Xenografting, is it a realistic possibility?

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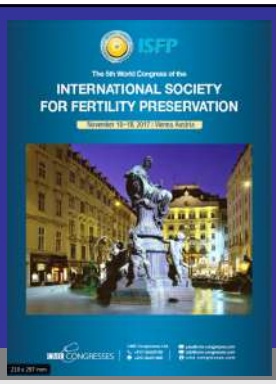

Disclosure information: Nothing to declare.

Session 7: Reimplantation of ovarian tissue, 17.11.17, 17.30 – 19.30
Chairs: Jehoshua Dor, Israel; Tommaso Falcone, USA

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CCC Comprehensive Cancer Center Erlangen-EMN Europäische Metropolregion Nürnberg

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The questions are: Can we do it? Should we do it?

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Agenda:

- Short introduction
  - How to get pregnant with cryopreserved ovarian tissue?
  - Indication for xenotransplation
- Technic of xenotransplantation of ovarian tissue.
- Outcomes so far
- What speaks against the use of this method for humans?
- Is xenotransplantation a realistic option?

Once the patient wants to get pregnant the question is how?

At the moment the only possibility to get pregnant with cryopreserved ovarian tissue is to transplant the tissue back into the patient:

• into the pelvic wall

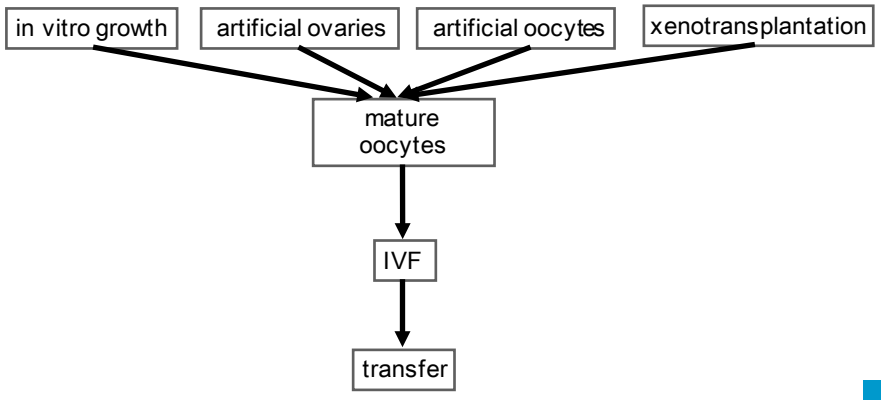
• onto the ovary

Retransplantation of ovarian tissue – is it a technology that is now available for everyone?

- But there are some patients where the transplantation back into the patient cannot help:
  - if malignant cells are present in the ovarian tissue.
- The question is therefore, can we also help these patients?

What can we offer to patients where the retransplantion is not possible due to malignant cells in the ovarian tissue.

Alternatives to retransplantation



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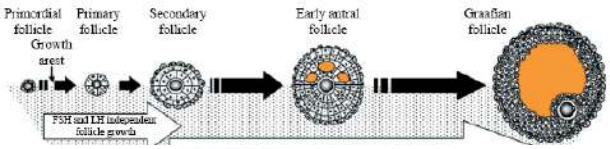
- In vitro growth: growth of primordial follicles to mature antral follicles in an incubator.



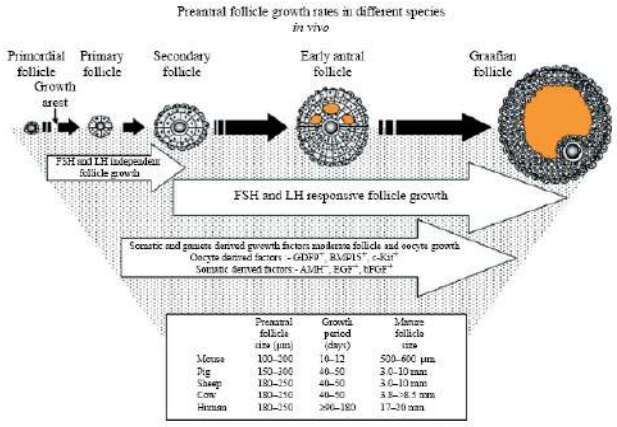
Development In Vitro of Mouse Oocytes from Primordial Follicles[1]

BIOLOGY OF REPRODUCTION **54**, 197–207 (1996)

John J. Eppig[2] and Marilyn J. O'Brien

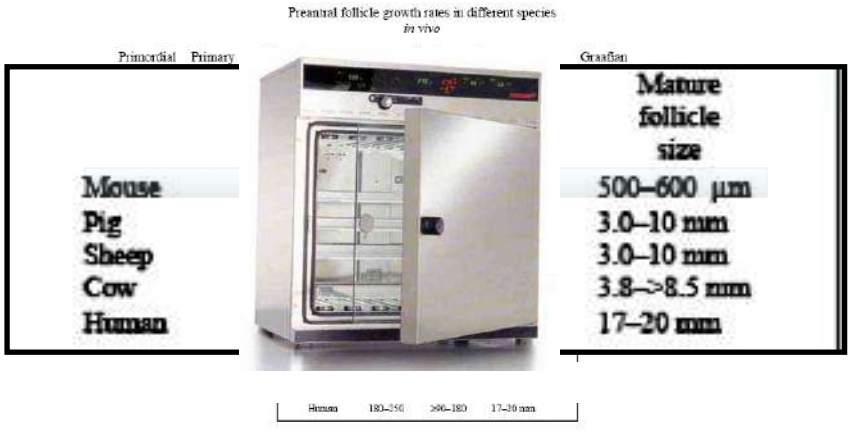
Nature **424**, 364-366)

In vitro growth: growth of primordial follicles to mature antral follicles.



Picton H.M. et al. Reproduction (2008) 136:703-715

In vitro growth: growth of primordial follicles to mature antral follicles.



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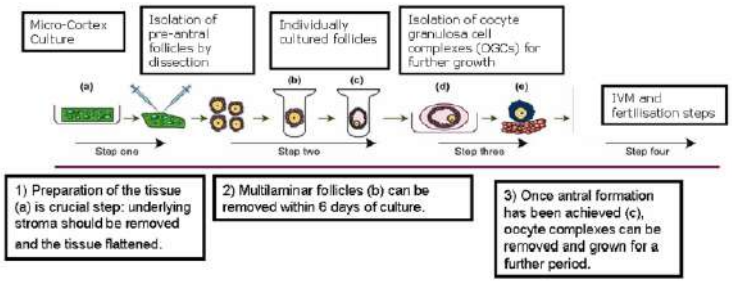
Int. J. Dev. Biol. 56: 901-907 (2012)
doi: 10.1387/ijdb.130001et

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

Strategies to support human oocyte development *in vitro*

EVELYN E. TELFER* and MARIE MCLAUGHLIN

Institute of Cell Biology and Centre for Integrative Physiology, University of Edinburgh, Scotland



1) Preparation of the tissue (a) is crucial step: underlying stroma should be removed and the tissue flattened.

2) Multilaminar follicles (b) can be removed within 6 days of culture.

3) Once antral formation has been achieved (c), oocyte complexes can be removed and grown for a further period.

Mc Laughlin, Albertini, Wallace, Andersen & Telfer:

- The group around Telfer has already had a Metaphase II oocyte. These oocytes even had intact spindels, which was demonstarated by this group.

  (personal communication, paper under review)

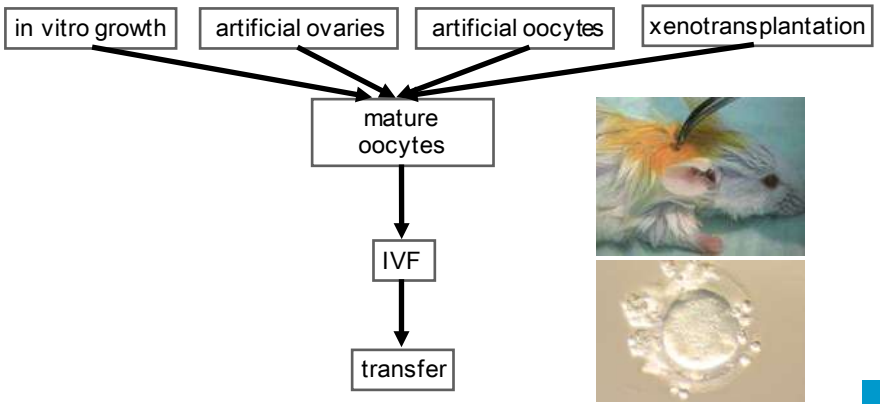
→ **Progress in culture systems should make the in vitro culture possible in the future also in humans. But unfortunately it is not working for humans at this time.**

Artificial ovary and artificial production of oocytes from stem cells:

- Artificial ovary: It‘s the same, progress has been achieved, especially from the Dolmans group, but unfortunately it is not possible at this time to produce functional human mature oocytes.
- This is the case also with the **artificial production of oocytes** from stem cells were we are only at the beginning.

What can we offer to patients where the retransplantion is not possible due to malignant cells in the ovarian tissue.

Alternatives to retransplantation



The most often used immundeficient animals are mice:

- Graft rejection is due to
  - Acute rejection (humoral immune response)
  - Delayed rejection (cellular immune response)
- Animal models:
  - Nude mice, athymic, can not produce AB
  - SCID mice, severe combined immunodeficient mice, mutation in the Prkdc or "protein kinase, DNA activated, catalytic polypeptide", which is necessary for V (D) J chain recombination and therefore these mice don't have a humoral or cellular immune system. NOD-SCID also have no NK and no complement activity.
  - Others (rag-deficient, higher-order, multigenic), are often not used for ovarian tissue xenotransplantation due to their sensitivity to diseases)

Fig. 1: Von Kuebi = Armin Kübelbeck - Eigenes Werk, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=432 1416 .
Fig. 2: National Cancer Institut, US

Dittrich et. al., not published

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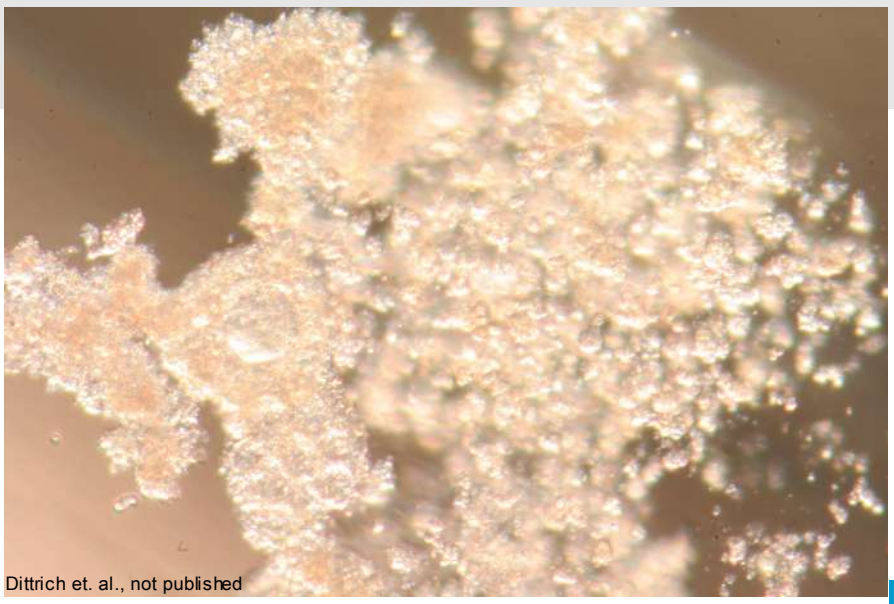

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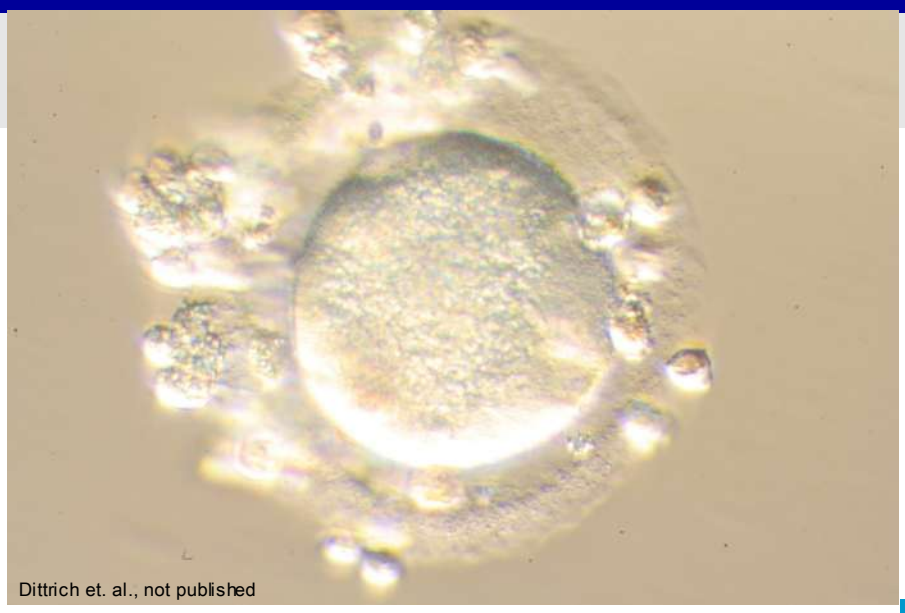
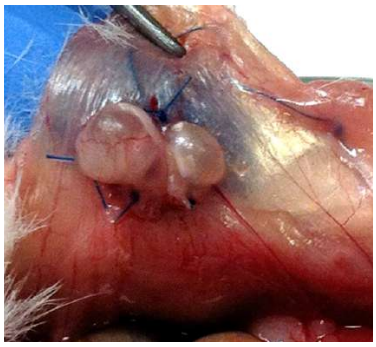


Dittrich et. al., not published

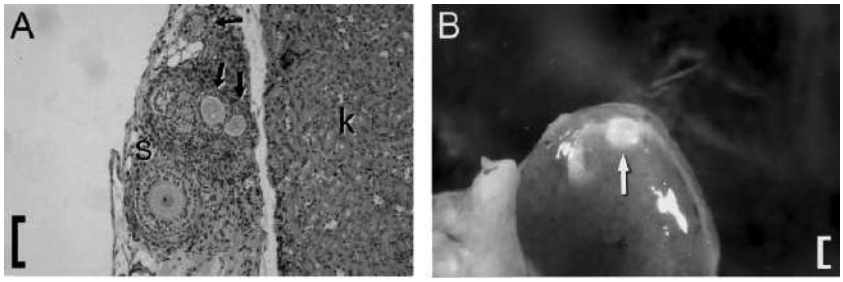
Different locations to transplant the tissue (neck, intraperitoneally, kidney, bursa)

- Intraperitoneal (Luyckx V., …, Dolmans MM. Fertil Steril 2013;100:1350–7)



Different locations to transplant the tissue (neck, intraperitoneally, kidney, bursa)

- kidney (Gook D, et al. Human Reproduction Vol.20, No.1 pp. 72–78, 2005)



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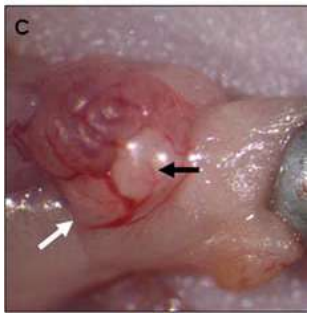
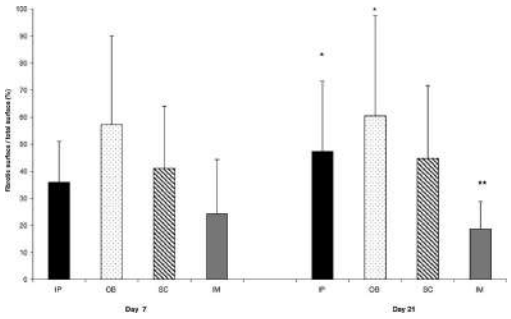
Different locations to transplant the tissue (neck, intraperitoneally, kidney, bursa)

- bursa (Dath C. et al. Human Reproduction, Vol.25, No.7 pp. 1734–1743, 2010)





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History of xenotransplantation of ovarian tissue into immundeficient mice (and other animals)- milestones

- Gosden was the first in 1994, cats and ewes, SCIDs
- Candy in 1995, marmoset, nude mice
- Helen Newton (Gosden) in 1998, first human OT, SCID, to compare different freezing protocols (she found EtGL the best).
- Then Oktay (1998); **Weissman (1999) first who used NOD-SCID**; **Gook (2001), antral follicles without stimulation**; Van der Broeke (2001), **Wolvekamp (Trounson), nude rat**; **Gook (2003), first human Met-II oocyte after xenotransplantation**.
- Currently: PubMed in November 2017: 481 hits

How many mature oocytes will be harvested after xenotransplantation per animal:

- There are only a few papers reporting the development and retrival of Metaphase II oocytes after xenotransplantation:

TABLE 1

Overview of human metaphase II (MII) oocytes from ovarian tissue xenotransplants.

Author (ref.)	SCID mice	Transplantation site	Exogenous stimulation	MII oocytes	Evaluation of the MII oocytes
Gook et al. (15)	Female	Kidney capsule	1 IU recombinant FSH	5	Morphologic
Gook et al. (19)	Female	Kidney capsule	1 IU recombinant FSH	9	Morphologic
Kim et al. (18)	Female and male	Subcutaneous space	5 IU PMSG	2	Morphologic and immunocytochemical
Soleimani et al. (16)	Female	Back muscle and kidney capsule	1 IU FSH	24	Morphologic (stereomicroscopic evaluation and MRI)
Lotz et al. (17)	Female	Intramuscular pocket in the neck muscle	hMG, hMG and triptorelin, or no stimulation	3	Morphologic
Lotz et al. (64)	Female	Intramuscular pocket in the neck muscle	No stimulation	1	Morphologic

Dittrich. Ovarian tissue xenotransplantation. Fertil Steril 2015.

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How many mature oocytes will be harvested after xenotransplantation per animal?

	Met II/Met II[1]	Out of grafts/ animals	Duration (weeks)	
Gook (1)	5	24/12 piece/mice in each kidney)	≥ 27	
Gook (2)	9	90	27	
Kim (3)	2	60	22	
Soleimani (4)	24	40	≥ 28	*
Lotz (5)	3	48	20 and 24	one without stimulation/hCG
Lotz (6)	1	7	18	without stimulation/hCG

[1] after IVM
* All from one ovary (of a 22 year old female to male transgender), preselection of tissue-slices before transplantation

Is stimulation of the tissue with exogenous gonadotropins necessary?

- Not necessarily
- Gook (Hum Reprod (2001) 16:417-22)
- Soleimani (Hum Reprod (2010) 25:1458-70)
- Lotz (Fertil Steril (2014) 101:1477-84)
- Lotz (Reprod Biol Endocrinol (2014) 12:41-9)

This is really astonishing. It means that:

- The LH/FSH secretion of the mouse pituitary is enough for the stimulation of human ovarian tissue
- LH/FSH of mice bind to human LH/FSH receptors
- The human ovarian tissue and the mouse pituitary build an independent feedback-system
- The human ovarian tissue is the (im)pulse generator in this feedback system (not the mouse hypothalamus)
- Maybe (data is too scarce for a final conclusion) the xenotransplantation is more comparable to in vitro culture than to the in vivo situation (hCG-trigger not necessary).

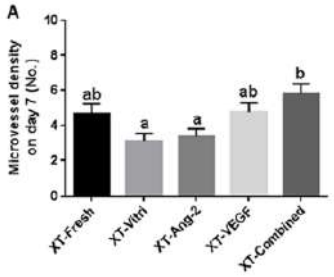
Improvement for Xenotransplantation success:

- Choosing a more „immundeficient“ mouse strain (e.g. the NODscid Gamma (e.g. NSG™ from Jackson Lab.)
- Transplantation of the ovarian tissue together with a tissue scaffold which promotes neovascularization
- Stimulation with growing factors before/during transplantation

Stimulation with growing factors before/during transplantation

- e.g.: Effect of treatment with angiopoitin-2 and VEGF on quality of xenografted bovine ovarian tissue in mice
- Kong HS, et al. PLOS ONE (2017) doi.pone0184546

Groups	Grafts No.	Primordial
XT-Fresh	12	73.9% (139/188)[a]
XT-Vitri	12	48.6% (51/105)[b]
XT-Ang-2	12	65.6% (101/154)[a]
XT-VEGF	12	70.5% (105/149)[a]
XT-Combined	10	69.7% (129/185)[a]



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Improvement for Xenotransplantation success:

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- Transplantation together with an tissue scaffold which promotes neovascularization
- Stimulation with growing factors before/during transplantation
- To increase the amount of medulla tissue when freezing and transplanting ovarian tissue (for a longer graft survival; Meirow (Gavish Z … Meirow D (2014) Hum Reprod 29:989).
- Fusion with cytoplasmic fragments (Kaneko H et al. 2013)

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Currently no embryos have been formed after the xenotransplantation of human ovarian tissue.

- But in animals
- Mouse tissue in nude rat (Snow et al. Science 2002; 297:2227) even living pups
- Pig tissue in nude mice (Kaneko et al. Therigenology 2013;80:887-92) blastocystes

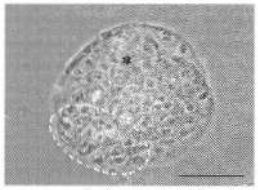

Fig. 1. An expanding (well-developed) blastocyst with 100 cells, which developed from an oocyte in the ICSI-3C group. The photograph was taken after fixation and staining. Trophectoderm (dotted circle, inner cell mass. Scale bar, 50 μm.

When you see such nice oocytes (after xenotransplantation of human ovarian tissue), the question arise why not use them for IVF?

When you see such nice oocytes (after xenotransplantation of human ovarian tissue), the question arise why not use them for IVF?

- Because of biological problems (e.g. the risk of zoonosis, developmental capacity of the oocytes)?
- Ethical issues?

When you see such nice oocytes (after xenotransplantation of human ovarian tissue) the question arise why not use them for IVF?

- Because of the risk of zoonosis?
- Ethical issues?
- We tried to give answers in a review article with this topic:

Xenotransplantation of cryopreserved human ovarian tissue — a systematic review of Met-II oocyte maturation and discussion about it as a realistic option for restoring fertility after cancer treatment?
Fertil Steril. 2015 Jun;103(6):1557-65.
Ralf Dittrich Ph. D.,[a] Laura Lotz M.D.,*[a], Tanja Fehm Ph.D.,*[b] Jan Krüssel Ph.D.,*[b] Michael von Wolff Ph.D.,*[c] Bettina Toth Ph.D.,*[d] Hans van der Ven Ph.D.,*[e] Andreas N. Schüring M.D.,*[f] Wolfgang Würfel Ph.D.,*[g] Inge Hoffmann,*[a] Matthias W. Beckmann Ph. D.*[a]

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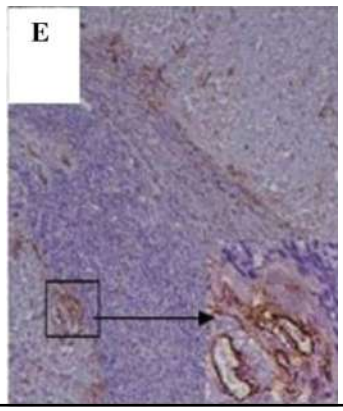
Biological Problems (Zoonosis I):

- Zoonosis
- Potential risk (especially retroviruses), but the risk is low.
- There is no single case reported of the transmission of any disease in humans exposed to live pig cells (Fishman and Patience Am J of Transplant (2004) 4:1383)
- There is no direct contact of mouse tissue cells with the oocytes in the human tissue (always human cells surrounding the oocyte) – but maybe with blood cells.

The vessels in human ovarian xenografts in SCIDs are of human origin.

- Shilong FU, et al. Exp Ther Med. 2014 Sep;8(3):742-746
- CD34 staining demonstrates the human-derived vessels



Biological Problems (Zoonosis II):

- And between collecting the oocytes and transfer of the embryo will be the IVF with several cleaning steps,
- Nevertheless, it is not impossible that a previously unknown microorganism might be transferred, and this risk cannot be totally excluded.

Ethical issues (the four principles of medical ethics):

- Non maleficence (does not harm the patient)
  - For the mother: low (zoonosis, transfer of embryo)
  - For the child: comparable to IVF **or** in vitro growth of ovarian tissue in a metal incubator **or** oocyte donation or surrogacy? This question cannot be answered easily. But, human reproduction follows the principle of all or nothing. As we know for IVF-methods: an embryo which is implanted carries no or nearly no additional risk for malformations of the child.
- Justice
  - There is no alternative at present for cancer patients, so it is justified.
- Beneficence
  - All patients who may become infertile should receive proper consideration of their interests for future options to preserve ovarian function. Xenotransplantion may be a solution.
- Respect of autonomy
  - All patients should be informed about all of the technical, ethical, and safety issues.

Of course in the public discussion the xenotrans-plantation method has a high risk of misunderstanding.

The risk of popular discussion in the tabloids:

- It may look strange to read such potential headings:
- Baby from the mouse
- Half mouse have human baby
- Dr. Frankenstein and his mouse baby
- etc.

Legal Problems in Germany:

- German Ethic Council recommended making a law about:
  „prohibition of procedures potentially resulting in the formation of human egg or sperm cells in an animal.“
  (Human-Animal Mixtures, 2011 p 120)
- Thus making it nearly impossible to use this method in Germany in the future.

Take Home Messages:

1. Xenotransplantation of human ovarian tissue results in mature oocytes (Met II).
2. Several papers demonstrated that the method is reproducible, but the outcome is low (only in about 10% of grafts Met-II oocytes develop).
3. The shape of these oocytes is good.
4. There is no report in the literature about a fertilization attempt in humans.
5. There is a big ethical concern about the use of this method to produce human „offspring“.
6. On the other hand: It could be a method to restore fertility of patients where direct transplantation of ovarian tissue into the patient (due to malignant contamination of tissue) is not possible.

Thank you for your interest

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