Gynecological Endocrinology, May 2006; 22(5): 235–238

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ASSISTED REPRODUCTIVE TECHNOLOGY

Gonadotropin-releasing hormone antagonist and metformin for treatment of polycystic ovary syndrome patients undergoing *in vitro* fertilization–embryo transfer

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Abstract

Aim. The combination of gonadotropin-releasing hormone (GnRH) antagonist and gonadotropin represents a valid alternative to the classical protocol with GnRH agonist for ovulation induction in patients with polycystic ovary syndrome (PCOS). The use of metformin is of benefit to women with PCOS. The aim of the present study was to compare the stimulation characteristics and *in vitro* fertilization (IVF)–embryo transfer (ET) outcomes of the standard short GnRH antagonist protocol for ovarian stimulation with or without metformin.

Materials and methods. We recruited 40 PCOS patients. The population studied was divided into two groups (A and B). Group A was pretreated for 2 months with metformin 1.5 g/day (Glucophage®; Merck Pharm), and then stimulated with recombinant follicle-stimulating hormone (rFSH) 150 UI/day (Gonal F® 75 UI; Serono). GnRH antagonist, cetrorelix acetate 0.25 mg/day (Cetrotide®; Serono), was started when the leading follicle reached 14 mm diameter on ultrasound scan. Group B was treated only with rFSH 150 UI/day and GnRH antagonist 0.25 mg/day when the leading follicle was $\geq$14 mm in diameter.

Results. In group A we found a statistically significant ($p < 0.05$) decrease in the number of ampoules of rFSH (A vs. B: 18 ± 6 vs. 24 ± 8) and estradiol levels (A vs. B: 2400 ± 600 vs. 3370 ± 900 pg/ml) (all values mean $\pm$ standard deviation). Group A had significantly fewer cancelled cycles (A vs. B: 1 vs. 3; $p < 0.05$). The incidence of ovarian hyperstimulation syndrome was 5% in group A and 15% in group B ($p < 0.05$). In patients treated with metformin, the total number of follicles on the day of human chorionic gonadotropin treatment (23 ± 1.2 vs. 33 ± 2.6) was decreased with no change in the number of follicles $\geq$14 mm in diameter (A vs. B: 18 ± 1.2 vs. 19 ± 1.7). However, the mean number of mature oocytes (A vs. B: 8.4 ± 1.5 vs. 5.0 ± 1.5) was increased with metformin treatment ($p < 0.05$). No difference was found in the number of cleaved embryos (A vs. B: 2.5 ± 0.5 vs. 2.2 ± 0.3).

Conclusions. The use of metformin with GnRH antagonist improves the outcome of ovarian stimulation in IVF-ET cycles in PCOS patients.

Keywords: *Polycystic ovary syndrome, in vitro fertilization, gonadotropin-releasing hormone antagonist, metformin*

Introduction

Polycystic ovary syndrome (PCOS) is the most common cause of infertility due to anovulation [1,2]. It is a very heterogeneous syndrome in both its clinical presentation and laboratory manifestations. The majority of PCOS women with anovulation have menstrual irregularities, usually oligomenorrhea or amenorrhea, associated with clinical and/or biochemical evidence of hyperandrogenism. Restoration of ovulation might be achieved by ovarian stimulation or by reducing insulin and luteinizing hormone (LH) concentrations.

Clomiphene citrate is the first-line ovulation-inducing agent, usually followed by direct stimulation with follicle-stimulating hormone (FSH) if unsuccessful. The prevalent complications of ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies can largely be avoided by administering FSH in a low dose and individualized regimen.

Hyperinsulinemia can be corrected by weight loss or insulin-sensitizing agents, such as metformin, which alone or in combination with other agents are capable of restoring ovulation. Metformin is an oral biguanide, well established for the treatment of diabetes as it is an antihyperglycemic which inhibits hepatic glucose production and increases the number of insulin receptors. Insulin concentrations are therefore decreased as a secondary phenomenon,

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ISSN 0951-3590 print/ISSN 1473-0766 online © 2006 Taylor & Francis
DOI: 10.1080/14767050600761893

with a resulting decrease in androgen and LH concentrations and an increase in sex hormone-binding globulin. Metformin may also have a direct action on theca cells, reducing androgen production. Most studies have demonstrated that metformin administered at a dose of 500 mg three times daily (1.5 g/day) increases menstrual cyclicity, improves spontaneous ovulation and promotes fertility [3–10].

In patients undergoing assisted reproductive technologies, gonadotropin-releasing hormone (GnRH) antagonists inhibit gonadotropin release within several hours through binding competitively to pituitary GnRH receptors, preventing or successfully interrupting the LH surge without the flare-up effect found with GnRH agonists [11–13]. The aim of the present study was to compare the stimulation characteristics and *in vitro* fertilization (IVF)–embryo transfer (ET) outcomes of PCOS women, using the standard short GnRH antagonist protocol for ovarian stimulation with or without metformin.

Materials and methods

Patients

We recruited 40 PCOS patients in our IVF unit. All patients signed in an informed consent form. The diagnosis of PCOS included at least two of the following criteria [14]: chronic anovulation manifested by the symptoms of oligomenorrhea (>40 days per cycle), amenorrhea or irregular menstrual cycles; clinical or biochemical (serum testosterone concentration >0.8 ng/ml) signs of hyperandrogenism; and ultrasonographic evidence of polycystic ovaries.

Each patient was screened with an oral glucose tolerance test (75 g glucose load) given the high prevalence of impaired glucose tolerance and type 2 diabetes in PCOS patients. No test of insulin resistance was performed because it is not necessary to make a diagnosis of PCOS or to select treatments [14]. Congenital adrenal hyperplasia, Cushing's syndrome, androgen-producing tumor, hyperprolactinemia and thyroid dysfunction were all excluded. Exclusion criteria included patient age older than 40 years, serum FSH level >12 mIU/ml and the presence of other pathology. The patients did not take any ovulation drugs or hormones for at least 3 months prior to the trial.

Treatments

The population was randomly divided into two groups (A and B). Group A was pretreated for 2 two months with metformin 1.5 g/day (Glucophage®; Merck Pharm, Milan, Italy), and then stimulated with recombinant FSH (rFSH) 150 UI/day (Gonal F® 75 UI; Serono, Rome, Italy) from day 3 of menstrual cycle. The menstrual cycle was spontaneous or obtained by a progestative. The dose of gonadotropin was adjusted according to the serum estradiol (E_2) levels and follicular response. GnRH antagonist, cetrorelix acetate 0.25 mg/day (Cetrotide®; Serono), was started when the leading follicle reached 14 mm diameter on ultrasound scan and stopped on the day of administration of human chorionic gonadotropin (hCG). Metformin treatment was stopped on the ET day.

Group B was treated only with rFSH 150 UI/day from day 3 of the menstrual cycle (spontaneous or obtained with a progestogen). GnRH antagonist 0.25 mg/day was started when the leading follicle reached 14 mm diameter on ultrasound scan and stopped on the day of hCG administration.

When the diameter of two or three follicles reached at least 16 mm on ultrasound scan, recombinant hCG (Ovitrelle®; Serono, Rome, Italy) was administered in both groups. Oocyte retrieval was performed approximately 36 h after hCG administration using transvaginal ultrasound guidance. In accordance with Italian law, we fertilized no more than three oocytes. ET was performed 48 h after oocyte retrieval. No embryos were cryopreserved or discarded. Progesterone (Crinone 8®; Serono, Rome, Italy) 90 mg was given for luteal support starting on the day of oocyte retrieval and was continued until menstruation or a positive pregnancy test.

Measurement of hormones in serum

E_2 concentration on the day of hCG administration was measured using a competitive enzyme assay, AIA – PACK (Tosoh Bioscience, Inc., S. Francisco, CA, USA).

Statistical analysis

The number and quality of oocytes, fertilization rate, number of embryos and cases of OHSS were analyzed and compared in the control and metformin groups. All data are expressed as mean $\pm$ standard deviation. Data were analyzed by the Student t test. A value of $p < 0.05$ was considered statistically significant. Data were tabulated and analysed using Instat 3 (GraphPad software, San Diego, CA, USA).

Results

In group A we found a statistically significant decrease in the number of ampoules of rFSH (A vs. B: 18 ± 6 vs. 24 ± 8) and estradiol levels (A vs. B: 2400 ± 600 vs. 3370 ± 900 pg/ml). No difference was

found in the duration of stimulation (A vs. B: 9.9 ± 2.1 vs. 9.8 ± 1.9 days) or in the number of follicles ≥14 mm in diameter (A vs. B: 18 ± 1.2 vs. 19 ± 1.7) (Table I).

Table II shows the outcomes of stimulation. We did not find any statistically significant difference in the number of oocytes retrieved (A vs. B: 13 ± 4.4 vs. 14 ± 5.1). Metformin treatment was associated with a statistically significant increase in the number of mature oocytes as defined by the presence of a polar body (A vs. B: 8.4 ± 1.5 vs. 5.0 ± 1.5). No difference was found in the number of grade A embryos (A vs. B: 2.5 ± 0.5 vs. 2.2 ± 0.3). Group A had fewer cancelled cycles (A vs. B: 1 vs. 3) and a lower incidence of OHSS (A vs. B: 5 vs. 15%); these data were statistically significant.

Table I. Comparison of ovulation induction in the two groups of patients.

	Group A	Group B	*p* Value (A vs. B)
n	20	20	
Duration of stimulation (days)	9.9 ± 2.1	9.8 ± 1.9	NS
No. of rFSH ampoules	18 ± 6	24 ± 8	<0.05*
Serum E_2 on hCG day (pg/ml)	2400 ± 600	3370 ± 900	<0.05*
No. of follicles ≥14 mm diameter	18 ± 1.2	19 ± 1.7	NS

Group A, standard short gonadotropin-releasing hormone (GnRH) antagonist protocol for ovarian stimulation with metformin pretreatment; Group B, standard short GnRH antagonist protocol for ovarian stimulation without metformin pretreatment; rFSH, recombinant follicle-stimulating hormone; E_2, estradiol; hCG, human chorionic gondadotropin; NS, not significant; data are expressed as mean ± standard deviation; *statistically significant difference.

Table II. Comparison of *in vitro* fertilization–embryo transfer outcomes in the two groups of patients.

	Group A	Group B	*p* Value (A vs. B)
No. of oocytes retrieved	13 ± 4.4	14 ± 5.1	NS
No. of mature oocytes	8.4 ± 1.5	5.0 ± 1.5	<0.05*
No. of grade A embryos	2.5 ± 0.5	2.2 ± 0.3	NS
No. of cancelled cycles	1 (5)	3 (15)	<0.05*
OHSS incidence	1 (5)	2 (15)	<0.05*

Group A, standard short gonadotropin-releasing hormone (GnRH) antagonist protocol for ovarian stimulation with metformin pretreatment; Group B, standard short GnRH antagonist protocol for ovarian stimulation without metformin pretreatment; OHSS, ovarian hyperstimulation syndrome; NS, not significant; data are expressed as mean ± standard deviation or *n* (%); *statistically significant difference.

Discussion

The GnRH antagonists have some advantages in the treatment of PCOS. Antagonists act by the mechanism of competitive binding, and this allows a modulation of the degree of hormonal suppression by adjustment of the dose. Furthermore, antagonists suppress gonadotropin release within a few hours, have no flare-up effect and gonad function resumes without a lag effect following their discontinuation [15]. The use of an antagonist prevents premature luteinization and protects the oocyte from the deleterious effects of high LH concentrations. Compared with agonist-treated cycles, the use of an antagonist gives the advantages of more conceptions and fewer miscarriages, reduces the amount of gonadotropin required for ovarian stimulation, and decreases the prevalence of OHSS. Only a few trials employing a GnRH antagonist with rFSH, specifically for women with PCOS, have been published to date [16].

It is widely accepted that PCOS is a condition of relative insulin resistance. This gives rise to chronic hyperinsulinemia, which results in abnormal ovarian androgen metabolism and altered gonadotropin responses [17].

Some authors suggest that metformin should one of the first lines of therapy for ovulation induction in PCOS patients [18]. It appears to be effective not only in inducing ovulation, but also may increase the number of pregnancies, reduce the prevalence of miscarriage and give a higher live birth rate. This may be due to metformin's reduction of elevated levels of insulin-like growth factor-I and hyperinsulinemia in PCOS patients [19]. Metformin is free from side-effects and requires minimal monitoring.

Metformin therapy offers additional beneficial effects on ovarian function. The reduction of hyperandrogenemia and insulin resistance in PCOS woman facilitates FSH stimulation of ovulation. This was confirmed in the present study by the reduced number of rFSH ampoules and the shorter duration of stimulation. We also found that the use of metformin with a GnRH antagonist improved the outcome of ovarian stimulation in IVF-ET cycles, reducing gonadotropin dose, serum E_2 on hCG day, the incidence of OHSS and the number of cancelled cycles. Furthermore, it was associated with an increased mean number of mature oocytes. However, more data are needed on pregnancy rate and potentially adverse effects on endometrium or implantation.

References

1. Adams J, Polson DW, Franks S. Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. Br Med J 1986;293:355–359.

2. Hull MG. Epidemiology of infertility and polycystic ovarian disease: endocrinological and demographic studies Gynecol Endocrinol 1987;1:235–245.
3. Velazquez E, Acosta A, Mendoza SG. Menstrual cyclicity after metformin therapy in polycystic ovary syndrome. Obstet Gynecol 1997;90:392–395.
4. Vandermolen DT, Ratts VS, Evans WS, Stovall DW, Kauma SW, Nestler JE. Metformin increases the ovulatory rate and pregnancy rate from clomiphene citrate in patients with polycystic ovary syndrome who are resistant to clomiphene citrate alone. Fertil Steril 2001;75:310–315.
5. Morin-Papunen LC, Koivunen RM, Ruokonen A, Martikainen HK. Metformin therapy improves the menstrual pattern with minimal endocrine and metabolic effects in women with polycystic ovary syndrome. Fertil Steril 1998;69: 691–696.
6. Nestler JE, Jakubowicz DJ, Evans WS, Pasquali R. Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome. N Engl J Med 1998;338: 1876–1880.
7. Ibanez L, Valls C, Potau N, Marcos MV, de Zegher F, Sensitization to insulin in adolescent girls to normalize hirsutism, hyperandrogenism, oligomenorrhea, dyslipidemia, and hyperinsulinism after precocious pubarche. J Clin Endocrinol Metab 2000;85:3526–3530.
8. Kolodziejczyk B, Duleba AJ, Spaczynski RZ, Pawelczyk L. Metformin therapy decreases hyperandrogenism and hyperinsulinemia in women with polycystic ovary syndrome. Fertil Steril 2000;73:1149–1154.
9. Pasquali R, Gambineri A, Biscotti D, Vicennati V, Gagliardi L, Colitta D. Effect of long-term treatment with metformin added to hypocaloric diet on body composition, fat distribution, and androgen and insulin levels in abdominally obese women with and without the polycystic ovary syndrome. J Clin Endocrinol Metab 2000;85:2767–2774.
10. Seale FG, Robinson RD, Neal GS. Association of metformin and pregnancy in the polycystic ovary syndrome. A report of three cases. J Reprod Med 2000;45:507–510.
11. Albano C, Smitz J, Camus M, Riethmuller-Winzen H, Van Steirteghem A, Devroey P. Comparison of different doses of gonadotropin-releasing hormone antagonist cetrorelix during controlled ovarian hyperstimulation. Fertil Steril 1997;67:917–922.
12. Olivennes F, Belaisch-Allart J, Emperaire JC, Dechaud H, Alvarez S, Moredu L, Nicollet B, Zorn JR, Bouchard P, Frydman R. Prospective, randomized, controlled study of *in vitro* fertilization–embryo transfer with a single dose of a luteinizing hormone-releasing hormone (LH-RH) antagonist (cetrorelix) or a depot formula of an LH-RH agonist (triptorelin). Fertil Steril 2000;73:314–320.
13. Hwang JL, Huang LW, Hsieh BC, Tsai YL, Huang SC, Chen CY, Hsieh ML, Chen PH, Lin YH. Ovarian stimulation by clomiphene citrate and hMG in combination with cetrorelix acetate for ICSI cycles. Hum Reprod 2003;18: 45–49.
15. Homburg R. The management of infertility associated with polycystic ovary syndrome. Reprod Biol Endocrinol 2003; 1:109.
14. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19:41–47.
16. Elkind-Hirsch KE, Webster BW, Brown CP, Vernon MW. Concurrent ganirelix and follitropin-β therapy is an effective and safe regimen for ovulation induction in women with polycystic ovary syndrome. Fertil Steril 2003;79:603–607.
17. De Leo V, la Marca A, Petraglia F. Insulin-lowering agents in the management of polycystic ovary syndrome. Endocr Rev 2003;24:633–667.
18. Palomba S, Orio F Jr, Nardo LG, Falbo A, Russo T, Corea D, Doldo P, Lombardi G, Tolino A, Colao A, et al. Metformin administration versus laparoscopic ovarian diathermy in clomiphene citrate-resistant women with polycystic ovary syndrome: a prospective parallel randomized double-blind placebo-controlled trial. J Clin Endocrinol Metab 2004;89: 4801–4809.
19. Berker B, Emral R, Demirel C, Corapcioglu D, Unlu C, Kose K. Increased insulin-like growth factor-I levels in women with polycystic ovary syndrome, and beneficial effects of metformin therapy. Gynecol Endocrinol 2004;19:125–133.