- ***The company report on the procedure included a small sample of nine women with no long-term reporting on the safety of this technique to delay the menopause or how effective it is.***

The safety concerns may relate to: 1) The operation (retrieval and transplantation). 2) Development of cancer in the transplanted ovarian tissue. 3) The effects of continued release of reproductive hormones on the risk of conditions such as breast cancer, heart disease, stroke and thromboembolism. 4) There may also be concerns about the risks of declining ovarian function and development of early menopause as a result of removing a third to half of one of the ovaries for the purpose of cryopreservation.

Whilst ovarian tissue retrieval and transplantation for the indication of postponing menopause is new, the use of this approach for preserving fertility in cancer patients is not new. The safety of ovarian tissue transplantation has been established over the past 20 years in Belgium, Germany, Denmark, France, Norway, Italy, Israel and USA, where ovarian tissue cryopreservation has been available for cancer patients. Although the purpose of tissue retrieval and subsequent transplantation later in life was primarily for fertility preservation, the transplanted ovarian tissues continued to function for up to 10 years with no relapses, and did not result in increased risk of cancer or medical conditions over and above what may be expected in the general population[1,2].
In terms of long-term medical risks, the fact that the hormones are natural reproductive hormones as opposed to the synthetic hormones used in HRT would suggest the risks may be lower, compared with HRT. Furthermore, the hormone production would be physiological: controlled cyclically and rhythmically by the body's natural mechanisms as in pre-menopausal women, as opposed to the static hormonal doses used in HRT; it is plausible this may reduce the long-term risks further.

- ***The report suggests that this treatment 'can delay the menopause by 20 years. However, the series does not appear to include long-term*** **follow up information to support this conclusion.**

Ovarian tissues have been transplanted in 318 patients (360 ovarian transplants) over the past 15 years [3,4,5]. The tissue survival has been reported in peer reviewed publications and can be from 2 to 10 years, with a mean duration of 5.5 years[3,4,5]. This data is based on a range of patient conditions and technical variances from different centres.
ProFaM removes a third to half of the outer cortex of an ovary using advanced, state of the art technologies resulting in as consistent as possible prepared strips. We store from a minimum of 4 to 8 or more strips depending on the case. If, based on published work, only a few grafted strips are required to restore ovarian function for a mean of 5.5 years there will be enough stored tissue for episodic grafting that makes 20 years of continued hormone production plausible; especially as ProFaM will work with healthy patients of all ages within the reproductive years. We shall follow up every

consenting patient in our cohort, and will report our own data on tissue survival.

- **This is particularly relevant as research on the use of this technique in infertility has shown that re-implanted samples do not always function and the duration of tissue activity / viability is variable**.

Whereas we provide references and a body of evidence that is in contradistinction to this statement, no evidence was provided by the authors of the British Menopause Society for this comment. However, we accept that there is variation in outcome in different parts of the world due to patient mix and, not least, the skill and technique of the practitioners; and that some of this work is carried out without the necessary degree of scrupulous regulation. But no technique offers guarantees of success, whether surgical, or for example, IVF.

While the use of this technique to restore fertility has a success rate up to 35%,[6] studies have showed that restoration of ovarian function with production of female steroids is achieved in more than 90% of the transplanted women[7-9]. These findings demonstrate that transplantation of cryopreserved ovarian tissue is indeed very successful to restore ovarian function. It is correct that the duration of transplanted tissues may vary from 2–10 or more years[1,3-6,8]. Such variation is due to techniques involved in the ovarian tissue cryopreservation and transplantation and as well as ovarian reserve.
Regarding the technique: we have developed our technologies, which have been validated and licensed by the HTA (Human Tissue Authority). We continue to research and refine ovarian tissue preparation, storage and transplantation techniques to improve on the already well-developed, trialled and tested techniques that we employ. The original techniques were developed from rigorous research in Professor Amorim's laboratory in Belgium. Concerning the ovarian reserve, all patients are submitted to medical tests designed to calculate their initial follicle population. It is important to highlight that we will ensure women are fully counselled, separate to the consultation, about risks and likely outcome before they proceed with the procedure.
It is well known that HRT may not resolve menopausal symptoms for all women, but this does not prevent us from offering a trial with HRT if a woman is debilitated with menopausal symptoms. Similarly, in IVF practice, we may offer IVF to women with a very small chance of a pregnancy, if the woman is fully informed and potential benefits outweigh risks. We believe the same principles and shared decision-making should apply for patients seeking ovarian tissue cryopreservation.

- **The biopsies are obtained through a surgical procedure that while safe and commonly performed can be associated with potential surgical risks and requires a general anaesthetic.**

In terms of surgical tissue retrieval, the risks relate to laparoscopy. The risks of laparoscopy are well documented and considered acceptable in routine clinical practice. The transplantation may be in the pelvis (if fertility is desired), in which case laparoscopy will be necessary. Laparoscopic procedure to obtain the ovarian biopsy has been performed in more than 5000 patients

worldwide and risks related to this surgery are lower than 1%, according to the main groups in Europe. For instance, *Ferti*PROTEKT had only one complication in 500 laparoscopies.[10,11]. Laparoscopy has been recommended for many life quality problems such as infertility and endometriosis. However, for hormonal restoration the ovarian tissue could be transplanted under the skin in an outpatient setting, in which case the procedure would be straight forward with minimal risks. As the graft is always traceable the removal of the graft (if required) would be an easy process.
Regarding the risk of adhesions, there is no evidence or existing practice support any concern about infertility secondary to adhesion in case of unilateral ovarian surgery. There is strong evidence that laparoscopic access and micro-surgical technique reduce significantly risk of adhesions.
We believe, therefore, there is strong body of literature providing supportive evidence of safety, and no evidence of significant risks from the proposed procedure. However, as a credible research group we shall continue to gather effectiveness and safety data meticulously and make this available in the public domain through scientific publications.

- **In addition, the potential impact of removing ovarian tissue on long-term ovarian function including future fertility, especially in women who do not have a clear indication to do so, requires further assessment and evaluation**.

In terms of removal of a third to half of one of the ovaries, the available evidence does not suggest any decline in ovarian function or an increase in the risk of early menopause. It is not uncommon for women to have part of an ovary excised, for example during surgery for ovarian cysts, or indeed whole of the ovary, for example from complications of ovarian torsion. Such loss of ovarian tissue, and even a whole ovary, does not appear to significantly influence fertility or the time of onset of menopause [12, 13-16].

To minimise this theoretical risk, we have refined our surgical procedure so that only a small amount of ovarian cortex is removed. Furthermore, we take meticulous care in the use of electrosurgical instruments as these can cause inadvertent injury to the ovarian tissue. We, therefore, believe this theoretical risk is small, but we shall study this risk rigorously in consenting women from our cohort.

- **There is also a need to assess the theoretical unknown risk of ovarian cancer that may be associated with re-implanting ovarian tissue and the potential risk of breast cancer with delayed menopause as well as the contraceptive requirements in women who do not desire a pregnancy.**

318 women have had ovarian tissue transplantation worldwide (with over 150 reported successful deliveries), with no reported cases of ovarian cancer in the transplanted tissue [4,10,16,17]. Indeed, Gellert et al reported on 230 cases of transfer where there was a former malignant diagnosis with no reports on the reoccurrence of the original cancer in connection with OTT[18]. However, we will meticulously follow-up consenting women in our cohort to generate further data on safety. We anticipate the risks of ovarian cancer to be similar

to that of the general population. As for breast cancer, we anticipate the risk to be lower than for women on HRT, as the hormones from transplanted ovarian tissues are the patient's physiological reproductive hormones, released with natural monthly cyclicity and physiological circadian rhythm, mimicking the pre-menopausal state; as pre-menopausal state is not associated with a particular increase in breast cancer, we do not believe ovarian transplantation represents a particular risk. Finally, for women who do not desire a pregnancy, the ovarian tissue will be transplanted under the skin (and not in the pelvis); therefore, there will be no risk of pregnancy and no need for contraception.

- ***The procedure should also be compared against the standard more controllable ways of managing the menopause that are currently used including HRT. The latter has been well studied and its safety has been demonstrated in numerous studies over many years.***

We entirely agree that ovarian tissue cryopreservation should be compared against the current standard of HRT. HRT is associated with a small but significant risk of thromboembolism, stroke, heart disease (in older women) and breast cancer. The NHS information site states:
"*Taking combined HRT (oestrogen and progestogen) is associated with a small increased risk of breast cancer – some studies have suggested that for every 1,000 women taking combined HRT, there will be around 5 extra cases of breast cancer (from a normal risk of 22 cases of breast cancer per 1,000 menopausal women to 27), and 'Oestrogen-only HRT can increase the risk of womb cancer (also called endometrial cancer), which is why it's only used in women who do not have a womb.*" [19]
However, the latest study in the Lancet[20] indicates the breast cancer risk may be significantly higher (from 5/1000 to 20/1000 for oestrogen plus daily progestagen preparations). The authors conclude: "*In western countries there have been about 20 million breast cancers diagnosed since 1990, of which about 1 million would have been caused by MHT*" (HRT). This has resulting in the latest issue from the MHRA: https://www.gov.uk/drug-safety-update/hormone-replacement-therapy-hrt-further-information-on-the-known-increased-risk-of-breast-cancer-with-hrt-and-its-persistence-after-stopping#new-study-on-the-increased-risk-of-breast-cancer-with-hrt
Furthermore, HRT does not resolve menopausal symptoms in all women; and not all women can tolerate HRT. It has been reported that 18% of patients of premature menopause discontinue the HRT because of side effect. Furthermore many, for clinical reasons, are prevented from considering HRT. Our procedure has the added benefit of preserving fertility, should a woman need this later in life due to unexpected early loss in her ovarian function. Approximately 1 million women in the UK enter premature menopause. Our hypothesis is that women with ovarian tissue transplantation will have a better long-term prognosis compared with those on HRT, as ovarian tissue transplantation produces natural reproductive hormones, physiologically; unlike HRT which uses synthetic, albeit apparently bio-identical, hormones in an artificially static dosing regimen. We shall study long-term risks in consenting women from our cohort.

There is also the view that as "HRT is based on oestradiol which is bio-identical**",** it is physiological; and as a result, "highly effective, simple and very safe for most women"[21]. It is important to put such views into context. A recent Editorial, 'BMS Consensus Statement' in the Post Reproductive Health Journal5 states: "'*bioidentical' is often used as a marketing term by clinics*". they are "*precise duplicates of hormones such as estradiol E2, estriol E3, estrone E1, progesterone*..." but there are concerns around "*absence of medical evidence to support the practice of combining E1 and E3 with E2*", absence of warnings on the products regarding potential risks and side effects."; the dosage of oestrogen, and "*issues related to purity, potency and safety*"[22].
Furthermore, the presentation and dosages of such pharmacological compounds, however 'identical' to the tissue-produced product(s) cannot be said to be delivered to the organism "physiologically", with all the circadian and rhythmical controls underlying physiology; hence some of the concerns raised by the usage of HRT raised above.

- ***This report explores a potentially promising concept for women at risk of medically or surgically induced menopause. However, when it comes to considering this in the context of delaying the menopause, further evaluation is needed to assess the safety of this technique, its effectiveness and the length of time such re-implanted tissue continues to function. Such assessment should also include a benefit / risk analysis particularly when applied in otherwise healthy women***.

We agree with the need for meticulous ongoing study of ovarian tissue cryopreservation and transplantation. We will be conducting a large prospective cohort study to gather and report key effectiveness and safety outcomes. However, given all the studies to date, as described above, the ProFaM position regarding the opportunity for the current and future generations of fertile women has been ethically deliberated; and, perhaps, the potential benefits to these women, along with the probability to ameliorate medical healthcare costs in the future can only be resolved by responsible, cautious action[2].

Last, but not least, there is the comparison to egg freezing, with regard to fertility preservation. Egg freezing is indeed an option but it is well recognised that a minimum of 20-30 eggs need to be stored (live birth per egg around 8% in women under 36 years and 3% in women 36–39 years; equating to 29.6 oocytes per live birth). This would require on average three cycles of stimulation, at a cost of over £15,000[23]. Plus, there is the necessary several rounds of egg collection, with attendant risks on each occasion, as well as multiple gonadotrophin stimulation rounds and the associated visits to the clinic for blood tests and monitoring. This compares to OTC which does not require any drug regimen, and a single visit to the hospital at a mutually agreed time at any point in the cycle.

References

1. Jensen, A.K., Kristensen, S.G., Macklon, K.T. Jeppesen, J.V. Fedder, J., Ernst, E., et al. Outcomes of transplantations of cryopreserved ovarian tissue to 41 women in Denmark. Hum Reprod. 30(12):2838–2845; *2015*
2. Amorim C.A., Leonel, E.C.R., Afifi Y., Coomarasamy, A., Fishel, S. Cryostorage and retransplantation of ovarian tissue as an infertility treatment. Best Practice & Research Clinical Endocrinology & Metabolism. https://doi.org/10.1016/j.beem.2018.09.002
3. Silber, S.J., 2012. Ovary cryopreservation and transplantation for fertility preservation. Mol. Hum. Reprod. 18, 59–67
4. Greve, T., Schmidt, K.T., Kristensen, S.G., Ernst, E., Andersen, C.Y., 2012. Evaluation of the ovarian reserve in women transplanted with frozen and thawed ovarian cortical tissue. Fertil. Steril.
5. Donnez J, Dolmans M. Fertility Preservation in Women. New England Journal of Medicine. 2017;377(17):1657-1665.
6. Meirow, D., Ra'anani,H., Shapira, M., Brenghausen, M., Derech Chaim, S., Aviel-Ronen, S., et al. Transplantations of frozen-thawed ovarian tissue demonstrate high reproductive performance and the need to revise restrictive criteria. Fertil Steril 2016;106:467–74.
7. Andersen CY, Mamsen LS, Kristensen SG. Freezing of ovarian tissue and clinical opportunities. Reproduction. 2019. doi: 10.1530/REP-18-0635.
8. Donnez J, Manavella DD, Dolmans MM. Techniques for ovarian tissue transplantation and results. Minerva Ginecol. 2018;70:424-431.
9. Liebenthron J, Montag M, Reinsberg J, Köster M, Isachenko V, van der Ven K, van der Ven H, Krüssel JS, von Wolff M. Overnight ovarian tissue transportation for centralized cryobanking: a feasible option. Reprod Biomed Online. 2019;38:740-749.
10. Beckmann MW, Lotz L, Toth B, Baston-Büst DM, Fehm T, Frambach T, et al. Concept paper on the technique of cryopreservation, removal and transplantation of ovarian tissue for fertility preservation. Geburtshilfe Frauenheilkd. 2019;79:53-62.
11. Jadoul P, Giulmain A, Squifflet J, Luyckx M, Votino R, Wyns C, Dolmans MM. Efficacy of ovarian tissue cryopreservation for fertility preservation: lessons learned from 545 cases. Hum Reprod. 2017;32:1046-1054.
12. Wilkosz P, Greggains GD, Tanbo TG, Fedorcsak P. Female reproductive decline is determined by remaining ovarian reserve and age. PLoS One 2014;9(10):e108343.
13. Bellati F, Ruscito I, Gasparri M, Antonilli M, Pernice M, Vallone C et al. Effects of unilateral ovariectomy on female fertility outcome. Archives of Gynecology and Obstetrics. 2014;290(2):349-353.
14. Bjelland E, Wilkosz P, Tanbo T, Eskild A. Is unilateral oophorectomy associated with age at menopause? A population study (the HUNT2 Survey). Human Reproduction. 2014;29(4):835-841.
15. Rosendahl M, Simonsen M, Kjer J. The influence of unilateral oophorectomy on the age of menopause. Climacteric. 2017;20(6):540-544.
16. Younis J, Naoum I, Salem N, Perlitz Y, Izhaki I. The impact of unilateral oophorectomy on ovarian reserve in assisted reproduction: a systematic review and meta-analysis. BJOG: An International Journal of Obstetrics & Gynaecology. 2017;125(1):26-35.

17. Gellert S, Pors S, Kristensen S, Bay-Bjørn A, Ernst E, Yding Andersen C. Transplantation of frozen-thawed ovarian tissue: an update on worldwide activity published in peer-reviewed papers and on the Danish cohort. Journal of Assisted Reproduction and Genetics. 2018;35(4):561-570.
18. Gellert, S.E., Pors, S.E., Kristensen, S.G., Bay-Bjørn, A.M., Ernst, E. Yding, Andersen, C.Y. Transplantation of frozen-thawed ovarian tissue: an update on worldwide activity published in peer-reviewed papers and on the Danish cohort. Journal of Assisted Reproduction and Genetics (2018) 35:561–570
19. Hormone replacement therapy (HRT) - Risks [Internet]. nhs.uk. 2019 [cited 21 August 2019]. Available from: https://www.nhs.uk/conditions/hormone-replacement-therapy-hrt/risks/
20. The Lancet. August 29, 2019 http://dx.doi.org/10.1016/S0140-6736(19)31709-X
21. Davies, M. Ovarian tissue grafts to combat the menopause. BioNews, 9 August 2019: https://www.bionews.org.uk/page_144472
22. Panay, N (in collaboration with the Medical Advisory Council of the British Menopause Society). Post Reproductive Health 2019, Vol. 25(2) 61–63.
23. Bracewell-Milnes, T., Norman-Taylor, J and Nikolaou, D. British Journal Obstetrics and Gynaecology, 2018; 125: 1580

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