GAVIN PUBLISHERS

OPEN ACCESS

Advances in Reproductive Sciences and Reproductive Health Infertility

Temur I, et al. Adv Reprod Sci Reprod Health Infertil 03: 110.

DOI: 10.29011/ARRHI-110.100010

Review Article

Allo and Xenografted Ovarian Tissue Transplantation in Rabbits: Assessment and Comparison of the Endocrine Characteristics

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Citation: Temur I, Ermutlu CS, Cihan M, Atakisi O (2020) Allo and Xenografted Ovarian Tissue Transplantation in Rabbits: Assessment and Comparison of the Endocrine Characteristics. Adv Reprod Sci Reprod Health Infertil 03: 110. DOI: 10.29011/ARRHI-110.100010

Received Date: 20 March, 2020; **Accepted Date:** 08 April, 2020; **Published Date:** 13 April, 2020

Abstract

Objective: To assess and compare of the endocrine characteristics of the allografted and xenografted ovarian tissue transplantation in rabbits.

Design: Experimental prospective study.

Setting: An academic research environment.

Animal(s): Fourteen rabbits and seven rats.

Intervention(s): Allografted ovarian tissue transplanted subcutaneously on seven rabbits and xenografted ovarian tissue(from seven rats) transplanted subcutaneously on seven rabbits.

Main Outcome Measure(s): Estradiol (E2), Follicule Stimulating Hormone (FSH), and Progesterone(P4) levels of all animals were measured in blood samples on 7th, 14th, 21st, 28th days.

Results: Each group included seven rabbits. Group I (allografted group) underwent freshly subcutaneous allografted heterotopic ovarian tissue transplantation (n=7) (from group II to group I). Group II (xenografted group) underwent freshly subcutaneous xenografted heterotopic ovarian tissue transplantation from rats (n=7). The levels of serum follicle-stimulating hormone (FSH) were very significant on the 2nd week ($p<0.01$; $p=0.002$), the levels of serum Estradiol (E2) were significant on the 3rd week ($p<0.05$; $p=0.017$) than during other weeks, and the levels of serum progesterone (P4) were not significant ($p>0.05$; $p=0.441$) in group I.The levels of serum follicle-stimulating hormone (FSH) were significant on the 3rd week ($p<0.05$; $p=0.02$), the levels of serum Estradiol (E2) were significant on the 1st week ($p<0.01$; $p=0.005$) than during other weeks, and the levels of serum progesterone (P4) were not significant ($p>0.05$; $p=0.065$) in group II according to the ANOVA.

Conclusion: We observed that there is remarkable difference between allografted and xenografted heterotopic freshly transplanted ovarian tissue, when estradiol and fsh values were taken into consideration, but not progesteron values.

Keywords: Rabbit; Rat; Ovarian tissue; Allograft; Xenograft; FSH; E2; P4

Introduction

Experimental allografted, auotografted and xenografted ovarian transplantation in heterotopical or orthotopical was performed approximately 100 years ago. This procedures has gained great momentum because of having advances in assisted reproductive techniques. But, still the optimal conditions for ovarian transplantation have not been established. It has been described in

many different transplantation techniques of the ovarian tissues or whole ovary with or without micro-vascular anastomoses or pedicles and normal location (othotopic) or abnormal location (heterotopic) in women and in many different animal models. The whole ovary and ovarian tissues (fresh or thawed) have been successfully reported by many authors in human [1-10].

Paul Bert, et al., in 1893, were described the first experimental ovarian transplantation (OT). Wiston and McClure were published the first experimental study of OT using micro-vascular surgery that resulted in pregnancy following the autografted transplantation of fallopian tubes and ovaries in a rabbit in the Lancet in 1974 [1]. Von Theobald, et al. were reported the first successful heterotopic ovarian auto-transplant using microsurgery that showed normal endocrine function and follicular development in this case in a woman in 1987 [1]. In this study, we evaluate and compare the endocrine characteristics in subcutaneously heterotopıcal allografted and xenografted of the transplantation whole ovary in rabbits.

Material and Methods

This study was approved by the Ethics Committee of Animal Experiments of Kafkas University. In this study, fourteen mature New Zealand white female rabbits and 7 Wistar rats were purchased from the Experimental Animal Investigation Center of Ataturk University in Erzurum, Turkey. These animals were maintained in a temperature controlled environment, illuminated for 12 h daily and fed with commercial pellets and water ad libitum. During the follow-up period, before and after surgical procedure, the female rabbits and rats received food and filtered water ad libitum in separate containers and were maintained in individual cages.

All procedures were carried out under aseptic conditions in the Laboratory of Experimental Surgery, Department of Veterinary Surgery of Kafkas University. One day before surgery, and for 3 days after surgery, all animals were intramuscularly injected with 1.000 mg of cefazolin sodium (Cefamezin; Eczacibaşii Drug Co, Turkey.).All animals undergoing transplantation (in group I and group II) were also received daily an intramuscular injection of 25 mg/kg cyclosporine-A (Sandimmune, Novartis Drug Co., Swiss) throughout the three week period to prevent graft-versus-host rejection.We also administered cyclosporine-A to the all received animals to eliminate any differences between the groups due to the effect of cyclosporine. Anesthesia was achieved by the intramuscular injection of 25 mg/kg ketamine HCl (Ketasol 10%, Richter Pharma Drug Co., Austria) and 5 mg/kg Xylazine HCl (Rompun 2%, Bayer Drug Co. Animal Health, Germany). Each animal's abdomen was shaved and then disinfected with a Povidone-iodine (PVP-I) solution followed by a 2% alcohol solution of iodine.

Rabbits were randomly divided into two experimental groups. Bilateral ovariectomies were performed on group I rabbits (n=7). The ovaries retrieved from the donor group and [xenografted (group II)] were immediately grafted to lower neck under the skin subcutaneously into group I (allografted group). During four weeks after surgery, blood samples were taken weekly for the analysis FSH, E2, and P4. One rabbits died due to immunosuppression on the twenty-ninth day. Bilateral ovariectomies were performed on Wistard rats (n=7), and those ovaries were immediately subcutaneously grafted into the lower neck of the [group II rabbits]. During four weeks after surgery, blood samples were taken weekly for the analysis FSH, E2, and P4. Blood samples were taken on days 7. 14. 21. 28. after transplantation. Unfortunately, one rabbit in group II died as a result of immunosuppression on the tenth day.

Blood samples were collected from the marginal artery of the rabbit's ear after surgery, on days 7, 14, 21, 28 after surgery in all animals and centrifuged immediately at 2.500 rpm for 10 min. Serum samples were obtained to measure the FSH, E2 and P4 levels. Then, the serum was frozen at-20ºC until the hormone tests were performed using commercially available ELISA kits (Cusabio Biotech Co. Ltd. Hubei Province 430223, P.R. China). Statistical analyses (SPSS package version 11.5) were performed using Student's t-test for parametric data and ANOVA variance for multiple groups. Tukey's test was performed to identify the source of significant differences revealed by ANOVA.

Result

We measured serum levels of FSH, E2 and P4 on days 7. 14. 21. 28. After transplantation in all animals. We started the study with fourteen rabbits and seven rats, but one rabbit from group I and one rabbit from group II died due to immunosuppression before the end of the study. The serum FSH levels were very significantly different (P=0.005; P=0.001) between group I (allograft) and group II (xenograft), but the serum E2 levels and progesterone were not significantly different between these two groups, respectively (P=0.55; p=0.55), p=0.94; p=0.94 (Table 1).

Table 1. *Serum FSH, E_2, P4 levels of group I (allografted and group II (xenografted)*

Days	Group I (Allografted Group)			Group II (Xenografted Group)		
	FSH m IU/ml	E_2 pg/ml	P_4 ng/ml	FSH m IU/ml	E_2 pg/ml	P_4 ng/ml
Day 7 (post-transp.)	3.0±0.1	142.3±7.6	65.8±4.9	4.3±0.6	125.7±4.1.	54.2±3.2
Day 14 (post-transp.)	**1.9±0.1**	153.2±9.6	68.1±7.3	4.4±0.8	146.0±12.4	64.6±4.2
Day 21 (post-transp.)	2.3±0.2	**143.8±2.4**	59.0±2.2	2.4±0.4	156.4±7.5	**66.4±4.0**
Day 28 (post-transp.)	1.9±0.1	145.5±4.5	59.5±4.3	**3.0±0.2**	**170.8±5.1**	68.1±3.5
Mean	2.27±0.12	146.2±6.0	63.1±4.6	**3.5±0.5**	**149.7±7.2**	63.3±3.7

** P<0.01, ** P<0.05*

The difference in the FSH level between the two groups was very significant according to a Student's t- test used (P=0.000). The levels of FSH were very significant on especially in the 1st and 2nd weeks (p<0.01; P=0.002) than during other weeks in group I, and also were significant on especially in the 3rd week (p<0.05; P=0.02) in group II according to the results of the ANOVA (Figure 1).

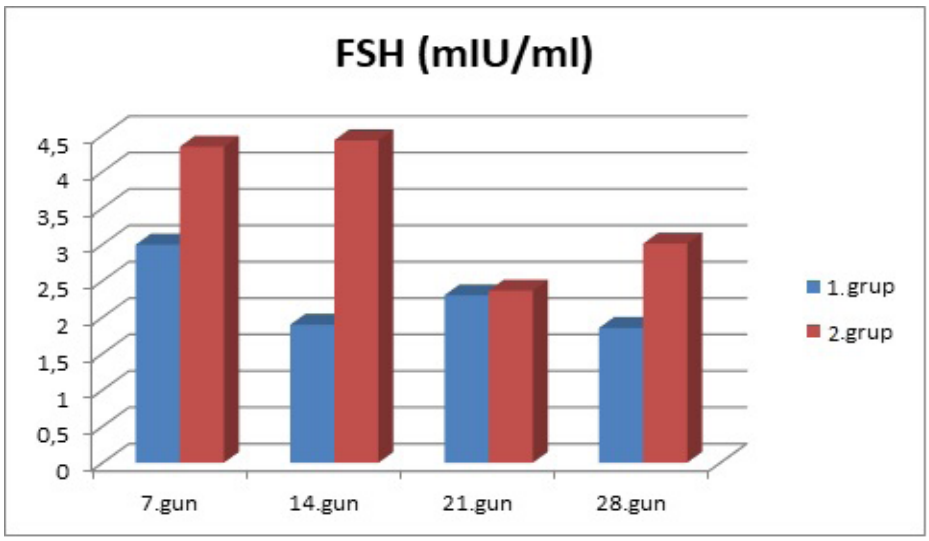

Figure 1: Variation of the serum level of FSH in allografted (group I) and xenografted (group II).

The difference in the E2 level between the two groups was not statistically significant according to a Student's t-test (P=0.55, P=0.55). The levels of serum 17-β estradiol (E2) were very significant on especially in the 3rd week (P=0.017) than during the 1st and 2nd weeks in group I, and also were significant during whole study weeks (p<0.01; P=0.005) in group II according to the ANOVA (Figure 2).

The difference in the P4 levels between two groups was not statistically significant according to the Student's t-test (P=0.94 in both groups). The levels of serum progesterone (P4) were not vary significantly in between both groups, respectively (p>0.05, P=0.277; p>0.05, p=0.065 according to the ANOVA (Figure 3).

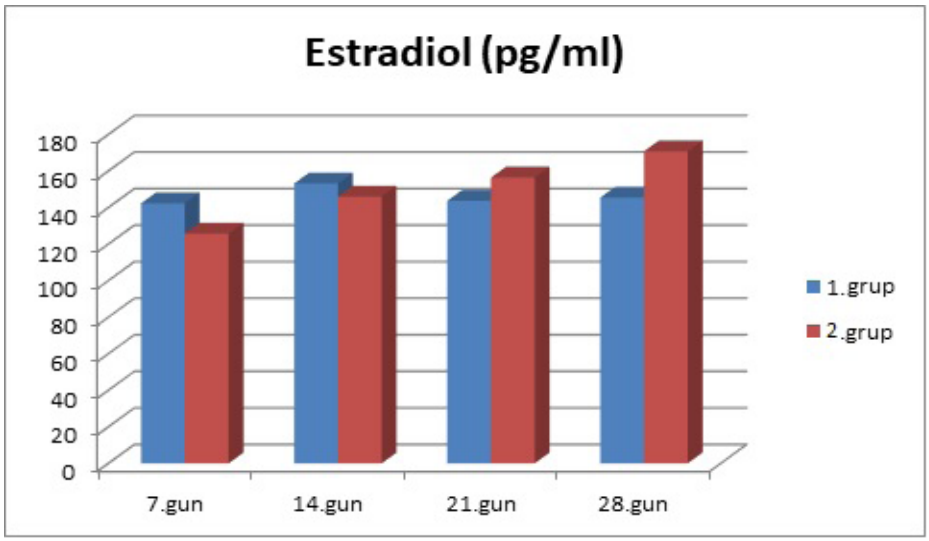

Figure 2: Variation of the serum level of E2 allografted(group I) and xenografted (group II).

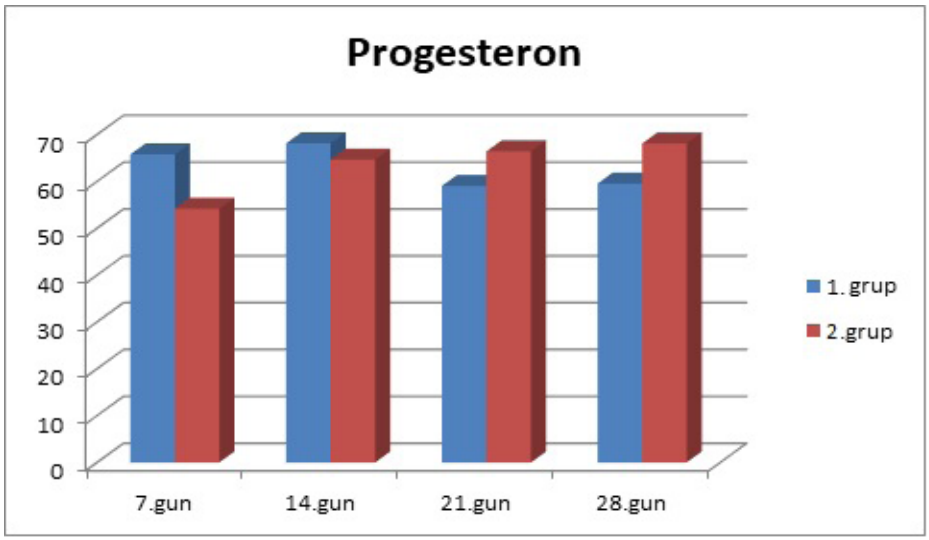

Figure 3: Variation of the serum level of P4 allografted (group I) and xenografted (group II).

Discussion

Experimental ovarian transplantation (OT) in animals was performed firstly in about one century ago. In recently, great progress has been established in ovarian transplantation in animals and in humans. But, there are limited satisfactory results in a few reports on this subject, it has great shortcoming of the restoration of ovarian function of its in todays. The auto-transplantation of fresh or frozen-thawed ovarian tissue allows the preservation of the fertility of girls or women whose ovaries are damaged due to treatment for diseases such as cancer. Meraz et al. [1] were reported in the autografted and allografted heterotopic ovarian trans-plantation with micro vascular technique in rabbits.1. We noticed in this study, after hCG administration, the values of E2 and P4 were reported in remarkably lower in allografted group than our values, respectively, (0.25 pg/ml versus 135.1 pg/ml in allografted group and 0.20 ng/ml versus 58.3 ng/m) [l,5].

Petroianu et al. [2,3] were reported the orthotopic allografted transplantation of intact and sliced ovarian tissue without any vascular pedicle [2,3]. They were analyzed the hormone differencies that received intact ovaries in first group, that received sliced ovaries in second group, received an intact ovary on one side in thrid group, and that recieved a sliced ovary on the other side in fourth group and control animals. After observing he rabbits for nine months, the hormone levels were compared between the groups and subgroups, were reported in either intact or sliced orthotropic allografted OT without vascular anastomosis was viable in rabbits [2,3]. We noted that the hormone values for allogenic the values of FSH, E2 and P4 were reported in especially lower in allografted group than our values, respectively, (0.13 IU/l versus 3.02 mIU/L; 4.13 pg/ml versus135.1 pg/ml; 0.103ng/ml versus 58.3 ng/ml) [l-5].

Callejo et al. [4] were reported the hormone levels and follicular development after heterotopic ovary transplant without any anastomosis in syngeneic Lewis rats [4]. They observed the recovery of hormone levels to preoperative values within 28 days, and the lowest values were observed at 4 and 7 days after transplantation. According to the report of this study, in only autogenic heterotopic group, was reported in lower than our values that those were the values of E2 on days 7, 14, 21, 28 were 23.4 pg/ml (versus 163.2 pg/ml), 45.2 pg/ml (versus 146.4 pg/ml), 56.6 pg/ml (versus 147.4 pg/ml) and 83.6 pg/ml (versus 112.3 pg/ml), respectively [1-5].

Stefanie M Nichols-Burns et al. [6] was described that transplantation of nude rat whole ovary was able to preserve the ovarian function for at least four weeks [6]. Imhof et al. [7] were reported in a pregnancy and delivery was achieved after the autotransplantation of whole cryopreserved sheep ovaries with microanastomoses [7].

Risvanli et al. [8] were published a study of ovarian autotransplantation without vascular pedicles in sixteen female rats. The authors noted that the estradiol concentrations of sub peritoneal transplanted ovary were significantly higher ($p< 0.001$) than those placed subcutaneous near the inguinal plexus in rats [8]. Gosden RG et al. [9] were reported that ovarian xenografs in SCID mice are suitable process for evaluating the follicules development and viability [9]. Lee et al. [10] were reported the pregnancy that retrieved oocytes from a primate had undergone transplantation any anastomosis fresh ovarian tissue without any anastomosis [10]. Kim et al. [11] were reported in a 37-years old woman had undergone the transplantation of the heterotopic crypreserved ovarian tissue that survived the endocrine function during 14 weeks after the transplanatation, and they verified in decreasing the ovarian fuction within 28 weeks after transplantation [11]. Camboni A et al. [12] were reported that primordial and primary follicles after orthotropic auto-transplantation of frozen thawed human ovarian tissue were highly protected throughout by using transmission electron microscopy throughout 13 months. They explained that those follicules were to be more resistant than other follicles (secondary and antral) against to freeze-thaw procedures and ischemia [12].

Donnez et al. [13,14] were reported the first case that resulted in a pregnancy and live birth after successfully transplantation of cryopreserved ovarian tissue and The same researcers (2013) were reported up to 2014, 24 live births have been achieved after orthotropic re-implantation of cryopreserved ovarian tissue [13,14]. Oktay et al. [15] were reported (2000) one case in which frozen-thawed ovarian tissue was transplanted laparoscopically into a 29 years-old patient who had under-gone bilateral oophorectomy due to a nonmalignant disease [15]. Meirow et al [16] were also reported in live birth after the orthotropic auto-transplantation of cryopreserved ovarian tissue in a patient who suffered from premature ovarian failure after chemotherapy [16]. Mhatre et al. [17] were reported two cases who had been diagnosed with ovarian dysgenesis undergone the transplantation of orthotopic allografted ovarian tissue with (case 1) and without (case 2) vascular anastomosist. The authors were showed that the patient with vascular transplantation gained the spontaneous menstruation and, ovulation, and excellent secondary sexual characters during the 2.5 year follow-up period. But, in other case, they showed high values of the measurements of the serum E2 level rised a significant increase from 20 pg/ml to 50 pg/ml [17]. Kikuchi K et a. [18] were explained in autgrafted ovarian transplantation may occur the transfer risk or relaps of cancer, xenotransplantation can remove it. Xenotransplantation of ovarian tissue acts providing not only accessing for gamet for reproduction from thawing its tissue but also shows undertandingthe mechanism of follicules development [18].

Conclusion

In conclusion, our results were showed that heterotopic allografted and xenografted fresh ovarian tissue transplantation were likely similar characteristics, when estradiol and fsh values were taken into consideration, but not progesteron values.

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