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Semi-programmed ovarian stimulation as the first choice in in-vitro fertilization programmes

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The objective of this work was to evaluate the results obtained with a protocol of semi-programmed ovarian stimulation (low-dose contraceptive pill + clomiphene citrate + human menopausal gonadotrophin + dexamethasone) used as the first-choice method for in-vitro fertilization (IVF). A total of 207 punctures was performed for oocyte collection from 168 patients (mean age 31.0 ± 4.0 years); mean infertility duration was 5.81 ± 3.30 years. The infertility factors indicating IVF for this population were as follows: tubo-peritoneal factor, 68%; pure or associated male factor, 9.2%; endometriosis, 11.1%; ovulatory factor, 4.3%; idiopathic factor, 11.6%; others, 2.4%. No oocyte was found on aspiration in five procedures (2.4%), with the mean number of oocytes collected per cycle being 5.87 ± 3.3 (range 0–18). The cancellation rate per puncture was 5%. The mean embryo cleavage rate was 60.2 ± 36.8%, with transfer of at least one embryo occurring in 82.6% of all punctures. The mean number of transferred embryos was 2.52 ± 1.60 (range 1–5). The clinical pregnancy rates per started cycle and per puncture were 22.4 (218 ovarian stimulation cycles) and 23.6% (a total of 49 clinical pregnancies, 36 single, nine twins and four triplets) respectively. The clinical pregnancy rate per embryo transfer was 28.6%. The embryo implantation rate was 12.6%. The abortion rate was 16.3%. The index of deliveries per puncture was 19.8%. There were no cases of moderate or severe ovarian hyperstimulation syndrome. The favourable results obtained, in addition to the low operational costs, confirm the validity of the use of semi-programmed cycles as the first choice for patients undergoing the IVF process.

Key words: in-vitro fertilization/ovarian stimulation/semi-programmed cycle

Introduction

Clinical pregnancy rates per ovarian puncture normally range from 15 to 30% in programmes of in-vitro fertilization (IVF; Medical Research International *et al.*, 1992). This requires repetition of the process to obtain higher pregnancy rates in an infertile population. In developing countries, the cost of the cost of the procedure is a decisive factor for the application of the IVF technique.

Many studies have emphasized the importance of the use of gonadotrophin-releasing hormone analogues (GnRHa) in combination with human menopausal gonadotrophin (HMG) as a routine protocol for ovarian stimulation in IVF programmes (Rutherford *et al.*, 1988; Meldrum *et al.*, 1989; Lejeune *et al.*, 1990). Most reports have indicated the superiority of the use of GnRHa + HMG over traditional regimens of ovarian stimulation. However, these studies were retrospective and non-randomized (Rutherford *et al.*, 1988; Meldrum *et al.*, 1989; Lejeune *et al.*, 1990). Prospective and randomized studies comparing the use of GnRHa + HMG to standard protocols of ovarian stimulation are few and have produced contradictory results. Some investigators have reported higher pregnancy rates with the use of GnRHa + HMG (Neveu *et al.*, 1987; Ron-El *et al.*, 1991), and others have not detected this superiority over traditional schemes (Ferrier *et al.*, 1990; Maroulis *et al.*, 1991; Kingsland *et al.*, 1992). Despite this divergence, two facts are clear when GnRHa are used in combination with HMG: the high cost of treatment and the higher incidence of ovarian hyperstimulation syndrome in its moderate and severe forms (Ron-El *et al.*, 1991; Dickey *et al.*, 1993).

On the other hand, Kemeter and Feichtinger (1989) have described a standard protocol of ovarian stimulation which differs from the previous ones (Frydman *et al.*, 1986) by being based on the use of a low-dose contraceptive pill during the cycle preceding the IVF one, a 5 day interval between discontinuation of the pill and the beginning of ovarian stimulation with clomiphene citrate, HMG and a daily corticoid, and variation of the day of human chorionic gonadotrophin (HCG) administration as a function of the individual response of each patient (semi-programmed protocol).

The objective of this study was to evaluate the results of ovarian stimulation with a semi-programmed cycle as the first-choice method in an IVF programme.

Materials and methods

A total of 168 female patients and their husbands participated in an IVF programme between January 1992 and May 1993, with a total of 207 punctures performed for oocyte uptake (218 ovarian stimulation cycles). Patient age ranged from 23 to 44 years (mean ± SD 31.4 ± 4.0) and duration of infertility was 1–15 years (5.81 ± 3.30). The indications for IVF were as follows: tubo-peritoneal factor, 68%; pure or associated

male factor, 9.2%; endometriosis, 11.1%; ovulatory factor, 4.3%; idiopathic factor, 11.6%; others, 2.4%.

The semi-programmed protocol consisted of blockage with a low-dose contraceptive (30 µg ethinyl-oestradiol and 75 µg gestodene) used from the first day of the menstrual cycle preceding the day of ovarian stimulation and continued for at least 21 and at most 28 days, with the last pill always being taken on a Monday. Ovarian stimulation was obligatorily started on the fifth day after discontinuation of the pill (obligatorily on Saturday) with 100 mg/day clomiphene citrate for 5 days, 150 IU HMG on alternate days and 0.5 mg/day dexamethasone (from the first day of ovarian stimulation to the time of embryo transfer).

Monitoring of follicular development was started on the eighth day of stimulation using an Ultramark 4-ATL ultrasound apparatus equipped with a 5 MHz vaginal transducer (Advanced Technology Laboratories, Bothel, WA, USA). Daily doses of 150 IU HMG were administered until two or more follicles ≥17 mm in diameter were identified, at which time HCG was administered i.m. at the dose of 10 000 IU (Oliveira *et al.*, 1993). Aspirative puncture was performed 34–36 h after HCG, with the patient under ethomidate sedation (Hypnomidate, Janssen, São Paulo, Brazil). Vaginal cleansing was performed with Ringer's solution and oocytes were collected with a 17 gauge needle guided by the vaginal transducer until follicle penetration, when follicular fluid was aspirated with a 5 ml syringe (Feichtinger and Kemeter, 1986). After identification in the follicular fluid, the oocytes were classified for maturity using an inverted microscope (Marrs *et al.*, 1984) and placed on Nunc dishes (Nunclon Delta, Roskilde, Denmark) containing Menezo B2 medium (Apy-System, Montalieu-Vercieu, France) enriched with 10% patient serum (Franco *et al.*, 1993).

Semen samples were obtained by masturbation after a period of 2–5 days sexual abstinence. The spermatozoa were recovered using a modification of the classic sedimentation–migration technique. In this technique, liquefied spermatozoa were deposited below the Menezo B2 culture medium plus inactivated patient serum (30%) in the proportion of 1:1 (spermatozoa:medium) in 5.0 ml polyethylene tubes (Falcon, Becton Dickinson Co., NJ, USA) and incubated at 37°C in a 5% CO_2 atmosphere for 45 min. After incubation, the culture medium was removed, together with the spermatozoa that had migrated, and centrifuged at 200 *g* for 5 min; the resulting supernatant was discarded. The sediment obtained was resuspended in 0.5 ml Menezo B2 + 30% serum and centrifuged again as described. The supernatant was then removed and the final sediment covered with Menezo B2 + 30% serum and incubated at 37°C in a 5% CO_2 atmosphere for 15 min. In cycles in which the male factor was present, semen was prepared using a discontinuous Percoll gradient.

Within a time interval of 4–6 h after oocyte collection, ~50 000–150 000 motile spermatozoa/ml were added to the culture medium. The embryos were identified within ~40 h of insemination, transferred to pure inactivated patient serum and deposited ~0.5 cm from the fundus of the uterus. For luteal phase maintenance, 1000 IU HCG were used on days 4, 6, 8 and 10 after embryo transfer. β-HCG was measured once on day 14 after embryo transfer. When the test was positive, a vaginal ultrasound examination was performed 2 weeks later to confirm clinical pregnancy.

Table I. Results obtained with the use of a semi-programmed ovarian stimulation in 207 punctures for IVF

No. of cycles	207
Patient age (years)	31.0 ± 4.0
No. of oocytes collected per cycle	5.87 ± 3.30
Cleavage rate (%)	60.2 ± 36.8
No. of embryos transferred per cycle	2.52 ± 1.68
Clinical pregnancy per started cycle (%)	22.4
Clinical pregnancy rate per puncture (%)	23.6
Clinical pregnancy rate per embryo transfer (%)	28.6
Embryo implantation rate (%)	12.6
Abortion rate (%)	16.3
Index of deliveries per puncture (%)	19.8

Values are means ± SD unless otherwise indicated.

Results

The frequency of ovarian punctures varied according to the day of the week as follows: 41.29% for Monday, 26.45% for Tuesday, 18.06% for Wednesday, 2.58% for Thursday, 3.87% for Friday, 2.58% for Saturday and 5.16% for Sunday. In general, HCG was administered between days 8 and 9 of stimulation, i.e. Saturday and Sunday according to our protocol. Thus, ~92% of the punctures were performed during the week. The mean number of days of ovarian stimulation was 9.27 ± 1.61 (range 8–14). The cancellation rate per puncture was 5%.

Table I shows the data of 207 punctures for IVF. There was no oocyte collection in five procedures (2.4%) and the mean number of oocytes collected per puncture was 5.87 ± 3.30 (range 0–18). The mean embryo cleavage rate was 60.2 ± 36.8%, with embryo transfer of at least one embryo occurring in 82.6% of the collections. The mean number of transferred embryos was 2.52 ± 1.68 (range 1–5). The clinical pregnancy rates per started cycle and per puncture were 22.4 (a total of 218 stimulation cycles) and 23.6% (a total of 49 clinical pregnancies, 36 single, nine twins and four triplets) respectively. The clinical pregnancy rate per embryo transfer was 28.65%. The embryo implantation rate was 12.6%. The abortion rate was 16.3%. The index of deliveries per puncture was 19.8%. There was no case of ovarian hyperstimulation syndrome, either of the moderate or severe type.

Discussion

A semi-programmed protocol of ovarian stimulation permits the physician to perform all punctures during the week, with the weekend being virtually free of laboratory activity.

The mean number of oocytes taken up per puncture (5.87 ± 3.30) was higher than that reported in several standard protocols using clomiphene citrate + HMG, e.g. 3.10 reported by Watrelot and 2.30 reported by Barriere (for a review see Demoulin *et al.*, 1989), but was lower than the mean values of ≥7 commonly cited with the use of protocols based on a GnRHa + HMG (Ferrier *et al.*, 1990; Ron-El *et al.*, 1991). The collection of a lower number of oocytes may reduce the total number of embryos transferred per puncture and

consequently the clinical pregnancy rate, especially in groups having access to efficient programmes of embryo cryopreservation.

The mean embryo cleavage rate was 60.2 ± 36.8%, similar to that reported by Demoulin *et al.* (1989) with schedules of clomiphene citrate + HMG (65.6 ± 26.8%) or with short-term (62.0 ± 24.1%) or long-term (67.0 ± 26.2%) GnRHa.

In 1990, analysis by the American IVF/Embryo Transfer Registry (Medical Research International *et al.*, 1992) of general IVF/embryo transfer results showed that the clinical pregnancy rate was 22.6% (137 collections and 31 clinical pregnancies) in standard protocols based on HMG alone, 13.4% (363 collections and 49 clinical pregnancies) in protocols based on clomiphene citrate + HMG, 14.3% (251 collections and 36 clinical pregnancies) in protocols based on HMG + follicle-stimulating hormone (FSH), 17.1% (1671 collections and 287 clinical pregnancies) in protocols based on GnRHa + HMG, and 18.1% (1451 collections and 264 clinical pregnancies) in protocols based on GnRHa + FSH + HMG. Although 97% of American clinics obligatorily use GnRHa in protocols of ovarian stimulation, there was no difference (χ^2 test) in clinical pregnancy rates between groups using GnRHa (3133 collections and 551 clinical pregnancies) and groups that did not use them (751 collections and 116 clinical pregnancies). The American Registry (Medical Research International *et al.*, 1992) does not cite the results obtained by the use of ovarian stimulation in programmed or semi-programmed cycles.

The semi-programmed protocol of ovarian stimulation described here is easy to administer, requires little monitoring and a lower use of medications, and virtually eliminates the risk of ovarian hyperstimulation syndrome.

On the other hand, Kemeter and Feichtinger (1989) used corticosteroid daily from the first stimulation day to prevent any excessive release of adrenal androgens caused by stress in the patients undergoing IVF. Also, the immunosuppressive doses of dexamethasone could have a beneficial effect on the pregnancy rates (Lobo, 1991; Polak de Fried *et al.*, 1993).

Furthermore, its low operational cost makes this one of the few options of ovarian stimulation for IVF, especially in developing countries. Thus far, prospective and randomized studies and even national registries with large numbers of cases have not been able to define the real advantages of the routine use of protocols based on GnRHa compared with standard models.

In view of the above considerations, semi-programmed ovarian stimulation can be indicated as a first-choice method for patients undergoing IVF, even though this information was obtained in the absence of a randomized control group.

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