Ovarian cortex transplantation in the baboon: comparison of four different intra-abdominal transplantation sites

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BACKGROUND: Several sites have been used for ovarian cortex transplantation (OCT) in humans. The present study was designed to evaluate different intra-abdominal transplantation sites in the baboon to gain further knowledge about alternative transplantation sites in a human setting.

METHODS: Autologous fresh OCTs were performed in 12 baboons (Papio anubis). Four different sites were tested: the free portion of the omentum (OMF), the portion of the omentum adjacent to the spleen (OMS), the pouch of Douglas (D) and the pelvic wall on the psoas muscle (PW). Cortex survival, follicle density, cyclicity and hormonal levels were compared between the different sites, 3 and 6 months after transplantation.

RESULTS: Macroscopically, antral follicles were only found in the OMS and OMF locations, which also showed a higher proportion of follicle-containing cortex at light microscopy (OMF 71.4%, OMS 83.3% versus PW 58.8% and D 40%, P < 0.05). Higher densities of primordial [OMF: 3.54 (0–13.18) follicles/grid, OMS: 3.85 (0–8.53), PW: 0 (0–13.25), D 0 (0–1.33), P < 0.05] and primary follicles [OMF: 3.54 (0–18.52), OMS: 3.85 (0–1), PW: 0 (0–4.58), D 0 (0–0.25), P < 0.05] was also found in the omental locations.

CONCLUSIONS: Omental locations provide a better site, in terms of follicle survival, for intra-abdominal OCT in the baboon compared with the pelvic wall and the D.

Key words: baboon / infertility / transplantation / ovary / fertility preservation

Introduction

During the last decades, there has been a considerable increase in survival rates after cancer treatment (Linabery and Ross, 2008), especially concerning cancer during childhood or at a young age. As one consequence of this, there is an increasing demand from society and patients to also ensure that the fertility of these young survivors of cancer can be preserved or restored. Fertility preservation in patients with cancer is required owing to the gonadotoxicity of many types of treatments. Thus, chemotherapy regimens with alkylating agents and radiotherapy over the pelvis are extremely gonadotoxic, leading to rapid and irreversible ovarian failure (Oktem and Oktay, 2007; Blumenfeld and von Wolff, 2008).

The research/clinical area of oncofertility, with the aim of harvesting and preserving ovarian tissue in young women facing cancer, has emerged and it has quickly moved from an experimental/laboratory setting into the clinical arena. In younger females of prepubertal age and in those women urgently requiring chemotherapy, the only current fertility preservation option is cryopreservation of ovarian tissue. In view of the fact that the bulk of the follicle pool is localized in the cortex of the ovary, either cortical biopsies or cortical strips have been retrieved and cryopreserved for several years with the aim of avascular retransplantation when the women is considered free from the cancer and wishes to plan for pregnancy. To date, around 15 healthy babies have been born since the first case in 2004 (Donnez et al., 2011).

The procedure of ovarian cortex cryopreservation and retransplantation was introduced in the human after some research in animal models, including the mouse (Weissman et al., 1999; Van den Broecke et al., 2001), rat (Dissen et al., 1994) and sheep...
However, few of these preparatory animal studies were performed in a non-human primate model (Schnorr et al., 2002; Lee et al., 2004), which should be the natural last step before introduction of any procedure in the human. Thus, many factors relating to graft survival and function after ovarian cortex transplantation (OCT) were not thoroughly investigated in a research setting before human application. Few well-designed comparative studies on heterotopic OCT have been published, although some exist in rodents (Callejo et al., 2002; Hernandez-Fonseca et al., 2004; Dath et al., 2010). To our knowledge only three studies on OCT in non-human primates have been published (Schnorr et al., 2002; Lee et al., 2004; Igarashi et al., 2010) with the first primate pregnancy and live birth reported in the rhesus macaque (Lee et al., 2004). There is one limited study (two animals) in a non-human primate species that has compared different OCT sites (Igarashi et al., 2010) and for ethical reasons these studies cannot be performed in women (Demeestere et al., 2009).

Nevertheless, in humans several heterotopic sites, such as subcutaneously in the abdominal wall or forearm (Oktay, 2005) or rectus abdominis muscle (Kim et al., 2004a,b), were tested and resulted in proven restored ovarian function, fertilized oocytes and biochemical pregnancy (Rosendahl et al., 2006) but so far no clinical pregnancy has been achieved after heterotopic transplantation.

A non-human primate species offers the possibility of performing this type of study in an anatomical and physiological setting close to the human. In the present study, we utilized the baboon model to compare four different intra-abdominal transplantation sites for OCT, with long-term follow-up.

### Materials and Methods

#### Animals

The present study was carried out at the Institute for Primate Research (IPR) in Nairobi, Kenya. Twelve (B1–B12) healthy adult female olive baboons (*Papio anubis*) were included. These baboons were also used as donors in uterus transplantation (UTx) experiments (our unpublished data). The animals were fed commercial monkey diet daily with additional supplementation of fruits and vegetables three times a week and they were housed in single cages during the experiment. The study protocol was approved by the Institutional Scientific Evaluation and Review Committee and the Animal Care and Use Committee of the IPR, Nairobi, Kenya.

#### Study design

The 12 monkeys were divided into two groups of six. A schematic of the experimental design is shown in Fig. 1. An initial study was performed (first series: B1–B6) to evaluate four different transplantation sites. In these initial experiments, which were auto-UTx experiments, the left ovary remained connected to the uterine graft and was transplanted back by vascular connection, but a right-sided oophorectomy was performed. From the right ovary, strips of fresh ovarian cortex were autotransplanted to four different locations: (i) free caudal portion of the omentum (OMF); (ii) portion of the omentum immediately adjacent to the spleen (OMS); (iii) pelvic wall, just lateral to the bifurcation between the internal and external iliac arteries and on the psoas muscle (PW) and (iv) the pouch of Douglas (D). Comparisons between different transplantation sites according to morphologic criteria were carried out on biopsies taken during the second-look laparotomy 3 months after surgery.

**Figure 1**  Schematic outline of the experimental design for comparison of OCT at four different intra-abdominal transplantation sites in the baboon. OMS, portion of the omentum immediately adjacent to the spleen; OMF, free caudal portion of the omentum; PW, pelvic wall; D, pouch of Douglas. The crosses denote the organs that were not transplanted.
The second set of experiments (B7–B12) consisted of donors for allogenic UTx. In these animals bilateral oophorectomy was performed as part of the uterus retrieval procedure. The animals underwent ovarian cortex autotransplantation only in two different locations (OMF and PW), which were selected after a preliminary analysis of the results of the first set (B1–B6). Functionality of the grafts was assessed by monitoring peri-neal skin cyclicity, hormonal levels and by morphological evaluation of biopsies from the transplanted tissue taken 3 and 6 months after transplantation (Fig. 1).

**Anaesthesia and post-operative care**

The anaesthetic procedures have been described in detail elsewhere (Enskog et al., 2010). Post-operative care included antibiotic therapy (15 mg/kg/day i.m. trimethoprim sulphamethoxazole, GlaxoSmithKline, Mississauga, Canada, 80 mg/400 mg and 20 mg/kg/day p.o. metronidazole, Sanofi-Aventis, Midrand, South Africa), antithrombotic prophylaxis (1500 IU/day s.c. dalteparin sodium, Pfizer, Sollentuna, Sweden) and analgesics (0.3 mg/kg meloxicam, Boehringer Ingelheim Vetmedica, Mississauga, Canada, 80 mg/400 mg and 20 mg/kg/day p.o. metronidazole, Sanofi-Aventis, Midrand, South Africa) for a duration of one week.

**Surgery and preparation of cortical strips**

A midline incision from the pubic bone up to 2–3 cm above the umbilicus was performed and the intestines were packed into the upper abdomen. Under gentle upward traction of the uterus, the terminal branches of the ovarian artery and ovarian branch of the distal uterine artery were isolated and ligated using 5–0 poliglactin sutures (Vicryl®; Johnson & Johnson, New Brunswick, NJ, USA). The ovaries were harvested without the ovarian veins, which remained with the uterus, and then the UTx surgical procedure continued (Enskog et al., 2010).

The preparation of the cortical strips took place in a room beside the operating room. The ovaries were weighed after dissection of the surrounding fat and then placed in a glass containing chilled (4°C) Hank’s Buffered Salt Solution (HBSS, Invitrogen AB, Lidingö, Sweden). A 1 mm portion of each ovarian cortex was kept in 4% formaldehyde for later histology (see below) and to use as a control. The ovaries were divided into two hemi-ovaries with the division along the long axis of the ovary, and the main portion of the medulla was pulled out using fine forceps and micro-surgical scissors (S&T Microsurgery, Neuhausen, Switzerland). Each hemi-ovary was cut again twice and the remaining medulla was scratched from the cortex using a surgical blade tip and fine forceps. The whole de-cortication process was performed with the help of magnifying loupes (×4 magnification). Eight cortex fragments measuring ~4 mm length × 2 mm width × 0.8 mm depth were obtained from each ovary.

All the fragments were kept at 4°C in HBSS until the uterine graft had been retrieved from the donor. Then the ovarian tissue blocks were reintroduced to different locations according to the study design (see paragraph above and Fig. 1). Two fragments of cortex were placed in each location (Fig. 2) using 6-0 polypropylene sutures (Prolene®; Johnson & Johnson). All the transplanted sites were referenced using a 1-0 non-absorbable suture (Tycron®; Covidien Sverige AB, Solna, Sweden). The abdomen was closed by continuous sutures of the fascia (1-0 polydioxanone; Johnson & Johnson) and the skin (3-0 Vicryl®; Johnson & Johnson).

**Monitoring of cyclicity**

The menstrual cycle in the baboon can be divided into eight distinct and different stages (D’Hooghe et al., 1991), with stage 7 being the menstrual phase. Stages 1–5 are characterized by progressive inflation of the perineum and they match the follicular phase of the cycle, with ovulation taking place during stage 5. Stages 6 and 0 are characterized by skin deflation and they equate to the luteal phase. If pregnancy occurs, stage 0 is followed by an additional stage (stage 8) which is similar to stage 0 but with a more reddish perineum. The perineal degree of inflation was monitored daily by experienced animal technicians and cyclicity charts were filed-in. The animals (B7–B12) included in the second series of the study lacked stages 7 and 8 by definition, as they had all been hysterecto-mized. Since the animals from the first series (B1–B6) retained their left ovary, connected by vascular anastomosis, cyclicity was not monitored for these animals.

**Hormone levels**

Blood samples were collected before surgery and then fortnightly until the end of the experiment. If cyclicity was resumed the sampling was adapted to take place every second and 15th day after sex skin deturgescence. Blood samples (5 ml) were collected in glass tubes and left for 1 h at room temperature, followed by centrifugation at 1000g for 20 min at 4°C. The supernatant was removed and stored at −20°C. A chemiluminescent microparticle immunoassay was used to measure progesterone and estradiol (E2) (Abbot Architect, Abbott Scandinavia AB, Solna, Sweden). The within-run coefficient of variation (CV) for this progesterone assay varies between 1.8 and 5.5%. The within-run CV for E2 varies between 1.4 and 6.4%. The analytical sensitivity was 0.1 ng/ml and 10 pg/ml for progesterone and E2, respectively.

**Second-look laparotomy**

Second-look laparotomies were performed 3 and/or 6 months after surgery. After entering the abdominal cavity, the marking sutures were identified in the different transplantation sites. The presence and size of
visible ovarian cortex was noted and biopsies were taken. Antibiotics or anti-coagulants were not given. After the last second-look surgery, the animals were euthanized during anaesthesia using iv phenobarbital (Fenenal Recip™, Recip AB, Solna, Sweden).

**Histology**

Biopsies were taken from the ovaries before decortication, after cold ischemia just before transplantation and during the second-look laparotomies. They were fixed in 4% buffered formaldehyde, dehydrated and embedded in paraffin. Sections were taken every 4 μm, with three consecutive sections stained with haematoxylin–eosin and the next three prepared for immunohistochemistry to detect active caspase-3. Randomly selected sections, positioned c.50 μm apart, were examined for histology in order to avoid counting the same follicle twice. Caspase-3 is a marker of early events in cells undergoing apoptosis (Porter and Janicke, 1999). The immunohistochemical staining of the samples was carried out using a rabbit anti-human polyclonal antibody against caspase-3 (AB4051; Abcam, Cambridge, UK) according to the methodology previously described (Martinez-Madrid et al., 2007) with slight modifications. Sections were deparaffinized in xylene, rinsed in ethanol and brought to water through a series of decreasing concentrations of ethanol. Antigen retrieval with 10 mM citrate buffer pH 6.0 was performed using a pressure cooker. The slides were incubated using a 1:200 dilution of the primary antibody in 0.02 M Tris-buffered saline containing 0.05% Tween 20 (Scharfau, Barcelona, Spain). For detection of the antibody a MACH 3™ rabbit alkaline phosphatase-polymer detection kit (Biocare Medical Inc. LLC, Concord, CA, USA) with the chromogen Vulcan Fast Red (Biocare Medical Inc.) was used. The slides were co-stained with haematoxylin.

**Results**

**General**

The animals included in this study weighed 11–14.3 kg and all of them showed regular menstrual cycles before surgery. The median weight of the ovaries was 510 mg (470–980), with those being heavier than 500 mg presenting large antral follicles measuring more than 5 mm. The surgical time for complete removal of the ovarian cortex was 9 min (8–20) and the cold ischemic time between retrieval and re-transplantation was 189 min (165–315). One of the monkeys (B7) died of peritonitis after the first (3 months) second-look laparotomy.

**Cyclicity**

Four of the six monkeys in the second series (B7–B12) resumed cyclicity during the study. The time that elapsed between surgery and the initiation of the first complete cycle ranged between 28 and 105 days. Two of the monkeys cycled once (B8 and B9) while the other two went through two cycles (B10 and B11). All the baboons in the second series had undetectable levels of progesterone and E2 during the first 4 weeks after surgery. Between 30 and 110 days after surgery an increase in the hormone baseline level was detected in baboons B8–B11 [E2 43 pg/ml (21–57), progesterone 0.6 ng/ml (0.4–1.4)]. Nevertheless, both E2 and progesterone remained under the detection limits of the assay for large parts of the follow-up period.

**Second-look laparotomy**

All the animals except B7 showed slight to mild adhesions at the first (3 months) second-look laparotomy. In the first series (B1–B6), fibrotic tissue was observed in the PW and D sites of all six monkeys. Ovarian tissue pieces (two pieces from each location) retrieved from omental locations measured between 4 and 12 mm (length) and 3 and 10 mm (width) after removing the surrounding fat, with Graafian follicles present in three animals. Ovarian tissue pieces retrieved from the PW sites had a maximum size of 5 × 3 mm after removing the surrounding tissue. In the second series of transplanted animals, B7 showed severe adhesions that required extensive adhesiolysis in order to find the transplanted tissue. The size of macroscopically visible ovarian cortex-like tissue was greater in OMF (6–12 mm length × 4–9 mm width) than PW (5 mm length × 3 mm width, including a small portion of fibrotic tissue) sites in animals B7–B12. Graafian follicles were found at 6 months (Fig. 2) in the OMF site for B9, B10, B11 and B12 (Table I). No follicles were macroscopically visible in other locations. None of the 12 baboons showed macroscopically identifiable ovarian cortex in the D site but a biopsy including all the fibrotic tissue was taken for histology.

**Histology**

The intra and inter-observer reliability analysis performed on the total, primordial, primary, secondary and antral follicle density showed intraclass correlation coefficients ranging from 0.832 (95% confidence interval (CI): 0.640–0.935) to 0.983 (95% CI: 0.878–0.998). A Cohen’s k of 1 was found for assessment of the presence/absence of follicles by the observers. In the fresh biopsies and biopsies after cold ischemia both small follicles and antral follicles were seen (Fig. 3). The location of primary and primordial follicles was...
Table I Follow-up and findings at second-look laparotomy of baboons B7–B12 (n = 6) following OCT.

<table>
<thead>
<tr>
<th>Baboon</th>
<th>First day of complete cycle</th>
<th>Cycles (n)</th>
<th>Findings during second-look laparotomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>B7</td>
<td>—</td>
<td>—</td>
<td>OMF—no macroscopic follicles, PW—fibrosis, Died after first second-look laparotomy</td>
</tr>
<tr>
<td>B8</td>
<td>94</td>
<td>1</td>
<td>OMF—no macroscopic follicles, PW—fibrosis</td>
</tr>
<tr>
<td>B9</td>
<td>47</td>
<td>1</td>
<td>OMF—macroscopic follicles (first and second), PW—cortex-like tissue visualized</td>
</tr>
<tr>
<td>B10</td>
<td>28/53</td>
<td>2</td>
<td>OMF—macroscopic follicles (first and second), PW—cortex-like tissue visualized</td>
</tr>
<tr>
<td>B11</td>
<td>105/129</td>
<td>2</td>
<td>OMF—macroscopic follicles (second), PW—fibrosis</td>
</tr>
<tr>
<td>B12</td>
<td>—</td>
<td>—</td>
<td>OMF—macroscopic follicles (second), PW—fibrosis</td>
</tr>
</tbody>
</table>

First and second refer to second-look laparotomy at 3 and 6 months, respectively. OMF, free caudal portion of the omentum; PW, pelvic wall.

subcortical, c.400 μm below the ovarian surface. Caspase-3 positive cells were found scattered in the stroma and occasionally in small follicles. There were no differences in follicle density between fresh controls and biopsies taken after cold ischemia preservation and no difference was seen regarding the number of apoptotic follicles (caspase-3+) or the number of grids showing any apoptotic stromal cells when these biopsies were compared (data not shown).

In the first series (B1–B6), we found that biopsies taken from omental locations showed a higher proportion of sections presenting cortex with follicles than PW and D sites (OMF 71.4%, OMS 83.3% versus PW 58.8% and D 40%, P < 0.05; Fig. 4), and we also found a higher follicle density, although the differences in follicle density were not significant (Fig. 3 and Table II). When the 12 animals were analysed together, the fresh non-transplanted cortex (control) had a higher density for all categories of follicle when compared with the cortex in the four transplantation sites (Table II). Primordial and secondary follicles were more abundant in OMF and OMS compared with PW and D. Density of primary follicles was higher in OMF but not in OMS, when compared with PW/D. Antral and secondary follicles were not seen in D but were present at the other transplantation sites.

Discussion

The major findings of the present study were that intra-abdominal OCT is feasible in non-human primate species and that transplantation sites of the omentum provide advantages compared with other sites in terms of survival of primordial and primary follicles as well as follicle development.

In the present study, we used the baboon (Papio anubis) as an OCT model due to its close similarities to the human regarding ovarian functionality, histology and anatomy. The average menstrual cycle length of the baboon is 33.4 days (SD: 2.1 days) (Stevens, 1997), with a steroid hormonal profile close to that of the human although lower progesterone levels are observed during the luteal phase (Stevens, 1997; Nyachiao et al., 2009). The correlation between the hormonal changes during the menstrual cycle and the perineal skin oedematization (D’Hoooge et al., 1991) makes the baboon an excellent model in which cyclicity can be studied even in hysterectomized subjects.

In the present study, we tested different intra-abdominal transplantation sites which have not been fully explored before. These locations present different physiological environments and may all have certain advantages. All the locations tested were intra-abdominal in order to avoid major differences from the normal situation in the ovary concerning pressure, temperature and microenvironment of the ovaries. The degree of vascularization of the transplantation site was also taken into consideration for the experimental protocol (Busing et al., 1981; Dath et al., 2010), since the revascularization process remains a critical step to condition the graft for survival with an active participation of paracrine factors of the graft and the site of transplantation (Van Eyck et al., 2010).

In a clinical context, omental locations are easily accessible sites for grafting by laparoscopy and they are also highly vascularized sites that have been proven to support engraftment and growth of transplanted tissue, such as pancreatic islets (Kriz et al., 2010). The choice of the two omental locations was based on the potential effects on the grafts caused by the portal venous drainage and the growth factors released by the spleen (Petroianu et al., 2005; Yamamoto et al., 2010). The PW site was chosen as this is a heterotopic location close to the natural orthotopic site and the PW is highly vascularized. The D was selected because of its potential accessibility for oocyte retrieval following transplant through the vaginal posterior fornix. Although a pregnancy was reported in rhesus macaque after transplantation of fresh ovarian tissue to a subcutaneous location of the abdominal wall (Lee et al., 2004), we decided not to use this site in our experiments owing to human data which demonstrate a poor outcome in this location (Demeestere et al., 2009) and lack of follicle development after fresh s.c. OCT in two cynomolgus macaques (Igarashi et al., 2010).

In the present study all cortical strips were freshly transplanted but after a cold ischemic period of ~3 h. We evaluated morphology after this cold ischemic period alone but found no differences compared with the control biopsies taken before the ischemic period. This finding is in line with those showing that ovarian cortex transplanted to severe combined immunodeficiency (SCID) mice after 3–4 h maintain the follicle pool (Schmidt et al., 2003). In our study, the rate of apoptosis was not altered after 3 h of cold ischemia. This is in line with the finding that apoptosis seems to occur mainly during the first days post-transplantation (Israely et al., 2006) and is related to the low oxygen pressure until complete neovascularization of the tissue is achieved (Van Eyck et al., 2009, 2010).

The proportion of transplanted cortex that contained follicles after 3 (OMS/D sites) and 6 months (OMF/PW sites) was above 70% in...
the omental locations but as low as 40% in the D. In the cortical slices with no, or a low number of, follicles the tissue was whitish at macroscopic examination and histologic examination of the tissue showed a high rate of fibrosis. In a recent study using a SCID mouse model to compare four different heterotopic sites (i.m. in the limb, subcutaneous in the abdomen, intraperitoneal and in the ovarian bursa) at xenotransplantation of human ovarian cortex grafts (Dath et al., 2010), the i.m. location presented less (~18%) fibrosis in the short-term (7–21 days) compared with an intraperitoneal site (~42%) or the bursa ovariaca (~55%). An interesting observation was that the higher degree of fibrosis was not associated with a lower follicle density in the transplanted tissue. In our study, higher follicle densities were found in both omental locations compared with the pelvic wall (PW) and the D. These apparently opposing results from the two studies may be because different time points of a similar degenerative process were studied. The omental tissue is extremely well vascularized and this may reduce the time for neovascularization, which would increase follicle and stroma survival. Nevertheless, even in omental sites the degree of fibrosis reached up to 28.6% and the follicle densities were significantly lower than the fresh control tissue, confirming the deleterious effect of the prolonged ischemia related to the delay in reperfusion events (Callejo et al., 2002; Kim et al., 2004a,b). Since the PW is also well vascularized, there must be other factors that explain the high fibrosis rate and low follicle density. The effect of mechanical forces on the transplanted tissue may play a deleterious role (Dath et al., 2010). The low proportion of viable cortical tissue in D could be related to the acute healing process of the sutured vaginal vault because the animal had already been involved in another experiment.

The density of primordial follicles in the transplanted cortical tissue was considerably lower than in the fresh tissue. The variability of primordial follicle density after transplantation was also large. The median primordial follicle density decreased approximately 50% for omental locations while it decreased over 95% for PW and D when transplanted tissue was compared with fresh control 3 months after

Figure 3: Histological sections of biopsies taken after (PW, D, OMF and OMS) and before (Ov cortex) OCT. Upper four panels: a lower follicle density as well as increased fibrosis are observed in D and PW locations when compared with omental locations (OMF/OMS) OMS, portion of the omentum immediately adjacent to the spleen; OMF, free caudal portion of the omentum (haematoxylin and eosin staining). The asterisk indicates an intense fibrotic area in the graft. The arrow heads point at small primordial and primary follicles. Lower two panels: Follicular lymphocytes in caspase 3-positive control tissue (Tonsil) and ovarian cortex after cold ischemia showing an apoptotic secondary follicle which is stained positive for caspase-3 in the oocyte (Ov cortex).
transplantation. Our results are consistent with those showing 70% loss of the follicle pool after transplantation to a subcutaneous site (Gosden et al., 1994; Nisolle et al., 2000). The extremely high loss of primordial follicles in PW and D clearly indicates that these are not suitable transplantation sites in this model.

Large antral follicles (visible macroscopically) were only found in the OMF sites. Secondary and antral follicles were rarely found in biopsies from all the heterotopic locations implying that the normal follicle dynamics is altered. This finding supports the conclusions from human clinical experiences and other animal data (Demeestere et al., 2009) suggesting that the orthotopic sites would provide a better environment for follicle development, although retrieval of fertilizable oocytes from heterotopic locations has been reported in three different primate species (Lee et al., 2004; Oktay et al., 2004; Rosendahl et al., 2006; Igarashi et al., 2010). Resumed cyclicity is caused by ovarian hormone production and for the baboon the visual inspection of perineal skin is a validated method of monitoring cyclicity (D’Hooghe et al., 1991). Cyclicity resumed in four out of six of the monkeys which had been previously devoid of ovarian tissue. Macroscopic follicles were seen in three out of four monkeys with demonstrated cyclicity. In one baboon (B12) large follicles were seen but cyclicity was not observed, suggesting that this graft was undergoing development of its first follicle. The short time for resumption of cyclicity in two of the baboons, together with the fact that hormone levels were undetectable after surgery, demonstrates that secondary or antral follicles present in the transplanted cortex had survived the ischemic period following the transplantation and that they had continued to develop. The low number of cycles observed may have several explanations, such as low follicle survival in non-omential sites, a restricted observation time which was also too short and the presence of longer inter-cycle intervals, as described (Donnez et al., 2010; Silber et al., 2010) after human OCT.

In baboons, the E2 peak level is seen around 3 days after sex skin deturgescence (Stevens, 1997), and the progesterone levels rise around 5 days after perineal deturgescence reaching up to 15 ng/ml during the luteal phase. E2 levels up to 57 pg/ml were observed in our study, which is consistent with values obtained during both the follicular and luteal phase (Stevens, 1997; Nyachieo et al., 2009). Progesterone levels remained below 1.4 ng/ml, which is below the normal range of c.5 ng/ml in the luteal phase as reported by Stevens (1997), although the present data are in concordance with other results reported in baboons from the same research centre (IPR, Nairobi, Kenya) (Nyachieo et al., 2009). These low hormone levels may be explained by the fact that only a small portion of the original follicle pool survived. Taking into account that high follicle density was only observed in omental locations, we can assume that only around 50–60% of half an ovary remained potentially functional. Noteworthy is that resumption of cyclicity, menstruation and oocyte fertilization were described in another non-human primate OCT model with low progesterone levels (0.03 ng/ml) (Igarashi et al., 2010) but it is still unclear whether these low progesterone levels could support early pregnancy.

A limitation of our study was that resumed cyclicity could not be traced to a specific transplantation site because two locations (PW/OMF) were grafted at the same time. Nevertheless the fact that two of the monkeys which resumed cyclicity displayed a complete absence of follicles in the PW location highlights the main role of the OMF in the restored functionality after transplantation. Another

![Figure 4](image-url) Proportion of ovarian cortical grafts containing follicles at second-look surgery in baboon 3 months after transplantation.

### Table II Follicle density in transplanted ovarian cortex according to morphological classification and transplantation site in baboons.

<table>
<thead>
<tr>
<th>Site</th>
<th>Control (n = 12)</th>
<th>OMF (n = 12)</th>
<th>OMS (n = 6)</th>
<th>PW (n = 12)</th>
<th>D (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primordial</td>
<td>8.50 (3.71–14.80)</td>
<td>3.54 (0–13.18)*</td>
<td>3.85 (0–8.53)*</td>
<td>0 (0–13.52)*</td>
<td>0 (0–13.25)*</td>
</tr>
<tr>
<td>Primary</td>
<td>2.92 (1.25–13.00)</td>
<td>2.45 (0–18.52)</td>
<td>0.75 (0–1.00)*</td>
<td>0 (0–4.58)*</td>
<td>0 (0–0.25)*</td>
</tr>
<tr>
<td>Secondary</td>
<td>2.00 (0.57–3.12)</td>
<td>0.29 (0–5.04)*</td>
<td>0.20 (0–1.03)*</td>
<td>0 (0–0.67)*</td>
<td>0 (0–0.26)*</td>
</tr>
<tr>
<td>Antral</td>
<td>1.23 (0–1.81)</td>
<td>0 (0–0.21)*</td>
<td>0 (0–0.40)*</td>
<td>0 (0–0.26)*</td>
<td>0 (0–0.26)*</td>
</tr>
</tbody>
</table>

The numbers in parentheses indicate the number of animals with sections showing follicles for each type of classification and location (number of animals showing follicles/total number of animals analysed for each specific site).

The follicle density is expressed as number of follicles/grid (median [range]). Data were compared using the Friedman test. Multiple paired comparisons were performed using the PAIRSET module (11) of WINPEPI/OMS, portion of the omentum immediately adjacent to the spleen; D, pouch of Douglas.

*a Versus control ovaries (P < 0.05).
*b Versus control ovaries (P < 0.01).
*c Versus OMF (P < 0.05).
*d Versus OMS (P < 0.05).
limitation is the fact that the animals used in this study were also used previously for uterus retrieval in another experiment. It should be emphasized that the experimental design, performing ovarian transplantation experiments in the hysterectomized donors, reduced the number of animals subjected to captivity and experimental procedures, in accordance with general recommendations. However, the previous hysterectomy surgery in these animals could have introduced an external source of stress, which cannot be measured, that could affect viability after OCT.

In conclusion, our study demonstrates that omental locations provide a better site for heterotopic OCT compared with the PW and the D in the baboon. Further research must be carried out on the clinical application of these potential transplantation sites. Although we do not propose that OCT to the omentum would replace orthotopic OCT for human reproductive purposes, the former could enhance the performance of the latter, as heterotopically transplanted cortex can activate remnant ovarian tissue (Demeestere et al., 2006; Oktay et al., 2011). Moreover, in cases with no available orthotopic site, a transposed omental flap to the posterior leaf of the broad ligament could be considered as a potential alternative transplantation site.

Authors’ roles
C.D.-G. and M.B. are responsible for the design of the experiment, surgical procedures, evaluation of the results and writing of the article. M.M. participated in the design of the study, evaluation of the results and writing of the article. K.G., M.M. and C.D.-G. did the follicle count. P.D.K. and M.O. participated in the surgical procedures.

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