Fertility preservation by ovarian transplantation: Multiparameter mapping of the graft neo-vasculature

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Introduction:

Ionizing radiation or chemotherapy are often associated with loss of ovarian function and infertility [(1)]. One of the possible options for preservation of fertility is to cryopreserve part or the entire gonadal reservoir prior to initiation of destructive treatments [(2)]. This procedure should allow retention of hundreds of immature oocytes kept within the protective environment of the original ovarian tissue. While cryo-preservation is successful in structural preservation of ovarian specimens, the major obstacle in oocyte recovery in ovarian grafting and restoration of ovarian function is hypoxic damage due to delayed revascularization of the implanted graft. The grafts cannot be directly reanastomosed to the blood supply and therefore remain hypoxic until new blood vessels emerge and nourish it. The degree of ischaemic-reperfusion injury after transplantation was found to be the most crucial factor influencing tissue survival [(3)]. The aim of this study was to study the angiogenic processes that occur after ovary xenotransplantation. Grafts were transplanted superficially in the muscle of CD-1 nude mice, thereby allowing good graft survival and easy detection by MRI.

Materials and Methods:

Ovary retrieval and transplantation: Recipient mice were ovariectomized 7 days prior to implantation of the graft. Ovaries were retrieved from 15d old Wistar rats and grafts of half rat ovary were immediately transplanted on the surface of the gluteus superficialis muscle of the ovariectomized CD1-nude mice.

MRI studies: Data was acquired at 4.7 T using a whole body excitation coil and RF decoupled 1.5 cm surface coil. Anesthetized mice, at different times after transplantation, were examined. Macromolecular contrast material: biotin-BSA-Gadolinium-DTPA (MMCM) was prepared as reported previously [(4,5)] and was administered by bolus injected through a tail vein catheter. Precontrast R_1 was determined by spin echo images, with repetition time (TR) values of 1000-100 ms. Dynamic postcontrast imaging: T_1 weighted spin echo images were obtained from time of injection of contrast material to 14 or 40 min after it, (TR = 200 ms, echo time = 10.6 ms, 2 averages, field of view = 3.5 cm, matrix 128 X 128). 3D-GE images were acquired prior to the MMCM administration and at the end of the follow-up.

Data analysis: Pixel-by-pixel analysis was used to generate concentration maps of the MMCM [(4)]. Concentration maps were used for derivation of: 1.Blood-plasma volume fraction (fPV). 2. Apparent permeability surface area product (APS). 3. Macromolecular convection – Time2max maps, the time in which the rate of accumulation of the MMCM was maximal.

Histology: Fixed tissues were sectioned serially at 4 μm thickness. Slides were stained by eosin-hematoxylin, for smooth muscle cells and for the biotinylated MRI contrast material.

Results:

Grafts were easily detected in precontrast gradient echo images. Immediately after intravenous administration, the contrast material was detected in the vasculature and in the graft. Both APS and fPV were significantly elevated in the graft relative to the adjacent muscle ($P<0.0001$). The elevated accumulation of the MMCM in the graft was evident also by fluorescence microscopy ($P<0.0001$). Histological sections revealed development of ovarian follicles and regeneration of the ovarian vasculature. The MMCM distribution in the grafts transplanted into non-ovariectomized animals (non-OVX) correlated with diminished follicle development compared to the grafts transplanted into the OVX animals.

Grafts were examined at different time points after transplantation in order to examine whether the macromolecular drainage pathways changed with time (8, 10, 21 and 34d). Grafts transplanted for longer periods, showed interstitial convection and interstitial drain of the contrast material away from the graft (Fig.1: A, D, G), as can been seen in Time2max maps (Fig.1: C, F, I). Changes in the properties of the vasculature within the grafts can also be observed in the fPV and APS maps (Fig.1: B, E, H).

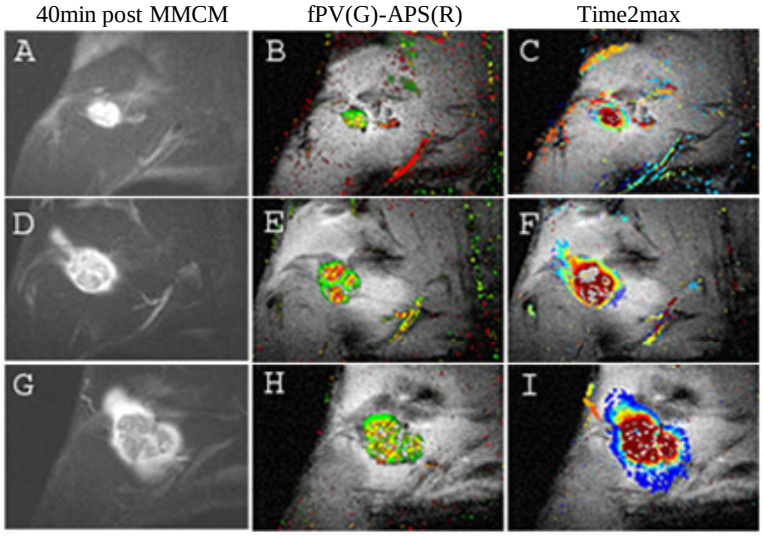
Oocytes retrieved from the grafts showed progression from germinal vesicle to germinal vesicle breakdown and polar body extrusion and thus could possibly be used for fertilization.

Conclusions:

The aim of this study was to reveal the properties of the neovasculature created in ovarian xenografts and its surroundings. Changes in blood volume and permeability were observed with time after transplantation reflecting the gain in ovarian function, along with induction interstitial drain. Optimization of perfusion at the early hours post transplantation remains a challenge for improving preservation of the graft.

Fig.1:

Longitudinal analysis of interstitial convection and drain in ovarian grafts. Macromolecular contrast material (MMCM) was intravenously injected and followed-up for 40 min in animals after 10d (A-C), 21d (D-F) and 34d (G-I) from transplantation. A, D, G) spin echo images after 40min. B, E, H) Blood-plasma volume fraction (fPV; red) and apparent permeability surface area product (APS; green) overlaid on gradient echo image. C, F, I). Macromolecular interstitial convection – Time2max maps overlaid on gradient echo image. Red represent the early time points while blue the late time points.



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