The Effect of Bilateral Salpingectomy and Tubal Ligation on Bone Mass

BLATERAL SALPENJEKTOMİ VE TUBAL LİGASYONUNUN KEMİK KÜTLESİ ÜZERİNE ETKİSİ

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Abstract

Objective: To evaluate the effects of bilateral salpingectomy and tubal ligation on bone mass, in a rat model.

Material and Methods: Forty-eight female Wistar-albino rats weighing 200-250 g were divided equally into 3 groups underwent laparotomy, while no specific intervention was made to the 1. group. Bilateral tubal ligation by Pomeroy's technique, bilateral salpingectomy was done to the 2. and 3. groups, respectively. Tissue samples taken from lumbar vertebrae and femoral neck were analysed to find out trabecular bone volume (TBV) in these study groups**.** TBVs were calculated according to the ratio of trabecular bone area to total bone area in X100 power field microscope area for each slide. Statistically, Kruskal-Wallis test was performed to analyse differences and correlations between groups.

Results: No statistical difference was found in TBV values of lumbar vertebra and femur neck in laporotomy, tubal ligation and salpingectomy groups (p= 0.119 for vertebrae, p= 0.625 for femur neck).

Conclusion: Our findings suggest that tubal ligation or salpingectomy does not have an increased short term risk for osteopenia.

Key Words: Salpingectomy, tubal ligation, osteopenia

Özet

Amaç: Bilateral salpenjektomi ve tubal ligasyonunun kemik kütlesi üzerine etkisinin bir sıçan modelinde incelenmesi.

Gereç ve Yöntemler: Ağırlığı 200-250 g arasında değişen ve laporotomi uygulanan 48 dişi Wistar-albino sıçanı eşit olarak 3 gruba ayrıldı. İlk gruba, laporotomi dışında bir işlem uygulanmadı. İkinci gruba, Pomeroy tekniği ile tüp ligasyonu, 3. gruba bilateral salpenjektomi uygulandı. Lumbar vertebra ve femur boynundan alınan doku örnekleri analiz edilerek trabeküler kemik hacmi hesaplanmıştır. Trabeküler kemik hacmi 100 büyütmelik ışık mikroskobunda her sahada trabeküler kemik alanının total kemik alanına oranına göre hesaplandı. Gruplar arasındaki farkın ve korelasyonların analizi için Kruskal-Wallis testi uygulandı.

Bulgular: Laparotomi, tüp ligasyonu ve salpenjektomi grupları arasında lumbar vertebra ve femur boynu total kemik hacmi değerleri açısından istatistiksel açıdan anlamlı bir fark bulunmadı (p= 0.119 vertebra için, p= 0.625 femur boynu için).

Sonuç: Bulgularımız, tüp ligasyonu veya salpenjektomi sonrası osteopeni açısından kısa dönem bir risk olmadığını göstermektedir.

Anahtar Kelimeler: Tüp ligasyonu, salpenjektomi, osteopeni

Turkiye Klinikleri J Gynecol Obst 2006, 16:6-11

Estrogen deficiency is one of the major risk factors in the pathogenesis of osteoporosis. Despite the popular use of tubal ligation, there is continuing debate that tubal sterilization may be associated with increased long-term risks of menstrual symptoms including heavy and irregular bleeding, cramps most probably due to anatomical and hormonal changes following this procedure, particularly progesterone deficiency.[1-4]

The decreased ovarian reserve may in turn lead to bone loss as demonstrated by some investigators.[5-7] Osteopenia due to tubal ligation was found to be more evident in trabecular bone.[5] Although not statistically significant, low axial bone density was detected in another study involving women with bilateral partial tubal resection.[6]

Geliş Tarihi/*Received:* 11.08.2005 **Kabul Tarihi/*Accepted:*** 14.11.2005

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Subsequent to negative findings for endocrine sequelae of salpingectomy, we aimed to investigate if fallopian tube contributes in any endocrine way to ovulation regulation and lead to osteopenia in an experimental rat model.

Material and Methods

Forty eight female Wistar-albino rats, each weighing 200-250 g and 3 months old, were used for the study. The rats were randomized into 3 groups, each consisting 16, based on their body weights, so that the mean body weight of each group was comparable. All groups underwent laporotomy. While no specific intervention was made to the 1. group. Bilateral tubal ligation by Pomeroy's technique, bilateral salpingectomy was performed to the 2. and 3. groups respectively. The rats were housed in wire cages, maintained at 22.2 ± 1.7 C on a 12 h light, 12 h dark cycle and had access to food [TekVad Diet,TD89222 (0.5% calcium and 0.4% phosphorus), Tek Vad Madison,WI] and water ad libitum. This research protocol was approved by the Akdeniz University Animal Care and Use Committee.

Following induction of general anesthesia by intraperitoneal ketamine HCl 40 mg/kg and Xylazine HCl 50 mg/kg, abdominal skin was shaved and antisepsis was obtained by 10% povidone iodine solution. Laparotomy was performed via 3 cm mid-line vertical incision. After then, no special intervention was made to the 1. group, whereas bilateral tubal ligation by Pomeroy's technique surgically 1cm away from both uterine horns using 2/0 silk was performed to the 2. group. The animals in the 3. group underwent bilateral salpingectomy 1 cm away from both uterine horns using 2/0 silk. In both tubal procedures, careful microsurgical techniques were used, so as not to interfere with ovarian vasculature, only the vasculature supplying the fallopian tubes was severed.

Peritoneal cavity was then closed en bloc with 2/0 catgut. Abdominal skin was closed en bloc with 2/0 silk. The total duration of laparotomy was 10 min for every individual rat, including the ones in the 1. group. The rats were observed for 3 months, and then sacrificed in estrus phase of the menstrual cycle by heart puncture under sodium pentobarbitital anesthesia (40 mg/kg).

From each rat, femur and two lumbar vertebra (L4-L5) were obtained. All adhering tissue was carefully removed from the femur and vertebrae using a scalpel and scissors. The vertebrae were then seperated through the discs with a scalpel.

Tissue samples taken from lumbar vertebrae and femoral neck were analysed to find out trabecular bone volume (TBV) in different groups. Before decalcification, fresh bone samples were treated with following steps, respectively; treatment with AgN03 (3%) for 24 hours, washing with distilled water 3 times (totally 3 minutes), holding in flowing water for 2-3 hours, treatment with formalin (10%) for 20 hours, holding in flowing water for an hour, treatment with Na2S2O3 (5%) for 4 hours, holding in flowing water for 2-3 hours. Finally, samples were decalcified by applying formic acid (10%) for 20 hours. After that, tissue samples were blocked with paraffin and 5 μm thick slides were stained with hematoxilen & eosin. Sections were evaluated histomorphometrically using SAMBA 2000 image analysing system. TBVs were calculated according to the ratio of trabecular bone area to total bone area (trabecular bone area + bone marrow area= total bone area= 691217.43μ²) in X 100 power field microscope area for each slide. The area measured was defined by the cortical bone on both sides and by a line 1 mm inside the epiphyses at each end of the vertebral bodies (the primary spongiosa was thereby avoided).

The results of the descriptive statistics were expressed as mean ± standard deviation. The level of significance was set at $p< 0.05$. Analysis of variance was used for the comparison of basal and final body weight values within the same group. One-way analysis of variance was used for the comparison of the basal and final body weights of the three groups. Statistically, Kruskal-Wallis test was performed to analyse differences and correlations between groups.

Results

Baseline and final body weights are summarized in Table 1. There were no differences be-

Tablo 1. Weight and age of the sham-operated, tubal ligated and salpingectomized groups.

Group	n	months	Weight (g)	p* value	p** value
Sham operated	16	3 Month	187.47 ± 0.34	0.89	0.98
		7 months	190.51 ± 0.18		
Salpengectomized	16	3 Month	192.51 ± 0.23	0.58	0.75
		7 months	189.43 ± 0.15		
Tubal ligated	16	3 Month	189.47 ± 0.31	0.91	0.96
		7 months	187.41 ± 0.15		

*Analysis of variance for within group differences,
**One way analysis of variance for between group differences.

tween these groups. As far as weight assessment is concerned, there was no difference between baseline and final body weight.

After the first laparotomy, the postoperative period was normal in all rats and no notable complications were seen in any of them. All rats in 3 groups completed the study. Endometrial phases of all rats in study groups were in accordance with the estrus phase.

Mean and median TBV values are demonstrated in Table 2 (Figure 1). No statistically significant difference was observed between TBV values of the 3 groups.

Discussion

The possibility of reduction in ovarian reserve after tubal occlusion or salpengectomy is of great importance since female sterilization is a widespread form of birth control throughout the world and salpengectomy is a well established management in infertile patients with hydrosalpinx or previous ectopic pregnancy.

Radwanska and Donnez have reported that after coagulation or Pomeroy sterilization subsequent ovarian function is altered as measured by midluteal progesterone levels in sterilized humans.[7] Alvarez-Sanchez et al found no difference in the progesterone levels but lower preovulatory LH and 17β estradiol peaks in sterilized women as compared to normally menstruating women.[8,9]

Either sterilization itself or the method of sterilization has been considered to interfere with ovarian function, which is determined by hormonal assays and/or endometrial dating.[10,11] Corson and Levinson found no correlation between midluteal progesterones in two cycles after hysterectomy or tubal ligation by Pomeroy coagulation or in normal women.[9]

Beyth et al noted decreased number of corporea lutea in rabbits after partial salpingectomy.[12] Halme et al found no difference in salpengectomized rats vs normal rabbits in the number of the corporea lutea subsequent to mating or in progesterone levels.[13]

There has also been debate on simple hysterectomy to affect ovarian function. Most studies of the short-term (1-12 months) effect of hysterectomy on ovarian function have not found evidence of altered steroidal hormone production.[14-16] In some small studies involving small selected samples or measures difficult to assess objectively, hysterectomy with ovarian cryopreservation was found to be associated with ovarian failure relying on menopausal symptoms.[17]

The significance of salpingectomy in the outcome of IVF is also controversial. Romeu et al found a detrimental effect, Whereas Oehninger et al and Verhulst et al did not.[18-19] Lass et al mentioned that there was no detrimental effect of salpingectomy on the total ovarian performance during IVF-ET treatment or on the outcome of IVF-ET and pointed out that ipsilateral ovary could be adversely affected.[20] They suggested to perform salpingectomy only in selected patients, in whom the second ovary is not compromised or missing.[20]

Tablo 2. Trabecular bone volume(TBV) of the sham-operated, tubal ligated and salpingectomized groups.

	Mean±SD	Median	p* value
Sham operated group TBV (vertebra)	1.11 ± 0.13	0.13	0.119
Salpingectomized group TBV (vertebra)	1.39 ± 1.46	0.4	
Tubal ligated group TBV (vertebra)	1.3 ± 1.09	0.2	
Sham operated group TBV (femur)	0.13 ± 0.08	0.2	0.625
Salpingectomized group TBV (femur)	1.73 ± 2.11	0.1	
Tubal ligated group TBV (femur)	1.15 ± 1.7	0.2	

*Kruskal-Wallis test was performed to analyse differences and correlations between groups.

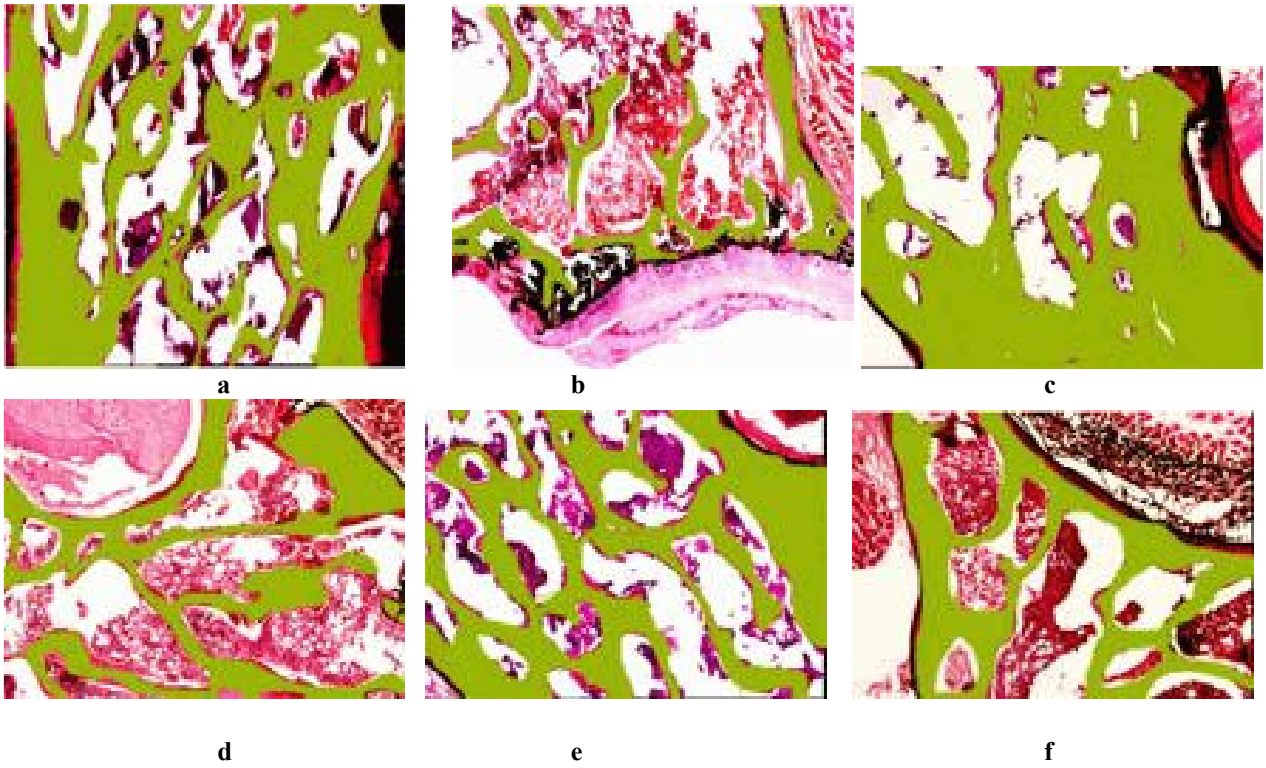

Figure 1. Trabecular bone volume (TBV) was handled according to the ratio of trabecular bone (green areas) to total bone area (trabecular bone + bone marrow) in X100 power field microscope area. H&E X50. **a.** Femur of the sham operated rats; **b**. Lumbar 4 vertebra of the sham operated group. **c.** Femur of the salpengectomized group. **d.** Lumbar 4 vertebra of the salpengectomized rats. **e.** Femur of the tubal ligated rats. **f.** Lumbar 4 vertebra of the tubal ligated group.

We believe the reduction in ovarian reserve may be explained by two possible mechanisms, either a decreased level of response of ovaries (i.e. increased threshold) to tropic hormones or a diminished ovarian reserve. The first possible mechanism may involve feedback signals transmitted from uterus to ovaries by means of paracrine, endocrine factors or nervous stimuli that may be hampered by the procedure of sterilization. These signals may constitute a component in determining the level of response of ovulatory cohort of follicles to tropic hormones and consequently ovulation. The interruption of the signals after sterilization may cause a decrease in ovarian response, resulting in a diminished number of tertiary follicles.

The second mechanism, i.e. diminished ovarian reserve may result from partial interruption of ovarian perfusion by sterilization. Although any gross change in uterine or ovarian arterial flow has not been reported by Doppler flowmetry following sterilization in humans, microvascular perfusion changes may occur in ovarian blood flow, as is shown for endometrium. [21,22] Such a reduction in ovarian perfusion may decrease ovarian reserve in rats, in medium to long-term. Tubal ligation might cause a similar impairment of ovarian blood flow to that of salpingectomy, in rats.

Subsequent to negative findings for endocrine sequelae of salpingectomy, the decreased ovarian reserve might in turn lead to osteopenia. Among the limited number of animal studies performed to investigate bone loss after sterilization, there is paucity of data regarding rat model.

There are conflicting reports regarding the effects of female sterilization on bone mass, in humans. Rico et al found that ligation of the uterine horns induced bone loss in the axial skeleton (vertebra) and peripheral skeleton (femur) similar to that observed with ovariectomized rats.[1,2,23]

Previous studies of women with tubal ligation or bilateral tubal resection have not shown peripheral bone loss in single photon absorbtiometry of the distal and proximal radius and os calcis or in DXA of the spine or hip.[6,24]

Our study failed to demonstrate any disruption in bone mass after bilateral salpingectomy or bilateral tubal ligation.

The bone disorders that appear after tubal ligation or similar techniques seem to be dependent mainly on the technique used and its effect on the ovary. In the present study, the microsurgical removal of the fallopian tube was designed to be performed carefully so as not to interfere with the blood supply of the ovary.

The rat model may not constitute an ideal model to study the possible effects of sterilization on ovarian function in humans because of the differences that exist between two species regarding reproductive physiology such as cycle length (long versus short) or ovulatory pattern (monoovu-latory versus polyovulatory) or less intracortical remodelling.[25-28] However, our study still constitutes a model to demonstrate the effects of sterilization and salpengectomy on bone mass in rats.

Our findings suggest that tubal ligation does not have an increased short term risk for osteoporosis. The presence of the reduced ovarian response/reserve and in turn leading to osteopenia due to sterilization or salpengectomy need further investigation.

Acknowledgement

We would like to thank Erdem Karabulut for his assistance in statistical analysis of our data.

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