# Gene banking and transplantation of (mammalian) ovarian tissue

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Samenvatting NL Hele gonaden van dieren (of weefsels daarvan) kunnen worden ingevroren en opgeslagen in genenbanken om later te worden gebruikt om een verloren gegaan ras of foklijn terug te krijgen. Dat dit een heel effectieve en efficiënte methode is was al gedemonstreerd in pluimvee (Silversides e.a., 2013; Liptoi e.a., 2013) en in zoogdieren (Huang e.a., 2010). Voor grotere landbouwhuisdiersoorten, zoals het varken, moet de mogelijkheid voor deze methode nog worden aangetoond. Wij (Egerszegi e.a., niet gepubliceerd) hebben een klein pilot experiment gedaan waarin (delen van) eierstokken van pas geboren biggen naar andere gecastreerde (gesteriliseerde) vrouwelijke biggen zijn getransplanteerd. Het experiment was niet geslaagd. De resultaten worden kort beschreven in dit rapport. Daarnaast bevat dit rapport een uitgebreid overzicht van de wetenschappelijke literatuur dat de huidige mogelijkheden van eierstoktransplantatie in diverse zoogdiersoorten beschrijft. Verder worden suggesties aangegeven van hoe mogelijk in toekomstig onderzoek de methoden kunnen worden aangepast en verbeterd voor het varken en andere zoogdier landbouwhuisdiersoorten.

Summary UK The use of cryopreserved gonadal tissue to reconstitute breeds or breeding lines has been demonstrated in birds (Silversides et al., 2013; Liptoi et al., 2013) and mammals (e.g. Huang et al., 2010) as a highly effective, efficient method. For large domestic mammals, such as the pig, proof of principle needs to be demonstrated. We (Egerszegi et al., unpublished) have undertaken a small pilot experiment in which orthotopic homografting of fragments of juvenile pig ovaries into neonate ovariectomized recipient piglets was attempted. This attempt was not successful. The results of the study are briefly presented in this report. This report further presents a literature review to describe current possibilities in various mammalian species and to provide suggestions for potential future improvements of methods in pigs or other mammalian farm animal species.

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# Preface

The use of cryopreserved gonadal tissue to reconstitute breeds or breeding lines has been demonstrated in birds (Silversides et al., 2013; Liptoi et al., 2013) and mammals (e.g. Huang et al., 2010) as a highly effective, efficient method. Gonadal transfer is in principle more invasive than some other reproductive technologies, but also has the ethical advantage of reducing numbers of animals needed. Moreover, in some macrolecithal species (birds) there may be no other effective method for ex situ genetic conservation. For bird species, further development and validation of the method and its animal welfare implications are needed, which was successfully undertaken in the EU Horizon 2020 project IMAGE by Liptói and co-workers (2020). For large domestic mammals, such as the pig, proof of principle needs to be demonstrated. We (Egerszegi et al., unpublished) have undertaken a small pilot experiment in which orthotopic homografting of fragments of juvenile pig ovaries into neonate ovariectomized recipient piglets was attempted. This attempt was not successful. The data of the study was obtained at the start of the EU Horizon 2020 project IMAGE and are briefly presented in this report. This report further presents the results of a comprehensive literature review of studies on cryopreservation and transplantation of ovaries or ovarian tissue in various (mammalian) species. The purpose of the review is to describe current possibilities in various mammalian species and to provide suggestions for potential future improvements of methods in pigs or other mammalian farm animal species.

# Summary

The use of cryopreserved gonadal tissue to reconstitute breeds or breeding lines has been demonstrated in birds (Silversides et al., 2013, Liptoi et al., 2013) and mammals (e.g. Huang et al., 2010) as a highly effective, efficient method. For large domestic mammals, such as the pig, proof of principle needs to be demonstrated. We (Egerszegi et al., unpublished) have undertaken a small pilot experiment in which orthotopic homografting of fragments of juvenile pig ovaries into neonate ovariectomized recipient piglets was attempted. This attempt was not successful. The results of the study are briefly presented in this report. This report further presents a literature review to describe current possibilities in various mammalian species and to provide suggestions for potential future improvements of methods in pigs or other mammalian farm animal species.

# 1 A brief history of cryopreservation and use of ovarian tissue in various taxa

As early as 1949, Smith (1949) reported survival of frozen-thawed rabbit granulosa cells, and in 1951, Smith and Parkes (1951) reported functional autografts of frozen-thawed rat ovarian tissue fragments. 'Functional' meant that the autografted rats obtained a regular cycle. In mice, Deanesly, (1954), also reported functional post-thaw grafts. Fertility (offspring) of mice after orthotopic grafting of frozen-thawed ovaries was first reported in 1958, described in more detail in 1960 (Parrott). In the rat, transplantation of cryopreserved ovaries resulted in a pregnancy in 2002 (Wang et al.), and live born offspring (Dorsch et al., 2007). Regarding non-rodents, almost 3 decades after the success in mice, bovine ovary slices were frozen but only survival of cells in in vitro culture was studied (Daniel Jr and Juneja, 1987). And, early studies in non-mammalian species were reported around that time, e.g. silkworm (Shinbo, 1989), and fish (Thomas, 1989).

In sheep, Gosden and co-workers (1994) reported restored fertility after autologous grafting of frozen-thawed ovary tissue (live born lamb). The right ovary was removed and cortex pieces were frozen. Three weeks later, the thawed cortex pieces were grafted onto the ovarian pedicle of the contralateral ovary. Also in marmoset monkeys, the feasibility of freezing ovary pieces was shown (Candy et al., 1995). Fertility of frozen-thawed (whole foetal) mouse ovaries was demonstrated by heterotopic and orthotopic (in the bursal cavity) transplantation of recipient mice by Cox and co-workers (1996). After orthotopic transplantation, 33% of the recipients became pregnant. That same year, also human ovary fragments were frozen-thawed, and were found to be functional when inspected histologically (Hovatta et al., 1996). Live offspring in mouse was again reported in 1997 (Gunasena et al., 1997) after autologous grafting of cryopreserved mouse ovaries. Again, live offspring in mice was shown after orthotopic grafting of frozen-thawed half-ovaries (Sztein et al., 1998) and later also by other groups (Candy et al., 2000, Snow et al., 2004). Vitrification of (rat) ovaries was first reported in 1996 (Sugimoto et al., 1996). The same group then reported functional autografts (follicle development and endocrinology) of vitrified rat ovaries in 2000 (Sugimoto et al., 2000). Vitrification of ovaries was performed in mouse, Chinese hamster, rabbit, and Japanese monkey ovaries. These ovaries were xenografted into rat recipients in the ovarian cavity and assessed by histology (Kagabu and Umezu, 2000). Live offspring from vitrified mouse ovaries was reported by several groups (Takahashi et al., 2001, Migishima et al., 2003, Chen et al., 2006, Hani et al., 2006, Bagis et al., 2008, Liu et al., 2008, Huang et al., 2010).

Also African elephant ovarian tissue was cryopreserved and xenografting was studied. (Gunasena et al., 1998). Cryopreservation (but no grafting) of bovine ovarian tissue was again studied by Paynter and co-workers (1999). Wombat (xenografted frozen-thawed ovarian tissue) was studied by Wolvekamp and co-workers (2001) and Cleary and co-workers (2003, Cleary et al., 2004). In human, a first case report of what at first appeared to be successful orthotopic autografting of cryopreserved ovarian cortical strips was reported in 2001 (Radford et al., 2001), but the follicle development and hormone function was soon lost. That same year, temporary re-established ovarian function was reported in a woman after heterotopic transplantation (Callejo et al., 2001). Later studies clearly demonstrated successful human autografting, resulting in many healthy born babies. All in all, live offspring in mammalian species was obtained in mouse (first in 1958), sheep (first in 1994), human (first in 2004), and rat (first in 2007).

# 2 Progress in specific (farm) animal species

**Sheep**

As mentioned above, live offspring after grafting frozen-thawed ovarian tissue was obtained in sheep as early as 1994 (Gosden et al.). Live born offspring in sheep was later also reported by the Lyon group (Salle et al., 2002) after orthotopic autografting (right frozen-thawed hemi-ovarian cortex was sutured on the hilum of the removed left ovary). Live offspring with frozen-thawed autografts in sheep was also reported in the UK (Baird et al., 2004). The Lyon group again used slow-freezing and grafting of hemi-ovaries in 2010 (Massardier et al.). They also studied vitrification of sheep ovarian cortex, reporting live offspring from vitrified hemi-ovary autograft group (Salle et al., 2002, Salle et al., 2003). Vitrification of sheep ovarian fragments was also studied by Fathi and co-workers (2011). Slow freezing of sheep ovarian cortex was also studied by the Brazil/Dutch/Norwegian group (Oskam et al., 2011). Sauvat and co-workers (2012) studied slow freezing of cortex from prepubertal ewes.

**Goat**

Many studies on goats were published by a Brazilian/Dutch group (Rodrigues et al., 2004a, b, Luz et al., 2009, Santos et al., 2009, Faustino et al., 2010, Santana et al., 2012, Carvalho et al., 2013, Carvalho et al., 2014). The Utrecht group published successful autografting in the goat (Santos et al., 2009), with complete follicular development and recovery of ovarian function of frozen-thawed, autotransplanted caprine ovarian cortex but with no (attempt to obtain) offspring. After complete ovariectomy, goats received autotransplantation of 15 fresh ovarian cortical fragments (approximately 1 mm3), sutured together and grafted with Prolene 5/0 onto the curvature minor region of the uterine horn (left side). In other goats, ovaries were removed and cortex fragments were frozen/thawed and were autografted with the same transplantation procedure 14 days after ovariectomy. In 2013, the Brazilian/Dutch group also published vitrification of ovarian tissue fragments (and hemiovaries and whole ovaries), but no grafting, only histology was done (Carvalho et al., 2013).

**Cattle**

Cryopreservation of bovine ovarian tissue was reported in a series of papers from the same Brazilian/Dutch group that had worked on goat and sheep ovaries (Celestino et al., 2008, Celestino et al., 2010, Santana et al., 2012, Campos et al., 2016). Also other groups have reported on cryopreservation of bovine ovarian tissues e.g. (Daniel Jr and Juneja, 1987, Paynter et al., 1999, Isachenko et al., 2002, Lucci et al., 2004, Lamaita et al., 2005, Kagawa et al., 2009, Gerritse et al., 2010, Gerritse et al., 2011, Isachenko et al., 2013, Gao et al., 2016, Westphal et al.). A number of groups applied vitrification to cryopreserve bovine ovarian tissue (Kagawa et al., 2009, Celestino et al., 2010, Campos et al., 2016). Cryopreservation after whole ovary perfusion, to load the tissue with cryoprotectants, was reported by three groups (Isachenko et al., 2013, Gao et al., 2016, Westphal et al., 2017). The study by Kagawa and colleagues (2009) reported grafting of the cryopreserved bovine ovarian tissue, showing no loss of oocyte viability after autotransplantation of vitrified–warmed tissue. However, no (attempts to obtain) live offspring after grafting cryopreserved bovine tissues are reported.

**Pig**

Cryopreservation of pig ovaries was studied by Thomas and co-workers (1997). These authors measured permeation of CPAs into the tissue and showed that CPA entry was lower than theoretically expected, which may affect cryopreservation outcome. In a later study, Bedaiwy and co-workers (2003) cryopreserved porcine ovarian tissue as a model for human ovarian tissue, using histology of fresh versus frozen/thawed ovarian cortex tissue as response variable. Also Borges and co-workers (2009) studied slow freezing of porcine cortex fragments, comparing various cryoprotectants. Imhof and co-workers (2004) studied slow-freezing of whole, perfused, pig ovaries. Hashimoto and co-workers (2013) applied vitrification of ovarian fragments. All these studies did not attempt grafting. In 2016, Damasio and co-workers (2016) published orthotopic and heterotopic grafting of fresh and slow-frozen/thawed ovarian cortex fragments in mini pigs. Heterotopic (subcutaneous in the groin

fold), and orthotopic (peri-infundibular intraperitoneal site) both appeared to give good results, as based on histology, with somewhat better results for subcutaneous grafting.
Regarding grafting, there had been earlier studies by Brian Heap and co-workers (e.g. Binns et al., 1969, Harrison, 1982) and by Phil Dziuk and co-workers (e.g. Hagen et al., 1981) in which grafting (non-frozen) was performed to study endocrinology. In these studies, the ovary remained connected with the blood stream (the pedicle) but was grafted onto another tissue. So, there was ample time for vascularization at that new location, after which the pedicle was severed. Kagawa and co-workers (2005) studied xenografting of porcine cortex (non-frozen) into SCID mice (kidney capsule) as tool for the study of follicular growth and development. Recently (2012-2013), we performed a pilot experiment in neonate piglets (Egerszegi et al., unpublished), in which piglets were ovariectomized and the removed ovaries were allografted orthotopically into ovariectomized neonate piglets. After reaching puberty, piglets became cyclic, and farrowed live young after artificial insemination, but all young were found to have derived from the remaining host ovarian tissue, as the recipient ovaries had not been removed completely in order to provide a 'grafting bed' for the donor ovary (Egerszegi et al., unpublished).

**Human**
Donnez and co-workers (2004) reported the first live birth of a baby after natural conception following orthotopic autotransplantation of cryopreserved (slow-frozen/thawed) cortex in a woman with premature ovarian failure after cancer treatment. Other authors (e.g. (Bath et al., 2004)) argued that even with confirmed ovarian failure it cannot be excluded that the conception and birth reported by Donnez and co-workers (2004) could have been obtained from the remaining ovaries, instead of the autograft. Later, other groups (Meirow et al., 2005, Andersen et al., 2008, Sánchez-Serrano et al., 2010) reported live born babies, but involving IVF and embryo transfer in women who had received frozen/thawed autograft. These results at least show that the oocytes obtained from the grafted ovarian tissue could be fertilized and could result in a pregnancy. In addition, Demeestere and co-workers (2007), reported the second published spontaneous pregnancy and live birth after an orthotopic and heterotopic transplantation of cryopreserved ovarian tissue, and provided several arguments for the graft origin of the conception. Silber and colleagues (2008) also reported live births and pregnancies after orthotopic sister-to sister transplants of fresh cortical tissues, and in one case also a second transplant of cryopreserved cortex tissue, in a number of monozygotic twin pairs. In one recipient, a (fresh) whole ovary transplant was performed with microvascular anastomosis, and this recipient was continuing to cycle regularly seven months post-surgery and, as reported by the same group in 2010 (Silber et al., 2010), this led to a pregnancy by natural conception, and the delivery of a healthy child. Live born babies after (auto)grafting of cryopreserved ovarian cortex tissue have now been obtained in several countries/centres (as reviewed by several authors (Dittrich et al., 2015, Donnez and Dolmans, 2016, Silber, 2016). Donnez and Dolmans (2015a, b) reviewed 60 live births after transplantation of frozen-thawed ovarian cortex and these authors argued that "It is time to stop considering this procedure as experimental".
Other cryopreservation approaches have also been studied. Kagawa and colleagues (2006) used cryo-top vitrification of human cortex, but only looked at post-thaw histology. In a later publication by that group (Kagawa et al., 2009) a somewhat different approach was used, which they called cryo-tissue, designed to ensure uniform minimal thickness of the tissues which may contribute to the success both of the vitrification process as well as the grafting, while Huang and co-workers (2008) introduced solid state vitrification of human cortex tissue. The Falcone group (Bedaiwy et al., 2006) studied whole ovary perfusion and slow-freezing, as had been done by that group in sheep (Bedaiwy et al., 2003). These ovaries were studied post-thaw but were not grafted. Also the Donnez group did similar whole ovary freezing studies, without grafting (Martinez-Madrid et al., 2007). Vitrification of ovarian cortex fragments was again addressed (no grafting) by the Donnez group (Amorim et al., 2011), and others (Chang et al., 2011, Oktem et al., 2011, Klocke et al., 2015). The latter paper stated that functional quality variables are similar in vitrified and freeze/thaw ovarian fragments.

**Other species**
Other species than the ones mentioned above for which cryopreservation was studied (with or without xeno- or auto- or allografting) include Syrian hamster (Suzuki et al., 2009), agouti (Praxedes et al., 2018), Guinea pig (Xu et al., 2012), cats (many studies, e.g. (Mouttham and Comizzoli, 2016), dogs e.g. (Lopes et al., 2016), horse e.g. (Gastal et al., 2017), dromedary camel (Madboly et al., 2017),

macaque e.g. (Ting et al.), cynomolgus monkey e.g. (Dolmans et al., 2015), baboon e.g. (Lu et al., 2014), birds (e.g. quail and chicken (Liu et al., 2010, Liu et al., 2012, Liptoi et al., 2013), as well as non-mammalian species, e.g. silkworm, fish species.

# 3 Whole large (adult) ovaries

For mouse, whole ovary transplantation of frozen-thawed or vitrified ovaries was successful (Cox et al., 1996, Huang et al., 2010). Also in the rat, orthotopic ovary transplantation of whole slow-frozen-thawed ovaries into the emptied bursa yielded offspring (Dorsch et al., 2007). And, similar success in birds was reported by the Agassiz group  and by Liptói and co-workers (2013). However, for large animals, whole (adult) ovary cryopreservation seems to require perfusion for loading and removal of CPA and grafting requires vascular reanastomosis, to prevent damage due to ischemia. Bedaiwy and co-workers (2003) reported orthotopic (auto)grafting by microvascular anastomosis of whole, large, sheep ovaries that had been slow frozen after perfusion with 1.5M DMSO. There were no (attempts to get) live offspring reported in this study. Later, also Revel and co-workers (2004) used perfusion of whole sheep ovaries. Then, in pigs, Imhof and co-workers (2004) applied perfusion of whole pig ovaries to load CPA. The Cleveland (Falcone) group also studied slow-freezing of perfused whole sheep ovaries and subsequent reanastomosis (e.g. Banerjee et al., 2006). Whole ovary perfusion and subsequent vitrification was studied by the Lyon group (Courbiere et al., 2006, Baudot et al., 2007, Courbiere et al., 2009). Wallin and co-workers (2009) also froze whole sheep ovaries after perfusion, but performed no grafting. Whole sheep ovaries were also slow-frozen using directional solidification (Maffei et al., 2013). Perfusion was also applied in freezing whole ovaries of pig, as mentioned above (Imhof et al., 2004), and in human (Bedaiwy et al., 2006), guinea pig (Xu et al., 2012), and cattle (Isachenko et al., 2013, Westphal et al., 2017). In sheep, live offspring was obtained by Imhof and co-workers (2006) after whole frozen-thawed sheep ovary reanastomosis. Later, also Campbell and co-workers (2014) reported live offspring after reanastomosis of frozen whole sheep ovaries. Despite these studies with perfusion of whole ovaries and, in a number of studies, subsequent grafting by reanastomosis, only a few studies in larger animals/human showed live offspring with whole ovary grafting. In human, virtually all live babies born from autografts (or sister to sister monozygotic twin grafts) resulted from transplanted cortex fragments (Dittrich et al., 2015, Donnez and Dolmans, 2015b, 2016). Silber and colleagues (Silber et al., 2008, Silber et al., 2010) reported one case of a woman that was cyclic after twin sister grafting of whole ovary by anastomosis, followed by a pregnancy by natural conception, and the delivery of a healthy child.

# 4 How to improve ovary transplantation in pigs in a future experiment

**General**

As described above, our attempt of orthotopic transplantation of pig ovaries failed as offspring obtained appeared to derive from the remaining host ovarian tissue which had been left in the recipient piglets as 'grafting bed' for the donor ovary (Egerszegi et al., unpublished). A future experiment may nevertheless follow a similar approach, i.e. orthotopic grafting of juvenile ovaries or ovarian cortex fragments, without vascular reanastomosis, because of the following reasons. First, only a few studies in larger animals/human showed live offspring with whole ovary grafting (with re-anastomosis). In human, all but one of the live babies born from autografts (or sister to sister monozygotic twin grafts) were obtained after grafting cortex fragments. Further, grafting of ovarian cortex fragments into the peritoneal cavity, onto an appropriate tissue close to the infundibulum, appears an easier approach than vascular anastomosis. Also cryopreservation of small juvenile ovaries or ovarian fragments is easier than cryopreservation of larger whole ovaries after vascular CPA perfusion.

Orthotopic grafting seems a logical choice, as recipients - if grafting is successful - can become pregnant after artificial insemination (as demonstrated in the human), which may be an easy way to obtain (donor-genotype) offspring. The early grafting experiments in pigs by the Heap and Dziuk groups (Binns et al., 1969, Harrison and Heap, 1972, Hagen et al., 1981) suggest that grafting should be successful, provided that ischemia damage can be minimized. In addition to these studies, the grafting technique as used in human (Donnez and Dolmans, 2015b, 2016) could be taken as a guideline. Damásio and co-workers (2016), working with fresh and slow-frozen/thawed ovarian tissue of mini pigs, obtained better results (based on histology of the graft) after heterotopic grafting (subcutaneous in the groin fold) than after orthotopic grafting (to the parietal peritoneum fold, near the left uterine tube). But then, in human, Donnez and co-workers (2006) state that: "The pelvic cavity (orthotopic site) provides the optimal environment for follicular development compared with heterotopic sites, as temperature, pressure, paracrine factors, and blood supply are similar to those observed in a physiological situation. Even if transplanting ovarian tissue to heterotopic sites has some advantages, only one pregnancy has been reported following this procedure, making this approach somewhat questionable".

Many papers state, or suggest, that ischemia is a more important factor than cryo-injury. For instance, Damásio and co-workers (2016) in their study on mini pigs said ".... (our) data are in agreement with the current concept that ischemia may have more influence on follicle loss than the process of cryopreservation prior to transplantation". Ischemia may be already a factor after removing and handling of the ovaries, but surely after transplantation of the cortex strips. Donnez and Dolmans (2015b) stated that ischemia is responsible for loss of follicles, as the graft needs 4–5 days to be reoxygenated. These authors therefore advocate the use of compounds that may stimulate revascularization of the graft, such as vascular endothelial growth factor (VEGF) or sphingosine- 1-phosphate (S1P), as well as inhibitory hormones such as anti-Müllerian hormone (AMH). Other growth factors (e.g. erythropoietin (EPO) may also influence revascularization after grafting (Demeestere et al., 2009). Donnez and Dolmans (2015b) further indicated that the host vascular bed may be prepared prior to grafting by addition of encapsulated vascular endothelial growth factor. VEGF111, a recently described isoform, seems to have advantages, as it does not bind to the extracellular matrix, diffuses extensively, and is resistant to proteolysis (Labied et al., 2013).

**Techniques for orthotopic grafting**

In humans (autografting), in case a (failing) ovary is still available, it makes sense to graft ovarian tissue onto the medulla of that ovary, using sutures, or simply fixing the tissue with Interceed (a cellulose surgical fabric) or fibrin glue (Donnez and Dolmans, 2016). For our purposes of recovery of donor genotypes, however, it is necessary to remove the recipient ovaries and not leave part of the recipient ovary as 'grafting bed'. More appropriate may therefore be an approach similar to that advised by Donnez and Dolmans (2016) in case of absence of ovaries. These authors describe that cortex pieces are transplanted over a peritoneal window, which may be created in two steps to induce

angiogenesis before the grafting procedure (Donnez et al., 2004). Donnez and Dolmans (2016): "The incision for this peritoneal window is made on the anterior leaf of the broad ligament in an area where a vascular network is visible (retroperitoneal vessels, see Fig. 1 in (Donnez et al., 2012) or figure 6.3 in (Donnez and Dolmans, 2016). The fragments are placed in the window and subsequently covered with Interceed, the edges of which are fixed with fibrin glue."
A perhaps similar, and also effective, method (live births) was described by Dittrich and co-workers (2015), who transplanted post-thaw human cortex fragments into a 1.5 cm deep pouch of peritoneum in the region of the broad ligament, below the fallopian tube. Fragments of ovarian tissue 1–3 mm in size were introduced into this pouch, and the pocket was closed with a Vicryl suture if necessary.

**Other considerations**
In our pilot experiment (Egerszegi et al., unpublished), we performed allografting. In a future experiment one could include a few piglets that receive autologous grafts. This would allow to estimate the relative importance of immunological rejection versus that of other variables. For instance, Smith and Parkes (1951) compared autografts and homografts in rats, in what seemed a very simple procedure: they removed the ovaries, chopped them, froze them (or not), and transplanted the ovary parts back in the same animal (in their case in subcutaneous tissues), or in different rats. Grafts in the same rat (autografts) resulted in regular cyclicity in 100% of used rats, both with 'fresh' (14/14) as with frozen/thawed (5/5) ovary pieces, whereas homografts of frozen or unfrozen ovarian tissue between different rats "take less readily than the corresponding autografts, but it is possible to obtain active grafts with frozen ovarian tissue from another individual" (Smith and Parkes, 1951).
Juvenile pig ovaries are small, but they may nevertheless be cut in halves or quarters (or one may try and obtain cortex fragments) so that the wound surface (the cut surface of the ovary) perhaps can play a role in revascularization. For instance, Dziuk and co-workers (1999) in pigs, described that the tissue onto which ovaries were grafted was deliberately 'abraded' in an apparent effort to improve contact and/or angiogenesis. Also, it may be beneficial for reducing risk of ischemia if the (cortex) fragment is thin (e.g. 0.75 mm), which, of course, may also improve cryopreservation.

**Note**
The literature review was performed in 2017, so does not include studies published in 2018-2020.

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