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# Transplantation of mouse ovarian tissue: Comparison of the transplantation sites

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ABSTRACT

Many studies have shown that ischemic injuries during the transplantation process were more detrimental than cryoinjuries for follicle survival and death, and it has been reported that transplantation sites can affect the outcomes of grafted ovarian tissue (OT). The purpose of this study was to assess the impact of different OT transplantation sites on follicular integrity and function of OT grafts. B6D2F1 mice were randomly assigned to control (sham) and four experimental groups according to transplantation sites (back muscle [BM], fat pad [FP], kidney capsule [KC], and subcutaneous [SC]). The ovaries from four groups were autotransplanted to each site. The OT recovery ratios on Days 2, 7, and 21 were significantly decreased in the FP group. The mean numbers of follicles were significantly lower in all the grafting groups compared with the sham group, except in the KC group on Days 7 and 21 and the BM group on Day 21. On Day 2, all the experimental groups showed low intact (G1) follicle ratio when compared with the sham group; however, the BM, KC, and FP groups recovered their morphologic integrity on Day 7, and only the SC group presented a significant decrease in G1 follicle ratios. On Day 21, the G1 follicle ratios of the FP and KC groups were greater than the sham control group. The proportion of apoptotic follicles of the four OT graft groups was higher than in the sham group on Day 2, followed by a significant decrease in the KC group and an increase in the SC group on Day 7. The serum follicle-stimulating hormone levels were significantly increased in all grafting groups on Day 2. On Day 7, only the SC group showed the high follicle-stimulating hormone level compared with the other groups. The mean numbers of oocytes from OT grafts were the highest in the KC group, except in the control group, and the lowest in the SC group. The ratios of mature oocytes were also significantly greater in the sham and KC groups. However, the ratios of normal spindle did not differ among the five groups. In conclusion, the KC was the optimal site for OT transplantation in this murine model, whereas the SC site was unfavorable for this procedure. In this study, we confirmed that the different grafting sites influenced the outcomes of transplantation.



## 1. Introduction

Recently, there has been a remarkable increase in the numbers of the patients with cancer and cancer survivors; however, cancer treatment such as high-dose chemotherapy and radiotherapy can negatively affect the ovarian follicular reserve, resulting in infertility [1]. In clinical practice, ovarian tissue transplantation (OTT) and cryopreservation have been used to restore the fertility of women with infertility caused by chemo/radiotherapy or ovarian loss and is used in many reproductive centers. Many reports on transplantation of cryopreserved–thawed ovarian tissues (OTs) have been

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published, and as a result of such advances, almost 30 live births have been achieved by transplanted human OTs [1,2]. Outside the body, follicles have to withstand physical restrictions such as ischemia, oxygen tension, unfavorable temperature, and nutrient depletion. Many follicles can be damaged and lost by cryoinjuries during the cryopreservation process. Nevertheless, many recent studies have shown that ischemic injuries during the transplantation process were more detrimental than cryoinjuries for follicle survival and death [3,4]. However, the optimal transplantation conditions of OT for follicular survival and graft continuity are not well documented yet. According to several studies, neovascularization after transplantation occurred within 48 hours in rats [5], 1 week in sheep [6,7], and 5 days in human xenotransplantation [8]. While neovascularization occurs, the OT grafts are vulnerable to ischemia and hypoxic environments. Therefore, the reduction of the ischemic period after transplantation is essential for the successful recovery of OT graft functions.

Many studies have reported that OT could survive and achieve folliculogenesis after xenotransplantation into different species and sexes. This has become a practical tool for the study of *in vivo* follicular development and evaluation of malignancy recurrence in cancer patients. Additionally, because of the limited availability of humans and primates for transplantation research, the murine model has been widely used for OT xenotransplantation in many studies [8,9].

Before OTT, it should be considered whether the different grafting sites lead to different results; if so, it is necessary to determine which site is the most suitable for OT survival and recovery of function. In previous reports, several heterotopic sites for OTT have been investigated, including the back muscle (BM) [10,11], fat pad (FP) [12,13], kidney capsule (KC) [14,15], and subcutaneous (SC) tissue [16,17]. Soleimani et al. [18] recently found that the BM had some advantages as a mouse grafting site because of its angiogenic conditions. Dath et al. [9] stated that the intramuscular site was good for grafting because of the preservation of the stroma and reduction of fibrosis. The BM site is convenient and less stressful for surgery. However, muscle activity can cause movement of the OT grafts followed by escape from the grafting site. The FP site is usually used when cancer cells are injected into mice because of the good blood supply and the small changes in temperature and pressure. Lee et al. [12] reported that transplantation of vitrified-warmed mouse OT to the FP site was a useful procedure for fertility preservation. However, OTs transplanted into the FP site can be less functional and have increased risk of hemorrhage [13]. The KC site is popular as a platform for OT because of rapid revascularization [14]. Wang et al. [19] reported that successful production of healthy offspring was achieved using oocytes taken from OT grafted in KC. Yang et al. [20] showed that the KC and ovarian bursal cavity yielded more grafts and oocytes than the SC site owing to the relatively good blood supply. The SC as a grafting site has been used in several centers. This site is located close to the body surface, and it is very easy to apply OT grafting surgery and observe the follicle growth [21]. Schubert et al [17] reported that the grafts of fresh and cryopreserved ovarian cortex into the SC site of SCID mice were able to sustain OT function. However, several other articles reported that the environment is sensitive to the change of external temperature and pressure so that could disrupt ovarian processes; furthermore, the grafts can be moving inside the SC pocket because of the spacious environment [7,9]. However, few studies have compared the graft sites directly, so the optimal site for OTT has not been well determined yet [9,22].

The present study was performed to compare and assess the efficacy and influence of the four different transplantation sites (BM, FP, KC, and SC) by evaluating the graft recovery ratios, follicular density and integrity, the follicle-stimulating hormone (FSH) concentration, follicle apoptosis, and the quantity and quality of oocytes obtained from OT grafts.

## 2. Materials and methods

### 2.1. Experimental animals

This study was carried out with the approval of the Institutional Animal Care and Use Committee (IACUC) of Seoul National University Bundang Hospital. Four-week-old B6D2F1 female mice (Orient bio, Seongnam, Korea) were used for the OT autotransplantation model. The mice were housed in groups of five per cage, maintained at 22 °C under controlled sterile conditions, with a 12-hour light/dark cycle and free access to an autoclaved pellet diet and water.

### 2.2. Autotransplantation of OTs

The mice (n = 175) were randomly assigned to five groups by OTT sites (sham, BM, FP, KC, and SC). At first, the mice were anesthetized by intraperitoneal injection of 30 mg/kg of zolazepam + tiletamine (Zoletil, Virbac, France) and 10 mg/kg of xylazine (Rompun, Bayer, Germany). After analgesia, bilateral dorsal incision was made, and both ovaries were extracted. The excised ovaries were placed in Dulbecco's phosphate-buffered saline (D-PBS: Gibco, Paisley, UK) and transplanted into four different transplantation sites, immediately. The procedure for OTT is shown in Figure 1.

#### 2.2.1. Sham

The dorsal–horizontal skin was incised bilaterally and closed immediately by suture.

#### 2.2.2. Back muscle

Both ovaries were grafted into the BM after making muscle pockets using a fine forceps, and then, the skin incisions were sutured.

#### 2.2.3. Fat pad

The inguinal mammary FP located 1 to 2 cm below the ovariectomy site was taken out. After making 1- to 2-mm-length incisions on FP, the ovaries were inserted into the FP, and the FP and skin incisions were sutured.

#### 2.2.4. Kidney capsule

The kidney was exteriorized through a dorsal–horizontal incision on the ovariectomy site. The ovaries were