Peer Review File

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Reviewer A:

Comment 1: Page 4 Line 23-32 and section about fixation techniques: It should be mentioned that transplantation of tissue into a peritoneal pocket does not necessarily require fixation and that even if in the study of Meirow presented otherwise comes to good pregnancy rates with this technique. The better circulation in the pelvic wall and the easier surgicalfeasibility (and the associated low surgical risk for the patient) arereasons for transplantation under the pelvic peritoneum as com-pared to the situation in the generally significantly atrophied ovary.

Reply 1: Thank you – we have highlighted this.

Changes in the text: "... or peritoneal pockets, with the incisions closed if required..."

Comment 1: Page 4 line 33-35: The poor outcome in this study is because it involves ovarian tissue from patients with premature ovarian failure (i.e., hardly any follicles present in the tissue) and therefore cannot be directly compared with the studies mentioned above.

Reply 2: Thank you – we have removed this study from this section.

Changes in the text: P4 line 33-35 removed.

Reviewer B:

Comment 3: I could recommend corresponding author to look a .pdf manuscript before upload. Superscripts are presented as normal numerals that make it difficult to read the text.

Reply 3: Thank you for noticing this. We have changed it to make it easier to read.

Changes in the text: Superscripts fixed.

Comment 4: The volume of analyzed literature is very poor. It would be better now to mention the existing today information appeared after the first publication regarding baby born after transplantation of cryopreserved ovarian tissue instead to cite some old publications. The spectrum of respective surgical manipulations is brighter than presented in this review.

Reply 4: Thank you – the old publications were intended to provide historical perspective but we aim to present new techniques within the last decade. Some of the older publications are also plastic surgery papers that discuss skin grafting principles.

Changes in the text: New references added throughout.

Comment 5: I know no publications regarding transplantation of ovarian tissue without illustrations. In this review there are no pictures that for such type of publication is not appropriated.

Reply 5: We have now added a table for ease of viewing.

Changes in the text: See Table 1.

Reviewer C:

Comment 6: Page 1, Line 36-37: Numerous protocols are available for safe ovarian stimulation in presence of hormone sensitive tumors and currently used. This therefore does not represent anymore an indication for ovarian tissue cryopreservation as the only strategy to preserve fertility.

Reply 6: Thank you – we have removed the line about hormone sensitive tumors.

Changes in the text: Removed "... and if there are concerns about safety, as may be the case for hormone sensitive tumours."

Comment 7: Page 2, lines 12-15: The statement is too general. Several authors published their surgical techniques for OTT, grafting selection, tissue strips size. This part should be properly developed in the introduction or in a dedicated chapter, describing data available in the literature about surgical techniques for OTT.

Reply 7: Thank you – we have added a bit more detail in the introduction section and will also expand on these techniques in the subsequent sections.

Changes in the text: Added detail to Page 2 introduction section and added a "Surgical Techniques" section as part of the "Fixation Techniques" section.

Comment 8: Page 3, lines 31-33: If the authors state that cortical strip preparation is not the only variable for OTT success, but also transport, freezing media and, I would add, freezing technique play a role, then they must provide extensive information present in the literature about all these aspects.

Reply 8: Thank you – we have expanded on these topics.

Changes in the text: Added some recent evidence around freezing and transport into this section.

Comment 9: Page 3, lines 33-37: this statement is extremely confusing, and mixes use of drugs in freezing media as possible cytoprotection with approaches to reduce hypoxia-related follicle loss after OTT. These are different studies, trying to address different issues in the context of ovarian tissue cryopreservation and transplantation for fertility restoration. In the present chapter about tissue preparation only the first should be included, and the latter should be moved to the chapter about transplant outcomes. Moreover, for each topic original research papers should be included in the references, and not only other reviews about fertility preservation.

Reply 9: Thank you – we have separated them as suggested.

Changes in the text: Hypoxia-related follicle loss line moved to transplant outcomes.

Comment 10: Chapter about tissue preparation: there is no parallel with plastic surgery principles in this chapter: Please find some and implement the present chapter or consider to exclude it as it may be out of the aim of the review.

Reply 10: Thank you – we have added some.

Changes in the text: See additional paragraph in Tissue Preparation section at the end.

Comment 11: Page 4, line 20: what do the authors mean with density of follicular tissue? If they meant follicle density in ovarian tissue, they must explain how to have these data prior to transplantation. Follicle distribution is noy homogenous in the ovarian cortex, leading to high variability in follicle density among strips.

Reply 11: Thank you – this was specified by the study as being analysed at pre-transplant by fixing and viewing the strip(s) histologically.

Changes in the text: None.

Comment 12: Page 4, line 27: Meirow's study was published in 2005. A lot of other papers have been published afterward, by the same group and other groups as well, about cortical strip size and revascularization and ovarian fragmentation for follicle activation. This part should be rewritten after a careful revision of the recent literature.

Reply 12: Thank you – we have revised this to reflect recent literature.

Changes in the text: See revised 3rd paragraph of "Quantity" section.

Comment 13: Page 5, lines 30-32: the parallel with skin transplantation is not clear. Please explain it better.

Reply 13: Thank you – we have clarified.

Changes in the text: Changed to "Like skin grafts,..."

Comment 14: Page 6, lines 7-9: the use of extracellular matrix has been experimentally used by one group, and it is not part of routine clinical application for OTT. If the purpose of this chapter is to present potential strategies to improve OTT, the authors should include many other papers including the use of stem cells, growth factors and antiapoptotic.

Reply 14: Thank you – we have removed this.

Changes in the text: Removed page 6 lines 7-9.

Comment 15: Page 6, lines 13-22: what is it known about risk of hematoma in the context of OTT? Is there literature available about this specific issue? The authors must state clearly what it is known for OTT and what for skin grafting, in order to make the comparison clear.

Reply 15: Thank you, we have clarified this paragraph.

Changes in the text: See revised paragraph on haematoma.

Comment 16: Page 6, lines 28-32: ischemia lasts around 72 hours in mice but was widely demonstrated to last more in large animals like ewes and humans (around 5-7 days). Please carefully revise the literature about this aspect, which is abundant, and correct the present statement.

Reply 16: Thank you – we have revised the paragraph.

Changes in the text: Changed paragraph to read "Skin grafts are avascular and rely on the graft bed to provide perfusion via imbibition, which typically occurs after the first 24 hours and continues the process of graft take over the next 5-7 days.[34] Similarly, ovarian tissue fragments can sit in a hypoxic environment with nutrients provided via diffusion for approximately 3-5 days but up to 10 days before full revascularization.[47] During this time, the grafted tissue undergoes ischaemic reprfusion injury, leading to follicle loss; methods aimed at reducing this hypoxic period have shown improved primordial follicle survival.[47]"

Comment 17: Chapter Fixation technique: this part should describe systematically all the techniques published for OTT (use of stitches, fibrin glue…).

Reply 17: Thank you – we have listed these techniques partly to compare to plastic surgery, but there are a number of different described uses of these products. We believe it may be outside of the scope of this review to describe these techniques in further detail.

Changes in the text: None.

Comment 18: Page 7, lines 17: gynecological disease is not an accurate description. Do the authors mean malignancies, including BOT, or also benign condition like endometriosis?

Reply 18: Thank you – this description has been altered.

Changes in the text: See revised 1st paragraph of "Patient factors" section.

Comment 19: Chapter patients' factors should be at the beginning of the review. Taking in consideration risk factors like diabetes, smoking and anticoagulation may be interesting, but no information is provided by the authors about how these risk factors impact ovarian tissue transplantation. If there is no data available in the literature, it should be stated, otherwise the authors must included them in the manuscript.

Reply 19: Thank you – we have revised this.

Changes in the text: Added "So far, no specific evidence has been found regarding the effect of patient comorbidities such as diabetes or smoking on OTT success." and moved the initial sentence to the plastic surgery section.

Comment 20: Page 8, lines 19-25: Information on risk of reintroduction of malignancies should be implemented. The parallel with skin transplantation is not clear. For ovarian tissue, the risk is to reintroduce malignant cells present in the cortical strips at the time of cryopreservation, and it is not related to the risk of poor healing (as the authors state regarding skin transplantation).

Reply 20: Thank you, we have clarified this.

Changes in the text: "... flaps and other reconstructive options are carefully selected from healthy skin as free as possible from sun damage or previous trauma to prevent grafting malignant or pre-malignant skin changes onto the recipient site."

Comment 21: No figures or tables were included in the manuscript. It would be useful, for exqmple, to have 1 table about the available OTT techniques (with references, number of patients treated, complications…) and 1 table or summary figure about the parallels between plastic surgery principles and OTT.

Reply 21: Thank you, we have now included a table.

Changes in the text: See table 1.

Comment 22: Page 9, lines 15-17: the choice of robotic surgery versus laparoscopy or minilaparotomy should be discussed earlier in the manuscript, while describing the available surgical techniques for OTT.

Reply 22: Thank you, we have moved this to the appropriate section.

Changes in the text: Relevant sentence moved to "Surgical Techniques" section.

Comment 23: Conclusions are not clear. The authors state what is already known by the present literature. The conclusions should be about the information contained in the present manuscript, namely what can we learn from the parallel between plastic surgery principles and OTT and therefore apply in the future to improve the outcomes.

Reply 23: Thank you, we have clarified the conclusion to reflect the point of the paper.

Changes in the text: See revised conclusion.

<u>Reviewer D</u>:

Comment 24: P1, Line 15: "tissue grafting has been performed for thousands of years " – I don't think so.

Reply 24: Thank you - free skin grafting has been performed for thousands of years and was first documented in India 3000 years ago. Reference: Hauben DJ, Baruchin A, Mahler A. On the histroy of the free skin graft. Ann Plast Surg. 1982 Sep;9(3):242-5. doi: 10.1097/00000637-198209000-00009. PMID: 6753699.

Changes in the text: None

Comment 25: P2, Line 32: A pubmed search of "ovarian tissue transplantation" alone gave 2114 hits, how did you end up with only 48 included references?

Reply 25: Thank you - while there is a large number of literature relating to ovarian tissue transplantation, we sought to include only the most recent and relevant articles in order to discuss relevant modern techniques.

Changes in the text: None

Comment 26: P5, line 12: Consider including that heterotropic transplantation sides is often chosen when the remining ovary is to poor (due to e.g. radiation) or absent. Radiated areas are very poor grafting sides, which may also explain the low success rate in heterotropic grafting.

Reply 26: Thank you - this is actually mentioned in the following paragraph: "Heterotopic transplantation can be used in cases where the pelvis is unsuitable for transplantation, for example due to radiation..."

Changes in the text: None.

Comment 27: P6, line 29-31: I don't understand this parallel to plastic surgery. Only ovarian GCs have FSHR, I don't believe it is relevant to consider FSH levels in plastic surgery at all.

Reply 27: Thank you – we have removed this.

Changes in the text: Removed P6 line 29-31 (last sentence of "Characteristics of graft site").

Comment 28: P8, line: Almost all reported pregnancies derive from tissue transplanted to the remaining ovary, not to peritoneal sides. Why is intraperitoneal and subperitoneal sides considered excellent first choices?

Reply 28: Thank you – we will clarify this.

Changes in the text: P8: "Transplantation to the remaining ovary is the best first option, followed by intraperitoneal or subperitoneal sites. For alternative sites, truncal or abdominal muscle are preferable due to a rich blood supply and low mobility."