Human Reproduction vol.14 no.3 pp.683–688, 1999

Revival of the natural cycles in in-vitro fertilization with the use of a new gonadotrophin-releasing hormone antagonist (Cetrorelix): a pilot study with minimal stimulation

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Natural cycles were abandoned in in-vitro fertilization (IVF) embryo transfer, due to premature luteinizing hormone (LH) surges–and subsequent high cancellation rates. In this study, we investigated the administration of a new gonadotrophin-releasing hormone antagonist (Cetrorelix) in the late follicular phase of natural cycles in patients undergoing IVF and intracytoplasmic sperm injection (ICSI). A total of 44 cycles from 33 healthy women [mean age 34.1 ± 1.4 (range 26–36) years] were monitored, starting on day 8 by daily ultrasound and measurement of serum concentrations of oestradiol, LH, follicle stimulating hormone (FSH) and progesterone. When plasma oestradiol concentrations reached 100–150 pg/ml, with a lead follicle between 12–14 mm diameter, a single injection (s.c.) of 0.5 mg (19 cycles) or 1 mg (25 cycles) Cetrorelix was administered. Human menopausal gonadotrophin (HMG; 150 IU) was administered daily at the time of the first injection of Cetrorelix, and repeated thereafter until human chorionic gonadotrophin (HCG) administration. Four out of 44 cycles were cancelled (9.0%). No decline in follicular growth or oestradiol secretion was observed after Cetrorelix administration. A total of 40 oocyte retrievals leading to 22 transfers (55%) was performed. In 10 cycles (25%), no oocyte was obtained. Fertilization failure despite ICSI occurred in six cycles (15%). In two patients the embryo was arrested at the 2 pronuclear (PN) stage. The stimulation was minimal (4.7 ± 1.4 HMG ampoules). A total of seven clinical pregnancies was obtained (32.0% per transfer, 17.5% per retrieval), of which five are ongoing. Thus, a spontaneous cycle and the GnRH antagonist Cetrorelix in single dose administration could represent a first-choice IVF treatment with none of the complications and risks of current controlled ovarian hyperstimulation protocols, and an acceptable success rate.

Key words: gonadotrophin-releasing hormone antagonist/in-vitro fertilization–embryo transfer/spontaneous cycle

Introduction

In-vitro fertilization (IVF) and embryo transfer is currently a widely used treatment in infertility. The number of countries and centres offering IVF–embryo transfer has increased significantly in recent years. The International Working Group for Registers on Assisted Reproduction collecting the IVF–embryo transfer cycles from national registries has recently reported over 220 000 cycles of IVF–embryo transfer for 1995, these data representing a 41% increase in such cycles compared with 1994 (de Mouzon and Lancaster, 1997).

Oocyte retrieval in spontaneous cycles during IVF has been replaced by stimulated cycles with clomiphene citrate and/or gonadotrophins to increase oocyte and embryo numbers as well as pregnancy rates (Fishel *et al.*, 1985). Mild ovarian stimulation with clomiphene citrate has been changed to controlled ovarian hyperstimulation (COH) with the combination of gonadotrophin-releasing hormone agonists (GnRH-a) and human menopausal gonadotrophins (HMG) which represent more than 80% of the IVF stimulation protocols (Assisted reproductive technology, 1996; FIVNAT, 1997).

GnRH-a induce a reversible medical hypophysectomy which prevents the occurrence of premature luteinizing hormone (LH) surges and allows the programming of treatment cycles such that the activities of large IVF centres can be organized. The protocols combining GnRH-a and gonadotrophins are producing a large number of oocytes and embryos, theoretically to increase the chance of pregnancy by allowing transfers of multiple embryos (Liu *et al.*, 1992). However, pregnancy rates reported in national or international reports have not increased in recent years and the mean delivery rate per cycle reported by the International Working Group for Registers on Assisted Reproduction for 1995 was 14.7% (similar to that observed in 1993), with major disparities between countries (de Mouzon and Lancaster, 1997). The number of embryos transferred is still high to compensate for the poor implantation rate per embryo, which remains almost unchanged (Edwards *et al.*, 1996). Meanwhile, with this type of intense ovarian stimulation protocol, patients are exposed to many complications.

Ovarian hyperstimulation syndrome (OHSS) is a well-recognized complication of COH which on rare occasions may be fatal (Cluroe and Synek, 1995). Thus, the morbidity due to OHSS and hospitalization of patients undergoing COH must be taken into account (Brinsden *et al.*, 1995). The present use of GnRH-a in the vast majority of IVF–embryo transfer cycles has increased the frequency of OHSS (Rizk and Smitz, 1992).

The occurrence of multiple pregnancies is probably the major complication of IVF (Tupin *et al.*, 1993). Perinatal complications of multiple pregnancies, including twins

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(Olivennes *et al.*, 1996a), increase the morbidity for the mother and fetus. Despite the progress in neonatal intensive care, prematurity and small birth weight associated with IVF predispose the newborn to mortality or handicaps (Tallo *et al.*, 1995). In the last report of the International Working Group for Registers on Assisted Reproduction, the overall mean rate of multiple pregnancies was 29.1%, reaching 35.3% in the USA. The overall incidence of premature IVF deliveries was 28%. In addition, 31.5% of the babies had a birth weight <2500 g (de Mouzon and Lancaster, 1997).

These severe treatment regimens—because of their complications—increase the human and financial costs of IVF–embryo transfer and represent a clear public health burden in countries where infertility is included in national health coverage. It also prevents some patients from having access to these procedures, especially in countries where couples have to pay for treatment costs (Gissler *et al*., 1995; Goldfarb *et al.*, 1996; Collins *et al.*, 1995). Finally, more serious complications might be related to ovarian stimulation, as indicated by the conflicting reports on the risk of ovarian or breast cancer (Whittemore *et al.*, 1992; Rossing *et al*., 1994; Venn *et al.*, 1995; Bristow and Karlan, 1996).

The physical, social and financial burden, as well as the complications and suspected (or unknown) risks for the couples, require the reappraisal of procedures designed to obtain oocytes for IVF (Edwards *et al.*, 1996). Improvements in the handling of gametes and embryos *in vitro*, together with high success rates of intracytoplasmic sperm injection (ICSI), could increase implantation rates and thus obviate the need to collect a maximum of oocytes per cycle. Ovarian stimulation protocols should be reduced to safe, cost-effective stimulation regimens with a view to producing one or two embryos to achieve an acceptable success rate.

Among the possible mild stimulation protocols, the natural cycle offers many advantages. Natural cycles have been proposed as an alternative to simplify IVF treatment procedures, to reduce its costs, and to avoid the risks of OHSS and multiple pregnancies (Foulot *et al*., 1989; Garcia, 1989; Paulson *et al*., 1990a, 1992; Aboulghar *et al*., 1995). However, spontaneous cycles are rarely used nowadays because of the alleged low pregnancy rates, the high cancellation rate (up to 30%) mainly related to frequent spontaneous LH surges which cannot be prevented by the use of GnRH-a (Lenton *et al.*, 1992; Claman *et al.*, 1993), and the difficulties in programming oocyte retrievals.

The GnRH antagonist Cetrorelix (ASTA-Medica, France) recently became available for clinical studies in humans; such newly developed compounds are devoid of the side effects described with earlier versions of these drugs (Karten *et al*., 1990). We have recently developed a single-dose protocol in patients undergoing IVF with COH (derived from previous work with Nal-Glu; Frydman *et al.*, 1991, 1992), in which Cetrorelix is administered in the late follicular phase at a time when LH surges are most feared. This protocol has an efficacy similar to that for GnRH-a, but is more flexible and allows a significant reduction in the dose of gonadotrophins (Olivennes *et al*., 1994, 1995, 1998).

The simplicity and efficacy of the single-dose antagonist protocol led us to propose a new alternative to ovarian stimulation in IVF, which combines the possible prevention of LH surges by GnRH antagonists and the simplicity of the natural cycle. We therefore investigated the administration of a new GnRH antagonist (Cetrorelix) in the late follicular phase of a natural cycle in patients undergoing IVF and ICSI.

Materials and methods

Ethics

This study protocol was reviewed and approved by the ethical committee of the University of Paris-Sud, France. Patients were included after having signed appropriate informed consents.

Population characteristics

This study was carried out in couples with severe male factor infertility in which ICSI was indicated. This group was selected since these women are often fertile and the implantation rate is expected to be higher than in classical IVF. Successful results have been published with the use of ICSI in the natural cycle (Norman *et al*., 1995). The severe stimulation protocols routinely used in IVF–embryo transfer are often feared by these women, who are not the cause of the infertility (Perez *et al*., 1998).

Severe male factor indicating the ICSI procedures were defined as patients presenting <250 000 motile spermatozoa after sperm preparation, and without curable pathology.

The study population included 33 healthy women of mean (± SD) age 34.1 ± 1.4 (range 26–36) years with normal menstrual cycles, day 3 follicle stimulating hormone (FSH) <8 IU/l, day 3 oestradiol <50 pg/ml and having undergone fewer than three previous IVF procedures. A total of 44 cycles was performed.

Before admission to the study, the blood pressure and pulse rate were measured and a gynaecological examination was performed in each patient. An ultrasound examination was also carried out on day 3 of the studied cycle to rule out any abnormalities.

Cycle monitoring was started on day 8 by daily pelvic ultrasounds. Plasma concentrations of oestradiol, LH, FSH and progesterone were assessed daily until Cetrorelix administration, and then twice per day until human chorionic gonadotrophin (HCG) administration.

When plasma oestradiol concentrations reached 100–150 pg/ml, and a lead follicle of 12–14 mm diameter was obtained, a single subcutaneous injection of 1 mg ($n = 25$) or 0.5 mg ($n = 19$) Cetrorelix was administered in order to assess the minimal effective dose.

Since studies with Nal-Glu (Kettel *et al.*, 1991) and Cetrorelix (Leroy *et al*., 1995) have shown that oestradiol secretion can be altered following GnRH antagonist administration in non-stimulated women, daily administration of 150 IU of HMG was performed at the time of the first injection of Cetrorelix until HCG administration. Triggering of ovulation was achieved by intramuscular administration of 5000 IU of HCG (Gonadotrophin Chorionique 'Endo', Organon, St Denis, France), and instigated when the lead follicle diameter reached 16–20 mm and the plasma oestradiol concentration was >200 pg/ml.

Oocyte retrieval was performed 36–40 h later without anaesthesia (Ramsewak *et al.*, 1990). Follicles were aspirated and flushed with a syringe under vaginal ultrasound guidance as an outpatient procedure. ICSI was performed according to the technique previously described (Olivennes *et al*., 1996b).

Embryo transfers took place 48 h after oocyte collection using a Frydman catheter (CDD Laboratories, Paris France). The luteal phase was supported by daily vaginal administration of 300 mg micronized progesterone (Utrogestan, Besins Iscovesco Pharmaceuticals, Paris,

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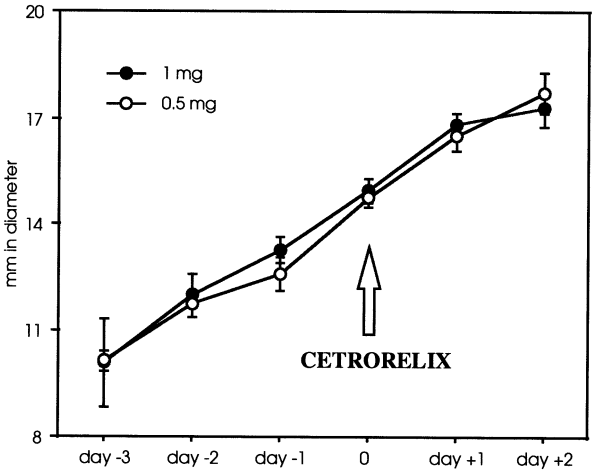

Figure 1. Follicular diameter in the two Cetrorelix dose groups [0.5 mg (n = 19) and 1 mg (n = 25)].

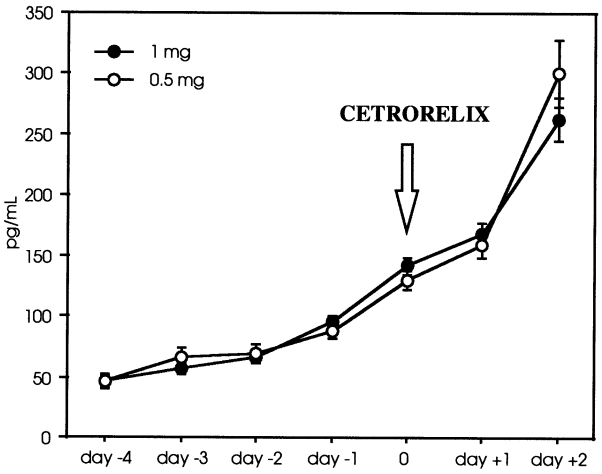

Figure 2. Plasma oestradiol concentrations in the two Cetrorelix dose groups [0.5 mg (n = 19) and 1 mg (n = 25)].

France), beginning on the day of the transfer. This luteal support was systematically proposed as no data are yet available on the necessity of luteal support in GnRH antagonist cycles. Thus, no risks were taken in this series of patients.

Hormone assays

Plasma oestradiol, progesterone, LH and FSH concentrations were determined by automated and direct chemoluminescent methods (ACS:180; Chiron Diagnostics Corp., USA). Sensitivity (minimum detectable concentration) was: 10 pg/ml (conversion factor to SI units, 3.671) for oestradiol; 0.1 ng/ml (conversion factor 3.180) for progesterone; 0.1 mIU/ml (conversion factor 1.00) for LH; and 0.3 mIU/ml (conversion factor 1.00) for FSH. Intra- and inter-assay coefficients of variation over the concentration range were <7% for oestradiol, <10% for progesterone and <5% for both LH and FSH.

Results

A total of 44 cycles was studied in 33 patients. Four cycles were cancelled (9.0%); in one case, the patient omitted to perform a second antagonist injection as normally scheduled 3 days after the first one, and an early LH surge was detected 72 h after the administration of 0.5 mg Cetrorelix. In the other three cases no visible follicle was observed at the time of oocyte retrieval, although LH surges were not detected.

Follicular growth in the two patient groups was not affected by the antagonist administration (Figure 1).

The secretion of oestradiol in patients receiving either 1 mg or 0.5 mg Cetrorelix is shown in Figure 2, there being no decline in oestradiol secretion following antagonist administration.

IVF results are presented in Table I. A total of 40 oocyte retrievals leading to 22 transfers (55%) was performed. In 10 cycles (25%), no oocyte was obtained. Fertilization failure despite ICSI occurred in six cycles in which one oocyte was collected (15% of cycles). The fertilization rate was 80.0%. In two patients, the transfer was not performed because of a developmental arrest of the embryo at the 2PN stage. A total of seven clinical pregnancies was obtained (32% per transfer, 17.5% per retrieval), of which five are ongoing (22.7% per transfer). These pregnancy rates are preliminary since they were calculated on only 22 transfers. Further studies and a greater number of patients are required to assess the real pregnancy rates after ICSI in the natural cycle.

Table I. Results of in-vitro fertilization in natural cycles with a single administration of Cetrorelix (1 mg or 0.5 mg)

Cycles	44
Retrieval	40
Fertilization rate	24/30 (80)
Transfer	22
Clinical pregnancy per transfer	7/22 (32)
Clinical pregnancy per retrieval	7/40 (17.5)

Values in parentheses are percentages.

The mean number of HMG ampoules used was 4.7 ± 1.4, and the mean period between Cetrorelix and HCG administration 2.0 ± 0.7 days.

Discussion

The use of GnRH antagonists in unstimulated cycles has been previously reported in five oocyte donors, the GnRH antagonist (Nal-Glu) having been administered in multiple doses for 4.0 ± 0.3 days with a mean HMG dose of 11.0 ± 0.6 ampoules (Meldrum *et al.*, 1994).

This study reports for the first time the use of a single-dose GnRH antagonist (Cetrorelix) in spontaneous cycles in IVF–embryo transfer. The use of Cetrorelix has been reported previously in IVF with controlled ovarian stimulation in protocols where the antagonist was administered either as small multiple doses commencing on day 6 of the stimulation until the day of HCG (Diedrich *et al.*, 1994; Felderbaum *et al.*, 1996; Albano *et al.*, 1997), or as a single dose administered in the late follicular phase (Olivennes *et al.*, 1994, 1995, 1998). The simplicity of the single-dose protocol and the possibility of a late follicular phase administration allowed us to adapt this protocol for the natural cycle.

In healthy volunteers, Leroy *et al.* (1995) studied the administration of Cetrorelix in natural cycles, and showed that antagonist administration did not produce atresia of the dominant follicle, but caused a transient decline in gonadotrophin and oestradiol concentrations. This effect was also seen

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in some cases of our first study carried out in IVF patients treated with controlled ovarian stimulation (Olivennes *et al*., 1994). The transient decline in oestradiol concentrations was not observed when the amount of HMG administered was increased on the day of the Cetrorelix injection (Olivennes *et al*., 1995). Thus, we decided in this study to sustain oestradiol secretion by administering HMG, starting on the day of Cetrorelix administration, until HCG application. No changes in either plasma oestradiol concentrations or follicular growth were thus observed after administration of the antagonist in the patients treated in this study.

The number of patients without transfers was surprising. In eight cycles (22%), no oocytes were obtained despite flushing of the follicle, compared with a rate of 10% reported elsewhere (Lenton and Woodward, 1993). Recent data obtained from patients with 'empty follicle syndrome' have suggested that a problem in HCG administration could be at the origin of the failed retrieval (Ndukwe *et al*., 1997). The repetition of HCG administration was also proposed by Ubaldi *et al.* (1997) and should be studied if this observation is to be confirmed in a larger number of patients.

In six patients, no embryo was obtained despite the fact that ICSI was performed on a mature (metaphase II) oocyte. This fertilization rate (24/30; 80%) is comparable with that reported in large studies (Nagy *et al.*, 1995; Palermo *et al*., 1996a). However, in spontaneous cycles a fertilization failure is very disappointing for the patient, as no transfer can occur. Nevertheless, IVF–embryo transfer with a natural cycle is a very soft protocol with very few injections, no side effects, and no anaesthesia or hospital stay—a regimen appreciated by the woman, especially when no female factors are involved in the couple's infertility. Consequently, the cancellation rate is less dramatic, and IVF or ICSI can theoretically be tried again in the next cycle. Moreover, the inclusion of other infertility factors for which treatment does not involve ICSI might reduce the rate of oocyte damage and could improve the fertilization rate.

The number of patients in whom the cycle had been cancelled was very low (9.0%) compared with previous reports on natural cycles, and underlines a clear benefit of the antagonist administration.

In IVF–embryo transfer, the vast majority of patients undergo COH which increases the number of oocytes and embryos (Balen, 1995). However, COH is associated with various side effects (Frydman *et al.*, 1988), a risk of OHSS and a high rate of multiple pregnancies (Edwards *et al*., 1996); ovarian stimulation could also have an adverse effect on endometrial receptivity (Paulson *et al.*, 1990b). The financial cost of IVF–embryo transfer treatment is also very high (Collins *et al*., 1995), while the physical, emotional, financial and social burdens of infertile couples are high, with up to 30% of IVF patients stopping all forms of medical treatment after the first IVF failure. The development of new treatment regimens is clearly necessary, possibly by a simplification of the stimulation protocols and hence a reduction in the multiple pregnancy rate (Edwards *et al.*, 1996; Olivennes and Frydman, 1998).

The spontaneous cycle would be of course the 'gold standard' of a simple and safe IVF–embryo transfer cycle. Indeed, that is how IVF–embryo transfer began in the 1970s, though better results were obtained with ovarian stimulation and the natural cycle was abandoned. Some teams regularly report their experience with the natural cycle (Foulot *et al*., 1989; Garcia *et al*., 1989; Paulson *et al*., 1990a, 1992; Lenton *et al.*, 1992; Aboulghar *et al*., 1995). The absence of GnRH-a leads to high cycle cancellation rates due to premature LH surges. Moreover, programming of the oocyte retrievals is impossible, and spontaneous cycles cannot be used easily in large IVF–embryo transfer units.

In these preliminary results, the administration of the GnRH antagonist Cetrorelix allowed us almost to suppress premature LH surges, and oocyte retrieval could be easily programmed. The pregnancy rate (17.5% clinical pregnancy rate per retrieval; 32% per transfer) needs to be confirmed in larger series, but appears interesting in these selected cases. The clear benefit of the GnRH antagonist should be demonstrated in a study including a control group without antagonist. However, it is not easy to obtain controls for a natural IVF cycle. Progress in gamete handling and embryo culture has improved implantation rates, and some teams have presented results with an implantation rate of >25% per embryo (Palermo *et al*., 1996b; Schoolcraft *et al.*, 1996). In the present group of patients, we obtained an implantation rate of 32%. This could represent a clear choice to repeat two or three of these spontaneous cycles with minimal gonadotrophin administration, since cumulative pregnancy rates might reach those of COH protocols, but without their potential adverse effects. Daya *et al.* (1995) claimed that even with a live birth rate of 3.8%, natural cycles were more cost-effective than stimulated IVF. It has already been shown that, in a total of 280 patients with tubal infertility, natural cycles gave a lower delivery rate per egg collection but a comparable delivery rate per transfer as compared with stimulated cycles (Lenton *et al.*, 1992). Monitoring of the cycle could start on day 7 or 8, with oocyte retrieval performed on an outpatient basis without anaesthesia. The amount of gonadotrophins administered was very low (4.7 ± 1.4 ampoules). The recent introduction of recombinant FSH is unlikely to modify the endocrine parameters observed with HMG, since endogenous LH remains detectable following the injection of the antagonist, allowing the two cells–two gonadotrophins machinery to be operational. The burden for the couples is therefore greatly reduced, as well as the costs of the procedure. The multiple pregnancy rate is reduced virtually to zero, which represents a major benefit of the method considering the potential adverse outcome of multiple pregnancies (Olivennes *et al.*, 1996a). Of course, patients who fail to conceive after a few cycles could be directed to more severe procedures as a second choice. It is also clear that natural cycles will not be possible for all the patients included in IVF–embryo transfer programmes, and selection of patients who could benefit from this regimen will be mandatory (Lenton *et al.*, 1992). For example, patients with dysovulation or polycystic ovary syndrome would probably not benefit from these treatment schemes, despite their representing a minority of IVF patients as tubal and male factors account for the majority of assisted reproductive therapy indications (FIVNAT,

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1997). For these patients, new conservative stimulation protocols could be proposed (Edwards *et al.*, 1996).

In conclusion, the use of the GnRH antagonist Cetrorelix in natural cycles allowed us to reduce the rate of premature LH surges and therefore the cancellation rate. The stimulation was minimal, and pregnancy rates in this preliminary report were satisfactory. If a larger study should confirm these results, the association of spontaneous cycles and GnRH antagonists in a single-dose administration regimen could represent a very interesting first-choice IVF treatment scheme in selected indications. This treatment regimen offers an acceptable success rate, a reduced incidence of the complications and risks associated with COH protocols, a very small dose of gonadotrophins, a reduction in the length of the treatment period (compared with long protocols), the possibility of successive cycles, and a reduction in the costs of the procedure.

Acknowledgements

We would like to thank Drs M.Marzetto and N.Froelich (ASTA-Medica, France), in addition to ASTA MEDICA-AG (Germany) for help and support for this study.

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Received on April 29, 1998; accepted on November 17, 1998

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