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Fertility Preservation with Gonadotoxic Cancer Therapy

Review

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Abstract – Malignant haematological conditions have recognized an expanded rate and require aggressive medicines. Designated chemotherapy, accompanied or not by radiotherapy, raises the possibility overcoming the disease, yet cancer protocols frequently associate long term gonadal consequences, for example, reduced or damaged ovarian reserve. The negative consequence is directly proportional to the types, doses, time of administration of chemotherapy, and irradiation. Moreover, follicle damage relies upon characteristics of the disease and patient, like age, concomitant diseases, previous gynaecological conditions, and ovarian reserve. Patients ought to be enough educated while continuing to gonadotoxic treatments; consequently, fertility conservation ought to be in the long run viewed as a first-aim procedure. This procedure is most valuable when performed before the beginning of cancer treatment, with the recommendation for embryos' or oocytes' cryopreservation. If not possible or satisfactory, a few choices can be accessible during or after the cancer treatment. Albeit not supported by medical practice, promising outcomes after in vitro studies increment the possibilities of future patients to protect their fertility. This review means to accentuate the mechanism of action and effect of chemotherapy, particularly the one demonstrated to be gonadotoxic, upon ovarian reserve and future fertility. Reduced fertility or infertility, as long term outcomes of chemotherapy and, especially, following bone marrow transplantation, is frequently associated with a negative effect of recovery, social and personal life, as well as exceptionally diminished personal satisfaction.

Keywords – Fertility Preservation; Ovarian Reserve; Gonadotoxic Therapy; Cancer

I. INTRODUCTION

Malignant conditions, among them haematological ones, have recently demonstrated an expanded occurrence among young, reproductive age people. Leukaemia, lymphoma, breast disease, sarcoma, cervical malignant growth, and melanoma are among the most well-known malignant diseases that happen before the age of forty [1]. Onco-hematological conditions face the most aggressive treatment protocols, for example, bone marrow transplants, with terrible consequences over the reproductive tissue [2].

The impact of aggressive cancer treatment upon reproductive tissue is frequently not thought of while confronting a life threatening condition. Unfortunately, the destruction could be extensive and permanent because of the non-renewable characteristics of ovarian reserve. If not permanent, temporary infertility happens with high incidence [3]. The diminishing in follicle pool influences the window for the patient to procreate. Pregnancy, as well fertility preservation strategies, are not suggested within the initial two years following aggressive chemotherapy treatment, as bone marrow transplantation. For a patient with a diminished ovarian reserve, this measure of time decreases, significantly more, the open door to utilize the excess follicles. Beside the follicle influence, harm to the genital tract is likewise common. Extra adverse consequence ponders both general genital function and pregnancy outcome [4,5].

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Taking into account that chemotherapy, regardless of association with radiation, oftentimes prompts the massive or absolute destruction of the follicular pool, fertility preservation ought to stand these days on the priority list before, during, or following cancer protocol [6]. The best quality level in preserving fertility is embryo cryopreservation. Essentially oocyte preservation ought to be performed before the beginning of cancer treatment, however the time outline before gonadotoxic treatment [7]. The patient's condition and general health too address a challenge for the medical team. Different medications have been proposed to be integrated during cancer treatment to protect the ovarian reserve, yet research is still expected before human use [8,9].

This review means to raise the familiarity with the multidisciplinary medical team in regards to fertility consequences for cancer patients while administrating specific and proven gonadotoxic treatment. The negative impact upon the genital tract is challenging to survive following cancer treatment. Chemotherapy and irradiation are associated with permanent damage to the genital tract and follicle pool [10]. Mechanism of action is significant and drug-specific, sometimes challenging to both understand and prevent [5,11].

The psychological effect of childbearing interruption is in many cases found to have profound and extensive negative consequences results. It can weaken disease recovery, social reinsertion, personal and family life, as well as an extraordinary diminishing in personal satisfaction. Women will generally report a better acceptance of procreation issues when earlier educated by the medical team, rising the significance of proper medical care before the treatment onset [9,12].

1. The Impact of Cancer Treatment on Genital Tract

The ovarian tissue might be profoundly responsive to various types of drug. The follicular pool is predetermined, limited, and non-renewable and ought to be protected against external adverse impacts. Environmental factors influence fertility and genital function overall. Cancer treatment influences both, directly and indirectly, the ovarian tissue, endocrine, and fertility function [13,14].

2. Ovarian and Follicular Pool Characteristics

To have a better comprehension of cancer treatment's effect on gonads, it is significant to see the genital tract in general and to recognize the complexity of the ovarian tissue and its functions. Histologically, the ovarian cortical area holds the follicles, both growing and dormant. The surrounding stroma from the cortex is a mage of fibroblasts, which are profoundly sensitive to hormonal secretion, dissimilar to the fibroblasts from the remainder of the body. The medulla, the internal area of the ovary, keeps the vascular network [15,16].

Follicles form from the primordial germ cells in the intrauterine foetal period. The1000 germ cells migrate to the future gonadal area, enter initial rapid mitosis, followed by the first meiosis and arrest in a few steps of prophase. Forming the initial 5 to 7 million follicles after the fifth month of intrauterine life, simply 1 to 2 million are available afterbirth in light of a process of apoptosis and atresia [17]. The rest oocytes will be surrounded by a layer of somatic pre-granulosa cells, in this manner forming primordial follicles. Just high quality oocytes further develop and fertilize during adult life [18]. Communication and signalling between the oocytes and the outside granulosa cells, also as the regulation of the primordial follicle assembly in the fetal period, are dependable for this specific mechanism, as shown by recent studies. The follicular atresia goes on over the childhood and adult life, permitting just 400-500 follicles to change into mature follicles and ovulate [19].

Folliculogenesis experiences in the ovarian cortex, and continually there is an entirely reasonable balance among dormant and active follicles. Among the central processes that characterize the mechanism are the recruitment rand activation of the primordial follicles, the development and reaching the preantral stage, the selection over the antral stage, and then ovulation or atresia [20]. Folliculogenesis divides into two parts in view of hormonal sensitivity. The preantral part is gonadotropin-independent and described by oocyte growth, differentiation under the stimulus of local growth factors, cell and subcellular mechanisms. The second, gonadotropin-dependant antral stage, is described by the fast growth of the follicles under the feedback mechanism of FSH (Follicle-stimulating hormone), LH (luteinizing hormone), and gonadotropins. The onset of folliculogenesis begins with the recruitment of a primordial follicle orientated towards growth and differentiation [21].

This interaction takes around ten menstrual cycles or roughly 290 days for the follicle to arrive at the second stage and one year until the ovulatory stage. Actually, the ovarian function ought to be assessed one year after the cessation of gonadotoxic treatment, meaning the important time for the growing follicle pool to be totally renewed [22]. The recruitment of dormant follicle is affected by very sensible mechanisms [23]. The purpose is to keep the ovarian reserve with primordial follicle sparing and an

ideal harmony among inactive and active follicles. When the level of growing follicles diminishes, the recruitment is activated [24]. The dormant follicles are kept inactive through subcellular pathways that inhibit the activation process. The inhibiting mechanism can be inactivated by the lack of AMH (anti-müllerian hormone) secreting follicles, consequently relying upon serum AMH [25]. Furthermore, several medications, for example, alkylating agents regularly utilized in cancer treatment, directly inactivate the pathways, so the recruitment is accelerated [26-28].

A few pathways were described, like PI3K/PTEN/Akt, mTOR, and FOXO3A.Phosphatidylinositol-3-kinase (PI3K), phosphatase and tensin homolog (PTEN), protein kinase B (Atk), mammalian target of rapamycin (mTOR) [29,30], and fork head box classO 3a (FOXO3A) are among the major pathways and signalling methods associated with cell growth, proliferation, differentiation, metabolism, as well as apoptosis and stress management [23,31]. In the primordial follicle pool, those pathways are responsible for recruitment, growth, keeping a high proportion of the inactive follicle, and contributing to follicle growth, survival, development, and response to DNA aggression and damage [32]. The activities and functions of these pathways upon ordinary cells activity and cancer development were strongly examined. Other than keeping the homeostasis of the follicle, focusing on those inhibitory pathways [33] is the way to protecting the ovary during gonadotoxic chemotherapy, ovarian aging, and ovarian tissue transplantation [29,34]. During a gonadotoxic treatment, the negative effect follows up on the two variations. Right away, systemic administrated drugs supress the growing follicles, prompting a reduction in serum AMH. Following this, the rapid activation of follicular recruitment re-establishes the AMH serum concentration and balances the two types of follicular pools [35]. If Cyclophosphamide, an alkylating agent, is administrated, the inhibiting mechanisms are directly inactivated and the activated of dormant follicle speeds up, consequently exposing more follicles at risk. If the exposure to gonadotoxic treatment is prolonged, more follicles destroy, and at last, the burnout impact takes over the ovary, leaving no ovarian reserve [36,37].

2.1 Cell Apoptosis

The central mechanism responsible for cell death is apoptosis, otherwise called the interaction of programmed cell death. Most of external factors influence the structure of cell DNA and lead to chain ruptures. The altered cells will be removed through apoptosis, while the damage can't be restored [28]. This direct action is the impact of chemotherapy and irradiation on ovarian tissue. Active and growing follicles are at high risk during aggressive medicines, with massive cell modification and induced apoptosis, leading to temporary amenorrhea [38]. The more extended the treatment, the greater the follicles exposure what's more, subsequent death, followed by a fast diminishing in serum estrogen, AMH, and increased FSH [39,40].

2.2 Acute Vascular Toxicity

Stromal cell injury is one of the systemic consequences of administrating chemotherapy. Heterogeneous changes upon blood vessels, decrease in blood flow and volume, vascular spasm, architecture disturbances with vessels disintegration, as well as fibrosis would therefore appear [13,14]. Vascular toxicity on ovarian tissue for the most part influences the cortical region and is trailed by acute follicular ischemia of the growing follicles. Primordial follicles were believed to be protected against direct vascular toxicity in light of the fact that they don't depend upon blood supply like the growing ones [41]. Late investigations affirmed that the follicular pool is additionally sensitive to this destructive mechanism [42].

2.3 Ovarian Burnout

Ovarian burnout addresses the best example of indirect damage related with cancer treatment. The ovarian follicular pool is in a perfect homeostasis state. Both internal and external factors share to a dormant state that protects the ovarian reserve. The activation of the primordial follicle is very complicated [33]. Researchers have attempted to cover the underlying mechanism that prompts massive or even total follicle depletion during continuous and prolonged chemotherapy [43]. From the dormant state, a primordial follicle activates while expecting to partake in the growing pool. External factors like AMH serum concentration, known to be secreted by small growing follicles, additionally, contribute [37]. The dormant reserve isn't sensitive to varieties of FSH, LH, or serum estradiol concentrations but is responsive to subcellular pathways that keep them in a non-active state. The activation of the PI3K/PTEN/Akt pathway is essential for the oocyte reserve [44]. This pathway is inhibited by the AMH concentrations, with resulting activation of the mechanism in the absence of small AMH secreting follicles [45]. This stimulation interaction followed by ceaseless activation of the dormant follicles is relative to the steady growing follicle destructions because of prolonged chemotherapy. Diminished AMH serum concentration gives this feedback reaction. Similarly, some chemotherapy prescriptions, for example, alkylating agents, directly actuate the PI3K/PTEN/Akt pathway followed by follicle awakening and additional growing follicles [33,37]. Those would be sensitive to the direct activities of cancer treatment, with prominent damage

and subsequent need to enact dormant follicles to re-establish the primordial/growing follicle balance [32]. The two mechanisms are dose-dependent and aggregate, in the long run prompting primordial follicle complete depletion and related ovarian failure [46].

2.4 Oxidative Stress

It is notable and recently affirmed that antioxidants assume a significant role in follicles' survival. Cell oxidative stress is related with exposure to Cyclophosphamide (an alkylating specialist generally used to treat onco-hematological conditions) [43]. It is present both inside granulosa cells and follicles. The mechanism is related with increased oxygen-derived free radicals and diminished antioxidants, prompting apoptosis of the damaged cell [5,13,47].

3. Irradiation

Whether as a hematopoietic stem cell transplantation regimen (HSCT) or as a direct and, limited procedure on the genital tract, total body irradiation associates destroying impacts upon ovarian tissue [48]. Primordial follicles are highly sensitive to ionizing irradiation. The greater part of them can't restore the irradiation incited damage and will go through phagocytosis [49]. The impact is connected with cell development, and the more mature, the greater the damage induced. The remaining functional ovarian reserve relies upon age, dose, and number of radiation procedures. The cut off limit for long-lasting damage is 10 Gy, commonly reached during transplant protocol [50]. A dose of 4 Gy prompts damage in close to half of the ovarian reserve, while sterility lays out at 20 Gy in young women and just 6 Gy in over 40 patients. Despite the fact that the rate of destruction relies upon age, the regimen got after the age of 25 is exceptionally harmful paying little heed to dose or fractioning [47,51]. Beside follicle impact, irradiation likewise damages the uterus, with decreased distension of the cavity and thinning of the endometrium. Those changes are related with increased abortion rate, premature deliveries, and low for gestational age foetuses [52,53]. Other endocrine glands are additionally impacted because of complete body irradiation. Diminishes in gonadotropic hormones release, hyperprolactinemia, secondary infertility, and abnormal steroid hormones secretion are likewise results of irradiation of the hypothalamic and pituitary areas [54,55].

4-Fertility Preservation Guidelines

Anticancer therapies might have significant consequences upon the genital system through chemotherapy, associated or not with irradiation exposure [56]. Secondary amenorrhea and infertility, either temporary or permanent, are the main concerns regarding cancer treatment impacts [13]. The level of destruction relies upon the age, medical condition, treatment protocol, and required procedures, including types and dosages of agents, irradiation, and the clinical profile, prior and concomitant clinical history [39,57].

The remaining functional ovarian following gonadotoxic cancer treatment is difficult to measure, particularly in regards to future fertility and oocytes quality. Every patient ought to be extensively assessed and accurately informed prior to beginning cancer treatment [12]. Doctors should advice each woman about adverse and destructive impacts on genital, endocrine, and reproductive functions [58]. When available and requested by patients, fertility preservation procedures ought to be introduced and performed accordingly. The oncofertility specialist ought to assess and decide among types of fertility preservation procedures, as well as the appropriate ovarian stimulation protocol. Ultrasound characteristics, age, clinical condition and the menstrual cycle phase of the patient will decide the type and duration of the fertility preservation protocol [59]. Moreover, future fertility and procreation opportunity are firmly connected with the retrieved oocytes, both number and quality. The best outcomes are accomplished when the procedure is performed before cancer treatment, however now and again, this is not possible due to the disease, general conditions, or following risks [60,61].

4.1 Fertility Preservation before Cancer Treatment

However aggressive cancer treatment has demonstrated its advantages diminishing in general cancer related mortality and increasing the survival rate, the ovarian tissue long term results ought to be considered as malignancy's treatment. Expected fertility issues related to cancer treatment should be explained for patients, particularly when the required treatment is known to significantly affect reproductive function [48]. Preserving fertility with regards to cancer treatment has been shown to be a challenge for oncofertility specialists. It tends to be performed either by preserving oocytes, embryos, ovarian tissue, or transposition of the ovary. The available and suitable procedures rely upon different external and internal factors, as well as financial and logistic [6,62].

The gold standard in fertility preservation before cancer treatment is embryo' cryopreservation [8,9]. If embryos are not an option, mature oocytes ought to be focused on. Consent from partners and single patients are among the limits in regards to the chance of embryos' cryopreservation [63]. Ovarian biomarkers, like FSH, LH, AMH, and AFC (antral follicle count), assess the ovarian reserve [64-66]. The time frame before cancer treatment initiation is very narrow; subsequently, the ovarian phase ought to be thought about while choosing a protocol [67].

These days, fourteen days are adequate to stimulate and retrieve oocytes no matter what the ovarian cycle phase. Best case scenario, for future fertility is to get and collect a high number with good quality oocytes from the patient. Standard protocols rely upon spontaneous menstruations and include GnRH (gonadotropin-releasing hormone) agonists to lower the hyper stimulation syndrome, but generally involved stimulation protocols with regards to cancer emergency preservation presently use GnRH antagonists to diminish procedure duration [68]. Doses and type of stimulation medication are related to age and ovarian reserve and are associated to increase the number of resulting oocytes [69]. Following the procedure, oocytes could be frozen or fertilized with embryos cryopreservation [70]. In the event that no mature oocytes can be retrieved, immature oocytes could be obtained, and the procedure can be performed of the menstrual period. They will be subjected to in-vitro maturation procedures, as it happens when given hCG to small follicles or in the case of prepubertal patients. Unfortunately, the outcomes are considerably inferior to mature oocytes, considering that the maturation rate is around 50-60%, and the fertility rate reaches 60-70% [71].

In the event that cancer treatment can't be delayed to stimulate the ovary and retrieve oocytes, the patient can benefit from ovarian tissue cryopreservation [72]. This is the only available choice for prepubertal patients that would be exposed to gonadotoxic medication and patients with acute leukaemia [73]. Cryopreservation can be performed by harvesting the whole ovary, remembering that the ovarian cortical region contains primordial follicles. Harvesting will likewise incorporate immature oocytes, later to be subject to in vitro mature procedures. For functionality preservation, both the procedure and the grafts sizes are important. The actual recommendations are to slow freeze and fast unfreeze small ovarian tissue slices, either (8-10) mm, 5 mm or 2 mm [9,74]. This methodology has its limits, like the bad quality of the ovarian tissue but in addition relapses of cancer subsequent to presenting the graft in the ovarian area, particularly when the initial proposal was acute leukaemia [75,76]. Studies show that the ovarian graft function and life expectancy re-establish after roughly 4-6 months. The decline in serum FSH also, expanded estradiol affirm this. As to following the methodology, the positive outcome with pregnancies ascends to 50% after spontaneous menstrual onset [74].

While once again introducing graft isn't a choice, an artificial ovary might represent another option arrangement [77]. The method is promising, however up to this point, it has been utilized exclusively in lab mice models. This future procedure could bring down the risk of once again introducing cancer cells when auto-transplant the ovarian graft [78,79]. Transposition on the ovary into somewhere else inside the patient's body has its indications when pelvic irradiation is required [80]. Unfortunately, it doesn't protect when all the body irradiation or systemic gonadotoxic chemotherapy is part of the treatment regimen [75,81].

4.2 Fertility preservation procedures

At the 20th week of embryogenesis, women reach the maximal number of germ cells in the genital rides, with roughly 6-7 million potential oocytes, known as primordial follicles. Notwithstanding, just 1 million of these will remain at birth and just around 400,000 oocytes will survive at puberty. This number, known as the ovarian reserve, will diminish, arriving at 1,000 oocytes at the time of menopause, due to the around 450 monthly ovulatory cycles, during which process most oocytes go through atresia (degeneration and reabsorption) (82). The preservation of the ovarian reserve is important to maintain overall women' health, as it assumes a part in oocyte development and fertility, but in addition in other systems, as the cardiovascular system and bony system (83).

The degree of the depletion of the ovarian reserve varies between chemotherapy and radiotherapy. As respects chemotherapy, it differs relying upon the age of the patient (the younger the patient, the lesser the risk of ovarian failure), the chemotherapy agent utilized (alkylating agents being of great risk) and the duration of the treatment. Oocytes are exceptionally sensitive to radiation. Exposure to 20-30 Gy of radiation or total body radiation of 15 Gy lead to the loss of ovarian function [premature ovarian failure (POF)] (84).

The following procedures are available to women who will preserve their fertility before, or during chemo-and radiotherapy: Embryo cryopreservation, immature or mature oocyte cryopreservation, ovarian tissue cryopreservation (OTC) and ovarian transposition.

Other experimental methods, for example, the activation of ovarian follicles, in vitro follicle culture, artificial ovaries and novel fertoprotective agents, may give an impression of being promising, albeit further research is still required (85).

In males, the onset of production of spermatozoa starts at puberty and it is known as spermarche. In contrast to women, from the moment of the spermarche, spermatogenesis is maintained during the entire duration of a man's life, because of the spermatogonia type A (86). Testicular stem cells differentiate into spermatogonia, which will ultimately become spermatozoa under the process of spermatogenesis. Spermatogonia in the testicles are very sensitive to radiation, regardless of age. Leydig cells, are more sensitive to radiation before the onset of adolescence, while in adulthood, they become more resistant to radiation (87). Thus, adult patients might preserve Leydig cell function and testosterone production following radiotherapy despite being azoospermia. Moreover, if a population of spermatogonial stem cells (SSCs) stays after cancer treatment, as the impact is dose-dependent, the regeneration of spermatozoa may go on for years (88). Those at the most noteworthy risk of developing permanent sterility are children and adolescent with testicular cancer, leukaemia and Ewing's sarcoma. Sperm banking is the suggested FP procedure for males, albeit the cryopreservation of SSCs is likewise available.

4.2.1 Embryo cryopreservation

This technique has laid out success rates and is a broadly utilized and reliable method. It resembles an in vitro treatment (IVF) protocol, which has been performed for over of 30 years. Women go through growth. After 10-14 days, oocyte retrieval is performed, normally under conscious controlled ovarian stimulation (COS) with gonadotropin infusions to promote multifollicular sedation and with transvaginal ultrasound-directed needle aspiration (89). The oocytes are then fertilized in the laboratory and are cryopreserved for future use, usually in their blastocyst phase (82).

The disadvantages of this technique are chiefly three as follows: The need of a steady male partner, ethical issues with respect to embryo disposition and the time expected for ovarian stimulation. COS regularly starts during the early follicular stage. At the point when a patient is analysed in her initial follicular stage, ovarian stimulation with gonadotropin-releasing hormone (GnRH) antagonists begins immediately. Nonetheless, if the patient is in some other phase, the IVF standard conventions require the patient to hold on as long as 3 weeks before the cycle starts (89). Consequently, this strategy is certainly not a suitable choice for women whose aggressive cancer treatment is of highest priority, as the IVF standard protocols require the patient to wait up to 3 weeks before the process starts (89). It is additionally not suggested in women with hormone sensitive cancers and isn't feasible for prepubertal girls.

There are three principal cryopreservation procedures: Slow-freezing, super-fast and vitrification. Slow-freezing includes a step wise programmed decline in temperature (90), accomplishing a freezing harmony because of the exchange of the extra-and intracellular liquids without causing significant osmotic and deformity cell impacts. In any case, ice crystals can be shaped inside the cells, which can bring about very harmful impacts (91). The methodology is dependable (around 1 or 2 h) and requires costly instrumentation and large amounts of fluid nitrogen, among others. Vitrification changes over water into solid glass-like cells, avoiding ice crystal formation, both intracellular and extracellular (92). Expensive instrumentation isn't needed, and just several minutes are required. Moreover, a meta-analysis in 2013 revealed that the rates of oocyte survival, treatment and implantation where higher in vitrification than in slow freezing techniques (93). Hence, vitrification is these days the favoured method.

Information’s on pregnancy and live the both rates in cancer patients after frozen embryo

transfer is restricted. Live rates of birth in non-oncological patients <35 years amount to 38.7% per frozen embryo transfer and to 34,8% for oocyte donor cycles (94).

4.2.2 Oocyte cryopreservation

As an alternative to embryo cryopreservation, this strategy is the favoured choice for postpuberal and adolescent females, women without a stable partner, and for the people who don't wish to utilize a sperm donor. It overcomes the ethical and religious issues that rise out of the embryo preservation. Clinical results in the oocyte vitrification procedure are better than slow-freezing and thawing (95). With oocyte vitrification, women can conceive from now on and keep up with their reproductive autonomy. In any case, it isn't fitting for patients who are needing urgent therapy or patients with hormonal -sensitive cancers, as the procedure likewise incorporates COS. The oocytes can be cryopreserved as mature eggs or as immature germinal vesicle oocytes. Mature oocyte cryopreservation is performed with the oocytes whose development is ended in metaphase II. These days, this is the favoured strategy for postpuberal patients and for patients whose chemotherapy and radiotherapy can be deferred. Immature

oocytes got by aspiration and followed by in vitro maturation (IVM) strategies is a reasonable choice for prepubertal women and women with hormone sensitive cancers or with polycystic ovarian syndrome (PCOS), since COS isn't needed. This additionally permits the chance of immediate cancer treatment. Oocytes will be matured in vitro (through IVM) as the cryopreservation of mature oocytes has yielded preferable survival results over immature cryopreserved oocytes (96). The retrieval of immature oocytes can likewise be accomplished during an OTC procedure.

4.2.3 OTC

Albeit this strategy is as yet thought to be experimental, it is at present the main choice for paediatric patients and for patients with hormonal-dependant disease, as it is COS-independent and doesn't defer the oncological therapy. It doesn't need a male partner or a sperm donor.

OTC is an invasive procedure, as it requires general anaesthesia to surgically remove the ovarian tissue. This tissue, with a high content on follicles, is cryopreserved and can then be utilized concerning the following procedures: I) Orthotopic implantation (reimplantation into the pelvic cavity; e.g., staying ovarian tissue or peritoneum) or heterotopic implantation beyond the ovaries (e.g., rectum, pectoralis muscle, abdominal wall and chest wall); ii) isolation of follicles from the thawed tissue for in vitro growth, maturation and fertilization. During OTC, it is feasible to aspirate immature oocytes from antral follicles of the ovarian tissue. Isolated oocytes can be cryopreserved or matured in vitro (through IVM) for later vitrification (97).

Either ovarian cortical tissue cryopreservation (slow-freezing) or whole ovary cryopreservation can be performed. All egg-containing follicles are in the outer one-millimetre layer of the ovary, and subsequently the removal of this layer of tissue is adequate for cryopreservation. The achievement rate of live-birth after reimplantation is roughly 30% (85). The cryopreservation of the whole ovary stays a technical challenge because of the greater size of the tissue, which impedes a homogeneous and adequate scattering of cryoprotectant, and the vascular damage in type of ice crystals. Further studies are expected for this procedure to be utilized in standard clinical practice.

Up to 2015, 60 live birth cases had been accounted for with OTC in adult patients. In any case, the complete number of re-implantation acted in each center until that time was unknown; in this way, no success rates could be concluded (98). Before menarche, just one live birth following the auto grafting of cryopreserved tissue has been published (99), basically as far as we could possibly know. In 2015, Donnez et al (98) distributed a large case series (n=111) which uncovered a pregnancy rate extent of 29% (n=32). Two women delivered 3 babies each, proving the efficacy of the technique and the chance of conceiving naturally after just a single procedure.

The most worrisome concern of OTC is the chance of the re-introduction of carcinogenic cells into the cured patient or the ensuing malignant change of the ovarian tissue, which has been now detailed (100). Hence, a thorough examination of the ovarian tissue before cryopreservation and reimplantation is required.

4.2.4 Ovarian transposition (oophoropexy)

This procedure intends to prevent ovarian damage during radiation treatment by relocating the ovaries from the radiation field. Along these lines, it will be useful in women who will undergo pelvic or low abdominal radiation treatment without extra gonadotoxic chemotherapy (101). As per the radiation field outlined by the radiation oncologist, the surgeon will decide the optimal location in the abdominal wall for ovarian transposition. Altogether, the ovaries won't be harmed by the treatment and ovarian failure will be prevented. The strategy is regularly performed laparoscopically before the initiation of radiation. Success rates are not conclusive, as they vary from 16 to 90% (102).

4.2.5 Fertoprotective adjuvant agents

Another approach to preserving fertility is to protect the follicles during oncological treatment by administrating fertoprotective agents. One example is the utilization of GnRHa agonists, which are administrated 10 days preceding the beginning of the chemotherapy. GnRH analogues slow down the hypothalamic-pituitary-gonadal axis and inhibit the ovarian function by suppressing gonadotrophin levels to prepubertal levels (84). Two meta-examination of randomized preliminaries closed a diminished risk of POF in younge breast cancer patients (103,104), though its utilization was unclear in ovarian cancer and lymphoma (105). Another review exhibited no impact in young patients with lymphoma (105). The quality of the proof is

inadequate to reach significant determinations; high –quality studies are expected to look at the drawn out impacts of the utilization of GnRHa on premature ovarian insufficiency (POI).

4.2.6 Arising techniques

A. Activation of ovarian follicles

Cryopreserved ovarian tissue from prepubertal patients and patients with POF contains immature primordial follicles, which should be activated to start developing. This can be induced either in vivo [by interfering with the Hippo signalling pathway (106)] Or in vitro, preceding auto-transplantation, by activating the phosphatidylinositol 3-kinase (PI3K)/phosphatase and tensin homolog (PTEN)/protein kinase B (AKT)/Fork head box O3 (FOXO3) pathway, which manages primordial follicle activation in oocytes (106). This pathway likewise assumes a significant role in the follicle-stimulating hormone (FSH) stimulation of granulosa cell differentiation in antral follicles and in oocyte maturation of preovulatory follicles (106). This might be a promising fertility choice for prepubertal patients and patients with primary ovarian insufficiency, whose cryopreserved tissue contains immature primordial follicles suitable for this procedure. In vitro protocols including the PTEN/AKT pathway are being developed to expand the pool of viable activated follicles accessible for in vitro growth (IVG) procedures (107).

B. In vitro follicle culture

This method might be a possibility for patients who require urgent oncological treatment, and consequently are not good candidates for oocyte or embryo cryopreservation, for example, patients with acute leukaemia or acute myeloblastic leukaemia (AML). OTC is the accessible choice for these patients. In any case, the chance of re-seeding original cancer cells from the ovarian tissue exists, and in this manner different options should be raised. The ovarian follicle culture in vitro, expects to mitigate the risk of re-implantation malignant cells from the cryopreserved ovarian tissue. It is in this manner helpful in patients with cancers whose metastasis show up frequently in the ovary or patients with BRAC1 and BRAC2 mutations, because of the increased risk of an ovarian disease, which wouldn't make possible the transplantation of cryopreserved ovarian cortex (108). In any case, the complete maturation of early stage follicles has not been accomplished in humans yet (109).

In this procedures, individual follicles are isolated from the patient's bank tissue, which will subsequently be developed in vitro to turn into a functioning oocyte. These will be fertilized, and the embryo will be transferred to the uterus. The follicles can be cultured in two-layered (2D) or three-layered (3D) systems. These 3D culture methods are the best in keeping up with the sphericity and the communications between cells (108) and have additionally shown more prominent follicular activity, follicle and oocyte diameters and hormone production (84).

C. Artificial ovaries

The creation of an artificial ovary for transplantation is an extremely encouraging fertility re-establishing method. Isolated preantral follicles got from ovarian cryopreserved tissue, together with other ovarian cells in a 3D-matrix, or framework, bring about an ovary-like environment, which could permit the development of follicles and subsequently could re-establish both fertility and endocrine function of the ovary whenever they are transplanted (84). Luyckx et al (110) accomplished the survival and growth of murine ovarian follicles (primary, secondary and antral follicles) within one week following the transplantation of ovarian cells in a fibrin matrix. Besides, Laronda et al (111) achieved the commencement of puberty in ovariectomized mice following an artificial ovary transplant.

D. Explicit target tissue drugs

Both nanoparticles and fertopotective agents share the point of safeguarding ovarian cells during gonadotoxic oncological medicines:

I) Nanoparticles

This technique involves the encapsulation of the therapeutic agent to diminish its plasma clearance and consequently its toxicity. For such a reason, a Nano particulate formulation of the therapeutic agent is developed and encapsulated inside liposomal vesicles or 'Nano bins' (NB) (112). Ahn et al (113) showed a predominant antitumor efficacy of the Nano particulate formulation of arsenic trioxide (As2O3) in Nano bins [NB (Ni, As)] in a murine model of lymphoma as well as a decreased fertotoxicity.

ii) Fertoprotective agents

Current research focus around two distinct pathways: a) Anti-apoptotic agents, for example, imatinib, sphingosine-1-phosphatase (AS101), granulocyte colony stimulating factor (G-CSF), thyroid hormone (T3) and tamoxifen (107), and they have displayed to lessen follicle loss in animal models (114); and b) agents which prevent follicle activation, like AS101, an immunomodulator interacting with the PI3K/PTEN/AKT follicle activation pathway (115) and the anti- Mullerian hormone (114). In summary, various novel fertoprotectives agents to protect oocytes against gonadotoxic medicines are being investigated and might be available soon (84).

5. Fertility conservation procedures in males

In men going through gonadotoxic treatment, both sperm cryopreservation or testicular tissue cryopreservation are presently available (84,85). The American Society of Clinical Oncology (ASCO) rules suggest that oncologists inform about the risk regarding infertility in patients with cancer during their reproductive phases of life, as well as to refer them to experts in fertility treatment.

5.1 Cryopreservation of spermatozoa

The cryopreservation of semen is the recommended FP method for adult males and pubertal boys, who will go through gonadotoxic treatment (116). For patients getting radiation treatment just, gonadal shielding might be a choice in the event that sperm collection is not possible. The spermarche starts at puberty, however it isn't precisely known when this onset starts, since clinical parameters, for example, Tanner stage or increment on reproductive hormones, don't always associate with spermermatogenesis onset (117,118).

The strategy incorporates the collection of, preferably, of at least 3 semen tests, with a restraint time of somewhere around 48hrs in between tests, and the accompanying cryopreservation of the sperm samples, albeit frequently more than one semen sample should be taken around the same day to keep away from the oncological treatment delay (7). On account of ejaculation failure or when no spermatozoa are found in the ejaculate, sperm can be recovered by epididymal sperm aspiration, either percutaneous (PESA) or with microsurgery (MESA), testicular sperm extraction (TESE) or electro-discharge (119,120).

Assisted reproductive treatment like IVF and intracytoplasmic sperm infusion (ICSI) are thereafter required. ICSI has the advantage of likewise permitting reproduction when the semen is of exceptionally low quality or with a few spermatozoa (86).

The pregnancy rates change from 12% for intrauterine insemination to 32% for ICSI.To date, no subsequent information for large cohorts of children born after assisted reproductive therapy utilizing frozen-thawed sperm of men with cancer are available in the literature, to the best of our knowledge. It is worth focusing on that the European Germ Cancer Consensus Group and ASCO firmly suggest educating patients about the chance regarding cryopreservation methods prior to going through orchiectomy or gonadotoxical therapy (130). Unfortunately, such suggestions are regularly not followed by health care professionals, and numerous patients stay without directing regarding this situation.

5.2 Cryopreservation of SSCs in prepubertal children

Prepubertal children don't go through spermatogenesis yet, and thusly they don't have mature sperm in their testicles. Subsequently, the cryopreservation of spermatozoa is unimaginable. The main opportunities for them is to protect testicular tissue, which contains SSCs. In a similar way to the cryopreservation of ovarian tissue in women, the testicular tissue can be obtained (through a testicular biopsy) and cryopreserved in form of spermatogonia or in form of testicular tissue (utilizing slow-freeze or ultrarapid techniques). This will be from that point accessible to utilize when the patient is free from oncological disease and wants to have children. When the tissue is defrosted, it would permit in vitro spermatogenesis (121) or auto transplantation of the cryopreserved tissue, either by infusion of a cell suspension into the seminiferous tubules or intra testicular grafting of the tissue (86).

The renewed introduction of testicular stem cells into the seminiferous tissue could restart the sperm production (86). Orthotopic transplantation involves the risk of re-seeding malignant cancer cells (e.g., in patients with leukaemia), as occurs with ovarian auto transplantation. To mitigate the issue, a decontaminated cell suspension could be a potential arrangement (122). In vitro culture of testicular cells to acquire mature spermatozoa likewise bypasses the risk of reseeding malignant cells in the auto-transplant of testicular tissue, being one more part of research right now (123). It means a lot to pressure that fertility restoration

techniques via auto-transplantation of cryopreserved testicular tissue have not been tried at this point for safe clinical use in humans and consequently it is as yet viewed as exploratory (85). More research is as yet required with respect to the utilization of frozen-defrosted tissue to acquire mature spermatozoa in vitro (88).

6. Fertility Preservation during Cancer Treatment

Malignant haematological conditions frequently require immediate treatment to save the patient's life, abandoning fertility preservation [124]. The fourteen days required for cryopreservation of oocytes or embryos could once in a while not be accessible for some patients, despite the fact that there is the urgency of treatment onset or the characteristics of the disease, as is the situation for leukaemia. In the case of acute leukaemia, the only accessible choice is ovarian tissue cryopreservation. Regarding this medical condition, it is known that both deferring the treatment and auto transplantation of the ovarian graft isn't in many cases a choice [56]. In all those cases, when the treatment has started, choices narrow particularly in light of the fact that of the negative effect of chemotherapy on growing follicles. Many studies and researchers authenticate that fertility protection can be accomplished during gonadotoxic medicines [125].

GnRH is presumably the most utilized and referred to, yet the results are not as expected. It is the first drug to be utilized to preserve fertility during cancer treatment, and the first studies began in 1981. An animal study on affirmed the gonadal protection of GhRH agonists administration during Cyclophosphamide treatment. likewise affirmed superior gonadal protection of prepubertal patients compared with adults, however followed by various researchers that revealed conflicting data, a large portion of them confirming follicular assurance during gonadotoxic cancer treatment. GnRH mechanism of action isn't exactly known, obviously both direct and indirect on the ovarian function [126]. Their protection relies upon the medical condition and type of chemotherapy. Inducing a menopausal state with low FSH and hypoestrogenemia, we should keep in mind that follicle activation isn't reliant upon those ovarian biomarkers [127]. There is a beneficial impact related to the menopausal state induced with mild protection of the follicle pool, particularly following a four weeks of treatment when the AMH has expanded by 30% after an initial decrease. Another protective effect related to GnRH administration is the decrease in ovarian perfusion and exposure to chemotherapy [68]. Lowering the blood flow to the ovarian tissue, prompts local ischemia and additional damage [128]. Another repressing mechanism related with GnRH is accepted to be upon follicular subcellular activation pathways, like PI3K/Akt/mTOR [68]. This pathway may some way or another prevent ovarian burnout, but clinical experience has not given at this point sufficient experience [129,130].

However not proven to be valuable for the ovarian reserve, GnRH administration protects patients from vaginal bleeding with regards to cancer treatment that frequently relates thrombocytopenia [131]. Chemotherapy decreases follicle pool through numerous and complex mechanisms, influencing primarily growing follicles and inducing local fibrosis. Considering that mature follicles are difficult to be protected, the main concern of the clinical staff is to protect the dormant reserve. Primordial follicles are activated and recruited utilizing subcellular activation pathways that might rely upon the serum AMH, as proven already [132]. Taking into account that AMH harms those pathways, the lower the serum concentration, the less negative impact on those mechanisms. AMH secreting follicles are damaged by gonadotoxic treatment, and primordial follicles are recruited to keep the active/dormant follicle balance [32]. Researchers have developed a technique to protect oocyte reserve by administrating recombinant AMH during cancer treatment [133]. This drug has been demonstrated advantageous in offering protection for future fertility in vitro, even in mix with Cyclophosphamide, which is known to be the most aggressive gonadotoxic agent [44].

The in vivo strategy has its constraints however, essentially on the grounds that alkylating agents affect the PI3K pathway, trailed by its activation and start of the follicle recruitment. Administrating recombinant AMH doesn't block the process of recruitment by all mechanisms included, some being active and primordial follicle activated despite serum AMH [128,134]. Another disadvantage is the restricted bioavailability of the medicine that proved to be undetectable17 h after administration. Other than many lab clinical studies that have demonstrated protection on ovarian reserve, more research is expected prior to presenting the drug in cancer protocol for human subjects [132,133].

The requirement for fertility protection during gonadotoxic drug drove the researchers to assess one more treatment for some time later [135]. AS101, otherwise called tricolour ammonium tellurate, is a non-poisonous immunomodulator frequently utilized in cancer treatment [30,40]. Other than other general impacts, the drug inhibits PI3K/PTEN/Akt activation pathway and is liable for follicular activation, keeping the ovarian reserve at a higher level looked at to previous drug. Studies have affirmed the protective activity of AS101 in association with Cyclophosphamide [45,136]. Also, serum concentrations of AMH were estimated during the

treatment, and they were affirmed to be higher while on AS101treatment, showing that both growing and dormant follicles are protected. Those in vitro studies on still need extra research before human use [37,134].

7. Fertility Preservation after Cancer Treatment

It is challenging to evaluate ovarian capacity function gonadotoxic medicines. There is

no standardization in regards to ovarian insufficiency or premature ovarian failure following cessation of cancer treatment. Different clinical preliminaries have attempted to make an assessment method to anticipate the recovery or premature failure of endocrine ovarian function, as well as fertility [68]. The common ovarian biomarkers, like FSH, estradiol, AMH, and ultrasound follicle count (AFC), can superficially orientate us in regards to present ovarian function [137]. Age is likely one of the most important factors in terms of future fertility on the grounds that the younger the patient, the higher the number and quality of the remaining oocyte [74]. It is additionally significant the moment of assessment for both paracrine biomarkers and ultrasound findings. The ovarian function ought to be assessed after at least six months following the end of treatment. This is related with how much time that is expected for a follicle to be activated from the ovarian pool and afterward to accomplish a growing, ultrasound assessment state. It is viewed as that 290 days are expected for a primordial follicle to turn into a secondary follicle and one year to reach to the ovulatory state. Hence, no spontaneous menstruation within a year is often associated with permanent ovarian damage and induced menopause [39]. All patients with gonadotoxic treatment for disease, with transitory ovarian damage, experience premature menopause [138]. The difference relies upon the number of remaining primordial follicles. The level of the primordial follicle destruction could anticipate the time before the onset of menopause, however the ovarian biomarkers are found to have low serum concentrations. The time period for fertility is restricted, in any event, for patients who re-established their menstruations [139].

Fertility is firmly related to the remaining ovarian reserve, but post-cancer treatment medication could moreover influence the both oocytes quality and the patient's chance of procreation. Furthermore, we ought to remember that the best oocytes are basically to be utilized, and those with the poorest quality are the ones that remain. While tending to ovarian reserve, AMH serum concentrations can be useful, but the quality of the remaining follicles is by a long shot the most significant factor with respect to fertility and future pregnancies [140]. Following aggressive cancer treatment, choices are restricted and not promising. Oocytes or on the other hand embryo cryopreservation could be considered for a patient with a small ovarian reserve, not before a year following cancer drugs or procedures cessation [141]. The system isn't similar women without gonadotoxic medicine exposure. [142] An ovary exposed to chemotherapy has a lower response to ovarian stimulation, as well as a lower number and a poor quality of resulting oocytes [75,143]. Another perspective that ought to be considered is the general damage of the genital system related with cancer treatment exposure. The effect of chemotherapy and irradiation is reflected in the whole genital system. Impede of the vascular system, the architecture, temporary atrophy, particularly related with irridiation, also influence the number of pregnancies and the outcome, significantly lowering the resulting full-term live births [53]. If poor quality or no oocytes are accessible, patients could go through egg donnation 'In vitro' preparation (IVF). This is a substantial choice for women presented to disease treatment, despite the fact that reviews detailed that patients seldom spoke to this technique compared with the normal population [144,145].

II. Conclusion

Oncological medical care is nowadays a long way from being exclusively the solely the cure of cancer. Providing hope for future fertility following oncological therapy, significantly increases the quality of life of the patients and assists them with adapting emotionally with cancer (30). FP in both female and male oncological patients is now days conceivable and ought to be coordinated as a component of the oncological health care. Various methods exist and the most fitting ought to be picked relying upon the characteristics of the patients: Male, female, prepubertal or post pubertal. Some of these have previously demonstrated successful results while others, newer and more imaginative, are still needing further improvement and development.

Sperm banking is presently viewed as the primary line FP choice for male patients; oocytes vitrification is as of now thought to be the main line choice for postpuberal female patients in which it is feasible to postpone chemotherapy and hormonal stimulation is approved. Embryo banking gets in moral struggle with regards to preserving fertility, as health care's point is to exclusively preserve the women's fertility, which is the motivation behind why it isn't viewed as the first-line therapy any longer. Moreover, developing proof of safety and efficiency progress in oocyte vitrification, maintains this procedure to be the favoured one. While confronting a therapeutic emergency, or contraindication for hormonal stimulation exists, OTC or puncture of immature oocytes

are accessible. Immature oocytes will then be cryopreserved, directly or after being matured in vitro, to be vitrified as full mature oocytes or as embryo after a fertilization technique.

Abbreviations

AKT	protein kinase B
As_2O_3	arsenic trioxide
ASCO	American Society of Clinical Oncology
AYAs	adolescent and young adults
COS	controlled ovarian stimulation
ESGO	European Society of Gynaecological Oncology
FEPNC	Spanish Federation of Parents of Children with Cancer
FOXO3	Fork head box O3
FP	fertility preservation
G-CSF	granulocyte colony-stimulating factor
GnRH	gonadotropin-releasing hormone
IVF	*in vitro* fertilization
IVM	*in vitro* maturation
ICSI	intracytoplasmic sperm injection
INCIP	International Network on Cancer, Infertility and Pregnancy
NB	Nano bins
OTC	ovarian tissue cryopreservation
PCOS	polycystic ovarian syndrome
PGD	pre-implantation genetic diagnosis

PI3K phosphatidylinositol 3-kinase

POF premature ovarian failure

POI primary ovarian insufficiency

PTEN phosphatase and tensin homolog

SSCs spermatogonial stem cells (SSCs)

Conflict Of Interest

All authors declare no conflicts of interest.

Authors Contribution

Authors have equally participated and shared every item of the work.

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