Use of GnRH antagonists in ovarian remnant syndrome experimentally induced in rats

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Summary

Purpose: The objective of this study was to demonstrate the efficacy of cetrorelix, a GnRH antagonist, in rats with experimentally induced ovarian remnant syndrome. *Methods:* 25 Wistar female rats at seven to eight weeks of age and weighing 200-250 g were used. The rats were randomly divided into five groups: the first group was used as a control group; the second and third groups underwent a sham operation; and the fourth and fifth groups underwent bilateral hemiovariectomy. At the first proestrus detected by vaginal cytology from postoperative day 2, the animals in groups 1, 2 and 5 received placebo and the animals in groups 3 and 4 received cetrorelix subcutaneously. In the study, the Kruskal-Wallis analysis of variance was used for comparison of the results of vaginal irrigation, histopathological examination, and of blood FSH and LH values, and the Mann Whitney U-test was used for determination of the differences between the groups. *Results:* It was determined that according to vaginal cytology results, estrus-like cytological changes disappeared in a shorter time and according to histopathology results, the number of follicles were fewer in the ovarian remnant syndrome-induced and cetrorelix-injected group 4 ($p < 0.05$), but there was no difference between the groups for FSH and LH concentrations. *Conclusions:* Ovarian remnant syndrome is a complication of bilateral ovariohysterectomy. In cases with this syndrome, certain treatment is possible with re-operation. However, it may not always be possible to perform an operation, or even if operated, it is difficult to determine the place of the residual ovarian tissue. In this study, it was determined that the use of cetrorelix as a GnRH antagonist in rats with ovarian remnant syndrome reduced the duration of estrogenic affect.

Key words: GnRH antagonist; Ovarian remnant syndrome; Rat.

Introduction

GnRH analogues are derived by deletion of the primery structure of GnRH or by shifting the location of one or more amino acids. These analogues include GnRH agonists and antagonists. GnRH antagonists bind to GnRH receptors in the hypophysis with a great affinity; however, they cannot cross-bind to the GnRH receptor and thus cannot induce calcium-mediated gonadotropin release. Consequently, they depress the LH secretion in a short time, strongly and reversibly [1-4].

Up to date, three different types of GnRH antagonists have been developed. The first two are not much used as they cause local and systemic reactions by inducing histamine release. Third generation GnRH antagonists induce a lower release of histamine while strongly depressing ovulation. Cetrorelix and ganirelix are the most frequently used third generation antagonists. The effects of GnRH antagonists on LH release and ovulation have shown variation according to dose and sexual stage in studies on both women and rats [4-8].

Ovarian remnant syndrome is a complication of ovariectomy and ovariohysterectomy, which occurs when functional tissue of the ovary remains after the operation. Breed, race, age, physical condition of the female, difficulty of the operation, or the experience of the surgeon are not related with the occurrence of this syndrome. The most important symptom of ovarian remnant syndrome is the continuous estrus activity in animals and menstrual activity in women following ovariectomy and ovariohysterectomy. This syndrome is important in females because follicular activity can show an increase in the residual ovarian tissue, consequently resulting in follicular cyst development, which will lead to an increase in the estrogen concentration that will cause pelvic pain, vaginal bleeding and more importantly, aplastic anemia in bitches [9-11].

The objective of this study was to determine whether GnRH antagonists can be used as a temporary treatment alternative in the termination of symptoms related to ovarian remnant syndrome.

Materials and Methods

Animals

In this study, 25 Wistar female rats of at seven to eight weeks of age and weighing 200-250 g were used. The animals were obtained from the Experimental Research Center of the Faculty of Medicine of Firat University. Throughout the study, the animals were housed in cages, each containing five rats; a 12-hour dark and 12-hour light regime was followed. The animals were given feed and water *ad libitum*. Animals with regular sexual cycles, confirmed by four consequent vaginal irrigations, were used for the experiments.

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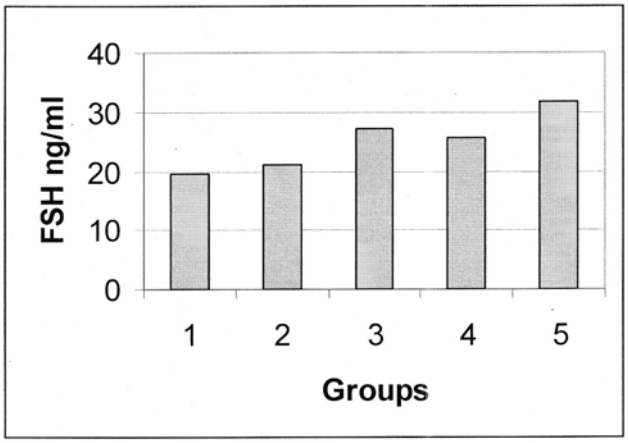

Figure 1. — Distribution of FSH results according to groups (not significant).

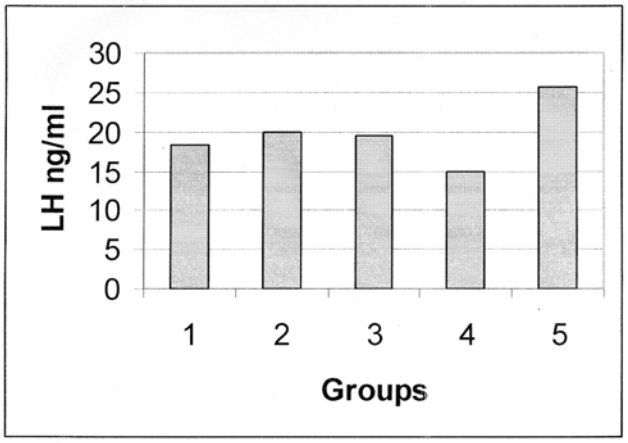

Figure 2. — Distribution of LH results according to groups (not significant).

Operations

Rompun (10 mg/kg IM) – ketalar (90 mg/kg IM) anesthesia was induced during the operations and the animals were randomly divided into five groups.

The animals in the first group (n = 5) were selected as the control group and did not undergo any operation.

Animals in group 2 (n = 5) and group 3 (n = 5) underwent the sham operation.

Animals in group 4 (n = 5) and group 5 (n = 5) underwent bilateral hemiovariectomy

Treatment protocol

At the first proestrus detected by vaginal cytology carried out as of postoperative day 2, the animals in groups 1, 2 and 5 received placebo, and the animals in groups 3 and 4 received cetrorelix (Cetrotide, Serono, Istanbul Turkey) subcutaneously.

The dose of cetrorelix was 0.46 mg/kg/single administration [12].

Vaginal irrigations

The sexual cycles of all animals were followed by Giemsa-stained preparations prepared from the vaginal irrigations taken in 4-hour intervals following the injections until the estrus signs disappeared cytologically. The intensities of cell types in the prepared samples were assessed as +, + +, + + + [13].

Histopathological Examination

The residual ovarian tissues of rats sacrificed by decapitation at the cytological start of diestrus were sent to the laboratory in 10% buffer formaldehyde. Following the routine tissue procedures, tissue sections with 4 µg thickness were prepared and stained with hemotoxylin-eosin for histopathology examinations. The preparations were examined for the presence of follicles under magnification (40 x) of an Olympus Bx50 light microscope. The primordial follicles were confirmed by the presence of at least one-fold of cubical follicle cells, while the antral follicles were confirmed by the presence of an antrum within the follicle [14].

Laboratory analysis

Blood samples were obtained from rats at the time when diestrus began cytologically and the sera were analyzed by ELISA for FSH and LH concentrations [15].

Statistical analysis

In the study, the Kruskal-Wallis analysis of variance was used for comparison of the results of vaginal irrigation, histopathological examination, and blood FSH and LH values, and the Mann Whitney U-test was used for determination of the differences between the groups [16]. These statistical analyses were carried out using the SPSS statistical package (Release 9.0, 1999).

Results

Results obtained from the study are presented in Table 1, Figure 1 and Figure 2. According to vaginal cytology and histopathology results, a difference was found among the groups ($p < 0.01$, Kruskal-Wallis analysis of variance), and cytological estrus signs disappeared earliest in group 4 (6.40 ± 1.60 hours) ($p < 0.05$, Mann Whitney U-test). In addition, group 4 had the minimum follicle number according to histopathology results (6.00 ± 1.31 piece) ($p < 0.05$, Mann Whitney U-test). However, no difference was found among the groups for FSH and LH concentrations ($p > 0.05$, Kruskal-Wallis analysis of variance).

Table 1. — *Distribution of results according to groups.*

Group	Cytological estrogenic time (hours)		Follicle (piece)	
	n	$\overline{X} \pm S_{\overline{x}}$	n	$\overline{X} \pm S_{\overline{x}}$
Group 1	5	39.20 ± 5.43^{b}	5	84.00 ± 5.14^{c}
Group 2	5	28.80 ± 4.80^{b}	5	71.00 ± 4.93^{bc}
Group 3	5	8.00 ± 1.79^{a}	5	59.00 ± 7.17^{b}
Group 4	5	6.40 ± 1.60^{a}	4	6.00 ± 1.31^{a}
Group 5	4	25.00 ± 1.00^{b}	4	21.00 ± 4.21^{a}
p		**		**

**: $p < 0.01$; a, b, c: The differences between the means with different letters in the same column were statistically significant ($p < 0.05$).

Discussion

A certain alternative treatment for ovarian remnant syndrome is possible by extirpation of residual ovarian tissue by reoperation. However, difficulty in the determination of residual ovarian tissue is the primary problem in such operations. In addition, in cases where the operation is a

contraindication due to general condition disorders arising from generalized infections, alternative treatments can be applied with medicines such as hCG, megesterol acetate and mibolerone [17-19].

Rivier and Vale [20] have reported that in rats with a one-week pregnancy, GnRH antagonists led to a decrease in LH concentrations, concurrently leading to a decrease in progesterone concentration at a degree that caused abortions following the application in the 7th-12th days of pregnancy. In the present study, the LH concentration in the hemiovariectomized cetrorelix group (group 4) showed a 42% reduction compared to the placebo group. However this reduction was not statistically significant. It has been suggested that in ovarian remnant syndrome, the use of cetrorelix leads to a decreasing tendency of LH concentration.

Although not yet used in ovarian remnant syndrome, the antagonistic effect of ganirelix has been investigated in many studies in both males and females. It has been reported that its administration at a 1.4 ug/kg dose to female rats at the proestrus stage depresses ovulation. Furthermore, it has been suggested that ganirelix administration at doses as high as 0.7-5.0 mg/kg/day depress estrus completely and irreversibly [21]. In the present study, cetrorelix was used for the first time in rats with experimentally-induced ovarian remnant syndrome. According to the vaginal cytology and histology results, it was determined that estrogenic signs in the cetrorelix rat group (group 4) disappeared in a shorter time than in the other groups.

GnRH antagonists can rapidly inhibit the release of FSH and LH in all stages of the sexual cycle. This situation will assist in termination of the negative effects of endogenous estrus by blocking the follicular development in cases with ovarian remnant syndrome where surgery is a contradiction. In addition, termination of antagonist use will lead to an increase in hypophysal activity, which will consequently promote follicular development in the ovaries in cases with ovarian remnant syndrome, which will make the determination of residual ovarian tissue easy during the operation. In conclusion, in the present study, it has been determined that the use of cetrorelix as a GnRH antagonist in rats with ovarian remnant syndrome reduced the duration of the estrogenic effect, reduced the number of follicles in the ovary, and led to a decreasing trend of LH concentrations. In light of the obtained results, more advanced studies are needed on the efficacy and benefits of GnRH antagonists in cases with ovarian remnant syndrome.

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