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

Ismail Temur¹, Celal Şahin Ermutlu², Mete Cihan², Onur Atakisi²

¹University of Sanko, Department of Obstetrics and Gynecology, School of Medicine, Gaziantep, Turkey
²University of Kafkas, Veterinary Medicine, Kars, Turkey

Corresponding author: Ismail Temur M.D., University of Sanko, Department of Obstetrics and Gynecology, School of Medicine, Gaziantep, Turkey

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), Follicle 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, autografted and xenografted ovarian transplantation in heterotopic or orthotopic 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 heterotopical 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; Eczacibaşıi 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 rabbit died due to immunosuppression on the twenty-ninth day. Bilateral ovariecotomies 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).

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
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).

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).
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/ml) [1,5].
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 differences that received intact ovaries in first group, that received sliced ovaries in second group, received an intact ovary on one side in third group, and that received 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)-[1-5].

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 xenografts in SCID mice are suitable process for evaluating the follicles 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 heterotropic cryopreserved ovarian tissue that survived the endocrine function during 14 weeks after the transplantation, 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 orthotrophic auto-transplantation of frozen thawed human ovarian tissue were highly protected throughout by using transmission electron microscopy throughout 13 months. They explained that those follicles 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 researchers (2013) were reported up to 2014, 24 live births have been achieved after orthotrophic 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]. Meirov et al [16] were also reported in live birth after the orthotrophic auto-transplantation of cryopreserved ovarian tissue in a patient who suffered from premature ovarian failure after chemotheraphy [16]. Mhatre et al. [17] were reported two cases who had been diagnosed with ovarian dysgenesis undergo the transplantation of orthotopic allografted ovarian tissue with (case 1) and without (case 2) vascular anastomosis. 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 autografted 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 understandingthe mechanism of follicles 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.

References

1. Meraz MM, Gracida C.J, Melchor JLO, Revilla CM, Buen ND, et al. (2006) Restoration of endocrine function and ovulation after a heterotopic ovarian transplant in the inguinal region of rabbits using a vascular microsurgical technique. Transplant Proc 38: 952-957.
2. Petroianu A, Aberti LR, Vasconcellos LS (2007) Morphologic, endocrinologic and natural pregnancy assessment of allogeneic ovarian orthotopic transplant without a vascular pedicle in Rabbits. Eur J Obstet Gynecol Reprod Biol 133: 70-75.
3. Petroianu A, Alberti LR, Vasconcellos LS (2006) Allogeneic ovarian orthotopic transplantation in Rabbits without a vascular pedicle: Morphological, endocrinologic, and Natural Pregnancy Assessment. Transplant Proc 38: 3092-3093.
4. Callejo J, Vilaseca S, Medina M, Salvador C, Valls C (2003) Inhibin and follicular development in heterotopically grafted ovary transplants without vascular pedicle in syngeneic Lewis rats. Fertil Steril 79: 743-748.

5. Temur I, Ulker K, Ermutlu CS, Gul A, Huseynoglu U (2013) Heterotopic allogenic and autogenic ovarian transplantation in rabbits: Assessment and comparison of morphological and endocrine characteristics. Kafkas univ Vet Fak Derg 19: A79-A85.

6. Nichols-Burns SM, Lotz L, Schneider H, Adamek E, Daniel C, et al. (2014) Preliminary observations on whole-ovary xenotransplantation as an experimental model for fertility preservation. Reproductive BioMedicine Online 29: 621-626.

7. Imhof M, Bergmeister H, Lipovac M, Rudas M, Hofstetter G, et al. (2006) Orthotopic microvascular re-anastomosis of whole cryopreserved ovine ovaries resulting in pregnancy and life birth. Fertil Steril 85: 1208-1215.

8. Risvanli A, Timurkan H, Akpolat N, Gulacti I, Ulakoglu E (2006) A study of ovarian auto-transplantation without vascular pedicle in rats. J Assist Reprod Gen 23: 401-406.

9. Gosden RG, Boulton MI, Grant K, Webb R (1994) Follicular development from ovarian xenografts in SCID mice. J Reprod Fertil 101: 619-623.

10. Lee DM, Yeoman RR, Battaglia DE, Stouffer R, Zelinski-Wooten MB, et al. (2004) Live birth after ovarian tissue transplant. Nature 428: 137-134.

11. Kim SS, Hwang IT, Lee HC (2004) Heterotopic auto-transplantation of cryo-banked human ovarian tissue as a strategy to restore ovarian function. Fertil Steril 82: 930-932.

12. Camboni A, Martinez-Madrid B, Dolmans MM, Nottola S, Langendonck AV, et al. (2008) Auto-transplantation of frozen-thawed ovarian tissue in young woman: ultrastructure and viability of grafted tissue. Fertil Steril 90: 1215-1218.

13. Donnez J, Dolmans MM, Pellicer A, Diaz-Garcia C, Sanchez Serrano M, et al. (2013) Restoration of ovarian activity and pregnancy after transplantation of cryopreserved ovarian tissue: a review of 60 cases of reimplantation. Fertil steril 99: 1503–1513.

14. Donnez J, Dolmans MM, Demytte D, Jadoul P, Pirard C, et al. (2004) Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet 36: 1405-1410.

15. Oktay K, Karlikaya G (2000) Ovarian function after transplantation of frozen, banked autologous ovarian tissue. New Engl J Med 342(25): 1919.

16. Meirow D, Levron J, Eldar-Geva T, Hardan I, Fridman E, et al. (2005) Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. New Engl J Med 353(3): 318-321.

17. Mhatre P, Mhatre J, Magotra R (2005) Ovarian transplant: A new frontier. Transplant Proc 37(2): 1396.

18. Kikuchi K, Nakai M, Kashiwazaki N, Kaneko H (2011) Xenografting of gonadal tissues into mice as a possible method for conservation and utilization of porcine genetic resources. Anim Sci J 82(4): 495-503.