Harvesting and autotransplantation of vascularized ovarian grafts: approaches and techniques

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Abstract

The objective of this study was to describe the different surgical approaches involved in harvesting and heterotopic autotransplantation of intact ovaries with microvascular anastomosis. Twenty-one synchronized Merino sheep underwent harvesting of their intact ovaries with vascular pedicles. Autotransplantation was performed with fresh (n = 6) and cryopreserved–thawed (C−T; n = 15) ovaries. The ovarian vessels were anastomosed to the deep inferior epigastric vessels using end-to-end (n = 8), end-to-side (n = 6) and fish-mouth modification (n = 7) techniques. Patency of the anastomosis, ischaemia time, hormonal functions and histology were evaluated. In addition, ovarian harvesting techniques in two human subjects were described. Possible autotransplantation sites in humans were suggested. In all, 33.3% (7/21) of all fresh and C−T transplants showed patency after 10 days of follow-up. Patency was observed in 5/8, 2/6 and 0/7 animals using end-to-end, end-to-side and fish-mouth modification for completion of the microvascular anastomosis respectively. Use of the fish-mouth modification technique was associated with significantly higher ischaemia time compared with end-to-end (P < 0.01) and end-to-side (P = 0.05) anastomosis. A laparoscopic approach appears to be convenient for ovarian harvesting in humans. The inferior epigastric vessel is probably the most suitable heterotopic vascularizing vessel. End-to-end anastomosis yields the highest patency rate of vascularized grafts.

Keywords: deep inferior epigastric vessels, ovarian harvesting, transplantation, vascular anastomosis, vascular discrepancy

Introduction

Autotransplantation, xenografting and follicular culture (Scott et al., 2004) have been suggested as experimental strategies for restoring ovarian functions in female cancer survivors to make use of their own cryopreserved–thawed (C−T) ovarian tissue. Many orthotopic and heterotopic transplantation techniques have been described for fresh and C−T ovarian cortical strips (Oktay et al., 2001a). Two pregnancies and deliveries have been reported after orthotopic autotransplantation of C−T ovarian cortical strips in patients with Hodgkin’s and non-Hodgkin’s lymphoma (Donnez et al., 2004; Meirow et al., 2005). On the other hand, only one 4-cell stage embryo was developed from 20 oocytes retrieved from heterotopically transplanted C−T ovarian cortical strips in a breast cancer survivor (Oktay et al., 2004).

Various orthotopic autotransplantation techniques have been described. The technique described by Donnez and associates was based on creating a peritoneal window very close to the ovarian vessels and fimbria and the thawed strips were placed in the created furrow (Donnez et al., 2004). A second technique
was adopted by Meirow who inserted ovarian strips in cavities created by blunt dissection beneath the ovarian cortex on one side or smaller fragments immersed in oocyte wash buffer beneath the cortex in the other ovary (Meirow et al., 2005).

A third technique was described for transplantation of fresh ovarian cortex donated by a fertile monozygotic 24-year-old twin to her sister who presented with discordant ovarian function. The sterile twin received a transplant of ovarian cortical tissue from her sister by means of a minilaparotomy, where the stump left after excision of the streak ovaries was used as the recipient site. During her second cycle, she conceived, and subsequently delivered a healthy infant (Silber et al., 2005). All techniques described above followed the pioneering technique for autotransplantation of the ovarian cortical strips described in sheep by Gosden and associates (Gosden et al., 1994).

The limited longevity of ovarian function in some human ovarian transplants using non-vascularized grafts may be partially due to the initial ischaemic injury (Oktay et al., 2001a,b; Radford et al., 2001) because of the time needed for re-vascularization of the grafts. Consequently, immediate revascularization and ischaemia time reduction may be essential to maintain the different functions of the autografts (Aubard et al., 1999). In a trial of the use of vascularized grafts instead, a series of experiments was performed with several observations. First, it was demonstrated that larger ovarian cortical strips could withstand ischaemia for variable durations (Jeremias et al., 2003) without changing the histological architecture or inducing molecular damage (Hussein et al., 2006). Second, an intact fresh ovary could be transplanted with its vascular pedicle using microvascular anastomosis without jeopardizing the graft function (Jeremias et al., 2002). Third, cryopreservation of an intact sheep ovary with its vascular pedicle followed by transplantation could restore ovarian function (Bedaiwy et al., 2003). Lastly, an intact human ovary with its vascular pedicle could be cryopreserved without affecting the follicular viability, vascular density or molecular integrity of different ovarian components (Hussein et al., 2006).

From all these observations, it was concluded that transplantation of fresh as well as C–T intact ovaries to heterotopic sites is technically feasible, with easy accessibility and shorter operative time. A detailed technique for orthotopic autotransplantation of an intact frozen–thawed ovary together with the upper genital tract using microvascular anastomosis in rats was provided (Wang et al., 2002). So far, there has been no detailed description of the different techniques of heterotopic autotransplantation of an intact fresh or frozen–thawed ovary together with its vascular pedicle using microvascular anastomosis in animal models. If autotransplantation of intact ovaries with their vascular pedicle proved effective in animal models, there will be a need for identification of heterotopic locations that could be used to vascularize human ovarian grafts.

With this background in mind, the broad objective of this study was to describe the different surgical approaches involved in the harvesting and heterotopic autotransplantation of intact ovaries with microvascular anastomosis. The specific aims of this study were: (i) to describe the different surgical techniques of harvesting and heterotopic transplantation of intact ovaries with their vascular pedicle; (ii) to provide information on additional experiments performed to validate this technique; and (iii) to describe techniques for harvesting human ovaries for the same purpose and provide a model for human intact ovary autotransplantation based on the human vascular anatomy guided by the results of animal experiments.

**Materials and methods**

**Animal experiments**

The Institutional Animal Research Committee of the Cleveland Clinic Foundation approved all the animal experiments. Twenty-one adult, non-pregnant merino ewes, weighing 55–70 kg, were included. Surgical procedures were performed at the Cleveland Clinic Foundation Biological Resources Unit, in accordance with the facility’s Standard Operating Procedures [IACUC (Institutional Animal Care and Use Committees) protocol]. The animals were cared for according to the standards of the US Public Health Policy of the Humane Care and Use of Laboratory Animals (PHS Manual, Ch. 143). Animals were synchronized by using megestrol acetate (Ovaban; Schering Plough, Kennilworth, NJ, USA) at a dose of 0.55 mg/kg per day for 10–12 days, followed by 5000–10,000 IU Profasi (Serono Randolph, MA, USA) 34–36 h before surgery. Two groups of animals were used. Group I (n = 6) underwent autotransplantation of fresh ovary and (group II, n = 15) underwent intact ovary cryopreservation and transplantation.

**Harvesting the ovaries**

The procedure was performed with sterile techniques using Storz surgical laparoscope under general endotracheal anaesthesia. The animal was secured on the operating table. The abdomen was shaved, prepared, and draped surgically. A 1-cm horizontal umbilical skin incision was made. A 10-mm trocar was inserted and a pneumoperitoneum was created with CO2 at 10 l/min and 12 mmHg of intraperitoneal pressure. The animal was placed in a slight Trendelenburg position. A 10-mm 0° laparoscope was inserted through an umbilical incision and inspection of the pelvis was performed.

Secondary 5-mm trocars were inserted through the right and left lateral skin incisions 5 cm below and 8 cm lateral to the umbilicus. A left upper quadrant 5-mm trocar was placed at the level of the camera port. The ovarian vessels were identified, and their course was traced from the hilum cephalad. The ovary was dissected off the uterine horn; oviduct and the infundibulopelvic blood vessels were dissected off the surrounding tissue. The skeletonized blood vessels were double ligated as proximal as possible with non-absorbable 1–0 multifilament silk suture using intracorporeal ligature technique. The connective tissues around the vessels were carefully isolated, and the ovary was then resected and extripated. The ovary was removed through one of the 5-mm trocars. The same process was repeated on the other side. After homeostasis was ascertained, the umbilical and the other incisions were closed using a single 1–0 silk stitch.

Cryopreservation, thawing and evaluation were performed as described earlier (Bedaiwy et al., 2003). Briefly, the grafts were perfused with a mixture of Leibovitz L-15 medium (Irvine Scientific, Santa Anna, CA, USA), 10% fetal calf serum (Irvine Scientific) and 1.5 mol/l dimethyl sulphoxide (DMSO) (Sigma, St Louis, MO, USA) immediately after oophorectomy.
via the ovarian vessels. The perfusion rate was maintained at 1.3 ml/min with continuous replenishment of the reservoir. After perfusion, the ovary was transferred to a 5 ml, 12.7 × 92 mm cryovial (Corning Coaster Corporation, Cambridge, MA, USA) containing the cryoprotective mixture for controlled freezing using a Planer cryochamber (Planer Freezer Ltd, Middlesex, UK) following the freezing protocol described earlier. On thawing, the ovary was washed and immediately perfused with Leibovitz L-15 medium supplemented with 10% fetal calf serum using the same flow rate as described earlier for 20 min. The cryoprotectant was gradually eliminated by pumping Leibovitz L-15 supplemented with 10% fetal calf serum into the reservoir.

**Autotransplantation**

**Dissection of the deep inferior epigastric vessels and preparation of the transplantation site:** The animal was prepared as mentioned previously. An incision (3–4 cm) was performed on the anterior abdominal wall near the site of the 5-mm trocar to expose the deep inferior epigastric vessels (Figure 1A). The deep inferior epigastric artery and vein were identified and dissected. The dissection was performed using standard microsurgical instruments and Acland vascular microclamps.

**Microvascular anastomosis:** The anastomosis of the ovarian vessels to the deep inferior epigastric vessels was performed using 8–10 interrupted sutures (9–0 or 10–0 prolene). All steps were performed under magnification using Zeiss surgical microscope (Carl Zeiss, Germany). Three different approaches were used to have the anastomosis completed based on the calibres of the ovarian and the deep inferior epigastric vessels: (i) End-to-end anastomosis. Vessels with matching calibres (eight animals; two fresh and six C-T) were anastomosed to the deep inferior epigastric vessels using end-to-end anastomosis (Figure 2A). The selected segment of the deep inferior epigastric vessel was double clamped and transected. The ovarian vessels were prepared and trimmed to match exactly the calibre of the recipient vessel. The vein was anastomosed first, followed by the artery. The anastomosis process was started on the dorsal aspect of vessels and the process was completed by suturing the ventral side at the end. The sutures were placed equidistant from each other. (ii) End-to-side anastomosis. Vessels with calibre discrepancy were anastomosed using end-to-side anastomosis (six animals; two fresh and four C-T). If the calibre of the ovarian vessel looked smaller than any accessible portion of the deep inferior epigastric vessels, the latter was clamped and an opening matching the calibre of the ovarian vessels was created on the ventral aspect of the epigastric vessels. The anastomosis was then completed as shown in Figure 2B. (iii) Fish-mouth modification. Vessels with size discrepancy were also anastomosed using a different approach called the fish mouth modification (seven animals; two fresh and five C-T). The terminal end of the ovarian vessel was split open to expand on the opening to make it match the calibre of the terminal opening of the deep inferior epigastric vessel. The anastomosis was then completed as shown in Figure 2C.

**Testing the immediate patency and finishing the procedure:** Diluted heparin was applied topically as required. Immediate vascular patency was tested for 20 min in all transplants (Figure 1B). The ovary was then fixed by a single stitch to the rectus muscle. The rectus sheath, subcutaneous tissue and the skin were closed using interrupted stitches. All animals were received anticoagulant using 5000 IU sodium heparin (Eli Lilly, Indianapolis, IN, USA) subcutaneously, twice a day, for 3 days.

**Harvesting the reimplanted ovary:** After 8–10 days, the transplant was inspected for macroscopic appearance, pulsations at the arterial anastomosis and patency of microvascular anastomosis. Viability of the ovarian transplant was also evaluated by checking the bleeding from edges at a small incision. The transplant was then bisected (Figure 1C), removed for further evaluation.

**Hormonal assay and histological evaluation**

Hormonal assay and histological evaluation were performed following the protocol described earlier. Briefly, serum oestradiol quantitative measurement was performed using the Automated Chemiluminescence Immunoassay System (ACS: 180; Bayer, Tarrytown, NY, USA). FSH concentrations were measured by radioimmunoassay. This measurement was done using NIH anti-ovine FSH (A780) as FSH antibody and A853 (ARGG) as second antibody. The data were analysed using the RIANAL program (from Colorado State University, USA for RIA analysis). Oestradiol and FSH concentrations were obtained on the day of surgery prior to operating and on the day the transplant was removed.

The ovarian tissues were Bouin-fixed, paraffin-embedded and haematoxylin and eosin stained for histological evaluation of the primordial follicle count, the presence of primary and secondary follicles, infarction and apoptosis following previously established criteria. To account for the non-uniform distribution of primordial follicles, a thorough examination of representative sections of all aspects of the ovarian cortex was performed. However, follicle counts were not performed on a small sample of fresh ovary prior to freezing, in order to ensure more efficient cryopreservation and thawing. Using the infusion pump system requires a closed circuit to ensure adequate distribution/washing of the cryoprotectants. Breaching the continuity of the ovarian cortex would disturb the closed circuit and could lead to uneven perfusion/wash of the cryoprotectant.

**Human experiments**

So far, four intact ovaries have been harvested with their vascular pedicles from two patients for cryopreservation purposes after institutional board approval. The first patient was 46 years old, and presented with menorrhagia and symptomatic fibroid uterus. She underwent total vaginal hysterectomy with bilateral salpingo-oophorectomy, McCall culdoplasty and cystoscopy. Intraoperative findings included bulky uterus and normal-appearing ovaries. The second patient was 44 years old, and presented with severe premenstrual dysphoric disorders. She underwent laparoscopic bilateral salpingo-oophorectomy. Normal appearing uterus, tubes and ovaries were detected. Both ovaries and their pedicles, from both patients, were processed immediately for intact ovary cryopreservation. The results of all in-vitro studies on these ovaries as well as the controls were presented in an earlier publication (Bedaiwy et al., 2006). The current paper describes the technical
**Figure 1.** Technique for ovarian transplantation to the deep inferior epigastric vessels with microvascular anastomosis. The deep inferior epigastric vessels were skeletonized (A). Immediate vascular patency was tested for 20 min in all transplants (B). The transplant was then bisected (C) and removed for further evaluation.
precautions that should be taken on harvesting the ovaries prior to freezing or transplantation. It also reports a proposed model of autotransplantation for intact human ovaries with microvascular anastomosis based on animal experiments and available comparative anatomy data.

Harvesting the ovaries

**Laparoscopy:** Laparoscopic oophorectomy is the preferred approach for the purposes of cryopreservation and subsequent transplantation, to maintain the minimally invasive nature of the procedure. In the present case, it was performed using the standard described elsewhere. For the purposes of subsequent cryopreservation and possible transplantation, several precautions should be taken into consideration. First, the procedure should start with severing the utero-ovarian ligament of the ovary and then advanced cephalad through the mesosalpinx and ends at the infundibulopelvic ligament (Figure 3). Second, sharp dissection and ligation with sutures should be used in preference to electrocoagulation to avoid destruction and thermal injuries of the vascular walls and desiccation of the ovarian tissue. Third, the infundibulopelvic ligament containing the ovarian vessels should be dealt with last to avoid prolonged ischaemia of the ovary, as it is the main source of the blood supply. Fourth, the ovarian vessels should be ligated as proximal to the origin as possible to provide longer and wider calibre vessels to facilitate subsequent perfusion and transplantation with microvascular anastomosis. Lastly, the ovary with its vascular pedicle should be taken outside the peritoneal cavity in an Endobag through the 10-mm trocar and if needed through an extended port incision, to avoid crushing the ovary and the blood vessels against the narrow port site.

**Transvaginal harvesting:** If oophorectomy is to be combined with vaginal hysterectomy, then the preferred approach for harvesting will be the vaginal route. That is what was done in the other case. Obtaining the ovary with its vascular pedicle transvaginally is technically challenging. Devascularization of the uterus performed from below the cephalad will save the infundibulopelvic ligament and the ovarian vessels until the very end of the procedure, minimizing the ischaemia time. Having a longer portion of the ovarian vessels via the vaginal route is not as easy as by laparoscopy. A longer portion of the pedicle could be obtained after releasing the ovary and the uterus from one side. This will provide more room and easy accessibility to the contralateral infundibulopelvic ligament at a more proximal location. Experience suggests handling one ovary at a time for cryopreservation, to minimize the ischaemia time. The surgeon should not wait for both ovaries to be harvested concomitantly.

**Autotransplantation**

Although no autotransplantation of intact human ovaries has been performed yet, extensive research has been carried out, and a model for potential recipient sites created. The selected sites were picked based on several inclusion criteria: (i) the accessibility of the recipient site for transplantation with microvascular anastomosis; (ii) the calibre of the recipient vessels at the target site and their compatibility with the ovarian vessel calibre; (iii) how amenable the recipient site is for frequent blood sampling, ultrasound monitoring and possible egg retrieval; (iv) the proximity to other sensitive structures; (v) proximity of the selected site to pressure surfaces (e.g. bones) and weight bearing areas; and (vi) the recipient site should be cosmetically acceptable to the patient, because there will be a scar and bulge at the transplantation site.
Surgical anatomy of inferior epigastric vessels in humans

The inferior epigastric and the deep circumflex iliac arteries are branches of the external iliac artery at the level of the internal inguinal ring. The inferior epigastric artery arises from the anterior surface of the external iliac, passing forward and upward on the anterior abdominal wall between peritoneum and fascia transversalis. It penetrates the fascia below the arcuate line, entering the rectus abdominis muscle or coursing along its inferior surface to anastomose with the superior epigastric which is the terminal branch of the internal mammary artery. The inferior epigastric artery forms the lateral boundary of the Hesselbach’s triangle. It frequently gives off a branch to the inguinal canal, as well as a branch to the pubis (pubic artery), which anastomoses with twigs of the obturator artery. The pubic branch of the inferior epigastric often becomes the obturator artery.

Statistical analysis

Analysis of variance (ANOVA) was used to compare the pre-operative to post-operative values \( (P < 0.05) \) using SAS version 11 software (SAS Institute Inc, Cary, NC, USA).

Results

Animal experiments

Cryopreserved–thawed transplants

The first six animals were used to test the feasibility of the autotransplantation technique of intact fresh ovaries with their vascular pedicles to the deep inferior epigastric vessels with microvascular anastomosis. The vascular patency was documented in 50% (3/6) of these transplants at the end of the follow-up period. The results of this experiment have
been reported previously and will therefore not be discussed here (Jeremias et al., 2002). The subsequent 11 animals were used to test the applicability of the above technique using intact C–T ovaries. Vascular patency was documented in 27% (3/11) of these transplants at the end of the follow-up period (Bedaiwy et al., 2003).

The same techniques were adopted in the second experiment on four more animals, to explore further the best approach to perform microvascular anastomosis using C–I intact ovaries, particularly when there is vascular discrepancy between the ovarian vessels and the deep inferior epigastric vessels. The results of the four new C–T transplants were added to the results of the previous 11 animals in group 2 and analysed. A total of four transplants out of the 15 C–T transplants were found to have patent anastomosis at the end of the follow-up period (26.7%; Table 1). In these cases, arterial bleeding occurred at the incision made in the ovarian cortex, documenting the long-term patency of the anastomosis. In animals with occluded anastomosis (n = 11), the anastomosed vessels were occluded completely, leading to significant tissue loss. The ischaemia time, defined as the sum of the time from removal of the ovary to cryopreservation and from thawing to transplantation, was not significantly different between patent and occluded vessel groups (3.84 ± 0.33 versus 4.03 ± 0.17 h; Table 1).

In C–T transplants, there was no significant change in serum oestradiol concentrations before and after transplantation in either the patent vessel group or the non-patent vessel group, indicating that a small remnant of surviving ovarian cortex tissue was sufficient for the resumption of oestradiol production (Table 1). Serum FSH in the patent vessel group did not change significantly from pre- to post-transplantation, whereas a significant rise was observed in the non-patent vessel group (93.65 ± 77.06 versus 260.83 ± 9.47 ng/ml, P = 0.009), suggesting ovarian failure.

Growing follicles were detected in the surviving grafts. That could in part explain the hormonal perturbations observed by monitoring. However, most of the growing follicles are more vulnerable to freeze–thaw injury as documented by many previous publications (e.g. Baird et al., 1999) It was considered more practical to count the primordial follicles to indirectly estimate the extent of damage to the ovarian reservoir. The surviving primordial follicles are the ones to be recruited and undergo growth within several weeks after transplantation.

The mean count of primordial follicle number, per high magnification field, after transplantation revealed a significantly higher number of follicles in the patent group as compared with the non-patent vessel group (3.25 ± 1.9 versus 0.23 ± 0.44, P = 0.005). Scattered areas of necrosis were observed on histological assessment of the patent vessel group, whereas transplants in the non-patent vessel group showed severe necrosis with thin peripheral rim of viable tissue.

### Fresh and C–T transplants: anastomosis techniques

In order to evaluate the impact of the anastomosis technique on the outcome, the outcome of all fresh and C–T transplants was evaluated as one group. Despite the fact that short-term vascular patency was observed in all transplants, patency at the end of the follow-up period was different according to the way the anastomosis was performed. When end-to-end anastomosis was performed, long-term vascular patency was the highest as 62% (5/8; group I) of the transplants showed patency at the time of the transplant removal (Table 2). On the other side, when there is disparity between the calibre of the ovarian vessels and the deep inferior epigastric vessels, patency at the end of the follow-up period was much lower. When this discrepancy was dealt with by end-to-side anastomosis, long term patency was documented in 2/6 (33.3%, group II) of the transplants, as compared with 0/7 (0%, group III) when fish mouth modification was used. On comparing all three groups, vascular patency in group I was significantly higher than groups II and III (P = 0.04 and <0.01) respectively. Group II had significantly higher patent transplants than group III as well (P < 0.01). Similarly, the ischaemia time reflecting, in part, the duration of the procedure was significantly higher in group III than in groups I and II (P < 0.01 and = 0.05) respectively.

### Human studies

Following the harvesting technique described above, it was possible to obtain all ovaries with adequate length of the vascular pedicle using the laparoscopic approach and a shorter pedicle using the vaginal route. Successful cryopreservation of intact human ovaries with their vascular pedicles was also performed, adopting the same protocol used in the animal experiments (Bedaiwy et al., 2006). The next step will be the identification of a suitable heterotopic transplantation site; the following target regions have been identified because of their rich blood supply; the neck, pectoral region, antecubital fossa, lower part of the anterior abdominal wall and the inguinal region (Figure 4). The carotid vessels, the cutaneous branches of the internal mammary vessels, the antecubital vessels, the inferior epigastric vessels and the femoral vessels are the feeding vessels to be anastomosed to the ovarian vessels in their respective regions.

Based on the selection criteria set above in materials and methods section, the advantages and the disadvantages of each transplantation site are shown in Table 3. From that table, it is obvious that the deep inferior epigastric vessels are probably the most suitable vessels to vascularize human ovarian grafts, should heterotopic vascularized ovarian transplantation be an option. This is in line with the results of the animal experiments. The surgical anatomy of that vessel is provided above in the methods section.
Table 1. Assessment of baseline and post-grafting endocrine functions in cryopreserved-thawed transplants. Values are mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>Patent vascular anastomosis (n = 4)</th>
<th>Occluded vascular anastomosis (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post-grafting</td>
</tr>
<tr>
<td>Ischaemia time (h)</td>
<td>3.84 ± 0.33</td>
<td>n/a</td>
</tr>
<tr>
<td>Primordial follicular count</td>
<td>3.25 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n/a</td>
</tr>
<tr>
<td>FSH (pg/ml)</td>
<td>202.44 ± 70.34</td>
<td>180.36 ± 38.1</td>
</tr>
<tr>
<td>Oestradiol (ng/ml)</td>
<td>151.25 ± 50.22</td>
<td>164.91 ± 28.9</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values with the same superscript are significantly different (P = 0.005 and 0.0009 respectively). All other comparisons were not statistically significant.

Table 2. Assessment of vascular patency and ischaemia time in fresh and cryopreserved-thawed ovarian transplants.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>End-to-end (group I; n = 8)</th>
<th>End-to-side (group II; n = 6)</th>
<th>Fish mouth (group III; n = 7)</th>
<th>P-value</th>
<th>I versus II</th>
<th>I versus III</th>
<th>II versus III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate patency (%)</td>
<td>8/8 (100)</td>
<td>6/6 (100)</td>
<td>7/7 (100)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Patency at the end of the follow-up period (%)</td>
<td>5/8 (62.5)</td>
<td>2/6 (33.3)</td>
<td>0/7 (0)</td>
<td>0.04</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Ischaemia time (h)</td>
<td>2.1 ± 0.2</td>
<td>3.8 ± 0.4</td>
<td>4.7 ± 0.5</td>
<td>0.05</td>
<td>&lt;0.01</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

NS = not statistically significant.
Figure 4. Proposed transplantation sites in humans: the neck, pectoral region, antecubital fossa, lower part of the anterior abdominal wall and the inguinal region could be recipient sites. The carotid vessels, the cutaneous branches of the internal mammary vessels, the antecubital vessels, the inferior epigastric vessels and the femoral vessels are the ones to be anastomosed to the ovarian vessels in their respective regions as shown in the figure.
Table 3. Criteria of the vessels proposed to vascularize ovarian grafts with microvascular anastomosis.

<table>
<thead>
<tr>
<th>Vessels</th>
<th>Accessibility</th>
<th>Calibre match to the ovarian vessels</th>
<th>Safety</th>
<th>Amenability to monitoring</th>
<th>Liability to trauma</th>
<th>Hard surfaces behind</th>
<th>Cosmetic factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotid vessels</td>
<td>Accessible</td>
<td>Not matched</td>
<td>Close to sensitive structures. May be risky</td>
<td>Amenable but could be uncomfortable</td>
<td>Liable</td>
<td>None</td>
<td>Scar and bulge in the neck</td>
</tr>
<tr>
<td>Cutaneous mammary branches</td>
<td>Accessible</td>
<td>Matched</td>
<td>No sensitive structures around</td>
<td>Amenable and comfortable</td>
<td>Liable</td>
<td>Chest wall</td>
<td>Hidden scar and bulge</td>
</tr>
<tr>
<td>Antecubital vessels</td>
<td>Accessible</td>
<td>Matched</td>
<td>No sensitive structures around</td>
<td>Amenable and comfortable</td>
<td>Liable</td>
<td>None</td>
<td>Scar and bulge in the forearm</td>
</tr>
<tr>
<td>Inferior epigastric vessels</td>
<td>Accessible</td>
<td>Matched</td>
<td>No sensitive structures around</td>
<td>Amenable and comfortable</td>
<td>Not liable</td>
<td>None</td>
<td>Hidden scar and bulge</td>
</tr>
<tr>
<td>Femoral vessels</td>
<td>Accessible</td>
<td>Not matched</td>
<td>No sensitive structures around</td>
<td>Amenable and comfortable</td>
<td>Liable</td>
<td>None</td>
<td>Hidden scar and bulge</td>
</tr>
</tbody>
</table>

Discussion

This study describes surgical techniques for ovarian harvesting and heterotopic ovarian transplantation to the anterior abdominal wall using microvascular anastomosis. The main focus was to provide the different surgical tips on how to deal with ovaries at the time of harvesting to optimize the subsequent cryopreservation and autotransplantation. In addition, two different approaches were tested to overcome size discrepancy in the calibre between the ovarian and the epigastric vessels. Size discrepancy was defined as the inequality of vessel diameters at a ratio of 1:1.5 or greater. Size discrepancy was found in approximately one-third of anastomoses in reconstructive surgeries (Cakir et al., 2003), the most frequent being in vein anastomoses performed during free flap transfers. The sudden change of calibre may cause turbulence to the blood flow, and predisposes to platelet aggregation.

Discrepancies in the cut end diameters have been managed by many geometrical methods in order to reduce the risk of thrombosis (Lopez-Monjardin and de la Pena-Salcedo, 2000). Simple discrepancies less than 1:1.5 are usually dealt with by dilatation with the use of a jeweller’s forceps (Cakir et al., 2003). Discrepancies exceeding 1:1.5 are usually dealt with the oblique cut, fish mouth cut, or end-to-side anastomosis (Cakir et al., 2003). When a great discrepancy may be anticipated and the upstream donor vessel is smaller than the recipient one, a sleeve anastomosis can be performed (de la Pena-Salcedo et al., 2000). Other geometrical designs (oblique cut or Y cut), devices, glues or adhesives and laser-assisted anastomosis are to be considered according the surgeon’s experience (Lopez-Monjardin and de la Pena-Salcedo, 2000). In one review, it has been concluded that there is not an ideal technique to manage every size discrepancy (Lopez-Monjardin and de la Pena-Salcedo, 2000). However, the authors suggested looking for the best method with least complications and the best procedure to fit a specific case or body area.

Ovarian tissue viability was demonstrated both by oestrogen production and significantly higher primordial follicle count in transplants with patent anastomosis at the end of the follow up period. Although no transplants were patent when fish-mouth modification was used to overcome vascular discrepancy, one out of four C–T and one out of two fresh transplants were patent when end to side anastomosis was adopted. However, partial vascular damage due to cryopreservation insults cannot be excluded in the C–T ovaries. This could be easily extrapolated from the overall 50% patency rate at the end of the follow-up period when fresh ovaries were transplanted compared with only 26.6% when C–T ovaries were transplanted (Jeremias et al., 2002). Consequently, survival of C–T transplants is almost half the survival of fresh transplants.

Obviously, end-to-end anastomosis appears to be the ideal approach to perform the anastomosis procedure, with a patency rate >60%. However, if vascular discrepancy between the ovarian vessels and the inferior epigastric vessels is inevitable, end-to-side anastomosis, rather than fish-mouth incisions should be adopted. Further advances in the anastomosis technique may help to improve the overall patency of the transplants. Sutureless approaches, glues or adhesives and laser-assisted anastomosis are to be considered according the surgeon’s experience or in consultation with a plastic surgeon (Lopez-Monjardin and de la Pena-Salcedo, 2000).

In addition, there is increasing interest in the use of the more sophisticated microvascular free flap techniques instead of the standard local or regional flap technique to provide reconstructive options for urogenital indications (Ninkovic and Dabernig, 2003). Free tissue transplantation (transfer) is a procedure that involves microvascular transplantation of a flap (a fasciocutaneous, muscle or composite flap) in one stage from a donor site in the body to a distant recipient site. The viability of the transplanted flap is maintained by microvascular
anastomosis between the flap’s vessels (at least one artery and one vein) and recipient vessels (Hoetter et al., 2005). With this fact in mind, it is expected to obtain optimal anatomical and functional reconstruction with minimal donor site morbidity particularly if an interdisciplinary approach is adopted.

Follicular loss after transplantation was found by Baird et al. to be during the initial ischaemia after re-implantation, and not due to the cryopreservation procedure (Baird et al., 1999). In addition, a primate study comparing heterotopically transplanted fresh and C–T ovarian cortical strips did not show significant differences in outcome (Schnorr et al., 2002). Hormonal functions were equally restored and mature oocytes were recovered in both groups. Hence, the majority of the primordial follicular loss is due to ischaemic injury rather than cryopreservation insults. Consequently, immediate revascularization could limit this accelerated follicular loss. However, this was not the case in a recent study which evaluated contralateral orthotopic autotransplantation of cryopreserved whole ovaries with microanastomosis of the ovarian vascular pedicle (Imhof et al., 2006). Although four sheep showed post-operative luteal function and one sheep conceived after spontaneous intercourse and delivered a healthy lamb 545 days after transplantation, histological examination of the ovaries 18–19 months after transplantation showed that follicular survival rate in the grafted ovaries was only 1.7–7.6% (Imhof et al., 2006).

The ischaemia time in this study was relatively long. This was probably because it incorporated the time since the ligation of the ovarian vessels, the microdissection of the pedicle under the microscope, cannulation of the ovarian vessels, perfusion of the cryoprotectant for almost half an hour, slow-freezing time, perfusion during the thawing process and the microanastomosis time. A progressive decrease in the ischaemia time was noted throughout the experiments. The ischaemia time could be further shortened in future experiments.

In transplants with an occluded blood supply, no revascularization from the surrounding tissue to any ovary was observed to compensate for the loss of the main blood supply. However, survival of some cortical tissue in transplants with occluded anastomosis could not be ruled out as perfusion of the peripheral ovarian tissue from the surroundings could occur.

Transplantation of intact ovary with its vascular pedicle to the anterior abdominal wall could have some advantages compared with other heterotopic and orthotopic techniques previously reported (Oktay et al., 2001a, 2004): (i) the ovarian graft over the rectus abdominis muscles gives better protection to the graft compared with other more exposed sites as the forearm; (ii) the anterior abdominal wall transplantation technique could be performed under local infiltration anaesthesia rather than general anaesthesia; (iii) the transplantation technique is much easier and more practical than orthotopic sites particularly if microvascular anastomosis is adopted; (iv) graft monitoring is easier and the graft could be removed if infracted; and (v) if fertility is desired, oocytes could be retrieved percutaneously without anaesthesia. However, the main disadvantage is that IVF is necessary to achieve pregnancy.

On trying to duplicate these findings in humans, it was found that the ovarian harvesting technique should probably be modified to nullify mechanical and ischaemic damage of the ovary. Laparoscopic harvesting should be the first option for this purpose. Using the transvaginal route, enough pedicle with adequate length should be obtained. In addition, unnecessary trauma to the ovary and prolonged ischaemia should be avoided. Achieving successful cryopreservation of an entire human ovary with its vascular pedicle has been shown to be associated with reasonable post-thaw survival rates of follicles, small vessels, and stromal cells and a normal histological structure in all the ovarian components after thawing (Martinez-Madrid et al., 2004). It was also shown that adopting a different slow freezing protocol, freezing an intact ovary was not associated with significant molecular alterations (Bedaiwy et al., 2006).

With all these data in mind, it was thought time to consider intact human ovary transplantation. Until a willing candidate can be found, the possibilities of defining a suitable recipient vessel for this technique in future are being explored. It is expected that anastomosis of the ovarian vessels in humans would be less technically challenging because of the straight course and wider calibre of the ovarian vessels provided that the recipient vessels have a matching calibre. From the criteria set before, it was found from all vessels evaluated that the deep inferior epigastric vessels stand as the best available option. Studying the surgical anatomy of that vessel highlighted its suitability for such a procedure, as shown in Table 3. However, this proposition should be tested in a patient who is willing to volunteer to try this approach.

The main limitation of this study was the short follow-up duration. In addition, the size discrepancy ratio between the donor and the recipient vessels was not quantified. However, the sharing of these surgical tips might be helpful for future experiments.

In conclusion, it has been shown that the adoption of a microvascular technique could help intact fresh and C–T ovaries retain reasonable function upon reimplantation. End-to-side anastomosis is more efficient in overcoming size discrepancies than the fish mouth modification. Inferior epigastric vessels were proposed to be the most suitable transplantation site in humans. Long-term survival, endocrine function and fertility of oocytes derived from transplanted intact ovaries with microvascular anastomosis should be investigated.

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