GnRH Agonist and GnRH Antagonist in Intracytoplasmic Injection Cycles

M R Zainul Rashid, DM*, F B Ong, Ph.D*, M H Omar, MOG*, S P Ng, MOG *, A Nurshaireen, BSc*, N S M N Sharifah-Teh, BSc*, A H Fazilah, BSc*, M A Zamzarina , BSc*

*Department of O&G, Medical Faculty, Universiti Kebangsaan Malaysia, Jalan Yaacob Latiff, Bandar Tun Razak, 56000, Cheras, Kuala Lumpur

SUMMARY

The long agonistic protocol for controlled ovarian hyperstimulation (COH) is effective and used most often, thus is considered the gold standard. Therefore any new regimen has to be compared in its results with those obtained with the long protocol. This report compares the efficacy of GnRH agonist and antagonist in a retrospective study of IVF/ICSI carried out in a tertiary teaching hospital from 2003 to 2006. Only the first COH cycle followed by IVF-ICSI from 200 couples (agonist=120 and antagonist=80) were analysed. The end points studied included the number of oocytes recovered, number of mature (MII) oocytes, fertilization, cleavage, morphology based embryo quality, pregnancy rate, quantity and cost of gonadotrophin. The average age of female subjects was 35.1±4.7 years with 50% being 35 years and above. Major infertility factors were tubal blockage, male factor and endometriosis altogether comprising 68%. GnRH agonist and antagonist cycle parameters were comparable except lesser amount of gonadotrophin was used with lower resultant costs (both p<0.0005) in antagonistic regime. Antagonist regime produce somewhat more good quality embryos (p=0.065), an insignificant difference. A clinical pregnancy rate per embryo transfer of 16.3% in agonist and 20.6% in antagonist regime was achieved respectively. In conclusion, GnRH antagonist protocol produced a COH response, embryonic development and pregnancy rates on par to GnRH agonist regime. Moreover GnRH antagonist protocol required a shorter stimulation period plus fewer complications. Hence GnRH antagonist regime provided means for a friendlier, convenient and cost effective protocol for patients.

KEY WORDS:
Ovarian stimulation, GnRH agonist, GnRH antagonist, Assisted reproduction, Intracytoplasmic sperm injection

INTRODUCTION

Ovarian stimulation to initiate multiple dominant follicular development has improved the clinical outcome of in-vitro fertilization (IVF). The mechanisms involved in controlled ovarian stimulation (COH) for GnRH agonist and antagonist protocol are well described [1, 2].

The long agonistic protocol for COH is generally the most effective and is used most often, therefore becoming the gold standard. Thus any new regime has to be compared in its results with those obtained with the long protocol [3]. Meta analyses comparison of GnRH agonist and antagonist regimens have shown comparatively lower pregnancy rate for GnRH antagonist [4], which may have discouraged its adoption by clinicians [5].

To address the issue, a comparison was done between GnRH agonist and antagonist in 200 first attempt COH followed by In-vitro fertilization-Intracytoplasmic Sperm injection (IVF-ICSI) cycles in local subjects.

MATERIALS AND METHODS

This was a retrospective study of IVF/ICSI carried out in a tertiary teaching hospital from 2003 to 2006. Only first COH cycle using GnRH analogues followed by IVF-ICSI from 200 couples was analysed. The end points studied included the number of oocytes recovered, number of mature (MII) oocytes, fertilization, cleavage, morphology based embryo quality, pregnancy rate, quantity and cost of gonadotrophin. Azoospermic, epididymal aspiration and testicular biopsy cases were excluded. All couples must be married and no donor program was carried out in accordance with the law. Basic infertility evaluation included semen parameters, ovulation assessment, ultrasonography of female pelvic anatomy and tubal patency via laparoscopy or hysterosalpingography. The couples were grouped according to their major infertility diagnosis.

COH with GnRH agonist and antagonist was carried using a standard protocol [6]. Briefly, GnRH agonist was started on first day (d1) of the menstrual period or in the previous midluteal phase (nasal spray: Buserelin, Aventi Pharma, Germany; i.m.: Goserelin, Astra Zeneca, UK). Daily GnRH antagonist (s.c.: 0.2mg Cetrorelix, Serono or Ganirelix, Organon) were given beginning on day 6 or when the largest follicle reached 14mm in diameter. Gonadotrophin (daily s.c: Puregon, Organon; Gonal-F, Serono) was started on second day of the menstrual cycle. Follicular tracking by serial ultrasound began from day 5 of the cycle. Human chorionic gonadotrophin (hCG) 10,000 IU (s.c: Pregnyl, Organon) was used to trigger ovulation when the two or more leading follicles were >18mm in diameter. Endometrial thickness was measured one day prior or on the day of HCG injection.

This article was accepted: 18 March 2008
Corresponding Author: Ong Fee-Bee, Department of Obstetrics & Gynaecology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latiff, Bandar Tun Razak, 56000, Cheras, Kuala Lumpur, Malaysia Email: ongfb@mail.hukm.ukm.my

On the morning of oocyte retrieval, husbands were asked to provide fresh semen samples in situ except in two cases when frozen semen was used. They were instructed to abstain from ejaculation for 2-7 days prior to collection. Semen analysis was performed according to WHO criteria[7] and sperms were prepared either by swim up or two density gradient protocol [GIII series, Colorado, USA].

IVF/ICSI was carried out in the conventional manner[8]. Oocyte retrieval was carried out transvaginally using an aspiration needle [Cook, Australia]. ICSI was performed with a semi-automated micromanipulator [Narashige, Japan]. Only MII oocytes were injected with single motile sperm in commercial PVP [Fertipro, Ferticult, Belgium]. ICSI was carried out in oligozoospermic couples and in those with less than five oocytes retrieved. ICSI was also performed in couples who had previous intra-uterine inseminations without pregnancies which comprised one fifth overall. Combined IVF/ICSI was carried out in the event that more than ten mature oocytes were recovered and when semen quality permitted IVF. IVF was carried out with 1-2 x 10^6 motile sperms per ml, incubated overnight. Commercial sequential culture media [GIII series, Colorado, USA] was used in culture.

Fertilization was observed 16-18 hours after insemination and calculated based on the number of zygotes with two or more pronuclei over MII eggs. Cleavage rate was based on the number of two cell embryos over fertilized eggs. Embryos were graded prior to transfer. Excellent-good embryos included those with even sized blastomeres (0-25% fragmentation) or uneven blastomeres with no fragmentation. Fair embryos had >25% fragmentation and poor embryos were highly fragmented (>50%). Three embryos were transferred to the uterus under ultrasound guidance three to five days after oocyte retrieval [Janssen-Andersen, Cook, Australia; Edward-Wallace, Smith, UK]. More embryos were replaced in older women (35+) if the embryos were deemed unsuitable for freezing.

Progesterone for luteal phase support was given orally or in the form of vaginal pessaries [Duphaston 10mg; Laboratories Besins Iscovesco, Paris; Utrogestan 100mg, Solvay Pharmaceuticals BV, Holland] with 2000 IU hCG injection s.c. on day 5, 8 and 13. Biochemical pregnancy was determined on day 21 and confirmed by presence of gestational sac on ultrasound 6-7 weeks later.

Statistical analysis was carried out using SPSS version 10. ANOVA, t-test, χ^2 and Pearson's correlation were used where appropriate. Significance was set at $p< 0.05$.

RESULTS

The average age of female subjects was 35.1±4.7 years old with half (50%) being 35+ years old. Subjects undergoing agonist cycles were slightly younger than those treated with antagonist, 34.8±4.4 vs. 35.4±5.2. Three quarters (78%) comprised of Malays and over four fifths (81%) presented with primary sub-fertility. Major infertility factors were tubal blockage, male factor and endometriosis altogether comprising 68% [Table I].

Pituitary down regulation by GnRH agonist comprised three fifths of all cycles [Table II]. Subjects undergoing agonist cycles and antagonist cycles were similar in age, distribution of infertility factors [Table I] and types of gonadotrophin used. Recombinant FSH was used in most (96.5%) stimulation cycles. All cycle parameters were comparable except the amount of gonadotrophin used and the resultant costs (both $p<0.0005$). On the average antagonist regime required 760 IU lesser gonadotrophin and saved nearly RM800 per subject compared to agonist protocol.

Table I: Sociodemographics and presenting infertility factor of subjects

Characteristics	Overall N=200	Agonist N=120	Antagonist N=80
	N (%)		
Ethnicity			
Malay	156 (78.0)	93 (77.5)	63 (78.8)
Chinese	33 (16.5)	20 (16.7)	13 (16.2)
Indian	7 (3.5)	6 (5.0)	1 (1.2)
Others	4 (2.0)	1 (0.8)	3 (3.8)
Age distribution (years)			
<30	18 (9.0)	11 (9.2)	7 (8.8)
30-<35	82 (41.0)	50 (41.7)	32 (40.0)
35-<40	63 (31.5)	43 (35.8)	20 (25.0)
40-<45	28 (14.0)	11 (9.2)	17 (21.3)
45+	9 (4.5)	5 (4.2)	4 (5.0)
Primary sub-fertility	162 (81.0)	97 (80.8)	65 (82.3)
Presenting infertility factors			
Normal	8 (4.0)	4 (3.3)	4 (5.0)
Tubal	48 (24.0)	37 (30.8)	11 (13.8)
Male factor	45 (22.5)	28 (23.3)	17 (21.3)
Endometriosis	44 (22.0)	29 (24.2)	15 (18.8)
Idiopathic	28 (14.0)	12 (10.0)	16 (20.0)
PCOS/Ovarian	16 (8.0)	7 (5.8)	9 (11.3)
Fibroid (≥2, >2.5cmØ)	4 (2.0)	1 (0.8)	3 (3.8)
Uterine abnormalities	2 (1.0)	1 (0.8)	1 (1.3)
Others *	5 (2.5)	1 (0.8)	4 (5.0)

*2 cases peritoneal TB, 1 multiple sclerosis, 1 cancer, 2 endocrine

Table II: Characteristics of assisted reproduction cycles

Cycle variables	Overall N=200	Agonist N=120	Antagonist N=80
		N (%)	
Type of follitrophin used			
uFSH (Metrodin)	5 (2.5)	5 (4.2)	0 (0)
rFSH (Puregon)	119 (59.5)	73 (60.8)	46 (55.7)
rFSH (Gonal-f)	74 (37.0)	40 (33.3)	34 (44.3)
LH/FSH combination	2 (1.0)	2 (1.7)	0 (0)
Fertilization method			
ICSI only	184 (92.0)	107 (89.2)	77 (96.3)
IVF+ICSI	16 (8.0)	13 (10.8)	3 (3.8)
Semen distribution (million per ml)			
Very severe oligozoospermia (<1)	33 (16.5)	19 (15.8)	14 (18.0)
Severe oligozoospermia (1-5)	35 (17.5)	24 (20.0)	11 (14.8)
Oligozoospermia (>5-20)	54 (27.0)	34 (13.3)	20 (14.8)
Normozoospermia (>20)	78 (36.0)	43 (50.8)	35 (52.5)
No. embryos per transfer			
1 embryo	19 (9.5)	11 (11.2)	8 (11.8)
2 embryos	47 (23.5)	28 (25.6)	19 (27.9)
3 embryos	63 (31.5)	33 (33.7)	30 (44.1)
4+ embryos	37 (18.5)	26 (26.5)	11 (16.2)
No. embryo transfer cycles	166 (83.0)	98 (81.7)	68 (85.0)
Pregnancy rate per embryo transfer	30 (18.1)	16 (16.3)	14 (20.6)
		Mean ± sd	
Semen parameters			
Density (x10^6/ml, range)	50.2 ± 53.9	49.6 ± 55.1	51.0 ± 52.4
Progressive motility (%, range)	43.8 ± 20.8	42.7 ± 21.1	45.5 ± 20.3
Morphology (%, range 0.0-45.0)	14.9 ± 6.5	15.6 ± 7.4	14.3 ± 5.5
Volume (ml, range 0.5-6.9)	2.6 ± 2.6	2.6 ± 2.7	2.7 ± 2.6
Oocytes			
No. oocytes retrieved	11.8 ± 8.6	12.5 ± 9.2	10.6 ± 7.6
No. mature oocytes (MII)	9.2 ± 6.6	9.8 ± 7.4	8.3 ± 5.8
No. oocytes fertilized	5.0 ± 3.9	5.0 ± 4.0	5.0 ± 3.4
Maturation rate (%)	80.8 ± 18.7	79.8 ± 18.6	81.5 ± 18.8
Fertilization rate (%)	57.0 ± 26.0	55.0 ± 26.0	60.1 ± 25.9
Cleavage rate (%)	95.0 ± 16.5	95.3 ± 15.0	94.5 ± 18.6
Embryos			
No. embryos developed	5.0 ± 3.8	5.0 ± 3.9	5.1 ± 3.8
Excellent-good quality	3.1 ± 2.1	2.9 ± 1.9	3.5 ± 2.3
Fair quality	3.1 ± 3.0	3.3 ± 3.3	2.8 ± 2.4
Poor quality (>50% fragmentation)	2.4 ± 2.0	2.5 ± 2.3	2.2 ± 1.2
Mean score of embryos transferred	3.0 ± 0.7	3.0 ± 0.7	3.0 ± 0.7
No. embryos transferred	2.8 ± 1.0	2.8 ± 1.0	2.7 ± 1.0
No. embryos cryopreserved	4.8 ± 3.4	4.8 ± 3.8	4.70 ± 2.7
Endometrial thickness (mm)	12.2 ± 2.3	12.4 ± 2.2	11.9 ± 2.5
Amount of gonadotrophin used (IU) *	3406 ±1201	3710± 1166	2949 ±1110
Total cost of stimulation regime (RM)$	5168 ±1515	5485± 1506	4693 ±1408

*p=0.0005; $p=0.0005

Table III : Age, quantity of gonadothropin (Gn) and number of oocytes retrieved

Variables	Overall N=200		Agonist N=120		Antagonist N=80	
Age group (years)	Mean±sd					
	Oocytes[a]	Gn[a]	Oocytes[b]	Gn[a]	Oocytes[c]	Gn[d]
a. <30	14.7 ± 8.8	2933 ± 1009	14.0 ± 8.4	3300 ± 852	15.9 ± 9.9	2357 ± 1020
b. 30-34.9	14.7 ± 9.8	3161 ± 1168	16.8 ± 10.5	3537 ± 1172	9.5 ± 4.8	2573 ± 899
c. 35-39.9	9.8 ± 6.5[e]	3426 ± 1052	9.1 ± 5.4	3637 ± 1041	9.4 ± 7.6	2974 ± 948
d. 40-44.9	7.7 ± 6.6[f]	3994 ± 1262[h]	9.3 ± 9.0	4695 ± 1165[l]	6.8 ± 4.1[n]	3511 ± 1133[q]
e. =/>45	5.1 ± 3.2[g]	4609 ± 1609[j]	4.0 ± 1.2[k]	4821 ± 1391[m]	7.7 ± 4.7[p]	4344 ± 1596[r]

[a]p<0.0005, [b]p=0.004, [c]p=0.001, [d]p=0.042 (ANOVA)
[e]p=0.026, [f]p=0.005, [g]p=0.004, group a vs. c, d, e (overall); [k]p=0.030, group a vs. e (agonist); [n]p=0.007, [p]p=0.045 group a vs. d, e (antagonist), oocytes post-hoc tests
[h]p=0.002, [j]p=0.0005, group a vs. d, e (overall); [l]p=0.004, [m]p=0.012 [q]p=0.011, [r]p=0.002 group a vs. d, e (antagonist), gonadotrophin post-hoc tests
The number in each age category was 11, 50, 43, 11 and 5 vs. 7, 32, 20, 17, and 4 for agonist and antagonist respectively.

Table IV: Pregnancy rate per embryo transfer versus infertility factor

Presenting infertility factors	Overall N=166	Agonist N=98	Antagonist N=68
		% (N)	
Normal	20.0 (1/5)	50.0 (1/2)	0.0 (0/3)
Endometriosis	18.2 (6/33)	17.4 (4/23)	20.0 (2/10)
Tubal	19.5 (8/41)	19.4 (6/31)	20.0 (2/10)
Male factor (severe)	23.7 (9/38)	13.6 (3/22)	37.5 (6/16)
Idiopathic	11.5 (3/26)	8.3 (1/12)	14.3 (2/14)
PCOS/Ovarian	15.4 (2/13)	0.0 (0/5)	25.0 (2/8)
Fibroid	0.0 (0/4)	0.0 (0/1)	0.0 (0/3)
Uterine abnormalities	50.0 (1/2)	100.0 (1/1)	0.0 (0/1)
Others	0.0 (0/4)	0.0 (0/1)	0.0 (0/3)

The fraction in parentheses denote pregnancies over embryo transfers. No statistical difference was observed between treatment groups

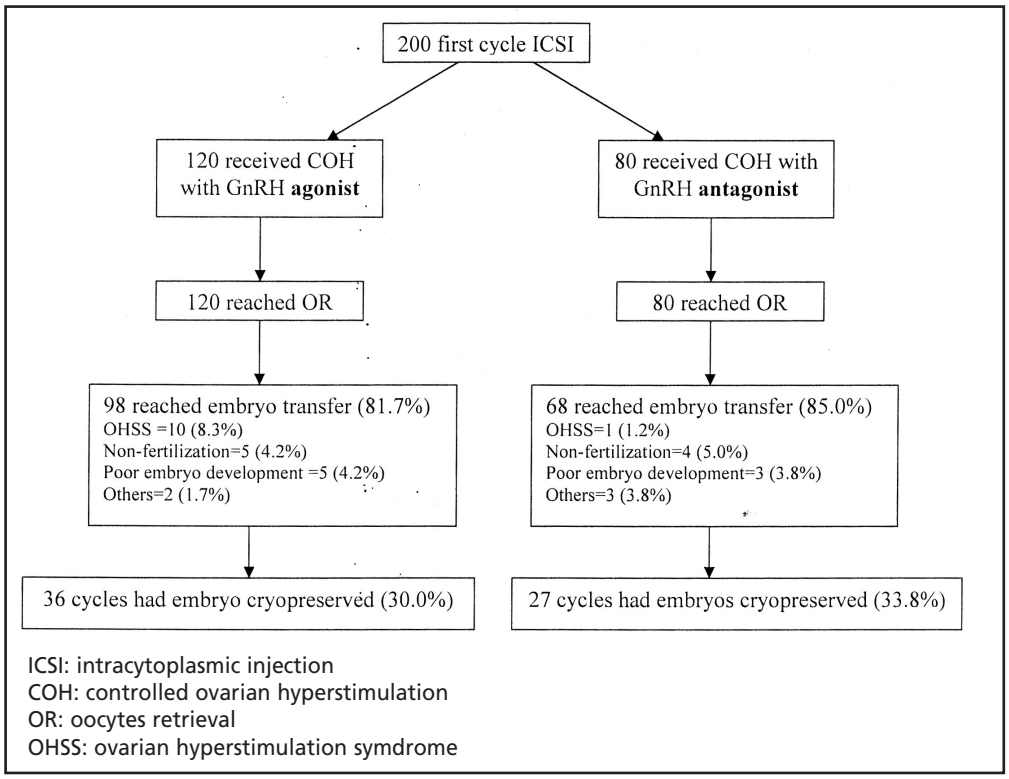

Fig. 1: Flow chart of subjects in study

Oocyte maturation, fertilization and cleavage rate showed that antagonist regime synchronized oocytes development and generated quality oocytes on par to the agonist regime. Antagonist regime produced more good quality embryos (p=0.065) that did not achieve statistical significance. Given that the average morphological score and number of embryos transferred were comparable, a corresponding PR per embryo transfer of 16.3% in agonist and 20.6% in antagonist regime was achieved. Overall PR per started cycle was 15.0%, 13.3% and 17.5% in agonist and antagonist regime respectively.

There were excess embryos for cryopreservation in 31.5% of all cycles, a similar proportion in both treatment groups. Non-fertilization occurred in eight couples, five on agonist and three on antagonist regimes. Other reasons for no embryo transfers included poor embryo development (10), feverish patients (2) and fluid in endometrial cavity (3), similarly distributed between the treatment groups. Eleven cases of OHSS (5.5%) occurred overall, 10 and 1 respectively in agonist and antagonist regime, an incidence rate that did not reach statistical significance (p=0.083).

Significantly fewer oocytes were recovered from women aged 35+ whereas women aged 40+ required significantly more gonadotrophin to induce a quantitative ovarian response [Table III]. The trend was similar in agonist and antagonist cycles although the quantity of gonadotrophin required was significantly lower in antagonist cycles (a, p=0.05; b, p=0.0005; c, p=0.019; d, p=0.015) for all age groups except in the oldest subjects. In the oldest group, the amount of gonadotrophin used in agonist vs. antagonist cycles to induce an ovarian response was statistically indifferent. The number

of oocytes recovered were statistically similar in each age category except in those aged 30-34 years old (p=0.015).

Age is a considerable factor in ovarian stimulation as age of the subjects was positively correlated to the quantity of gonadotrophin used (r=0.324, p<0.0005) and negatively to the number of oocytes recovered (r=-0.314, p<0.0005). The number of oocytes retrieved as expected was strongly correlated to the total number of embryos generated (r=0.774, p<0.0005).

There was a trend towards lower PR in older women which did not reach statistical significance. The PR by age was 18.8%, 19.4%, 18.2%, 15.4% and 14.3% for age category a, b, c, d and e respectively. The trend was similar between agonist (33.3%, 10.5%, 21%, 0%, 25%) and antagonist cycles (0%, 33.3%, 11.8%, 23.5%, 0%).

An almost three fold higher PR was achieved in couples who presented with only male factor infertility on antagonist treatment [Table IV]. However, PR for all factors was statistically indifferent.

DISCUSSION

The findings indicated that ovarian response, embryo development and PR were comparable between GnRH agonist and antagonist cycles. The findings concurred with the more recent studies on GnRH antagonist versus agonist protocol rather than the adverse outcome of older studies [9, 10].

Only the first COH attempt was taken into analysis to avoid management bias as a consequence of poor ovarian response. A fifth (20.0%) of all women in the study were poor responders, recovering four or less oocytes after adequate COH, consistent with the finding of other centers [11]. Even when ovarian response was poor, the subjects proceeded to oocyte pickup as cancellation was avoided whenever possible. The conservative management had in effect reduced the overall PR.

Analysis was restricted to ICSI cycles which had the advantage of overcoming minor sperm and/or oocyte anomalies that impede fertilization. Serious sperm abnormalities associated with testicular aspiration and biopsy cases were excluded to avoid downstream effect on embryonic development confounding the study. We observed that GnRH antagonist cycles produced more better-quality embryos compared to GnRH agonist [12], yielding a slightly higher PR. The latest study suggests milder ovarian stimulation reduces aneuploidy in the resultant embryos, corresponding to better PR [13].

The limitation of the study was due to the retrospective nature of the analysis consisting of a medium size patient population with mixed infertility factors. All GnRH antagonist cycles were analysed as a group as no differentiation was made between the fixed and flexible protocol. At the point of fresh embryo transfer, the study did not detect a significant difference between the two protocols. However, there were 63 cycles with cryopreserved embryos that potentially have a bearing on the final outcome although some studies have indicated that frozen embryos transfer have a relatively modest impact on success rates. To date, cryopreservation programs have remained debatable and the resulting increase in specific pregnancy rates is less than generally perceived [14].

Given this, GnRH antagonist offered a less expensive, less time consuming and patient friendly COH protocol associated with fewer complications[2]. GnRH antagonist reduces the duration of COH by two to three weeks and could be fitted into the stimulation cycle with no need for pretreatment. GnRH antagonist regime could be refined with addition of oral contraceptive to match the organizational regimen of IVF units, thus reducing the uncertainty in the timing of oocyte retrieval [5, 6].

In conclusion, GnRH antagonist protocol produced a comparable ovarian response, embryo development and pregnancy rates to GnRH agonist regime requiring lesser amounts of gonadotrophins. Moreover GnRH antagonist protocol required a shorter stimulation period plus fewer side-effects. Hence GnRH antagonist regime provided means for a friendlier, convenient and cost effective protocol for patients.

REFERENCES

1. Lunenfeld E. Application of GnRH analogues in the management of female infertility. In: Lunenfeld E (eds) GnRH analogues: the state of the ART at the millennium. A summary of the 5th International Symposium on GnRH Analogues in Cancer and Human Reproduction, Geneva. London: Parthenon Publishing Group, 1999; 65-72.
2. de Jong D, Macklon NS, Fauser BCJM. Current status of GnRH antagonist in ART. In Allahbadia GN (ed) Manual of Ovulation Induction. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd. 2003; 230-41.
3. Felderbaum RE, Diedrich K. The use of GnRH antagonist in assisted reproduction technologies. In: Lunenfeld E (eds) GnRH analogues: the state of the ART at the millennium. A summary of the 5th International Symposium on GnRH Analogues in Cancer and Human Reproduction, Geneva. London: Parthenon Publishing Group, 1999; 47-64.
4. Al-Inany H, Aboulgar M. GnRH antagonist in assisted reproduction: a Cochrane review. Hum Reprod 2002; 17: 874-85.
5. Griesinger G, Felberbaum R, Diedrich K. GnRH antagonists in ovarian stimulation: a treatment regimen of clinicians's second choice? Data from the German national IVF registry. Hum Reprod 2005; 20: 2372-75.
6. van Loenen ACD, Huirne JAF, Schats R, Hompes PGA, Lambalk CB. GnRH agonists, antagonists, and assisted conception Semin Reprod Med 2002; 20: 349-64.
7. World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction (4th ed.) Cambridge: Cambridge University Press, 1999.
8. Allahbadia G, Basuray R (eds) The art and science of assisted reproductive techniques (ART). London: Taylor and Francis, 2004.
9. Lindhem SR and Morales AJ. Gn RH antagonist followed by decline in serum estradiol results in adverse outcomes in donor oocytes cycles. Hum Reprod 2003; 18: 2048-51.
10. Ricciarelli E, Sanchez M, Martinez M, *et al.* Impact of gonadotrophin-releasing hormone antagonist in oocytes donation cycles. Fertil Steril 2003; 79: 1461-63
11. Panzan M, Motta ELA, Olive DL, *et al.* Poor responders in assisted reproductive technology: a blue print for management. In Allahbadia GN (ed) Manual of Ovulation Induction. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd. 2003; 207-22.
12. Takahashi K, Mukaida T, Tomiyama T, *et al.* GnRH antagonist improved blastocyst quality and pregnancy outcome after multiple failures of IVF/ISCI-ET with GnRH agonist protocol. J Assist Reprod Genet. 2004; 21: 317-22.
13. Baart FB, Martini E, Eijkemans MJ *et al.* Milder ovarain stimulation for in-vitro fertilization reduces aneuploidy in the human preimplantation embryo: a randomized controlled trial. Hum Reprod 2007; 22: 980-88.
14. Jones HWJ, Out HJ, Hoomans EH, Driessen SG, *et al.* Cryopreservation: the practicalities of evaluation. Hum Reprod 1997; 12: 1522-24.