Improved oocyte quality is obtained with follicle stimulating hormone alone than with follicle stimulating hormone/human menopausal gonadotrophin combination

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Introduction

The use of gonadotrophin has increased the number of oocytes retrieved, number of embryos replaced, and pregnancy rates in in-vitro fertilization (IVF). Both pure follicle stimulating hormone (FSH) and human menopausal gonadotrophin (HMG) have been used effectively for ovulation induction even in women with hypogonadotropic amenorrhoea (Couzinet et al., 1988; Shoham et al., 1991).

Although pure FSH was originally developed for the treatment of patients with polycystic ovarian syndrome, it is now widely used in the treatment of almost all patients applying for assisted reproduction. Pure FSH also can be administered s.c. and this does not alter the pharmacodynamic response of the patients (Howles et al., 1994). Although the precise amount of luteinizing hormone (LH) required for normal follicular development is still unknown, it has been demonstrated that only a minimal amount of LH is required for ovarian steroidogenesis and normal follicular development (Hillier, 1990; Bergh et al., 1993). Furthermore, elevated LH concentrations during the late follicular phase and peri-ovulatory period have detrimental effects on oocyte quality, fertilization, cleavage, embryo quality and pregnancy rates (Stanger and Yovich, 1985; Howles et al., 1987). It also increases the miscarriage rates (Homburg et al., 1988; Regan et al., 1990). Howles et al. (1987) found that pregnancy rates were lower in patients with elevated LH concentrations 2 days prior to human chorionic gonadotrophin (HCG) administration. HMG increases LH and follicular fluid testosterone concentrations that may impair oocyte quality and implantation (McNatty et al., 1979; Polan et al., 1986), whereas pure FSH results in a decrease in LH concentrations (Kamrava et al., 1982; Venturoli et al., 1984; Anderson et al., 1989). It has been suggested that higher LH concentrations may prematurely allow resumption of meiotic maturation (Jacobs et al., 1987). Premature luteinization can be observed despite routine use of gonadotrophin-releasing hormone analogues (GnRHa) for pituitary desensitization and this may be due to the HCG content of HMG. Therefore it has been recommended that exogenous LH should be used only for patients with hypogonadotrophic hypogonadism (Shoham et al., 1991).

Although several previous prospective randomized studies demonstrated that there was no difference between pure FSH and HMG regarding pregnancy rates (Polan et al., 1986; Scoccia et al., 1987; Lavy et al., 1988; Edelstein et al., 1990; Tanbo et al., 1990), a recent meta-analysis revealed that pure FSH increased the pregnancy rates by 50% (Daya, 1995).

The aim of this study was to compare pure FSH and FSH/HMG combination with regard to oocyte quality, fertilization, cleavage and pregnancy rates.

Materials and methods

A total of 337 patient IVF cycles between October 1994 and October 1995 at our institution was evaluated retrospectively. Two hundred and eighteen patients received FSH alone and 119 received FSH/HMG (Metrodin and Pergonal respectively; Serono Laboratories, Inc., Randolph, MA, USA) combination. The choice of protocol was based on the availability of medications and the patients’ prior stimulation experience. No bias was applied in choosing patients for a particular protocol. All patients were suppressed with leuprolide acetate (LA) (Lupron; TAP Pharmaceuticals, Abbott Park, IL, USA) using the long protocol. Lupron was commenced in the mid-luteal phase of the

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preceding cycle at the dose of 1 mg/day and reduced to 0.5 mg/day at the first day of menses. In patients demonstrating poor response in a previous IVF cycle, Lupron was commenced in the mid-luteal phase of the preceding cycle and stopped on day 1 of menses. FSH (Metrodin, Serono Laboratories) alone (218 patient cycles) or in combination with HMG (Pergonal, Serono Laboratories), (119 patient cycles) were initiated on day 3 of the menstrual cycle with two to six ampoules (75 IU/ampoule) and then the dose was adjusted in an individualized fashion using a step-down protocol. In the combination protocol when four ampoules were used, two were pure FSH and two were HMG, and when six ampoules were used, four were pure FSH and two were HMG. For both protocols, when at least three follicles \( \geq \)16 mm were seen on ultrasound, human choric gonadotrophin (HCG) 10 000 IU i.m. was administered. Follicular aspiration was performed 34-36 h later and three or four embryos were transferred 2 days after oocyte retrieval. Extra embryos were frozen at the two pronuclear (2-PN) stage using 1,2-propanediol as cryoprotectant. Luteal phase was supported using progesterone 50 mg i.m. daily beginning on the day of embryo transfer. All oocytes were assessed at aspiration for corona/oocyte morphology (corona density, ooplasm colour, oocyte shape and nuclear morphology) and fertilization outcome. Oocytes were classified into mature (metaphase II, or metaphase I), immature (presence of a germinal vesicle), or atretic. Typical desired morphology for mature oocytes was defined as metaphase II oocytes (presence of first polar body), with expanded cumulus and corona, round even shape, and light, uniform coloration of the ooplasm. Oocyte identification and classification were performed by the two senior embryologists (J.M. and D.W.) who were unaware of the type of stimulation protocol used at the time. All patients underwent an initial serum test for \( \beta \)-HCG 12–14 days after embryo transfer. Clinical pregnancies were defined as the presence of a gestational sac by ultrasound at 6–7 weeks gestation. The implantation rate was defined as the number of gestational sacs seen by first trimester ultrasound divided by the total number of embryos transferred.

**Statistical analysis**

All data were compared using Fisher exact test and \( P \)-values \( < 0.05 \) were considered significant.

**Results**

A total of 337 patient cycles was studied: in 119, the FSH/ HMG combination and in 218, pure FSH were used. There was no difference between the two groups in mean age of patients, number of new and repeat patients and aetiology of infertility (Table I). There were no significant differences in the aetiology of infertility within each group of new or repeat patients (data not shown). Similarly, both groups were comparable for total oocytes aspirated, mature oocytes, number of ampoules used and oestradiol concentrations at the day of HCG (Table II). Cancellation rates and cycles with freezing were 6.4 and 48%, 7.5 and 41% in the pure FSH and FSH/ HMG groups respectively, and the difference was found to be insignificant. Also, the mean number of embryos frozen was comparable between two groups (6.5 \( \pm \) 1.1 in the FSH group and 7.9 \( \pm \) 5.7 in the FSH/HMG group). Although there was no difference in the number of mature oocytes between the two groups (Table III), mature oocytes with typical desired morphology were significantly higher in the FSH-only group (\( P < 0.0001 \)). In contrast, the percentage of immature oocytes matured in vitro was significantly higher in the combination protocol (\( P = 0.001; \) Table III). Fertilization rates, the number of 2-PN from mature oocytes and immature oocytes, the number of 3-PN from mature oocytes, and miscarriage rates (Table IV) were not different between two stimulation protocols. Although clinical pregnancy rates per attempt and retrieval, and delivery rate appeared higher in the FSH alone group, the difference was not significant; however, clinical pregnancy rate per transfer was significantly higher in FSH alone group (40 versus 28%, \( P < 0.05 \)). The implantation rate also appeared higher in the FSH group (11.6 versus 9.3%) (Table IV) but the difference was not statistically significant.
The incidence of severe ovarian hyperstimulation was similar in the FSH ($n = 3$) and the FSH/HMG ($n = 2$) groups.

### Discussion

The first clinical pregnancy using pure FSH was reported by Flamigni et al. (1985) in a woman with polycystic ovarian disease and the first pregnancy using GnRHa suppression and Metrodin was achieved in 1985 (Shaw et al., 1985).

Several prospective randomized studies demonstrated that there was no difference between Metrodin and HMG regarding fertilization and pregnancy rates (Polan et al., 1986; Scoccia et al., 1987; Lavy et al., 1988; Edelstein et al., 1990; Tanbo et al., 1990). However, most of these studies were small and included <20 cycles in each group. Also, in some other recently published and larger studies (Howles et al., 1994; Hull et al., 1995) comparable results were obtained between FSH and HMG. Howles et al. (1994), in a multicentre European study, found that there was no difference regarding oocytes recovered, cleavage and pregnancy rates between s.c. highly purified FSH and HMG and reported results with HMG. Also, Hull et al. (1995), in a study including 2204 cycles with use of FSH alone, found that the overall results were comparable with HMG, but both of these studies used historic data as the control groups. Similarly, Check et al. (1995), in a prospective randomized study, found comparable results between HMG and FSH. Devroey et al. (1995) in a randomized study including 158 patients compared Normegon (75 IU FSH and 25 IU LH) and Metrodin and found no difference regarding the number of oocytes recovered, fertilization, embryo transfer and pregnancy rates.

Recently a meta-analysis showed that Metrodin results in >50% improvement over HMG in clinical pregnancy rates (Daya, 1995). This meta-analysis included eight prospective, randomized studies, and seven of them showed that the pregnancy rates were higher in Metrodin group. In an another recent study including 232 patients it was found that fertilization rate was significantly higher in the Metrodin than in the HMG group (Daya et al., 1995). Furthermore, a higher pregnancy rate was obtained in the Metrodin group, although the difference was found to be insignificant. They concluded that the advantage of using HMG should be re-evaluated. Similarly, Wikland et al. (1995) in a study including 500 couple, found that pregnancy rates were significantly higher in the Metrodin group than in the HMG group (35 versus 18% per cycle, 40 versus 23% per transfer). Also, fertilization rate was found to be higher in the Metrodin group, but the difference lacked significance. In all the aforementioned studies, FSH was compared with HMG, but in this study we compared FSH alone with the FSH/HMG combination.

In the present study, in contrast to previous studies, we obtained a significantly higher clinical pregnancy rate per transfer in the FSH alone group (40 versus 28%) and our results are in agreement with those of meta-analysis and Wikland et al. (1995). Although pregnancy rates per attempt and retrieval were higher in the Metrodin group, the difference was not significant. Also no difference was observed in number of oocytes retrieved, number of mature and immature oocytes, fertilization rates, miscarriage and ongoing pregnancy rates, but the number of mature oocytes with typical morphology was significantly higher in the Metrodin group. Because there was no difference in other parameters, the higher pregnancy rates may be due to a higher rate of mature oocytes with desired morphological appearance in the pure FSH group. It has been reported that the oocytes from patients with high LH were darker in colour but did not show signs of degeneration (Stanger and Yovich, 1985). Similarly, Imthurn et al. (1996) demonstrated that highly purified FSH resulted in a higher proportion of mature oocytes and fewer oocytes with dark cytoplasm than HMG. The higher percentage of immature oocytes that mature in vitro suggests that the LH present in the combination protocol may influence the in-vitro maturation process.

Although it is generally reported that ovulation induction using FSH alone results in lower oestradiol concentrations on the day of HCG (Edelstein et al., 1990; Fried et al., 1996), this has no practical value, because it has been demonstrated that oestradiol concentration was not a good indicator for pregnancy potential (Howles et al., 1994). In the present study no difference was observed in oestradiol concentrations on the day of HCG between the two groups. It should be emphasized that our study used pure urinary FSH. The results may be different with newer preparations such as highly purified (HP) urinary FSH or recombinant FSH. Westergaard et al. (1996), in a prospective randomized trial, reported a lower fertilization rate in down-regulated HP-FSH patients and lower oestradiol concentrations on cycle day 8 compared to HMG-stimulated patients. However, the duration of stimulation, mean number of ampoules used, and clinical pregnancy rate were similar in the two groups. Fleming et al. (1996) reported higher circulating concentrations of FSH with s.c. administration of HP-FSH, but lower circulating oestradiol concentrations and longer duration of stimulation compared to i.m. administration of HMG. The duration of stimulation was notably longer in patients with reduced LH concentrations in the follicular phase. In stimulating oocyte donors, Soderstrom-Antilla et al. (1996) reported a higher fertilization rate with i.m. HMG compared to s.c. HP-FSH stimulation. However, the duration of stimulation, number of ampoules used, and clinical pregnancy rates were the same in the two groups. Tomlinson et al. (1996) reported a higher, but not statistically significant, pregnancy rate, in patients stimulated with s.c. HP-FSH and undergoing intrauterine insemination (IUI) compared with i.m. HMG stimulated patients. Balasch et al. (1996)}
reported no significant differences between FSH and FSH/HMG or between HP-FSH and HP-FSH/HMG stimulated cycles with regard to the number of ampoules used, day of HCG administration, peak oestradiol concentrations, number of oocytes aspirated, embryos transferred, and clinical pregnancy rates. As more studies are reported with s.c. administration of HP-FSH or recombinant FSH, the issue of clinical efficacy, ovarian response, and pregnancy rates with the use of these preparations, and any differences with i.m. HMG treatment will be further clarified.

In conclusion, previous studies and the present study showed that there is a trend toward better oocyte quality, fertilization and pregnancy rates with pure FSH protocols. However, further studies are required to confirm these findings and to determine the best method for ovulation induction for assisted reproduction. Also, it remains to be determined if the detrimental effects of LH are dose dependent or if there is a threshold value at which that oocyte quality is decreased.

References

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