Experimental
&
Clinical Article

Comparison of the Effects of Gonadotropin Releasing Hormone Agonists and Antagonists on Endometrial Development in Women who Had Inadequate Endometrial Development in a Previous Assisted Reproduction Treatment Cycle; A Randomised Parallel Group Pilot Trial

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OBJECTIVE: To analyse the effects of GnRH agonist or antagonist administration on endometrium of patients with poor prognostic feasures of endometrium undergoing IVF
STUDY DESIGN: A randomised controlled trial was done in 152 women undergoing an assisted reproduction treatment cycle subsequent to an unsuccessful cycle in which they had demonstrated poor endometrial growth. Assited reproduction treatment using standard stimulation protocols were used, implantation, clinical and ongoing pregnancy rates were compared in the two groups.
RESULT: A total of 152 patients were included in the trial. 76 women were allocated to stimulation with the long GnRH agonist protocol while 76 women were allocated to stimulation with the flexible GnRH antagonist protocol. The total oocyte number, the number of excellent quality embryos and the number of embryo transfered were not significantly different between the groups. Implantation rate of cetrotide group was higher than leuprolide group which was not statistically significant (24.1% versus 15.3, p=0.068). The clinical and ongoing PRs rates in the cetrotide group were significantly higher than in the leuprolide group (clinical pregnancy rate 55.2% versus 32.8%, p=0.054, ongoing pregnancy rate 44.7% versus 27.6 %, p=0.028, respectively).
CONCLUSION: Cetrorelix seems to provide better outcome than leuprolide acetate in IVF cycles with poor endometrial responders.

Key Words: GnRH antagonist, GnRH agonist, Endometrium, Assisted reproduction, Pregnancy rate, Randomized controlled trial

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Introduction

The influence of ovarian stimulation with agonist or antogonist on endometrium receptivity has been inadequately addressed in medical literature. The available data in the field indicate that endometrial changes have an impressive negative influence on the potential of embryonic implantation. Poor endometrial responders have the morhology of the endometrium is good as the normal responders but the thickness is lower than 6mm. Regaring the poor endometrial reponders, use of a GnRH agonist or antogonist is likely to have a significant impact on pregnancy rates. Recomendations on the use of GnRH agonist/antogonist in the management of endometrial response are not described with absolute criteria. Suppresion of sex steroids in long protocol and in antogonist protocol is not depend on the patients characteristics accurately. We think cetrorelix is favorable than leuprolide acetate in poor endometrial responders. GnRH is expresed in human endometrial cells and in the trophoblast.[1,2,3] GnRH is potent regulator of matrix metalloproteinases and tissue inhibitors of metalloproteinases, thus important for the overall proteolytic activity of trophoblasts during human implantation.[4] GnRH acts directly on the endometrial cells altering the expression and activation of Smads in human endometrial epithelial and stromal cells.[5] Also Cetrorelix has negative direct effect on the endometrium[6,7] but, within hours of administration, the secretion of gonadotropins is reduced and this is not earlier than the stimulation day 6 onwards. So the endometrium is undergone cyclic developmental changes in preparation for implantation within sixdays. Due to the immediate suppression of gonadotropins, the unwanted and the prolonged endometrial effects of the LHRH agonists can be avoided. On the other hand

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standard or high dose GnRH administration does not make an alteration in the development in the early and mid-luteal phases.[8] Whereas GnRH agonist induces apoptosis and reduces cell proliferation in eutopic endometrial cultures from women with endometriosis.[9]

The main goal of this prospective cohort analysis was to investigate the impact of GnRH antogonist on endometrium, if women undergoing IVF cycles with sonographic evidence of endometrial insufficiency benefit from GnRH antogonist therapy rather than GnRH agonist ones

Material and Methods

The trial was performed at Suzan Health Care Center and American Hospital between January 2006 and November 2007. Informed consent was obtained from all women before recruitment. The Ethic Committee of American Hospital and Suzan Health Care Center approved the study protocol.

152 consecutive women undergoing an assisted reproduction treatment cycle following a previous attempt that had not resulted in pregnancy were included. Causes of infertility were tubal factor, male factor, endometriosis and polycystic ovarian disease.

Inclusion criteria

Couples undergoing assisted reproduction treatment with their own gametes.

Women who had an endometrial thickness less than 6mm, but had the triple line appearance as demonstrated with a transvaginal ultrasound scan, in the previous cycle.

Randomization

Women were randomized to pituitary suppression with either a long gonadotropin releasing hormone (GnRH) agonist protocol or a flexible GnRH antogonist protocol according to a previously constructed computer generated randomization list.

Stimulation protocols, oocyte retrieval, in vitro fertilization, and embryo transfer.

Long GnRH agonist protocol

0.1 mg/day leuprolide acetate (Lucrine, Abbot) sc was commenced on the 20th day of the to and 150-450 IU/day depending on the anticipitated ovarian response. Human chorionic gonadotropin (HCG) 10.000 IU was administered to trigger oocyte maturation when there were at least 3 follicles measuring greater than or equal to 17mm in the mean diameter.

Flexible GnRH antogonist protocol

FSH injections at doses varying between 150 and 450 IU/day were commenced on the second day of menstruel bleeding. 0.25mg GnRH antogonist (Cetrotide, Serono) injections were started when the leading follicle reached a mean diameter at 10mm. Patients were received 10.000 IU of HCG as soon as > or = 3 follicles > or = 17mm were present on ultrasound.

Oocyte retrieval was performed 36 hours after HCG administration. Fertilization was achieved by intracytoplasmic sperm injection (ICSI) in all couples.

Defining characteristics of top quality embryo

On day 3 of culture, the quality of the embryos was evaluated. The group of 'excellent' quality consisted of grade I embryos which had 6-8 cells, without fragmentation, equal sized blastomers with an absence of multinucleation and grade II embryos defined as with 6-8 cells, lower or equal to 25% fragmentation, an even blastomers with an absence of multinucleation. Embryo transfers were performed under direct ultrasound guidance on day the third day after ICSI.

Evaluation of the endometrium

All patients were examined after spontaneous emptying of the urinary bladder, lying supine with the knees slightly bent, and a small pillow under the buttocks. Patients were examined vaginally with an Antares, Siemens 5 MHz transducer. Wall filter was set to 25-50 Hz. Assessment of endometrial paterns and vascularization was always done on the day of human chorionic gonadotropin administration by two treatment staff with together for the same patient. Endometrium was scanned and the double thickness of the endometrium was measured in the midsagittal plane. The sonographic appearance of the endometrium was defined as trilineer if it had three phasic line (multilayered patern) and as not trilineer in all other conditions (atrophic and homogene ones were excluded form the study).

A colour flow map was superimposed on the 2-D picture and doppler studies were performed on selected areas. Adjacent to the endometrium sub-endometrial region was studied. Spectral analysis was made and the lowest values for resistance to flow were selected as representative. 2-dimensional(2-D), colour and doppler gains were set to the minimum. A better picture was achieved by decreasing the frame rate and setting at least three focal zones. Sector angle was settled as wide as possible. Volume was around 1-2 mm. Pulsatility and resistance indexes were measured, and the presence of end diastolic blood flow was evaluated by the formulation of Jaffe and Warson[10] as the following:

Resistance index (RI): the difference between maximal systolic blood flow and minimal diastolic flow divided by the peak systolic flow (S-D/S),

Pulsatility index (PI): the difference between maximal systolic blood flow and minimal diastolic flow divided by the mean flow throughout the cycle (S-D/mean).

Outcome measures

The primary outcomes measured were implantation rate, clinical and ongoing pregnancy rate; secondaries were endometrial thickness, endometrial morphology and subendometrial blood flow studies.

Endometrial thickness and sonographic appearance of endometrium was evaluated as previously described.

Getational sac was located 21-24 days after transfer (gestational age=5 weeks) by transvaginal ultrasound. Implantation rate was calculated as the number of fetal sacs on transvaginal ultrasound at 5 weeks post-transfer/number of embryos transfered and multiplied by 100. It was calculated separately for each subject and treated as continuous variable to address the issue of multple implantations in a woman. Fetal heart beat was evaluated at 6 weeks of pregnancy. Clinical pregnancy was defined as the presense of at least one fetus with a heartbeat, and ongoing pregnancy was defined as a pregnancy proceeding beyond the 12th gestational week.

Serum levels of estradiol, progesterone, LH, FSH and β-hCG were determined by using commercially available kits (electrochemiluminescence immunassay, Roche Elecsys, Roche Diagnostics, Mannheim, USA)

Continuous variables were compared with t-test for independent samples and binary variables were compared with the chi square test. A p value <0.05 was considered significant.

Results

A total of 152 patients were included in the trial. 76 women were allocated to stimulation with the long GnRH agonist protocol while 76 women were allocated to stimulation with the flexible GnRH antagonist protocol. Baseline and treatment cycle characteristics of the study groups are given in Table 1. Except the mean serum estradiol level all were statistically not significant between the two groups.

Endometrial thickness was significantly higher in the cetrotide group as compared to the leuprolide group (7.50±0.73mm versus 7.10±0.74mm, respectively, p=0,001) (Table 2). However, the difference between the proportion of women with a trilineer endometrium did p=0.26) (Table 2). Sub-endometrial blood flow pulsatility index and resistance index were significantly lower in the cetrotide group than in the leuprolide group, (1.28±0.48 versus 1.33± 0.26, p=0.017 and 0.62± 0.07 versus 0.67± 0.1, respectively, p=0.001) (Table 2). End diastolic blood flow was more frequently observed in women stimulated with the antagonist protocol than in women stimulated with long protocol (%80.3 versus %64.5, p=0.029). Characteristics of endometrial blood flow in the two groups are summarized in Table 2.

The total oocyte number, the number of excellent quality embryos and the number of embryo transfered were also not significantly different between the groups (Table 3). There were 207 transfers for cetrotide group and 206 transfers for leuprolide group. The implantation rate was higher in the cetrotide group than in the leuprolide group but this was not statictically significant (15.3% versus 24.1%, p=0.068). The clinical and ongoing PRs rates of cetrotide group were significantly higher than leuprolide group (clinical pregnancy rate 55.2% versus 32.8%, p=0.054, ongoing pregnancy rate 44.7% versus 27.6 %, p=0.028, respectively). The number of twin pregnancy was higher in the cetrotide group (n=8) than in the leuprolide group (n=5), which was not statisticaly significant (p=0,388). IVF outcomes are presented in Table 3.

Table 1: Demographic characteristics and blood hormonal levels

	Leuprolide	Cetrotide	P
Age	32.28 ± 4.36	33.55 ± 4.18	0.071
BMI (kg/m^2)	26.54 ± 2.46	26.26 ± 2.23	0.468
Days	9.53 ± 0.87	9.57 ± 0.92	0.787
Duration	12.53 ± 4.04	12.22 ± 4.04	0.656
Dosage	3018 ± 09	3271 ± 05	0.064
Basal E_2	29.97 ± 5.34	31.46 ± 4.93	0.077
FSH	5.87 ± 1.71	6.31 ± 1.53	0.101
LH	6.077 ± 2.76	5.722 ± 1.87	0.355
Progesteron	0.60 ± 0.14	0.62 ± 0.15	0.38
hCG day E_2	2118.5 ± 957	1808.3 ± 326.5	0.034

BMI, body mass index.

Table 2: Endometrial characteristics

	Leuprolide	Cetrotide	P
Endometrial thickness	7.10 ± 0.74	7.50 ± 0.73	0.001
Endometrial multilayered pattern	% 88.2	% 93.4	0.261
End diastolic flow	% 64.5	% 80.3	0.029
Endometrial pulsality index	1.332±0.26	1.286±0.48	0.017
Endometrial resistance index	0.67 ± 0.104	0.62 ± 0.079	0.001

Table 3: IVF cycle outcomes

	Leuprolide	Cetrotide	P
No of oocycte	10.51 ± 4.21	9.47 ± 3.35	0.095
Fertilization	7.03 ± 2.93	6.25 ± 2.52	0.077
Excellent quality embryos	2.78 ± 1.20	2.51 ± 1.34	0.183
No of embryo transferred	2.57 ± 0.54	2.72 ± 0.45	0.077
No of all embryo transferred	206	207	
No of twin pregnancy	5	8	0.388
Implantation rate	%15.3	%24.1	0.068
Clinical pregnancy rate	%32.8	% 55.2	0.054
Ongoing pregnancy rate	% 27.6	% 44.7	0.028

Discussion

GnRH antagonists are an effective, safe and well tolerated alternative to agonists for controlled ovarian stimulation.11 For window of implantation genes, expression patterns were closer to those in the naturel cycle following standart or high dose ganirelix than after buserelin administration.8 No relevant alterations was observed in the endometrial development in the early and mid-luteal phases in women undergoing controlled ovarian stimulation for oocyte donation following daily treatment with a standard or high dose GnRH antogonist. In addition, the endometrial development after GnRH antogonist mimics the natural endometrium more closely than after GnRH agonist.5 Hofmann had shown that women with inadequate endometrial maturation on the pelvic ultrasound (thickness <5mm), after estrogen replacement, ovarian stimulation with hMG and a GnRH antogonist can yield appropriate endometrial maturation for pregnancy through ovum donation.12 The clinical significance of gonadotrophin-releasing hormone agonists is well recognised, but the potential use of GnRH antagonists is not designed with some criteria in ovarian stimulation. Our results showed that the minimum endometrial thickness associated with pregnancy was 6.9 mm. The existence of a homogeneous endometrial pattern after FSH stimulation seems to be a prognostic sign of an adverse outcome in IVF. A tripple-line pattern after FSH stimulation appear to be associated with conception. We excluded atrophic and homogene endometrium in the study as this tissue does not respond to the treatment. Endometrial tissue blood flow was significantly greater in morphologically normal than abnormal uteri. A good blood supply to the endometrium is usually considered as an essential requirement for implantation. Quantitative assessment of subendometrial blood flow by sonograpy is an important predictive factor of implantation in IVF programme.13 We think that the vascularity of subendometrial layers measured by power doppler ultrasound is a good predictor of ability of transvaginal power doppler ultrasonography to assess the releationship between pregnant and not pregnant women. We tested our observation with the measurement of endometrial thickness and doppler flow study in pregnant and non-pregnant patients. Our results showed that the mean ET and the subendometrial blod flow PI and RI demonstrate significant differences between the women who conceived and those who did not ($p<0.05$). In addition, subendometrial blood flow pulsatility and resistance indexes were significantly lower in the cetrotide cycles than that in the leuprolide cyles ($p=0.001$). End diastolic flow is more frequently observed in women stimulated with the antagonist protocol than in women stimulated with long protocol ($p=0.029$). Antagonist group was also found to have higher than leuprolide group with respect to the thickness of endometrium ($p=0,001$). Furhermore, antagonists were associated with significantly higher clinical ($p=0.054$) and ongoing ($p=0.028$) pregnancy rates than agonists in our subgroup of patients consisting thin endometrium. Implantation rates were not statistically significant ($p=0.68$). However, we assume that in case of higher number of patients treated the difference of implantation between the groups will gain significance. Devaux had shown a decrease of the pregnancy rate after antogonist treatment in their retrospective meta–analyses, but this study group consisting the ART attempts for good prognosis women (<35 years, IVF range 1 or 2) and the begining of antogonist was later than the current starting day.14 To our preliminary experience, one of the two important factor in usage of the antogonist is to start as early as you can do when the leading follicle reaches around the size of 10mm, the other is to choice it for the women of poor endometrial features. Cochrane database was resulted with a high pregnancy rate in the agonist group than in the antogonist group.15 Again these groups have not poor endometrial features and as mentioned in the reviewer's conclusions fixed antogonist protocol (with antogonist start fixed on day 6 of gonadotrophin stimulation) is not suitable for all cases regarding the outcomes of pregnancy rate. Endometrium of PCOS patients have diminished reproductive potential in terms of hormonal miliue, receptor and uterine perfusion.16,17,18 Use of an antogonist protocol in policysistic ovarian disease is comparable with the use of an agonist regarding ART outcome.19 For the same reason antogonist protocol is valuable for implantation in the PCOS patient's ART. The efficacy of GnRH antagonists in poor ovarian responders is good and preferable than GnRH agonists.20,21 Taking into consideration of the stimulation for the poor ovarian resonders, GnRH antagonists succeed in the prevention of premature LH surge, further they have no detrimental effects on ovary and endometrium. On the other hand, agonists have high affinity binding and direct antiproliferative effects on human endometrial cancer cell lines.22 Gonadotropin-releasing hormone agonist induces apoptosis and reduces cell proliferation in eutopic endometrial cultures from women with endometriosis.9 Radowicki had used agonists in the treatment of hyperplasic endometrium.23 If used long duration, agonists suppresses cell proliferation of the endometrium and effects negatively on it. Prologation of follicular phase by delaying hCG administration results in a higher incidence of endometrial advancement on the day of oocyte retrieval in GnRH antogonist cycles.24 But this is not the case in GnRH agonist cycles. In this point of view, its logical to use GnRH antogonist in poor endometrial responders.

Poor endometrial responders have also a functioning endometrium. But they have insufficient biological data for endometrium. Our findings on a specific subgroup of patients suggest an evidence for poor endometrial responders that an association is existed between endometrial perfusion and reproductive outcome following the use of a GnRH antagonist

versus agonist in the IVF treatment.

Use of a GnRH antogonist is likely to have a significant impact on pregnancy rates for poor endometrial responders. A well designed prospective, randomized multicentered trial in this selected population would help to settle the question clearly.

Daha Önceki IVF Siklusunda Yetersiz Endometrial Gelişimi Olan Kadınlar da Gonadotropin Agonist ya da Antagonistlerin Endometriyuma Olan Etkilerinin Karşılaştırılması; Randomize Paralel Grup, Pilot Çalışma

IVF uygulanan kötü prognostic endometriyuma sahip hastalarda GnRH agonisti veya antagonistinin endometriyuma etkisinin araştırılması amaçlandı.

Zayıf endometrial gelişim göstererek başarısız bir siklusa ardışık olarak IVF uygulanan 152 kadında kontrollü randomize bir çalışma yapıldı.

Yardımcı üreme tedavisinde standart stimülasyon protokolleri kullanıldı, her grupta implantasyon, klinik ve devam eden gebelik oranları karşılaştırıldı.

Total olarak 152 hasta çalışmaya dahil edildi. Bunlardan 76 tanesi uzun GnRH agonist protokolüne alındı, 76 kadın da esnek GnRH antagonist protokollüne alındı. Gruplar arasında total oosit sayısı, iyi kalite embiryo sayısı ve transfer edilen embiryo sayısı açısından anlamlı olarak farklılık yoktu. İmplantasyon oranı cetorelix grubunda leuprolide grubundan daha fazla olup istatistiksel olarak anlamlı değildi (%24.1 karşılık %15.3, p= 0.68). Klinik ve devam eden gebelik oranları cetrorelix grubunda leuprolide grubundan anlamlı olarak yüksekti (sırasıyla, %55.2 karşılık %32.8, p=0.054; %44.7 karşılık %27.6, p= 0.028).

Kötü endometrial cevaplı IVF siklüslarında cetrorelix'in leuprolid'den daha iyi sonuç sağlayabileceği görünmektedir.

Anahtar Kelimeler: GnRH antagonist, GnRH agonist, Endometrium, Yardımcı üreme, Gebelik oranı, Randomize kontrollü çalışma

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References

1. Raga F, Casan Em, Kruessel JS, Wen Y, Huang HY, Nezhat C, Polan M. Quantitative gonadotropin-releasing hormone gene expression and immunohistochemical localization in human endometrium throughout the menstrual cycle. Biol Reprod 1998; 59: 661-9.
2. Stojilkovic SS, Catt KJ. Expression and signal transduction pathways of gonadotropin-releasşng hormone receptors. Recent Prog Horm Res 1995; 30: 161-205.
3. Wolfahrt S, Kleine B, Rossmanith WG. Detection of gonadotropin-releasing hormone and its receptor mRNA in human placental trophoblasts using in-situ reverse transcription-polymerase chain reaction. Mol Hum Reprod 1998; 4:999-1006.
4. Raga F, Casan EM, Wen Y, Huang HY, Bonilla- Musoles F, Polan ML. Independent regulation of matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1 (TIMP-1), and TIMP-3 in human endometrial stromal cells by gonadotropin-releasing hormone: implications in early human implantation. J clin Endocrinol Metab 1999; 84: 636-42.
5. Luo X, Xu J, Chegini N. Gonadotropin releasing hormone analogue (GnRHa) alters the expression and activation of smad in human endometrial epithelial and stromal cells. Reprod Biol Endocrinol 2003; 1: 125.
6. Hermandez ER.Embrio implantation: the rubicon for GnRH antagonists. Hum Reprod 2000; 15: 1211-16.
7. Devroey P, Bourgain C, Macklon NS, Fauser BC. Reproductive biology and IVF: ovarian stimulation and endometrial receptivity. Trends Endocrinol Metab 2004; 15: 84-90.
8. Simon C, Oberye J, Bellver J, Vidal C, Bosch E, Horcajadas JA, Murphy C, Adamormal S, Riesewijk A, Mannaerts B, Pellicer A. Similar endometrial development in oocyte donors treated with either high-or standard-dose GnRH antagonist compared to treatment with a GnRH agonist or in natural cycles. Hum Reprod 2005; 20: 3318-27.
9. Meresman GF, Bilotas M, Buquet RA, Baranao RI, Sueldo C, Tesone M. Gonadotropin-releasing hormone agonist induces apoptosis and reduces cell proliferation in eutopic endometrial cultures from women with endometriosis. Fertil Steril 2003; 2:702-7.
10. Jaffe R, Warsof SL. Color Doppler Imaging in Obstetrics and Gynecology. Newyork, McGraw-Hill press, 1992.
11. Xavier P, Gamboa C, Calejo L, Silva J, Stevenson D, Nunes A, Martines-de-Oliveira J. A randomised study of Gn RH antagonist (cetrorelix) versus agonist (busereline) for controlled ovarian stimulation: effect on safety and efficacy. Eur J Obstet Gynecol Reprod Biol 2005;120:185-9.
12. Hofmann GE, Sosnowski JP, Warikoo P, Bertsche A. Use of a gonadotropin-releasing hormone antagonist for endometrial maturation and synchronization in an ovum recipient. Fertil Steril 2003; 80: 1508-9.
13. Schild RL, Holthaus S, d'Alquen J, Fimmers R, Dorn C, van Der Ven H, Hansmann M. Quantitative assessment of subendometrial blood flow by three-dimensional-ultrasound in an important predictive factor of implantation in an in-vitro fertilization programme. Human Reprod 2000; 15: 89-94.

14. Devaux A, Pouly JL, Bachelot A, Mourouvin Z, de Mouzon J. Biological effects of GnRH antagonists. Gynecol Obstet Fertil 2004; 32: 741-7.

15. AI-Inany H, Aboulghar M. Gonadotropin-releasing hormone antagonists for assisted conception. 2001; 4: CD 001750.

16. Gregory CW, Wilson EM, Apparao KB, Lininger RA, Meyer WR, Kowalik A, Fritz MA, Lessey BA. Steroid receptor coactivator expression throughout the menstruel cycle in normal and abnormal endometrium. J Clin Endocrinol Metab 2002; 87: 2960-6.

17. Chekir C, Nakatsuka M, Kamada Y, Noguchi S, Sasaki A, Hiramatsu Y. Impaired uterine perfusion associated with metabolic disorders in women with polycystic ovary syndrome. Acta Obstet Gynecol Scand 2005; 84: 189-95.

18. Giudice LC. Endometrium in PCOS: Implantation and predisposition to endocrine CA. Best Pract Res Clin Endocrinol Metab 2006; 20: 235-44.

19. Bahceci M, Ulug U, Ben-Shlomo I, Erden HF, Akman MA. Use of a GnRH antagonist in controlled ovarian hyperstimulation for assisted conception in women with polycystic ovary disease: a randomised, prospective, pilot study. J Reprod Med 2005; 50: 84-90.

20. Fasouliotis SJ, Laufer N, Sabbagh-Ehrlich S, Lewin A, Hurwitz A, Simon A. Gonadotropin-releasing hormone (GnRH)-antagonist versus GnRH-agonist in ovarian stimulation of poor responders undergoing IVF. J Asist Reprod Genet 2003; 20: 455-60.

21. Cheung LP, Lam PM, Lok IH, Chiu TT, Yeung SY, Tjer CC, Haines CJ. GnRH antagonist versus long GnRH agonist protocol in poor responders undergoing IVF: a randomized controlled trial. Hum Reprod 2005; 20: 616-21.

22. Emons G, Schroder B, Ortmann O, Westphalen S, Schulz KD, Schally AV. High affinity binding and direct antiproliferative effect of luteinizing hormone-releasing hormone analogs in human endometrial cancer cell lines. J Clin endocrinol Metab 1993; 77: 1458-64.

23. Radowicki S, Skorzewska K. Treatment of hyperplasia endometrium with GnRH agonists. Ginekol Pol 2003; 74: 836.

24. Kolibianakis EM, Bourgain C, Papanikolaou EG, Camus M, Tournaye H, Van Steirteghem AC, Devroey P. Prolongation of follicular phase by delaying hCG administration results in a higher incidence of endometrial advancement on the day of oocyte retrieval in GnRH antagonist cycles. Hum Reprod 2005; 20:2453-6.