Chapter 7
Heterotopic Ovarian Tissue Transplantation

Michelle Soares, Marie-Madeleine Dolmans, and Jacques Donnez

Abstract Restoration of ovarian function has been consistently observed after transplantation of ovarian tissue to heterotopic sites, and recent reports now confirm that ovarian function following heterotopic transplantation can last more than 7 years, depending on the initial ovarian reserve. The possibility of long-term restoration of ovarian function, combined with the advantage of a less invasive and more cost-efficient procedure (especially when considering repeated transplantations), makes heterotopic transplantation the technique of choice when the main goal of grafting is restoration of endocrine function.

Although follicle development, oocyte retrieval and fertilization, and embryo development have been demonstrated after heterotopic transplantation of frozen-thawed ovarian tissue to various heterotopic sites, oocyte quality, and hence embryo quality, appear to be compromised. This is probably attributable to the nonoptimal environment for follicular development. Teams who have simultaneously grafted ovarian tissue to orthotopic as well as heterotopic sites report superior results with orthotopic sites. Therefore, when fertility restoration is the goal, there is no doubt that orthotopic sites in the pelvic cavity (ovarian medulla or pelvic peritoneum), although more invasive, are much more effective, as evidenced by the number of live births.

Moreover, grafting of ovarian tissue to a subperitoneal pocket in the abdominal wall could potentially be an option in case of severe pelvic adhesions that preclude pelvic surgery, or in addition to grafting to orthotopic sites, and should be investigated further.

Keywords Heterotopic • Ovarian tissue transplantation • Grafting site

M. Soares
Pôle de Recherche en Gynécologie, Institut Expérimental et Clinique, Université Catholique de Louvain, Avenue Mounier 52, bte B1.52.02, 1200 Brussels, Belgium

M.-M. Dolmans (✉)
Pôle de Recherche en Gynécologie, Institut Expérimental et Clinique, Université Catholique de Louvain, Avenue Mounier 52, bte B1.52.02, 1200 Brussels, Belgium

Département de Gynécologie, Cliniques Universitaires Saint-Luc, Brussels, Belgium
e-mail: marie-madeleine.dolmans@uclouvain.be

J. Donnez
Société de Recherche pour l'Infertilité, Brussels, Belgium

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7.1 Introduction

Ovarian tissue transplantation has a long history, dating back to 1895, when the first graft was performed by Robert Morris. However, due to the lack of good clinical indications, interest in human ovarian tissue grafting gradually waned until the development of new technologies for assisted reproduction and cryopreservation. Almost 100 years after the first transplant, interest in human ovarian tissue transplantation was once again reignited in 1994, when Gosden et al. demonstrated fertility restoration after frozen-thawed ovarian tissue transplantation in sheep [1]. Since then, ovarian tissue cryopreservation and transplantation has become a key topic in reproductive medicine.

Today, cryopreservation of ovarian tissue is gaining ground as an established fertility preservation technique. It is the only currently available option for prepubescent girls and women with cancer who cannot delay the start of cancer treatment, and can be combined with immature oocyte retrieval followed by in vitro maturation.

Reimplantation of cryopreserved ovarian tissue has become clinically feasible and is practiced in a number of centers worldwide. Since the first live birth following frozen-thawed ovarian tissue transplantation in a woman with Hodgkin's lymphoma in 2004 [2], 60 more live births ([3], + see chapter by J. Donnez in the present book) have been reported in the literature to date, showing that this technique should no longer be considered experimental.

Ovarian tissue has been transplanted back to orthotopic as well as heterotopic sites, and the choice of transplantation site constitutes an essential factor in future graft viability and oocyte quality and competence. Before proceeding any further, it is important to clearly define what is meant by orthotopic and heterotopic, as the use of different terminology can lead to misunderstandings.

Strictly speaking, orthotopic ovarian tissue transplantation implies grafting of ovarian cortical strips to their natural site, namely the remaining ovary. However, grafting of this tissue to pelvic sites in general, including the pelvic peritoneum and broad ligaments where environmental conditions are very close to natural, is also widely considered to be orthotopic [4]. While restoration of endocrine function and fertility have been demonstrated following orthotopic transplantation, efficacy and success rates are still not really known because the denominator is unknown. In a recently published report (5) combining results from 5 fertility centers [5–8], the pregnancy rate (based on the number of women who conceived) following orthotopic transplantation was 29 % (32 out of 111).

Heterotopic sites, namely sites that have nothing to do with the natural localization or environment of the ovary, like the forearm (subcutaneous (SC)), abdominal wall (SC, intramuscular (IM), just below the fascia), and chest wall (pectoralis muscle), have also been used for grafting of ovarian cortical strips and are associated with restoration of ovarian function and follicular development with oocyte retrieval. Nevertheless, just one (twin) live birth has been reported to date [9].

After a general overview of the history of ovarian tissue grafting, this chapter will address the specifics of heterotopic transplantation, including different grafting sites, advantages and limitations, experimental and clinical results, and factors affecting graft function after transplantation.

7.2 History of Ovarian Tissue Transplantation

Ovarian tissue grafting has a long history, beginning with a small account that appeared in the thesis of Paul Bert in 1863 [10, 11]. The initially disappointing results apparently dulled interest for the next 30 years. These early studies often used implants of ovarian tissue slices, showing their capacity to survive following rapid revascularization. Early studies on human ovarian tissue transplantation for fertility restoration or as treatment for menopausal symptoms described menstrual cyclicity, pregnancies, and even deliveries after grafting, as reported in a review by Nugent et al. [10]. Some also claimed success with ovarian tissue allografts, believing the ovary to have a certain degree of immunological privilege. However, current knowledge of immunology indicates that these claims were somewhat overstated, and we now know that immunosuppressive therapies are essential if ovarian allografts are to survive. Although the scientific validity of this early work is questionable, the concept of ovarian implants remains attractive.

In humans, the first ovarian implants were reported by Robert Morris in a woman suffering from ovarian failure as far back as 1895 [12]. The patient reportedly became pregnant, but it ended in spontaneous miscarriage. Other similar transplants were described in the years that followed, with variable outcomes.

Orthotopic transplantation to the broad ligament was then the procedure of choice to obtain pregnancy due to its simplicity. Nonetheless, it proved disappointing from a fertility point of view, and ovarian transposition (including the vascular pedicle) to the uterine cavity [13] or uterine horn [14] appeared to yield better results.

As potential treatment for menopausal symptoms, the graft site was not an issue, and many heterotopic sites were tested: the rectus muscle, skin of the abdomen, omentum, cervix, and saphenous vein [10]. Cycles of normal length could be restored by grafting to these heterotopic locations, as long as a satisfactory blood supply was available, the only exception being portal circulation. Indeed, in this site, hepatic clearance of steroid hormones reduces negative feedback on gonadotropin secretion, causing graft hyperstimulation. With the advent of high-resolution ultrasound and in vitro fertilization (IVF) techniques, heterotopic ovarian transplantation began to be considered a potential option for fertility preservation, either by reimplanting slices of frozen-thawed tissue or by grafting fresh tissue to sites outside the radiation field. In 1987, Le Porrier et al. heterotopically transplanted the ovary of a patient with subdiaphragmatic Hodgkin's disease to the SC tissue of the arm [15]. This resulted in regular menstrual cycles, with observable cyclic changes

to the circumference of the arm. A year after surgery, a secondary oocyte was recovered.

Advances made in the field of cryobiology over these last 30 years now allow successful cryopreservation techniques for human ovarian tissue and led to the introduction of ovarian tissue freezing as an option to preserve female fertility in the late 1990s.

Transplantation of cryopreserved ovarian tissue has been successfully performed in different animal models (rodents, rabbits, sheep, and marmoset monkeys [1, 16, 17]). In 2000, the first case of successful orthotopic autotransplantation of frozen-thawed ovarian tissue was reported in humans [18]. Ovarian tissue was transplanted to peritoneal pockets in the pelvic peritoneum of a woman who had previously undergone bilateral oophorectomy, resulting in follicle development and ovulation 15 weeks later, after gonadotropin administration. The following year, Callejo et al. described the same after autografting frozen-thawed tissue to a heterotopic site (rectus muscle of the abdomen) [19]. The first human live birth issuing from the orthotopic technique was reported in 2004 [2] and, since then, at least 42 more live births have been achieved by several centers worldwide after orthotopic transplantation (see chapter on orthotopic transplantation). On the other hand, just one twin delivery has been documented after grafting to a subperitoneal pocket in the abdominal wall, while no pregnancies have been obtained with SC or IM sites.

7.3 Optimal Heterotopic Site

7.3.1 Animal Studies

A number of experiments conducted in animal models after ovarian tissue autografting or xenografting have evaluated follicle development potential in various heterotopic sites, allowing comparison with orthotopic sites.

Different studies in rodents show the kidney capsule or ovarian bursa to have an advantage over SC or intraperitoneal (IP) locations [20–22]. Using a xenografting model, Israely et al. showed better follicle preservation and less tissue damage in rat ovaries transplanted to an IM site in mice compared to an SC site. In a xenotransplantation study of human ovarian tissue to nude mice, four grafting sites (peritoneum, ovarian bursa, SC, and IM) were found to equally support early follicular growth and preserve some quiescent follicles, although a lower degree of fibrosis was observed in the IM site [23]. In another study comparing the kidney capsule to the back muscle as a grafting site in mice, Soleimani et al. reported better follicle survival in grafts from the back muscle.

Since the primary aim of ovarian tissue transplantation is fertility restoration, evaluation of oocyte quality and competence and normal embryo development are important prerequisites for human application. Different studies to date have shown that oocytes collected from grafted ovarian tissue, depending on graft site, exhibited

lower embryo development potential than controls [24–27]. Subcutaneously grafted ovaries in mice had a lower oocyte yield compared to ovaries placed in the kidney capsule or ovarian bursa, while more embryos developed from oocytes retrieved from tissue grafted to the ovarian bursa, even though implantation rates did not differ [27]. Another study demonstrated that oocytes recovered and further matured in vitro after xenografting of human ovarian tissue subcutaneously to SCID mice showed aberrant microtubule organization and chromatin patterns.

Several reports of live young obtained after IVF of oocytes retrieved from SC [27, 28] and IM [29] grafts in mice have been published. In monkeys, the only live birth derived from an SC graft (fresh tissue) occurred in an animal whose resulting embryos were transferred to a gestational surrogate [30]. Other grafting sites, like the pouch of Douglas, omentum, retroperitoneal iliac fossa, mesosalpinx, uterine serosa, and pelvic wall, have also been tested in primates [31, 32]. One of these studies revealed that an omental location was associated with better follicle survival and development than the abdominal wall or pouch of Douglas [31].

7.4 Clinical Experience of Heterotopic Ovarian Tissue Transplantation in Humans

In humans, different heterotopic sites have been investigated since the first graft in 1895. These include the forearm [33, 34], SC tissue of the abdomen [19, 35], breast tissue [36], rectus muscle [36–38], and the anterior wall of the abdomen, just beneath the peritoneum [9, 39, 40].

Clinical studies on heterotopic autotransplantation of cryopreserved ovarian tissue are still limited and summarized in Table 7.1. In 2001, Callejo et al. evaluated the long-term function of fresh (three patients) and frozen (one patient) ovarian tissue grafted subcutaneously to the arm or rectus muscle of the abdomen [19]. Ovarian activity resumed 3–4 months after the procedure in three of the four patients. However, as the patients were premenopausal (46–49 years) at the time of ovarian tissue cryopreservation, no conclusions could be drawn.

In the same year, Oktay et al. described SC fresh ovarian tissue transplantation to the forearm in a 35-year-old patient with stage III squamous cell carcinoma of the cervix and a 37-year-old patient with recurrent benign serous ovarian cysts [33]. Ovarian function resumed 10 weeks and 5 months after transplantation respectively. One MII oocyte obtained after ovarian stimulation in patient 1 failed to fertilize. Graft function lasted more than 21 months in both cases.

These early studies confirmed that simple ovarian grafts, either fresh or cryopreserved, could form functional tissue when grafted to heterotopic sites in women, without any vascular anastomosis.

Later, Kim et al. reported transplantation of cryopreserved ovarian tissue to the rectus muscle (abdominal site, technique shown in Fig. 7.1) and pectoralis muscle (breast site) in a 37-year-old patient cured of squamous cell carcinoma of the cervix

Table 7.1 Heterotopic transplantation of cryopreserved ovarian tissue

Reference/ group	Age before freezing	Chemotherapy before freezing	Indication	Graft site (1st and 2nd transplants)	Grafted strips (size and number per transplant)	Time to recovery of ovarian function: 1st/2nd transplant	Life-span of the graft (months): 1st/2nd transplant	Outcome from heterotopic site
Callejo et al. [19]	47	No	Uterine leiomyoma	Rectus abdominis muscle	Size: 2–3 mm^3 Pcs: 40–45	3–4 months	5–6	↑ E2, FD after stimulation (follicle of 20 mm)
Kim et al. ([36], [65])	37	No	Cervical cancer	1st: A (between rectus muscle and sheath) + B (between breast tissue and pectoralis muscle)	Size: 5 × 5 × 1–2 mm	14 weeks	4/14	↑ E2, ↓ FSH
				2nd: A (rectus muscle)	Pcs: 1st: 20 (B) + 20 (A) 2nd: 20 (A)			FD (follicle of 11–16 mm) only in A site, ovulation
	NA	No	Cervical cancer	A (rectus muscle)	NA	3–5 months/ 2–4 months	NA	Disease relapse, patient expired 5 months after the graft
	28	No	Cervical cancer	1st and 2nd: A (rectus muscle)	Size: 5 × 5 × 1–2 mm Pcs: 1st: 9 2nd: 8		6/84	↑ E2, ↓ FSH, FD *3 IVF cycles: 6 oocytes (1 GV, 4 MI, 1 MII) -> 4 embryos (6-cell, 3-cell, 2-cell, and pronuclear stage*
	29	No	Breast cancer	1st and 2nd: A (rectus muscle)	Size: 5× 5× 1–2 mm Pcs: 1st: 11 2nd: 8		5/9	All embryos cryopreserved
	30	No	Hodgkin's lymphoma	1st and 2nd: A (rectus muscle)	Size: 5 × 5 × 1–2 mm Pcs: 1st: 10 2nd: 8		9/→	↑ E2, ↓ FSH, FD

Oktay et al. ([41], [42], [43])	30	No	Breast cancer	A (SC)	Size: 5 × 5 × 1–15 × 5 × 2 mm Pcs: 15	3 months	62→	FD after gonadotrophin stimulation 20 oocytes -> 8 IVF-ICSI ->2 embryos: one 4-cell (transferred) + one aneuploidic
	28	Yes	Hodgkin's lymphoma	A (SC)	Left ovary in pieces	2 months	42→	FD, ovulation 1 MII oocyte recovered → no fertilization N.B. Spontaneous pregnancy with 3 LB after transplantation
Wolner-Hanssen et al. [34]	30	No	Pure red cell aplasia + BMT	Forearm (SC)	Size: 1–2 × 1–2 × 0.5–1 mm Pcs: 10	18 weeks	NA	FD (follicles of 12.6 mm and 6.7 mm) after local gonadotrophin stimulation
Rosendahl et al. [9] and Schmidt et al. [44]	32	No	Hodgkin	1st: O 2nd: O + A (SP)	Size: 5 × 5 × 1 mm Pcs: 1st: 4 (O) 2nd: 8 (O) + 4 (A)	8/12 weeks	45/23	2 IVF cycles: no oocyte from A
	28	Yes	Hodgkin's relapse	1st: O + P + A (SP) 2nd: O + A (SP)	Size: 5 × 5 × 1 mm Pcs: 1st: 4 (O) + 4 (P) + 4 (A) 2nd: 4 (0) + 4 (A)	19 weeks/1st still functioning	54→/12→	9 IVF cycles: I empty zona + 2 MII oocytes retrieved -> 2 embryos -> 1 biochemical pregnancy
	25	Yes	Hodgkin's relapse	1st: O + P + A (SP) 2nd: O + A (SP)	Size: 5 × 5 × 1 mm Pcs: 1st: 3 (O) + 4 (A) + 5 (P) 2nd: 5 (O) + 1 (A)	18/16 weeks	26/39→	12 IVF cycles: no oocyte from A
	19	No	Paroxymsal nocturnal hemoglobinuria (PNH)	O + A (SP)	Size: 5 × 5 × 1 mm Pcs: 7 (O) + 5 (A)	15 weeks	18→	3 IVF cycles: I empty zona + 3 MII oocytes retrieved -> 1 embryo -> no pregnancy
	25	No	Aplastic anemia	O + A (SP)	Size: 5 × 5 × 1 mm Pcs: 5 (O) + 2 (A)	26 weeks	13 →	3 IVF cycles: I irregular +1 MII oocyte retrieved -> failed to fertilize

(continued)

Table 7.1 (continued)

Reference/ group	Age before freezing	Chemotherapy before freezing	Indication	Graft site (1st and 2nd transplants)	Grafted strips (size and number per transplant)	Time to recovery of ovarian function: 1st/2nd transplant	Life-span of the graft (months): 1st/2nd transplant	Outcome from heterotopic site
Demeestere et al. [35], [45]	24	Yes	Hodgkin's	1st: O + P (2 step) + A (SC)	Size: 5 × 5 × 2 mm	3 months	~12/NA	↑ E2, ↓ FSH, FD at all sites
				2nd: O + P (2 step)+ A (SC)	Pcs:			Follicles at A site reached a max of 13 mm
					1st: 3 (O) + 9 (P) + 6 (A)			N.B. LB from spontaneous ovulation at O site
					2nd: 2 (O) + 2 (A)			
Stern et al. [39], [40], 2014 [46]	17	Yes	Non-Hodgkin's lymphoma	1st: O + P + A (SP)	Size: 4 × 2 × 1 mm	8.5/3 months	17/NA	FD at all 3 graft sites
				2nd: O + P + A (SP)	Pcs: 80 mm^3			9 IVF cycles: at least 1 oocytes from A site
	21	No	Granulosa cell tumor	1st: P + A (SP)	Size: 4 × 2 × 1 mm	4.5 months	24/NA	↑ E2, ↓ FSH
				2nd: A (SP)	Pcs:			*1st graft*: Multiple mild stimulation cycles -> 3 oocytes -> 2 embryos -> no pregnancy
					1st: 30 (P) + 30 (A)			FD seen at Lps 2 years after Tx
					2nd: 60 (A)			*2nd graft:* Two IVF cycles: 2 MII oocytes from A -> 8-cell and 5-cell embryo -> twin delivery
								N.B. Disease recurrence observed at C-section

"→" in life span column = still ongoing at the time of submission
NA not available
In graft site column: *A* anterior abdominal wall, *SC* subcutaneous, *SP* subperitoneal, *B* breast, *O* ovary, *P* pelvic peritoneum
FD follicle development, *E2* estradiol, *GV* germinal vesicle, *Pcs* pieces, *LB* live birth

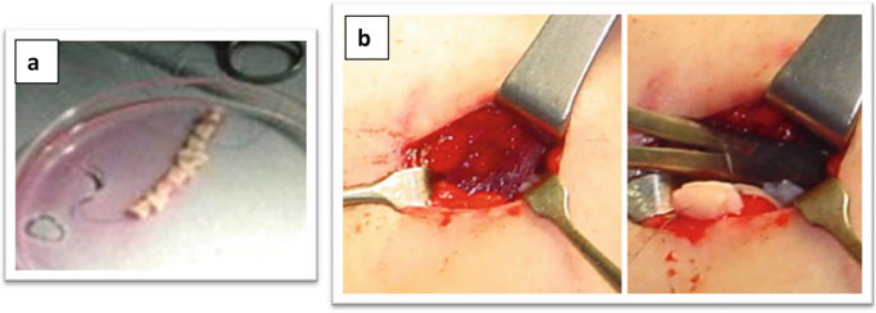

Fig. 7.1 Process of transplantation of frozen-thawed ovarian tissue to a heterotopic site in the abdominal wall. (**a**) Thin ovarian cortex is threaded onto a 3-0 Vicryl suture and (**b**) transplanted between the rectus muscle and its sheath through a small skin incision (2 cm) (pictures taken from **a** Kim et al. [37] and **b** Kim et al. [38]

[36]. Restoration of ovarian function was detected 14 weeks after transplantation, but unfortunately activity ceased around 28 weeks after transplantation, as evidenced by high follicle-stimulating hormone (FSH) and very low estradiol levels.

Around the same time, Oktay et al. reported a case of embryo development from an oocyte retrieved after implantation of frozen-thawed ovarian tissue beneath the skin of the abdomen in a woman with breast cancer. After eight retrieval attempts, just 8 of the 20 oocytes obtained were suitable for IVF and only one fertilized to give a four-cell embryo, but no pregnancy was achieved after transplantation. This study indicated that ovarian follicles with viable and functional oocytes can develop in frozen-thawed ovarian tissue transplanted to a heterotopic site, but also suggested that oocyte quality may be compromised in this heterotopic location. Although freezing and thawing procedures and initial ischemia could be partially responsible for this, these poor results are also attributable to suboptimal environmental factors for follicle development, like pressure, temperature, and paracrine factors in heterotopic sites, compared to those in the peritoneal cavity.

In 2005, Wolner-Hanssen et al. [34] reported the development of two follicles after transplantation of thawed ovarian tissue to the forearm. However, these follicles reached a maximum diameter of only 12.6 mm and 6.7 mm respectively, and the life span of the tissue was around 7 months.

More recently, Kim et al. published their 8-year [37] and subsequently 10-year experience in heterotopic transplantation of ovarian tissue in cancer patients, evaluating long-term graft function and fertility restoration ([38]). Between 2001 and 2011, thawed ovarian tissue was transplanted to the space between the rectus muscle and fascia in the abdomen of five cancer survivors (three with cervical cancer, one with breast cancer, and one with Hodgkin's lymphoma) aged between 28 and 35 years. Restoration of ovarian function was observed in all five patients 12–20 weeks after transplantation, as evidenced by the decrease in FSH levels to less than 20 mIU/ml. Graft function lasted only 3–5 months, but a second

transplantation in four of these patients was able to restore long-term ovarian function (9–84 months). These patients were stimulated with gonadotropins until a dominant follicle of 14–16 mm was obtained, before oocyte retrieval for IVF. Seven oocytes (two germinal vesicles, four MI, one MII) were recovered from two patients. The MI oocytes were matured in vitro, and three of them developed to full maturity. Four MII oocytes were then fertilized, and the resulting embryos after 2–3 days of culture (six-cell, three-cell, two-cell, and pronuclear stage) were frozen for transfer to a surrogate.

When ovarian tissue was transplanted to an SC site or orthotopically to the pelvic peritoneum or ovaries, follicles were found to develop more often in ovarian sites than in peritoneal or SC sites [35, 45]. Furthermore, follicle development was limited in heterotopic sites (<15 mm), and oocyte recovery and fertilization rates obtained from SC sites were low [35].

In 2006, a Danish group reported a biochemical pregnancy resulting from transfer of a four-cell embryo obtained after intracytoplasmic sperm injection, following retrieval of an MII oocyte from tissue grafted to a midline subperitoneal pocket on the lower abdominal wall [9]. Two months later, a second MII oocyte was recovered and fertilized, and a five-cell embryo was transferred, but no pregnancy was achieved. In their series of 12 autotransplantations of cryopreserved ovarian tissue published in 2011 [44], ovarian tissue was grafted to this same heterotopic site in five patients, in addition to other orthotopic sites (remaining ovary and pelvic peritoneum). After stimulation and IVF, tissue grafted to the subperitoneal pocket was shown to yield mature oocytes after IVF in three patients, but no pregnancy was reported from this site, compared to two live births and a higher number of embryos obtained from orthotopic locations.

A second biochemical pregnancy following ovarian grafting to this same site was reported by Stern et al. in 2011. Recently, the same group published their first pregnancy and live birth (twins) [39, 46] following heterotopic grafting of frozen-thawed ovarian tissue. However, in this case, the tissue was laparoscopically transplanted to a subperitoneal area of the anterior abdominal peritoneum (along with the lateral pelvic peritoneum) in a patient who had previously undergone bilateral oophorectomy for a granulosa cell tumor. This site, just below the peritoneum, was considered heterotopic by this team. However, laparoscopic visualization of the grafts confirms the presence of cystic follicular structures protruding into the peritoneal cavity (Fig. 7.2), very similar to what is observed after orthotopic grafting of tissue to the pelvic peritoneum (orthotopic site). It is therefore questionable whether this graft can truly be considered heterotopic.

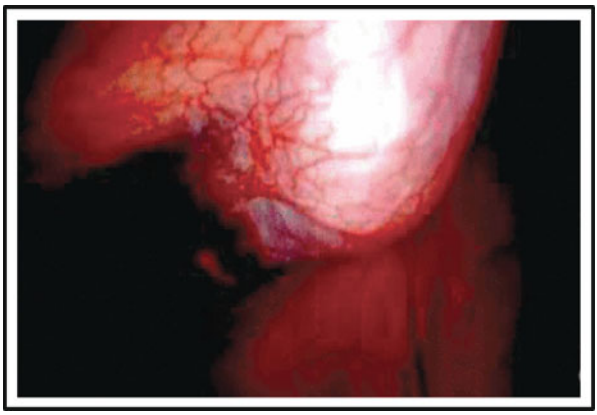

Fig. 7.2 Laparoscopic view of the abdominal wall ovarian tissue graft two years after transplantation, showing cystic follicular structures protruding into the peritoneal cavity (picture from Stern et al. [39])

7.5 Factors Influencing Graft Function After Transplantation

The choice of grafting site is an important factor when considering future graft viability and subsequent follicle development and oocyte competence. Apart from graft site (environment), factors influencing graft function after heterotopic transplantation are partially the same as for orthotopic transplantation. They include the following:

7.5.1 Clinical Considerations

The life span of an ovarian graft is influenced by various clinical factors, like the number and size of grafted tissue and the surgical technique used. However, the most important among them is follicle density in the cryopreserved tissue, which depends on the age of the patient at the time of ovarian tissue cryopreservation and any prior gonadotoxic treatment she may have undergone. Previous gonadotoxic treatment could decrease the follicular pool, but also interfere with the revascularization process after grafting, as chemotherapy causes injury to blood vessels and cortical fibrosis [47].

7.5.2 Vascularization

Since ovarian tissue grafting is an avascular procedure, survival of the transplant is hugely dependent on the revascularization process. Indeed, ischemic injury occurring immediately after transplantation is associated with massive initial follicle loss

[48, 49]. Some experimental studies report loss of at least 25 % of primordial follicles [50], while others put the figure as high as 50–65 % of the primordial follicle pool [48, 51] and even >90 % in one study [25].

In mice, perfusion was observed in autografts 3 days after grafting [52]. A study analyzing angiogenic events following rat ovary transplantation to nude mice by dynamic contrast-enhanced magnetic resonance imaging (MRI) detected functional vessels in grafts from day 7 onward [53]. In humans, neovascularization was observed 3 days after transplantation to chick chorioallantoic membrane [54]. Another study using electron paramagnetic resonance (EPR) oximetry showed a hypoxic period before day 5 after xenotransplantation of human ovarian tissue, followed by progressive reoxygenation during the subsequent 5 days [55]. The same group demonstrated that host and graft vessels contributed sequentially to graft revascularization; host (murine) angiogenesis initiated reperfusion from day 5, but by day 10, human angiogenesis was found to participate in graft revascularization [56].

Prevention of ischemic tissue damage, which remains one of the main limitations of ovarian cortical strip use, is therefore essential to reduce follicle loss.

7.5.3 Addition of Exogenous Factors

Addition of exogenous factors has also been investigated, with the aim of shortening the ischemic period following transplantation and increasing the viability and reproductive potential of grafts.

7.5.3.1 Growth and Angiogenic Factors

Various angiogenic and growth factors known to play a role in revascularization processes have been tested in xenotransplantation models. Sphingosine-1-phosphate (S1P) was shown to promote neoangiogenesis in ovarian transplants and reduce ischemia-reperfusion injury in the days following transplantation in a mouse xenograft model [57]. Erythropoietin was found to improve early follicle morphology, stromal cell density, and blood vessel density [58, 59]. Although earlier studies with vascular endothelial growth factor (VEGF) failed to demonstrate any positive effect on graft function [24], encapsulation of ovarian tissue in a collagen matrix in the presence of VEGF-165 before grafting was recently shown to have a positive impact on functional blood vessel recruitment [60]. Another study demonstrated that administration of VEGF and basic fibroblast growth factor (bFGF), especially combined, was found to trigger angiogenesis, reduce apoptosis and fibrosis, and increase survival of human ovarian tissue transplanted to rabbits [61]. Use of growth factors to improve ovarian tissue survival after grafting has progressed beyond the preclinical stage. One group reported a live birth after impregnation

of frozen-thawed ovarian cortex with platelet-rich plasma and orthotopic reimplantation [62].

The gonadotropins, luteinizing hormone (LH) and FSH, are follicle survival factors with possible antiapoptotic action and are known to upregulate angiogenic factors like VEGF. Gonadotropin administration for a few days increases the number of growing follicles in grafts when initiated 2–4 days before transplantation [20, 63]. However, no effect is observed on follicle populations when started immediately after grafting [52].

7.5.3.2 Antioxidants

Reactive oxygen species (ROS) produced during the ischemia-reperfusion period are widely implicated in causing damage to cells and massive activation of primary follicles. Endogenous antioxidant molecules are produced to neutralize these free oxygen radicals, but are rapidly overwhelmed. Nugent et al. [52] reported an improvement in follicle survival rates after local vitamin E injection before grafting, but these findings were not corroborated by others [64, 65]. Abir et al. [65] compared incubation of ovarian tissue with VEGF-A and vitamin E before transplantation, and treatment of host mice with vitamin E and gonadotropins before and after grafting, with control groups. Although apoptosis was higher in the non-treated group, no difference was found in the number of total and atretic follicles between the groups.

Melatonin and oxytetracycline, two more antioxidants, were shown to decrease graft necrosis when administered locally during intraperitoneal ovarian tissue grafting in rats [66]. The efficacy of ascorbic acid was also tested on bovine ovarian cortex deprived of a blood supply [67]. Primordial follicles were found to be less sensitive to ischemic injury than stromal cells, and incubating the tissue in ascorbic acid reduced apoptosis for up to 24 hours.

A recent study evaluating the impact of adding N-acetylcysteine (NAC) to freeze/thawing solutions showed reduced ROS production and better preservation of morphological characteristics, proliferation, and DNA integrity of ovarian tissue with NAC [68].

Even if these results are encouraging, further studies are required to establish the positive effects of such growth factors and antioxidants on follicle development and fertility restoration, and to precisely define the cytokines that may be of significant clinical benefit in terms of ovarian grafting efficiency.

7.6 Advantages and Disadvantages of Heterotopic Ovarian Transplantation

The advantages and disadvantages of heterotopic ovarian transplantation are summarized in Table 7.2. In theory, the optimal grafting site should have a rich blood supply, mimic the environmental conditions of the ovary in the peritoneum, and offer easy access for both implantation and oocyte recovery.

Transplantation to a heterotopic site is a relatively simple and minimally invasive procedure. It can also be performed in patients with severe pelvic adhesions and those in whom orthotopic transplantation is precluded. These aspects, along with easy accessibility for follicle monitoring and oocyte retrieval (in more superficial sites), are its major advantages compared to orthotopic transplantation. Another benefit of this technique is the ability to closely monitor the graft for possible recurrence of malignancy. Heterotopic sites also allow grafting of larger amounts of tissue.

However, unlike orthotopic transplantation, heterotopic transplantation does not allow natural conception, and patients are obliged to undergo IVF in order to achieve pregnancy. Moreover, obtaining healthy and competent oocytes from these grafts for IVF still remains a considerable challenge. Although restoration of ovarian activity and oocyte retrieval after grafting to heterotopic sites have been confirmed by several teams, follicular growth appears to be compromised, and recovered oocytes are mostly of inferior quality. This is probably due to the suboptimal environment of heterotopic sites. Indeed, environmental conditions in most heterotopic locations (pressure, temperature, cytokines, space for follicular

Table 7.2 Advantages and disadvantages of heterotopic ovarian tissue transplantation

	Heterotopic sites (e.g. SC)	Orthotopic sites (ovary and pelvic peritoneum)
Advantages	Simple and less invasive transplantation procedure	Possibility of natural pregnancy
	Easy access for follicle monitoring and oocyte recovery	Favorable environment for follicular development
	No limit to the number of grafted fragments	Restoration of fertility demonstrated with follicle development and oocyte quality superior to heterotopic sites
	Possibility to closely monitor for recurrence of malignancy in the graft	
Disadvantages	IVF procedure required	Invasive transplantation procedure requiring general anesthesia
	Probable suboptimal environmental conditions for oocyte development	For the ovarian site, number of grafted fragments limited by ovarian size
	Cosmetic issues	
	Restoration of fertility not demonstrated	

growth, angiogenic and hormonal factors ([37, 38]) are not equivalent to those in an orthotopic site in the pelvis.

7.7 Conclusion: The Future of Heterotopic Ovarian Tissue Transplantation

It is important to choose well vascularized grafting sites, as the major cause of follicle loss after ovarian transplantation is ischemic injury. Concerning restoration of ovarian function, this has been consistently observed after transplantation of ovarian tissue to heterotopic sites. Recent reports confirm that ovarian function following heterotopic transplantation can last more than 7 years, depending on the initial ovarian reserve [38]. The possibility of long-term restoration of ovarian function, combined with the advantage of a less invasive and more cost-efficient procedure (especially when considering repeated transplantations), makes heterotopic transplantation the technique of choice when the main goal of grafting is restoration of endocrine function.

Although follicular development, oocyte retrieval and fertilization, and embryo development have been confirmed after heterotopic transplantation of frozen-thawed ovarian tissue to various heterotopic sites, oocyte quality, and hence embryo quality, appear to be compromised [9, 36, 37, 38]. Teams who have simultaneously grafted ovarian tissue to orthotopic as well as heterotopic sites report superior results with orthotopic sites [35, 44]. Alterations in oocyte quality described in heterotopic sites are probably attributable to the nonoptimal environment for follicular development. Therefore, when fertility restoration is the goal, there is no doubt that orthotopic sites in the pelvic cavity (ovarian medulla or pelvic peritoneum), although more invasive, are much more effective, as evidenced by the number of live births.

Moreover, grafting of ovarian tissue to a subperitoneal pocket in the abdominal wall could also potentially be an option in case of severe pelvic adhesions that preclude pelvic surgery, or in addition to grafting to orthotopic sites, and should be investigated further.

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