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REVIEW

Allografting and Xenografting of Testis Tissue and Using Possibilities in the Future [1]

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Summary

The allografting or xenografting of testis tissue pieces into immunodeficient host mice is a new approach for germ cell conserving in livestock production as well as in human medicine. This technique also presents a new approach as a functional assay to study the spermatogenesis process and physiology in domesticated animals and humans; however, some problems observed in xenografted testis tissues remain to be unknown. Xenografts develop a dilated lumen in their seminiferous tubules during grafting period, particularly in xenografting. In addition, xenografts especially have a marked degeneration in advanced meiotic germ cells at the beginning of the grafting and spermatogenesis is reinitiated from spermatogonia stem cells or differentiating spermatogonia. In some species such as human and marmoset, this technique does not result in a complete spermatogenesis, indicating that outcomes of the allografting or xenografting of the testis tissue change according to the species. Despite these main challenges of xenografting of testis tissue, this technique implies a new approach to investigate spermatogenesis process and to solve the infertility problems both in livestock production and human medicine in the future.

Keywords: *Spermatogenesis, Spermatogonia, Transplantation, Testis xenografting*

Tür İçi ve Türler Arası Testis Doku Parçacıklarının Transplantasyonu ve Gelecekte Kullanım Olanakları

Özet

Testis doku parçacıklarının bağışıklık sistemleri zayıf alıcı farelere transplantasyonu çalışmaları, hem hayvansal üretimde hem de insan tıbbında, erkek gamet hücrelerinin korunmasında yeni bir yaklaşımdır. Bu teknik aynı zamanda çiftlik hayvanlarında ve insanda spermatogenesis süreçleri ve fizyolojisinin araştırılmasına ilişkin yeni bir yaklaşım sunmaktadır. Bununla birlikte transfer edilen testis doku parçacıklarında gözlemlenen bazı problemler bilinmezliğini korumaktadır. Özellikle türler arasında transfer edilen testis dokularının seminifer tubullerinde genleşmiş lumen gözlemlenmektedir. Ayrıca, transplantasyon başlangıcında ileri mayotik gamet hücrelerinin dejenerasyona uğraması ve spermatogenesisisin spermatogonial kök hücrelerinden veya farklılaşan spermatogonyumlarda yeniden başlatılmaktadır. Bu teknikte insan ve maymunlarda olduğu gibi, tür içi ve türler arası testis doku sonuçlarının türe göre değiştiğini göstererek tam bir spermatogenesis elde edilmemektedir. Bu önemli sorunlara rağmen testis doku transplantasyonu, gelecekte hayvansal üretimde ve insan tıbbında spermatogenesis süreçlerinin araştırılması ve infertilite problemlerinin çözümünde önemli bir yaklaşım olarak ortaya çıkmıştır.

Anahtar sözcükler: *Spermatogenesis, Spermatogonia, Transplantasyon, Testis xenografting*

INTRODUCTION

Allografting or xenografting of testis tissue into immunodeficient (naked mouse) host mice is a novel tool for germ line conservation in livestock production and in pre-pubertal male oncology patients not having mature sperm to be frozen. In addition to these patients it has not already been developed any effective method

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to preserve their fertilities for the duration of cancer therapy. In immature pig and goat testis, xenografts which contain only primitive germ cells were reported by Honaramooz et al.[1]. In that study, testicular fragments from pigs and goats were transferred to immunodeficient host mice and thus testicular tissues were developed other than natural local places. Then, the technique has broadly been applied in humans and in various animal species. Allografting or Xenografting of testis tissue pieces into immunodeficient nude mice presents the opportunities for several applications in livestock production as well as in human medicine [1-5]. First, allografting or xenografting of testis tissue potentially provides an unlimited source of male gametes including immature gonads, and offers a valuable tool for conserving endangered species by allowing sperm production from immature males. Moreover, with this technique it is possible to study germ cell development and to produce gametes from animals with poor viability. Second, contrary to the transplantation of isolated germ cells, allografting or xenografting of the testis tissue does not have the potential risk of transmitting tumor cells. Third, spermatogenesis and steroidogenesis can be manipulated in a controlled manner and the cytotoxic effects of the chemical matter on germ cell damage can be investigated by using testicular allografts/xenografts as a bioassay model [6-13].

We aimed to introduce the allografting/xenografting of testis tissue for both animal genetic sources conserving and human medicine, especially in children being treated for cancer. Thus, it is expected that a contribution will provide for developing of this technology in our country.

GRAFTING PROCEDURE

Grafting is a simple procedure and principally small pieces cut from testis of a donor male are inserted under the back skin of immunodeficient host mice, and thus these testicular small pieces continue growth and differentiation in host mice other than the original local places *(Fig. 1)*. In *Fig. 1* it clearly shows that there is an increase in the size of the recovered xenografts, and that spermatozoa are obtained from recovered xenografts after xenogafting procedure. It is reported that the host mice's endocrine system effectively supports the development of the germ cells and maturation of the transplanted testicular tissues grafted under back skin of the immunodeficient host mice from different animal species. As a biological incubator, the blood supply of the host mice to the grafted tissue is restored two weeks after grafting process, but the time passed for this restoration varies according to species [1, 14].

Preparation of the Recipient Mice and Appropriate Age of Donor

For the grafting procedure, immunodeficient host mice are used to establish the compatibility between donors and recipients. In addition, recipient mice are generally castrated to provide the high levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH). In the same way, host mice's endocrine system can be induced by exogenous hormone administration or growth factors, these treatments usually result in the improvement of xenograft properties [12,15,16]. Xenografting of testis tissue can be also affected from the age of recipient because of the differences respect with immunosensitivity. Therefore, it is generally recommended that old recipient host mice are proper for a successful xenografting [17]. The successful grafting process also depends on the age of the donors used. Studies showed that testicular xenografts from immature animals are more successful than from adult donors. It is reported that the best appropriate donor age is just before the onset of puberty [10,18-20]. However, testis tissues from very young donors may be unsuitable for grafting, despite the biggest graft growth potential in that age period [4,18].

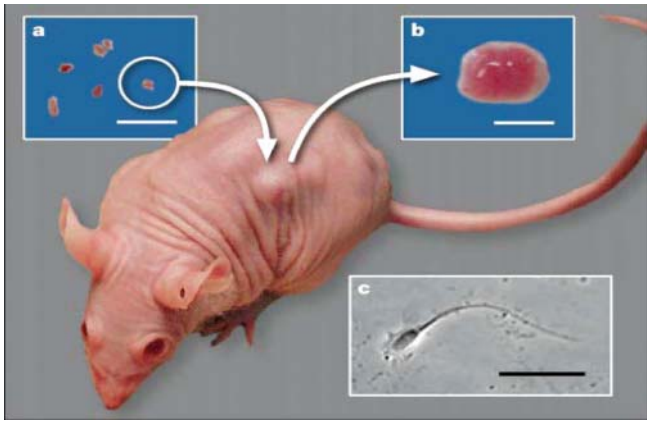

Fig 1. Grafting of testis tissue [(1)].a: testicular fragments before grafting procedure b: testicular fragments after grafting procedure c: sperm recovery from xenografts

Şekil 1.Testis doku transplantasyonu[(1)]. a: grafting uygulaması öncesi testis doku parçacıkları b: grafting uygulamasından sonra testis doku parçacıkları c: sperma alımı

Time of the Graft Removal in Immunodeficient Host Mice

The researchers' findings indicated that a feedback between the endocrine cells of the allograft or xenograft and the recipient mouse pituitary is established in 2 weeks after grafting. This changes based on the species of donor. The suitable removal time of the graft is also significant to achieve a complete spermatogenesis in

recovered xenografts. A minimum of 24 weeks is required to obtain the maximal number of sperm in post natal bovine xenografts [4,15]. On the other hand, in cats, by 36 weeks after grafting xenografts of testis tissue has a complete spermatogenesis. To describe a suitable time for the removing of the grafts, which can change according to the species, further investigations are needed [19,21]. After removing of xenografts, several analyses can be performed to assess the outcomes of grafting in recovered allografts/xenografts. To evaluate of the recovered xenografts, recovered graft weight and seminal vehicle weight which is an indicator for bioactive testosterone in host mice can firstly be determined, and blood can be collected for hormone concentrations. In the same way histological analysis of the recovered xenografts can be done to evaluate the spermatogenesis. In addition, the sperm analyses can be performed from allografts/xenografts as well [14].

TRANSPLANTATION of TESTIS TISSUE

Although responses to xenografting are changeable according to species, percentage of recovered xenografts from various donor species such as bull, pig, goat, sheep, horse, cat, hamster, monkey, marmosets and human is mostly high *(Table 1)*. All of these donor species mentioned above a complete spermatogenesis are also obtained except for human and marmosets.

To understand why the grafting technique do not work in marmosets, Wistuba et al.[27] investigated the effects of the treatment with the exogenous administration of Human Chorionic Gonadotropin (hCG) and co-grafting technique in marmoset and hamster species. In that study, although a high proportion of meiotic and post-meiotic germ cells in the tubules of hamster grafts are observed, the marmoset tubules are only populated with gonocytes and pre-meiotic spermatogonia. It clearly shows that neither the normal androgen serum levels nor the high local testosterone levels are sufficient to begin marmoset spermatogenesis. Likewise, co-grafting does not enough to start the graft development in marmosets [27]. In the same way, in some donor species such as bull and horse, spermatogenesis has a low efficiency in recovered testicular xenografts. The cause of this low efficiency of bull xenografts were investigated by Rathi et al.[23]. They reported that the low percent of germ cells of bull testicular grafts can be a result of low germ cell division rate and relatively higher sertoli cell division. It has been reported that one possible cause of this low spermatogenesis efficiency in horses is that equine somatic or endocrine environment is less responsive to murine endocrine system. But this explanation is not enough alone and it requires to be supported with further investigations [18].

Before grafting, several manipulations can be created both in xenografts and in site of the grafting of host mice. In order to increase angiogenesis in postnatal bovine testis grafts, Vascular Endothelial Growth Factor (VEGF) supporting the development of the blood supply of the xenografts can be used. Schmidt et al.[15] reported that the treatment with VEGF positively affects the graft properties such as the graft weight and the percentage of seminiferous tubule cross sections with elongating spermatids at the time of graft removal even though cultured testis tissue grafts are smaller and have fewer seminiferous tubule cross sections than

Table 1. *Allografting or xenografting of testis tissue: Summary of literature*
Tablo 1. *Testis dokusunun tür içi ve türler arası transplantasyonu: Literatür özeti*

Donors	Recipients	Outcomes
Banteng	Mouse	Meiotic cells [22]
Cat	Mouse	Spermatogenesis (complete) [19,21]
Cattle	Mouse	Spermatogenesis (low efficiency) [4,6,10, 21,23]
Donkey	Mouse	Spermatogenesis (complete) [10]
Hamster	Mouse	Spermatogenesis (complete) [17,24]
Horse	Mouse	Spermatogenesis (low efficiency) [16,10,18]
Human	Mouse	Spermatogonia [8,25,26]
Goat-sheep	Mouse	Spermatogenesis (complete) [1,3,10]
Marmoset	Mouse	Spermatogonia [24,27]
Mouse	Mouse	Offspring (allograft) [28]
Monkey	Mouse	Spermatogenesis (complete) [2,12,29]
Pig	Mouse	Spermatogenesis (complete) and sperm recovery [1,3,10,30]
Rabbit	Mouse	Offspring (no ectopic) [31]

control grafts. Nevertheless, they reported that there are no significant differences respect with the number of seminiferous tubule cross sections, seminiferous tubule diameter and serum testosterone concentrations between VEGF and control groups [15]. To improve graft properties in xenografted testis tissues, other options can be presented as an alternative. One of these attempts, with supplementing of exogenous gonadotropins equine xenografts is restored to pre-castration values. As a different approach, it is showed that spermatogenesis are restored and progressed to at least meiosis and even production of haploid germ cells following grafting in cryptorchid testes [16]. Similarly, the sustained gonadotrophin stimulation of immature monkey testis supports sertoli cell maturation and stimulates a complete spermatogenesis in testicular xenografts from infant Rhesus Monkey [12]. However, in the same species, it is indicated that testicular maturation is achieved without supplementing exogenous hormones to the host mice [2]. Furthermore, Rathi et al.[23] reported that there is no significant difference in graft maturation and spermatogenic differentiation in mice that are treated with Growth hormone at the dose and time course tested compared with untreated controls. The allografting or xenografting of the testis tissue into immunodeficient nude mice is presented as a tool to correct the testicular disruptions. However, it is clearly confirmed that testicular disruption is not corrected with testis tissue xenografting techniques [8,15].

To investigate the possibilities for conserving of the testis tissue xenografts following the grafting event is also very significant. Therefore the cryopreservation is an alternative to protect testicular allografts/xenografts [24,32,33]. Schlatt et al.[24] found that androgen concentrations are comparable in intact (control) mice and in mice receiving fresh and cryopreserved hamster grafts, whereas it is markedly lower in crypreserved grafts and adult-regressed hamster and marmoset testis tissue xenografts. It was additionally reported that adult mouse and adult photo-regressed hamster testis tissue are in part able to recover function of grafting, but showing signs of severe atrophy. In allografted mice testes, Van Saen et al.[34] investigated two diverse approaches concerning with germ line conservation. In the beginning, they were compared to the grafting technique and the germ cell transplantation evaluating concerning donor-derived spermatogenesis. In the second experiment, they researched the differences respect with donor derived spermatogenesis between in fresh grafts and cryopreserved grafts. It is particularly noticed that there is no significant difference between colonization efficiencies using both fresh and frozen thawed grafts.

CONCLUSION

Xenografting of testis tissue clearly shows that the host's endocrine system supports the development of the donor testicular grafts and even accelerates their puberty events [1-3,35]. However, some problems in xenografted testis tissues remain to be elusive. First, during grafting period, xenograft clearly develops as a dilated lumen in seminiferous tubules of the xenograft particularly in inter-species application. This dilation of seminiferous tubules and asynchronous development is not observed in mouse to mouse grafts. However, in almost all species studied so far a dilated lumen in their testicular xenografts has been seen [1,4,10,23,28]. This abnormal situation of xenografts is resulted from the absence of rete testis of xenografts. Rete testis has an important role to set up the seminiferous tubule fluid stability. In the normal testis tissues the STF (seminiferous tubule fluid) is reabsorbed in the rete testis. The absence of the rete tissue in the grafts likely impairs the absorption of STF and it accumulates in the tubules leading to tubuler distension. Interestingly, these histological defects are very similar to those occurring after efferent duct ligation or in the estrogen receptor knockout mouse [36].

As a result, the xenotransplantation of testis tissue pieces into immunodeficient nude host mice is a novel approach showing that it is possible to achieve a complete spermatogenesis in recovered xenografts from immature donors. However, further researches to reveal problems relating to xenografting of testis tissue mentioned above are required, which is particularly the germ cell degeneration and dilation of seminiferous tubules. Similarly, the development new effective approaches changing according to species for the donor age, the time of removal of the testicular xenografts, the administration of exogenous hormone and the castrating of recipients animals in testis tissue xeno-transplantation technique are needed for the further investigations.

REFERENCES

1. Honaramooz A, Snedaker A, Boiani M, Schöler H, Dobrinski I, Schlatt S: Sperm from neonatal mammalian testes grafted in mice. *Nature*, 418, 778-778, 2002.

2. Honaramooz A, Li M, Cecilia M, Penedo T, Meyers S, Dobrinski I: Accelerated maturation of primate testis xenografting into mice. *Biol Reprod,* 70, 500-1503, 2004.

3. Zeng W, Avelar GF, Rathi R, Franca LR, Dobrinski I: The length of the spematogenic cycle is conserved in porcine and ovine testis xenografts. *J Androl,* 27, 527-533, 2006.

4. Oatley JM, Reeves JJ, McLean DJ: Establishment of spermatogenesis in neonatal bovine testicular tissue following ectopic xenografting varies with donor age. *Biol Reprod,* 72, 358-364, 2005.

5. Orwig KE, Schlatt S: Cryopreservation and transplantation of spermatogonia and testicular tissue for preservation of male fertility. *J Natl Cancer Inst ,* 34, 51-56, 2005.

6. Oatley JM, de Avila DM, Reeves JJ, McLean, DJ: Spermatogenesis and germ cell transgene expression in xenogrfated bovine testicular tissue. *Biol Reprod,* 71, 494-501, 2004.

7. Jahnukainen K, Ehmcke J, Schlatt S: Testicular xenografts: A novel approache to study cyttoxic damage in juvenile primate testis. *Cancer Res,* 66 (7): 3813-3818, 2006.

8. Shclatt S, Honaramooz A, Ehmcke J, Goebell PJ, Rübben H, Dhir R, Dobrinski I: Limited survival of adult human testicular tissue as ectopic xenograft. *Human Reprod,* 21 (2): 384-389, 2006.

9. Hou M, Andersson M, Eksborg S, Söder O, Jahnukainen K: Xenotransplantation of testicular tissue into nude mice can be used for detecting leukemic cell contamination. *Hum Reprod,* 22 (7): 1899-1906, 2007.

10. Arregui L, Rahi R, Megee SO, Honaramooz A, Gomendio M, Oldan ERS, Dobrinski I: Xenografting of sheep testis tissue and isolated cells as a model for preservation of genetic material from endangered ungulates. *Reprod*, 136, 85-93, 2008.

11. Arregui L, Rathi R, Zen W, Honaramooz A, Gomendio M, Roldan ERS, Dobrinski I: Xenografting of adult mammalian testis tissue. *Anim Reprod Sci,* 106, 65-76, 2008.

12. Rathi R, Zeng W, Megee S, Conley A, Meyers S, Dobrinski I: Maturation of testicular tissue from infant monkeys after xenografting into mice. *Endocrinology*, 149, 5288-5296, 2008.

13. Schlatt S, Ehmcke J, Jahnukainen K: Testicular stem cells for fertility preservation: preclinical studies on male germ cell transplantation and testicular grafting. *Pediatr Blood Cancer,* 53 (2): 274-280, 2009.

14. Dobrinski I, Rathi R: Ectopic grafting of mammalian testis tissue into mouse hosts. *Meth Mol Biol,* 450, 139-148, 2008.

15. Schmidt JA, de Avila JM, McLean DJ: Effect of vascular endothelial growth factor and testis tissue culture on spermatogenesis in bovine ectopic testis tissue xenografts. *Biol Reprod,* 75, 167-175, 2006.

16. Turner RM, Rathia R, Zeng W, Honaramooz A, Dobrinski I: Xenografting to study testis function in stallions. *Anim Reprod Sci,* 94, 161-164, 2006.

17. Ehmcke J, Gassei K, Schlatt S: Ectopic testicular xenogafts from newborn hamster (Phodopus sungorus) sow better spermatogenic activity in aged compared with young recipients. *J Exper Zoology,* 309, 278-287, 2008.

18. Rathi R, Honaramooz A, Zeng W, Turner R, Dobrinski I: Germ cell development in equine testis tissue xenografted into mice. *Reprod*, 131, 1091-1098, 2006.

19. Kim Y, Selvaraj V, Pukazhenthi B, Travis AJ: Effect of donor age on success of spermatogenesis in feline testis xenografts. *Reprod Fert and Develop,* 19, 869-876, 2007.

20. Huang S, Sartini BL, Parks JE: Spermatogenesis in testis xenografts grafted from pre-pubertal Holstein bulls is re-established by stem cell or early spematogonia. *Anim Reprod Sci,* 103, 1-12, 2008.

21. Snedaker AK, Honaramooz A, Dobrinski I: A game of cat and mouse: Xenografting of testis tissue from domestic kittens results in complete cat spermatogenesis in a mouse host. *J, Androl,* 25 (6): 926-930, 2004.

22. Honaramooz A, Zeng W, Rathi R, Koster J, Ryder O, Dobrinski I: Testis tissue xenografting to preserve germ cells from a cloned banteng calf. *Reprod Fert Develop,* 17, 247, 2005.

23. Rathi R, Honaramooz A, Zeng W, Schlatt S, Dobrinski I: Germ cell fate and seminiferous tubule development in bovine testis xenografts. *Reprod*, 130, 923-929, 2005.

24. Schlatt S, Kim SS, Gosden R: Spermatogenesis and steroidogenesis in mouse, hamster and monkey testicular tissue after cryopresevation and heterotopic grafting to castrated hosts. *Reprod*, 124, 339-346, 2002.

25. Geens M, Goossens E, Gert DB, Ning L, Van Saen D, Herman T: Autologous spematogonial stem cell transplantation in man: Current obstacles for a future clinical application. *Human Reprod,* 14 (2):121-130, 2008.

26. Yu J, Cai ZM, Wan HJ, Zhang FT, Ye J, Fang JZ, Gui YT, Ye JX: Development of neonatal mouse and fetal human testicular tissue as ectopic grafts in immunodeficient mice. *Asian J Androl,* 8 (4): 393-403, 2006.

27. Wistuba J, Mundry M, Luetjens CM, Schlatt S: Co-grafting of Hamster *(Phodopus sungorus)* and Marmost *(Callithrix jacchus)* testicular tissues into nude mice does not overcome blockade of early spermatogenic differentiation in primate testis. *Biol Reprod,* 71, 2087-2091, 2004.

28. Schlatt S, Honaramooz A, Boiani M, Schöler HR, Dobrinski I: Progeny from sperm obtained after ectopic grafting of neonatal mouse testes. *Biol Reprod,* 68, 2331-2335, 2003.

29. Jahnukainen K, Ehmcke J, Hegenrother SD, Schlatt S: Effect of cold storage and cryopreservation of immature non-human primate testicular tissue on spermatogonial stem cell potential in xenogfats. *Human Reprod,* 13, 1-8, 2006.

30. Zeng W, AK, Snedaker S, Megee S, Rathi R, Chen F, Honaramooz A, Dobrinski I: Preservation and transplantation of porcine testis tissue. *Reprod Fertil Dev,* 21 (3): 489-497, 2009.

31. Shinohara T, Inoue K, Ogonuki N, Kanatsu-Shinohara M, Miki H, Nakata K, Kurome M, Nagashima H, Toyokuni S, Kogishi K, Honjo T, Ogura A: Birth of offspring following transplantation of cryopeserved immature testicular pieces an *in-vitro* microincemination. *Human Reprod,* 17 (12): 3039-3045, 2002.

32. Wyns C, Curaba M, Martinez-Madrid B, Langendonckt AV, François-Xavier W, Donnez J: Spermatogonial survival after cryopreservation and short-term orthotopic immature human cryptorchid testicular tissue grafting to immunodeficient mice. *Human Reprod,* 22 (6): 1603-1611, 2007.

33. Cezayirli T, Tuğlu Mİ, Vural K, Varol T: *In vitro* effects of culture medium and serum on germ cells in testis and epididymis of male wistar rats. *Kafkas Univ Vet Fak Derg,* 15 (5): 661-668, 2009.

34. Van Saen D, Goosses E, De Block G, Tournaye H: Regeneration of spermatogenesis by grafting testicular tissue or injecting testicular cells into the testes of sterile mice: A comparative study. *Fertil and Steril,* 91 (5): 2264-2272, 2008.

35. Honaramooz A, Megee SO, Rathi R, Dobrinski I: Building a testis: Formation of functional testis tissue after transplantation of isolated porcine (Sus scrofa) testis cells. *Biol Reprod,* 76, 43-47, 2007.

36. Lee KH, Hess RA, Bahr JM, Lubahn DB, Taylor J, Bunick UD: Estrogen receptor α has a functional role in the mouse rete testis and efferent ductules. *Biol Reprod,* 63, 1873-1880, 2000.