Evidence of revascularization in cryopreserved whole ovine ovaries after transplantation

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Objective: Young patients undergoing treatment of cancer with chemotherapy or radiotherapy predispose ovaries to failure and loss of fertility potential. Procedures are being developed to improve ovarian tissue cryopreservation. The objective of this study was to examine the morphology, vascularization, expression of vascular endothelial growth factor (VEFG), and Factor VIII in cryopreserved-thawed autotransplanted whole ovaries following vascular anastomosis in an ovine model.

Design: Prospective study

Materials and Methods: Dorset/Suffolk cross bred ewes were divided into two experimental groups; Control group (n = 5 ewes) had intact ovaries. The treatment group consisted of 5 ewes undergoing laparoscopic oophorectomy with intact vascular pedicle. One ewe served as a positive control where the ovaries were directly transplanted at the preferred site without cryopreservation. Of the remaining 4 ewes, ovaries were cryopreserved using dimethylsulfoxide in Leibovitz medium followed by slow freezing before being transferred to liquid nitrogen. The ovaries with intact vascular pedicle were thawed after 1 week and transplanted back in the same ewe at a heterotopic site within the rectus sheath with the deep epigastric vessels. The ewes were stimulated for ovulation, and ovaries were removed surgically from the transplanted site on day 15 of the estrous cycle. The ovarian tissue was fixed in 10% formalin and or Carnoy’s solution. The test ewes were examined for presence of stimulated ovaries, retrievable oocytes, histopathology and presence of vascular growth markers (VEGF) expressed by pericytes and smooth muscle cells, and detection of blood vessels by immunolocalization of Factor VIII, a marker of endothelial cells.

Results: Two stimulated ovaries (one each) were retrieved from the positive control and cryopreserved ovaries. An additional ovary that was cryopreserved failed to show evidence of ovarian stimulation but provided adequate tissue for histological assessment. Good staining pattern was seen for VEGF and Factor VIII demonstrating viability of vascular tissue and minimal ischemic insult, whereas complete atrophy of the remaining ovaries was seen probably as a result of ischemic insult.

Conclusion: Vascular anatomy and neo vascularization can be preserved in cryopreserved whole ovary after anastomosis and vitality of the follicles in sheep ovary maintained. Further efforts are needed to optimize the technique and minimize ischemia time.
Financial Support: Cleveland Clinic

Author Disclosure Block: J. Banerjee, None; R.K. Sharma, None; W. Choi, None; A. Agarwal, None; T. Falcone, None; A.T. Grazul-Bilska, None.

Category (Complete): Reproductive Surgery (SRS)
Topic (Complete):
  Topic: Fertility preservation

Additional Information (Complete):
  Presenting Author Fellow: No
  ACCME Disclosure: I will not be discussing non-FDA approved products
  I agree: True

Status: Complete

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