Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 21, issue 3, Jul.-Sep. 2011, pp. 539-545

COMPARATIVE THERAPEUTICAL STUDY AT THE PATIENTS WITH POLYCHISTIC OVARS

Mirela Voicu[1], Carmen Cristescu[1], Liana Drăgan[2], Liana Suciu[1], A. Voicu, B. Bumbăcilă[1]
University of Medicine and Pharmacy "Victor Babeş" Timişoara, Faculty of Pharmacy
[1]Department of Pharmacology-Clinical Pharmacy
[2]Department of Pharmaceutical Chemistry

ABSTRACT

The objective. The purpose of this study is the comparison of the clinical effects gained as the result of the administration of an analogues of gonadorelin - triptorelin at the patients that have been diagnosed with the syndrome of polychistic ovary (SOP), against the administration of an oral combined contraceptive - Diane 35.

Material and method. The study included a number of 24 patients, with the average age of 23,2 that were diagnosed with SOP. The patients were divided aleatory into 2 groups: group A, formed by 12 women (50%) used Diane 35, and group B, 12 patients (50%), used Diphereline 3,75.

Results. The introduction of Diphereline therapy 3,75 in injectable administration improved the clinic symptoms (acne, hirsutism, amenorrhea, the morphology of ovaries). The results of the study show the therapeutically efficiency of the preparate Diane 35 on the symptoms of androgen nature - especially acne.

Conclusions. The utilisation of preparate Diphereline 3,75 at the patients that have been diagnosed with SOP represents the most efficient therapeutically variant, aspect that had been proved by clinical, hormonal and morphological data. Additionally this formula permits the maintenance of the obtained results on a long time.

Keywords: syndrome of polychistic ovary, therapeutically efficiency, Diane 35, triptorelin.

INTRODUCTION

The syndrome of polychistic ovary (SOP) is an affection characterised by abnormal gonadotrophinic secretions, chronic anovulation, hiperandrogenism and a variety of metabolic effects, as obesity (30-60 %) and insulino-rezistance (present at 30-46% among the normoponderal women and at 57-78% among obese women).

In SOP, hiperandrogenism is masked from a clinically point of view at approximate 30% of European patients and 80-90% of Oriental patients it induces hirsutism at 60-83% of cases and acne at 11-43% of cases. The clinical expression depends of the events the have been produced at the level of pilosebaceous unit, where the individual sensibility for androgens is different. The patients with SOP were applied a few therapeutically methods, inclusive dietetic formulas and pharmacological agents[1].

Taking in consideration that the purpose of the treatment, especially for the associated hirsutism, is the decrease of production, of biodisponibility or of linking the androgens by the aimed organs, the traditional treatment is the combination between the oral contraceptives (CO) and antiandrogens, as for instance ciproteron acetate (CPA). The results of the treatment with CO have often been assigned as being unsatisfactory, not only due to incomplete suppression, but also that the progesterone component of the majority of contraceptive oral products is a derivative of 19-nor-testosterone and, as a result, it presents a kind of androgen activity. That is why CPA was registered as being efficient, as monotherapy, or associated with an oral contraceptive, or in combination with estrogens, in a oral contraceptive product. This combination, (CPA+EE estrogens) may decrease the production of androgens, simultaneously with the blockage of their action and it seems proper for the treatment of hiperandrogenism (hirsutism and/or acne), as part of polychystic ovary syndrome[1,7].

In the last years there have been introduced the analogues agonists of the gonadotropins release hormone (GnRH) for the evaluation and the treatment of patients with SOP. These produce the complete suppression, however reversible, of the pituitary gonadotrophins. This has as the result the suppression of both ovarian functions, that is ovulation and steroidogenesis. It was concluded that GnRH presents a great efficiency in cases of ovarian hiperandrogenism. GnRH with a long time action can be used in monotherapy - although these produce symptoms of hipoestrogenic nature and decrease the content of minerals in bones - or in combination with a treatment of substitution estro-progestative: either standard oral contraceptives, or oral contraceptives with a content of etynilestradiol (EE) and CPA. GnRH offers an

answer superior in comparison with the standard oral contraceptives and resembles with that with Diane 35.

OBJECTIVE

The objective of the study is the comparison of clinical effects obtained as a result of administration of a analogues of gonadoreline - triptorelin at the patients diagnosed with the syndrome of polychystic ovary (SOP), against the administration of a combined contraceptive oral - Diane 35.

MATERIAL AND METHOD

The study took place in the Clinic of Obstretics and Gynaecology Bega, Timisoara in 2008 January 30 - 2009 January 30 and included a number of 24 patients, with an average age of 23,2 years (between 14-32 years), diagnosed with SOP, with clinical manifestations of hirsutism (23 patients - 96%), obesity (15 patients 62,5%), menstrual disorders (17 patients - 70,83%), amenorrhea (7 patients - 29,17%), seborrhea (9 patients - 37,5%) or acne (4 patients -16,7%). Initially, there were another 5 patients that had been part of the study, but they were excluded later, because they give up treatment from subjective reasons.

The SOP diagnostic was established by: the clinical history of the patients; the objective results of the laboratory examinations and investigations (serial concentrations of androgens that seemed to be high and exceeded values of the rapport LH/FSH); the paraclinic evidence, by transvaginal echography, of the polyclistic ovary morphology (the bilateral presence of more periphery follicles, with reduced dimensions: 2 - 8 mm, without the dominance of a special one, around a dense stromatic nucleus)[11].

The criterion of the diagnosis based on ultrasonographic aspect includes the bilateral increase in volume of the ovaries (the maximum diameter >9cm), 10 follicles, or more, with a diameter de 2 - 10 mm and the increase of stroma density. There has been made the differential diagnosis against other affections with similar manifestations: corticosuprarenal dysfunction, tumor androgensecretory, and tiroidian dysfunction.

For all the patients that made basic biochemical tests (inclusive lipids concentrations) and serial concentrations for gonadotrophins, prolactin, insuline, ovarian hormones, thyroidiens and suprarenal. The stimulation test at the adrenocorticotrop hormone and the test of insulinorezistance have been made, too (ACTH).

Other screening criteria: the absence of any other treatment for this affection, in the last 3 months; the absence of systemic affections; the lack of interest for pregnancy in the period allocated for study; the absence of any other contraindication for the use of oral contraceptives and GnRH.

After the diagnosis has been established by clinical, hormonal and echographic methods, the patients were re-examined in the precocious follicular phase (days 5-10 of the menstrual cycle); in any other day, if they have ameonorrhea; in the period of an anovulatory cycle, proved by the plasmatic level of the progesterone.

The patients were asked not to smoke, in addition they had to do alimentary abstinence with 12 hours before the prevelation of the samples for the laboratory tests: haematological and biochemical analysis, the beginning concentrations for gonadotrophins, prolactin, estradiol, progesterone, 17-OH- progesterone, cortyzol, dehidroepiandrosteron sulphate (RHEAS), testosterone, androstendion and insulin. Later, there have been introduced tips for SHBG (sex-hormone binding globulin) and free testosterone.

The insulin and the glucose were determined by a jeun, after 2 consecutive days of diet with > 300g carbohydrates. All the patients were investigated for the resistance at insulin in the same time with the glicemy tests a jeun, at an oral filling of 100 glucose - the administration of insulin doses with a short duration of action.

The acne forms were classified in function of number of lesions (comedos and papules/pustules) and of their spreading on face, back and chest: low acne - characterised by a number of comedos <10 for each of the body surface, without, or with a few inflammatory pustules/ papules; moderate acne - with a number of comedos between 10 and 25 and with a lot of inflammatory lesions (pustules or papules between 10 and 20 for each of the body surface), but with a low frequency of chystic activity; severe acne - with many comedos (>25) and inflammatory pustules/papules, as well as cysts (>20), spread on the entire face, back and chest areas. For the study the patients with moderate and low acne were included.

Hirsutism was evaluated by the system of global index Ferriman-Gallwey, in which a single observer evaluated the degree of hairiness on 11 body surfaces, after a scale from 0 to 4. There were also evaluated the height, weight, the body mass index (calculated in kg/ m^2) and the percentage of the ideal body weight (for calculation there were used the tables of Association of Life Insurance Directors and Actual Society of America)[3].

It was made a transabdominal or transvaginal echographic exam, before, at the end of the treatment and after 3, 6 months from the end of treatment, using an ultrasonic system and an vaginal transducer of 6,5 MHz, an angular sector of 160 degrees, focalised at 3 cm.

The patients were distributed after the following treatment protocols:

Group A: the contraceptive pills, with a content of 0,035 mg EE and 2 mg CPA/cp (Diane 35), starting with the first spontaneous cycle or induced by progesterone,

administrated on the duration of 10 consecutive cycles, according with a standard regime of oral contraceptives;

Group B: bottles with injectable powder i.m. with 3,75 mg triptorelin - analogue of releasing hormone D-Trp-6-LH (Diphereline 3,75), with the administration of a bottle at every 28 days, starting with the first day of spontaneous or induced cycle, in total 8 bottles - 8 cycles. Additionally, there were administrated oral contraceptives, Diane 35 for avoiding the hipoestrogenic effects induced by GnRH - hot flash and the decrease of bone density.

The patients were asked to present at the Clinic at trimestrial intervals (3 months) for a clinic evaluation (hirsutism, body weight, symptoms associated with hipoestrogeny, psychological status). There were repeated the hormonal and biochemical screenings, by randomising, after 3 or 6 months of treatment and after the treatment ending, at the next spontaneous menstruation, or, in case of amenorrhea maintenance, at 3 months, then 6 months after the treatment ending.

The group A - 12 patients - received a treatment with Diane 35 on duration of 10 months. 5 patients repeated the hormonal screenings during the third or fourth treatment cycle, and the other 7 were tested during 6 or 7 cycle.

The second group - 12 patients - treated with Diphereline 3,75 + Diane 35, made the laboratory doses during 3 or 4 cycle (6 patients) and at 6 or 7 cycle (the other 6 patients). From this point of view there were not obtained differences between the results, so that all the values were classified as "values during the treatment". From all the patients, only 9 from group A and 10 patients from group B presented for the biochemical analysis and of clinical and hormonal evaluation after the treatment ending. The remaining patients decided to continue the administration with Diane 35, as contraceptive method. The study was made without having a reference group (witness).

Therapy beginning parameters

Table 1. Clinical hormonal and biochemical parameters before the treatment beginning for groups A and B

Parameters	Normal values	Group A (Diane 35) n=12	Group B (GnRH-a) n=12
Age (years)		23,7	22,4
Heigh (m)		1,58	1,61
Weight (kg)		67,2	78,1
Index Ferriman-Gallwey	< 8	12,0	15,7
Insulin resistance	0	2/12 (16,7%)	3112 (25%)
FSH (mUI/mL)	3- 14	4,4	4,8
LH (mUI/mL)	3-20	8,9	7,0
Prolactin (ng/mL)	2-20	15,2	13,4
Oestradiol (pg/mL)	10-90	66,7	56,1
Cortisol (pg/dL)	5-25	21,8	17,2
17-OH-progesteron (ng/mL)	0,3-3,0	1,63	1,36
Testosteron (ng/mL)	0,3-0,8	1,01	1.04
Androstendion (ng/mL)	0,4-4,5	4,5	4,7
DHEAS (pglmL)	0,7-3,9	3,6	3,1
Insulin (NUI/mL)	8-25	14,6	19,4
Giycohaemia (mg %)	70-105	90,0	I 94,5
Cholesterol (mg %)	120 - 220	174,4	i 177,0
HDL cholesterol (mg °a)	30-70	50,0	51,4
Triglycerides mg %)	40 - 128	I 98,5	115,0

The both lots of patients presented similar values, without any significant variations.

Table 2. Hormonal values and hirsutism's evaluation during and after the treatment at groups A and B

	During treatment		After treatment	
	Group A Diane 35 n=12	Group B GnRH-a n=12	Group A Diane 35 n=9	Group B GnRH-a n=10
Index Ferriman-Gallwey	9,0	10,4	8,5	10,9
FSH (mUI/mL)	2,6	0,9	4,2	4,3
LH (mUI/mL)	2,9	0,5	7,0	6,2
Oestradiol (pg/mL)	21,3	21,2	73,5	53,2
17-OH-progesteron (rig/ml-)	0,9	0,65	1,5	1,3
Testosteron (ng/mL)	0,3	0,3	0,76	0,7
Androstendion(ng/mL)	3,3	2,9	4,4	3,6
DHEAS (ug/mL)	3,1	2,7	3,8	3,3
Insulin (pUhmL)	13,3	16,1	12,1	14;8

The two patients groups presented close values, without significant variations. There were not recorded significant differences between the clinical and hormonal parameters in case of the two groups.. The patients from group B present obesity degrees, insulinorezistance and hirsutism a little more increased, with the ' hormonal values a little depreciated, in comparison with group A.

RESULTS

From a clinically point of view, there were not recorded significant changes of the body weight although, during the treatment, there was a tendency of weight increase in both groups (6 patients - 24% reported a weight increase of 5 - 11 kg, and 4 patients - 16,67% decreased in weight with 3 - 6 kg).

The subjective indices of acne reduction, the hairiness degree and the psychological state, were more satisfactory for group B then for group A (91,7% in comparison with 58,3% for amelioration during the treatment), but these differences are not significant from a statistically point of view

In an objective way, at all the patients disappeared the acne after the therapy ending, but the hirsutism persisted at 7 patients (29,16%). The hirsutism indices decreased significantly for both treatment variants and mentained low after the therapy ending in both groups. The low hirsutism disappeared during the 10 cycles, while the moderate and severe hirsutism reduced substantially, but remained present. After the therapy ending, in case of group B hirsutism remained unchanged another 6 months, then its intensification was noticed.

The ovarian volume, the number of microchysts and the stomatic proportion decreased significantly in both groups, with an equal distribution of its amelioration. At 6 months from the therapy ending, the endocrine parameters were identical with the initial ones, the acne and the hirsutism reappeared, and the ovarian morphology was between the initial and final stage, with a better situation in case of group B. At 5 patients (20.83%) - with the most severe initial situation- were recorded polychystic ovaries and at 19 patients (79,17%), multifollicular ovaries.

The results of the study show the therapeutically efficacy of Diane 35 on androgen nature symptoms - especially acne - and suggest that hirsutism and acne are induced by different peripherical mechanisms. Undoubtedly Diane 35 contributed to ovarian morphology change and it can be appreciated that Diphereline 3,75 had a superior contribution because in group B there were more patients (3) with a severe situation then in group A (2 patients).

The FSH, LH, estradyol, 17-OH-progesterone, testosterone and androstendion concentrations decreased significantly, and the cortyzol concentration increased in both groups during the treatment. At group A the trigliceride concentrations increased significantly during the treatment. The differences between the two groups, during the treatment, were significant only for FSH and LH concentrations, which were lower in group B. After the treatment, there were not noticed significant differences between the final and initial values of parameters, with the exception of androstendion, which remained significantly low in group B, but without significant differences between the group A and B. There was noted no significant variation during or after the treatment, at no patients group, for prolactin, DHEA-S, insulin, glicaemia, cholesterol or HDL-cholesterol. The rest of the parameters mentained at constant values in both groups.

The hirsutism indices recorded a more significant decrease in case of group B, both during the treatment (79,5% at group B in comparison with 68,0% in group A), as well as after the treatment (87,2% at group B in comparison with 69,0% in group A). There were taken in calculation only the patients with moderate or severe

hirsutism (the hirsutism indices > 12, in case of group A at 7 patients during the treatment and 5 after the treatment and 10, respective 9 patients in group B). There were no significant differences of the biochemical parameters, except of the gonadotropins, increased during the treatment and of 17-OH-progesterone, increased after the treatment, in group A.

In a similar way, if are taken in consideration only the obese patients, (with a body weight indices ≥12%: 5 patients, during and after the treatment, in group A; 8 patients during the treatment and 6 after the treatment, in group B), during the treatment there were significant procentual differences between group A and B, for hirsutism indices and for FSH, LH and DHEA-S concentrations. That is why it results that the Diphereline treatment is more efficient. After the treatment, this protocol maintained efficiently (b_y lowering the testosterone and androstendion), but the differences were not very high. It worth mentioning that at the obese patients in group A, the androstendion and DHEAS concentrations increased a little during Diane 35 treatment[2].

After the therapy ending, the menstrual cycles were become regulated in the first 4 months, at all 19 patients (100%), that continued the study. After this period, it appeared menstrual deviations at 14 patients (73,68%) and amenorrhea at 5 patients (26,31%).

At the obese patients in group A the DHEAS level (androgen in majority of suprarenal origin) increased during the treatment with Diane 35. In the other hand, chortyzol increased in both groups. These are confirmed by glucocortichoid activity proved for CPA at people and animals, but without being observed symptoms of adverse effects of this nature.

Although there was not used any particular method of recording for comparing the adverse effects between the groups, it was recorded no major adverse effects and there were no case of abandoning for these hind of reasons. There were not recorded changes of hepatic enzymes, and the coagulation parameters were not evaluated. Although the treatment with GnRHa was accompanied by estrogenic treatment, no patient reported vaginal mucous membrane atrophy, hot flash, or other hipoestrogenic symptoms[5]. During the treatment, 2 patients (8,33%) presented moderate cephalee, and in group A it was reported occasionally the breast congestion.

DISCUSSIONS

According to previous studies *(Belisle - Love 1986),* a prolonged cyclic treatment (minimum 10, 12 months), with oral contraceptive pills, EE and CPA (Diane 35) is efficient in gonadotrophins and androgens concentrations reduction, in hirsutism, acne, and subjective symptoms amelioration (seborrhea, psychological status, degree of hairiness), at SOP patients.

The introduction of therapy with agonist of gonadotrophins releasing hormone, with prolonged action, (triptorelin, under the form of Diphereline 3,75) in injectable administration (i.m., a bottle at 28 days, on a period of 3 menstrual cycles) improved the clinical results (acne, hirsutism, amenorrhea, the ovaries morphology) and the subjective methods.

A significant change is the gonadotrophin reduction in a proportion that is higher during the treatment (especially LH reduction). After the treatment, the hirsutism indices and androgens level (testosterone and androstendion) mentained lower at group B, in comparison with group A and on a longer period.

The research studies of triptorelin effects on SOPS revealed the similar results by therapy with standard oral contraceptives, or with GnRH or by their association. In severe cases of SOP that did not answer to an anterior treatment with etynilestradiol - ciproteron acetate it was observed the hirsutism reduction after GnRH administration, mentaining oral contraceptives.

At the utilisation of GnRH without the association of a estro-progestative in hirsutism treatment and hiperandrogenism within SOP[7], it was obtained the complete suppression of hipofizar gonadotrophins, with the consequence of the significant decrease of estradyol and the appearance of a state similar with menopause. This situation results to the simultaneous inhibition of steroids hiperproduction, proved by significant decrease of 17-OH-progesterone, androstendion and testosterone concentrations[5]. The treatment formula with a GnKH in monotherapy presents serious adverse effects: hot flash, changes in mood (depression), irritability, vaginal mucous membrane atrophy, the libido decrease, as well as a significant decrease in bone density. These are not appearing when is added a substitutive therapy (estroprogestative or oral contraceptives with etynilestradiol - ciproteron acetate).

The strategy of avoiding or iminimalizing the adverse effects recommends the use of this long time medication not only in SOP treatment, but also represents an advantage in other indications of GnRH - precocious puberty, endometriosis, mammary neoplasm.

During pregnancy it is used no therapeutically antiandrogen or antigonadotrop manevrous, with the mention that at the appearance of a pregnancy immediately after such a treatment, there is no pathology induced to conception product, moreover as both medications present also the indication of anovulatory infertility treatment (by a major effect of rebound hopotalamo-hipofizar, that determines the increase of fecundity rate, in the following months after the treatment ending)[9].

This study shows that the introduction of a agonist GnRH - Diphereline 3,75 - in the treatment of polychystic ovary improves the therapeutically result (clinical and hormonal) realised by use of oral contraceptives (decrease LH, 17-OH-progesterone, androstendion and testosterone), without the appearance of adverse effects and, additionally, it mentains on a longer term the gained benefit[8].

In spite of all these, at 6 months from the therapy ending, the endocrin, clinical and ultrasonografic profiles of SOP reappeared, proving the genetic origin, probably on a family line, of this syndrome.

The combination EE/CPA is efficient on androgen symptoms, by their capacity to reduce LH concentrations and LH-dependent androgens. In addition, by its estrogenic component, it increases the SHBG and IGFB-1, with the result of reducing the serial concentrations, of biodisponibility and IGF-1 and free testosterone activity. The insuline/ IGF-1 system acts sinergically with LH in sense of androgen production stimulation at tecal level. The low androgen and IGF-1 concentrations reduce the activity 5α-R and consecutively, the DHt production in sensitive tissues. In addition, IGF-1 stimulates the increase and the development of pilosebaceous units. Estrogens, on the whole, and also, the progesterone's of oral contraceptives, inhibit the 5α-R activity in the skin and contributes to the clinical impact of oral contraceptives on hirsutism.

The oral contraceptives have the priority to decrease the suprarenal androgen production. Particularly, CPA component, with the moderate glucocorticoid activity, reduces significantly the DHEAS levels, probably by an inhibit effect on the secretion of adrenocorticotrop hormone and/or by a direct effect on adrenal cortex. In this study, RHEAS decreased progressively and significantly, much more then other androgens. It is possible that EE/CPA pill, by its mechanism, to inhibit intensenly 5α-R1 (predominant in sebacee glands) then 5α-R2 (predominant in pilous follicles).

There is the possibility that in sebaceous glands and pilous follicles to be different quantities of androstendion, testosterone or DHT. The acne and/ or hirsutism could be induced concomitantly or separately, depending on the prevalent androgen and on individual tisular sensibility. Beyond these, the sebum production proved to be reduced at the subjects that have been exposed intensely to androgens, but not also to those with congenital deviance of 5α-R. Some data sustain the idea that sebaceous glands are sensitive at androstendion and/or testosterone, and the pilous follicles, at DHT[7]. In fact, it was proved that the androstendion and its metabolites have the property to stimulate selectively the sebaceous cells multiplication and the production of sebum in higher degree then the testosterone. In conclusion, it is possible that sebaceous glands can use directly androstendion, without the conversion in testosterone.

Because the cyclic prolonged treatment with oral contraceptives EE-CPA is an efficient formula and presents the advantage of a antiandrogenic action offered by CPA on hirsutism and, taking in consideration that addition of a GnRH analogue, adds improvements of the results, but it is more expensive, it seems that such an association is not justified at all SOP cases[10]. The association GnRH with an action of long duration can be recommended at the patients with severe obesity and hirsutism.

CONCLUSION

1. The use of Diphereline 3,75 at the patients diagnosed with polychystic ovary symptom represents the most efficient therapeutically variant, aspect that had been proved by clinical, hormonal and morphological data, even if the differences toward the gained results with Diane 35 are not very high. In addition, this formula permits the maintenance of the gained results on a longer term then other well-known therapies.
2. The therapeutically formula that includes Diphereline 3,75 more complex and very expensive in comparison with monotherapy with Diane 35, is justified in severe cases of hirsutism and/or obesity and in those refractory to the traditional treatment.
3. The compliance at the treatment improved by the absence of significant adverse effects and by the increased psychological motivation of the patients.
4. The use of therapy with agonists of GnRH presents an important advantage by fertility stimulation, taking in consideration that the majority of SOP patients' present amenorrhea and infertility.

REFERENCES

1. Acien P., Mauri M., Gutierrez M., Clinical and hormonal effects of the combination gonadotrophin-releasing hormone agonist plus oral contraceptive pills containing ethinyl-oe;[radial (I-l: and cyproterone acetate (CPA) versus the EE=CPA pill alone on polycysdc ovarian disease-related hyperandrogenisms. Hum. Reprod. 1997, 12,423-429.
2. Andreyko J.L., Monroe S.E. and Jaffe R.B., Treatment of hirsutism with a gonadotrophin-releasing hormone agonist (Naferelin). J. Clin. Endocrinol. Metab., 1986 63, 854-859.
3. Belisle S. and Love E.J., Clinical efficacy and safety of cyproterone acetate in severe hirsutism: results of

a multicentered Canadian study, Fertil.Steril. 1986, 46, 1015-1020.

4. Carr B.R., Breslau NA., Givens C. et al. Oral contraceptive pills, gonadotropin-releasing hormone agonists, or use in combination for treatment of hirsutism: a clinical research center study. J. Clin. Endocrinol. Metab.1995, 80, 1169-1178.
5. Chang R.L., Laufer L.R., Meldrum D.R. et al. Steroid hormone secretion in polycystic ovarian disease after ovarian suppression by a long-acting gonadotrophin-releasing hormone agonist. *J.* Clin. Endocrinol. Metab. 1983, 56, 897-903.
6. Ciotta L., Cianci A., Giuffrida *G.* et al. Clinical and hormonal effects of gonadotropin-releasing hormone agonist plus an oral contraceptive in severely hirsute patients with polycystic ovary disease. Fertil.Steril. 1996, 65, 61-67.
7. Falsetti L. and Galbignani E. Long-term treatment with the combination ethinylestradiol and cyproterone acetate in polycystic ovary syndrome. Contraception, 1990, 42, 611-619.
8. Goni M., Markussis V and Tolis G. Efficacy of chronic therapy with the gonadotrophin-releasing hormone agonist Diphereline in patients with polycystic ovary syndrome. Hum. Reprod. 1994, 9, 1048-1052.
9. Morcos R.N., Abdul-Malak M.E. and Shikora E. Treatment of hirsutism with a gonadotropin-releasing hormone agonist and estrogen replacement therapy. Fertil.Steril. 1994, 61, 427--431.
10. Steingold K., De Ziegler D., Cedras, M. et al. Clinical and hormonal effects of chronic gonadotrophin-releasing hormone agonist treatment in polycystic ovarian disease. J Clin Endocrinol. 1987, 65, 773-778.