Journal of Cancer Biology and Therapeutics

Review Article

Should you be putting All Your Eggs into One Basket? A Look into the Current State and Future of Ovarian Tissue Cryopreservation

Rauff S^{1,2*}, Khan Z³ and Andersen CY¹

¹Laboratory of Reproductive Biology, Section 5712, Rigshospitalet, Juliane Marie Centre for Women, Children and Reproduction, University Hospital of Copenhagen, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 9, DK-2100 Copenhagen, Denmark

²Department of Obstetrics and Gynaecology, Division of Reproductive Endocrinology and Infertility and Minimally Invasive Surgery, National University Hospital Singapore, 5 Lower Kent Ridge Road, Singapore 119074, Singapore

³Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Mayo Clinic, 200 First Street SW Rochester, MN 55905, USA

Received: Oct 25, 2015; Accepted: Nov 30, 2015; Published: Dec 3, 2015

Abstract

Ovarian tissue cryopreservation has its primary goal in fertility preservation for women diagnosed with a malignancy and who may be rendered infertile because of the potential gonadotoxic chemotherapy and/or radiotherapy involved in treating their disease. Unlike the standard and endorsed methods of fertility preservation like mature oocyte and embryo cryopreservation, ovarian tissue freezing not only conserves the reproductive capacity of the woman but additionally, maintains the steroidogenic competence of the ovary due to the fact that the frozen cortex contains numerous ovarian follicles – the functional units of the ovary. Not every follicle is fated to aid procreation. In fact more than 99% are destined to end up in atresia, which may be viewed as an enormous waste of inherent resources. In light of this, there have been propositions to expand the scope of ovarian tissue cryopreservation and transplantation beyond its traditional purpose of fertility preservation for medical indications. Some of these ideas include utilizing cryopreserved ovarian tissue for induction of puberty, delaying menopause and fertility preservation for social motives. Needless to say, these novel ideas will evoke questions, controversy and a plethora of criticism about the safety, superiority, cost-effectiveness, implications and necessity of these different utilities. In this article, we aim to explore some of the issues that shroud these new indications and discuss the advantages for and diatribe against these evolving suggestions.

Keywords: Ovarian tissue cryopreservation; Fertility preservation; Menopause; Puberty; Deferring reproduction

***Correspondence:** Rauff S, Department of Obstetrics and Gynaecology, Division of Reproductive Endocrinology and Infertility and Minimally Invasive Surgery, National University Hospital Singapore, 5 Lower Kent Ridge Road, Singapore 119074, Singapore, Tel: +65-67724267; E-mail: srauff1@gmail.com

Introduction

The foremost indication for ovarian tissue cryopreservation (OTC) and reimplantation is for fertility preservation in females undergoing potentially gonadotoxic therapy, particularly in cases when mature oocyte and embryo cryopreservation may not be feasible options. The first birth employing the above technique was reported by Donnez and co-workers in 2004 in a woman who entered premature ovarian insufficiency following treatment for Hodgkin's lymphoma [1]. Since then, there has been rapid progress in this field of fertility preservation with numerous publications and reports of similar successes. Although unlike mature egg and embryo cryopreservation, OTC has not yet been endorsed by the American Society of Reproductive Medicine as a valid method of fertility preservation [2].

Briefly, ovarian tissue cryopreservation involves harvesting the ovarian cortex surgically – either by a unilateral oophorectomy or decortication of the ovary and this is usually performed laparoscopically, before the patient undergoes cancer treatment. The ovarian cortex harvested is cut up in smaller fragments and these pieces are cryopreserved, the most common method of cryopreservation being the slow-freezing technique [3]. When the patient is eventually declared “disease-free”, desirous to conceive and unable to do so naturally, the pieces of ovarian cortical tissue are thawed and reimplanted preferably into the remaining ovary or pelvic region (“orthotopic”) or less commonly, transplanted to an area outside the pelvis (“heterotopic”). More than 90% of the follicles are located in the ovarian cortex and with revascularization, the ovarian graft can recover its function. Steroid production will be restored and along with it, cyclical monofollicular development, menstrual status and ultimately, fertility. The patients have a chance to conceive spontaneously although some may still require assisted reproductive techniques to increase their chances of conception.

Whilst oocyte and embryo freezing only preserve a woman's fertility, one can see that OTC has a further advantage over merely securing reproductive potential – sex steroid function is maintained as the ovarian follicular pool is preserved. It is based on this premise that other suggestions to utilize frozen ovarian tissue

have evolved. Reports on applying the functional unit of the ovarian follicle for inducing puberty in children with ovarian failure post-treatment for malignancy, postponing menopause and fertility preservation to defer reproduction have kindled provocative discussions on how the future might change [4-6]. The extrapolation of ovarian tissue cryopreservation and reimplantation beyond its initial principal indication to these ancillary uses is undoubtedly food for thought. In this article, we aim to discuss the issues and controversies that shroud these new aspects.

Fertility Preservation for Medical Indications – The Original Goal

At the time of writing, there have been 60 live births resulting from ovarian tissue freezing and reimplantation for women who underwent the procedure for the reason of fertility preservation due to obligatory gonadotoxic treatment reported, and this number will naturally increase [7]. Although the true denominator, which is the total number of transplants performed worldwide, is not well-established, reviews by Donnez et al. in 2013 and 2015 estimated the conception rate to be 25% based on results from four large centres in Belgium, Denmark, Germany and Spain [8,9]. Both the numerator and denominator are difficult to define precisely. Furthermore, only when a large cohort of women who have had tissue transplanted with a pregnancy wish and the tissue has stopped working, will it be possible to calculate the true efficacy of OTC [10]. It was also demonstrated that the graft function could provide long term efficacy and not just a ‘one-off’ chance as more than one woman conceived and delivered more than once.

From these studies, information on the length of graft function has been obtained. The average duration of graft function is approximately 5 years when follicular density is adequate and well-preserved but this duration of follicular activity can exceed 5 years and has even survived until 7 years if the ovarian tissue was cryopreserved when the patient was young and prior to commencement of chemotherapy [8,9,11,12]. The graft function will be compromised when the follicular density is low and the amount of tissue transplanted is little, resulting in a reduced duration of function or minimal

steroid activity. When sufficient amounts of ovarian tissue are stored, the ovarian transplantation procedure may be repeated multiple times thus prolonging the duration of ovarian activity and extending the lifespan of ovarian endocrine activity up to 11 years or more [9]. This may have further implications on utilizing the banked ovarian tissue for hormone replacement therapy once menopause ensues and when the woman does not desire fertility anymore.

The advantages of ovarian tissue cryopreservation are that having a partner/donor sperm is not a prerequisite. It is the only method that can be performed in pre-pubertal girls, there is minimal delay in initiating cancer treatment when time is of the essence, and no hormonal stimulation is necessary. However, it is not without jeopardy and besides the requirement for an albeit minor, surgical route twice, along with its attendant peri-operative risks, the major fear is the possibility of reintroducing undetected metastatic malignant ovarian cells to the patient who has otherwise been declared disease-free or cured, resulting in disease recrudescence. Thus, it may not be able to be performed when there is a high risk of ovarian metastasis present, for example in very advanced stage cancer or some cancer subtypes like leukaemia. Ovarian tissue cryopreservation is also still considered an experimental technique in many countries which may not be acceptable to some patients [2].

All in all, the benefits of ovarian tissue cryopreservation and reimplantation in this group of patients are likely to outweigh the liabilities and has thus, become a worthwhile option for them.

Ovarian Tissue Cryopreservation as a Fertility Preservation Method to Defer Reproduction (“Social Reasons”)

Nowadays, women have the option of preserving their fertility when they wish to postpone childbearing due to personal reasons – in order not to hinder career progression, financial difficulties or simply because they have not found “Mr. Right” yet among other explanations. The traditional method of fertility preservation for these social indications is mature oocyte cryopreservation

via vitrification. However, even in the most experienced centres, the live birth rate per vitrified oocyte is only about 5 to 6 % and most recommend obtaining 20 mature oocytes for vitrification to achieve a reasonable chance of a live birth [13-16]. This would typically entail more than one controlled ovarian stimulation (COS) cycle in most women, even with aggressive stimulation and mandates subsequent in-vitro fertilization (IVF) when the woman needs to use her frozen oocytes to conceive. If the patient is naturally fertile, she may conceive spontaneously and the need to use banked eggs may never arise, leaving a surplus of frozen eggs that will potentially be wasted.

A proposition would be to use OTC and transplantation as an alternative to social egg freezing. One of the valuable points that come with this method is that the woman has the opportunity to conceive spontaneously without IVF as more than half the pregnancies reported in the above section were conceived naturally [9]. Many women would appreciate this possibility as opposed to undergoing a more “artificial” method of conception.

If a woman is intrinsically fertile and is ultimately able to conceive on her own but has banked her ovarian tissue earlier as a back-up plan, the option of not letting the frozen ovarian cortex go to waste is a prospect she might want to consider. If not used for fertility, the potential of using the frozen ovarian tissue for postponement of menopause later on or avoiding premature ovarian insufficiency (POI) in life could be discussed with her. Of course, when a woman approaches a reproductive specialist at the initial consultation to discuss social fertility preservation, it will be overwhelming for her to have to entertain a deliberation on menopause and its implications as well. This, compounded with the thought of having to go under the knife to harvest ovarian tissue, may for some, be daunting and excessive. Thus, whether or not this supplementary knowledge should be brought up at the preliminary meeting with a woman seeking social fertility preservation remains debatable.

In certain groups of patients, ovarian tissue freezing may be a preferable or more suitable method over oocyte cryopreservation and another approach to the matter would be to stratify patients according to their ovarian reserve markers. The anti-Müllerian hormone

(AMH) level and ultrasonographic measurement of the antral follicle count (AFC) are frequently used in the field of assisted reproduction to predict response to controlled ovarian stimulation [17-19]. Those patients who have polycystic ovary syndrome and a high AFC and those who demonstrate good AMH levels may succeed in retrieving the ideal number of mature oocytes within one or two controlled ovarian stimulation cycles. On the contrary, those patients who are likely to exhibit a poor ovarian response and require more than two cycles of COS, which would be costly and time-consuming, may have a better outcome with OTC and reimplantation with or without COS.

In particular, women with a relatively poor ovarian reserve may need to undergo a number of stimulation cycles in order to collect enough oocytes to have a good chance of conceiving later on. For this group of patients, their future use of frozen ovarian tissue may be enhanced by *in vitro* activation. It is now possible to activate the pool of dormant primordial follicles, representing the ovarian reserve, *in vitro* prior to transplantation. Once the follicles have been activated during a short culture period in the laboratory, the tissue can be transplanted and the follicles left to develop within the ovarian tissue *in situ*. After three to six months, a substantial number of follicles are likely to develop and full maturation of the follicles may require exogenous administration of gonadotropins. This is expected to augment her chances of conceiving. The suitability of the method has already been shown by the birth of two healthy children in Japan, although these studies used this technique on patients who had already undergone POI [20,21].

Although there is growing data on the efficacy of ovarian tissue cryopreservation and reimplantation, the statistics on the short-term and long-term outcomes of the children born from this strategy are limited and it is likely that more information is required before this takes off as a major social fertility preservation method.

Ovarian Tissue Cryopreservation and Reimplantation for Induction of Puberty

In 2012, Poirot et al. [6] published the first case report showing that puberty could be induced by grafting cryopreserved ovarian tissue, prior to puberty. This was performed in a 13-year old girl who incurred POI after being diagnosed with sickle-cell disease requiring myeloablative treatment and haematopoietic stem-cell transplantation at the age of 10 years old. The second case report was in a 13-year old girl who had been diagnosed with Ewing sarcoma at the age of 9 years and developed POI following chemotherapy, surgical resection and radiotherapy [5]. In both cases, a unilateral oophorectomy was performed prior to gonadotoxic therapy and a small proportion of the frozen ovarian cortical fragments were transplanted to a heterotopic location in the former case and to the remaining ovary in the latter (3 out of 23 pieces in the case by Poirot et al. [6] and 2 out of 10 pieces in the report by Ernst et al. [5]). Puberty was successfully induced in both girls with thelarche, adrenarche and menarche achieved although the graft function failed after nearly 2 years.

Although these case reports illustrate that frozen/thawed ovarian tissue from pre-pubertal girls do have the capacity to provide endogenous sex steroid hormone levels adequate to induce puberty, this naturally steers one to question the value of this treatment – especially when puberty has traditionally been initiated with exogenous hormone therapy and when there are a finite irreplaceable number of frozen ovarian tissue fragments available which maybe fully capitalized on for fertility wishes later on. Andersen and co-workers stated that the pre-pubertal ovaries contain an abundance of follicles compared to the young woman in her twenties [5,22]. Furthermore, by using just a small fraction (20% in the report by Ernst et al. and 13% in the case of Poirot et al. [6]), this would be unlikely to compromise the girls' chances of conceiving later on in life because of the wealth of follicles in the pre-pubertal ovary [5,22].

However, the issue of whether induction of puberty with endogenous sex steroid therapy is superior over exogenous induction is a matter of debate. In 2013, Anderson et al. [23] alluded to the fact that the autograft could not mimic the events and physiological time sequence of normal puberty and that the graft lasted less than 2 years, which is a suboptimal time

frame for pubertal induction. Nonetheless, Andersen et al. [22] do not favour the use of endogenous sex steroids for puberty induction as the patient's ovarian function is restored. This would allow her to avoid taking exogenous steroids in the form of pills or patches at least temporarily and the estradiol levels produced are not exceedingly high. In young girls, one of their key concerns is to be able to feel "normal" and function similarly to their peers and this entails avoidance of medications for many of them.

In patients desiring fertility, the preferred location to transplant the thawed ovarian cortex would be in an orthotopic environment. However, if the demand is merely sex steroid function, a less invasive surgery can be accomplished by grafting the thawed ovarian tissue to a heterotopic site like the abdominal wall or arm, which would obviate the need for a general anaesthesia or a laparotomy. It is important to recognize, as Andersen and co-workers acknowledged, that the risk of reimplanting malignant cells still remains and thus, proper pre-operative counselling and risk assessment are of paramount importance [22].

Ovarian Tissue Cryopreservation to Postpone Menopause – A Boon or Bane?

At the other end of a woman's reproductive spectrum, menopause is the dreaded inevitability that many women face with trepidation. This inexorable eventuality has numerous consequences on the woman's psychology, health, and quality of life and its ramifications encompass the healthcare system, increasing its financial burden. In many developed countries where the life expectancy of women is 80 years or older, the demographic structure is changing towards an increasingly ageing population where women will spend 30% or more of their lives in menopause. Along with menopause come the repercussions of osteoporosis, a higher risk of cardiovascular disease, sexual dysfunction, reduced quality of life, increased vulnerability to depression and cognitive decline among others [24]. The encumbrance of osteoporosis is expensive and pervasive in developed nations – it affects about 75 million people in Europe, Japan and the United States and osteoporosis-related

fractures cost the latter 19 billion USD in 2005 [25,26]. Given the increasing life expectancy and fraction of the population above 60 years old, this enormous figure will only augment.

The exhaustion of follicles and diminution of sex steroid (particularly estrogen) production that occurs at the menopause removes the cardiovascular protection that estrogen has provided during the reproductive years. This cardioprotective effect of estrogen reduces the overall prevalence and age of onset of coronary heart disease for women compared to men [24]. Although there are acute symptoms associated with menopause like warm flushes, fatigue, and mood changes, these chronic diseases arise about 10 years after the menopause. Thus, if the onset of menopause could be delayed, a chance might exist to mitigate the consequences of these chronic diseases. Exogenous hormone replacement therapy (HRT) has been the conventional method to combat the acute symptoms associated with menopause. Other medications like Tibolone, Raloxifene and bisphosphonates are used for indications of sexual dysfunction and osteoporosis. Despite their benefits, the duration of HRT is limited to about 5 years due to the fear of marginally increased risks of breast cancer, cardiovascular disease and venous thromboembolism as established by the controversial Women's Health Initiative study [24]. Furthermore, most exogenous hormonal therapy or non-hormonal therapy is associated with side effects that may preclude women from being compliant to their medications. However, in terms of cost, HRT is relatively inexpensive and in hysterectomized patients, has the advantage of existing in estrogen-only preparations which are less perilous in terms of breast cancer risk.

Andersen and Kristensen [4] proposed a novel use of cryopreserved ovarian tissue – ovarian tissue frozen in youth could be transplanted back at the time of menopause to provide continued endogenous, physiological levels of steroid production. Although the ovaries contain plentiful follicles, majority are destined to undergo atresia. These could otherwise provide sex steroid function if they are deemed incompetent for reproduction thus maximizing the woman's pool of follicles and minimizing the number "wasted". Frozen/thawed ovarian tissue has been demonstrated to last

5 years depending on the follicular density and amount of tissue transplanted; in some cases, this duration can be prolonged to more than 5 years and with multiple transplants, tissue hormone activity can exceed 7 years [12]. For steroid production only and not for reproductive potential, the amount of tissue to be reimplanted needs only to be a small fraction of one ovary thus facilitating repeated transplants if needed. Of course, the woman would not remain in a pre-menopausal status for the rest of her life but this might potentially delay the menopause onset long enough to reduce the burden of osteoporosis, cardiovascular diseases, etc. The graft would also provide physiological levels of hormones which is the concentration the woman has been accustomed to all her life, thus minimizing the side effects that accompany exogenous HRT. For women who dislike taking pills or using patches, this may be a more welcome method as it utilizes their natural tissue.

Needless to say, this innovative idea would be swathed in controversy. Women may want to delay their menopause, but most would also not want continued menstrual cycles and none would entertain the thought of any increased risk of malignancies that may be associated with prolonged steroid hormone exposure. To have to undergo a surgery just to remove the ovarian cortex and then another one or even two, at a later stage to replace the cortical tissue can clearly be intimidating to a significant number of females – would many concur that the end (delaying menopause) justifies the means (multiple surgeries)? Some will be hesitant to remove an ovary or ovarian cortical tissue as to them, the corollary would be an earlier menopause. It is possible too, that by transplanting ovarian tissue that was frozen when the patient was younger, a naturally menopausal woman would now be fertile necessitating the use of contraception to avoid an unwanted pregnancy.

The uncertainties surrounding the above issues have not been clarified as one cannot extrapolate the findings of the Women's Health Initiative study and the risks with exogenous HRT to that of endogenous levels of HRT. Although it seems intuitive that the incidence of estrogen-dependent tumors like breast cancer and endometrial cancer would rise with prolonged albeit physiological levels of sex steroids, this remains to be proven. One must also recognize that should an

estrogen-dependent malignancy arise in a patient who has ovarian tissue transplanted, the graft which is the source of steroid production, can only be removed by surgery and hopefully, in its entirety. Among the above concerns, one can address the layman's apprehension of the menopause being brought forward after a unilateral oophorectomy. Two large studies have shown that although women who had undergone a unilateral oophorectomy did encounter menopause earlier than those with two ovaries *in situ*, the clinical difference was only 1 year earlier than natural menopause and one must also bear in mind that the age of menopause does vary across individuals as well [27,28]. Unless they had ovarian tissue already cryopreserved in excess, majority of women would be doubtful to subject themselves to an elective surgery purely for the reason of wanting to delay their menopause especially when the debates surrounding this have not been fully resolved. The option of cryopreserving ovarian tissue for reimplantation later on may be more feasible if the woman was undergoing pelvic surgery for another reason for example, a Caesarean section or an appendectomy where ovarian tissue harvesting could be performed as an adjunct. Needless to say, the cost-benefit ratio also remains to be established – whether traditional HRT followed by several years of Tibolone and then bisphosphonates have a lower cost-benefit ratio compared to the surgeries required for ovarian tissue cryopreservation and reimplantation(s) is unproven. The financial outlay involved in surgery and medications vary hugely among the different economic statuses of countries and healthcare models making it necessary to calculate the expenditure of each individually.

To some, providing an innate source of sex steroid hormone therapy to postpone menopause is an attractive, original option. To others, the use of cryopreserved ovarian tissue solely for this purpose is still a realm shrouded in ambiguity as the long-term safety, cost effectiveness and efficacy need to be explored and verified further. It is however, a meaningful topic to ponder on.

Conclusion

Ovarian tissue cryopreservation and transplantation

is becoming increasingly well-established as a method of fertility preservation in women with cancer, which is its foremost indication. As we realize that storing ovarian tissue preserves more than merely reproductive potential, the realm of its utility may expand to other beneficial applications in the future for example, postponing menopause, puberty induction and fertility preservation for social intentions. It is a meaningful venture to explore these possibilities. However, before these practices can be deemed advantageous and worthwhile, greater endeavors need to be undertaken to ascertain the cost-benefit and risk-benefit ratios and to determine their social acceptability.

Declaration of conflict of interest

The authors have no conflicts of interest to declare and have not received any funding in the production of the manuscript.

References

1. Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, et al. (2004) Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. *Lancet* 364: 1405-1410.
2. ASCO Guidelines (clinical tools and resources): Fertility preservation for patients with cancer. Clinical practice guideline update.
3. Jensen AK, Krostensen SG, Macklon KT, Jeppesen JV, Fedder J, et al. (2015) Outcomes of transplantations of cryopreserved ovarian tissue to 41 women in Denmark. *Hum Reprod* Oct 30: 2838-2845.
4. Andersen CY, Kristensen SG (2015) Novel use of ovarian follicular pool to postpone menopause and delay osteoporosis. *Reprod Biomed Online* 31: 128-131.
5. Ernst E, Kjærsgaard M, Birkebæk NH, Clausen N, Andersen CY (2013) Stimulation of puberty in a girl with chemo- and radiation therapy induced ovarian failure by transplantation of a small part of her frozen/thawed ovarian tissue. *Eur J Cancer* 49: 911-914.
6. Poirot C, Abirached F, Prades M, Coussieu C, Bernaudin F, et al. (2012) Induction of puberty by autograft of cryopreserved ovarian tissue. *Lancet* 379: 588.
7. Donnez J, Dolmans MM (2015) Ovarian cortex transplantation: 60 reported live births brings the success and worldwide expansion of the technique towards routine clinical practice. *J Assist Reprod Genet* Aug 32: 1167-1170.
8. Donnez J, Dolmans MM, Pellicer A, Diaz-Garcia C, Sanchez Serrano M, et al. (2013) Restoration of ovarian activity and pregnancy after transplantation of cryopreserved ovarian tissue: a review of 60 cases of reimplantation. *Fertil Steril* 99: 1503-1513.
9. Donnez J, Dolmans MM (2015) Ovarian tissue freezing: current status. *Curr Opin Obstet Gynecol* 27: 222-230.
10. Andersen CY (2015) Successes and challenges in fertility preservation after ovarian tissue grafting. *Lancet* 385: 1947.
11. Macklon KT, Jensen AK, Loft A, Ernst E, Andersen CY (2014) Treatment history and outcome of 24 deliveries worldwide after autotransplantation of cryopreserved ovarian tissue, including two new Danish deliveries years after autotransplantation. *J Assist Reprod Genet* 11: 1557-1564.
12. Andersen CY, Silber SJ, Bergholdt SH, Jorgensen JS, Ernst E (2012) Long-term duration of function of ovarian tissue transplants: case reports. *Reprod Biomed Online* 25: 128-132.
13. Cobo A, Meseguer M, Remohi J, Pellicer A (2010) Use of cryo-banked oocytes in an ovum donation programme: a prospective, randomized, controlled, clinical trial. *Hum Reprod* 25: 2239-2246.
14. Cobo A, Garcia-Velasco JA, Domingo J, Remohi J, Pellicer A, et al. (2013) Is vitrification of oocytes useful for fertility preservation for age-related fertility decline and in cancer patients? *Fertil Steril* 99: 1485-1495.
15. Rienzi L, Cobo A, Paffoni A, Scarduelli C, Capalbo A, et al. (2012) Consistent and predictable delivery rates after oocyte vitrification: an observational

- longitudinal cohort multicentric study. *Hum Reprod* 27: 1606-1612.
16. Vajta G, Rienzi L, Cobo A, Yovich J (2010) Embryo culture: can we perform better than nature? *Reprod Biomed Online* 20: 453-469.
 17. Broer SL, Dolleman M, Opmeer BC, Fauser BC, Mol BW, et al. (2011) AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: a meta-analysis. *Hum Reprod Update* 17: 46-54.
 18. Broer SL, Dolleman M, van Disseldorp J, Broeze KA, Opmeer BC, et al. (2013) Prediction of an excessive response in in vitro fertilization from patient characteristics and ovarian reserve tests and comparison in subgroups: an individual patient data meta-analysis. *Fertil Steril* 100: 420-429.
 19. La Marca A, Sunkara SK (2014) Individualization of controlled ovarian stimulation in IVF using ovarian reserve markers: from theory to practice. *Hum Reprod Update* 20: 124-140.
 20. Kawamura K, Cheng Y, Suzuki N, Deguchi M, Sato Y, et al. (2013) Hippo signalling disruption and Akt stimulation of ovarian follicles for infertility treatment. *Proc Natl Acad Sci USA* 110: 17474-17479.
 21. Suzuki N, Yoshioka N, Takae S, Sugishita Y, Tamura M, et al. (2015) Successful fertility preservation following ovarian tissue vitrification in patients with primary ovarian insufficiency. *Hum Reprod* 30: 608-615.
 22. Andersen CY, Ernst E, Kjærsgaard M, Birkebæk NH, Clausen N (2013) Response to the letter to editor from Richard A. Anderson and co-workers. *Eur J Cancer* 49: 2962-2963.
 23. Anderson RA, Hindmarsh PC, Wallace WH (2013) Letter to the editor: induction of puberty by autograft of cryopreserved ovarian tissue in a patient previously treated for Ewing sarcoma. *Eur J Cancer* 49: 2960-2961.
 24. Lobo RA, Davis SR, De Villiers TJ, Gompel A, Henderson VW, Hodis HN et al. (2014) Prevention of disease after menopause. *Climacteric* 17: 540-556.
 25. Gullberg B, Johnell O, Kanis JA (1977) World-wide projections for hip fracture. *Osteoporos Int* 7: 407-413.
 26. Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, et al. (2007) Incidence and economic burden of osteoporosis-related fractures in the United States, 2005–2025. *J Bone Miner Res* 22: 465-475.
 27. Bjelland EK, Wilkosz P, Tanbo TG, Eskild A (2014) Is unilateral oophorectomy associated with age at menopause? A population study (the HUNT2 Survey). *Hum Reprod* 29: 835-841.
 28. Yasui T, Hayashi K, Mizunuma H, Kubota T, Aso T, et al. (2012) Factors associated with premature ovarian failure, early menopause and earlier onset of menopause in Japanese women. *Maturitas* 72: 249-255.

Copyright: ©**Rauff et al.** This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.