Early initiation of gonadotropin-releasing hormone antagonist in polycystic ovarian syndrome patients undergoing assisted reproduction: randomized controlled trial ISRCTN69937179

Mostafa I. Abuzeid · Mohamed Mitwally ·
Yasmine M. Abuzeid · Hammad A. Bokhari ·
Mohammad Ashraf · Michael P. Diamond

Received: 15 June 2012 / Accepted: 8 August 2012 / Published online: 8 September 2012
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Abstract

Purpose To compare the implantation rates in two groups of women with Polycystic Ovary Syndrome (PCOS) after embryo transfer based on the initiation time of GnRH antagonist. Secondary outcome measures included clinical pregnancy, delivery and miscarriage rates.

Methods This is a prospective, randomized trial in which 140 PCOS patients underwent ICSI, with 122 having ET performed. GnRH-antagonist was started on day 1 of stimulation in 69 patients (Group 1) or day 5 in 71 patients (Group 2).

Capsule In patients with polycystic ovary syndrome undergoing ICSI-ET, early initiation of GnRH antagonist on day 1 of ovarian stimulation may improve implantation rates, especially after blastocyst transfer.

M. I. Abuzeid (✉) · M. Ashraf
Center for Reproductive Medicine, Reproductive Endocrinology
and Infertility, Department of OB/GYN, Hurley Medical Center,
Flint, MI 48503, USA
e-mail: reprod1@hurleymc.com

M. I. Abuzeid · Y. M. Abuzeid · H. A. Bokhari · M. Ashraf
IVF Michigan PC,
3050 S. Rochester Road, Suite 2300,
Rochester Hills, MI 48307, USA

M. I. Abuzeid · M. Ashraf
Obstetrics and Gynecology and Reproductive Biology, Michigan
State University, College of Human Medicine,
Flint Campus, MI 48823, USA

M. Mitwally
Odessa Reproductive Medicine Center,
Odessa, TX, USA

M. P. Diamond
Kamran S. Moghissi, Obstetrics and Gynecology, Reproductive
Endocrinology & Infertility, Associate Dean for Clinical
and Translational Research, School of Medicine, Executive
Director for Clinical and Translational Research,
Wayne State University,
Detroit, MI, USA

Results The overall implantation rate in Group 1 (46.2 %) was clinically higher than Group 2 (35.5 %), although not statistically significant ($p=0.075$). For blastocysts transfer, the implantation rate in Group 1 was 55.1 %, compared to 40.4 % in Group 2 ($p=0.051$). There was a clinically, but not statistically, higher clinical pregnancy rate (68.3 % vs. 56.5 %) and delivery rate (60.0 % vs. 53.2 %) per transfer in Group 1 compared to Group 2, respectively. There was a statistically significant lower biochemical pregnancy rate in Group 1 (2.4 %) compared to Group 2 (18.6 %) [$p=0.015$]. There was no difference in miscarriage rates between the two groups. **Conclusion** Our data suggest that early initiation of GnRH antagonist on day 1 of ovarian stimulation in PCOS patients undergoing ICSI-ET may improve implantation rates, especially after blastocyst transfer.

Keywords Early initiation of GnRH antagonist · PCOS · ART

Introduction

Gonadotropin-releasing hormone antagonists (GnRH-ant.) were introduced in the late nineties and have since provided infertility specialists with an alternative approach during controlled ovarian stimulation (COS). Although initial reports suggested lower clinical pregnancy rates when compared to GnRH agonist, more recent large meta-analysis reported no significant difference in the odds for live birth [1, 2]. Despite the later findings, the use of GnRH-agonists (GnRH-a) continues to be more wide spread compared to the antagonists.

One particular clinical condition in which the use of antagonists has been reported to be beneficial is in patients with polycystic ovarian syndrome (PCOS). In such patients the use of antagonists was associated with reduction in the incidence of severe ovarian hyperstimulation syndrome (OHSS) compared to the agonists [3]. Although one

retrospective study reported a significantly lower pregnancy rate with GnRH ant. compared to GnRH-a protocol in PCOS patients who underwent IVF/ET, several prospective randomized studies reported no difference in pregnancy, clinical pregnancy or delivery rates between the two protocols [4–8].

There are two protocols for time of initiation of GnRH-ant.: the flexible protocol (when follicles are >14 mm) and the fixed protocol (treatment day 5 or 6). Two studies compared early initiation of GnRH- ant. in the follicular phase to the long agonists during ovarian stimulation of PCOS patients, and both reported similar reproductive outcome [9, 10]. The purpose of this pilot study was to test the hypothesis that in women with PCOS, early initiation of GnRH-ant. (day 1 of ovarian stimulation) is associated with higher implantation rate compared to conventional GnRH-ant. protocol (starting on day 5 of ovarian stimulation). Secondary outcome measurements included clinical pregnancy, delivery, and miscarriage rates.

Materials and methods

Two hundred twenty five infertile patients with PCOS were found to be eligible for this study, and all met the inclusion criteria. Eighty-five patients declined to participate in the study primarily due to financial constrains. One hundred forty women (age between 18 and 40 years) with PCOS, undergoing assisted reproductive technology (ART) participated in a physician-blinded, prospective, randomized, parallel-group trial. Informed consent was obtained for experimentation with human subjects from all participants. This study was approved by the Institutional Review Board at Hurley Medical Center, Flint, Michigan. Between January 2006 and December 2008, participants were recruited among patients evaluated for infertility at our clinics in Flint and Rochester Hills, MI. Interested patients were enrolled in the study by the physician (principal investigator). PCOS was defined according to Rotterdam criteria: At least 2 of the following 3 features: Oligo and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovary on ultrasound scan [11]. Patients with hypothyroidism, hyperprolactinemia, nonclassical form of congenital adrenal hyperplasia, Cushing's syndrome and androgen-secreting tumors were excluded. Infertility work up included complete semen analysis, hysterosalpingogram, transvaginal ultrasound (US) scan (2D and 3D), saline-sonohysterogram (2D and 3D), fasting glucose and insulin, and hormonal profile. Hormonal profile included serum day 3 Follicle stimulating hormone (FSH) and Luteinizing hormone (LH), prolactin, thyroid stimulating hormone, testosterone (T), dihydro-epiandrosterone sulfate (DHEA-S), and morning 17 OH progesterone (17-OHP). Exclusion criteria included: Age <18 or >40 years, BMI >40 kg/m², day 3 FSH >10 mIU/mL, anatomical abnormalities of the uterus,

and contraindication to treatment medications or procedures. All patients received oral contraceptive pills (OCP)[Desogen, Merck & Co., Inc., North Wales, PA 19454, USA] 21–35 days in the preceding cycle, Metformin hydrochloride (1,000–1,500 mg) (Glucophage, Bristol-Myers-Squibb, Princeton, NJ, 08543-4500, USA) starting 1 month before stimulation and continuing through treatment, and recombinant FSH (rFSH) for ovarian stimulation. Patients were randomly assigned to Group 1 or Group 2 based on a computer-generated list of random numbers, where even numbers represented one group and odd numbers represented the other group. Patients in Group 1 started GnRH-ant. (0.25 mg SC) on day 1 of ovarian stimulation (69 patients); patients in Group 2 started GnRH-ant. on day 5 of ovarian stimulation (71 patients). The subject group assignment was blinded from the all study staff (nurses, physicians) by placing the group assignment in sequentially numbered, sealed identical envelopes. The envelopes were prepared by a contracted research assistant who had no involvement with the recruitment, consent, assignment, or treatment of the subjects. The information inside the envelope could not be seen even when held up to the light. After the informed consent document was signed, an envelope with subject assignment inside was opened by the nurse coordinator, thereby determining which group the subject was in. Thereafter, only physicians remained blinded to subject assignment through the study. This was achieved by not including start time of GnRH ant. in the clinical chart. This information was recorded in a separate sheet kept in a study folder that was only accessible by the nursing staff in charge of patient education. This information became available to the physicians only after the study was **conducted**. Patients were stimulated with rFSH (150–225 IU) starting on day 2 or day 3 of the cycle. Baseline serum estradiol (E2), progesterone (P4), FSH, and LH were determined and baseline US scan was performed on day 2 or day 3 prior to starting rFSH and at the time of each subsequent visit. The patients were seen on the sixth day of treatment and the timing and frequency of subsequent visits were determined depending on the patients' responses. The initial dose of rFSH was determined based on BMI. If BMI was <28 kg/m², 150 IU of rFSH was administered daily, while if BMI >28, a dose of 225 IU was administered daily. After 5 days the dose of rFSH was adjusted according to ovarian response as determined by serial US scans and measurements of serum E2 levels. In patients with E2 levels significantly high (>1,000 pg/ml) and the size of the follicles was small on treatment day 5, the cycle was cancelled to avoid severe OHSS. If there were a large number of mature follicles (≥16) with a large number of small follicles and E2 levels exceeded 3,000 pg/ml on treatment day 7–8 or afterwards, then coasting was used for 1–3 days, until E2 level was less than 3,000 pg/ml.

After coasting, or if there was a potential for severe OHSS for other reasons, a liter of hydroxyethyl starch solution (HSS) [B. Braun Medical, Inc. Bethlehem, PA 18018, USA] was given intravenously on retrieval day and for up to two additional days, if needed. These measures are commonly used to reduce the incidence of severe OHSS when indicated. When three follicles were ≥ 17 mm, 5,000–10,000 IU human chorionic gonadotropin (HCG) was administered 36 h before oocyte retrieval. The dose of HCG was reduced to 5,000 IU if risk of severe OHSS was high, as outlined above. Intracytoplasmic sperm injection (ICSI) was performed on all mature oocytes 3 to 4 h after retrieval. Embryos were graded on both day 2 based on blastomere nuclear scoring and morphologic appearance of day 3 cleavage embryos [12, 13]. On day 3, if six to eight embryos were of good quality, the transfer was delayed until day 5; otherwise, the embryos were transferred on day three. Blastocyst transfer was performed in 46 patients (76.7 %) in Group 1, and 53 patients (85.5 %) in Group 2. Blastocysts were graded according to Gardner et al. [14] criteria. Ultrasound-guided ET was performed on either day 3 or day 5. Usually two top quality embryos/blastocysts were transferred. Extra good quality blastocysts were frozen. If patients presented with manifestations of early start of severe OHSS (within 3–5 days of oocyte retrieval) ET was cancelled and, therefore, total freeze was performed. Luteal phase support was the same in both groups. Starting on the second day after retrieval, Progesterone vaginal tablets T.I.D (Endometrin 100 mg Vaginal Insert, Ferring Pharmaceuticals, Inc., Parsippany, NJ, 07054 USA) or vaginal cream once a day (Crinone 8 % Vaginal Gel, Watson Pharmaceuticals, Morristown, NJ 07962 USA), Progesterone in oil 100 mg I.M every other day (Progesterone in Oil 50 mg/mL Vial, Watson Laboratories, Inc., Corona, CA 92880 USA) and Estradiol B.I.D (Estrace 2 mg, Warner Chilcott LLC, Rockaway, NJ 07866 USA) was utilized for luteal phase support. If pregnancy occurred, the same treatment continued until 6 weeks gestation. At that time Estrace and Progesterone in oil were discontinued, and vaginal progesterone was continued until 12 weeks gestation. In addition, if pregnancy was achieved, Metformin chloride was continued until 8 weeks gestation. Pregnancy was confirmed by measurement of β HCG 12 days after blastocyst transfer, or 14 days if ET was done on day 3. Biochemical pregnancy was defined as a transit rise in β HCG, or a positive pregnancy test in the absence of US scan evidence of pregnancy. Clinical pregnancy was defined as the presence of a gestational sac on transvaginal US scan at 6 weeks gestation. Miscarriage was defined as a clinical pregnancy that ended in pregnancy loss prior to 12 weeks gestation. The incidence of severe OHSS according to Golan et al. [15] criteria was documented in each group. If severe OHSS occurred, it was treated by insertion of a pigtail catheter and hydration on outpatient basis in the majority of patients [16, 17]. Follow

up for all patients was completed by the end of October 2009. Sample size was determined based on funding to enroll approximately 140 subjects. After 40 subjects were randomized, an interim analysis assessed the safety and effectiveness; similar and positive findings indicated the study should continue. Final statistical analysis was performed with independent *t*-test or a Chi-square analysis where appropriate, using SPSS version 11.5.

Results

Figure 1 illustrates a flow chart. A total of 140 patients signed the consent form and were randomized; 69 patients to Group 1 and 71 patients to Group 2. Nine patients did not reach ET in Group 1. One patient was excluded from the study due to an abnormal Pap smear (Papanicolaou test) result that required further evaluation. Such result was not discovered until after the patient was randomized, but before the patient started controlled ovarian hyperstimulation. Three patients were cancelled because of poor ovarian response. In two patients HCG was not administered and oocyte retrievals were cancelled due to ovarian hyper-response, for fear of severe OHSS. In these two patients E2 levels were significantly high ($>1,000$ pg/ml) and follicle size was small on treatment day 5. In three patients ET was cancelled; two of them because of manifestations of early onset of severe OHSS and therefore, total freeze was performed, and in one patient embryos were discarded because of contamination of the culture media. In Group 2 nine patients were cancelled. Six of those nine patients had their cycles cancelled; three due to poor ovarian response, one due to the occurrence of an LH surge, one as a result of an allergic reaction to GnRH-ant., and one due to ovarian hyper-response on treatment day 5, for fear of severe OHSS (E2 $>1,000$ pg/ml with small follicle size). ET was cancelled in the remaining three patients; two because of arrest of embryo development, and one because all oocytes were immature and failed to mature in vitro. Of the remaining patients, 122 completed the study, and 60 were randomized to Group 1 and 62 to Group 2.

There was no significant difference in age, duration and type of infertility, or body mass index between the two groups (Table 1). There was no difference in incidence of hirsutism, obesity (BMI >30 kg/m²) or the incidence of associated infertility factors between the two groups (Table 1). In addition, there was no significant difference in day 3 serum FSH and LH levels, serum T levels, serum DHEA-S levels, serum 17-OHP levels, or fasting blood sugar and insulin levels between the two groups (Table 1).

During COS there was no significant difference between the two groups with respect to baseline serum Estradiol (E2), Progesterone (P₄), FSH, and LH levels (Table 2). There was no significant difference in the duration of

Fig. 1 Participation flow chart

stimulation, total dose of rFSH, as well as endometrial thickness, number of follicles ≥ 14 mm, number of follicles ≥ 16 mm, and hormonal profile (E_2 , P_4 , FSH, and LH) on day of HCG between the two groups (Table 2). There was also no significant difference between Group 1 and Group 2 in the incidence of coasting (18.3 % vs. 18.0 %) or in the incidence of severe OHSS, requiring pigtail catheter placement (8.3 % vs. 6.5 %) respectively (Table 2). In addition, there was no significant difference in the percentage of patients who received 5,000 IU of HCG to mimic LH surge, or in the percentage of patients who received HSS for prophylaxis against severe OHSS (Table 2).

There was significantly higher numbers of mature oocytes, zygotes, and number of blastocysts on day 6 in Group 1 compared to Group 2 (Table 3). On the other hand, the number of excellent blastocysts that were transferred

(1.6 ± 0.5 and 1.9 ± 0.3) and the number of frozen blastocysts on day 5 (2.8 ± 1.5 and 4.6 ± 2.3) were significantly lower in Group 1 compared to Group 2 respectively (Table 3). Table 3 illustrates that there were no significant differences in the remaining embryology parameters between the two groups. There was no significant difference in the percentage of patients who had blastocyst formation (91.7 vs. 87.1 %) or the percentage of patients with frozen blastocysts (66.7 % vs. 58.1 %) between Group 1 and Group 2 respectively. Eighteen patients (12.9 %) had no embryo transfer (ET). In nine patients (50 %) the cycles were cancelled due to either poor ovarian response (33.3 %) or for fear of severe OHSS (16.7 %). In three patients (16.7 %) the cycles were cancelled for various reasons (Fig. 1). In six patients (33.3 %) no ET was performed (Fig. 1). There was no difference in the cancellation rate between Group 1 and Group 2 (13 %

Table 1 Demographic data

	Group 1 (n=60)	Group 2 (n=62)
Age (years)	30.3±3.6	30.4±3.5
Duration of infertility (years)	3.7±2.8	4.0±3.0
% Primary infertility	55.0 %	67.7 %
BMI (kg/m ²)	29.3±6.8	29.4±7.1
Hirsutism %	46.7 %	58.1 %
Obesity (BMI >30) (%)	43.3 %	48.4 %
Associated infertility factors * (%)	61.7 %	62.9 %
Day 3 FSH mIU/mL	5.6±1.4	5.3±1.3
Day 3 LH mIU/mL	8.8±7.8	7.4±5.3
Testosterone ng/dL	59.9±24.7	56.7±22.9
DHEA-S mcg/dl	171.2±98.5	147.7±69.4
17-OH Progesterone ng/dL	76.0±78.6	81.8±68.8
Fasting blood sugar mg/dL	87.3±8.7	86.9±9.5
Fasting insulin µIU/ml	10.0±6.6	10.0±8.6

* Percent of patients with two or more associated infertility factors

vs. 12.7 % respectively). Ultrasound-guided ET was performed on day 3 or day 5 depending on number and quality of embryos available on day 3. Fourteen patients in Group 1 (23.3 %) and nine patients in Group 2 (14.5 %) had ET on day 3, while 46 patients in Group 1 (76.7 %) and 53 patients in Group 2 (85.5 %) had blastocyst transfer on day 5. There was no difference in the number of embryos transferred on day 3 (1.8±0.8 vs. 1.8±1.1) or on day 5 (1.9±0.4 vs. 1.8±0.7) between Group 1 and Group 2, respectively.

For all embryos there was clinically higher implantation rate in Group 1 (46.3 %) compared to Group 2 (35.5 %); however, the difference was not statistically significant [$p=0.075$] (Fig. 2). The limits of the 95 % confidence

interval were 38.1 %–54.7 % in Group 1 and 28.1 %–43.7 % in Group 2. For blastocyst transfer the implantation rate in Group 1 was 55.1 % compared to 40.4 % in Group 2, a difference that approached statistical significance ($p=0.051$) (Fig. 2). The limits of the 95 % confidence interval were 44.7 %–63.0 % in Group 1 and 30.9 %–50.8 % in Group 2.

There were clinically higher clinical pregnancy and delivery rates per transfer in Group 1 (68.3 % vs. 56.5 %) compared to Group 2 (60 % vs. 51.6 %) (Table 4); however, the difference was not statistically significant. There was no difference in both first and second trimester miscarriage rates between the two groups (7.3 % vs. 8.6 and 4.9 % vs.

Table 2 Ovarian stimulation characteristics

	Group 1 (n=60)	Group 2 (n=62)
Base line serum E ₂	44.7±14.1	42.8±13.8
Baseline serum P ₄	0.4±0.5	0.3±0.2
Baseline serum FSH (mIU/mL)	5.8±1.9	6.2±2.1
Baseline serum LH (mIU/mL)	5.2±2.7	4.9±3.4
Total duration of treatment (days)	9.6±1.7	9.5±2.0
Total dose of rFSH (IU)	2116±594.2	1874.6±733.8
HCG serum E ₂ (pg/mL)	2469.2±1258.5	2274.4±1102.2
HCG serum P ₄ (ng/mL)	1.1±0.6	1.0±0.5
HCG serum FSH (mIU/mL)	12.6±5.0	12.2±6.9
HCG serum LH (mIU/mL)	1.9±1.8	1.5±1.3
HCG # follicles ≥14	15.2±8.0	16±8.0
HCG # follicles ≥16	9±5.7	9.7±6.1
HCG endometrial thickness (mm)	10.7±1.8	10.5±2.1
(%) Coasting	18.3 %	18 %
(%) HCG 5,000 IU	60.0 %	56.5 %
(%) Severe OHSS requiring pigtail catheter	8.3 %	6.5 %
(%) Prophylactic use of HSS *	66.7 %	69.4 %

*Hydroxyethyl starch solution

Table 3 Embryology data

	Group 1 (n=60)	Group 2 (n=62)
# Mature oocytes ^a	16.2±7.7	14.0±5.7
# 2 PN ^b	12.6±6.3	10.4±4.9
# Frozen zygote	7.5±4.0	7.1±3.4
Fertilization rate	77.7 %	74.2 %
# Day 3 embryos	10.0±2.9	9.7±3.7
# ≥8 Blastomeres	6.3±3.1	6.7±3.3
# <8 Blastomeres	4.0±2.3	3.5±2.2
# No fragments	7.1±3.3	6.3±3.6
# With fragments	3.5±2.3	4.2±2.8
# Day 5 blastocysts	5.5±2.0	5.7±2.9
# Day 6 blastocysts ^c	3.3±2.5	2.9±1.6
Total # blastocysts (day 5+ day 6)	7.5±4.0	7.1±3.4
Blastocyst formation (from day 3) (%)	69.2 %	63.7 %
# Day 5 frozen blastocyst ^d	2.8±1.5	4.6±2.3
# Day 6 frozen blastocysts	2.4±1.3	2.0±1.2
# Total frozen blastocysts	2.1±1.9	2.6±2.8
# Embryos transferred (day 3)	3.0±0.8	2.6±0.5
# Morula transferred (day 5)	2.1±0.7	2.3±1.5
# Blastocysts transferred (day 5)	1.9±0.3	2.0±0.2
# Excellent blastocysts ^e	1.6±0.5	1.9±0.3
# Good blastocysts	1.3±0.5	1.3±0.5
# Early blastocysts	1.3±0.5	1.4±0.5
Total # transferred on day 5 (morula and blastocyst)	2.1±0.3	2.1±0.4
(%) Assisted hatching	28.3 %	22.6 %

^a *P* value=0.039, ^b *P* value=0.017, ^c *P* value=0.006, ^d *P* value=0.011, ^e *P* value=0.006

0 % in Group 1 and Group 2 respectively) (Table 4). Table 4 illustrates that there was a statistically significantly lower biochemical pregnancy rate in Group 1 (2.4 %) compared to Group 2 (18.6 %) [*p*=0.015]. Intention to treat pregnancy, clinical pregnancy and delivery rates were 60.9 %, 59.4 %, and 52.2 % in Group 1, 60.6 %, 49.3 % and 45.1 % in Group 2, and 60.7 %, 54.3 % and 48.6 % in the total population studied, respectively.

Fig. 2 Illustrates implantation rate on day 3, day 5, and overall in group 1, group 2 and the total number of patients studied. **P*=0.051, ***P*=0.075

**P* = 0.051

***P* = 0.075

Discussion

Despite decades of experience, COS in patients with PCOS is still associated with several problems that have not been adequately resolved, including OHSS, lack of synchronization of developing follicles, and poor quality oocytes and embryos [18]. During COS for IVF- ET, GnRH-a protocol remains to be more popular compared to antagonist protocol

Table 4 Pregnancy outcome per ET

	Group 1	Group 2	Total
# Cycles with ET	(n=60)	(n=62)	(n=122)
# Pregnant (%)	42 (70 %)	43 (69.4 %)	85 (69.7 %)
# Clinical preg. (%)	41(68.3 %)	35 (56.5 %)	76 (62.3 %)
# Delivered (%)	36 (60.0 %)	32 (51.6 %)	69 (56.7 %)
# Biochemical preg. (%) ^a	1 (2.4 %)	8 (18.6 %)	9 (10.6 %)
# First trimester miscarriage (%)	3 (7.3 %)	3 (8.6 %)	6 (7.9 %)
# Second trimester miscarriage (%)	2 (4.9 %)	0 (0 %)	2 (2.6 %)
# Multiple pregnancy (%)	19 (46.3 %)*	15 (42.9 %)	34 (44.7 %)

^a *P* value=0.015

*All twins except for 2 sets of triplets

[18]. The use of GnRH-a protocol in PCOS patients has been associated with a significant improvement in the cumulative pregnancy rate and a reduction in the incidence of miscarriage, perhaps as a result of suppressing LH hormone before and during COS [19–21]. However, the use of GnRH-a during COS in PCOS patients, with its suppressive effect on LH, has been associated with multiple follicles and a high risk of severe OHSS [22]. Meanwhile COS using GnRH-ant. protocol in PCOS patients undergoing IVF-ET has been shown to be beneficial [23]. In addition, in a meta-analysis, Al-Inany et al. [18] reported that GnRH-ant. protocol was associated with reduced incidence of severe OHSS. The optimal stimulation protocol for PCOS patients undergoing IVF-ET is still debatable. However, there is now a consensus that a combination of pure FSH and GnRH-ant. is the best protocol for COS in such patients, particularly in view of the low incidence of OHSS [22].

Using such protocol (rFSH and GnRH-ant.), our data suggest a satisfactory overall implantation rate (40.7 %), excellent pregnancy rate (69.7 %) and excellent clinical pregnancy (62.3 %) and delivery (55.7 %) rates in PCOS patients undergoing ICSI/ET who were pretreated with OCP and Metformin hydrochloride. The latter was continued during COS and for up to 1 month if pregnancy occurred. In addition, the overall miscarriage rate (10.5 %) in our study is lower than what has been reported in literature [24–29]. However, if miscarriage included biochemical pregnancy together with both early and late miscarriages, the rate was 20 %.

Our data support the notion that early initiation of antagonist on the first day of stimulation may improve reproductive outcome in PCOS patients. This is judged by a statistically significant higher implantation rate after blastocyst transfer ($P=0.051$) and lower biochemical pregnancy rate ($P=0.015$). Blastocyst transfer accounts for the majority of patients in each group (76.7 % in Group 1 and 85.5 % in Group 2 respectively). In addition, there is a clinically higher overall implantation rate (46.2 % vs 35.5 %). However, the difference was not statistically significant. This is also judged by clinically significant higher clinical pregnancy and delivery rates per ET (68.3 % and 60.0 %) in Group 1

versus (56.5 % and 53.2 %) in Group 2, albeit the difference was not statistically significant. The mechanism for such improvement is not fully understood.

However, one possible mechanism could be early suppression of LH levels at the beginning of ovarian stimulation as illustrated by Lainas et al. [10]. LH levels tend to be higher in PCOS patients and may have deleterious effects on follicular growth and oocyte quality [30]. Therefore, it is feasible that an early start of antagonist could have reduced such levels and in turn may improve oocyte/embryo quality.

Currently there are two protocols for time of initiation of GnRH-ant. during COS: the flexible protocol (when follicles >14 mm) and the fixed protocol on treatment day 5 or 6. Tarlatzis et al. [31] suggested that earlier start of GnRH-ant. during COS for IVF purposes requires further evaluation. There are only two published prospective randomized studies on early initiation of GnRH-ant. in PCOS patients. In the first study, Hwang et al. [9] compared two groups of PCOS patients undergoing IVF-ET. The first group received GnRHant. one day before the start of COS and the second group received long protocol of GnRH-a. In their study, Hwang et al. [9] reported no significant difference in implantation or pregnancy rates per ET between the two groups (18 % and 40 % vs. 17.7 % and 41.7 % respectively). In a more recent study on PCOS patients undergoing IVF-ET, Lainas et al. [10] reported that when GnRH-ant. was initiated on day 1 of COS, there was no significant difference in ongoing pregnancy rate compared with GnRH-a protocol (46.2 % vs. 48.1 % respectively).

Contrary to previous reports, which suggest that rFSH and antagonist protocol reduce incidence of OHSS in PCOS patients, our data still showed high incidence of severe OHSS in both groups. This can be explained on the basis of how COS was fashioned in this study. There was no effort to step down the dose of rFSH in this study, as has been reported by others, or to use 5,000 IU of HCG as a triggering dose in all patients [2, 6]. However, we adopted the coasting protocol, reduction in dose of HCG to 5,000 I.U. and IV administration of HSS, in patients who were at higher risk of OHSS as indicated by serum E2 levels and total number of developing follicles, especially small

follicles [32, 33]. Except in two patients in group 1, in whom total freeze was performed because of manifestations of early onset of severe OHSS, all cases of severe OHSS were of late onset type (> 10 days after triggering dose of HCG). This type of severe OHSS is poorly correlated to the ovarian response and is rather more correlated to the endogenous HCG produced by an implanting embryo.

We elected pretreatment with both OCP and Metformin hydrochloride. The latter was also used during COS and for up to 8 weeks gestation if pregnancy occurred. Metformin hydrochloride has been shown to be beneficial in PCOS patients even in the absence of insulin resistance [34]. However, in a recent Cochrane review, Tso et al. [35] concluded that the use of Metformin before or during ART cycles did not improve pregnancy or live birth rates; however, the incidence of OHSS was reduced. In addition, the Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group [21] recommended that Metformin should only be used in PCOS patients with glucose intolerance.

OCPs have been used for programming oocyte retrieval and suppression of gonadotropins in an attempt to synchronize follicular growth [36]. However, during COS with GnRH-ant, the use of OCPs before the start of ovarian stimulation has been shown to be associated with conflicting results with respect to the number of oocytes and the pregnancy rates [37, 38]. Griesinger et al. [39], based on meta-analysis of six randomized controlled studies, reported a trend towards a reduction of ongoing pregnancy rates when OCPs were used prior to COS, albeit the size effect was low. In addition, in this study both normal and low responder patients were mixed. On the other hand, pretreatment with OCP prior to COS has been used extensively by many groups with satisfactory outcome [40, 41]. Other investigators reported no deleterious effects of pretreatment with OCP in PCOS patients undergoing IVF/ET [6, 8–10, 40]. In a recent prospective randomized controlled trial, Garcia-Velasco et al. [42] concluded that the use of OCP to schedule patients undergoing the antagonist protocol has no negative effects on pregnancy outcome. Our data is in agreement with the notion that pretreatment with OCP does not seem to have any deleterious effects on the outcome of IVF/ET in PCOS patients undergoing the antagonist protocol.

Our study has its limitations. The number of patients in both groups was relatively small, which affected the statistical power. For our primary outcome, we would have needed 343 embryos per group to detect the difference at an alpha of 0.05. This is approximately double the number of embryos per group that our study included. However, this pilot study was to examine if there was clinically significant differences between the groups, and the results should be interpreted in that context. In addition, most of the literature that we referred to in this manuscript that compared GnRH-a

and GnRH-ant. in PCOS patients were under powered and reported small numbers of patients [4–6, 8–10]. Therefore, more prospective randomized studies with a larger sample size are needed to confirm our preliminary data. One point of strength in our study is the fact that the physicians were blinded as to which protocol was being used. Another point of strength is the fact that the majority of ET was performed by the first author using identical techniques; thereby eliminating an important confounding factor that affects implantation rates. There were no significant differences between the two groups studied with respect to demographic data, ovarian stimulation, and embryology data with the exception of the few issues mentioned in the results section, which we believe had no bearing on the outcome. Therefore, the two groups studied were similar and, in turn, reduce the effects of other confounding factors that might affect the conclusion.

In summary, our data suggest that the initiation of GnRH ant. on day 1 (compared to day 5) of ovarian stimulation in women with PCOS undergoing ICSI-ET may improve implantation rates, especially after blastocyst transfer. More studies are needed to confirm our findings. Such studies may need to be multicenteric to be able to include a larger sample size. Also, future studies with a large sample size may look at pregnancy and delivery rates as primary outcome measures.

Acknowledgments The authors would like to thank Jenny LaChance, MS, Hurley Medical Center, Flint, Michigan USA, for her assistance in the statistical analyses, and Cheryl Anderson, AAS, IVF Michigan, Hurley Medical Center, Flint, Michigan, USA for her assistance in the preparation of this manuscript.

This study was supported in part by a research grant from the Investigator-Initiated Studies Program of Merck Sharp & Dohme Corp. The opinions expressed in this paper are those of the authors and do not necessarily represent those of Merck Sharp & Dohme Corp. The authors would also like to thank Merck Sharp & Dohme Corp. for their support of this research.

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