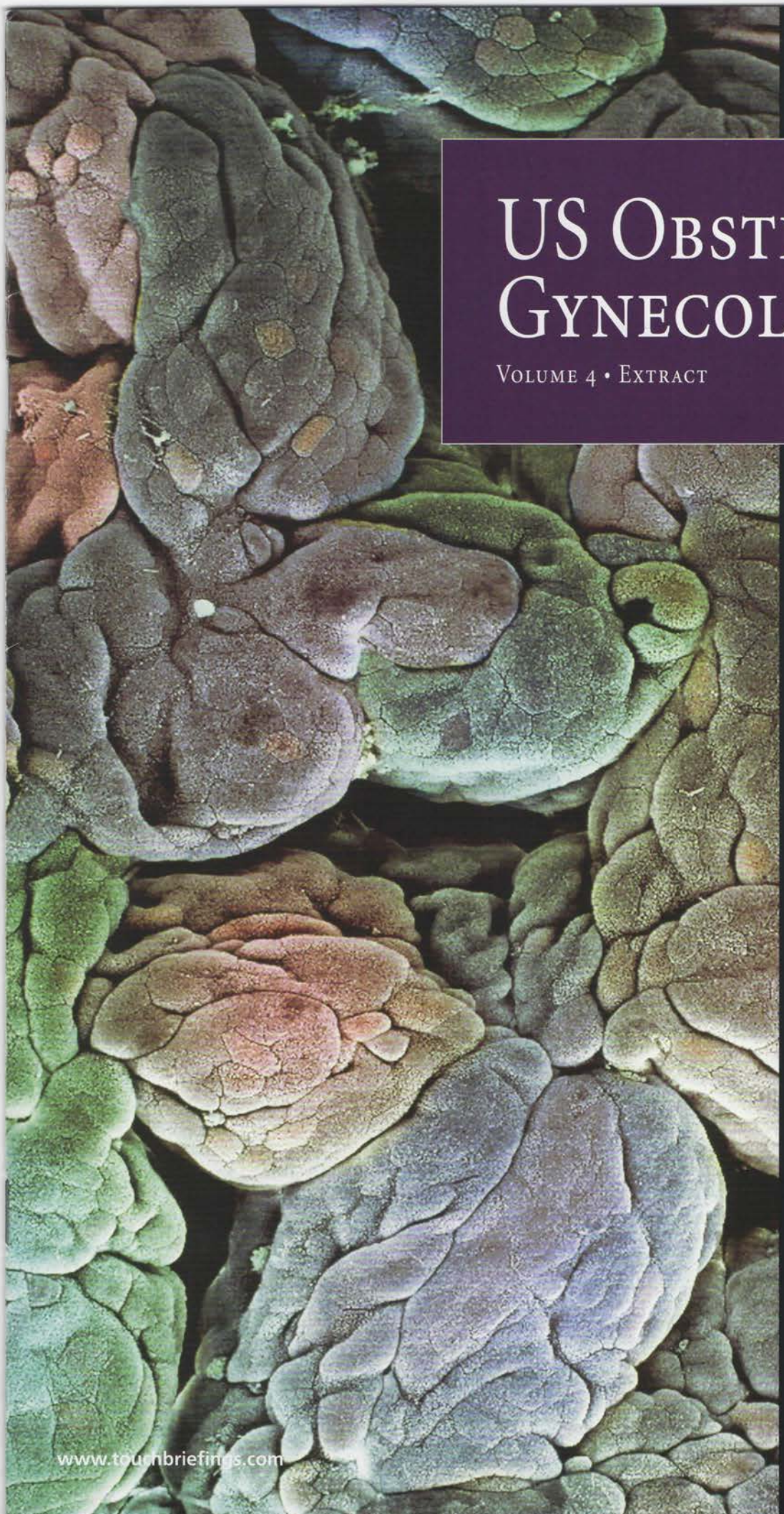


US Obstetrics & Gynecology

Volume 4 • Extract

Gonadotropins in the Treatment of Infertility

George T Koulianos, MD

Adjunct Assistant Professor, Department of Obstetrics and Gynecology, University of South Alabama, and Program Director, The Center for Reproductive Medicine, Mobile Infirmary Medical Center

Reprint Citation: Koulianos G, Gonadotropins in the Treatment of Infertility, *US Obstetrics & Gynecology,* 2009;4(1):29–36.

Gonadotropins in the Treatment of Infertility

George T Koulianos, MD

Adjunct Assistant Professor, Department of Obstetrics and Gynecology, University of South Alabama, and Program Director, The Center for Reproductive Medicine, Mobile Infirmary Medical Center

Abstract

Gonadotropins have been used for the treatment of infertility for approximately 50 years. The US infertility specialty has empirically chosen to incorporate greater amounts of human menopausal gonadotropin (hMG) into stimulation protocols. Following this trend to use more hMG, evidence has accumulated demonstrating the benefit of this unique gonadotropin formulation. Studies over the past decade have become more complex in their scope and more expansive in size as they have sought to identify the endocrinology and physiology underlying the increased use of hMG. This article will attempt to identify some of the unique attributes of hMG, and especially highly purified (HP)-hMG, that lead to better outcomes, focusing on the different aspects of ovarian stimulation physiology.

Keywords

Human menopausal gonadotropin (hMG), follicle-stimulating hormone (FSH), *in vitro* fertilization (IVF), ovarian stimulation, Menopur®, Repronex®

Disclosure: George T Koulianos, MD, PC, serves on the Organon National Speaker's Bureau, has completed clinical trials for Serono, and is conducting ongoing clinical trials for Pfizer.
Received: August 3, 2009 **Accepted:** October 12, 2009
Correspondence: George T Koulianos, MD, Mobile Infirmary Medical Center, Suite 213, Mobile, AL 36607. E: gkoulian@bellsouth.net

Support: This article has been supported by an educational grant from Ferring. The views expressed are those of the author, and not necessarily those of Ferring.

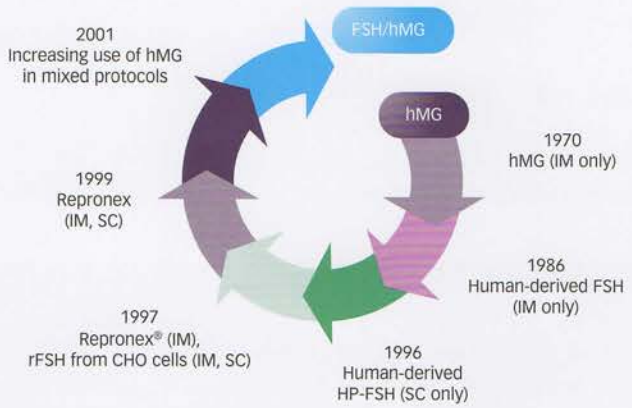
The use of gonadotropins in the treatment of infertility and especially assisted reproductive technologies (ART) has dramatically improved outcomes. Since Lunenfeld first reported the use of gonadotropins extracted from the urine of menopausal women to induce ovulation in 1963,[1] these compounds have undergone significant improvements in efficacy and purity.[2] These improvements are often under-appreciated by clinicians. The role of gonadotropins, and in particular the newer and purer formulations, have been especially important to the improvement of the efficiency of *in vitro* fertilization (IVF).[3] This article will discuss the evolution and current state of gonadotropins in IVF.

Initial extraction techniques were extremely crude, requiring high volumes of urine—about 30l—to extract enough gonadotropin for a single ovulation-induction treatment cycle.[1] The ratio of follicle-stimulating hormone (FSH) to luteinizing hormone (LH) in these initial crude preparations was ~1:1. These initial preparations were impure, with only 5% of the proteins being bioactive, and had considerable batch-to-batch variability. With improved extraction techniques, urinary products including human menopausal gonadotropin (hMG) and highly purified (HP)-FSH now demonstrate comparable efficacy to recombinant products and are used interchangeably.[4–7] Furthermore, the role of LH in IVF has been controversial and we can now demonstrate that we have come full circle on both the role of urinary products in IVF and the need for LH activity; therefore, the use of gonadotropins in the US over the past 50 years can be seen as an incomplete circle.

In the 1960s and 1970s, hMG was the only agent available for controlled ovarian hyperstimulation (see *Figure 1*).[8] With the development of recombinant FSH (rFSH), the role of LH in IVF cycles came into question. In the late 1970s through to the mid-1980s, there was a desire to do away with this LH activity component as it was seen as detrimental to outcomes.[8] The belief that LH is detrimental to outcomes was founded on two hypotheses that no longer hold true. First, unlike today, gonadotropin-releasing hormone (GnRH) analogs were not used, so patients tended to have high endogenous LH levels.[8] Second, patients with polycystic ovary syndrome (PCOS) have a poor prognosis, with a hallmark of PCOS being relatively high endogenous LH. One piece of this hMG body of evidence that is becoming apparent is that endogenous LH activity is not equivalent to exogenous human chorionic gonadotropin (hCG)-driven LH activity that is observed with HP-hMG. LH and hCG have the same bioactivity but dramatically different pharmacokinetics and pharmacodynamics, especially when using one as a pharmaceutical and the other endogenously.[9]

This desire to remove the LH activity component from the FSH activity component of hMG was realized in 1986 with a urinary FSH-only product that was indicated for intramuscular (IM) use only.[10] This IM limitation was a result of purification techniques that had not sufficiently reached the level where all contaminating proteins could essentially be removed from gonadotropin preparation. This resulted in substantial injection-site reactions when used subcutaneously (SC). A decade later, a highly purified

Figure 1: The Infertility Treatment Field Has Empirically Found a Need for Human Menopausal Gonadotropin



hMG = human menopausal gonadotropin; FSH = follicle-stimulating hormone; IM = intramuscular; SC = subcutaneously; rFSH = recombinant FSH; HP-FSH = highly purified FSH; CHO = Chinese hamster ovary.

Figure 2: The Human Menopausal Gonadotropin Continuum

hMG = human menopausal gonadotropin; FSH = follicle-stimulating hormone.

urinary FSH (HP-uFSH)-only preparation that could be used SC became available. This was followed one year later by rFSH products that were pure FSH with no detectable LH activity, but had the same amount of contaminating proteins from the recombinant production process as the HP urinary product, only the contaminating proteins were of animal origin.[11] Since the beginning of the decade there has been a steady increase in the use of hMG (both HP-hMG and hMG), indicating that the field has empirically chosen hMG as its basis of stimulation.[12,13] This article will describe the body of evidence that supports this empirical decision.

With improved extraction techniques, urinary products including human menopausal gonadotropin and highly purified follicle-stimulating hormone now show comparable efficacy to recombinant products and are used interchangeably.

Stimulation on the Right of the Human Menopausal Gonadotropin Continuum Is Superior

The utilization of rFSH alone in IVF cycles downregulated with GnRH agonists allows the role of LH to be better understood and also improves our understanding of the physiology of ovarian 17 beta-estradiol (E_2) production. As the follicle evolves, it is known that LH exerts a synergistic action with FSH. Theca cells are stimulated by LH to produce androstenedione (A) and testosterone (T) by cytochrome P450 and 3-beta-hydroxysteroid dehydrogenase. Induced by FSH, aromatase in granulosa cells converts A and T into estrone and estradiol. The need for both granulosa cells and theca cells and both LH and FSH has led to the 'two-cell, two-gonadotropin' theory.[14] FSH also induces granulosa cell LH receptors with roughly 10mm follicles demonstrating LH receptors.[15] Therefore, one can demonstrate a cellular need for exogenous LH in IVF cycles, although clinically this has been controversial. The emerging consensus is that some LH activity is needed and results in improved pregnancy rates. The utilization of hMG in stimulation protocols can be graphed as a linear continuum (see *Figure 2*). On the left only FSH vials are used, with hMG not started; it then moves through different permutations until the right-hand side of the continuum is reached, where hMG is utilized exclusively from day one of stimulation. A recent survey revealed that approximately 86% of cycles in the US—about 95,000 per year—fall in the middle of the continuum: either a 1:1 vial ratio is used starting from day six or one vial of hMG is used throughout stimulation. Of the approximately 14% remaining cycles, ~8% are FSH alone and ~6% are hMG alone.[16] The body of evidence presented demonstrates that the right-hand side of the hMG continuum is 5–8% higher in terms of pregnancy and live birth rates compared with the left-hand side. Unfortunately, little is known about the slope of the curve as it rises 5–8% from left to right (it would take an extraordinarily large study to describe such a slope), but it is known that the decision of infertility professionals to incorporate increasing levels of hMG in stimulation protocols, while empirical in nature, now has a wealth of supporting evidence.

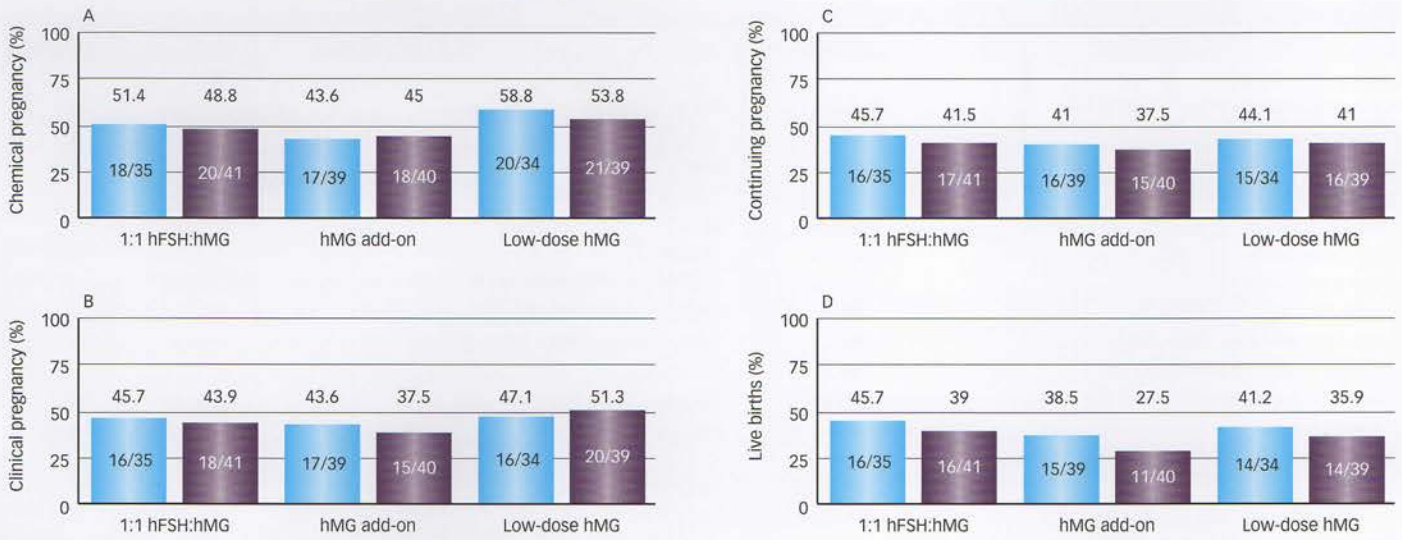
Success Rates in the Middle of the Human Menopausal Gonadotropin Continuum Trend Higher as More Human Menopausal Gonadotropin Is Used

Keye et al.[6] studied various ratios and start times for hMG in the Evaluation of Mixed protocols with Bravelle® (human-derived FSH) and Repronex® (hMG) to assess clinical efficacy (EMBRACE) I and II studies. These studies were stratified based on age. Although not statistically significant, the cumulative results demonstrate a trend toward higher pregnancy rates and fewer miscarriages (see *Figure 3*). This study was limited by the fact that there were no control groups for the direct comparison of FSH with hMG. Given the study's limited sample size examining the middle of the hMG continuum, the differences only reveal themselves as trends. Indeed, the difference anticipated in pregnancy and live birth rates between the two sides of the hMG continuum is slight in statistical terms—approximately 5–8%—but large in human terms, representing approximately 6,000–9,000 live births in the US every year. With the limited resources available it is nigh-on impossible to describe the middle of the hMG continuum, so we must study the two extremes. This is the approach utilized by the majority of studies in the hMG body of evidence.

The Approval Study for Human Menopausal Gonadotropin (Menopur®) Identifies Important Trends

Menopur® (HP-hMG) was introduced in the US in April 2005 following its introduction in Europe based on the data from the European-Israeli Study Group (EISG) study.[17] This study was the largest IVF trial conducted in the world. The EISG study compared the two extremes of the hMG continuum, that is rFSH alone versus hMG alone (see *Table 1*). Despite the inclusion of

Figure 3: Pregnancy and Live Birth Rates in the EMBRACE Study[6]



hMG = human menopausal gonadotropin; hFSH = human follicle-stimulating hormone.

Table 1: Pregnancy Outcomes Described by Completed Cycle or Embryo Transfer in Patients Treated with Either Highly Purified Menopausal Gonadotropin or Recombinant Follicle-stimulating Hormone in Per Protocol and All Patients Treated Population[17]

Outcomes	PP		APT	
	HP-hMG n (%)	rFSH n (%)	HP-hMG n (%)	rFSH n (%)
Started cycles	357 (100)	336 (100)	373 (100)	354 (100)
Cycles with ovum pick-up	346 (96.9)	321 (95.5)	361 (96.8)	339 (95.8)
IVF/ICSI cycles	344 (96.4)	317 (94.3)	359 (96.2)	335 (94.6)
Implanted transferred embryos	321 (89.9)	299 (89)	336 (90.1)	315 (89)
Ongoing pregnancy rate per completed cycle	85 (25)	71 (22)	87 (24)	73 (22)
Cycles with embryo transfer	321 (100)	299 (100)	336 (100)	315 (100)
Positive hCG test (biochemical pregnancies) per embryo transferred	114 (35.5)	98 (32)	119 (35.4)	101 (32.1)
Clinical pregnancy rate per embryo transferred	95 (29.6)	76 (25.4)	98 (29.2)	78 (24.8)
Ongoing pregnancy rate per embryo transferred	85 (26.5)	71 (23.7)	87 (25.9)	73 (23.2)

PP = per protocol; APT = all patients treated; HP-hMG = highly purified human menopausal gonadotropin; rFSH = recombinant follicle-stimulating hormone; IVF = in vitro fertilization; ICSI = intracytoplasmic sperm injection; hCG = human chorionic gonadotropin.

substantial numbers of patients in this study, statistical significance was not achieved. However, the trends favored hMG (the right-hand side of the hMG continuum) over rFSH. More importantly, this study provided the foundational database used by Platteau et al. in their re-analysis.[13]

The Platteau Study Reveals Significant Differences and Formulates Hypotheses

The re-analysis by Platteau et al.[13] of the EISG study data found two important differences: first, hMG significantly outperformed rFSH when used in conjunction with IVF, and second, HP-hMG did not outperform rFSH in conjunction with intracytoplasmic sperm injection (ICSI) (see *Table 2*). Therefore, in this analysis HP-hMG was not inferior to rFSH; when ICSI was used, the two treatments were equivalent. Within the limitations of the re-analysis, it was not possible to provide a satisfactory explanation for this. The author presented some hypotheses that will be addressed below. It is notable that this study is limited, as it is a re-analysis that compares treatment groups not included in the original study design (see *Table 2*).

In addition to the above determination, the re-analysis of the EISG study showed that serum hCG levels following a static 225 international units (IU) dose of hMG for five days correlated with pregnancy rate. The investigators also demonstrated lower pregnancy rates with lower LH levels.[13] The LH activity component of hMG (Menopur) is derived almost exclusively from hCG (10IU). Less than one immunoreactive IU of LH is detectable in hMG. It is generally accepted that due to its increased half-life and greater receptor affinity, hCG has seven times more LH activity than LH.[15] This can be interpreted as 1mol of hCG being seven times as potent in eliciting a receptor-mediated response than 1mol of LH. Even though the range of hCG values obtained is limited and the correlation is based on a trend analysis that uses categorical and not continuous variables, the existence of a difference in gonadotropic agents at an early stage in stimulation indicates the presence of an as yet unknown physiology and/or pharmacology. The re-analysis of Platteau provided valuable information about the two extremes of the hMG continuum that provides the foundation for the most extensive IVF study ever performed, the Menotrophin versus Recombinant FSH *in vitro* Fertilization Trial (MERiT).

Table 2: Clinical End-Points in the Platteau Study[18]

Parameter	*In Vitro* Fertilization			Intracytoplasmic Sperm Injection		
	HP-hMG	rFSH	p-value	HP-hMG	rFSH	p-value
Number of patients	121	112	–	237	221	–
Number of follicles ≥16mm on day of hCG	7.4±3.8	8±4.5	NS	8.4±4.6	8.5±5.7	NS
Number of oocytes retrieved	11.7±7.1	14.1±8.7	0.022	13.4±7.4	14.0±8.4	NS
Fertilization rate (% of retrieved)	59%	59%	NS	54%	57%	NS
Number of embryos transferred	2.1±0.9	2.1±0.8	NS	2.2±0.8	2.2±0.8	NS
Postitive beta-hCG	48 (40%)	30 (27%)	0.035	71 (30%)	70 (32%)	NS
Clinical pregnancy	42 (35%)	22 (20%)	0.009	56 (24%)	55 (25%)	NS
Ongoing pregnancy	38 (31%)	22 (20%)	0.037	49 (21%)	50 (23%)	NS
Implantation rate	21%	15%	0.054	12%	13%	NS
LH level on day 6 (IU/l) (mean ± SD)	1.6±1.2	1.4±1	NS	1.4±1.1	1.3±0.8	NS
hCG level on day 6 (IU/l) (mean ± SD)	1.4±0.5	–	–	1.4±0.6	–	–
E_2 levels on day 6 (pmol/l) (mean ± SD)	634±654	793±664	NS	769±829	686±648	NS
E_2 levels on day of hCG (pmol/l) (mean ± SD)	7,560±5,343	6,531±4,717	NS	8,381±6,170	6,262±4,311	<0.001
Ongoing pregnancy according to LH levels on day 6:						
<0.75IU/l	24% (6/25)	9% (2/23)	NS	22% (5/23)	21% (10/47)	NS
≥0.75IU/l	35% (32/92)	21% (17/81)	0.045	19% (37/193)	23% (36/157)	NS
Ongoing pregnancy according to hCG levels on day 6:						
<1IU/l	8% (2/24)	–	–	8% (4/51)	–	–
1 to <2IU/l	35% (27/77)	–	–	22% (29/133)	–	–
≥2IU/l	53% (8/15)	–	–	28% (9/32)	–	–
Ongoing pregnancy according to E_2 levels on day of hCG:						
<4,500pmol/l	18% (7/38)	19% (8/42)	NS	17% (10/58)	26% (21/81)	NS
≥4,500pmol/l	38% (30/79)	18% (12/67)	0.008	21% (34/164)	20% (25/127)	NS

HP-hMG = highly purified human menopausal gonadotropin; rFSH = recombinant follicle-stimulating hormone; hCG = human chorionic gonadotropin; LH = luteinizing hormone; E_2 = estradiol; NS = not significant.

MERiT Demonstrates Significant Differences in Stimulations

The MERiT trial was a randomized, open-label, assessor-blind, parallel-group, multicenter trial in 731 women undergoing IVF, comparing HP-hMG with rFSH, and was specifically designed to further investigate the differences detected by Platteau's re-analysis.[4] It examined the two extremes of the hMG continuum while taking the most systematic approach to an IVF study thus far. While there were multiple differences detected between HP-hMG and rFSH treatment, two important findings have been reported in the MERiT study. First, there were distinct differences in stimulations detected when large numbers of patients were studied. Second, hMG stimulations produced fewer follicles and oocytes compared with rFSH stimulations. Numerically, the difference was fewer than two oocytes, but this was still statistically significant when studying this large number of patients.[4]

MERiT Endocrine Study Demonstrates a More Natural Stimulation When Human Menopausal Gonadotropin Is Used

The MERiT endocrine paper compared differences in endocrine response when HP-hMG alone (the right-hand side of the hMG continuum) is used with rFSH alone (the left-hand side of the continuum). *Figure 4* presents endocrine values as a ratio between hMG and rFSH. From this study, it can be seen that HP-hMG usage at the time of oocyte retrieval results in relatively high estradiol concentrations and relatively low progesterone concentrations. The converse is true for rFSH when there is relatively low estradiol and relatively high progesterone. We also see an increase in the free androgen index (FAI), reflecting the increase in T and A. The absolute mean values of progesterone may fall below the level considered to be clinically significant by most (usually 1–2ng/ml), but still remain statistically higher when rFSH is used, indicating that the likelihood of falling over the clinically significant threshold is more probable when rFSH is used.[19] If the overall endocrine milieu is considered when hMG is used exclusively as the stimulation agent, it can be seen that at the time of oocyte retrieval, which is analogous to ovulation in the natural menstrual cycle, there is a more physiological environment present. These significant differences in endocrine parameters are being translated through to the endometrium where there is a greater incidence of hypoechogenic endometriums in the hMG-stimulated group. Given that echogenicity is a crude measure of endometrial staging, it is still a significant difference that provides another plausible explanation for the observed differences in pregnancy rates. Not only is the endocrine profile being reflected in the endometrium, but also other distinct differences in oocytes and their capacity to produce pregnancy and live birth, as reported in the MERiT Embryology Study (see *Figure 5*).[20]

MERiT Embryology Paper Demonstrates a Greater Capacity for Pregnancy and Live Births from Human Menopausal Gonadotropin-stimulated Oocytes

The MERiT embryology study describes the developmental potential of oocytes from HP-hMG- and rFSH-stimulated cycles. The HP-hMG-stimulated group provided fewer oocytes but with less fragmentation and of higher quality, yielding better-quality embryos to transfer and a higher pregnancy rate compared with rFSH. A given oocyte from an HP-hMG-stimulated cycle has a greater capacity to produce a pregnancy and live birth than an rFSH-stimulated oocyte. However, the

conclusions of this report address only one parameter of competence in embryology. The differences observed are a measure of oocyte quality, but do not reveal anything about the complement of the oocytes or embryos.[20]

The Weghofer I Study Shows a Greater Number of Cytogenetically Normal Embryos When Human Menopausal Gonadotropin Is Used

A major limitation of the MERiT study was the lack of information in terms of the cytogenetics of the embryos from each of the stimulation groups. To address this deficiency, Weghofer investigated the cytogenetics of embryos arising from stimulation in the right-hand side compared with the left-hand side of the hMG continuum.[21] A significantly greater proportion of euploid embryos were reported in the hMG-stimulated group compared with rFSH (69.8±26.7 versus 45.3±26.5; p<0.01). In addition, an explanation was provided as to why significant differences appear to be age-group-specific. Younger patients could be masking the benefit of hMG stimulation, as those differences are likely to be present in their augmented pregnancy rate. It is only in older patients, where reproductive potential is diminished, that the 5–8% difference with rFSH alone compared with hMG alone is immediately apparent. Younger patients derive some benefit from superior treatment, but they are more able to compensate for inferior treatment.

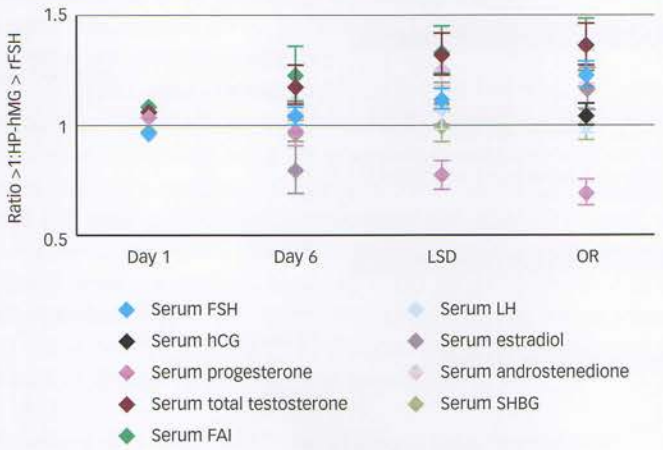
The Bosch Study Indicates Similar Trends with Antagonist Downregulation

The data discussed above show trends and significant differences between hMG- and rFSH-stimulated cycles when agonist downregulation is used. However, this does not address cycles in which an antagonist is employed to prevent a premature LH surge. These cycles account for approximately 30% of IVF cycles every year. Bosch et al.[5] provided evidence of why fertility specialists have concentrated on hMG over the past decade as the preferred choice for stimulations, and showed two interesting points in their data. First, the endocrine profile in these antagonist cycles at the time of hCG trigger is similar to that seen when an agonist was used in the earlier studies, with hCG-stimulated cycles having relatively high estradiol and relatively low progesterone, while in the rFSH-stimulated cycles the reverse was the case. In this study, not only was the endocrine profile similar when an antagonist was used, but there was also a greater number of oocytes retrieved when rFSH was used exclusively. The trend in pregnancy rates favoring hMG showed a decrease in the capacity of rFSH-treated oocytes to produce a pregnancy and live birth.

A Definitive Superiority of Human Menopausal Gonadotropin Was Observed in the Combined Analysis

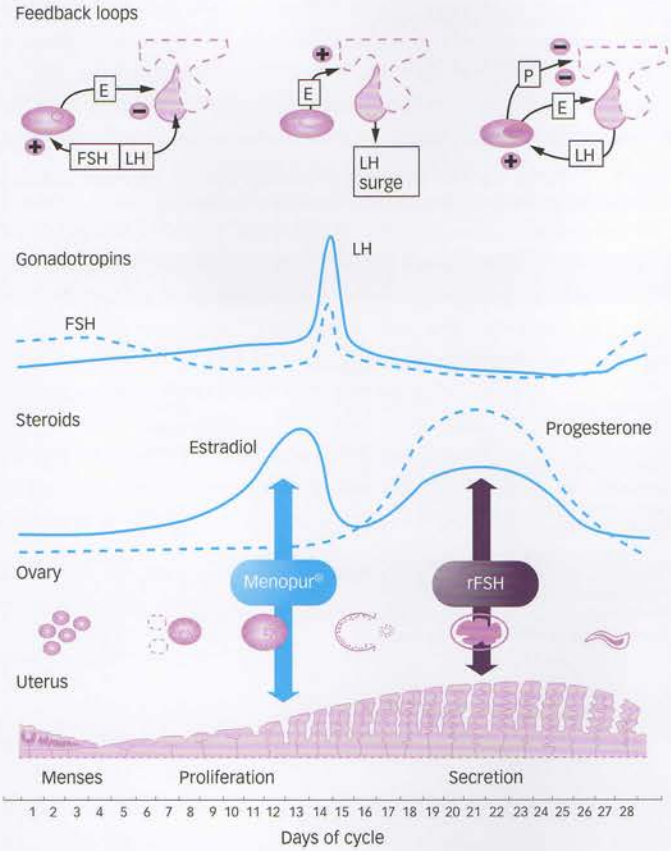
To date, significant differences in hMG compared with rFSH have been demonstrated in some secondary end-points, such as endocrine parameters, endometrial fitness, and embryo ploidy. There are trends to high pregnancy and live birth rates in some studies, but the only statistically significant difference was seen in a re-analysis of data,[6] not in a primary study. This is a reflection of the fact that the number needed to detect a difference of 5–8% in pregnancy rates is over 1,000 and not in the hundreds.

Figure 4: Serum Profiles During Stimulation[20]



LSD = last simulation day; OR = oocyte retrieval; FSH = follicle-stimulating hormone; hCG = human chorionic gonadotropin; HP-hMG = highly purified human menopausal gonadotropin; rFSH = recombinant FSH; LH = luteinizing hormone; SHBG = sex hormone-binding globulin; FAI = free androgen index.

Figure 5: Gonadotropin, Steroid Hormone, and Associated Feedback Loops During the Menstrual Cycle



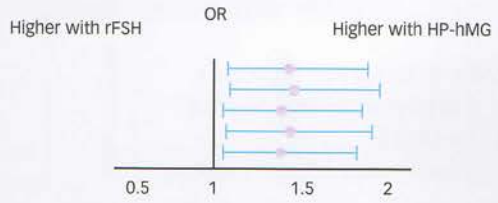
FSH = recombinant follicle-stimulating hormone; rFSH = recombinant FSH; LH = luteinizing hormone; E = estradiol; P= progesterone.

However, a more recent report included the requisite number of patients and was termed a 'combined analysis,' as it was not a meta-analysis in the classic sense.[13] The analysis included two studies, EISG

Figure 6: Combined Analysis of Pregnancy and Implantation Rates, Oocyte Retrieval, and Outcome and Safety for Highly Purified Human Menopausal Gonadotropin and Recombinant Follicle-stimulating Hormone[13]

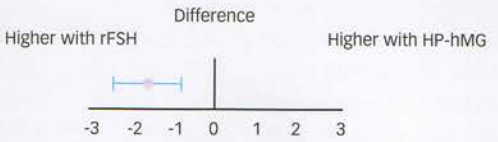
A. Pregnancy and implantation rates

	HP-hMG (%)	rFSH (%)	OR (95% CI)	p-value
Clinical pregnancy rate/cycle initiated	29	22	1.42 (1.06, 1.89)	0.017
Clinical pregnancy rate/cycle with ET	35	27	1.46 (1.08, 1.96)	0.013
Ongoing pregnancy rate/cycle initiated	27	21	1.38 (1.03, 1.86)	0.030
Ongoing pregnancy rate/cycle with ET	33	25	1.42 (1.05, 1.92)	0.024
Implantation rate	24	19	1.37 (1.03, 1.82)	0.030



B. Oocyte retrieval and outcome

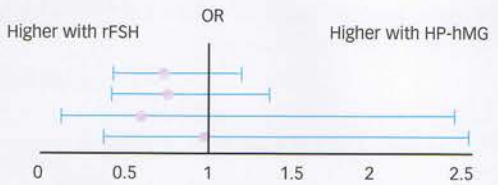
	HP-hMG (%)	rFSH (%)	Difference (95% CI)	p-value
Number of oocytes retrieved	10.5±5.9	12.3±6.7	-1.6 (-2.4, -0.8)	<0.001



Ongoing pregnancy rate	HP-hMG (%)	rFSH (%)	OR (95% CI)	p-value
0–3 oocytes retrieved	9	6	1.35 (0.28, 7.29)	0.704
≥4 oocytes retrieved	29	23	1.39 (1.03, 1.88)	0.031

C. Safety

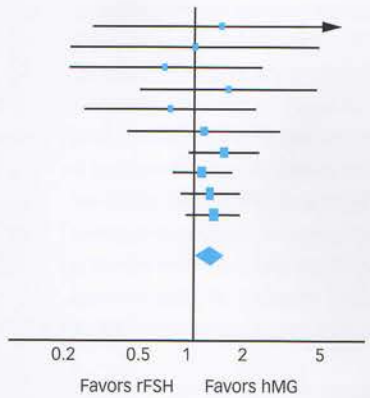
	HP-hMG (%)	rFSH (%)	OR (95% CI)	p-value
Miscarriage	24	31	0.73 (0.45, 1.20)	0.215
Multiple pregnancy/ongoing pregnancy	29	32	0.77 (0.43, 1.37)	0.372
Ectopic pregnancy	1	1	0.60 (1.03, 2.48)	0.616
Moderate to severe OHSS	2	2	0.99 (0.38, 2.56)	0.485



OR = odds ratio; CI = confidence interval; ET = embryo transfer; HP-hMG = highly purified human menopausal gonadotropin; rFSH = recombinant FSH; OHSS = ovarian hyperstimulation syndrome.

Figure 7: Odds Ratios Showing the Effect of Human Menopausal Gonadotropin and Recombinant Follicle-stimulating Hormone on Live Birth Rate[21]

Study or Subcategory	hMG n/N	rFSH n/N	Peto OR 95% CI	Weight %	Peto OR 95% CI
Rashidi, 2005	4/30	3/30		1.21	1.37 (0.29, 6.56)
Ng, 2001	4/20	4/20		1.26	1.00 (0.22, 4.62
Belash, 2003	6/30	8/30		2.09	0.69 (0.21, 2.27)
Gordon, 2001	9/29	9/39		2.52	1.50 (0.51, 4.41)
Jansen, 1998	6/35	12/54		2.66	0.73 (0.26, 2.10)
Kilani, 2003	12/50	11/50		3.43	1.12 (0.44, 2.03)
Westergaard, 2001	67/189	53/190		15.76	1.42 (0.92, 2.18)
Hompes, 2007	79/312	75/317		22.33	1.09 (0.76, 1.57)
Euro-Israel, 2002	80/395	67/386		22.90	1.21 (0.84, 1.73)
Anderson, 2006	96/363	82/368		25.85	1.25 (0.89, 1.76)
Total (95% CI)	1,453	1,484		100	1.20 (1.01, 1.42)
Total events: 363 (hMG), 324 (rFSH)					
Test for heterogeneity: χ^2 = 2.81, df = 9 (p=0.97); p=0%					
Test for overall effect: Z = 2.04 (p=0.04)					



HP-hMG = highly purified human menopausal gonadotropin; rFSH = recombinant FSH; OR = odds ratio; CI = confidence interval; df = degrees of freedom; Z = vector of other variables.

and MERiT, that essentially used the same treatment protocols and primary end- points. Unlike most meta-analyses in which there are typically different treatment protocols and primary end-points, this report has a much more homogenous patient population. The results of this combined analysis show that while significantly more oocytes are retrieved with rFSH irrespective of cycle initiated or patients with embryos transferred, there is a statistically favorable advantage to using hMG over rFSH (see *Figure 6*). This can be interpreted as oocyte stimulation and implantation rates on the right side of the hMG continuum are significantly more favorable than stimulation on the left-hand side of the continuum.

The Significant Advantage of Human Menopausal Gonadotropin Is Confirmed with a Meta-analysis

While the above analysis demonstrated a clear advantage to HP-hMG stimulations, it was limited by utilizing only two elements of a

company- sponsored study; therefore, Al-Inany et al. investigated the analysis of all the available information.[22] This showed that when all applicable studies are used in a meta-analysis, an advantage of hMG over rFSH is confirmed (see *Figure 7*).

Weghofer II Favors Recombinant Follicle-stimulating Hormone, But Reveals an Interesting Piece of Data

Weghofer recently reported a second investigation of ploidy of embryos based on stimulation protocol.[23] This study (Weghofer II), unlike the previous work detailed above, looked at antagonist not agonist cycles. There was a statistically significantly higher pregnancy rate in the rFSH group; however, the study raises some interesting points. First, there are the statistically significantly higher pregnancy rates favoring rFSH, which are contrary to the previously presented information. This illustrates the purpose of the body of evidence; that is, to cover all of the data supporting hMG that should be used to make evidence-based decisions, not to focus on individual observations from single studies. Second, this study has some faults that need to be addressed before it can be reliably utilized. This study, unlike Weghofer I,[21] used a mixed protocol, not hMG alone. The amount of hMG used and when it could have been titrated is poorly defined, so a comparison of the two studies is difficult. The same holds true for the mean age, which was approximately 40 years in Weghofer II[23] compared with approximately 35 years in Weghofer I.[21] An interesting note in this study is that the mean number of oocytes retrieved for the advanced maternal age population appears excessively high (~14).

> *It should be expected that the body would produce its most active follicle-stimulating hormones in post-menopausal women as that is when the body is struggling hardest to stimulate follicles.*

Even with these difficulties in making a comparison between the two Weghofer studies, an interesting point emerges. In Weghofer I, using an agonist and rFSH, there was a 9% miscarriage rate. In Weghofer II, using an antagonist and rFSH, there was a 27.8% miscarriage rate. These values can be compared with hMG alone in Weghofer I and the mixed protocol group in Weghofer II, in which no miscarriages were reported. The fact that these are euploid embryos makes this comparison more dramatic. This supports a conclusion that any exposure to hMG provides a more suitable maternal environment. While it would be advantageous to know which factor causes stimulations on the right-hand side of the hMG continuum to be superior to the left-hand side, it is probable that there is no single reason. It is most likely that hMG influences multiple parameters, such as the endocrine profile, embryo capacity, and endometrial fitness, that result in the significantly higher pregnancy rates. Therefore, it is necessary to investigate which factors are unique to the currently available HP-hMG formulation (Menopur).

The Only Appreciable Difference in the Manufacture of Urinary Gonadotropins from Recombinants Is the Cell that Produces Gonadotropin

The purification procedures used to concentrate FSH from the source material are similar for all gonadotropins, although there is an exclusive monoclonal antibody used by one manufacturer.[24-26] This is not surprising as the purification of the same protein would out of necessity use the same biochemical procedures. In the case of HP-hMG, its components are urinary FSH (the same as HP-uFSH, Bravelle), with LH activity driven almost exclusively by ~10IU of immunoreactive hCG. Given that the advantages of hMG have been observed, it is necessary to define whether it is only the hCG-driven LH activity component of hMG that distinguishes it from other gonadotropins.

Urinary-derived Follicle-stimulating Hormone Is Used by More Patients than Any Other Stimulation Agent

As noted earlier, approximately 86% of IVF cycles use a mixed protocol, i.e. incorporating urinary FSH in the form of hMG. This can be added to approximately 6% of IVF cycles that use hMG alone, so approximately 92% of all IVF cycles use urinary FSH. As the majority of the remaining 8% of cycles use rFSH, it is worth evaluating whether there is any evidence that this 8% is being put at a disadvantage as they are not exposed to urinary FSH. Various isoforms of FSH are made during the course of the natural menstrual cycle that have different receptor affinities affecting potency, and different half-lives, affecting the duration of action. The isoforms with the highest level of action are produced mid-cycle and in post-menopausal woman. It should be expected that the body would produce its most active FSH in post-menopausal women as that is when the body is struggling hardest to stimulate follicles. There are approximately 20 isoforms of FSH in nature.[27,28] Urinary FSH in HP-FSH and HP-hMG have 16 isoforms, compared with rFSH, for which only 11 isoforms are identified.[29] Less acidic isoforms are more biologically active. Almost 100% of HP-hFSH (Bravelle) isoforms have isoelectric point (pI) values >4.25, whereas for rFSH this figure is 56%.[30]

Some published evidence helps explain why infertility physicians have empirically incorporated urinary FSH into the majority of IVF cycles and why it is not just the hCG-driven LH activity of HP-hMG that distinguishes it but also the FSH component. The outcome data from the Endometrin® approval trial[31] compared with the concurrent Society for Assisted Reproductive Technology (SART) data[32] are revealing on this point. The Endometrin trial represents about 1% of

> *While it would be advantageous to know which factor causes stimulations on the right-hand side of the human menopausal gonadotropin continuum to be superior to the left-hand side, it is probable that there is no single reason.*

the IVF cycles performed during the time period of the trial. In this trial there was a requirement of at least a 75IU vial of hMG per day with only HP-hFSH allowed as the rounding-out FSH product. The SART data[32] can serve as a surrogate for rFSH and hMG, as that is the predominant protocol used to stimulate patients. Therefore, the pure urinary FSH stimulation used in the Endometrin trial is not contaminated with any recombinant product, unlike the SART data. The comparative analysis shows that, irrespective of age, the urinary only protocol outperforms with fewer embryos transferred.

While the Endometrin analysis[31] used a large number of patients, it was poorly controlled and permitted some liberal dose changes. This shortcoming was addressed by Kilani et al.,[7] who uncovered some differences between HP-hFSH and rFSH. In a randomized, controlled clinical trial they compared a fixed amount of 150IU of HP-hMG with 150IU of rFSH. The urinary FSH from hMG demonstrated higher serum FSH levels than rFSH for a fixed amount of dose, suggesting that there was decreased clearance of those isoforms from the urinary product. In addition, higher E_2 levels were observed with hMG.[7] This effect is usually attributed to LH activity, but an alternative explanation is that the urinary isoforms of the FSH component of hMG appear to be more potent in stimulating the granulosa-cell aromatase, as there was no increase in T. If this effect were purely derived from LH activity, we would expect to see an increase in theca-cell production of T. Therefore, the beneficial effects of hMG may be manifold, arising from both the urinary FSH component and the hCG-driven LH activity component.

Conclusion

We have reviewed evidence supporting the benefits of HP-hMG treatment. While the benefits are clear, considerable additional study is required to elucidate which component is the most beneficial and which parameters of treatment are most affected by this unique pharmaceutical treatment. ■

George T Koulianos, MD, is an Adjunct Assistant Professor in the Department of Obstetrics and Gynecology at the University of South Alabama and Program Director at The Center for Reproductive Medicine at the Mobile Infirmary Medical Center. He is a Fellow of the American College of Obstetricians and Gynecologists (ACOG) and a member of many professional societies, including the American Society for Reproductive Medicine (ASRM) and the Society for Assisted Reproductive Technology (SART). He has published over 20 abstracts, book chapters, and publications in the area of reproductive endocrinology.

1. Lunenfeld B, Rabau E, Sulomivici S, et al., Treatment of amenorrhea by gonadotropic substances from women's urine., *Harefuah*, 1963;64:289–92.
2. Jennings JC, Moreland K, Peterson CM, *in vitro* fertilisation. A review of drug therapy and clinical management, *Drugs*, 1996;52:313–43.
3. Macklon NS, Fauser BC, Gonadotropin therapy for the treatment of anovulation and for ovarian hyperstimulation for IVF, *Mol Cell Endocrinol*, 2002;186:159–61.
4. Andersen AN, Devroey P, Arce JC, Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: a randomized assessor-blind controlled trial, *Hum Reprod*, 2006;21:3217–27.
5. Bosch E, Vidal C, Labarta E, et al., Highly purified hMG versus recombinant FSH in ovarian hyperstimulation with GnRH antagonists—a randomized study, *Hum Reprod*, 2008;23: 2346–51.
6. Keye WR Jr., Marrs RP, Check JH, et al., Evaluation of mixed protocols with Bravelle® (human-derived FSH) and Repronex (hMG) to assess clinical efficacy (EMBRACE) in women undergoing *in vitro* fertilization, *Fertil Steril*, 2004;82:348–57.
7. Kilani Z, Dakkak A, Ghunaim S, et al., 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: 1194–9.
8. Zafeiriou S, Loutradis D, Michalas S, The role of gonadotropins in follicular development and their use in ovulation induction protocols for assisted reproduction, *Eur J Contracept Reprod Health Care*, 2000;5:157–67.
9. Baer G, Loumaye E, Comparison of recombinant human luteinising hormone (r-hLH) and human menopausal gonadotropin (hMG) in assisted reproductive technology, *Curr Med Res Opin*, 2003;19:83–8.
10. Sharma V, Williams J, Collins W, et al., Studies on the measurement and pharmacodynamics of human follicle-stimulating hormone, *Fertil Steril*, 1987;47:244–8.
11. Bergh C, Howles CM, Borg K, et al., Recombinant human follicle stimulating hormone (r-hFSH; Gonal-F) versus highly purified urinary FSH (Metrodin HP): results of a randomized comparative study in women undergoing assisted reproductive techniques, *Hum Reprod*, 1997;12:2133–9.
12. Al-Inany HG, Abou-Setta AM, Aboulghar MA, et al., Highly purified hMG achieves better pregnancy rates in IVF cycles but not ICSI cycles compared with recombinant FSH: a meta-analysis, *Gynecol Endocrinol*, 2009;1–7 (Epub ahead of print).
13. Platteau P, Nyboe Andersen A, Loft A, et al., Highly purified HMG versus recombinant FSH for ovarian stimulation in IVF cycles, *Reprod Biomed Online*, 2008;17:190–98.
14. Hillier SG, Whitelaw PF, Smyth CD, Follicular oestrogen synthesis: the 'two-cell, two-gonadotrophin' model revisited, *Mol Cell Endocrinol*, 1994;100:51–4.
15. Filicori M, Cognigni GE, Tabarelli C, et al., Stimulation and growth of antral ovarian follicles by selective LH activity administration in women, *J Clin Endocrinol Metab*, 2002;87: 1156–61.
16. IVF Treatment Protocol Survey 2008. Oasis Marketing, on behalf of Ferring.
17. Efficacy and safety of highly purified menotropin versus recombinant follicle-stimulating hormone in *in vitro* fertilization/intracytoplasmic sperm injection cycles: a randomized, comparative trial, *Fertil Steril*, 2002;78:520–28.
18. Platteau P, Smitz J, Albano C, et al., Exogenous luteinizing hormone activity may influence the treatment outcome in in vitro fertilization but not in intracytoplasmic sperm injection cycles, *Fertil Steril*, 2004;81:1401–4.
19. Smitz J, Andersen AN, Devroey P, et al., Endocrine profile in serum and follicular fluid differs after ovarian stimulation with HP-hMG or recombinant FSH in IVF patients, *Hum Reprod*, 2007;22:676–87.
20. Ziebe S, Lundin K, Janssens R, et al., Influence of ovarian stimulation with HP-hMG or recombinant FSH on embryo quality parameters in patients undergoing IVF, *Hum Reprod*, 2007;22:2404–13.
21. Weghofer A, Munne S, Brannath W, et al., The impact of LH-containing gonadotropins on diploidy rates in preimplantation embryos: long protocol stimulation, *Hum Reprod*, 2008;23:499–503.
22. Al-Inany HG, Abou-Setta AM, Aboulghar MA, et al., Efficacy and safety of human menopausal gonadotrophins versus recombinant FSH: a meta-analysis, *Reprod Biomed Online*, 2008;16:81–8.
23. Weghofer A, Munne S, Brannath W, et al., The impact of LH-containing gonadotropin stimulation on euploidy rates in preimplantation embryos: antagonist cycles, *Fertil Steril*, 2009;92:937–42.
24. Lee DW, Grasso P, Dattatreyamurty B, et al., Purification of a high molecular weight follicle-stimulating hormone receptor-binding inhibitor from human follicular fluid, *J Clin Endocrinol Metab*, 1993;77:163–8.
25. Lunenfeld B, Lunenfeld E, Ovulation induction with human menopausal gonadotropins. In: Seibel MM (ed.), *Infertility: A Comprehensive Text*, Stamford, CT: Appleton & Lange, 1997; 507–23.
26. Prevost RR, Recombinant follicle-stimulating hormone: new biotechnology for infertility, *Pharmacotherapy*, 1998;18: 1001–10.
27. Stanton PG, Robertson DM, Burgon PG, et al., Isolation and physicochemical characterization of human follicle-stimulating hormone isoforms, *Endocrinology*, 1992;130: 2820–32.
28. Wide L, Bakos O, More basic forms of both human follicle-stimulating hormone and luteinizing hormone in serum at midcycle compared with the follicular or luteal phase, *J Clin Endocrinol Metab*, 1993;76:885–9.
29. Cerpa-Poljak A, Bishop LA, Hort YJ, et al., Isoelectric charge of recombinant human follicle-stimulating hormone isoforms determines receptor affinity and *in vitro* bioactivity, *Endocrinology*, 1993;132:351–6.
30. Wolfenson C, Groisman J, Couto AS, et al., Batch-to-batch consistency of human-derived gonadotrophin preparations compared with recombinant preparations, *Reprod Biomed Online*, 2005;10:442–54.
31. Doody KJ, Schnell VL, Foulk RA, et al., Endometrin® for luteal phase support in a randomized, controlled, open-label, prospective in-vitro fertilization trial using a combination of Menopur® and Bravelle® for controlled ovarian hyperstimulation, *Fertil Steril*, 2009;91:1012–17.
32. Stern JE, Goldman MB, Hatasaka H, et al., Optimizing the number of cleavage stage embryos to transfer on day 3 in women 38 years of age and older: a Society for Assisted Reproductive Technology database study, *Fertil Steril*, 2009;91:767–76.

Saffron House
6–10 Kirby Street
London
EC1N 8TS

EDITORIAL
Tel: +44 (0) 20 7452 5232
Fax: +44 (0) 20 7452 5050

SALES
Tel: +44 (0) 20 7452 5332
Fax: +44 (0) 20 7452 5606

E-mail: info@touchbriefings.com
www.touchbriefings.com