Cukurova Medical Journal

Araştırma Makalesi / Research Article

Ovarian Autografting in Rabbits: Hormonal and Morphological Evaluations.

Tavşanlarda Overyen Otograft: Hormonal ve Morfolojik Değerlendirme

M. Turan Çetin[1]; İbrahim Ürünsak[1], Abdulhakim Arısoy[2]; Cihan Çetin[3], Taner Kafadar[3], İlhan Tuncer[4]

[1]Cukurova University Faculty of Medicine, Obstetrics and Gynecology Department, ADANA

[2]Obstetrician and Gynecologist, State Hospital, ADANA

[3]Obstetrician and Gynecologist

[4]Pathology Department, Medical Faculty of University of Çukurova, ADANA.

Cukurova Medical Journal 2013; 38 (3):403-411.

ABSTRACT

Purpose: The functional and histological changes in the ovarian tissue of 10 female mature rabbits were evaluated before and after ovarian autografting.

Material and Methods: Preoperative (one month before the autograft) and postoperative (two months after the autograft) estradiol (E2) and progesterone (P) levels were measured, and histological evaluations were performed by light microscope. One month later, ovarian autografting was performed on the rabbits.

Results: Ovarian tissue was observed in 80% of the transplanted ovarian samples. Both preoperative and postoperative P values increased from the basal to the late period and the differences were statistically significant. There was no significant difference between preoperative and postoperative E2 values from the basal to the late period. Preoperative P values were statistically significantly higher than postoperative P values in all three time periods. Preoperative E2 values were also higher than postoperative E2 values, but the differences were not statistically significant.

Conclusion: If autografting is performed by employing microsurgical techniques, ovarian autografts will have similar postoperative hormonal function and morphology as in preoperative period.

Key Words: ovarian autograft, ovarian hormonal function, ovarian morphology.

ÖZET

Giriş: 10 adet erişkin dişi tavşanda otograft öncesi ve sonrası ovaryumda oluşan fonksiyonel ve histolojik değişiklikler değerlendirildi.

Materyal ve Metod: Preoperatif (otografttan bir ay önce) ve postoperatif (otograftan iki ay sonra) östradiol (E2) ve progesteron (P) seviyeleri ölçüldü, akabinde histolojik değerlendirmeler ışık mikroskobuyla yapıldı. Bir ay sonra, tavşanlarda ovaryum otograftı yapıldı.

Sonuçlar: Ovaryum dokularının %80'inde transplante ovaryum örnekleri gözlendi. Preoperatif ve postoperatif P değerleri bazaldan geç periyoda doğru artış gösterdi ve farklar istatistiksel olarak anlamlıdır. Bazaldan geç periyoda doğru preoperatif ve postoperatif E2 değerleri arasında istatistiksel olarak anlamlı bir fark yoktur. Her üç zaman periyodunda da preoperatif P değerleri postoperatif P değerlerinden istatiktiksel olarak anlamlı şekilde yüksektir. Preoperatif E2 değeri postoperatif E2 değerine göre yüksektir fakat bu fark istatistiksel olarak anlamlı değildir.

Tartışma: Eğer otograft işlemleri mikrocerrahi yöntemleri kullanılarak gerçekleştirilirse, postoperatif periyottaki ovaryum otograftları preoperatif periyotta olduğu gibi hormonal fonksiyon ve morfoloji gösterirler.

Anahtar Kelimeler: Ovaryum otograftı, ovaryum hormonal fonksiyonu, ovaryum morfolojisi.

INTRODUCTION

In women requiring pelvic radiation, the implantation of the ovarian tissue from its normal anatomic place to another may be necessary to prevent any possible damage to ovaries due to radioactivity. Therefore, experimental studies were started to be conducted on animals (1,2,3). The most important problem faced in ovarian autografting is vascularization and refunctioning of the ovary. In this experimental study, hormonal functions, neovascularization and follicular growth were investigated in autografted ovaries.

MATERIALS and METHODS:

10 female mature rabbits were included in this study. The rabbits were separated into different cages one month before the study to eliminate any possible pregnancy. The rabbits' mean age was 10 (6-18) months and their mean weight was 3000 (2500- 3500) grams. Rabbits were chosen as subjects of this experiment because of the ease in inducing ovulation with gonadotropins at any time in these animals. For the hormonal studies, blood samples (5 ml) were taken from the rabbits' ear vein (preoperative basal value). Immediately after, 100 IU HCG (Pregnyl, Organon) I.M. was injected. 5ml. blood samples were taken from the rabbits' ear vein 2 hours after (preoperative early value) and 24 hours after (preoperative late value) the injection. The levels of estradiol (E2) (pg/ml) and progesterone (P) (ng/ml) were measured in these blood samples by the radioimmunoassay (RIA) method. Approximately one month (25-35 days) after taking the blood samples for hormonal evaluation, the same rabbits were operated for ovarian autografting. In this operation, Ketamin HCL (20 mg/kg) was injected I.M. as an anesthetic. Hair on the abdominal region of the rabbit was shaved in supine position. 10 ml of prilocaine 0.5% solution was injected under the skin through the median vertical line on which the incision was later performed. The abdomen was then opened with a median vertical incision. The intestines were moved away from the genital organs. The ovaries were carefully separated from the fimbrias and the ovarian arteries were ligatured with 5/0 vicryl. After the ovaries were separated from the mesosalpinx, they were grafted onto the iliac psoas muscle with 7/0 vicryl in such a way enabling touching the peritoneum. The parietal peritoneum was then sutured with 3/0 vicryl , fascia and skin were sutured with 0/0 silk sutures.

Approximately 2 months after the autografting, 5 ml of blood was taken from each rabbit for postoperative hormonal evaluation (postoperative basal) and immediately following this, 100 IU HCG (Pregnyl, Organon) I.M. was injected. 2 hours (postoperative early) and 24 hours (postoperative late) later, 5 ml of blood was retaken. E2 and P values were measured in these blood samples by the radioimmunoassay (RIA) method. Oophorectomy was then performed on the rabbits duringthe second laparotomy by employing the same operation and anesthesia techniques for grafting. The ovarian graft tissues were evaluated histopathologically.

In this operation, intra-abdominal organs were examined for adhesion. The macroscopic appearance of the ovaries which underwent grafting was evaluated for adhesion and follicular growth. Velamentous adhesion on the psoas peritoneum was regarded as a minor whereas the covering of the surface of ovaries with the peritoneum was considered as a major adhesion. Oophorectomy was later performed with a sharp dissection. The ovaries which were taken out were fixed in Bouin's solution for 4 to 12 hours. Then, 4 micron paraffin tissue sections were stained with Hematoxylene-Eosine and were examined by the same pathologist using a Nikon E600 light microscope. Microphotographs were taken using a Nikon NFX-35 camera. The hormonal values

were compared statistically by utilizing the "repeated measure of analysis of variance" method. Preoperative basal and postoperative basal, preoperative early and postoperative early, preoperative late and postoperative late values of E2 and P were analyzed with the "matched t-test". The guidelines for the care and use of animals approved by the Cukurova University and were followed throughout the experiments.

RESULTS

During the laparotomy which was performed 2 months after the autotransplantation, 8 of the 20 ovaries (40%) were found to be smaller. Macroscopic and/or microscopic follicular growth was seen in 12 (60%) of the 20 ovaries. (Fig.1). No adhesion was seen on the surrounding organs in any of the rabbits. Minor adhesions were found in 40% and major adhesions were found in 50% of the transplanted ovaries (Table I).

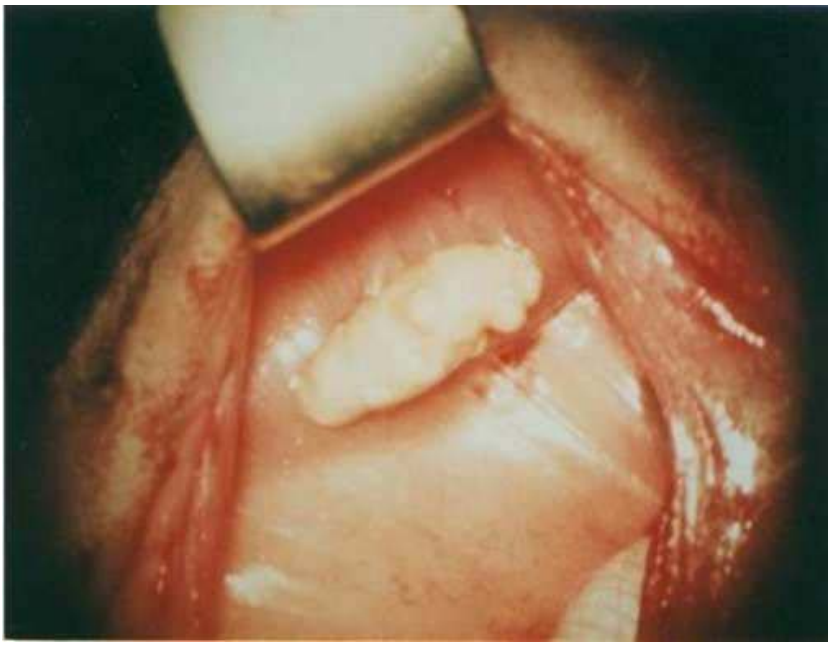


Fig. 1. Minor adhesions and follicles 2 months after autografts.

Table 1 : Macroscopic evaluation of the ovaries

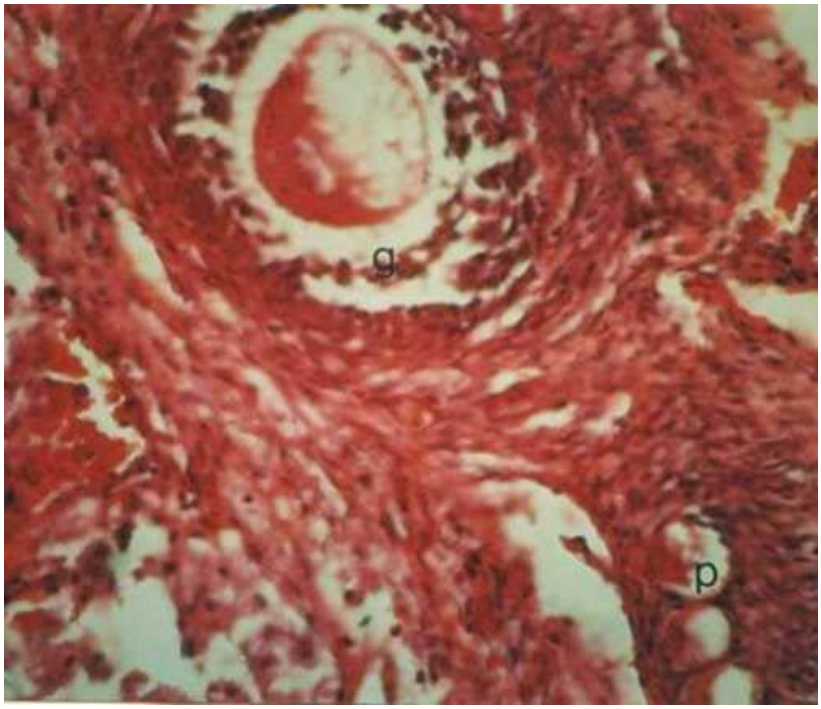
The macroscopic appearance of ovaries	Total ovaries 20	 %
Shrinkage in size	8	40
Macroscopic follicle	12	60
Minor adhesion	8	40
Major adhesion	10	50

Ovarian tissue was found in 80% of the transplanted ovaries. During the microscopic examination of the autografted ovaries; follicles of different maturation phases, regressed corpus luteum and active corpus luteum cysts and neovascularization were identified. Sclerosis, fibrosis, granulomatous reaction, cholesterol

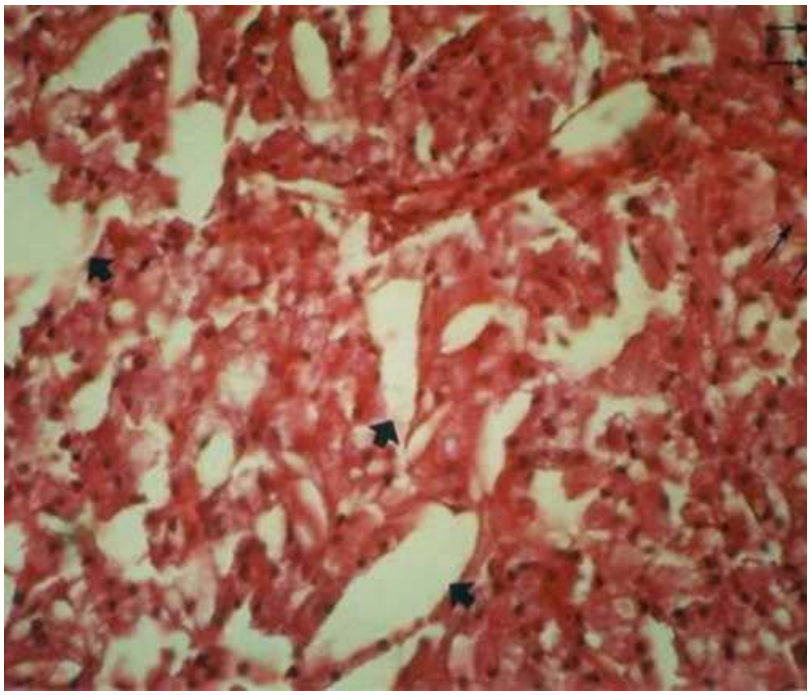
clefts, dystrophic calcification, foreign body giant cells, lymphoplasmacytic cell infiltration, bleeding areas were also identified (Fig. 2). Ovarian tissue was not found in 20% of the transplanted ovaries. Foreign body giant cells, granulomatous reaction, dystrophic calcification and fibrous and adipose tissue were observed on microscopic examination of the areas where there is not any ovarian tissue (Fig. 2 and 3).

The hormonal results of this study are given in Table 2. An increase in preoperative P values was found from the basal to the late period (Basal: 0.123±0.67, early: 0.565±0.414, late: 0.681±0.370 p=0.000). This difference was statistically significant. When postoperative p values were compared (Basal: 0.086±0.041, early: 0.407±0.330, late: 0.479±0.286, P=0.000), the increase was also statistically significant. However, when preoperative E2 values (Basal:386.9±143.7, early:514.1±309.2, late:539.0±353.4 p=0.388), and postoperative E2 values (Basal:381.2±150.6, early:359.5±149.2, late:376.2±217.8 p=0.79) were compared, the differences were not statistically significant.

Preoperative basal and postoperative basal, preoperative early and postoperative early, preoperative late and postoperative late p values were compared. Preoperative basal, early and late P values were higher than postoperative p values, and the differences were statistically significant. When preoperative basal and postoperative basal, preoperative early and postoperative early, preoperative late and postoperative late E2 values were compared, preoperative E2 levels were found to be higher compared to postoperative E2 levels but the differences were not statistically significant.



Appearance of Graaf follicle (g) and primordial follicle (p)



Histiocytes (sm. arrow), clefts incl. chol. crystals (lrg arrow)

Ovarian tissue was found in 80% of the transplanted ovaries. During the microscopic examination of the autografted ovaries; follicles of different maturation phases, regressed corpus luteum and active corpus luteum cysts and neovascularization were identified. Sclerosis, fibrosis, granulomatous reaction, cholesterol clefts, dystrophic calcification, foreign body giant cells, lymphoplasmacytic cell infiltration, bleeding areas were also identified (Fig. 2). Ovarian tissue was not found in 20% of the transplanted ovaries. Foreign body giant cells, granulomatous reaction, dystrophic calcification and fibrous and adipose tissue were observed on microscopic examination of the areas where there is not any ovarian tissue (Fig. 2 and 3).

The hormonal results of this study are given in Table 2. An increase in preoperative P values was found from the basal to the late period (Basal: 0.123±0.67, early: 0.565±0.414, late: 0.681±0.370 p=0.000). This difference was statistically significant. When postoperative p values were compared (Basal: 0.086±0.041, early: 0.407±0.330, late: 0.479±0.286, P=0.000), the increase was also statistically significant. However, when preoperative E2 values (Basal:386.9±143.7, early:514.1±309.2, late:539.0±353.4 p=0.388), and postoperative E2 values (Basal:381.2±150.6, early:359.5±149.2, late:376.2±217.8 p=0.79) were compared, the differences were not statistically significant.

Preoperative basal and postoperative basal, preoperative early and postoperative early, preoperative late and postoperative late p values were compared. Preoperative basal, early and late P values were higher than postoperative p values, and the differences were statistically significant. When preoperative basal and postoperative basal, preoperative early and postoperative early, preoperative late and postoperative late E2 values were compared, preoperative E2 levels were found to be higher compared to postoperative E2 levels but the differences were not statistically significant.

Table 2 : Hormonal results of the preoperative and postoperative periods.

Rabbits		Preoperative		Postoperative	
		E2 (pg/ml)	P (ng/ml)	E2 (pg/ml)	P (ng/ml)
1	Basal Early Late	409 412 1486	0.15 0.25 1.30	163 118 196	0.12 0.20 0.54
2	Basal Early Late	403 444 426	0.18 0.54 0.60	456 234 240	0.15 0.20 0.35
3	Basal Early Late	468 1062 553	0.08 0.80 0.50	719 351 233	0.07 0.50 0.45
4	Basal Early Late	633 1062 553	0.06 0.80 0.50	256 351 233	0.05 0.50 0.45
5	Basal Early Late	471 553 335	0.27 0.30 0.40	350 370 420	0.15 0.20 0.30
6	Basal Early Late	208 353 415	0.09 0.20 0.60	408 420 480	0.07 0.15 0.35
7	Basal Early Late	454 409 712	0.11 0.20 0.25	260 308 300	0.09 0.14 0.20
8	Basal Early Late	122 110 300	0.05 0.10 0.18	380 390 270	0.04 0.08 0.09
9	Basal Early Late	321 330 300	0.08 0.06 1.00	370 290 310	0.04 0.80 0.90
10	Basal Early Late	380 408 400	0.16 1.00 0.98	450 408 370	0.08 0.90 0.65

DISCUSSION

In this ovarian autografting experimental study which was performed on rabbits, eight (40%) of the 20 transplanted ovaries were found to be smaller. This was also shown in the study of G. Maye et al.[4] in which insufficient nutrition was regarded as the reason for the shrinking in size of the four ovaries. Adhesions were also seen in Maye et al's study[4] while in our study, we found minor adhesions in 8 (40%), and major adhesions in 10 (50%) of the transplanted ovaries. In the laparotomy, which was performed following the administration of 100 IU HCG, follicles of various phases were microscopically seen in 12 (60%) of the ovaries. In their study, G. Maye et al. stated that follicular reserves of transplanted ovaries were of different quality and quantity[4]. They also showed that the quality of stimulation was an important factor in determining the growth of ovarian follicles. In order to protect ovaries from pelvic radiation, M. Leporrier et al. performed forearm subcutaneous ovarian autotransplantation to women who had Hodgkin disease[1]. These patients were under close

clinical observation with USG. The researchers observed that the ovarian cycles continued. A year later, they got mature oocytes from the ovaries. YC Yamada transplanted ovaries to breast fat tissue in patients with cancer and he showed follicular growth and ovulation via USG examination, hormonal analysis and basal body temperature[2]. C.Müller et al. obtained mature oocytes one year after ovarian autotransplantation which was performed on patients who would undergo radiotherapy and chemotherapy because of subdiaphragmatic lymphoma[3]. Two years later, they discovered follicular growth in 2/3 of the transplanted ovaries.

While G. Maye et al. did not find any ovarian tissue loss in the area where ovaries were transplanted, ovarian loss was encountered in 20% of the subjects of this study[4]. Lesions seen in ovarian grafting such as fibrosis, sclerosis, granulomatous reaction, foreign body giant cells, lymphoplasmacytic cell infiltration, dystrophic calcification might be reactions which developed against dead cells resulting from insufficient nutrition of grafts or they might be reactions against damaged autografts. These histological findings were also observed by G.Maye et al, but neither the rejection nor the degeneration of grafting was proposed by their group. Since grafts had thin walls and no vascular anastomosis was done, they were nourished by neovascularization. Gunasena et al. reported high fertility rate in mice after autologous transplantation of ovaries, before or after cryopreservation[5]. There was also no report of significant loss in ovarian size. Bedaiwy MA et al reported that transplantation of an intact frozen-thawed ovary was technically feasible and that immediate restoration of vascular supply and hormonal function was possible[6].

Although the morphological appearance of the organs gives information about their functions, the levels of the hormones which were secreted by active organs is another sign of activity. Basal, early and late P values were compared in the preoperative period. It was found that following 100 IU HCG, there was an increase in preoperative early and late P values in comparison to basal p values. This increase was statistically significant. The same was also valid for p values in the postoperative period.

This shows that, after the injection of 100 IU HCG, ovarian follicles are stimulated, ovulation occurs and corpus luteum is formed. When P values of the preoperative and postoperative periods were compared, preoperative values were found to be significantly higher than postoperative values. This result indicates a decline in the functioning of the corpus luteum in transplanted ovaries. G.Maye et al. reached the same conclusion in their study4. When E2 levels in preoperative and postoperative periods were compared, no significant difference was found. G. Maye et al also reported similar results.

Although any major complication was not encountered in the ovarian autotransplantation processes, N.L. Davies faced some complications in 41 of the 1130 female dogs on which he performed portal vein drainage area ovarian autotransplantation[7].

Callejo et al suggested that hormonal production could be restored after both fresh and cryopreserved ovarian transplantation in women[8]. Oktay K and Buyuk E studied ovarian transplantation in humans and concluded that ovarian function could be restored by transplantation of the ovaries. They believe that the major improvement in the efficiency of ovarian transplantation is anticipated to come from research exploring the revascularization process[9]. The authors have, in this study, determined the refunctioning of the ovarian autotransplant histopathologically and hormonally with hormonal functions, neovascularization, and follicular growth.

Baird et al.[10] performed orthotopic transplantation on sheep and four lambs were born following grafting. Bordes et al.[11] reached the same conclusion with the orthotopic autograft of

vitrified- warmed hemi-ovaries into ewes and 3 pregnancies occurred. Tryde Schmidt et al reported restored ovarian function for several cycles and sustained development of mature oocytes in a woman cured of cancer who had an orthotopic autotransplantation of cryopreserved ovarian tissue[12]. Finally, Donnez et al. reported a live birth following human orthotopic autotransplantation of cryopreserved ovarian tissue[13]. In vitro fertilization procedures will be able to be performed with oocytes obtained from ovarian auto transplantations. In the future, women who suffer from streak gonad (Turner syndrome) or women who require chemotherapy or radiotherapy for the treatment of malignant diseases will be able to gain their normal hormonal function with the help of transplants which will be done with ovarian allografts.

COMMENT

In this study, the corpus luteum of ovarian autografts secreted less E2 and P. If autografting is performed by employing microsurgical techniques, ovarian autografts will have similar postoperative hormonal function and morphology as the preoperative period.

CONDENSATION

If autografting is performed by employing microsurgical techniques, ovarian autografts will have similar postoperative hormonal function and morphology as in preoperative period.

REFERENCES

1. Leporrier M, Von Theobald P,Roffe JL,Muller G :A new technique of protect ovarian function before pelvic irradiation. Cancer. 1987; 600: 2201-4.
2. Yamada Y: Clinical long term observations on the function of ovaries autotransplanted at radical hysterectomy on cervical carcinoma patients. Nokkaido-Igaku-Zasshi. 1985; 60: 38-47.
3. Muller G, Van-Theobald P, Levy G, Roffe JL, Le-Porrier M: First heterotopic ovarian autotransplantation in the female. J Gynecol Obstet Biol Reprod Paris. 1988; 17: 97-102.
4. G.Maye, J Michelot, M.Lagoutte et all: Autogreffe overienne chez lapine J Gyn Obst Biol Repr. 1979; 8:483-8.
5. Gunasena KT, Villines PM, Critser ES, Critser JK Live birhts after autologous transplant of cryopreserved Mouse ovaries. Hum Reprod. 1997;12:101-6
6. Bedaiwy MA, Jeremias E, Gurunluoglu R, Hussein MR, Siemianow M, Biscotti C, Falcone T. Restoration of ovaran function after autotransplantation of intact frozen-tahwed sheep ovaries with microvascular anastomosis.
7. Davies NL:Complications of ovarian aıtotransplantation in bitches.J S Afr Vet Assoc. 1989; 60:145.
8. Callejo J, Salvador C, Miralles A, Vilaseca S, Lailla JM, Balasch J. Long-term ovarian fuction evaluation after autografting by implantation with fresh and frozen thawed human ovarion tissue. The Journal of Clinical Endocrinology and Metabolism. 2001; 86: 4489-94
9. Oktay K, Buyuk E. Ovarian transplantation in humans: indications, techniques and the risk or reseeding cancer. Eur J Obstet Gynecol Reprod Biol. 2004; 113: 45-7
10. Baird DT, Campbell B, de Souza C, Tefler E. Long-term ovarian function in sheep after ovariectomy and autotransplantation of cryopreserved cortical strips. Eur J Obstet Gynecol Reprod Biol. 2004; 1:55-9
11. Bordes A,Lornage J,Demirci B,Franck M,Courbiere B,Francois Guerin J,Salle B.: Normal gestations and live births after orthotopic autograft of vitrified-warmed hemi-ovaries into ewes. Hum. Reprod. 2005.
12. Tryde Schmidt KL, Yding Andersen C, Starup J, Loft A, Byskov AG, Nyboe Andersen A.: Orthotopic autotransplantation of cryopreserved ovarian tissue to a woman cured of cancer- follicular growth, steroid production and oocyte retrieval. Reprod. Biomed Online. 2004; 8:448-53.
13. Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, Martinez-Madrid B, van Langendonckt A.: Livebirth after orthotopic

transplantation of cryopreserved ovarian tissue. Lancet. 2004; 364:1379-80

Yazışma Adresi / Address for Correspondence:
Dr. Mehmet Turan Çetin
Cukurova University Faculty of Medicine
Department Obstetrics and Gynecologyı
Balcalı-ADANA
e-mail: info@tupbebekmerkezi.net
Tlf: 0 322 2563177

geliş tarihi/received :25.12.2012
kabul tarihi/accepted:20.01.2013

.