—Mini Review—

A Systematic Review of Controlled Ovarian Stimulation (COS) with Recombinant Follicle-stimulating Hormone (rFSH) versus Urinary Gonadotropin in GnRH Protocols for Pituitary Desensitization in Assisted Reproduction Cycles

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Abstract: *This article systematically reviews the relevant clinical data of rFSH for COS in ART, which were mainly obtained from the Cochrane Library, PubMed, MEDLINE, and reference lists of articles. HMG and rFSH have both been used equally successfully for COS in ART. However the another review has concluded that there is a statistically significant increase in clinical pregnancy rate with rFSH compared to uFSH, when used for COS in standard IVF cycles but not in cycles in which ICSI was used. Recombinant FSH is a new treatment option for Japanese women undergoing COS for ART with several advantages over conventional urinary gonadotropin preparations. Since SC administration of rFSH is safe, efficacious, and acceptable, the availability of rFSH as a ready-for-use solution supplied in an injector system may make its administration, in particular self-administration by the patient or her partner, more convenient the current review concludes that the use of rFSH is not associated with a higher incidence of obstetrical and neonatal problems compared to urinary gonadotropins.*

Key words: *Controlled ovarian stimulation (COS), Recombinant follicle-stimulating hormone (rFSH), Self-administration, Urinary gonadotropin*

For more than thirty years, human menopausal gonadotropins (hMG) have been applied to ovulation induction and COS in ART. Most hMG preparations contain either equal amounts of follicle stimulatimg hormone(FSH) and luteinizing hormone (LH) activity (1:1) or three fourths of FSH activity with one fourth of LH activity (3:1). The production of these hormones depends on the collection of huge amounts of menopausal urine, and the use of urine sources implies limited product consistency and purity [1]. FSH has been manufactured by means of recombinant DNA technology using Chinese hamster ovary (CHO) cell lines transfected with the genes encoding human FSH. The extremely high purity and batch-to-batch consistency of rFSH make it an attractive alternative to urinary FSH (uFSH). Moreover rFSH has been shown to have a higher *in vitro* activity than uFSH. The first human exposure studies of rFSH go back to early 1991 [2, 3], when single and increasing multiple doses were administered to gonadotropin-deficient but otherwise healthy female and male volunteers. Recently, hMG and rFSH have both been used successfully for COS in *in vitro* fertilization and embryo transfer (IVF-ET). In late 1991, an efficacy study was initiated in women undergoing IVF-ET to evaluate whether rFSH therapy could be combined with various gonadotropin-releasing hormone (GnRH) agonist treatment regimens, inducing different degrees of pituitary suppression. That study confirmed that rFSH appeared to be a safe drug without any unexpected adverse effects [4]. So far, many investigation studies have been published on the clinical efficacy and safety of rFSH in assisted reproductive technology (ART) [5]. This article systematically reviews the relevant clinical data of rFSH which were mainly obtained from the Cochrane Library, PubMed, MEDLINE, and reference lists of articles.

Received: March 10, 2005
Accepted: March 16, 2005
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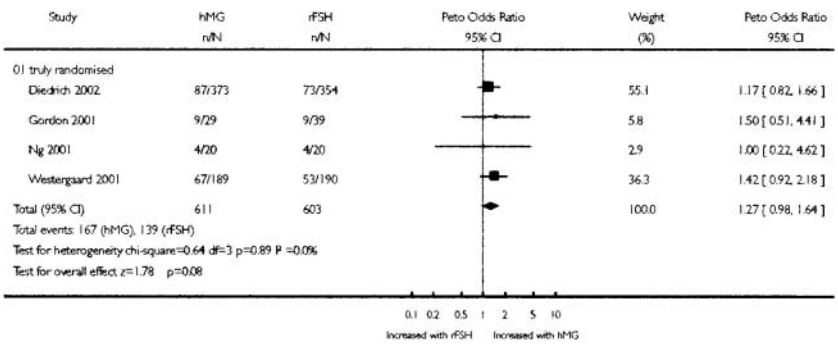

Fig. 1. Comparison 03 Down regulation with long GnRHa-protocol 03.01 Live birth or ongoing pregnancy per woman. Review: Human menopausal gonadotropin versus recombinant follicle stimulation hormone for ovarian stimulation in assisted reproductive cydes. Comparison: 03 Down regulation with long GnRHa-protocol. Outcome: 01 Live birth or ongoing pregnancy per woman.

hMG versus rFSH for COS in ART

To compare the effectiveness of hMG with rFSH in infertile women undergoing ART, the Cochrane Menstrual Disorders and Subfertility Group trials register, PubMed, MEDLINE, Web of Science and reference lists of articles were searched. In randomized trials comparing hMG with rFSH for COS in ART for treatment of infertility in normogonadotropic women, the main outcome measure was ongoing pregnancy/live birth per patient. Secondary outcomes included total gonadotropin dose used, cancellation, number of oocytes retrieved, implantation, clinical pregnancy per woman, multiple pregnancy, spontaneous abortion and ovarian hyperstimulation syndrome (OHSS). Odds ratios (OR) for hMG relative to rFSH were calculated after testing for homogeneity of treatment effect across all trials. Analyses were performed separately for the three different GnRHa protocols used: (1) without GnRHa down-regulation, (2) with GnRHa down-regulation using a short protocol and (3) with GnRHa down-regulation using a long protocol. Eight trials that met the inclusion criteria could be identified. One trial did not use down-regulation, one trial used a short protocol and six trials used a long down-regulation protocol. In the one trial without down-regulated patients and in the one trial that used a short down-regulation protocol there was no evidence of a difference between hMG and rFSH in any clinical outcome. Data of the four truly randomized trials in women down-regulated using a long protocol could be pooled. There was no evidence of a difference between hMG and rFSH in ongoing pregnancy/live birth per woman (OR 1.27; 95% CI 0.98 to 1.64) (Fig. 1). Furthermore there was no clear difference in any of the secondary outcomes, although the clinical pregnancy rate per woman was of borderline significance in favor of hMG (summary OR 1.28; 95% CI 1.00 to 1.64) (Fig. 2). The other secondary outcomes were comparable for both gonadotropins. For all three GnRHa protocols analyzed there was insufficient evidence of a difference between hMG and rFSH in ongoing pregnancy or live births. More large randomized trials are needed to estimate the difference between hMG and rFSH more precisely. Such trials should preferably use a consistent long GnRHa protocol and use a fixed dose of gonadotropin to prevent potentially subjective decisions of the clinician in dosing and take live birth as the primary endpoint. The gonadotropins studied in this review, hMG and rFSH have both been used successfully for COS in IVF-ET. However, there is insufficient evidence of a difference between hMG and rFSH on ultimate treatment effect, i.e., ongoing pregnancy or live births. At the present time, however, in prescribing gonadotropins for COS in ART, one should use the least expensive medication [6]. Another review demonstrated that the overall odds ratio for clinical pregnancy per cycle started was 1.21 (95% confidence limits (CL) 1.04, 1.42) for rFSH compared to uFSH (Fig. 3). The risk difference was a 3.7% (0.8, 6.7) absolute increase in clinical pregnancy rate with rFSH. The OR for ongoing pregnancy per cycle started was 1.29 (1.08, 1.54) (Fig. 4). There was no significant difference between rFSH and uFSH in the rates of spontaneous abortion, multiple pregnancy or OHSS. The total dose of FSH was lower by 406 (185, 627) IU with rFSH, but

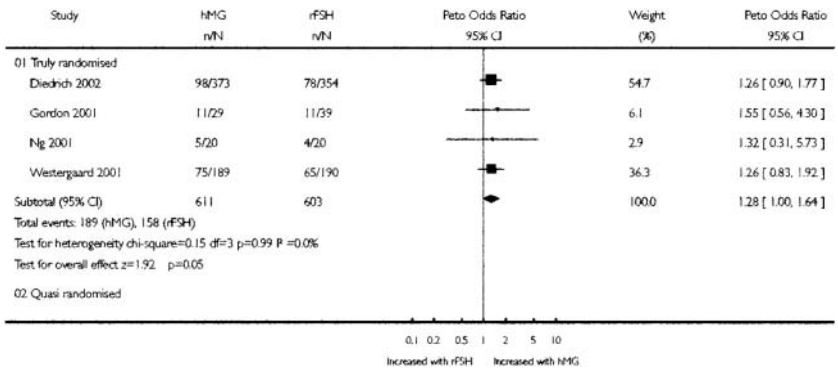

Fig. 2. Comparison 03 Down regulation with long GnRHa-protocol 03.02 Clinical pregnancy per woman. Review: Human menopausal gonadotropin versus recombinant follicle stimulation hormone for ovarian stimulation in assisted reproductive cydes. Comparison: 03 Down regulation with long GnRHa-protocol. Outcome: 02 Clinical pregnancy per woman.

there was no significant difference in the number of follicles or serum estradiol on hCG day or in the number of oocytes retrieved. This review concluded that there was a statistically significant increase in the clinical pregnancy rate with rFSH compared to uFSH, when used for ovarian stimulation in assisted reproduction. This benefit was observed only in standard IVF cycles but not in cycles in which ICSI was used. The review of trials found that taking rFSH instead of uFSH increased the chances of pregnancy by 14%. The review also found rFSH has a potentially unlimited supply, is very consistent and is also cost effective [7].

Comparative study of efficacy and safety of rFSH between Japanese and European infertile groups

The efficacy and safety of rFSH (Org 32489, follitropin-β) was assessed in pituitary-suppressed Japanese women undergoing ART. To compare the efficacy and safety of rFSH in Japanese women with those of Caucasian women, the study was designed as an open-label, multicenter, prospective trial similar to a previous European trial of rFSH [8]. The clinical data for 153 Japanese women were compared with these of 118 Caucasian women undergoing COS for ART. Daily SC administration of 150 IU or 225 IU of rFSH for the first 4 days was done, after which the daily dose was adjusted individually. Pituitary down-regulation subjects were pretreated daily with intranasal spray of GnRHa (buserelin) in a long protocol. Statistical analysis of the main efficacy parameter, the mean number of oocytes retrieved, showed similar effects in both races: 12.7 ± 9.6 oocytes in Japanese subjects vs. 11.7 ± 6.7 oocytes in Caucasian subjects. Other variables of clinical outcomes were also comparable: e.g. mean number of good quality embryos per subjects (3.8 ± 3.9 vs. 4.5 ± 3.8) and ongoing pregnancy rate per cycle (22.9 vs. 26.3%) [9]. The main differences observed between the two groups were: 1) a shorter treatment period in Japanese subjects (8.4 ± 1.6 vs. 9.8 ± 1.7 days), 2) a lower total rFSH dose (1,781 ± 562 vs. 2,063 ± 668 IU) and 3) higher serum FSH levels on the day of hCG (14.5 ± 5.0 vs. 12.3 ± 5.1 IU/L) (Table 1). The total dose per kg body weight was similar in both subject groups, and the differences in serum FSH levels were completely corrected after normalization by body weight. Therefore, the differences could be attributed to the lower body weight of Japanese subjects. The influence of body weight on clinical outcomes such as number of oocytes retrieved appeared to be completely corrected by individual dose-titration. The incidence of moderate (1.3 vs. 1.7%) or severe (0.7 vs. 1.7%) OHSS indicated similarity between Japanese and Caucasian subjects [10] (Fig. 5). Since rFSH was equally efficacious and safe in Japanese and Caucasian ART patients, the outcomes of foreign clinical data on rFSH to the Japanese population is applicable. Recombinant FSH is a new treatment option for Japanese women undergoing COS for ART with several advantages over conventional urinary gonadotropin preparations [11].

Intramuscular (IM) vs subcutaneous (SC) administration of rFSH

The safety and efficacy of rFSH when administered

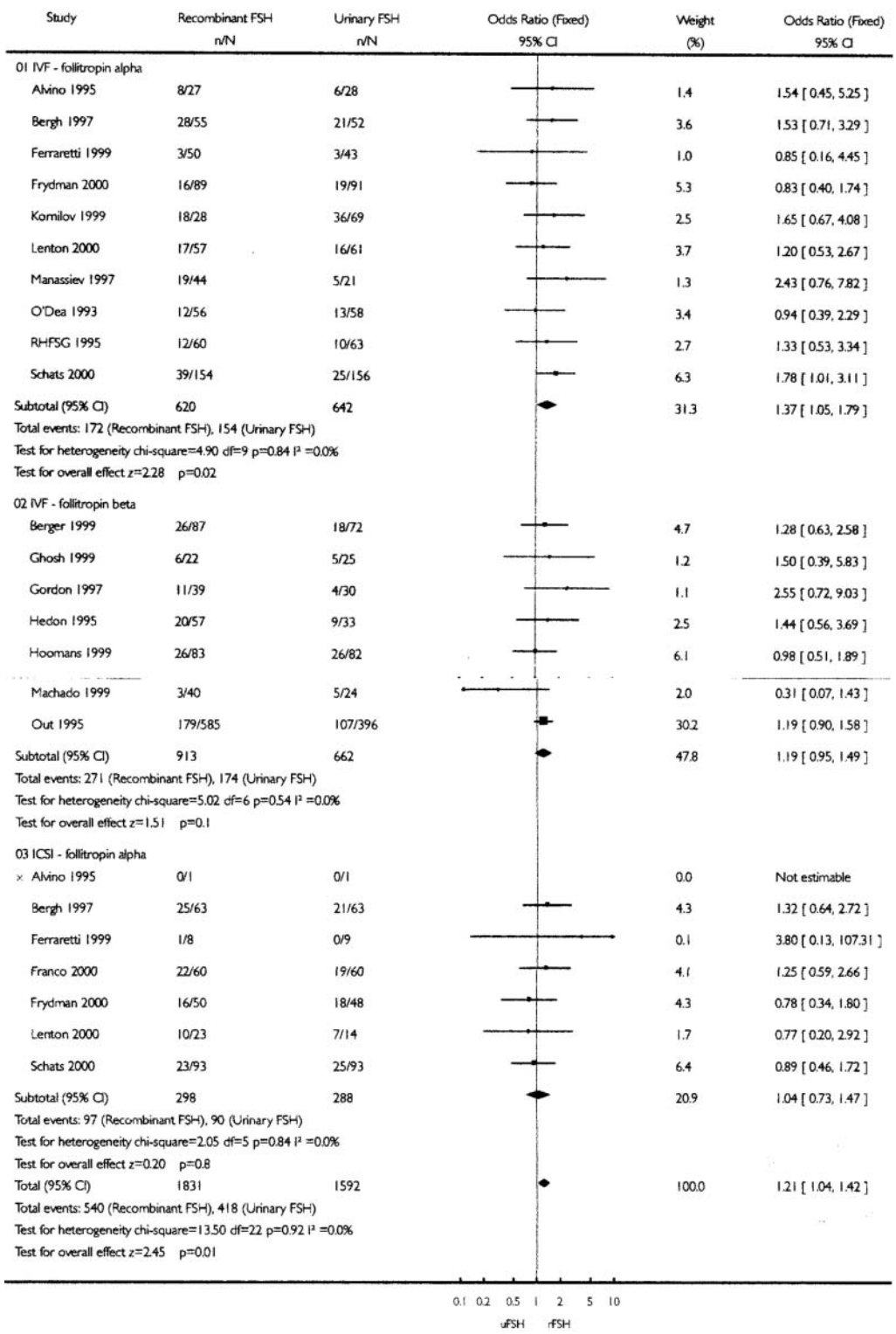

Fig. 3. Comparison 01 rFSH vs uFSH in ART 01.01 Clinical pregnancy per cycle started. Review: Recombinant versus urinary follicle stimulating hormone for ovarian stimulation in assisted reproduction cycles. Comparison: 01 rFSH vs uFSH in ART. Outcome: 01 Clinical pregnancy per cycle started.

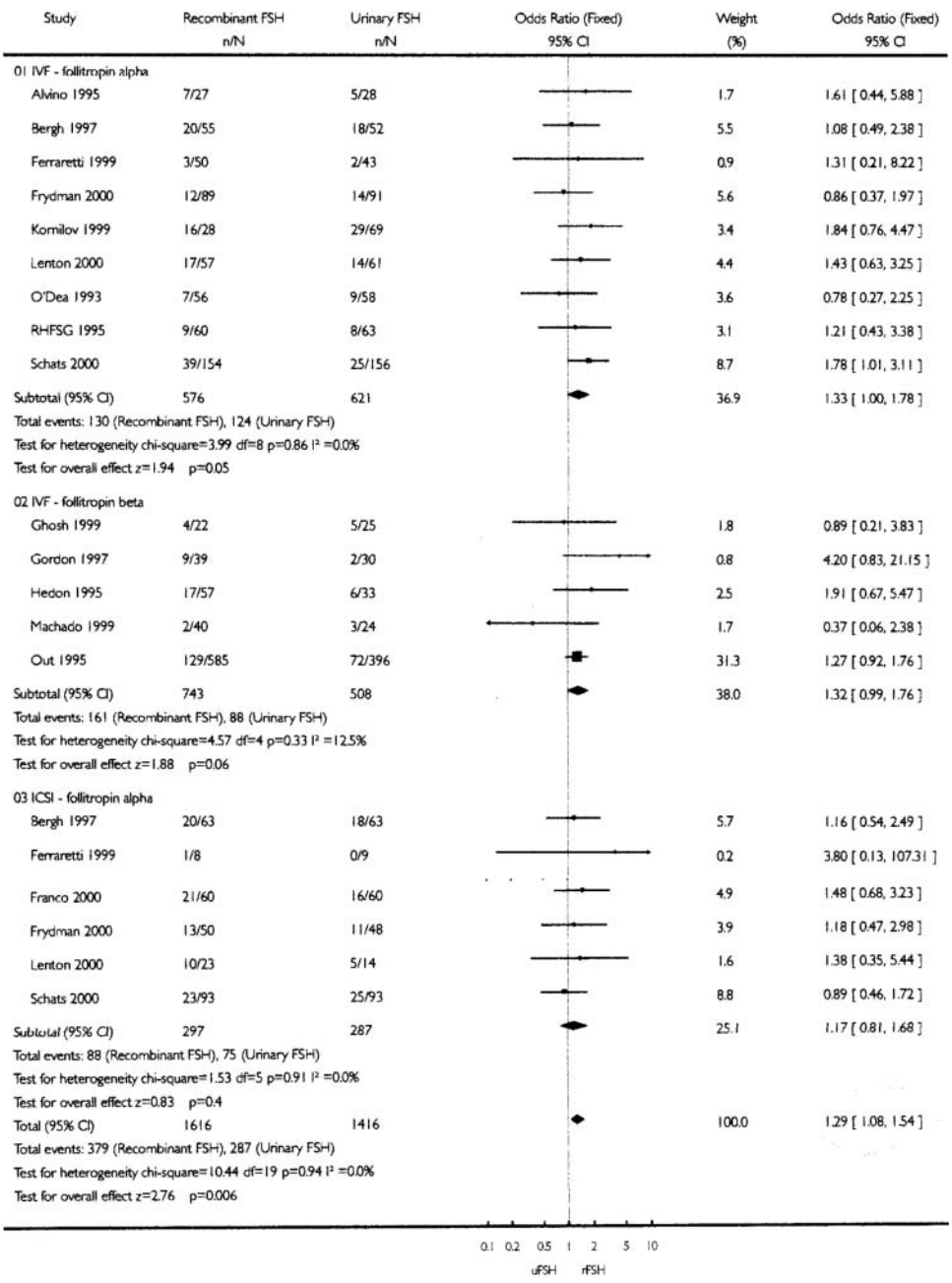

Fig. 4. Comparison 01 rFSH vs uFSH in ART 01.04 Ongoing/delivered pregnancy per cycle started. Review: Recombinant versus urinary follicle stimulating hormone for ovarian stimulation in assisted reproduction cycles. Comparison: 01 rFSH vs uFSH in ART. Outcome: 04 Ongoing/delivered pregnancy per cycle started.

via the IM and SC routes have been proven in multicenter studies, including a study with nearly 1000 infertility patients undergoing *in vitro* fertilization [12].

In most IVF studies, IM administered rFSH proved to be a safe and efficacious drug for the induction of COS in pituitary suppressed women. As a rule, the injections are given by qualified nurses or physicians, often requiring frequent visits to IVF clinics. Compared with the IM route, the SC route has as the main advantage that its self-administration is feasible, thus limiting the number of visits to a clinic.

The objectives of the present review were to compare

Table 1. Comparison of the efficacy of rFSH (Org. 32489) with Japanese and Caucasian infertile couples undergoing COS for ART

Parameter	Japanese mean ± SD	Caucasian mean ± SD	95% C.I.
mean FSH dose (IU)	212.0	210.4	n.a.
Total FSH dose (IU)	1,781 ± 562	2,063 ± 668	131.4 to 431.8
Duration of treatment (D)	8.4 ± 1.6	9.8 ± 1.7	1.04 to 1.87
No. of follicles ≥ 10 mm	10.5 ± 5.4	12.8 ± 7.3	0.80 to 3.90
Hormone concentrations at day of hCG administration			
E_2 (pmol/L)	7,651 ± 5,396	7,109 ± 5,143	–1,845 to 760.1
LH (IU/L)	1.7 ± 1.0	1.5 ± 1.1	–0.439 to 0.075
FSH (IU/L)	14.5 ± 5.0	12.3 ± 5.1	–3.51 to –0.97
P (nmol/L)	4.2 ± 2.0	3.6 ± 7.4	–1.84 to 0.66
No. of oocytes retrieved	12.7 ± 9.6	11.7 ± 6.7	–3.1 to 1.1
No. of mature oocytes	10.8 ± 8.4	10.0 ± 6.8	n.a.
Total No. of embryos	7.7 ± 5.9	7.3 ± 4.8	n.a.
No. of high quality embyos	3.8 ± 3.9	4.5 ± 3.8	–0.21 to 1.71
Abortion rate per attempt (%)	8.5	3.4	–0.106 to 0.0004
Abortion rate per clinical pregnancy (%)	16.7	11.4	n.a.
Ongoing pregnancy rate per attempts (%)	22.9	26.3	–0.070 to 0.138
Ongoing pregnancy rate per transfer (%)	26.1	29.2	n.a.

n.a.: not available.

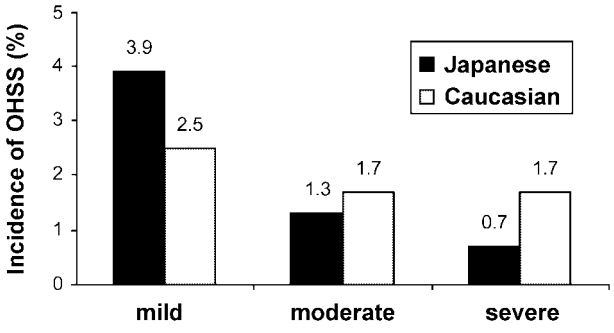

Fig. 5. The incidence of OHSS between Japanese and Caucasian women.

the clinical efficacy and the local tolerance after IM or SC injection of rFSH preparation in IVF patients.

The study was designed as an open-label, prospective, randomized, group comparative, multicenter study. Two hundred eighteen infertile women undergoing IVF-ET were randomized, of whom 195 (IM, n=77, SC, n=118) received rFSH for COS with GnRHa desensitization. The incidences after IM injection of bruising, pain, redness, swelling and itching were 37.7%, 31.2%, 13.0%, 7.8% and 6.5%, after SC injection, and the corresponding figures for SC were 54.2%, 28.0%, 16.1%, 5.9% and 3.4%, respectively. Only bruising was significantly lower in the IM group, which could be attributed to the more visible superficial injection site with SC administration. The overall occurence of local symptoms were 63.6% after IM injection and 68.6% after SC injection.

The mean numbers of oocyte recovered were 9.8 (IM) and 10.4 (SC) and the ongoing pregnancy rates per cycles were 27.1% (IM) and 26.1% (SC), respectively (Table 2). In the IM group, there were two cases (2.6%) of OHSS, whereas in the SC group there were seven cases (5.9%) of OHSS. In four cases (all in the SC group), the OHSS was severe, as defined by a hospitalization. There were no significant differences in local tolerance symptoms, OHSS and clinical efficacy between IM and SC administration of rFSH. Therefore, the results of the study confirmed that SC administration of rFSH is safe, efficacious, and acceptable. In addition, use of the self or non-self administration route may offer significant advantages for both patients and hospital staff in terms of convenience and work load. The availability of rFSH as a ready-for-use solution supplied in an injector system for the SC route may make its administration, in particular self-administration by the patient or her partner, more convenient, as both needle size and injection volume are smaller [13].

Depot vs. daily administration of GnRHa

GnRHa has been widely used for pituitary desensitization in cycles of ART. Among the various types of GnRHa ovarian stimulation protocols, the long protocol presents the best clinical pregnancy rates per

Table 2. FSH on efficacy parameters

Parameter	Mean adjusted for center	
	IM recombinant FSH	SC recombinant FSH
No. of 75 IU ampules administered	29.8	28.2
Duration of treatment (d)	9.9	9.7
Follicles with diameter ≥ 17 mm (n)	4.3	5.0
Follicles with diameter ≥ 15 mm (n)	8.1	8.5
Oocytes recovered (n)	9.8	10.4
Mature oocytes (n)	8.2	8.6
High quality embryos obtained (n)	4.3	3.8
Clinical pregnancy rate/attempt (%)	29.8	30.1
Clinical pregnancy rate/transfer (%)	33.2	33.8
Ongoing pregnancy rate/attempt (%)	27.1	26.1
Ongoing pregnancy rate/transfer (%)	30.1	29.3

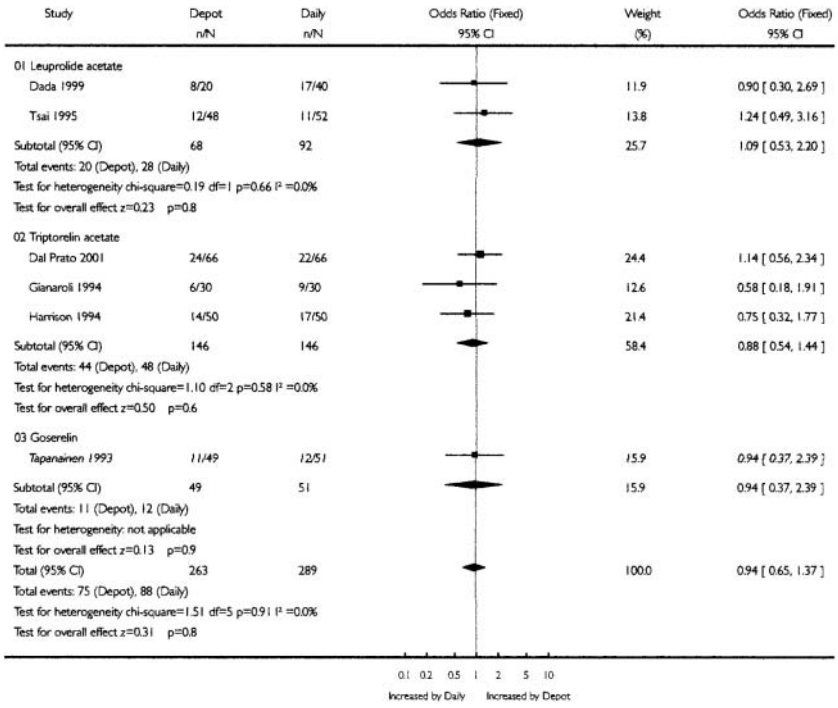

Fig. 6. Comparison 01 Comparison of GnRHa depot versus daily injection (According to analogues) 01.01 Clinical pregnancy rates per woman. Review: Depot versus daily administration of gonadotrophin releasing hormone agonist protocols for pituitary desensitization in assisted reproduction cycles. Comparison: 01 Comparison of GnRHa depot versus daily injection (According to analogues). Outcome: 01 Clinical pregnancy rates per woman.

cycle initiated with GnRHa administration until the suppression of ovarian activity is evident, within approximately 14 days. There are two types of GnRHa administration that can be used to lead to hypophysis desensitization in the ART cycle in the long protocol: one consists of daily GnRHa low doses, and the other of administration of analogues in higher long-acting doses (depot). There are controversies in the data regarding the number of ampoules to be used in the cycles with the depot GnRHa treatment, as well as regarding the number of follicles made available, the number of oocytes, fertilization, implantation and pregnancy rates.

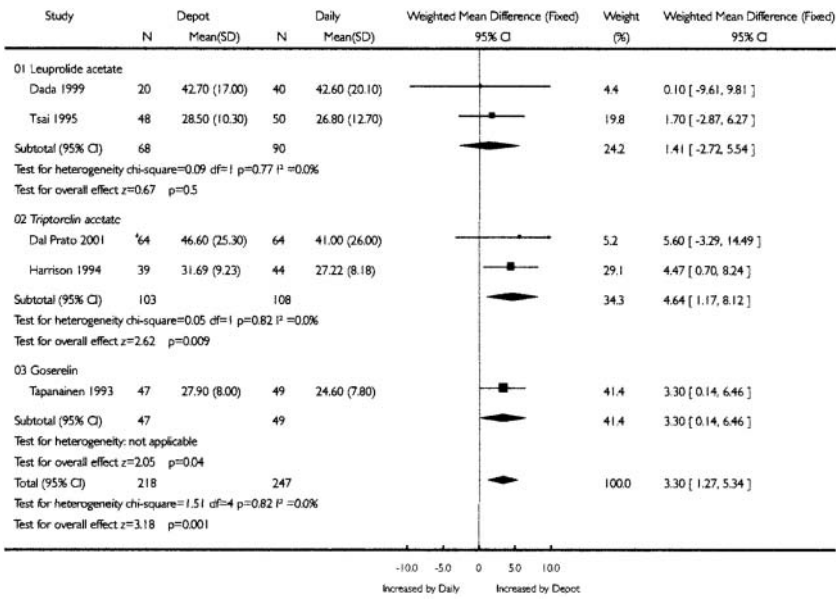

Fig. 7. Comparison 01 Comparison of GnRHa depot versus daily injection (According to analogues) 01.04 Number of ampoules of gonadotropin employed. Review: Depot versus daily administration of gonadotrophin releasing hormone agonist protocols for pituitary desensitization in assisted reproduction cycles. Comparison: 01 Comparison of GnRHa depot versus daily injection (According to analogues). Outcome: 04 Number of ampoules of gonadotropin employed.

The objective of the present review was to compare the use of a single long-acting depot dose to that of daily GnRHa doses in IVF cycles. Relevant RCTs were identified by electronic search of the following databases: MEDLINE, EMBASE, and the Cochrane Controlled Trials Register. One study analyses RCTs comparing depot and daily administration of GnRHa for long protocols in IVF treatment cycles. The participants of that study were couples with any cause of infertility. COS was performed with hFSH and/or hMG and/or rFSH in ART treatment cycles. Statistical analysis of the main efficacy parameters were clinical pregnancy rates per patient, per oocyte retrieval procedure, per embryo transfer, number of oocytes retrieved, oocyte fertilization rates, ongoing/delivered pregnancy rates per cycle started, abortion rates, multiple pregnancy rates, number of ampoules of gonadotropin employed, OHSS incidence rates, cost analysis and patient convenience. All analyses were performed according to the intention-to-treat method. Six studies, with a total of 552 women, were included and analyzed. The studies do not indicate that there is a statistically significant difference between the use of depot GnRHa or daily GnRHa in the primary outcome, clinical pregnancy rates per woman (OR 0.94, 95% CI 0.65 to 1.37) (Fig. 6). However, there was sufficient evidence that the use of depot GnRHa for pituitary desensitization in ART cycles increased the number of gonadotropins ampoules (WMD 3.30, 95% CI 1.27 to 5.34) (Fig. 7) and the duration of the ovarian stimulation (WMD 0.56, 95% CI 0.31 to 0.81), as compared with daily GnRHa. Although we recognize that the clinical pregnancy rates per patient are not the ideal primary outcome, we found no evidence of differences between the long protocol using depot or daily GnRHa for IVF cycles. However, the use of depot GnRHa is associated with increased requirements for gonadotropins and a longer time required for ovarian stimulation. If these differences could be shown to translate into economic benefit, depot GnRHa would increase the overall costs of IVF treatment [14].

Pregnancy and children follow-up study with rFSH in IVF-ET

Recombinant FSH has been shown to have a higher *in vitro* bioactivity than uFSH [15]. Furthermore, in a very large prospective randomized trial in IVF, it was shown

Table 3. Average gestational age and route/mode of delivery of ongoing pregnancies with live-born children

	rFSH		uFSH or hMG	
	singleton pregnancy (n=103)	multiple pregnancy (n=49)*	singleton pregnancy (n=48)†	multiple pregnancy (n=32)
Average gestational age (weeks)	39.6	36.6	39.5	35.6
Route/mode of delivery				
- spontaneous vaginal delivery	67 (65%)	13 (27%)	36 (75%)	6 (18%)
- artificial vaginal delivery	9 (9%)	8 (16%)	2 (4%)	4 (12%)
- cesarean section	27 (26%)	28 (57%)	10 (21%)	23 (70%)

*Excluding one subject for whom no follow-up data were available and two subjects who had a premature delivery (miscarriage) and for whom no delivery data were available. †49 cases with live-born children but in one case no follow-up data were available.

Table 4. Neonatal outcome of live-born children

	rFSH		uFSH/hMG	
	children from singleton pregnancies (n=103)	children from multiple pregnancies (n=100)	children from singleton pregnancies (n=48)*	children from multiple pregnancies (n=64)
Male (n, %)	50 (49%)	58 (58%)	13 (27%)	32 (50%)
Female (n, %)	53 (51%)	42 (42%)	35 (73%)	32 (50%)
No (%) of neonates ≤ 2,500 g	8 (8%)	58 (58%)	4 (8%)	40 (63%)
No (%) of children with weight < tenth percentile†	12 (12%)	18 (18%)	3 (6%)	22 (34%)
No (%) of children with Apgar score < 7 after 5 minutes‡	2/81 (2%)	8/83 (10%)	1/39 (3%)	5/54 (9%)
No (%) of children reported ot have a congenital malformation	5 (5%)	5 (5%)	0	4 (6%)

*49 live-born children; for one child no follow-up data were available. †According to Kloosterman (1970) curves. ‡As a consequence of missing data, denominators differ from the total number of children in each group.

that compared to uFSH, after treatment with rFSH significantly more oocytes were retrieved, more high quality embryos were obtained and, when the results of the cryoprogramme were included, more ongoing pregnancies were achieved after embryo transfer.

Also in other comparative trials [16], higher ongoing pregnancy rates were seen after rFSH treatment. In the present review, the obstetrical and neonatal data of the pregnancies obtained either after rFSH or uFSH treatment in IVF were compared.

Other studies indicated that the use of rFSH is not associated with a higher incidence of obstetrical and neonatal problems compared to urinary gonadotropins [17, 18]. Most complications were seen in multiple pregnancies. Therefore, obstetrical and neonatal outcome of pregnancies obtained with rFSH, were compared with traditional urinary gonadotropins in a comparative study. The study design pooled data derived from three prospective, randomized, multicenter trials. The subjects were 159 ongoing pregnancies by ART after rFSH and 83 after urinary gonadotropin stimulation.The average gestational age for singleton pregnancies in the rFSH and urinary gonadotropin groups were 39.6 and 39.5 weeks, respectively. For the multiple pregnancies, these ages were 36.6 and 35.6 weeks (Table 3). A significantly lower number of small-for-gestational age children was seen in the rFSH multiple pregnancy group (18%) as compared to the urinary gonadotropin group (34%). In multiple pregnancies, the percentage of neonates with a birth weight > 2,500 g was 58% and 63% in the rFSH and urinary gonadotropin groups, respectively. The overall malformation rate was 5.0% in the rFSH group and 3.6% in the urinary gonadotropin group (Table 4). Therefore, it was concluded that the use of rFSH does not result in increased adverse obstetrical and neonatal outcomes as compared to urinary gonadotropins [19].

Conclusions

HMG and rFSH have both been used successfully for COS in ART. One review has concluded a statistically

significant increase in clinical pregnancy rate with rFSH compared to uFSH, when used for COS in standard IVF cycles but not in cycles in which ICSI was used. Another review of trials found that taking rFSH instead of uFSH increased the chances of pregnancy by 14%. It also found that rFSH has a potentially unlimited supply, is very consistent and is also cost effective. Since racial differences have never been observed between Japanese and Caucasians in the outcomes of ART with rFSH, foreign data on rFSH is applicable to the Japanese population. Recombinant FSH is a new treatment option for Japanese women undergoing COS for ART with several advantages over conventional urinary gonadotropin preparations. SC administration of rFSH is safe, efficacious, and acceptable. In addition, use of self or non-self administration routes may offer significant advantages for both patients and hospital staff in terms of convenience and work load. The availability of rFSH as a ready-for-use solution supplied in an injector system for the SC route may make its administration, in particular self-administration by the patient or her partner, more convenient, as both needle size and injection volume are smaller. We found no evidence of differences between the long protocol using depot or daily GnRHa for IVF cycles. However, the use of depot GnRHa is associated with increased requirements for gonadotropins and a longer time required for ovarian stimulation. The present review found that the use of rFSH is not associated with a higher incidence of obstetrical and neonatal problems compared to urinary gonadotropins.

References

1) de Leeuw, R., Mulders, J., Voortman, G., Rombout, F., Damm, J. and Kloosterboer, L. (1996): Structure-function relationship of recombinant follicle stimulating hormone (Puregon). Mol. Hum. Reprod., 2: 361–369.
2) Mannaerts, B., Shoham, Z., Schoot, B., Bouchard, P., Harlin, P., Fauser, B., Jacobs, H., Rombout, F. and Bennink, C.H.J.T. (1993): Single-dose pharmacokinetics and pharmacodynamics of recombinant human follicle-stimulating hormone in gonadotropin deficient volunteers. Fertil. Steril., 59: 108–114.
3) Schoot, D.C., Harlin, J., Shoham, Z., Mannaerts, B., Lahlou, N., Bouchard, P., Bennink, C.H.J.T. and Fauser, C.J.M. (1994): Recombinant human follicle-stimulating hormone and ovarian response in gonadotropin-deficient women. Hum. Reprod., 9: 1237–1242.
4) Devroy, P., Mannaerts, B., Smitz, J., Bennink, C.K. and Van Steirteghem, A. (1994): Clinical outcome of a pilot efficacy study on recombinant human FSH (Org 32489) combined with various GnRH agonist regimens. Hum. Reprod., 9: 1064–1069.
5) Out, H.J., Mannaerts, B.M.J., Bennink, C.K. and Driessen, S.G.A.J. (1995): For the European Org 32489 Collaborative IVF Study Group. A prospective, randomized, assessor-blind, multicenter study comparing recombinant and urinary follicle-stimulating hormone (Puregon vs Metrodin) in in-vitro fertilization. Hum. Reprod., 10: 2534–2540.
6) van Wely, M., Westergaard, L.G., Bossuyt, P.M. and Van der Veen, F. (2003): Human menopausal gonadotropin versus recombinant follicle stimulation hormone for ovarian stimulation in assisted reproductive cycles. Cochrane Database Syst. Rev., 2003 (1): CD003973.
7) Daya, S. and Gunby, J. (1997): Recombinant versus urinary follicle stimulating hormone for ovarian stimulation in assisted reproduction cycles. The Cochrane Library 2005, 1: 278–283.
8) Out, H.J., Driessen, S.G.A.J., Mannaerts, B.M.J.L. and Bennink, C.H.J.T. (1997): Recombinant follicle-stimulating hormone (follitropin beta, Puregon) yields higher pregnancy rates in in-vitro fertilization than urinary gonadotropins. Fertil. Steril., 68: 138–142.
9) Hedon, B., Out, H.J., Hugues, J.N., Camier, B., Cohen, J., Lopes, P., Zorn, J.R., van der Heijden, B. and Bennink, C.H.J. (1995): 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, multicenter trial. Hum. Reprod., 10: 3102–3106.
10) Navot, D., Relou, A., Birkenfeld, A., Rabinowitz, R., Brzezinski, A. and Margalioth, E.J. (1988): Risk factors and prognostic variables in the ovarian hyperstimulation syndrome. Am. J. Obstet. Gynecol., 159: 210–215.
11) Kubo, H., Takeda, S., Kinoshita, K., Motoyama, H., Kobayashi, S. and Morimoto, Y. (2003): An open prospective multicenter study in pituitary-suppressed Japanese women undergoing controlled ovarian hyperstimulation with recombinant follicle-stimulating hormone (Org 32489) to compare Japanese and European IVF results. Jpn. Fertil. Steril., 48: 49–60.
12) Out, H.J., Reimitz, P.E. and Coelingh Bennik, H.J.T. (1997): A prospective, randomized study to assess the tolerance and efficacy of intramuscular and subcutaneous administration of recombinant follicle-stimulating hormone (Puregon). Fertil. Steril., 67: 278–283.
13) Voortman, G., van de Post, J., Schoemaker, R.C. and van Gerven, J.M.A. (1999): Bioequivalence of subcutaneous injections of recombinant human follicle stimulating hormone (Puregon) by Pen-injector and syringe. Hum. Reprod., 14: 1698–1702.
14) Albuquerque, L.E., Saconato, H. and Maciel, M.C. (2002): Depot versus daily administration of gonadotrophin releasing hormone agonist protocols for pituitary desensitization in assisted reproduction cycles. Cochrane Database Syst. Rev. 2002 (3): CD002808.
15) Matikainen, T., de Leeuw, R., Mannaerts, B. and

Huhtaniemi, I. (1994): Circulating bioactive and immunoreative recombinant follicle stimulating hormone (Org 32489) after administration to gonadotrophin-deficient subjects. Fertil. Steril., 61: 62–69.
16) Jansen, C.A.M., Van Os, H.C., Out, H.J. and Coelingh Bennink, H.J.T. (1998): A prospective, randomized clinical trial comparing recombinant follicle-stimulating hormone (Puregon) and human menopausal gonadotrophins (Humegon) in non-downregulated in-vitro fertilization patients. Hum. Reprod., 13: 2995–2999.
17) Kloosterman, G.J. (1970): On intra-uterine growth. Int. J. Obstet. Gynecol., 8: 895–912.
18) No authors listed (1990): MRC working party on children conceived by in vitro fertilization. Br. Med. J., 300: 1229–1233.
19) Out, H.J., Driessen, S.G.A.J., Mannaerts, B.M.J.L. and Coelingh Bennink, H.J.T. (1999): A pregnancy and children follow-up study of three randomized clinical trials with recombinant follicle-stimulating hormone (Puregon) in in-vitro fertilization. Middle East Fertil. Soc. J., 4: 28–34.