Ovarian function and fertility preservation for young people treated for cancer
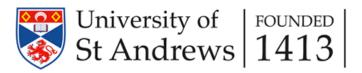
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INTRODUCTION

With further advances on technology and science, our understanding of the pathologies of cancer has helped design better treatment notably in chemotherapy and radiotherapy. However, although these treatments may help destroy the tumour, some can be gonadotoxic. In depth knowledge of gonadal physiology and function has encouraged researchers to design methods to protect the reproductive system from cancer-induced toxicity.

The aim of this chapter is to gain a better understanding of ovarian function. The two main roles of the ovary are to produce an oocyte which is essential for fertilisation and to secrete female sex steroids, oestrogen and progesterone. Although this chapter separates the two functions, they are intertwined and depend on one another as endocrinology stimulates oocyte release and ensures the formation of one dominant follicle, thus, maintaining the ovarian reserve. Moreover, this chapter explains how different cancer treatment concentrating on chemotherapy and radiation, disrupts normal ovarian function. As a follow up from gonadotoxic insult, this chapter concludes with the most recent approaches and techniques on preserving fertility for cancer patients enabling them to have the opportunity to have a family.

Concerning relevance, this chapter is important for the audience, regardless of medical background, to understand and raise awareness on the gonadotoxicity of cancer treatment and the approaches that have been investigated to save a patient's fecundity. In a clinical environment, this chapter emphasises to doctors the value of communicating with the patient and their family regarding the gonadotoxic risk of cancer treatment and explain the different fertility preservation techniques and their criteria.

OVARIAN FUNCTION

The ovary has two predominant functions; to produce oocytes and to secrete the key hormones oestrogen and progesterone. These two functions go hand-in-hand, as steroidogenesis regulates the oocyte release.

Formation of oocytes

One of the main ovarian functions is to form oocytes for fertilisation. Formation of oocyte only occurs *in utero*, thus, when born, girls have a set number of oocytes that will support them throughout reproductive age.

Pre-natal and pre-pubertal

In females, primordial germ cells, are known as oogonia, migrate to the gonadal ridge where the ovaries are formed. During the migration, the oogonium undergo continuous proliferation and upon reaching the ovaries, they stop dividing and form nests of germline cysts. These nests breakdown and the individual germ cells form associations with the somatic cells that are present in the area. This individual unit, known as a primordial follicle, comprises of a primary oocyte surrounded by a single layer of granulosa cells. As soon as follicles are formed, they start to grow but do not ovulate. The oocyte then starts meiosis but undergoes meiotic arrest in prophase I. During this arrest, the primary oocyte undergoes developmental competence, acquiring the ability to support a viable embryo. This arrest places constant strains on the oocyte increasing its susceptibility to DNA damage. Meiotic resumption occurs at ovulation. The nest breakdown is followed by a wave of oocyte atresia, indicating that the majority of the oocytes produced die before birth. Each

female has a fixed number of primordial follicles prior to birth which will last her reproductive lifespan.

Post- natal

When a girl reaches puberty (ovulation) her ovarian reserve, the population of primordial follicles, is much smaller than during gestation. Meiotic resumption in the oocyte occurs during ovulation, releasing the first polar body which is disregarded. After ovulation, the oocyte undergoes a second meiotic arrest. Only if fertilisation occurs will meiosis resume, resulting in a zygote and second polar body. Atresia mechanism continues after birth throughout a female's reproductive lifespan for example, the selection of a dominant follicle results in a wave of cell death, all of which further decreases the number of possible oocytes that can be successfully fertilised.

Importance of the Ovarian reserve

The ovarian reserve can predict ovarian function as it reflects the number and quality of follicles at a specific time. During oogenesis, the only time proliferation occurs is during gastrulation. This results in a set number of primary oocytes which will form a woman's ovarian reserve lasting her reproductive lifespan, contrasting to spermatogenesis. There is a consensus that this pool of follicles declines with age, as follicles will undergo atresia or ovulate. Therefore, given that the ovarian reserve represents their fertility, older women have a reduced chance of successful pregnancy.

In a clinical environment, premature ovarian insufficiency (POI) occurs when there is an early depletion of the ovarian reserve. Many insults notably cancer treatment can cause this early reduction, rendering a female infertile at an early age hence undergoing early menopause. The number of primordial follicles that undergo atresia at the time of the insult such as cancer treatment, will determine the number of follicles that survive, suggesting that the younger the woman at the time of treatment, more follicles will survive and the later the menopause. Therefore, it remains crucial to protect the ovarian reserve from any damage.

The main biomarkers for ovarian reserve are Follicle Stimulating Hormone (FSH) and Anti-Müllerian Hormone (AMH). AMH, secreted by the granulosa cells of the growing follicles, inhibits primordial follicular activation proving to be an accurate representation of the pool (Brougham et al., 2012). During infancy, AMH levels are high, plateauing during early adulthood and eventually is inversely correlated with increasing age (Brougham et al., 2012). Therefore, women with depletion of ovarian reserve would have low serum measurements of AMH. These biomarkers have been of particular use in cancer treatments, in order to analyse the effects of treatment on the ovaries.

Concerning chemotherapy, AMH can also be used as a predictor of fertility prior to treatment to analyse how many primordial follicles would survive treatment. AMH serum levels would indicate ongoing ovarian function following treatment. Similarly, high FSH levels in post-pubertal women accurately represents ovarian failure (Brougham et al., 2012) However, for pre-pubertal girls there is still no validated biomarker representing the ovarian reserve. FSH is not a reliable marker, in pre-pubertal girls undergoing treatment because its levels do not change throughout cancer treatment and after completion (Brougham et al., 2012). Similarly, it is uncertain if AMH is an accurate representation of ovarian reserve in pre-pubertal children as there is an increase follicular recruitment, hence, the levels of AMH would fluctuate prior to puberty (Wallace et al., 2013).

Hormonal secretion

Alongside oocyte production, hormonal secretion is equally as paramount and is controlled by the Hypothalamic Pituitary Gonadal axis. Pituitary secretions in the reproductive axis play a role in folliculogenesis and ovulation. The ovary itself secretes sex steroids which act locally on endometrial tissue in preparation for pregnancy as well as impacting systemically on both the hypothalamus and pituitary gland. The reproductive axis remains relatively quiescent until puberty and after it is active throughout reproductive age.

HPG axis

Hormonal secretion of the ovary is governed by the Hypothalamic Pituitary Gonadal (HPG) axis. This tightly regulated cycle is important for the selection of a dominant follicle for ovulation and preparing the endometrium for possible implantation. The hypothalamus releases Gonadotrophin releasing hormone (GnRH) which acts on the pituitary triggering the secretion of Luteinising hormone (LH) and Follicle stimulating hormone (FSH). These latter hormones act directly on the ovary to produce oestrogen: In the ovary, theca cells, directly stimulated by LH, produce androgens that travel to the granulosa cells. Under the influence of FSH, granulosa cells convert androgens to oestrogens via the enzyme aromatase and produce inhibin B. The ovary communicates to the pituitary and the hypothalamus via negative and positive feedback loops. Both oestrogen and inhibin B suppress the release of hormones from the pituitary and hypothalamus thus controlling the reproductive axis. However, above a certain threshold, oestrogen can also hyper-activate the HPG axis, stimulating the release of more GnRH and LH. This unique mechanism only applies to females and occurs at a specific point during the menstrual cycle. Consequently, there is a co-dependency of FSH and LH, unlike in males, to produce oestrogen. This female sex steroid is fundamental for ovulation and endometrium proliferation.

Mini Puberty & Puberty

Mini puberty

When the girl is 3-6 months old, the HPG axis becomes activated, an event known as the "mini puberty" (Lanciotti et al., 2018). The role of the mini puberty is less known in females compared to males, nevertheless, it is speculated that this activation stimulates the development of breast tissue (Lanciotti et al., 2018). During this event, FSH levels remain high, presumably to stimulate folliculogenesis whilst LH gradually decreases (Lanciotti et al., 2018). Although the mechanism is unknown, the HPG axis will subsequently switch off and remain quiescent until puberty.

Puberty

Puberty in girls is controlled by Leptin, a hormone secreted by adipose fat cells. During puberty, the HPG axis is activated and matures overnight releasing GnRH and LH in a pulsatile manner. These hormones will trigger menarche, indicating that a girl has started her menstrual cycle. Alongside menses, oestrogen will trigger secondary sex characteristics.

Adulthood

After puberty, the menstrual cycle is continuous throughout adulthood until menopause. This complex cycle shifts from preparing the endometrium for possible implantation each month to shedding and menses if pregnancy does not occur.

The hormones from the HPG axis influence folliculogenesis, oocyte development and stimulate ovulation.

Menstrual cycle

The first stage of the menstrual cycle, follicular phase, begins on the first day of menses. The fall of the corpus luteum, when a woman is not pregnant, and inhibin B lifts the suppression of the HPG axis, allowing the secretion of FSH. There is then complex network of signals notably FSH and AMH that act to either activate, suppress or maintain primordial follicles. Although not the predominant activator, FSH plays a role in supporting primordial follicular growth. Upon activation, primordial follicles start to grow and eventually will divide into two somatic cells, granulosa and theca cells.

Whilst growing, some of these follicles will be "dominant" and developmentally more mature, thus will contain more FSH receptors rendering them more sensitive to the influx of this hormone. These dominant follicles become antral follicles and the formation of two subtypes of granulosa cells occurs, cumulus and mural. Mural granulosa cells express LH receptors, therefore, they start to synthesize oestrogen and inhibin B. Both oestrogen and inhibin B suppress FSH secretion via the HPG axis triggering the subordinate follicles to undergo atresia as they purely rely on FSH for survival. This mechanism is key to ensure that only the dominant follicles will be ovulated not the entire pool of primordial follicles. Further maturation of the dominant follicle leads to the formation of the final follicular stage before ovulation, Graafian follicles.

Whilst the Graafian follicles continue to grow, they become more sensitive to LH as both granulosa and theca cells have more LH receptors. Because of this, the Graafian follicles secrete increasing amounts of oestrogen. This causes oestrogen to switch to a positive feedback effect on the HPG axis, leading a rapid increase of GnRH secretion resulting in a LH surge causing ovulation. The physiology of ovulation is as follows; the ovulatory stigma weakens the follicular wall, resulting in the breakdown of the connective tissue and the rupture of the follicular wall, causing the release of the oocyte. The oocyte then is picked up by the fimbria of the fallopian tube and transported via ciliary action to the oviduct where it waits for sperm.

The remnants of the follicle increase in vasculature and begin to form the corpus luteum, a process known as luteinisation. Luteinisation causes a switch in the production of sex steroids, shifting from oestrogen to predominantly progesterone. The corpus luteum produces progesterone which is the essential hormone for maintaining pregnancy. Progesterone changes the endometrium microenvironment, switching from a proliferative to secretory state. This stabilisation marks the ideal environment for implantation. Moreover, progesterone also relaxes the myometrium and suppresses maternal immune response in order to prevent the uterus rejecting the zygote if fertilisation occurs. If there is no fertilisation, luteolysis occurs, the corpus luteum degrades and the fall of progesterone results in endometrial shedding, thus, menses. Due to the reduction in inhibin B, the suppression on HPG axis is lifted leading to an increase in FSH secretion and the cycle is repeated.

Menopause

With each menstrual cycle, the pool of primordial follicles gets smaller, reducing the chances of getting pregnant. Eventually, when the ovarian reserve is too little, a woman enters menopause. The average age of menopause in the United Kingdom is 51. Menopause is defined as amenorrhoea for more than 12 months. The absence of menses is due to

insufficient amounts of oocytes to continue the normal ovarian cycle. Given that no follicles are growing oestrogen synthesis cannot occur. Therefore, the HPG axis remains activated and continues to secrete FSH but follicles no longer respond to FSH. Lack of oestrogen can have secondary causes outside of the reproductive system notably the brain and bones, increasing the risk of osteoporosis.

Conclusion

Given that the physiology and endocrine functions are dependent on each other, impairment in one compartment will negatively affect ovarian function as a whole. This cascade will impact a patient's fertility. Therefore, co-dependency makes the ovary susceptible to destruction. It has been recognised that cancer treatment, regardless of specific therapy, may induce damage to ovarian function.

HOW CANCER TREATMENT AFFECTS THE FEMALE REPRODUCTIVE SYSTEM

There is a consensus that cancer treatment, both chemotherapy and radiation, can be gonadotoxic. It can impair the reproductive endocrine axis which then impacts ovarian function but, equally, treatment can also directly affect the ovary resulting in early depletion of ovarian reserve and cell death.

Cancer therapy affecting the HPG

Ovarian function is dependent on the HPG axis. The HPG axis is the main trigger for puberty, hence, irradiation in any part of the axis can delay onset of puberty (Müller et al., 2003). Radiation may be required for adenoma tumours in the pituitary trigger excessive hormonal secretion which impacts ovarian function, leading to accelerated follicular growth. If the pituitary gland is affected, FSH and LH will no longer be secreted disrupting folliculogenesis and ovulation. Acquiring radiation near the hypothalamus will impair its ability to secrete GnRH which cascades into affecting ovarian function. Finally, radiotherapy may affect any components of the HPG axis causing hypogonadotropic hypogonadism (Müller et al., 2003). Given that the components of the axis work together and are intertwined, disruption in one area of the axis will have negative consequences on the ovaries which in turn, has great impacts on their function.

Cancer therapy affecting the ovary

Alongside the HPG axis, cancer therapy can also directly affect the ovary. Both ovarian architecture and function can be negatively impacted resulting in early depletion of ovarian reserve and a reduced fertility window. Chemotherapy is one of the most common cancer treatments. Although not all chemotherapeutic drugs are gonadotoxic, alkylating agents such as cisplatin reduce the pool of primordial follicles. For example, Cyclophosphamide, an alkylating agent, suppresses ovarian function by directly accelerating follicular growth (Spears et al., 2019). In addition, this drug also induces greater oocyte apoptosis as it rapidly stimulates DNA breaks in the ovary (Spears et al., 2019). Alongside ovarian function, Cyclophosphamide can also affect the architecture, notably vascular damage which, in turn, will negatively impact the health and function of the follicles (Spears et al., 2019). Consequently, chemotherapy proves to be extremely gonadotoxic having both

direct and indirect impacts on the ovarian reserve by accelerating folliculogenesis or increasing apoptosis.

Similar to chemotherapy, radiation directly on the ovaries induces premature reproductive ageing and premature amenorrhoea. Radiotherapy induces DNA breaks in the oocyte which, as a result, they either undergo DNA repair mechanisms or apoptosis (Anderson et al., 2015). Radio-sensitivity of the oocyte depends on its growth stage as it is more resistant to radiation when being quiescent in the primordial follicles. Radiation to the ovary can also affect the somatic cells, particularly when the granulosa cells mature and develop during folliculogenesis.

Consequently, cancer treatments can directly affect primordial follicles leading to reduced ovarian reserve. Therapy can indirectly damage follicles that are growing which causes increased recruitment of primordial follicles to replace the damaged dominant follicle. However, cancer therapy can also affect different cell types notably the oocyte itself by inducing DNA breaks and the surrounding somatic cells, both of which ultimately leads to cell death.

Different types of cancer treatment causing gonadotoxicity

Multiple cancer treatments have negative effects on the reproductive system. The majority of the gonadotoxic drugs utilised will have similar risk assessments in both males and females. In this context, risk for females is defined as the chances of developing premature ovarian insufficiency (POI), thus, reduced fertility window and early menopause. Table 1 highlights the different classifications of risks based upon percentage of early ovarian depletion. Table 2 show the different types of cancer treatment that can be gonadotoxic and the risk assessment in females.

Risk	Percentage (%)
Low	<10
Medium	10-60
High	60-80
Very High	>80

Table 1 showing the classifications in percentage of the risk assessment. Adapted from Oncofertility Consensus Document

	Cancer Subtype	Drugs that are gonadotoxic	Estimated Gonadotoxicity Risk in Female
LEUKAEMIA	Acute Lymphoblastic Leukaemia (First Line)	Cyclophosphamide or Ifosfamide	LOW - MEDIUM
	Acute Lymphoblastic Leukaemia (Relapse)	Cyclophosphamide Ovarian Irradiation	MEDIUM – HIGH VERY HIGH
	Acute Myeloid Leukaemia	Nil	LOW
	Acute Myeloid Leukaemia (Relapse)	Nil	LOW

LYMPHOMA	Non-Hodgkins Lymphoma (low risk)	Cyclophosphamide	LOW
	Non-Hodgkins Lymphoma (standard risk)	Cyclophosphamide	HIGH
	Non-Hodgkins Lymphoma (high risk)	Cyclophosphamide	HIGH
	T-Cell Non-Hodgkins Lymphoma	Cyclophosphamide	LOW - MEDIUM
	B-Cell Non-Hodgkins Lymphoma	Cyclophosphamide	MEDIUM –HIGH
	High Risk B cell	Cyclophosphamide	MEDIUM-VERY HIGH
	Hodgkins Lymphoma	Cyclophosphamide +/- Dacarbazine	LOW - MEDIUM
		Cyclophosphamide + Dacarbazine + Procarbazine	VERY HIGH
BRAIN TUMOURS	Ependymoma	Cyclophosphamide + Cisplatin	HIGH – VERY HIGH
	Embryonal tumours	Cyclophosphamide + Cisplatin +/- Lomustime	VERY HIGH
	Pineoblastoma	Cyclophosphamide	MEDIUM
	Atypical Teratoid/Rhabdoid Tumour	Cyclophosphamide + Ifosfamide	MEDIUM
		Cyclophosphamide + Ifosfamide + Thiotepa (NO RADIOTHERAPY)	VERY HIGH
	High grade Glioma	Temozolomide	HIGH
	Intracranial Germ Cell Tumour	Ifosfamide	LOW
		Ifosfamide Cisplatin	HIGH – VERY HIGH
BONE AND SOFT	Ewings Sarcoma	Ifosfamide +/- Cyclophosphamide Busulphan Melphalan	VERY HIGH

	Osteogenic Sarcoma	Cisplatin +/- Ifosfamide	HIGH - VERY HIGH
	Soft Tissue Sarcoma (low risk)	Nil	LOW
	Soft Tissue Sarcoma (standard risk)	Ifosfamide	HIGH – VERY HIGH
	Soft Tissue Sarcoma (high risk)	Ifosfamide +/- Cyclophosphamide	VERY HIGH
	MMT	Ifosfamide +/- Cyclophosphamide	VERY HIGH
	Synovial Sarcoma	Ifosfamide	HIGH –VERY HIGH
	'Adult-Type' Soft Tissue Sarcoma	Ifosfamide	VERY HIGH
	Neuroblastoma (low risk)	Cyclophosphamide +/- Cisplatin	MEDIUM
	Neuroblastoma (intermediate risk)	Cyclophosphamide +/- Cisplatin	HIGH
	Neuroblastoma (high risk)	Cyclophosphamide Cisplatin Busulphan Melphalan	VERY HIGH
WILMS TUMOUR	Wilms Tumour (low risk)	Nil	LOW
	Wilms Tumour (high risk/metastatic)	Cyclophosphamide + Pelvic Radiotherapy	HIGH – VERY HIGH
	Wilms Tumour (relapse)	Cyclohosphamide +/- Melphalan	HIGH- VERY HIGH
OTHERS	Hepatoblastoma	Nil	LOW
		Cisplatin +/- Carboplatin	HIGH – VERY HIGH
	Retinoblastoma	Nil	LOW
	Langerhans Cell Histiocytosis	Nil	LOW
		Fludarabine + Melphalan	HIGH
	Extra-Cranial Germ cell Tumour	Nil	LOW

		Cisplatin +/- Ifosfamide Vinblastine	HIGH – VERY HIGH
BMT	Allogenic Bone-Marrow Transplant	Cyclophosphamide Busluphan/ Melphalan/ Treosulphan	MEDIUM –HIGH
	Allogenic Bone-Marrow Transplant	Total Body Irradiation	VERY HIGH
	Allogenic Bone-Marrow Transplant	Fludarabine	LOW

Table 2 showing the different types of cancer followed by their respective treatment and the degree of gonadotoxicity in females. Adapted from Oncofertility Consensus Document

Therefore, from Table 2, there is a consensus that many different types of cancer treatment can be extremely toxic to the ovary, these patients have a high chance of having POI and a reduced fertility window if measures are not being taken prior to treatment. It is important to note that specific gonadal cancer will have direct effects on the ovary regardless of whether the treatment is gonadotoxic. Therefore, the consequences on the ovary depend on the type of cancer and which drug treatment is given.

As depicted in Table 2, most chemotherapeutic drugs present show a dose dependent gonadotoxicity risk in females. The effect of chemotherapy on the gonads has been confirmed by Chow et al. (2016) who compared live birth rates of childhood cancer survivors and their siblings who were used as a control. The rates of live births for the survivors are lower but follow a similar pattern as the control group. This supports the statement that chemotherapy induces damage to the ovary, however, if treatment occurs during childhood, the ovarian function will be replenished to a certain extent throughout adulthood. Moreover, chemotherapeutic drugs can be gonadotoxic alone but in combination with other drugs can cause a greater risk of premature ovarian insufficiency, highlighted in Table 2.

Furthermore, Table 2 is more predominantly centralised around chemotherapy agents. However, in some cases where radiation is required, the risk of POI is very high. This demonstrates that using radiation as a cancer treatment could potentially be more gonadotoxic than chemotherapy. Although it is established that early onset menopause is a long-term consequence of treatment for Hodkins Lymphoma, the risk of premature menopause and ovarian failure significantly increases when using a combination of chemotherapeutic agents and pelvic radiation (Swerdlow AJ et al., 2014). This suggests that radiation greatly raises the risk of early depletion of ovarian reserve.

Another factor to take into consideration upon analysing Table 2 is that risk assessment can be influenced by a variety of environmental factors notably age. Exposure to radiation at a younger age will have negative effects during adulthood. The uteri of patients who have undergone radiation will be smaller with an absent endometrium and poor blood flow, all of which affects patients' fertility (Bath LE et al., 1999). On the contrary, patients treated with chemotherapy show that the younger the patient is, the higher the chance they have at recovering full ovarian function and maintain a normal fertility window (Letouneau et al., 2012). This was further shown in Chow et al. (2016) comparison since the

fertility pattern was similar to the control suggesting that there is an age-specific correlation between the chemotherapeutic insult and the number of primordial follicles that survived.

Conclusion

Consequently, cancer treatment both radiation and chemotherapy prove to be extremely gonadotoxic. Counselling is paramount as undergoing cancer therapy in general is emotionally traumatic. Doctors need to support their patients, but most importantly, prior to treatment they need to discuss the age-specific impact treatment has on the ovaries as well as the potential techniques for fertility preservation. This is essential in order to restore ovarian function after treatment.

Fertility Preservation

Fertility preservation has been an emerging field of research. It is paramount that doctors speak to their patients about preserving their fertility prior to treatment regardless of the type of treatment. There are different approaches to fertility preservation all of which have specific criteria that need to be fulfilled. Although most of these techniques focus on post-pubertal women, more recent methods give pre-pubertal girls the opportunity to preserve their fertility for future use.

Ovarian shielding and Transposition

Non-pharmacologic approaches to protect ovarian structure and function have been used in the clinical environment for many decades. However, these techniques require specific criteria as it can only be utilised for females undergoing pelvic radiation. Nevertheless, if the approaches are executed correctly, they can successfully protect the ovary. One example of this appoarch is ovarian shielding which comprises of shielding the ovary during radiotherapy which protects them from damage. However, the ability to use this technique depends on the location of the tumour. If the tumour resides in approximation to the ovary, then shielding will not be possible. Moreover, accurate placing of the lead shield is essential for the ovary to be fully protected and well-covered by the lead, therefore, requiring precision and accuracy (Anderson et al., 2015). Improving radiotherapy machinery and techniques to enable specific target radiation would prevent damage to neighbouring cells, notably the ovaries (Anderson et al., 2015). Consequently, this technique is only effective to a certain extent as it is dependent on many variables.

Alongside ovarian shielding, moving the ovaries outside of the pelvic area, a process known as ovarian transposition, would also prevent radiation-induced damage to the ovarian function. This laparoscopic surgery is done prior to cancer treatment, however, can be painful and delay treatment. Again, this fertility preservation technique can only be applied to women undergoing pelvic radiation. Nevertheless, this procedure is successful and in one case study, after treatment, a woman had normal menstrual cycles and their hormones were within the normal range (Faber et al., 2005).

GnRH analogues

A more pharmaceutical approach of fertility preservation is to manipulate the HPG axis, suppressing ovarian function with GnRH analogues. The philosophy behind this medical concept is to inhibit GnRH and create a pre-pubertal hormonal environment, preventing early depletion of ovarian reserve. In this fashion, the adult ovary would mirror a pre-pubertal ovary, increasing the chances of successful fertility preservation. GnRH analogues have been proven effective in women undergoing chemotherapy for breast cancer (Halle et

al., 2015). Only 8% of the women with the analogue had ovarian failure compared to 22% in the chemotherapy only group (Halle et al., 2015). The differences highlight the effectiveness of GnRH analogues as a way of protecting the ovarian function during chemotherapy treatment. Concerning successful pregnancy outcome, more offspring were born to women in the GnRH analogue compared to the chemotherapy-alone group (21% versus 11%), proving that ovarian function is protected and fecundity level remains similar to pre-treatment (Halle et al., 2015). A key advantage of this technique is that it is accessible and cost-effective. However, GnRH analogues do cause menopausal-like symptoms, which can cause great discomfort.

Cryopreservation

Cryopreservation is a process that freezes organelles using vitrification as the main procedure because it is fast and inexpensive compared to the former procedure, slow freezing (Jang et al., 2017). However, the risk for malignant contamination is greater in vitrification than slow-freezing, thus, the procedure for cryopreservation would depend on the type of cancer. Cryopreservation can lead to long term preservation of cells notably oocytes and embryos. However, these latter approaches are only eligible for post-pubertal women, therefore, an emerging idea is ovarian tissue cryopreservation in order to preserve fertility in pre-pubertal girls.

Embryo freezing

The most historical technique of cryopreservation is embryo freezing. Ovaries are hyper-stimulated in order to increase the number of ovulatory mature eggs. These are collected and fertilised with partner or donor sperm to produce an embryo. The embryo is then vitrified for future use after the cancer treatment. However, ethical, religious and social issues are associated with embryo freezing. Additionally, given that a sperm is required to freeze an embryo, there is shared ownership and both biological parents need to consent in order to use this embryo (Anderson et al., 2015). Therefore, given the criteria, this technique may be useful for couples that are in a relationship or single women who are willing to use sperm donor. This dependency on a partner or donor restricts women's reproductive autonomy which may not be appealing to all.

Egg Freezing

Given the limitations and disadvantages of embryo freezing, a new technique, egg or oocyte freezing was introduced which offered women the opportunity to preserve both their fertility and reproductive autonomy as they would have sole responsibility of their egg freezing. Given the methodology of this technique, only post-pubertal women are allowed to cryopreserve their oocytes. The availability of this approach also depends on the advancement of cancer as it postpones chemotherapy by a few weeks which may not necessarily be possible if the cancer is advanced.

Oocyte cryopreservation comprises of daily gonadotrophin injections to stimulate multiple follicles to grow. The oocytes are retrieved and frozen prior to cancer treatment. However, this methodology has a few limitations. Firstly, vitrification negatively affects the oocyte physiology as it may induce osmotic stress (Jang et al., 2017). Secondly, hyper-activation of the HPG axis may risk the patient developing Ovarian Hyper-Stimulation Syndrome. This complication is due to the build-up of human chorionic gonadotropin causing ovarian inflammation.

After treatment, artificial reproductive technologies must be used for pregnancy with cryopreserved eggs which may not appeal to all women as these procedures may be expensive and are both emotionally and physically difficult. Data on cancer patients undergoing oocyte cryopreservation remains limited. However, Druckenmiller et al. (2016) vitrified oocytes from women with malignant cancers and they had similar fertility rates as non-cancer patients concluding that long term freezing or cancer does not impair on oocyte quality or function. The aforementioned case study is just one of a few, therefore, the majority of the data concerning egg cryopreservation is carried out with infertile non-oncologic women. The main determinant for successful fertilisation is oocyte quality, therefore, Cobo et al. (2008) compared the quality of fresh egg and thawed/ vitrified egg. There was no difference in fertilisation, embryo quality, and implantation between the two groups proving to be an effective way of preserving fertility and potentially allowing survivors to have a family after treatment (Cobo et al., 2008).

Tissue freezing
The two cryopreservation techniques aforementioned are only applicable to post-pubertal women, however, the techniques to preserve fertility in pre-pubertal girls remain unexplored. Moreover, patients who are unable to have hormonal stimulation or egg retrieval have no option for fertility preservation. Gathering from these limitations, onco-fertility have focussed on cryopreserving ovarian tissue in order to give an opportunity for patients to preserve their fertility regardless of age. The practice involves taking 3-5 ovarian cortical strips, removing approximately 70% of the ovarian cortex, cut into fragments and cryopreserved for future use (Wallace et al., 2014). Cancer treatment can start soon after this process with minimum delay. After cancer therapy, the ovarian tissue is transplanted back into the patient or undergoes *in vitro* maturation.

A wide window of patients can be eligible for this fertility preservation technique, however, to narrow this window down criteria known as the Edinburgh selection criteria, has been established. For example, patients under the age of 35 and who are at high risk of premature ovarian insufficiency, more than 50%, are eligible for this technique (Wallace et al., 2014). Additionally, the assessment of the eligibility of a patient to undergo ovarian tissue cryopreservation is based on both intrinsic, patient herself and health state, and extrinsic, estimated success rate of the treatment and time available, factors (Wallace et al., 2014). A retrospective study validated the selection criteria as the pre-pubertal girls that were offered to undergo ovarian tissue cryopreservation, 35% of them had developed premature ovarian insufficiency (Wallace et al., 2014).

Transplantation of frozen ovarian tissue can lead to normal restoration of hormonal patterns and the menstrual cycle within 20 weeks in patients who had cancer treatment (Anderson C.Y et al., 2008). Additionally, 2 live birth were recorded from these patients with transplanted ovarian tissue, proving to be a successful technique C.Y Anderson C.Y et al., (2008). To further confirm this, a recent report investigated 74 women, aged 34 years who were diagnosed with breast cancer and Hodgkin Lymphoma in Denmark (der Ven et al., 2016). These women were presented with ovarian tissue cryopreservation as their method of fertility preservation. One year after the transplant, 62% had evidence of normal ovarian function and 27.5% achieved a natural pregnancy (der Ven et al., 2016). This highlights that the likely successes of full restoration following transplantation, restoring both hormonal and fertility aspects of the ovary. However, the consequences of ovarian tissue cryopreservation are not fully established, urging researchers to conduct follow up data on

these patients to investigate the long term effects. Given that in the latter case study, pregnancy was achieved by natural insemination is an appealing characteristic that ovarian tissue cryopreservation presents in comparison to oocyte or embryo freezing.

Consequently, this technique is proven effective in adults, however, one of the main and initial aims was to devise a method to preserve fertility for children undergoing cancer treatment. Ovarian tissue freezing in pre-pubertal girls is still experimental. Having said that, Demeestere et al. (2015) reported the first live birth from a woman who underwent ovarian tissue cryopreservation at the age of 13 due to sickle cell anaemia. A few months after transplantation, ovarian activity was present with a normal menstrual cycle and a spontaneous pregnancy occurred 4 years following treatment (Demeestere et al., 2015). Although her diagnosis was not cancer, it still provides the possibility of using pre-pubertal cryopreservation as a fertility preservation technique. Additionally, it highlights that this method is successful in achieving a pregnancy and transplantation restores ovarian function. Whilst pregnancy would suggest normal ovarian function, the ovary is an endocrine organ as well, therefore, induction of puberty would also indicate full restoration of the ovary. A 9-year-old girl diagnosed with Ewing sarcoma underwent ovarian tissue cryopreservation prior to treatment (Ernst et al., 2013). This tissue was transplanted back into the patient and after 4 months, FSH levels were low and oestradiol increased (Ernst et al., 2013). The following year after transplantation, the patient had menarche and a regular menstrual cycle indicating that the thawed tissue had restored normal ovarian function (Ernst et al., 2013). Consequently, this demonstrates that cryopreservation of ovarian tissue can restore both ovarian functions.

Although this approach may be promising for fertility preservation in pre-pubertal girls, there is, however, a key risk that the ovarian tissue may contain malignant cells, hence, there is the possibility of transplanting the tumour back into the patient after treatment. This is of particular risk in malignant and blood-borne cancers notably leukaemia. This was shown in a clinical case study in which a 7-year-old patient with vertebral Ewing sarcoma underwent tissue cryopreservation prior to treatment (Anderson et al., 2017). The cortex of the removed tissue had no indication of malignancy, however, the ovarian tissue did, in fact, contain cancerous cells (Anderson et al., 2017). This precluded to discarding the ovarian tissue and discussing other fertility preservation techniques (Anderson et al., 2017). Whilst this technique is proven to be promising, its success rate depends on the malignancy of the cancer. Consequently, this case study proves that meticulous and extreme vigilance is required to detect any contamination in the ovarian tissue.

Artificial ovary and in vitro growth

With ovarian tissue cryopreservation comes the risk of malignancy contamination in the ovary rendering that tissue sample impossible to use. Much of onco-fertility research investigates how to bypass this limitation focussing on making an artificial ovary, free of contamination with the cryopreserved tissue. The follicles are removed from the ovarian cortex, isolated, cleansed from malignant cells and mixed within a matrix. This matrix would be transplanted back into the patient instead of the ovarian tissue, however, there is still the risk of malignancy.

Pioneer approaches have looked at growing in vitro a mature oocyte from the cryopreserved tissue. This oocyte would be of good quality, can be fertilised and support healthy embryos. In this way, cancer patients would have non-contaminated oocytes. However, this has been difficult as devising the correct environment in a culture dish is key

to induce primordial follicle and oocyte growth given that they communicate to each other via signals activating and/or suppressing development. Full *in vitro* growth from primordial oocyte to an ovulatory oocyte which can be fertilised has only be successful with mice (Anderson RA et al., 2014). *In vitro* oocyte growth has been achieved in humans with the oocyte reaching metaphase II, equivalent to an ovulated oocyte (McLaughlin et al., 2018). However, morphological changes notably in the size of the polar body suggests that the culture conditions were mal-established, further highlighting the importance of oocyte-somatic communications for successful growth (McLaughlin et al., 2018). This is reflected in another experiment as *in vitro* activation of primordial follicles resulted in poor oocyte quality (McLaughlin et al., 2014)

The main application of this technique would be for pre-pubertal girls as if their cryopreserved ovarian tissue contains malignant cells, they have no options for fertility preservation. However, this approach needs to be further investigated as Anderson RA et al. (2013) took biopsies from pre-pubertal girls and compared them with adult ovaries. Pre-pubertal ovaries are extremely different as they contain structures forming neuron nerve cells, blood vessels and have abnormal follicles all of which are absent in an adult ovary (Anderson RA et al., 2013). When pre-pubertal ovarian tissues were placed in culture, it was discovered that they grew differently depending on the age of the ovarian tissue (Anderson RA et al., 2013). Therefore, age impacts the ability of the oocyte and follicles to grow *in vitro* which complicates the process of using this technique clinically and implies that the culture system needs to be adapted for the type of ovarian tissue. Although this is still experimental, further research is required to understand these complex intricacies and upon understanding, in the future, *in vitro* growth could give hope for pre-pubertal girls to preserve their fertility even though they are diagnosed with malignant cancers.

Conclusion

There are a range of fertility preservation techniques available for females compared to males. These approaches, however, tend to focus on post-pubertal women and require specific criteria, notably location of the tumour and a woman's relationship status. Embryo cryopreservation is effective, however, the shared ownership of this embryo may pose problems if the woman is not in a stable relationship with the man's sperm she used or not wanting to use a sperm donor. Egg freezing enables more reproductive autonomy and although majority of the data was carried in non-oncologic patients, the live birth rates with oocyte cryopreservation are promising and hopeful. However, further studies are required to investigate a potential relationship between cancer and egg cryopreservation. This theme, the urgent requirement for further research is applicable throughout all fertility preservation techniques. It is especially important in the emerging field of ovarian tissue cryopreservation which gives pre-pubertal girls an opportunity to save their ovaries. However, the risk of malignant contamination has led to *in vitro* growth. *In vitro* growth enables pre-pubertal girls to preserve their fertility regardless of which cancer type they have, however, this in humans has not yet been achieved.

Concluding statement

This chapter discussed the two key functions of the ovary, production of an oocyte and hormonal secretion, generated by the reproductive axis, which work together to render a woman fertile. Given the co-dependency, disruptions in either compartments will inevitably affect the other causing complications in ovarian function and fertility. Cancer treatment-induced damage, regardless of whether it is a gonadotoxic drug or radiation, impairs normal ovarian function.

Our understanding of these gonadotoxic treatments has shaped the philosophy behind fertility preservation as protecting the ovarian reserve is more important than treating it after cancer therapy-induced destruction. The initial fertility preservation techniques, shielding, transposition and pharmaceutical manipulation, protect the pool of primordial follicles but are only suitable for a narrow window of patients. This has motivated researchers to investigate into cryopreservation of both embryos and oocytes. More recently, a pioneer technique of ovarian tissue cryopreservation gave the chance of pre-pubertal girls to preserve their fertility for the future. There is constant evolution within fertility preservation mechanisms as researchers are now focussing on *in vitro* growth of an oocyte, one that is of good quality, able to be fertilised and develop a viable embryo. Further investigations are still required as *in vitro* oocyte maturation can only go to a certain stage and activating primordial follicles in culture leads to a poor quality oocyte. Having said that, this evolution enables pre-pubertal girls undergoing cancer treatment due to malignant cancer to have an equal opportunity to preserve their fertility, reassuring both the patient and parents that it would be possible to restore ovarian function in the future.