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Original Citation:

Effect of highly purified urinary follicle-stimulating hormone on oocyte and embryo quality / Selman HA;De Santo M;Sterzik K;Coccia E;El-Danasouri I. - In: FERTILITY AND STERILITY. - ISSN 0015-0282. - ELETTRONICO. - 78:(2002), pp. 1061-1067.

Availability:

The webpage <https://hdl.handle.net/2158/782000> of the repository was last updated on

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(Article begins on next page)

Effect of highly purified urinary follicle-stimulating hormone on oocyte and embryo quality

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Objective: To determine the effects of ovarian stimulation with highly purified urofollitropin on oocyte and embryo quality.

Design: Parallel randomized open-label clinical study.

Setting: Assisted reproduction centers.

Patient(s): Two hundred sixty-seven infertile couples undergoing IVF/ICSI.

Intervention(s): All participants underwent standard down-regulation with GnRH analogue. One hundred thirty-three participants received highly purified urinary FSH and 134 controls received recombinant FSH.

Main Outcome Measure(s): Primary end points were number of morphologically mature oocytes retrieved, embryo quality, and pregnancy and implantation rates. Secondary end points were: total number of days of FSH stimulation, total dose of gonadotropin administered, fertilization rate per number of retrieved oocytes, embryo cleavage rate, live birth and miscarriage rates, endometrial thickness and estradiol level on the day of hCG administration, cancellation rate, and incidence of moderate or severe ovarian hyperstimulation syndrome.

Result(s): Pregnancy and implantation rates were nonsignificantly higher in the urinary FSH group than the recombinant FSH group (46.5% vs. 36.8% and 22.1% vs. 15.8%, respectively). The grade 1 embryo score was significantly higher in the urinary FSH group than the recombinant FSH (42.1% vs. 33.5%), and the live birth rate was nonsignificantly higher in the former group.

Conclusion(s): Highly purified urinary FSH is as effective, efficient, and safe for clinical use as recombinant FSH. (*Fertil Steril*® 2002;78:1061–7. ©2002 by American Society for Reproductive Medicine.)

Key Words: Embryo, oocyte, ovarian stimulation, recombinant human FSH, urinary human FSH

Received November 14, 2001; revised and accepted March 12, 2002.

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Induction of multifollicular growth in assisted reproduction programs is essential for successful treatment. Since pregnancy and live birth rates are correlated with the number of fertilized oocytes (1), IVF procedures have historically used protocols involving administration of gonadotropins to increase the number of oocytes available for eventual embryo transfer.

Ovarian stimulation regimens generally include pituitary desensitization (down-regulation) with a GnRH analogue followed by administration of gonadotropins (2). Pituitary down-regulation with GnRH agonist, which inhibits the mid-cycle LH surge, is one of the most effective ways to induce formation of enough preovulatory follicles (3–5).

Until recently, gonadotropins used for ovarian stimulation have been extracted from the urine of postmenopausal women. Since menopausal gonadotropin (hMG) is of human origin, the source is not consistent and the end product is always contaminated with >95% non-FSH, human proteins, and LH (6). Urinary proteins may negatively affect follicle recruitment and development (7).

The introduction of highly purified urinary FSH, which contained >95% FSH protein content and a negligible amount (0.1 per 1,000 IU of FSH) of LH (8), has substantially addressed the contamination problem. Some reports have indicated that use of highly purified FSH has

resulted in higher pregnancy rates than those obtained with hMG (9–11).

With the advent of recombinant DNA technology, two pure FSH preparations have become available: follitropin- α and follitropin- β . Recombinant human FSH has a specific activity of >10,000 IU/mg protein and lacks LH activity or extraneous human proteins (12–14). Its purity and *in vivo* bioactivity are thought to confer safety, efficiency, and tolerability advantages over urine-derived FSH (15, 16).

Recent clinical trials have shown that recombinant FSH is effective in terms of number of oocytes retrieved, number of embryos obtained, and total gonadotropin dose needed, without increasing the risk for the ovarian hyperstimulation syndrome (OHSS) (17–19). In addition, recombinant FSH has been shown to be as effective as urinary FSH or hMG (20–24), with or without GnRH agonists. However, in most of these trials, the main end points were the number of oocytes retrieved and embryos obtained, dosage and duration of stimulation, and rates of fertilization, pregnancy, and implantation.

Few trials have investigated the possible difference between recombinant FSH and highly purified urinary FSH in terms of the quality of oocytes and embryos in stimulated cycles. Recent data show that hMG compares favorably to recombinant FSH (25, 26) in terms of oocyte and embryo quality and subsequent pregnancy rates.

We sought to compare the effects of highly purified urinary FSH and recombinant FSH on oocyte and embryo quality and on pregnancy and implantation rates.

MATERIALS AND METHODS

Patient Selection

Two hundred sixty-seven infertile couples were recruited between December 1998 and November 2000. Women 18 to 38 years of age were included if they fulfilled the following criteria: [1] infertility attributable to tubal factor, male factor, or unexplained infertility; [2] serum levels of FSH, LH, and prolactin in the normal range; [3] regular ovulatory menstrual cycles of 25 to 35 days; [4] a normal uterine cavity; [5] no treatment with gonadotropins in the month before study entry; [6] presentation for the first IVF treatment cycle; [7] body mass index ≥ 18 but ≤ 26 kg/m²; and [8] willingness to participate in the study and to comply with the procedures.

Patients were excluded if they had gynecologic abnormalities or disease, previous poor response to gonadotropins used for IUI, history of severe OHSS, or current polycystic ovary syndrome or if the male partner had azoospermia or clinical signs of infection detected in semen analysis within 12 months before treatment.

Patients were extensively counseled about the nature of the study and gave written informed consent. The Institu-

tional Review Board of each participating center approved the study.

Study Design

We performed a prospective, open, randomized, parallel-group study at three hospital-affiliated IVF centers in Italy. The study was designed to compare the effectiveness of recombinant FSH (Gonal-F; Serono, Rome, Italy) and highly purified urinary FSH (Fostimon; AMSA, Rome, Italy) administered during a single IVF cycle. Evaluation was based on the number of mature oocytes and on embryo quality as a primary end point of the study.

Fostimon is a new preparation of highly purified urinary FSH obtained by an ion-exchange chromatography column method. It has a specific activity of >6,000 IU/mg protein and purity >90%. This high level of biological potency and purity allows safe administration by intramuscular or subcutaneous routes.

All patients underwent standard pituitary down-regulation with GnRH analogue (triptorelin) (Decapeptyl; Ipsen, Milan, Italy), 3.75 mg i.m. on day 21 of their cycle. Fifteen days later, patients were considered desensitized and gonadotropin administration was begun, provided that ultrasonography showed no follicles ≥ 10 mm in diameter, endometrial thickness <7 mm, and estradiol serum concentration <50 pg/mL.

After pituitary desensitization, patients were randomized to receive highly purified urinary FSH or recombinant FSH, administered once daily s.c., starting with a fixed dose of 225 IU/d for the first 6 days. Ovarian response was assessed on day 6 by ultrasonography and by measurement of serum estradiol to evaluate whether a change in the dose was required.

Patients with a poor response to gonadotropin treatment were withdrawn from the study. Patients with excessive response were counseled about the risk for OHSS and were advised to interrupt the stimulation cycle or to undergo oocyte retrieval with cryopreservation of any resultant embryos for replacement in the subsequent cycle.

Follicle-stimulating hormone was administered daily until the criteria for triggering final follicular maturation (leading follicle with a mean diameter ≥ 18 mm and at least two other follicles with a diameter >16 mm) were met. Ovulation was triggered by i.m. administration of 10,000 IU of hCG 12 to 16 hours after the last ultrasonogram that confirmed adequate follicular development.

Oocytes were retrieved 34 to 36 hours after hCG administration. The cumulus-oocyte complex was assessed according to the oocyte maturation score criteria described by Veeck (27). The oocytes were then inseminated *in vitro* by conventional IVF or ICSI, and the resultant embryos were scored according to the criteria of Veeck (28).

In brief, embryos were scored on the basis of morphologic appearance and fragmentation. Grade 1 embryos had

TABLE 1

Outcome of patients stimulated with highly purified FSH or recombinant FSH.

Characteristic	Highly purified FSH group	Recombinant FSH group	P value
No. of patients	133	134	
Age (y)	32 ± 4	31.8 ± 6	≤.928
Body mass index (kg/m ²)	21.2 ± 2.8	20.6 ± 3	≤.954
Duration of stimulation (d)	13.4 ± 1.5	13.7 ± 1.4	≤.869
No. of ampoules or vials of FSH, 75 IU	51.7 ± 15	60.5 ± 21	≤.591
17β-estradiol level on the day of hCG administration (pg/mL)	1,891.6 ± 975.5	1,698.5 ± 864.4	≤.368
Endometrial thickness on day of hCG administration (mm)	11.5 ± 1.6	11.6 ± 2	≤.830

Note: Values with the plus/minus sign are the mean (±SD).

Selman. Highly purified FSH and oocyte and embryo quality. *Fertil Steril* 2002.

equal-sized blastomeres and no fragmentation; grade 2 embryos had equal-sized blastomeres with minor cytoplasmic fragmentation; grade 3 embryos had unequal-sized blastomeres with variable fragmentation; grade 4 embryos had equal- or unequal-sized blastomeres with >10% fragmentation; grade 5 embryos had equal- or unequal-sized blastomeres and >20% fragmentation; and grade 6 embryos had few, small blastomeres and severe fragmentation comprising >50% of the embryo surface (28).

Embryos were transferred on day 3 after oocyte retrieval. No more than three embryos per patient were transferred. Luteal phase support was initiated on the day of oocyte retrieval by using a commercially available progesterone preparation (50 mg/mL i.m.). Surplus embryos were cryopreserved. Clinical pregnancy was confirmed 6 weeks after embryo transfer by ultrasonography.

Efficacy Measures

Primary end points were the number of mature oocytes retrieved, embryo quality, and clinical pregnancy and implantation rates. Secondary endpoints were total dose of FSH administered, total number of days of stimulation, serum estradiol level and endometrial thickness on the day of hCG administration, fertilization rate, embryo cleavage rate, live birth and miscarriage rates, cancellation rate, and incidence of moderate or severe OHSS. All end points except the cancellation rate, the incidence of OHSS, and the number of frozen embryos were analyzed statistically.

Statistical Analysis

The *t*-test, χ^2 square table of contingency, and analysis of variance were used where appropriate. Statistical significance was established if $P \leq .05$.

RESULTS

The patients were divided into two groups: One hundred thirty-three received highly purified FSH (urofollitropin), and 134 "controls" received recombinant FSH (follitropin- α). Of the 267 randomized patients, 264 completed the cycle

and had oocyte retrieval. Two couples in the urinary FSH group and one couple in the recombinant FSH group (1.5% and 0.7% of patients, respectively) were excluded owing to excessive ovarian response leading to high risk for OHSS.

The two study groups were similar in terms of age, body mass index, duration of stimulation, number of ampoules or vials of FSH administered, and estradiol level and endometrial thickness on day of hCG administration (Table 1).

Table 2 shows oocyte maturity, fertilization rate, and cleavage rate for all patients who underwent oocyte retrieval. No significant difference between groups was found for mean number of oocytes retrieved per patient, average number of morphologically mature oocytes, and fertilization rate. The embryo cleavage rate was nonsignificantly lower in the urinary FSH group than the recombinant FSH group.

The highest proportion of grade 1 embryos occurred in the urinary FSH group (42.1% vs. 33.5% in the recombinant FSH group; $P \leq .05$). The percentages of grade 2, 3, 4, or 5 embryos did not differ statistically between group, whereas the percentage of grade 6 embryos was significantly lower ($P \leq .05$) in the urinary FSH group than the recombinant FSH group (5.1% vs. 8.5%, respectively) (Table 3).

The rates of clinical pregnancy and implantation were nonsignificantly higher in the urinary FSH group than in the recombinant FSH group. However, the live birth rate per embryo transfer was nonsignificantly higher in the urinary FSH group than in the recombinant FSH group (56.5% vs. 38.3%). The multiple pregnancy rate was also nonsignificantly higher in the former group (29.5% vs. 22.4%). Frozen-thawed embryos were not included in this study (Table 4).

DISCUSSION

The recent availability of recombinant FSH has introduced an alternative to urine-derived FSH for ovarian stimulation regimens. Several comparison studies have shown that recombinant FSH is more effective than urinary FSH

TABLE 2

Oocyte maturity and fertilization and cleavage rates in patients stimulated with highly purified FSH or recombinant FSH.

Characteristic	Highly purified FSH group	Recombinant FSH group	P value
No. of patients with oocyte retrieval	131	133	
No. of retrieved oocytes	1,146	1,197	≤.878
Mean ± SD	8.7 ± 3.4	8.9 ± 4.7	
No. of morphologically matured oocytes (%)	978 (85.3)	1,029 (86)	≤.804
Mean ± SD	7.4 ± 1.8	7.7 ± 3.2	
No. of two-pronuclei oocytes (%)	741 (64.6)	788 (65.8)	≤.766
Mean ± SD	5.7 ± 1.7	5.9 ± 2	
Cleaved embryos (%)	589 (79.5)	668 (84.7)	≤.449

Note: Values with the plus/minus sign are the mean (±SD).

Selman. Highly purified FSH and oocyte and embryo quality. *Fertil Steril* 2002.

(hMG or highly purified FSH) and that the absence of LH activity in recombinant FSH does not inhibit follicular growth.

Studies have shown that FSH, even in the absence of LH, induces multiple follicular growth as well as meiotically and developmentally competent oocytes (17), although serum concentrations of estradiol remain lower than levels resulting from follicular stimulation with hMG (29–33). However, in agreement with our results, recent studies of urinary FSH or hMG and recombinant FSH had similar results in terms of oocyte and embryo quality and of implantation and pregnancy rates (25, 26, 34).

We aimed to compare highly purified FSH with recombinant FSH to evaluate possible differences in oocyte and embryo quality and in implantation and pregnancy rates. Highly purified FSH proved to be as effective and safe as recombinant FSH for inducing growth of multiple follicles in stimulation regimens. Subcutaneous administration of highly purified FSH had no side effects and was well tolerated, as reported in previous studies (35, 36).

Recombinant FSH contains a higher proportion of the less acidic isoforms (isoelectric point range of 3.5 to 6.1), whereas urinary FSH containing both acidic and mid-acidic isoforms (isoelectric point range of 3.5 to 5.2). It has been suggested that the less acidic isoforms have faster circulatory clearance and, thus, a shorter circulatory half-life (37) than the acidic isoforms (38, 39). A more recent study has shown that the slow clearance of the acidic isoform results in better follicular maturation and estradiol secretion than the less acidic isoform (40). Further investigation into the role of FSH isoforms in the modulation and regulation of follicular growth and maturation is needed.

In our study, the mean number of retrieved oocytes (8.7 ± 3.4 vs. 8.9 ± 4.7) and the number of morphologically mature oocytes (7.4 ± 1.8 vs. 7.7 ± 3.2) did not differ between the urinary FSH group and the recombinant FSH group. Of note, the serum estradiol level on the day of hCG administration was nonsignificantly higher in the urinary FSH group than in the recombinant FSH group ($1,891 \pm 975.5$ pg/mL vs. $1,698.5 \pm 864.4$ pg/mL). These findings contrast with those of some previous studies (18, 19, 41) but agree with those of Jacob et al. (42), who found a significantly lower estradiol level in the recombinant FSH group than the urinary FSH (or hMG) group. This observation might be explained by the fact that LH is required for normal steroidogenesis activity during follicular growth while increasing the number of FSH receptors on granulosa cells (43). Alternatively, the negligible amount of LH in highly purified urinary FSH may affect estrogen production (22, 42).

Patients treated with urinary FSH had a statistically higher number of grade 1 embryos than did patients treated with recombinant FSH group (42.1% vs. 33.5%; $P < .05$), but the number of morphologically mature oocytes was similar in the two groups. The number of grade 2, 3, 4, and 5 embryos was similar in both groups, whereas the number of grade 6 embryos was statistically lower in the urinary FSH group. These differences may reflect the slightly higher

TABLE 3

Embryo score after stimulation with highly purified FSH or recombinant FSH.

Embryo score ^a	Highly purified FSH group	Recombinant FSH group	P value
Grade 1 (%)	248 (42.1)	224 (33.5)	≤.05
Grade 2 (%)	183 (31)	203 (30.4)	≤.887
Grade 3 (%)	59 (10)	86 (12.9)	≤.186
Grade 4 (%)	40 (6.8)	63 (9.4)	≤.142
Grade 5 (%)	29 (5)	35 (5.3)	≤.910
Grade 6 (%)	30 (5.1)	57 (8.5)	≤.05

^a The embryos were scored according to the criteria established by Vecek (1988).

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TABLE 4

Clinical outcome of patients after treatment with highly purified FSH or recombinant FSH.

Characteristic	Highly purified FSH group	Recombinant FSH group	P value
No. of patients with embryo transfer	131	133	
No. of embryos transferred	380	385	≤.955
No. of embryos cryopreserved ^a	98	120	≤.353
Average of embryos transferred per patient	2.9	2.9	≤.688
No. of clinical pregnancies (%)	61 (46.5)	49 (36.8)	≤.360
Multiple pregnancies per clinical pregnancy (%)	18 (29.5)	11 (22.4)	≤.668
Twins	13	9	≤.937
Triplets	5	2	≤.665
Spontaneous abortion rate per clinical pregnancy (%) ^b	9/61 (14.7)	8/49 (16.3)	≤.946
Implantation rate per embryo transferred (%)	84/380 (22.1)	62/385 (15.8)	≤.099
No. of deliveries per embryo transfer (%)	52 (39.7)	41 (30.8)	≤.356

^a Only viable embryos were cryopreserved when requested by the patients.^b Spontaneous abortion includes one triplet for each group.Selman. Highly purified FSH and oocyte and embryo quality. *Fertil Steril* 2002.

pregnancy and implantation rates in patients treated with urinary FSH compared with recombinant FSH (46.5% vs. 36.8% and 22.1% vs. 15.8%, respectively). The slightly lower miscarriage rates and higher live birth rates in the urinary FSH group may indicate that urinary FSH has a better effect on embryo quality.

The rate of pregnancy, implantation, and live birth may not have differed significantly between the groups because of small sample. Assuming that the difference between recombinant FSH and urinary FSH is 5%, at least 1,212 participants for each group would be needed to reach 90% power and an α value of .05. However, our study has a statistical power of 15% to 20%.

Of the factors that affect oocyte quality in stimulated cycles, the most important appear to be patient age, basal FSH concentration, profound suppression of LH during down-regulation, and estradiol concentration per growing follicle. Estradiol and androgens are reported to affect oocyte nuclear maturation and fertilization and may contribute to embryo development (44–47).

Profound suppression of LH during the down-regulation protocols generally used for ovarian stimulation negatively affects treatment outcome (48–50). Recent studies demonstrated that low concentrations of endogenous LH (<3 mIU/mL) in the late follicular phase of an IVF cycle are associated with significantly lower fertilization rates and higher biochemical pregnancy rates. The authors suggested that when using recombinant FSH only, it may be of clinical benefit to add LH in the late follicular phase or to further reduce the dose of GnRH analogue (48, 51).

Conversely, recombinant FSH is reported to be more effective than urinary FSH for ovarian stimulation, even when used in combination with a long-acting GnRH agonist

(depot formulation) (20, 29, 52). Loumaye et al. (53) reported that patients with very suppressed serum LH levels respond similarly to those with moderately suppressed LH levels and that about 60% of patients might benefit from a more pronounced pituitary down-regulation, whereas only less than 6% of patients would benefit from exogenous LH administration (53).

Although LH plays an important role during ovarian stimulation (44), excessive LH during the follicular phase has been shown to have a detrimental effect on oocyte quality and thus fertility (54). However, other studies found that an elevated LH level on the day of hCG administration in a low-GnRH analogue protocol does not reduce cycle fecundity (55). These discrepant findings require further investigation. In a recent study, hMG (1:1 FSH/LH) produced comparable results to recombinant FSH when used alone for ovarian stimulation (25, 26, 56).

We conclude that highly purified FSH is as effective as recombinant FSH for ovarian stimulation protocols, thus offering a viable alternative to recombinant FSH.

References

1. Templeton A, Morris JK. Reducing the risk of multiple births by transfer of two embryos after in vitro fertilization. *N Engl J Med* 1998;339:673–7.
2. Porter RN, Smith W, Craft J. Induction of ovulation for in vitro fertilization using buserelin and gonadotrophins. *Lancet* 1984;2:1284–5.
3. Loumaye E. The control of endogenous secretion of LH by gonadotrophin-releasing hormone agonists during ovarian hyperstimulation for in vitro fertilization and embryo transfer. *Hum Reprod* 1990;5:357–76.
4. Hughes EG, Fedorkow DM, Daya S, Vagle MA, Van de Koppel P, Collins JA. The routine use of gonadotrophin-releasing hormone agonists prior to in vitro fertilization and gamete intrafallopian transfer: a meta-analysis of randomized controlled trials. *Fertil Steril* 1992;58:888–96.
5. Smitz J, Devroey P, Braeckmans P, Camus M, Staessen C, Van Waesbergh L, et al. Management of failed cycles in an IVF-GIFT programme with combination of GnRH analogue and HMG. *Hum Reprod* 1987;2:309–14.

6. Vandervorst M, Devroey P. Recombinant FSH: results in assisted reproduction. Ovulation induction update '98. In: Follicori M, Flamigni C (eds). *Proceedings of the 2nd World Conference on Ovulation Induction*. Bologna, Italy: Parthenon, 1998:137–44.
7. Giudice F, Crisci C, Eshkol Apapoian R. Composition of commercial gonadotrophin preparation extracted from human post-menopausal urine: characterization of non-gonadotrophin proteins. *Hum Reprod* 1994;9:2291–9.
8. Flamigni C, Venturoli S, Dal Parto L, Porcu E. Purified FSH: characteristics and applications. In: Follicori M, Flamigni C (eds). *Ovulation induction: basic science and clinical advances*. Amsterdam: Elsevier Science, 1994:125–34.
9. Howles CM, Loumaye E, Giroud D, Luyet G. Multiple follicular development and ovarian steroidogenesis following subcutaneous administration of a highly purified urinary FSH preparation (Metrodin High Purity) in pituitary desensitized women undergoing IVF-ET: a multicentre European phase III study. *Hum Reprod* 1994;9:424–30.
10. Wikland M, Borg J, Hamberger L, Sulander P. Simplification of IVF, minimal monitoring and the use of subcutaneous highly purified FSH administration for ovulation induction. *Hum Reprod* 1994;9:1430–6.
11. Daya S, Gunby J, Hughes EG, Collins JA, Sagle MA. Follicle-stimulating hormone vs. human menopausal gonadotrophin for in vitro fertilization cycles: a meta-analysis. *Fertil Steril* 1995;64:347–54.
12. Chaple E, Kelton C, Nugent N. Expression of human gonadotrophins by recombinant DNA methods. In: Genazzani AR, Petraglia F (eds). *Proceedings of the 3rd World Congress on Gynaecological Endocrinology*. Carnforth (United Kingdom): Parthenon, 1992:179–84.
13. Loumaye E, Campbell R, Salat-Baroux J. Human follicle stimulating hormone produced by recombinant DNA technology: a review for clinicians. *Hum Reprod Update* 1995;1:188–99.
14. Olijve W, De Boer W, Mulders JWM, van Wezenbeek PM. Molecular biology and biochemistry of human recombinant follicle stimulating hormone (Puregon). *Mol Hum Reprod* 1996;2:371–82.
15. Redfearn A, Hughes EG, O'Connor M, Dolovich J. Delayed-type hypersensitivity to human gonadotropin: case report. *Fertil Steril* 1995;64:479–82.
16. Albano C, Smits J, Camus M, Bennink HC, Van Steirteghem A, Devroey P. Pregnancy and birth in an in-vitro fertilization cycle after controlled ovarian stimulation in a woman with a history of allergic reactions to human menopausal gonadotrophin. *Hum Reprod* 1996;11:1632–4.
17. Out HJ, Mannaerts BMJL, Driessen SGJ, Coelingh-Bennink HJT. A prospective, randomized, assessor-blind, multicentre study comparing recombinant and urinary follicle stimulating hormone (Puregon vs. Metrodin) in in-vitro fertilization. *Hum Reprod* 1995;10:2534–40.
18. Out HJ, Mannaerts BMJL, Driessen SGJ, Coelingh-Bennink HJT. Recombinant follicle stimulating hormone (recombinant FSH, Puregon) in assisted reproduction: more oocytes, more pregnancies. Results from five comparative studies. *Hum Reprod* 1996;2:162–71.
19. Bergh C, Howles CM, Borg K, Hamberger L, Josefsson B, Nilsson L, et al. Recombinant human follicle stimulating hormone (r-FSH, Gonal-F) vs. highly purified urinary FSH (Metrodin highly purified): results of a randomized comparative study in women undergoing assisted reproductive techniques. *Hum Reprod* 1997;12:2133–9.
20. Devroey P, Mannaerts B, Smits J, Coelingh-Bennink H, Van Steirteghem A. Clinical outcome of a pilot efficacy study on recombinant human follicle-stimulating hormone (Org 32489) combined with various gonadotrophin-releasing hormone agonist regimens. *Hum Reprod* 1994;9:1064–9.
21. Hedon B, Out HJ, Hugues JN, Camier B, Cohen J, Lopes P, et al. Efficacy and safety of recombinant follicle stimulating hormone (Puregon) in infertile women pituitary-suppressed with triptorelin undergoing in-vitro fertilization: a prospective, randomized, assessor-blind, multicentre trial. *Hum Reprod* 1995;10:3102–6.
22. Recombinant human FSH study group. Clinical assessment of recombinant human follicle-stimulating hormone in stimulating ovarian follicular development before in-vitro fertilization. *Fertil Steril* 1995;63:77–86.
23. Out HJ, Reimnitz PE, Coelingh-Bennink HJT. A prospective, randomized study to assess the tolerance and efficacy of intramuscular and subcutaneous administration of recombinant follicle-stimulating hormone (Puregon). *Fertil Steril* 1997;67:278–83.
24. Jansen CAM, van Os HC, Out HJ, Coelingh-Bennink HJT. A prospective, randomized clinical trial comparing recombinant follicle stimulating hormone (Puregon) and human menopausal gonadotrophins (Humegon) in non-down-regulated in-vitro fertilization cycles. *Hum Reprod* 1998;13:2995–9.
25. Ng EHY, Lau EYL, Yeung WSBY, Ho PC. HMG is as good as recombinant human FSH in terms of oocyte and embryo quality: a prospective randomized trial. *Hum Reprod* 2001;2:319–25.
26. Strehler E, Abt M, El-Danasouri I, De Santo M, Sterzik K. Impact of recombinant follicle-stimulating hormone and human menopausal gonadotrophins on in vitro fertilization outcome. *Fertil Steril* 2001;75:332–6.
27. Veeck LL. *An atlas of human gametes and conception*. London: Parthenon, 1999.
28. Veeck LL. Oocyte assessment and biological performance. *Ann N Y Acad Sci* 1988;541:259–74.
29. Frydman R, Howles CM, Truong F. A double-blind, randomized study to compare recombinant human follicle stimulating hormone (FSH; Gonal-F) with highly purified urinary FSH (Metrodin HP) in women undergoing assisted reproductive techniques including intracytoplasmic sperm injection. *Hum Reprod* 2000;15:520–5.
30. Schats R, De Sutter P, Bassil S, Kremer JAM, Tournaye H, Donnez J. Ovarian stimulation during assisted reproduction treatment: a comparison of recombinant and highly purified urinary human FSH. *Hum Reprod* 2000;15:1691–7.
31. Zelinski-Wooten MB, Hutchinson JS, Hess DL, Wolf DP, Stouffer RL. Follicular stimulating hormone alone supports follicle growth and oocyte development in gonadotropin releasing hormone antagonist treated monkey. *Hum Reprod* 1995;10:1658–66.
32. Fried GM, Harlin J, Csemiczky G, Wramsby H. Controlled ovarian stimulation using highly purified FSH results in a lower serum estradiol profile in the follicular phase as compared with HMG. *Hum Reprod* 1996;11:474–7.
33. Agarwal R, Conway GS, Engmann L, Bekir JS, Jacobs HS. Implications of using follicular stimulating hormone preparation depleted of luteinizing hormone to achieve follicular growth in in vitro fertilization. *Gynecol Endocrinol* 1998;12:9–15.
34. Ravhon A, Lavery S, Aurell R. Clinical experience with recombinant follicle-stimulating hormone (FSH) and urinary FSH: a retrospective case-controlled analysis. *Fertil Steril* 2001;75:920–5.
35. Anserini P, Costa M, Remorgida V, Venturini PL. A prospective, randomized, controlled clinical study of a new subcutaneous, purified, urinary FSH preparation for controlled ovarian hyperstimulation in in vitro fertilization. *Gynecol Endocrinol* 2000;14:75–80.
36. Gerli S, Perino M, Abate A, Costabile L, Gholami H, Vitiello L. Ovarian stimulation using a new highly purified urinary FSH: a prospective randomized clinical study. *Clin Exp Obstet Gynecol* 1999;26:93–4.
37. Antonio MD, Borrelli F, Datola A, Bucci R, Mascia M, Polletta P, et al. Biological characterization of recombinant human follicle stimulating hormone isoforms. *Hum Reprod* 1999;14:1160–7.
38. Flack MR, Bennet AP, Froehlich J, Anasti JN, Nisula BC. Increased biological activity due to basic isoforms in recombinant human follicle-stimulating hormone produced in a human cell line. *J Clin Endocrinol Metab* 1994;79:756–60.
39. Galway AB, Hsueh AJ, Keene JL, Yamoto M, Fauser BC, Boime I. In vitro and in vivo bioactivity of recombinant human follicle-stimulating hormone and partially deglycosylated variants secreted by transfected eukaryotic cell lines. *Endocrinology* 1990;127:93–100.
40. West CR, Carlson NE, Lee JS, McNeilly AS, Sharma TP, Ye W, et al. Acidic mix of FSH isoforms are better facilitators of ovarian follicular maturation and E₂ production than the less acidic. *Endocrinology* 2002;143:107–16.
41. Lenton E, Soltan A, Hewitt J, Thomson A, Davies W, Ashraf N, et al. Induction of ovulation in women undergoing assisted reproductive techniques: recombinant human FSH (folitropin alpha) vs. highly purified urinary FSH (urofolitropin HP). *Hum Reprod* 2000;15:1021–7.
42. Jacob S, Drudy L, Conroy R, Harrison RF. Outcome from consecutive in-vitro fertilization/intracytoplasmic sperm injection attempts in the final group treated with urinary gonadotrophins and the first group treated with recombinant follicle stimulating hormone. *Hum Reprod* 1998;13:1783–7.
43. Shoham Z, Mannaerts B, Insler V, Coelingh-Bennink H. Induction of follicular growth using recombinant human FSH in two volunteer women with hypogonadotropic hypogonadism. *Fertil Steril* 1993;59:738–42.
44. Filicori M. The role of luteinizing hormone in folliculogenesis and ovulation induction. *Fertil Steril* 1999;71:405–14.
45. Zelinski-Wooten MB, Hess DL, Wolf D, Stouffer R. Steroid production during ovarian stimulation impairs oocyte fertilization but not folliculogenesis in rhesus monkey. *Fertil Steril* 1994;61:1147–54.
46. Wu TJ, Wang L, Wan YY. Detection of estrogen receptor messenger ribonucleic acid in human oocyte and cumulus oocyte complexes using reverse transcriptase polymerase chain reaction. *Fertil Steril* 1993;59:54–9.
47. Hild-Petito S, Stouffer RL, Brenner RM. Immunocytochemical localization of estrogen and progesterone receptors in the monkey ovary throughout the menstrual cycle. *Endocrinology* 1988;123:2896–905.
48. Westergaard LG, Laursen SB, Andersen CY. Increased risk of early pregnancy loss by profound suppression of luteinizing hormone during

- ovarian stimulation in normogonadotrophic women undergoing assisted reproduction. *Hum Reprod* 2000;15:1003–8.
49. Fleming R, Lloyd F, Herbert M, Fenwick J, Griffiths T, Murdoch A. Effects of profound suppression of luteinizing hormone during ovarian stimulation on follicular activity, oocyte and embryo function in cycles stimulated with purified follicle stimulating hormone. *Hum Reprod* 1998;13:1788–92.
 50. Fleming R, Rehka P, Deshpande N, Jamieson ME, Yates RWS, Lyall HL. Suppression of LH during ovarian stimulation effects differ in cycles stimulated with purified urinary FSH and recombinant FSH. *Hum Reprod* 2000;15:1440–5.
 51. Esposito MA, Barnhart KT, Coutifaris C, Patrizio P. Role of periovulatory luteinizing hormone concentrations during assisted reproductive technology cycles stimulated exclusively with recombinant follicle-stimulating hormone. *Fertil Steril* 2001;75:519–23.
 52. Dada T, Salha O, Baillie HS, Sharama V. A comparison of three gonadotrophin-releasing hormone analogues in an in-vitro fertilization programme: a prospective randomized study. *Hum Reprod* 1999;14:288–93.
 53. Loumaye E, Engrand P, Howles CM, O'Dea L. Assessment of the role of serum luteinizing hormone and estradiol response to follicle-stimulating hormone on in vitro fertilization treatment. *Fertil Steril* 1998;69 (Suppl 2):76s–85s.
 54. Chappel SC, Howles C. Reevaluation of the role of luteinizing hormone and follicle stimulating hormone in the ovulatory process. *Hum Reprod* 1991;6:1206–12.
 55. Lincoln SR, Sopelak VM, Long CA, Cowan BD, Whitworth NS. Elevated luteinizing hormone on the day of human chorionic gonadotropin administration does not reduce cycle fecundity in a low-dose flare-up in vitro fertilization protocol. *Fertil Steril* 1995;63:563–5.
 56. Agarwal R, Holmes J, Jacobs HS. Follicle-stimulating hormone or human menopausal gonadotropin for ovarian stimulation in in vitro fertilization cycles: a meta-analysis. *Fertil Steril* 2000;73:338–43.