Experimental
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Clinical Article

Reproductive Medicine; *Endocrinology & Infertility*

Effect of Ovulation Induction on Ovarian Histologies in a Rat Model

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OBJECTIVE: To examine the effect of human menopausal gonadotropin(HMG) and follitropinBeta (rFSH) on the ovarian histologies in a rat model.

STUDY DESING: Thirty-nine female, one-year old rats were enrolled for the trial. They were divided into three groups. In the first group, 13 rats received, six cycles of ovulation induction with human menapausal gonadotropin.In the second group, 13 rats received six cycles of ovulation induction with folitropin beta the third study group consisted of 13 rats which received six cycles of saline intramuscularly.

RESULT: The mean number of the cells that stained positive for Ki-67 was 42.3±20.6, 44.9±27.3and 42.5±24.8 in the folicles, respectively.The mean number of cells that stained positive for Ki-67 in epithelium was 0.15±0.42, 0.04±0.14,0.05±0.18, respectively. The mean dysplasiascore was 2.46±2.10, 1.69±13 and 1.62±1.89 in the ovarian epithelial cells respectively.

CONCLUSION: Development of malignant lesion were not found in any of the rat ovaries after ovulation induction. As a result of this study, we found out that human menapausal gonodotropin and folitropin beta used in treatment of infertility, when administered for six cycles in accordence with the dosage determined, do not have the potential to develop neoplasia in rats.

Key Words: Ovulation induction, Ki-67 expression, Dysplasia score

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Introduction

In the 1980 s, case reports of ovarian canser in women undergoing assisted conception raised cancern about the long-term effects of infertility treatment[1] subseguent epidemiological studies reported positive associations between exposure to fertility drung used to stimulate ovulation and the risk of ovarian cancer.However, more recent studies have not confirmed this association, nor have they found an association and the overall riks of breast cancer.[2]

The Ki-67 antigen, whin is coded by agene on chromosome l0 is expressed in G1, S and G2 phases in cycling cell but not in the resting phase G0. The Ki-67 score partly correlates with other proliferation markers like percentage of S-phase cell and mitotic count.[3,4]

High-grade ovarian carcinomas display higher Ki-67 expression.[5]

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Our purpose in this study is to investigate the effect of ovulation induction on dysplasia score and Ki-67 expression of the ovarian epithelium in a rat model.

Material and Method

With the permission of the local ethic committe of the Ondokuz Mayıs University school of medicine, one-year-old and nearly 160-270 g weiging, 39 sexually mature Wistar-Albino female rats were enrolled in the study.Rats were divided into three groups. In the first group, is rats received six cycle of ovulation induction with follitrop Beta.The third study group consisted of 13 rats which received six cycle of saline intramuscularly (im). Vaginal smears were obtained from bolh groups ever y morning for two consecutive cycles and 5- day cycles were divided in to phases as estrous; diestrous days 1,2,3 and proestrous day. On estrous day 2 of the six cycle, group 1 receved 150 IU7kg HMG im at 5 PM, group 2 received 150 IU/kg rFSH im at 5 PM, group 3 received saline intramuscularly. On the proestrous day ot the third cycle, 75 IU/kg human chorionic gonodotropin (hCG). Was injected im at 5 PM in group 2 and Group 3 received saline injection on the proestrous day of the six cycle at 5 PM. Each ovulation induction cycle was repeated evry 2 weeks in the three groups, given consideration to the fact that their menstruel cycle is 5 day long.

Laparatomy and bilateral oophorectomy were performed in all rats under ether anesthesia.Laparotomy was performed

at the seventh cycle, 2 days after the last human chorionic gonodotropin injection.

Laparatomy with midline incision to all the rats were done under the ketamine hydrochloride anesthesia (2 mlt/kg). The rats were killed after their ovaries were extracted. Ovarian tissue was fixed with 10% formaldehyde fore the histopathological examination. After the parafine blocks were prepared, 5 µm thick cross sections were taken and six cros sections prepared from each ovar y were dyed in hematoxylin and eosin. The preparations were examinned by a pathologist.

In the histopathological examination, the fallowing parameters have been investigated: a malignant lesion, ovarian cyst and its size, staratification of ovary epithelial cells, a local epithelial accumulation(tufting), a mitotic index in granulosa cells, polymorphism in epithelial cells and the chromatin intensity, nuclear atypia in ovarian cyst epithelium, mitotic actctivity, and papilary formation in the ovarian cyst epithelium. The most dysplastic region in the histopathological examination. The size of nucleus was measured with ocular micrometer.

Each inducted group was compared with its control group, the Mann-Whitney U test and chi-square test, Kruskal-Wallis test and Mann-Whitney U-test were used.

Results

In group 1,2,3, the mean number of the cell that stained for Ki-67 was 42.3±20.6, 44.9±27.3, 42.5±24.8 in the follicles.Respectively (p>0.05, Table I).

	Comparison of Ki-67immune reactivity of the folliculer cell in three groups	P Value	Comparison of Ki-67 immune reactivity of the epithelium	P Value
Group 1 HMG	42.3±20.6	>0.65	0.15±0.42	>0.05
Group 2 rFSH	44.9±47.3	>0.05	0.04±0.14	>0.05
Group 3	42.5±24.8	>0.05	0.05±0.18	>0.05

The data are presented as mean±standart deviasyon
**Statistically significant(p< 0.05), Mann-Whitney U test*

The mean number of the cell thet stained positive for Ki-67 in the epitelyumium, were not significantly in the ovulation induction group(0.15±0.42, 0.04±0.14, 0.05±0.18 respectively) compered to the control group p>0.005. The mean dysplasia score in the ovulation induction groups was not significantly(2.46±2.10, 1.69±2.13, 1.62±1.89 P>0.05 respectively)

The distribution of the histologic features of the dysplasia score is presented in table II.

Table II: Distributation of the histologic abnormalities in the three groups.

	Scoring of groups								
	Groups 1 n:13			Groups2 n:13			Groups3 n:13		
Histologic Features	1	2	3	1	2	3	1	2	3
Epithelial multilayerin	6	7	0	8	5	0	9	4	0
Tufting	12	1	0	13	0	0	13	0	0
Nuclear chromatin irregularity	12	1	0	13	0	0	13	0	0
Nuclear contour irregularity	12	1	0	13	0	0	13	0	0
Nuclear size	6	7	0	8	5	0	8	5	0
Nuclear/cytoplasmic ratio	6	7	0	8	5	0	8	5	0
Presence of nucleoli	13	0	0	11	2	0	13	2	0
Presence and number of mitotic figures	13	0	0	13	0	0	13	0	0

0: Normal, 1: Moderate abnormality, 2: Severe abnormality

There was not a positive correlation between the Ki-67 index and the dysplasia(P>0.05). Cell expressing Ki-67 identified in the epithelium, follicle (Fig I.II.III).

The follicular cells in many of the developing folicles of the ovulation induced ovaries not showed intense nuclear immunorecc tivity compared to the controls (Table II).

When number of the Ki-67 stained cells in tree groups are evaluated, results for group 1,2,3 are 1134.54±236.54, 1154.31±246.05 and 922. 15±170.31 respevtively. Not statistical ignificance was found between those three groups in terms of the folicules and surface epitelyumial Ki 67 index, ovarian displastic alteration and the average number of the Ki 67 stained cell. Not statistical significance was found between the individual evalustions of the groups

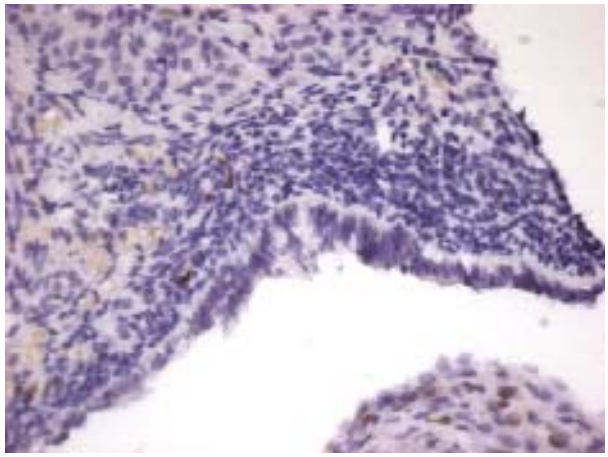

Figure I: Ki-67 İmmunostaining in the overian epitelium of group 1X100 (A), X 200 (B)

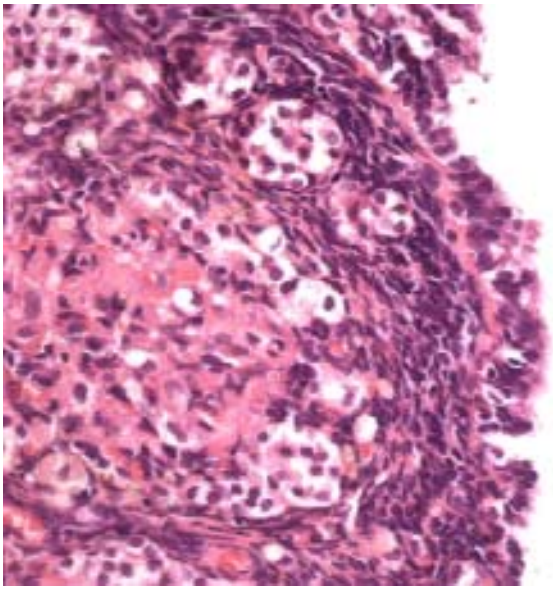

Figure II: Epitelial displasia in the rat ovarian epitelium in group 2. H-E, X 400

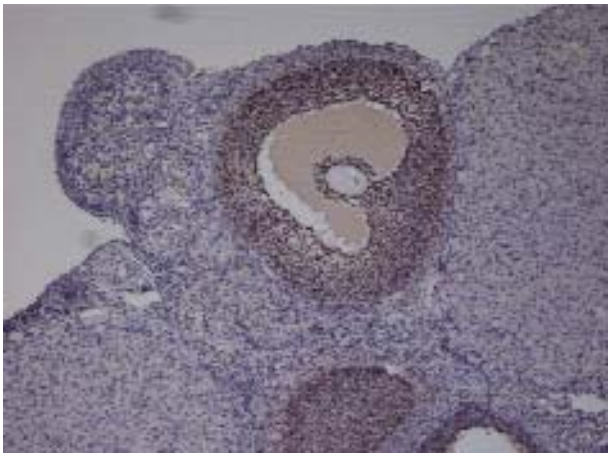

Figure III: Ki67 İmmunostaining in the overian follicle of group 1X100 (A), X 200(B)

Discussion

Currently available data in the literature suggest that an association bettween ovulation induction and ovarian cancer does not indicate necessarily a causal effect infertility alone is an independent risk factor fort he development of ovarian cancer. Nulliparous women with refractor y infertility may harbo a particularly high risk of ovarian cancer, irrepective of their use of fertility drugs. Furthermore, the apparent association between fertility drug use and ovarian cancer may arise because these women are the most likely to have used ovulation stimulating agents as part of their infertility treatment.[6] In 1971, fathalla proposed that the etiology of ovarian cancer was related to "with each ovulation the ovarian surface (epithelium) was thought to incur minor travma.The cumulative effect of repetitive surface injury was hypothesized to contribute to the development of ovarian neoplasm.[6,7]

Preliminary results of an ongoing case-control study in ltaly were published recently and didnot suggest a role for ovulation stimulation in the etiology of ovarian cancer.[6,8]

Gonodotropins were also blamed for inducing ovarian cancer at high an prolongged doses, as used in ovulation induction. Gonodotropins can induce Cyclooxygenase 2 and increease prostaglandin production, whinh in turn casuses the loss of basement membrane of the ovarian surface epithelium and alters the biology of the epithelial cells in cells in cell contact signaling and organization.[3,7] Our study is an experimental study using HMG and rFSH in rats has no noticeable effect on the ovary because the comparison of the groups 2,3 and controls did not show any pre-malign changes.

Corakci et al. Found increased Ki-67 expession in the ovulation induction group with increasing dysplasia score. This finding should be evaluated with caution as the ovaries were examined immediately after six cycles of ovulation induction increases epithelial dysplasia scores and Ki-67 expression in rat ovaries.[3,9]

Celik et al. No malignant ovarian lesion was found in the ovulation induction. Ovarian cyst development was most frequent in the rats that underwent six cycles of ovulation induction.

Significant difference was found between induction and control group for cellular and nuclear polymorphysm, presence of nucleolus, and nuclear chromatin density.[10]

In this study, neither the ovulation inducted rats nor the control group rats were found to have neoplastic lesion (Ki-67 expression and dysplastic features).

The etiology of ovarian cancersi multifactorial, and the genetic, enviromental, and endocrinologic factors or indirectly. Therefore, lange prospective studies consisting of muticulously selected proper control groups are needed.

Ovulasyon İndüksiyonunun Over Histolojisi Üzerine Etkilerinin Rat Modelinde İncelenmesi

AMAÇ: Bu çalışmanın amacı overian histoloji üzerine ovulasyon indüksiyonunu etkilerinin rat modelinde incelenmesidir. Bu amaçla otuz dokuz adet bir yaşında dişi rat kullanıldı.

GEREÇ ve YÖNTEM: Ratlardan üç gurup oluşturuldu. İlk guruptaki 13 rata ovulasyon indüksiynu için altı siklus human menapozal gonodotropin ikinci guruptaki 13 rata 6siklus follitropin beta verildi. Üçüncü guruptaki 13 rata intramuskuler olarak altı siklus salin uygulandı.Altı siklus sonra bilateral ooferektomi uygulandı.

BULGULAR: Over epitelinin displazi scoru ve Kİ-67 ekspresyonu incelendi.Ortalama olarak folliküllerde Ki-67 pozitif saptanan hücre sayıları sırasıyla 42.3±20.6, 44.9±27.3 ve

42.5±24.8 olarak bulundu. Epitelde Ki-67 pozitif saptanan hücre sayıları ortalama olarak sırasıyla 0.15±0.42, 0.04±0.14, 0.05±0.18 olarak saptandı. Over epitelindeki ortalama displazi skoru sırasıyla 2.46±2.10, 1.69±13 and 1.62±1.89 olarak saptandı. Ovulasyon indüksiyonu sonrasında hiçbir rat overinde malign lezyon gelişimi saptanmadı.

SONUÇ: Rat modelinde altı siklus boyunca tanımlanan dozlarda kullanılan human menopozal gonodotropin ve folitropin beta neoplazi gelişimi için potansiyel sebep oluşturmamıştır.

Anahtar Kelimeler: Ovulasyon indüksiyonu, Ki-67, Displazi skoru

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