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ARTICLE

Effects of HMG on revascularization and follicular survival in heterotopic autotransplants of mouse ovarian tissue

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Abstract Ovarian tissue transplantation is now considered as a procedure to preserve the fertility of young women patients undergoing cancer therapy. The present study investigated the effects and mechanism of human menopausal gonadotrophin (HMG) intervention on vascular remoulding in ovarian heterotopic autotransplantation. Ovaries of 8-week-old mice were cultured *in vitro* with different concentrations of HMG for 3 h for measuring the expression of vascular endothelial growth factor (VEGF). The cultured ovaries were implanted under the kidney capsule and removed 24, 36, 48 h or 1 month after transplantation. Revascularization, fluid exudation and the number of surviving ovarian follicles were observed. The results showed that VEGF was increased 1.6–6.5 times in the HMG intervention groups. Revascularization appeared 24–36 h after transplantation and was earlier than that of the control. Fluid exudation increased incrementally with increasing HMG concentrations. The total number of surviving ovarian follicles was increased by 1.2–1.5 times in the HMG 0.15 IU/ml group as compared with the other groups 1 month after transplantation. It is concluded that intervention with HMG *in vitro* before transplantation could improve the blood supply reconstruction and survival of the autotransplanted ovarian follicles, which might be associated with increased VEGF expression.

KEYWORDS: blood supply, heterotopic autotransplantation, human menopausal gonadotrophin, ovarian tissue, vascular endothelial growth factor

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Introduction

While early diagnosis and effective chemo- and radiotherapy have been improving the long-term survival rate of children, adolescents and young women suffering from malignant tumours (Linet et al., 1999; Young et al., 1986), these types of treatment commonly compromise the quality of life with impaired organ functions. In particular, premature ovarian failure with amenorrhoea and infertility is one of the most common long-term adverse effects affecting premenopausal patients treated with chemotherapy and/or radiation therapy (Larsen et al., 2003). Therefore, preserving fertility has been considered as part of treatment in young women (Donnez et al., 2004; Oktay et al., 2004; Smitz et al., 2010). Cryopreservation and transplantation of ovarian tissues have been employed as one of the methods for preservation and restoration of women's reproductive functions. This approach has provided hope for those who will lose their reproductive capacity as a result of the premature ovarian failure, especially as the only option for young patients is to preserve the ova. Although 13 live births after orthotopic transplantation of frozen—thawed ovarian tissue have been reported (Donnez et al., 2011), various problems still exist, such as the shorter lifespan of transplanted ovary, poor response to gonadotrophin and empty follicles without ovum (Dolmans et al., 2009; Donnez et al., 2006; Meirow et al., 2007), as well as ischaemia caused by the slow post-transplantation graft revascularization leading to substantial follicular loss.

Restoration of ovarian function after cryopreserved ovarian tissue transplantation is an important issue; however, studies concerning approaches to improve fertility restoration after this procedure are still very limited. Studies in murine hosts have demonstrated that grafts treated with vitamin E or gonadotrophins before transplantation have reduced ischaemia and improved follicular survival (Baird et al., 1999; Schubert et al., 2008). Considering that exogenous gonadotrophins can either improve the survival of the transplanted ovary directly or consume the primordial follicle which will affect the condition of transplanted ovary, it is still an open question as to whether gonadotrophin has any direct effect on the graft itself.

The present study was designed to investigate the effects of human menopausal gonadotrophin (HMG) on the blood supply, numbers of follicles and vascular endothelial growth factor (VEGF) expression in transplanted ovary of 8-week-old mice. It aimed to gain further insight into the effect of gonadotrophin on transplanted grafts and help to accelerate the clinical application of ovarian transplantation.

Materials and methods

Animals and treatments

All experimental procedures were approved by the animal committee of Ningxia Medical University. The Experimental Animal Centre of Ningxia Medical University (ID number SCXK, Ning 2005—001) provided 156 8-week-old ICR strain mice (20 ± 2 g). Mice were randomly divided into two groups: group one (n = 12) for VEGF immunohistochemistry and group two (n = 144) for ovarian transplantation. The animals were anaesthetized with an intraperitoneal injection of 0.3% napental (0.1 ml/10 g bodyweight; Beshide, Wuhan, China). Then the bilateral ovaries were removed through small dorsolateral skin incisions, placed in Dulbecco's phosphate-buffered solution (DPBS) with 20% calf serum (Sijiqing, Hangzhou, China) and were cut into two halves (2 x 1.5 x 1 mm; **Figure 1**).

Culture of ovarian tissues

The culture medium contained 1:1 (v/v) DMEM and F12 (Gibco), 20% calf serum, 1.19 g/l HEPES and 10 ml/l DMSO (Sigma) for hemi-ovary culture. To assess the expression of VEGF and the revascularization of transplanted ovaries treated with HMG, the ovaries were randomly divided into five groups: control group (not cultured); $HMG_{0.00}$ group (no HMG cultured control, cultured with HMG 0.00 IU/ml); $HMG_{0.15}$ group (cultured with HMG 0.15 IU/ml); $HMG_{0.30}$ group (cultured with HMG 0.30 IU/ml) and $HMG_{0.60}$ group (cultured with HMG 0.60 IU/ml). Ovarian tissue blocks were cultured under 5% CO_2 at 37 °C for 3 h.

Immunohistochemistry for VEGF

Six hemi-ovaries for the control group (from six mice) and 24 hemi-ovaries (from six mice, four hemi-ovaries from the

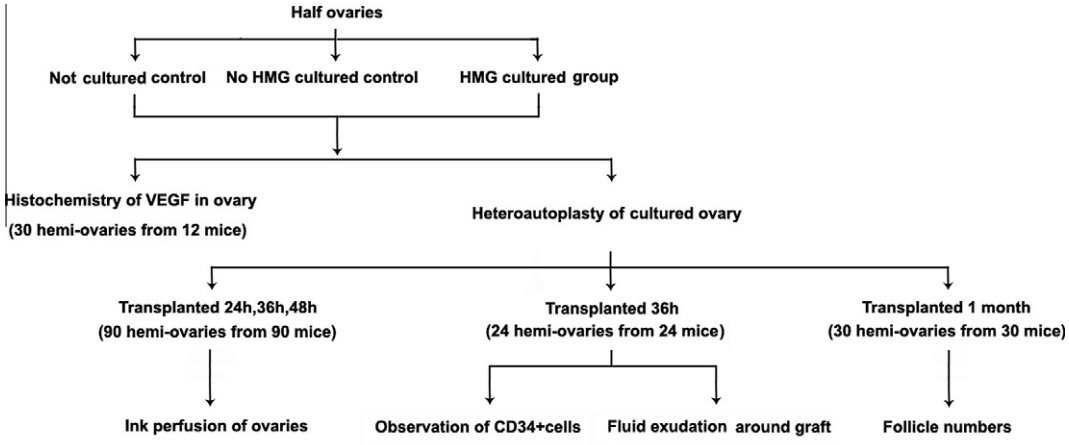

Figure 1 The flow chart of the study.