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REVIEW

Ovarian tissue transportation: a systematic review

BIOGRAPHY

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KEY MESSAGE

This review highlights the currently limited knowledge of ovarian tissue transport in different species. Ovarian tissue should be transported at 4°C to minimize ischaemic damage, and it is vital to create guidelines for ovarian tissue transportation to enhance ART outcomes. Xenotransplantation is the best means of evaluating ovarian tissue transportation.

ABSTRACT

In recent years, some countries and fertility preservation networks have started adopting 24 h transportation for ovarian tissue, a practice that has the potential to spread very quickly due to the high costs and bureaucracy involved in the establishment of ovarian tissue cryobanks. While pregnancies and live births have been reported after such long periods of transportation, this, however, remains an empirical procedure. This review aims to prompt reflection on ovarian tissue transport, highlighting the lack of knowledge in humans by providing a counterpoint looking into more than 40 studies published in different animal models. By discussing these studies in animals, the findings of various models can be deciphered, and light shed on the patterns identified. Like the development of different assisted reproductive technology procedures, this is an important step in creating guidelines for future studies on human ovarian tissue transportation.

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KEYWORDS

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Ovary
Temperature

INTRODUCTION

Assisted reproductive technology (ART) is vital for the preservation of animal and human gametes. In animals, techniques have been developed for the selection of species/breeds of economic interest and also the conservation of endangered species (*Comizzoli et al., 2009*; *Barberino et al., 2018*). In humans, ART has several applications, including fertility preservation in cancer patients (*Donnez and Dolmans, 2017*).

One of the most recent ART strategies, ovarian tissue cryopreservation and transplantation, is now considered an established procedure in a growing number of countries, yielding more than 130 live births to date (*Donnez and Dolmans, 2017*; *Liebenthron et al., 2019*). While this procedure is being increasingly applied all over the world (*von Wolff et al., 2015*; *Beckmann et al., 2019*), it is important to stress that there are still many challenges. Tissue cryopreservation requires specialized equipment, trained staff as well as designated time and space to prepare and freeze the ovarian cortex. Due to strict European rules, specific protocols and high costs, only large hospitals can maintain an ovarian tissue cryobank. One way of increasing access to this strategy is improving ovarian tissue transportation and creating a network for fertility preservation, which would allow small clinics and less developed countries to extend the availability of ovarian tissue cryopreservation and transplantation (*Duncan et al., 2016*; *Kyono et al., 2017*; *Liebenthron et al., 2019*). Currently, there are two such networks operating successfully: the Danish network and *Ferti*PROTEKT (*von Wolff et al., 2019*).

While kidney, heart and liver transportation protocols are well established and widely implemented (*Guibert et al., 2011*; *Ricci et al., 2016*), with adapted medium composition, temperature and time for transport, the same cannot be said for ovarian tissue. Indeed, there are still no standard procedures for ovarian tissue transportation (*Duncan et al., 2016*; *Beckmann et al., 2019*; *Liebenthron et al., 2019*), even though its impact can be considerable.

The effects of ischaemia, for example, have mainly been studied after tissue transplantation (*Van Eyck et al., 2009*; *Van Eyck et al., 2010*; *Van Langendonckt et al., 2014*; *Cacciottola et al., 2018*), when the blood supply is re-established, but it does not only occur after grafting. In fact, ischaemia begins when ovarian tissue is taken from the patient and continues until its cryopreservation and/or transplantation (*Jing et al., 2018*). Since the ovary is a complex organ, with a diversity of cells and structures of great importance for ensuring follicle survival and development, its transportation is not straightforward. Media used for human ovarian tissue transportation at present, such as alpha-minimum essential medium (α-MEM) (*Rosendahl et al., 2011*), IVF medium (*Schmidt et al., 2003*), Leibovitz-15 (L-15) (*Isachenko et al., 2012*), HEPES-MEM (*Van Langendonckt et al., 2014*) or Custodiol (Essential Pharmaceuticals, USA) (*Liebenthron et al., 2019*), were actually developed for different purposes. After understanding the current status of ovarian tissue transport in humans (*Duncan et al., 2016*) and animals (*Barberino et al., 2018*), the aim here is to systematically review studies on ovarian tissue transportation in both animals and humans. Species, media, timepoints, temperatures, analyses and optimal transport conditions were addressed for each study to better understand practices that are well established, as well as possible gaps in knowledge and future strategies that can be implemented to prepare guidelines for ovarian tissue transport.

MATERIALS AND METHODS

This review was carried out according to the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) (*Moher et al., 2009*), and registered on PROSPERO on 6 January 2020 (CRD42020155900).

Search strategy

A PubMed search was conducted of the literature in English, from 1990 to 2020, focusing on transportation of human and animal ovarian tissue, using the following keywords: 'ovary and transportation', 'ovarian tissue and transportation', 'ovary and transport', 'ovarian tissue and transport', 'ovary and storage' and 'ovarian tissue and storage'. Results were downloaded to EndNote software (Thomson Reuters, Canada), references were merged and duplicates were removed. Any risk of bias was avoided.

Screening and eligibility

Screening was carried out for article titles and abstracts containing the keywords by J.M.V.V. and C.A.A. Only full-text English reports that specifically compared different conditions for ovarian tissue transportation were included. Scientific articles reporting successful outcomes of cryopreservation were excluded, unless the main goal of the research was to analyse tissue transport.

A data-charting table was developed by the authors to extract the main variables. This table was continually updated while writing this review. Disagreements were resolved through discussion and consensus. Charting was implemented using Airtable (USA), a customizable online platform.

Data items

Data were extracted on article characteristics (such as authors, year, country and institution), tested temperatures, timepoints and media, and analyses and best conditions were scrutinized for each study. Data were cross-checked by the authors.

RESULTS

A total of 324 records were found through database searches and a further 28 were identified from studies' references. Of those, 293 were excluded and 58 were screened, based on the title and abstract that matched the study's criteria. From those, 52 full-text articles were considered as potentially eligible, of which 46 were included in the qualitative synthesis (FIGURE 1). Selected studies dated from 1992 to 2020 (Supplementary Figure 1). The studies were grouped by country, research group and species, outlined tested media, temperatures and timepoints, and considered applied analyses, as well as the main findings and best conditions in each study.

Description of studies

Results from the 46 studies selected for systematic review are summarized in TABLE 1 (humans) and TABLE 2 (animals). They involved 13 countries, namely Italy (n = 2), Brazil (n = 15), Germany (n = 4), Iran (n = 1), the USA (n = 7), Japan (n = 7), Belgium (n = 2), China (n = 1), Turkey (n = 3), South Korea (n = 1), the UK (n = 1), Spain (n = 1) and Argentina (n = 1), and 28 different research groups, the majority being from Brazil (six groups) and Japan (six groups) (Supplementary Table 1).

Four studies were conducted in humans (*Isachenko et al., 2009*; *Isachenko*

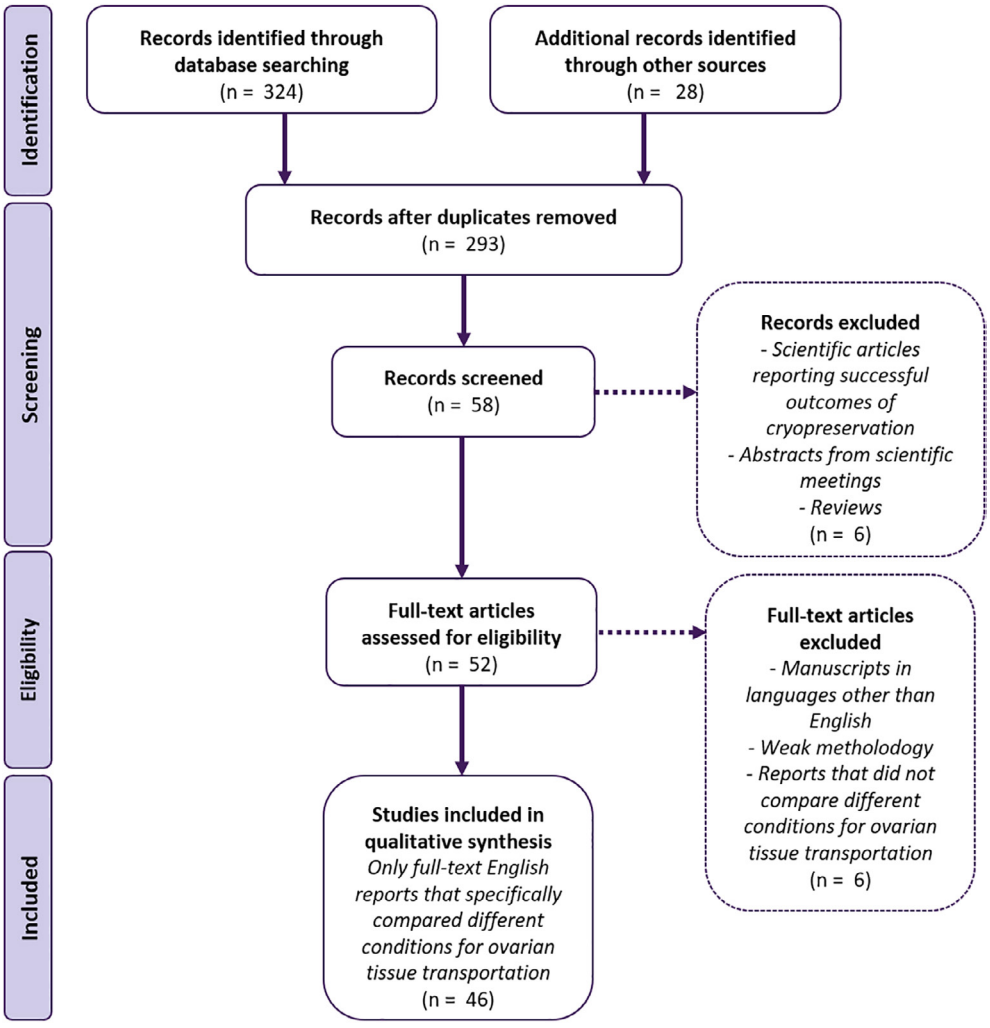

FIGURE 1 PRISMA flow diagram for ovarian transportation studies.

et al., 2013; *Klocke et al., 2014*; *Kyoya et al., 2014*), six in bovines (*Nakao and Nakatsuji, 1992*, *Lucci et al., 2004*; *Matsushita et al., 2004*; *Nagao et al., 2010*, *Wang et al., 2011*; *Vilela et al., 2020*), seven in cats (*Jewgenow et al., 1997*; *Wood et al., 1997*; *Naoi et al., 2007*; *Evecen et al., 2010*; *Cocchia et al., 2015*; *Martins et al., 2018*; *Piras et al., 2018*), six in goats (*Silva et al., 2000*; *Carvalho et al., 2001*; *Santos et al., 2002*; *Chaves et al., 2008*; *Gouveia et al., 2015*; *Correia et al., 2017*), six in dogs (*Lee et al., 2006*; *Tas et al., 2006*; *Hanna et al., 2008*; *Lopes et al., 2009*; *Evecen et al., 2010*; *Lima et al., 2010*), five in sheep (*Gonçalves et al., 2015*; *Barberino et al., 2016*; *Henry et al., 2016*; *Cavalcante et al., 2017*; *Goodarzi et al., 2018*), five in horses (*Love et al., 2003*; *Pedersen et al., 2004*; *Ribeiro et al., 2008*; *Gomes et al., 2012*; *Gastal et al., 2017*), four in pigs (*Wongsrikeao et al., 2005*; *Lucci et al., 2007*; *Tellado et al., 2014*; *Raffel et al., 2020*), one in mice (*Kamoshita et al., 2016*), one in the *Pecari tajacu* (musk hog) (*Lima et al., 2014*), and one in Iberian red deer (*García-Álvarez et al., 2011*) (Supplementary Table 2). A total of 26 studies evaluated transportation of whole ovaries, one of half-ovaries and 16 of ovarian fragments, and one compared whole ovaries and fragments.

Tested parameters and assessment

Of the 46 publications analysed, 28 evaluated different temperatures, 34 monitored the effect of time, 13 investigated different media, six assessed the impact of additives in media and only two considered the influence of tissue size (TABLE 3). Tested temperatures ranged from 0 to 39°C, 4°C (n = 34) and 20°C (n = 13) being the most frequently evaluated. Timepoints varied from 1 to 96 h, 4 (n = 16), 12 (n = 20) and 24 h (n = 24) being the periods most commonly investigated. In total, 20 different media were tested over all the studies, with saline solution (n = 14) and phosphate-buffered saline (PBS; n = 12) emerging as the most prevalent, followed by MEM (n = 8) (FIGURE 2). The best study conditions for each species are summarized in TABLE 4.

Analyses performed

Most studies focused their analysis on oocytes (43.7%, 20 studies) and ovarian tissue (50%, 23 studies) (Supplementary Figure 2). Regarding DNA/RNA analyses, one study applied the polymerase chain reaction (*Cocchia et al., 2015*) and one evaluated chromatin configuration (*Pedersen et al., 2004*). DNA fragmentation (using TdT (terminal deoxynucleotidyl transferase)-mediated dUTP nick-end labelling [TUNEL], in-situ end-labelling [ISEL] or other techniques) was performed in 10 studies (*Jewgenow et al., 1997*; *Wongsrikeao et al., 2005*;

TABLE 1 STUDIES ON OVARIAN TISSUE TRANSPORTATION IN HUMANS

Authors, year	Country	Sample size	Tissue size	Tested medium	Additives in media	Temperatures tested	Timepoints	Analyses in	Best conditions[a]
Klocke et al. (2014)	Germany	12	Ovarian fragments	Celsior		4°C	24 h, 48 h	Ovarian tissue, medium	Not applicable
Kyoya et al. (2014)	Japan	6	Whole ovary	L-15	Antibiotics, ascorbic acid, sodium pyruvate, glutamine	4°C	6 h, 18 h	Ovarian tissue, follicles	18 h
Isachenko et al. (2009)	Germany	5	Ovarian fragments	Brama I		4°C, 10°C	8 h, 15 h, 26 h, 36 h	Ovarian tissue	4°C, 26 h
Isachenko et al. (2013)	Germany	6	Ovarian fragments	L-15	Dextran serum substitute	5°C	24 h	Ovarian tissue	24 h

[a] Best conditions within the parameters observed in each study. Not applicable = the conclusion of this study was that transport duration should be limited to a minimum to avoid damage.

Gonçalves et al., 2015; *Gouveia et al., 2015*; *Kamoshita et al., 2016*; *Cavalcante et al., 2017*; *Gastal et al., 2017*; *Barberino et al., 2018*; *Piras et al., 2018*; *Vilela et al., 2020*). Four studies used embryo analyses (*Wongsrikeao et al., 2005*; *Naoi et al., 2007*; *Ribeiro et al., 2008*; *Wang et al., 2011*) and five assessed isolated follicles (*Lucci et al., 2007*; *Lopes et al., 2009*; *Lima et al., 2014*; *Correia et al., 2017*; *Raffel et al., 2020*). One study considered offspring after IVF (*Kamoshita et al., 2016*).

Four studies analysed media; Klocke and colleagues (*Klocke et al., 2014*) measured oestradiol concentrations in culture medium after vitrification/warming to assess transported tissue, Lima and colleages (*Lima et al., 2014*) measured pH values, while Correia and co-workers (*Correia et al., 2017*) evaluated reactive oxygen species (ROS) levels in culture medium after storage, and Vilela and co-workers (*Vilela et al., 2020*) evaluated glucose, pyruvate and lactate concentrations, as well as pH and lactate dehydrogenase activity in media used for transportation. Two other studies analysed follicular fluid; Tellado and colleagues (*Tellado et al., 2014*) quantified lactate, glucose and pH, and Wongsrikeao and co-workers (*Wongsrikeao et al., 2005*) calculated the pH of follicular fluid in antral follicles after storage of pig ovarian tissue.

In ovarian tissue, follicle morphology and ultrastructure were the focus of analyses, conducted using haematoxylin and eosin staining (23 studies) or transmission electron microscopy (TEM; 7 studies). For assessment of the transportation protocol, ovarian tissue fragments were cultured after storage in nine studies, and transplanted in one study (*Kamoshita et al., 2016*). In oocytes, investigations were largely related to oocyte development after in-vitro maturation (IVM; 16 studies), nine of which involved IVF (results summarized in TABLE 2).

Effects on ovarian tissue

Analyses of ovarian tissue were performed in humans (*Isachenko et al., 2009*; *Isachenko et al., 2013*; *Klocke et al., 2014*; *Kyoya et al., 2014*), cows (*Lucci et al., 2004*; *Vilela et al., 2020*), cats (*Jewgenow et al., 1997*; *Wood et al., 1997*; *Smitz et al., 2010*; *Martins et al., 2018*), goats (*Silva et al., 2000*; *Carvalho et al., 2001*; *Santos et al., 2002*; *Chaves et al., 2008*; *Gouveia et al., 2015*), sheep (*Gonçalves et al., 2015*; *Barberino et al., 2016*; *Henry et al., 2016*; *Cavalcante et al., 2017*), dogs (*Lima et al., 2010*), horses (*Gomes et al., 2012*; *Gastal et al., 2017*), mice (*Kamoshita et al., 2016*) and musk hogs (*Lima et al., 2014*).

Human ovarian tissue

In humans, ovarian tissue pieces cultured for 15 days after 26 h of transportation in Brama I medium at 4°C showed no evidence of morphological damage (*Isachenko et al., 2009*). Another study by Isachenko and colleagues (*Isachenko et al., 2013*) exhibited increased follicle viability after 24 h of transport at 5°C and culture of ovarian pieces *in vitro* and in the chorioallantoic membrane of chick embryos for 5 days. Kyoya and co-workers (*Kyoya et al., 2014*) transported ovarian tissue in L-15 for up to 18 h at 4°C and cultured vitrified fragments for 24 h. They analysed oxygen consumption rates by scanning electrochemical microscopy and found no difference in follicle morphology or oxygen consumption from fresh controls. After transport in Celsior medium at 4°C for 24 or 48 h, Klocke and colleagues (*Klocke et al., 2014*) performed vitrification and tissue culture for 18 days, reporting that malondialdehyde concentrations increased in the medium, indicative of oxidative stress. Oestradiol was also measured and its concentrations progressively fell with storage time. Apoptosis was assessed with anti-active caspase-3 after vitrification and warming, and the proportion of apoptotic follicles was not found to be significantly different from that in non-transported (fresh) tissue.

Animal ovarian tissue

Cow ovaries were stored at 4°C or 20°C for up to 18 h in saline or coconut water. Conservation at 4°C for up to 18 h and 20°C for up to 6 h kept the percentage of morphologically normal follicles similar to fresh controls, but the solution had little effect on the results (*Lucci et al., 2004*). Another study (*Vilela et al., 2020*) compared concentrations of glucose, pyruvate and lactate after transporting cow ovaries in IVF medium, L-15 medium, saline solution and PBS for up to 24 h at 4°C, and reported no glucose consumption, low pyruvate consumption and comparable lactate release in all media. Viability assays were performed using TUNEL and fluorescence-activated cell sorting, and showed low apoptosis and necrosis rates, indicating anaerobic metabolism during ovarian tissue transportation.

In cats, Jewgenow and co-workers (*Jewgenow et al., 1997*) stored ovarian tissue for up to 48 h in Dulbecco's PBS at 4°C and analysed follicle atresia by histology and DNA fragmentation using ISEL. They detected significant DNA degradation in granulosa cells after 12 h of storage. Using the same media and temperature, Wood and colleagues (*Wood et al., 1997*) observed that cold storage inhibited taphonomic changes to tissue for 48 h after removal from

TABLE 2 STUDIES ON OVARIAN TISSUE TRANSPORTATION IN ANIMALS

Authors, year	Species	Country	Sample size	Tissue size	Tested medium	Additives in media	Temperatures tested	Time-points	Analyses in	Best conditions[a]
Wang et al. (2011)	Cow	China	10	Whole ovary	PBS		15°C, 25°C, 35°C	4 h	Oocytes, embryos	15°C, 4 h
Nagao et al. (2010)	Cow	Japan	Not informed	Whole ovary	Saline solution, DPBS, UW solution	Antibiotics, GSH, EGCG	5°C, 15°C, 25°C, 35°C	24 h	Oocytes	15°C, 24 h, Use of additives
Lucci et al. (2004)	Cow	Brazil	4	Ovarian fragments	Saline solution, coconut water		4°C, 20°C	6 h, 12 h, 18 h	Ovarian tissue	4°C, 18 h
Matsushita et al. (2004)	Cow	Japan	Not informed	Whole ovary	Saline solution	Antibiotics	0°C, 10°C, 20°C	24 h, 48 h, 72 h	Oocytes	10°C, 24 h
Nakao and Nakatsuji (1992)	Cow	Japan	Not informed	Whole ovary	Ringer's solution, TCM-199		4°C, 20°C, 39°C	5 h	Oocytes	20°C
Vilela et al. (2020)	Cow	Belgium	15	Ovarian fragments	PBS, Saline solution, L-15, IVF medium	Antibiotics	4°C	1 h, 4 h, 24 h	Medium, ovarian cells, ovarian tissue	Not applicable
Martins et al. (2018)	Cat	Brazil	12	Whole ovary	DPBS		4°C	6 h, 24 h, 72 h	Ovarian tissue	24 h
Piras et al. (2018)	Cat	Italy	55	Whole ovary	PBS	Calcium dichloride hydrate, antibiotics	4°C	24 h, 48 h, 72 h, 96 h	Oocytes	24 h
Cocchia et al. (2015)	Cat	Italy	76	Whole ovary	PBS	SOD	4°C	3 h, 24 h, 48 h, 72 h	Oocytes, RNA	24 h, Use of additives
Evecen et al. (2009)	Cat	Turkey	25	Whole ovary	Saline solution		4°C	4 h, 24 h, 48 h	Oocytes	24 h
Naoi et al. (2007)	Cat	Japan	Not informed	Whole ovary	m-PBS	Antibiotics	4°C, 38°C, 25°C	24 h	Oocytes, embryos	4°C, 6 h
Wood et al. (1997)	Cat	USA	50	Whole ovary	DPBS	Antibiotics	4°C	4 h, 8 h, 12 h, 24 h, 48 h	Ovarian tissue	48 h
Jewgenow et al. (1997)	Cat	USA	12	Whole ovary	DPBS		4°C	8 h, 12 h, 24 h, 48 h	Ovarian tissue	12 h
Correia et al. (2017)	Goat	Brazil	30	Whole ovary	MEM	HEPES	4°C, 33°C	4 h	Medium, follicles, oocytes	4°C
Gouveia et al. (2015)	Goat	Brazil	10	Ovarian fragments	MEM	Antibiotics, *Amburana cearencis* extract	4°C	6 h, 12 h, 24 h	Ovarian tissue	4°C, 6 h, Use of additives
Chaves et al. (2008)	Goat	Brazil	5	Ovarian fragments	MEM	Antibiotics	4°C, 20°C, 35°C	2 h, 4 h	Ovarian tissue	4°C, 4 h
Santos et al. (2002)	Goat	Brazil	5	Ovarian fragments	Saline solution, PBS		4°C, 20°C, 39°C	4 h, 12 h, 24 h	Ovarian tissue	4°C, saline solution, 12 h, PBS, 24 h
Carvalho et al. (2001)	Goat	Brazil	5	Ovarian fragments	Braun-Collins solution, saline solution		4°C, 20°C, 39°C	4 h, 12 h, 24 h	Ovarian tissue	4°C, 12 h
Silva et al. (2000)	Goat	Brazil	5	Ovarian fragments	Braun-Collins solution, coconut water-based medium		4°C, 20°C, 39°C	4 h, 12 h, 24 h	Ovarian tissue	4°C, coconut water, 24 h
Goodarzi et al. (2018)	Sheep	Iran	Not informed	Whole ovary	PBS	Melatonin	4°C, 20°C	24 h	Oocytes	4°C, 24 h, Use of additives
Cavalcante et al. (2017)	Sheep	Brazil	8	Ovarian fragments	MEM	Antibiotics, *Morus nigra* extract	4°C	6 h, 12 h, 24 h	Ovarian tissue	6 h, Use of additives
Barberino et al. (2016)	Sheep	Brazil	15	Ovarian fragments	MEM	HEPES, antibiotics	4°C	6 h, 12 h, 24 h	Ovarian tissue	24 h
Henry et al. (2016)	Sheep	Belgium	4	Half ovary	L-15	Z-VAD-FMK, S1P	4°C	Not informed	Ovarian tissue	Use of antiapoptotic drugs

(continued on next page)

TABLE 2 *(continued)*

Authors, year	Species	Country	Sample size	Tissue size	Tested medium	Additives in media	Temperatures tested	Time-points	Analyses in	Best conditions[a]
Gonçalves et al. (2015)	Sheep	Brazil	5	Ovarian fragments	MEM, MEM+	Antibiotics, ITS, glutamine, hypoxanthine, BSA, FSH	35°C	6 h, 12 h	Ovarian tissue	MEM+, 6 h
Evecen et al. (2010)	Dog	Turkey	39	Whole Ovary	PBS		4°C, 37°C	4 h	Oocytes	4°C
Lima et al. (2010)	Dog	Brazil	9	Ovarian fragments	PBS, coconut water-based medium		10°C	12 h, 24 h, 36 h	Ovarian tissue	ACP medium, 24 h
Lopes et al. (2009)	Dog	Brazil	15	Ovarian fragments	Saline solution, MEM		4°C, 20°C, 38°C	2 h, 6 h, 12 h, 24 h	Follicles, ovarian tissue	4°C, MEM+, 12 h
Hanna et al. (2008)	Dog	USA	Not informed	Whole ovary	PBS, TCM-199, TL-HEPES	Hanks salts, HEPES	20°C, 37°C	Not informed	Oocytes	37°C, TL-HEPES
Lee et al. (2006)	Dog	South Korea	30	Whole ovary	Saline solution	Antibiotics	4°C, 38°C	5 h	Oocytes	38°C
Tas et al. (2006)	Dog	Turkey	23	Whole ovary	Saline solution		4°C, 38°C	4 h	Oocytes	4°C
Gastal et al. (2017)	Horse	USA	18	Whole ovary, ovarian fragments	α-MEM	Antibiotics, BSA	4°C	6 h, 12 h, 24 h	Ovarian tissue	24 h, ovarian fragments
Gomes et al. (2012)	Horse	Brazil	10	Ovarian fragments	PBS, MEM		4°C, 20°C, 39°C	4 h, 12 h, 24 h	Ovarian tissue	PBS, 4°C, 4 h
Ribeiro et al. (2008)	Horse	USA	2	Whole ovary	No medium		24°C, 35°C	10 h, 20 h	Oocytes, embryos	7 h
Pedersen et al. (2004)	Horse	UK	Not informed	Whole ovary	M-199	Hanks salts, HEPES	20°C, 30°C, 37°C	12 h	Oocytes, DNA	6 h, 37°C
Love et al. (2003)	Horse	USA	Not informed	Whole ovary	M-199	HEPES, Hanks salts	4°C, 25°C	9 h, 18 h	Oocytes	Not applicable
Tellado et al. (2014)	Pig	Argentina	15?	Whole ovary	Saline solution	Antibiotics	15°C, 25°C, 35°C	2 h, 4 h, 6 h	Oocytes	12 h, 25°C
Lucci et al. (2007)	Pig	USA	3	Whole ovary	Saline solution		4°C, 20°C	6 h, 12 h, 18 h	Follicles	4°C, 18 h
Wongsrikeao et al. (2005)	Pig	Japan	Not informed	Whole ovary	Saline solution		35°C, 4°C, 15°C, 25°C	3 h, 6 h, 9 h, 12 h	Oocytes, embryos	25°C, 6 h
Raffel et al. (2020)	Pig	Germany	10	Ovarian fragments	PBS		4°C, 38°C	4 h, 12 h, 24 h	Ovarian tissue, follicles	4°C
Lima et al. (2014)	*Pecari tajacu*	Brazil	8	Ovarian fragments	PBS, coconut water-based medium	Antibiotics	0°C	4 h, 12 h, 24 h, 36 h	Ovarian tissue, medium	ACP medium, 24 h
Kamoshita et al. (2016)	Mouse	Japan	Not informed	Whole ovary	Dulbecco's modified Eagle's medium		4°C, 14°C, 37°C, 25°C	4 h, 8 h, 24 h	Ovarian tissue, offspring	PBS, 4°C, 4 h
García-Álvarez et al. (2011)	Iberian red deer	Spain	236	Whole ovary	Saline solution		8°C, 0°C, 25°C	12 h	Oocytes	25°C

[a] Best conditions within the parameters observed in each study. Not applicable = the study aimed to verify metabolic activity in different media. The authors did not report best conditions

ACP, powdered coconut water; BSA, bovine serum albumin; DPBS, Dulbecco's PBS; EGCG, epigallocatechin gallate; GSH, glutathione; ITS, insulin–transferrin–selenium; MEM, minimum essential medium; m-PBS, modified PBS; PBS, phosphate-buffered saline; SOD, superoxide dismutase; UW, University of Wisconsin

the animal. The authors reported that atresia found in samples was natural and not a result of cold storage. In a recent study, Martins and co-workers (*Martins et al., 2018*) evaluated follicle morphology before and after storage followed by vitrification. They used MEM at 4°C for 24 or 72 h and encountered higher survival rates in the 24 h storage group, with no difference in pre-antral follicle survival rates between fresh tissue and ovaries stored for 24 h.

All studies using goat ovarian tissue were from the same research group in Brazil. They tested five different media at four temperatures (4, 20, 35 and 39°C). Using histology or TEM, they observed better preservation of follicles with coconut water at 4°C than with Braun-Collins solution (*Silva et al., 2000*). Similarly, saline solution yielded higher rates of morphologically normal follicles than Braun-Collins (*Carvalho et al., 2001*) or PBS (*Santos et al., 2002*) for up to 12 h. Coconut water was the only medium in which they could preserve follicle morphology for 24 h at 4°C (*Silva et al., 2000*). In another study, ovarian fragments cultured for up to 7 days after storage in MEM for 2 or 4 h exhibited follicle morphology similar

TABLE 3 ANALYSED PARAMETERS IN OVARIAN TISSUE TRANSPORT

Parameter	Human	Cow	Cat	Goat	Sheep	Dog	Horse	Pig	*Pecari tajacu*	Mouse	Iberian red deer	Total
Additives in media					1							1
Additives in media × time			1	1	1							3
Media × temperature		1				1						2
Media × temperature × additives in media		1										1
Media × temperature × time		1		3		1	1					6
Temperature		1	1	1		3	1				1	8
Temperature × time	1	1		1			2	4		1		10
Temperature × time × additives in media					1							1
Time	3		5									8
Time × media		1			1	1			1			4
Time × tissue size					1		1					2
Total	4	6	7	6	5	6	5	4	1	1	1	46

to the fresh controls (*Chaves et al., 2008*).

Studies in sheep were the first to demonstrate that tissue size during transport affects follicle morphology (*Barberino et al., 2018*), by comparing the transportation of a whole ovary with fragments of equivalent to half, a quarter and an eighth its size. In that study, the smallest fragments showed better preservation of the follicle capacity to grow *in vitro* for up to 24 h. Gonçalves and colleagues (*Gonçalves et al., 2015*) reported that sheep ovarian fragments can be stored for up to 6 h at 35°C in MEM supplemented with additives like insulin–transferrin–selenium, hypoxanthine, glutamine, bovine serum albumin and FSH (which they named MEM+). Other studies have described the positive impact of two anti-apoptotic drugs, Z-VAD-FMK (a pan-caspase inhibitor) and SP1 (a bioactive lipid in follicular fluid) (*Henry et al., 2016*), and extracts from the plants *Amburana cearensis* (*Gouveia et al., 2015*) and *Morus nigra* (*Cavalcante et al., 2017*) in the transportation of sheep ovaries.

In dogs, one study reported better preservation of follicle morphology when ovarian fragments were stored at 10°C in a coconut water-based medium compared with PBS. Indeed, while the percentage of morphologically normal follicles started to decrease after 24 h in PBS, it only started to fall after 36 h in coconut water-based medium (*Lima et al., 2010*). Another study on the transport of canine ovarian tissue fragments found follicle morphology to be preserved for 12 h in MEM at 4° or 20°C (*Lopes et al., 2009*).

In horses, transportation of ovarian tissue fragments at 4°C for 4 h yielded a higher rate of morphologically normal follicles in PBS than MEM (*Gomes et al., 2012*). Moreover, small fragments (2 × 2 × 12 mm) showed better preservation of follicle morphology over 24 h at 4°C than did transport of whole ovaries (*Gastal et al., 2017*).

The first report of reduced fertility after storage followed by transplantation of ovarian tissue comes from a study in mice (*Kamoshita et al., 2016*). Although histology evidenced no significant changes after transport in Dulbecco's modified Eagle's medium, there was a significant reduction in implantation and live birth rates with prolonged tissue storage (>8 h).

Effects on oocyte maturation and fertilization, and embryo production

Studies on oocyte maturation and fertilization, as well as embryo production after ovarian tissue transportation, have only been conducted in animals. At the time of writing this review, no studies had been completed in humans. IVM of oocytes followed by IVF was performed after transport of bovine (*Nakao and Nakatsuji, 1992*; *Matsushita et al., 2004*; *Nagao et al., 2010*; *Barberino et al., 2016*), feline (*Naoi et al., 2007*; *Cocchia et al., 2015*; *Piras et al., 2018*), ovine (*Goodarzi et al., 2018*) and porcine (*Wongsrikeao et al., 2005*; *Tellado et al., 2014*) ovarian tissue. IVM was also carried out in four studies in dogs (*Lee et al., 2006*; *Tas et al., 2006*; *Hanna et al., 2008*; *Evecen et al., 2010*), two in horses (*Love et al., 2003*; *Ribeiro et al., 2008*), one in goats (*Correia et al., 2017*) and one in cats (*Evecen et al., 2009*) after ovarian tissue transportation.

In bovines, oocyte meiotic competence and the number of cells per embryo were the main factors used to evaluate storage effects. Two studies (*Nakao and Nakatsuji, 1992*; *Nagao et al., 2010*) focused on the effects of media and temperature, while one (*Matsushita et al., 2004*) analysed the impact of time and temperature. Using PBS, saline or Ringer's solution, the studies suggest that bovine ovarian tissue is better preserved at 20°C for up to 5 h, but higher rates of maturation and blastocyst formation are obtained when storage was between 10 and 15°C when stored for 24 h. Moreover, Nagao and colleagues (*Nagao et al., 2010*) observed that use of glutathione and epigallocatechin gallate in the storage medium increased blastocyst formation.

In cats, three studies evaluated oocyte meiotic competence (*Naoi et al., 2007*; *Evecen et al., 2009*; *Piras et al., 2018*). Only one (*Naoi et al., 2007*) reported higher rates of meiosis resumption when storage was carried out at 4°C. At this temperature, these authors found decreased MII rates after 48 h of storage (*Naoi et al., 2007*; *Evecen et al., 2009*; *Piras et al., 2018*), but they all agreed that with PBS or saline solution, cat ovarian tissue can be stored for up to 24 h at 4°C. Use of superoxide dismutase increased the viability of cumulus–oocyte

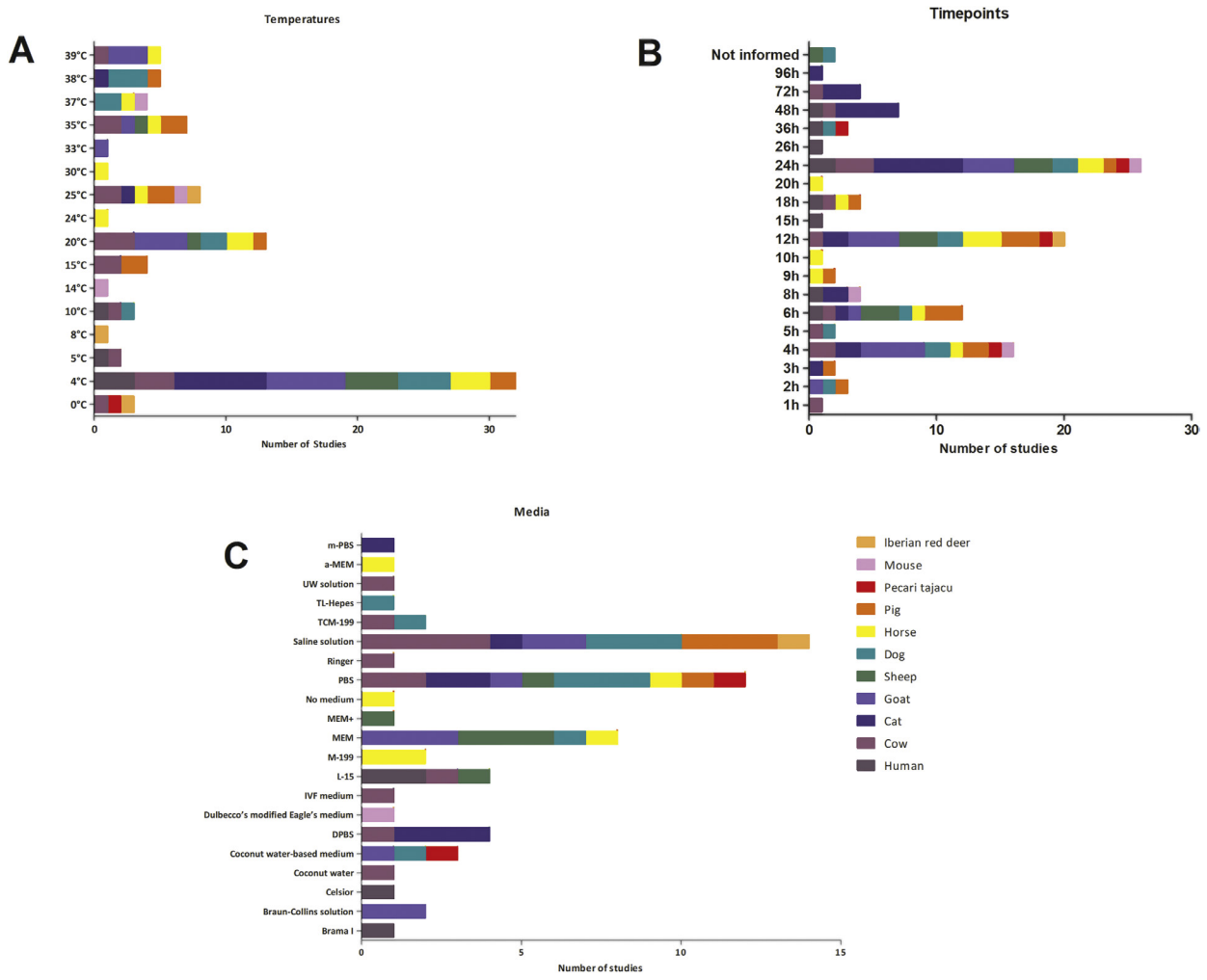

FIGURE 2 Temperature (A), timepoints (B), and media (C) tested in ovarian tissue transportation studies.

complexes, embryo production and expression of the antiapoptotic gene *Bcl-2*, while reducing the expression of *Bax*, an apoptosis regulator (*Cocchia et al., 2015*).

In pigs, studies reported reduced oocyte viability and maturation rates at low temperatures in saline solution, suggesting that porcine ovaries should be stored for a short time (<6 h) at 25–35°C (*Wongsrikeao et al., 2005*; *Tellado et al., 2014*). In PBS, follicle viability declined over time, even at 4°C, but follicle morphology remained similar to fresh controls for up to 12 h (*Raffel et al., 2020*).

There is no consensus on the influence of transport temperatures on canine ovarian tissue. While some authors found this tissue to be more sensitive to low temperatures (*Lee et al., 2006*; *Hanna et al., 2008*), others observed higher maturation rates when ovaries were transported at 4°C (*Tas et al., 2006*), or showed that the oestrous stage affects transportation more than temperature (*Evecen et al., 2010*).

Ovaries from sheep were stored in PBS with different concentrations of melatonin, and there was an improvement in embryo quality with increasing concentrations of melatonin in the medium after storage at 4°C for 24 h (*Goodarzi et al., 2018*). Goat oocytes showed higher meiotic resumption rates and a greater diameter of early antral follicles after 18 days of culture following ovarian tissue storage at 4°C for 4 h (*Correia et al., 2017*).

DISCUSSION

This is the first systematic review of the current state of ovarian tissue transportation. The authors identified 46 studies addressing optimal conditions for ovarian tissue preservation. Indeed, papers on this subject have been consistently published from 2004 to 2018 (an average of two or three per year), and continue to this day, testament to growing interest in this emerging topic of research.

While vital organ preservation has been developed and applied over the past 60 years (reviewed by *Petrenko et al., 2019*), preservation of ovarian tissue is relatively new. Hence, ovarian tissue transportation has only been recently discussed, with the concept of 'the woman stays, the tissue moves' established by the Danish network (*Jensen et al., 2015*). This practice has the potential to spread extremely quickly due to the costs and bureaucracy involved in the establishment of ovarian tissue cryobanks. Pregnancies and live births have been reported after such long periods of transportation, but this systematic review demonstrates that this remains an empirical procedure.

TABLE 4 **BEST CONDITIONS FOR EACH SPECIES**

	Best temperature							Best time								Best medium							
	4°C	10°C	15°C	20°C	25°C	37°C	38°C	4 h	6 h	7 h	12 h	18 h	24 h	26 h	48 h	ACP medium	Coconut water	MEM+	PBS	Saline solution	TL-HEPES	Use of additives	Use of antia-poptotic drugs
Human	1											1	1	1									
Cow	1	1	2	1				1				1	2									1	
Cat	1								1		1		4		1							1	
Goat	6							1	1		2		2				1		1	1		1	
Sheep	1								2				2					1				2	1
Dog	3					1	1				1		1			1		1			1		
Horse	1					1		1	1	1			1						1				
Pig	2				2				1		1	1											
Pecari tajacu													1			1							
Mouse	1							1											1				
Iberian red deer					1																		
Total	17	1	2	1	3	2	1	4	6	1	5	3	14	1	1	2	1	2	3	1	1	5	1

ACP, powdered coconut water; MEM, minimum essential medium; PBS, phosphate buffered saline.

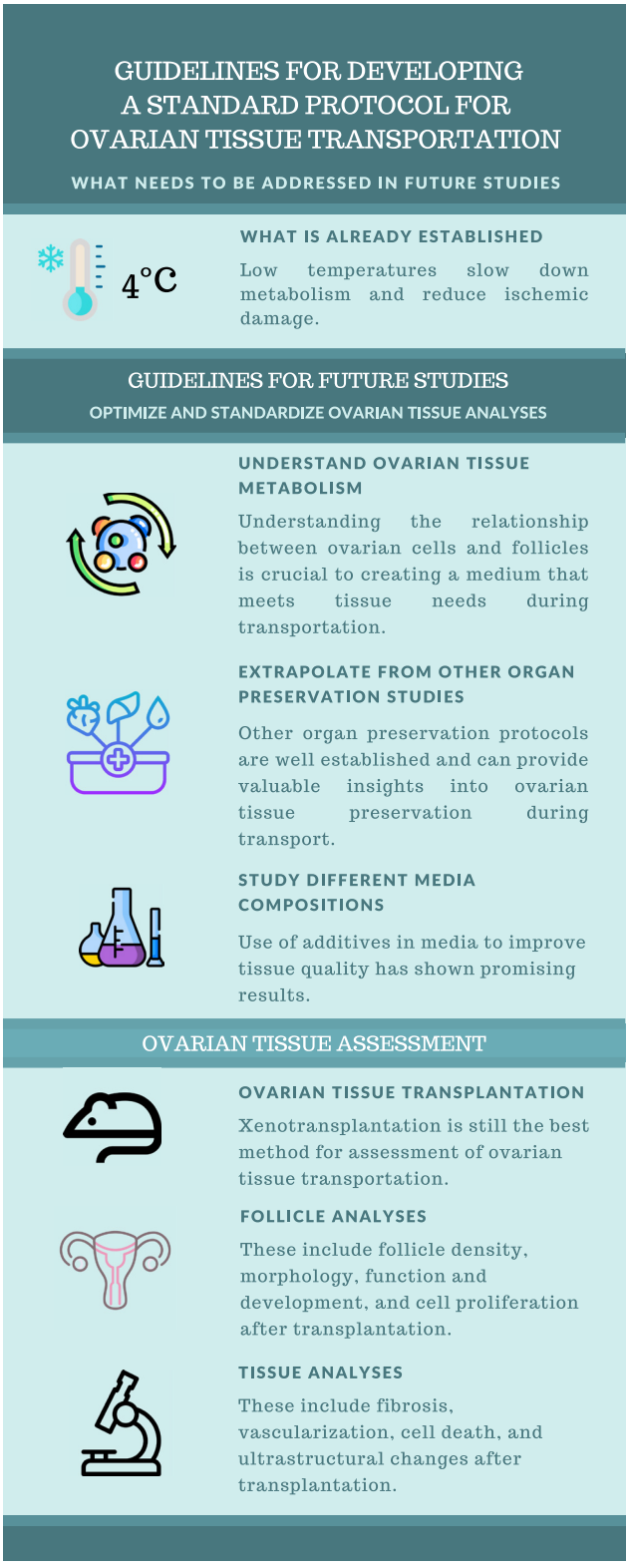

FIGURE 3 Guidelines for developing a standard protocol for ovarian tissue transportation.

The aim of this review was to prompt reflection on ovarian tissue transport, given the lack of knowledge there is in humans, by scrutinizing more than 40 studies published in different animal models. Among different domestic animal species, cows, sheep and goats have been used as models for humans thanks to their physiological ovarian similarities (*Fransolet et al., 2014*; *Lunardi et al., 2015*; *Sirard, 2017*). While no animal models can replace studies in our own species, they nevertheless provide valuable information that can be used in human medicine. By discussing

these results in animals, an attempt can be made to identify patterns within the studies. As in the development of different ART procedures, the authors believe this is an important step to create guidelines for future studies on human ovarian tissue transportation.

This review found that, when ovaries are stored for longer periods, lower temperatures can preserve follicle morphology and yield better oocyte maturation rates. Only two studies in pigs reported better IVM results after storage at over 25°C for 6 h (*Wongsrikeao et al., 2005*; *Tellado et al., 2014*). This may have been due to high lipid concentrations in pig oocytes, which makes them more sensitive to low temperatures (*Dunning et al., 2014*).

In fact, vital organs exposed to normothermic ischaemia remain viable for less than 1 h. Warm ischaemia leads to rapid depletion of ATP, accumulation of lactic acid, decreased pH, proteolysis, lipolysis and lipid peroxidation (*Finger, 2019*). Meanwhile, hypothermia lowers metabolism 12-fold (*Belzer and Southard, 1988*), reducing oxygen needs during the ischaemic period (*Calne et al., 1963*). However, it does not completely halt metabolic activity (*Connolly et al., 1996*; *Bellini et al., 2019*). Cold ischaemia causes an energy crisis, leading to injuries that are normally expressed after graft reperfusion, such as metabolic disruptions, oedema, autolysis and oxidative stress (*Storey, 2004*).

In ovarian follicle metabolism, it is known that primordial germ cells consume twice as much pyruvate as glucose and primordial follicles release lactate, indicating glycolysis and mitochondrial pyruvate oxidation (*Harris et al., 2009*). As follicles develop, they become predominantly glycolytic (*Boland et al., 1994a*; *Harris et al., 2007*). Amino acids, fatty acids and glycerol may also be used by follicles for ATP production (*Boland et al., 1993*; *Boland et al., 1994b*). While there is a lack of information about metabolism during ovarian tissue transport, it was shown that when pig ovaries were stored at 25°C for 6 h, follicular fluid exhibited lower pH values (*Wongsrikeao et al., 2005*; *Tellado et al., 2014*), glucose consumption, and lactate and ROS release (*Tellado et al., 2014*). Only one of the studies in this systematic review investigated metabolism immediately after storage at low temperatures (*Vilela et al., 2020*) and only a few analysed the medium for pH and/or ROS after IVM or in-vitro culture (*Lima et al., 2014*; *Correia et al., 2017*; *Gastal et al., 2017*; *Piras et al., 2018*). In a more recent study, Vilela and colleagues (*Vilela et al., 2020*) compared cow ovarian tissue storage in saline solution, IVF medium and L-15 at 4°C for 24 h and observed an increased lactate release in the media, even though there was low consumption of pyruvate and no consumption of glucose in IVF medium or L-15. However, these metabolic responses are associated with the assembly of different ovarian cell types (*Wagner et al., 2020*). It is important to stress that there is a lack of data on stromal ovarian cell metabolism, which, although not directly responsible for fertility, plays a significant role in the activation of primordial follicles and development of growing follicles (*Parrott and Skinner, 2000*; *Tagler et al., 2011*).

Overall, for most species, ovarian tissue should be transported at low temperatures to reduce metabolic activity and injuries during this period, allowing the tissue to be preserved for up to 24 h with minimal damage.

In terms of media, there is no consensus on the best medium for ovarian tissue transport and studies have been carried out with 20 different media, none of them specific for ovarian tissue. The main media used to transport ovarian tissue are PBS and saline solution. However, follicle morphology analyses in different species show that ovarian tissue requires nutrients that are not present in these solutions. Adenine nucleotide metabolism plays a key role in organ ischaemia, and preservation solutions that take this into account yield a better quality of preservation than saline solution (*Pegg, 2012*). Indeed, when ovaries stored in PBS or saline solution were compared with those in more complex media, the latter were able to maintain normal morphology for longer periods (*Silva et al., 2000*; *Lima et al., 2010*; *Lima et al., 2014*). Use of antiapoptotic (*Henry et al., 2016*) or antioxidant (*Nagao et al., 2010*; *Cocchia et al., 2015*; *Goodarzi et al., 2018*) drugs also appeared to improve the quality of ovarian tissue, pointing to important factors to be studied in future experiments. For kidney, heart, lung and pancreas preservation, many substances have been used to avoid damage caused by reperfusion, such as xanthine oxidase inhibitors (*Clavien et al., 1992*), interleukins and cytokine inhibitors (*Colletti et al., 1990*; *Strüber et al., 1999*), and other components that prevent cellular oedema, delay cell destruction and maximize organ function after revascularization (*Ali et al., 2015*; *Mundt et al., 2016*). Extrapolating knowledge acquired for different organs might well provide valuable insights into ovarian tissue preservation during transportation.

Most studies in humans were not part this systematic review because they reported outcomes of ovarian tissue cryopreservation and transplantation in clinical settings. In this context, the *Ferti*PROTEKT network validated Custodiol as a transportation medium for up to 22 h, even though there are no data on its efficacy for ovarian tissue (*Beckmann et al., 2019*; *Liebenthron et al., 2019*). In Denmark, 12 women underwent 18 transplantations and three babies were born to two women whose ovarian fragments were transported in α-MEM for 4–5 h prior to cryopreservation (*Rosendahl et al., 2011*). Only four studies were conducted on human ovarian tissue transport using three different media: Celsior (*Klocke et al., 2014*), L-15 (*Isachenko et al., 2013*; *Kyoya et al., 2014*) and Brama I (*Isachenko et al., 2009*). However, none of them is used in clinical practice for ovarian tissue transport prior to cryopreservation, stressing the importance of this topic for future research.

Ultimately, it is clear that different media compositions influence follicle viability and fertility outcomes, which could be related to cell metabolism. Although low temperatures reduce metabolism 12-fold, studies in animal models have shown lactate release in media (*Vilela et al., 2020*) and follicular fluid (*Tellado et al., 2014*), suggesting that cells might be using internal sources of energy instead of nutrients present in the media. These data indicate that ovarian tissue metabolism is affected by storage, even at low temperatures, and might impact fertility after transplantation. Moreover, it reinforces the importance of understanding whole tissue dynamics, beyond ovarian follicles.

The reliability of assessing transportation parameters was also considered in this review. Assessment of ovarian tissue

transport differs according to application. In this sense, using IVM, IVF and in-vitro culture to analyse oocytes isolated from transported tissue provides important clues to how fertility is affected by storage time, temperature and medium. Results from species that rely on these techniques for fertility preservation offer direct answers on how maturation and embryo development rates are impacted by transportation. Unfortunately, findings of this type do not apply to follicles included in ovarian tissue.

Most studies on ovarian tissue evaluated only follicle morphology after storage. While morphology is an important criterion to observe, it does not translate to normal follicle activity, so it is necessary to allow a re-establishment of tissue activity to gauge the effects of the procedure. Some studies have used in-vitro culture of ovarian fragments, but this system is not very well established for many species, yielding low rates of follicle survival in both short- and long-term culture (*Isachenko et al., 2006*; *Smitz et al., 2010*; *Vilela et al., 2016*). Since ovarian tissue transplantation has already been validated for cryopreservation assessment (*Nottola et al., 2008*; *Amorim et al., 2011*; *Luyckx et al., 2013*; *Ayuandari et al., 2016*; *Ruan et al., 2019*; *Vilela et al., 2019*), it might be a valuable technique to evaluate tissue transport. Indeed, transplantation of mouse ovarian tissue was used to assess ovarian tissue storage and revealed a significant reduction in both implantation and live birth rates, even though the morphology before transplantation did not show any difference in the percentage of morphologically normal follicles (*Kamoshita et al., 2016*).

This systematic review was, however, limited by the fact it was based on full-text publications in English. Studies published in different languages were not included in the investigation.

This review highlights the limited knowledge there is on ovarian tissue transportation conditions for all species addressed. Results from studies in this systematic review, as well as reports from clinical settings, demonstrate that finding a solution that meets ovarian tissue needs is essential to improving ART techniques. While there is mostly a consensus on maintaining ovarian tissue at low temperatures (namely 4°C), it is vital to consider different types of ovarian cells, as well as their interactions and needs, to be able to establish a standard protocol for ovarian tissue transport according to each species. Elucidating stromal cell metabolism and whole ovary dynamics is essential to developing a specific medium to transport ovarian tissue destined for transplantation. Moreover, interdisciplinary studies will play a crucial role in the development of ovarian tissue transport media; much has been learned and developed for vital organs, and this knowledge can be used to study how to improve the quality of ovarian tissue after transport. Assessment of follicle development and function should also be addressed in future studies to understand how fertility is affected by transport protocols once tissue vascularization has been restored (FIGURE 3).

With increasing demand for ovarian tissue cryopreservation and transplantation around the world, creating standard protocols for efficient transport is imperative in order to reach more patients, who can then have biopsies taken in small clinics and hospitals, and frozen in national tissue bank centres. It is also important to stress that improving transport may also affect the quality of the grafts and reproductive outcomes, which in turn can have a positive impact on birth rates after ART.

CONCLUSION

Upon review of the current literature, it is clear that there are no set standards for ovarian tissue transportation and, to date, its evaluation has been largely empirical. Ovarian tissue should be transported at 4°C to minimize ischaemic damage, and it is vital to create guidelines for ovarian tissue transportation to enhance ART outcomes in all species. To be able to develop a medium that meets the needs of ovarian tissue, it is essential to study the metabolic activity of this tissue, and not only isolated follicles, to discern how whole-tissue dynamics affect fertility. Moreover, assessment of transported tissue should be conducted after its return to activity. Finally, to shed light on the effects of procuring ovarian tissue for grafting, xenotransplantation is still the best means of evaluating ovarian tissue transportation.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2020.11.001.

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