Chapter 1

General introduction

General introduction

In context of the fact that the likelihood of survival after cancer treatment among young female cancer patients has increased considerably during the last decades, quality of life issues after treatment, has drawn more attention. Since cancer affects an increasing proportion of women during their fertile lifespan (approximately 3% of all Dutch cancer victims are younger than 40 years), fertility preservation inherently becomes an important issue with regard to the patients future quality of life.[1, 2]

Fertility preservation is even more important since the age for attaining the first pregnancy has increased in many countries. As a consequence, the proportion of women diagnosed with cancer who may have a children's wish after treatment, is increasing. Given the fact that many cancer treatment regimens will lead to iatrogenic ovarian insufficiency and infertility, measures to preserve fertility prior to the administration of these gonadotoxic cancer treatments are relevant for an increasing number of women. Apart from the inability to conceive, premature ovarian failure leads to other implications for women's health and quality of life, including climacteric symptoms such as hot flashes and night sweats, increased risks of osteoporosis, depression, cardiovascular disease and cognitive and sexual dysfunction.[3-5]

As outlined, preservation of ovarian function and fertility preservation is in daily practice of increasing importance in these patients. To understand the principles of fertility preservation, we will first comprehend the anatomy and physiology of the ovary, followed by the way cancer treatment threatens the ovarian function. Thereafter background of the options to preserve fertility are highlighted. This allows to introduce the specific methods of preservation of ovarian function and/or fertility prior to the administration of the scheduled gonadotoxic treatments. Once these techniques have been explicated, the research questions that we aimed to answer in this thesis will be formulated.

Anatomy and physiology of the ovary

The ovary, positioned in the pelvic area of a woman, consists of an inner layer, the medulla and an outer layer, the cortex (Figure 1). The medulla contains loose connective tissue, nerves and blood and lymphatic vessels and the ovarian cortical stroma constitutes the environment in which the oocytes reside. Moreover this is where essential events of early follicle development occur.

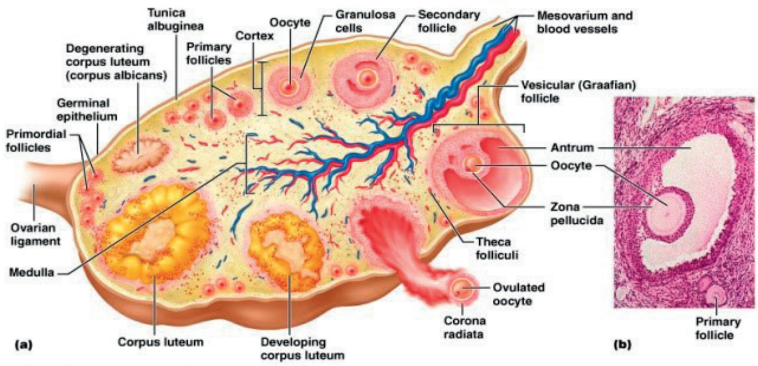

Figure 1

The ovary contributes to the development of the oocytes, called the process of oogenesis and harbors all resting primordial follicles (Figure 1). During oogenesis, follicles will enter different stages classified as: primordial follicles (the earliest stage) containing an oocyte surrounded by a single layer of flattened granulosa cells; primary follicles surrounded by at least one cuboidal layer of granulosa cells; secondary follicles with more than one layer of cuboidal granulosa cells and theca externa and interna cells which originate from ovarian stroma; tertiary follicles when a fluid filled antrum inside the granulosa cells followed by the preovulatory follicles.[(6)] In humans, the duration of the transition from a primordial into a preovulatory follicles is more than 200 days and from primary to preovulatory respectively 80 days.[(7)]

Oogenesis is a limited event which ceases during fetal development with a finite number of oocytes present at birth. At about 20 weeks in utero, a peak number of oogonia of approximately 6–7 million is present. During the years, most of the follicles are lost, with an drop of number by birth to about 1-2 million, at puberty only 200,000-500,000 oocytes will remain, with less than 1000 at menopause (Figure 2). [(8-10)]

The loss of oocytes is mediated by aging but also factors such as genetics or iatrogenic causes like smoking, high body mass index, parity and stress are described. Moreover, and of interest in the light of fertility preservation, gonadotoxic therapy can be an important cause of the loss of oocytes.[(11-18)]

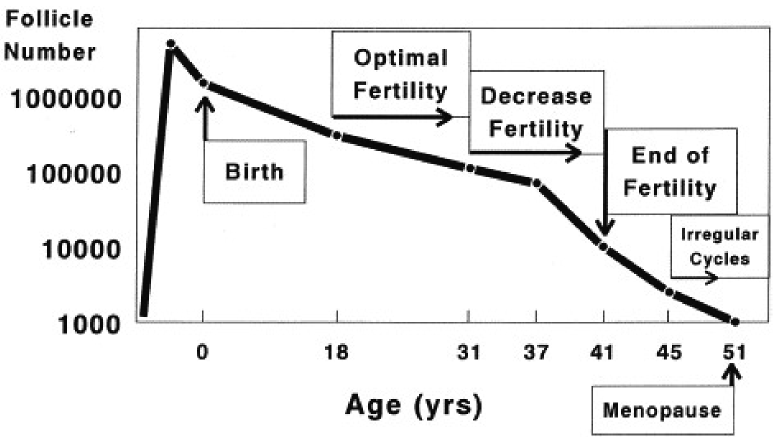

Figure 2
Adapted from E.R. te velde et al 1998[8]

Cancer treatment and ovarian toxicity

Cancer therapy usually involves treatment regimens including chemotherapy, radiotherapy or surgery. Each of these treatment options may impair ovarian function and reproductive function, and these different regimens influence the ovarian function at various levels.[15]

During gonadotoxic treatment, besides the type, dosage and length of gonadotoxic therapy, the age of the patient is also an important factor contributing to the ovarian reserve, and therefore to the risk of Premature Ovarian Insufficiency (POI).[19, 20] As described before, the number of follicles in the ovaries declines with increasing age, thus the higher the age, the higher the chance of ovarian insufficiency after gonadotoxic treatment.[21, 22]

A. Chemotherapy

Systemic applied chemotherapy is used in many types of cancer and even in non-malignant diseases. For the latter, examples are stem cell transplantation with pre-additional chemotherapy, can be part of the treatment for non-malignant or precancerous diseases, such as myelodysplasia, aplastic anemia, thalassemia and systemic lupus erythematosus.

The way chemotherapeutic agents will affects the ovary leading to ovarian failure differs but parallels the anti-tumour effect, like DNA-cross-linking, DNA-damage, DNA inhibition and thereby direct apoptosis of the oocyte, and apoptotic cascade by the mitochondria.[23-25] Apart from direct cytotoxic effect in the oocyte, chemotherapy can also provoke injury to the ovarian blood vessels and focal fibrosis of the ovarian cortex which will cause follicle apoptosis.[26, 27]

The level of gonadotoxicity is generally divided into high, moderate and low risk, of premature insufficiency (Table 1).[28] However, nowadays chemotherapy agents are often combined to achieve optimal cancer toxicity because of their additive or synergistic antineoplastic effects, which complicates the assessment of the gonadotoxic effects and increases the risk of ovarian failure.

Table 1: Cytotoxic agents to degree of gonadotoxicity

High Risk	Intermediate Risk	Low/ no Risk
Cyclophosphamide	Cisplatin	Methotrexate
Cholarambucil	Adriamycin	5-Fluorouracil
Melphalan		Vincristine
Busulfan		Bleomycin
Nitrogen Mustard		Actinomycin D
Procarbazine		

B. Radiation therapy

Radiation therapy is applied locoregional or as a total body irradiation. Local radiation treatment can be either administered as adjuvant or neo-adjuvant (prior to surgery) treatment or even combined with chemotherapy and is often used in cervical cancer (e.g. in case of positive pelvic lymph nodes or inadequate surgical margins) and rectal cancer.[29-32] Total body radiation is frequently used in preparation for allogenic hematopoietic stem cell transplantation.

The human oocyte is extremely sensitive to ionizing radiation: radiation therapy causes direct DNA damage to ovarian follicles, leading to follicular atrophy and consequently decreased ovarian follicular reserve. A dose less than 2Gy is enough to destroy half of the oocyte reserve, dose of more than 6Gy usually causes irreversible ovarian failure.[33, 34] In case of cervical cancer, a total dose of 45Gy will be used to destroy cancer cells (local protocol). Furthermore, the age of the patient is highly correlated to the gonadotoxic effect of radiation therapy. For example, at birth the sterilizing dose is 20.3Gy while at the age of 30 only 14.3Gy already sterilizes. Thus the number of primordial follicles present at the time of treatment, together with the dose applied to the ovaries, will determine the possible fertility ‘‘window’’ and influence the age of premature ovarian failure after radiation therapy.

With regard to the effect of radiation therapy on fertility, not only radiation at the ovaries has to be taken into account, but also the radiation effect on the uterus. Radiation therapy from 12Gy and higher, has a potential deleterious effect on fertility and pregnancy when applied to the uterus. For the latter, radiation causes uterine vasculature damage, endometrium damage and myometrial fibrosis. As a consequence, in case of pregnancy, an increased chance of abortion of premature labor occurs.[35-38]

1

Ovarian and fertility preservation

Several treatments and techniques to preserve fertility have been established: hormonal treatments to reduce the impact of chemotherapy on the ovarian reserve like the additional use of GnRH in case of breast cancer treatment,[39, 40] ovarian sparing surgery to reduce the toxic effect of radiotherapy at the ovaries and finally cryopreservation of ovarian tissue, germ cells and/or embryos. However, all these techniques harbor their own advantages and disadvantages. Hence each patient in whom gonadotoxic treatment will be started should be counseled by a well-informed fertility specialist who is familiar with both the possible fertility preserving techniques as well as the many different oncological treatments. Collaboration between these fertility specialists and the (medical) oncologists will, without doubt, improve the care for patients in whom fertility is a risk due to gonadotoxic treatment.

A. Cryopreservation of ovarian tissue.
Cryopreservation is a process where cells, gametes or tissue is preserved by cooling to sub-zero temperatures (-196°C) using liquid-nitrogen. At this temperature all biological activity is suspended and consequently cryopreserved tissue can be preserved indefinitely.[41] When ovarian tissue is removed before the gonadotoxic treatment is administered, the ovary, and thereby the primordial follicles, will not be exposed to this treatment. After cryopreservation this unexposed tissue can be used to restore fertility.

To harvest ovarian tissue for cryopreservation an uni- or bilateral oophorectomy can be performed preferably by the minimally invasive surgical approach. Ex vivo, the cortex of the ovary will be dissected into small parts ('chips') and cryopreserved. Once the patient has been treated successfully for her initial disease, either malignant or non-malignant, and is in (long term) remission, the ovarian tissue can be thawed and auto-transplanted. The cryopreserved ovarian can by re-implanted into the remaining ovary, or heterotopically (e.g. the abdominal wall).[42, 43] Now, the ovarian tissue will revascularize whereby the primordial follicles are able to grow and develop which will establish a normal menstrual cycle and thereby restore hormonal function and inherently the fertility.[44]

Over the last three decades, the technique to perform Ovarian Tissue Cryopreservation (OTC) has developed from the first successful report of live birth in sheep via the resumption of follicular activity and menstrual cycle in humans to the birth of more than 90 healthy babies reported until now.[45-47] However, despite these publications on the successes, there is minimal data on the usage rate of ovarian tissue cryopreservation. In other words: in how many women ovarian tissue has been cryopreserved but not been auto-transplanted because of either recurrent ovarian function after gonadotoxic treatment or recurrence of the initial disease.

Furthermore, auto-transplantation of cryopreserved ovarian tissue harbours the risk of reintroducing micro-metastases, from the stored ovary, into potentially cured cancer patients. That is why, for example, in the Netherlands, OTC is not performed in patients with leukaemia.(48) However, the precise risk of ovarian metastasis is unknown in many cancers and apart from the risk in general, for the individual patient the only interest is whether her stored ovary contains micro-metastasis.

B. Ovarian transposition

In an attempt to protect the ovaries from pelvic irradiation and to prevent POI, ovaries can be displaced outside the radiation field: Ovarian Transposition (OT). OT is a surgical technique where the ovaries are mobilized and positioned outside the radiation field to prevent the destructive effect of radiation. Since the vascular pedicle of the ovary can be dissected easily over a relatively long distance, the transposed ovary can be adhered to the abdominal wall as cranial as the lowest rib.(49, 50) Although theoretically this should result in preservation of ovarian function, the effectivity of OT is uncertain. On the other side, we have to consider that theoretically, similar to cryopreservation of ovarian tissue, ovarian micro-metastasis are left untreated whenever the ovary is transposed outside the radiation field.(51)

C. Embryo and Oocyte vitrification

Cryo-storage of either oocytes or embryos (IVF procedure) by-passes the before mentioned risk of reintroducing malignant cells in case of ovarian involvement at the time of cryopreservation of ovarian tissue. To optimize the success rate, at average, 15 oocytes are needed, which implies that multiple stimulations are needed which will delay the start of gonadotoxic treatment at least 2 weeks.(52, 53) During the development of the technique of oocyte vitrification, no generally method has been formulated to analyze practice and outcome within vitrification programs which can inform us about the problems during this procedure.(54)

Thesis outline

In this thesis we aim highlight most important questions concerning **Preservation of ovarian function and fertility**, prior to gonadotoxic treatment.

1

Objectives of the research described in this theses:

- In **Chapter 2,** we studied the usage rates of ovarian tissue cryopreservation and the life birth rate after auto-transplantation.
- In **Chapter 3**, a tailor-made approach was developed to minimize the risk of reintroducing minimal residual disease after ovarian tissue auto-transplantation.
- In **Chapter 4**, ovarian survival after Ovarian Transposition followed by pelvic radiation was evaluated by a retrospective case control study.
- In **Chapter 5**, we conducted a systematic review to analyze the effect of OT prior to pelvic radiation therapy.
- In **Chapter 6,** the learning aspects in implementing a vitrification program using mouse blastocysts was studied.
- Finally in **Chapter 7**, a general discussion about efficacy and safety is given.

References

1. Letourneau JM, Ebbel EE, Katz PP, Katz A, Ai WZ, Chien AJ, et al. Pretreatment fertility counseling and fertility preservation improve quality of life in reproductive age women with cancer. Cancer. 2012;118:1710-7.
2. Dutch Cancer Centers. 2012. [http://www.iknl.nl] 2012 updated 2017.
3. Absolom K, Eiser C, Turner L, Ledger W, Ross R, Davies H, et al. Ovarian failure following cancer treatment: current management and quality of life. HumReprod. 2008;23:2506-12.
4. Davies MC, Hall ML, Jacobs HS. Bone mineral loss in young women with amenorrhoea. BMJ. 1990;301:790-3.
5. Lubiszewska B, Kruk M, Broda G, Ksiezycka E, Piotrowski W, Kurjata P, et al. The impact of early menopause on risk of coronary artery disease (PREmature Coronary Artery Disease In Women--PRECADIW case-control study). EurJPrevCardiol. 2012;19:95-101.
6. Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. EndocrRev. 1996;17:121-55.
7. Gougeon A. Dynamics of follicular growth in the human: a model from preliminary results. HumReprod. 1986;1:81-7.
8. te Velde ER, Dorland M, Broekmans FJ. Age at menopause as a marker of reproductive ageing. Maturitas. 1998;30:119-25.
9. Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. HumReprod. 1992;7:1342-6.
10. Gougeon A. Ovarian follicular growth in humans: ovarian ageing and population of growing follicles. Maturitas. 1998;30:137-42.
11. Wallace WH, Kelsey TW. Human ovarian reserve from conception to the menopause. PloS one. 2010;5:e8772.
12. Singh RP, Carr DH. The anatomy and histology of XO human embryos and fetuses. AnatRec. 1966;155:369-83.
13. Snieder H, MacGregor AJ, Spector TD. Genes control the cessation of a woman's reproductive life: a twin study of hysterectomy and age at menopause. JClinEndocrinolMetab. 1998;83:1875-80.
14. van Asselt KM, Kok HS, Pearson PL, Dubas JS, Peeters PH, te Velde ER, et al. Heritability of menopausal age in mothers and daughters. FertilSteril. 2004;82:1348-51.
15. Meirow D, Biederman H, Anderson RA, Wallace WH. Toxicity of chemotherapy and radiation on female reproduction. ClinObstetGynecol. 2010;53:727-39.
16. Sun L, Tan L, Yang F, Luo Y, Li X, Deng HW, et al. Meta-analysis suggests that smoking is associated with an increased risk of early natural menopause. Menopause. 2012;19:126-32.
17. Bleil ME, Adler NE, Pasch LA, Sternfeld B, Gregorich SE, Rosen MP, et al. Psychological stress and reproductive aging among pre-menopausal women. HumReprod. 2012;27:2720-8.
18. Dratva J, Gomez RF, Schindler C, Ackermann-Liebrich U, Gerbase MW, Probst-Hensch NM, et al. Is age at menopause increasing across Europe? Results on age at menopause and determinants from two population-based studies. Menopause. 2009;16:385-94.

19. Warne GL, Fairley KF, Hobbs JB, Martin FI. Cyclophosphamide-induced ovarian failure. NEnglJMed. 1973;289:1159-62.
20. Letourneau JM, Ebbel EE, Katz PP, Oktay KH, McCulloch CE, Ai WZ, et al. Acute ovarian failure underestimates age-specific reproductive impairment for young women undergoing chemotherapy for cancer. Cancer. 2011.
21. Goodwin PJ, Ennis M, Pritchard KI, Trudeau M, Hood N. Risk of menopause during the first year after breast cancer diagnosis. JClinOncol. 1999;17:2365-70.
22. Whitehead E, Shalet SM, Blackledge G, Todd I, Crowther D, Beardwell CG. The effect of combination chemotherapy on ovarian function in women treated for Hodgkin's disease. Cancer. 1983;52:988-93.
23. Raz A, Fisch B, Okon E, Feldberg D, Nitke S, Raanani H, et al. Possible direct cytoxicity effects of cyclophosphamide on cultured human follicles: an electron microscopy study. JAssistReprodGenet. 2002;19:500-6.
24. Morgan S, Anderson RA, Gourley C, Wallace WH, Spears N. How do chemotherapeutic agents damage the ovary? HumReprodUpdate. 2012;18:525-35.
25. Matzuk MM, Burns KH, Viveiros MM, Eppig JJ. Intercellular communication in the mammalian ovary: oocytes carry the conversation. Science. 2002;296:2178-80.
26. Doll DC, Yarbro JW. Vascular toxicity associated with antineoplastic agents. Seminars in oncology. 1992;19:580-96.
27. Bar-Joseph H, Ben-Aharon I, Tzabari M, Tsarfaty G, Stemmer SM, Shalgi R. In vivo bioimaging as a novel strategy to detect doxorubicin-induced damage to gonadal blood vessels. PloS one. 2011;6:e23492.
28. Sonmezer M, Oktay K. Fertility preservation in young women undergoing breast cancer therapy. Oncologist. 2006;11:422-34.
29. Sedlis A, Bundy BN, Rotman MZ, Lentz SS, Muderspach LI, Zaino RJ. A randomized trial of pelvic radiation therapy versus no further therapy in selected patients with stage IB carcinoma of the cervix after radical hysterectomy and pelvic lymphadenectomy: A Gynecologic Oncology Group Study. GynecolOncol. 1999;73:177-83.
30. Green J, Kirwan J, Tierney J, Vale C, Symonds P, Fresco L, et al. Concomitant chemotherapy and radiation therapy for cancer of the uterine cervix. CochraneDatabaseSystRev. 2005:CD002225.
31. Grigsby PW, Perez CA, Chao KS, Herzog T, Mutch DG, Rader J. Radiation therapy for carcinoma of the cervix with biopsy-proven positive para-aortic lymph nodes. IntJRadiatOncolBiolPhys. 2001;49:733-8.
32. Folkesson J, Birgisson H, Pahlman L, Cedermark B, Glimelius B, Gunnarsson U. Swedish Rectal Cancer Trial: long lasting benefits from radiotherapy on survival and local recurrence rate. JClinOncol. 2005;23:5644-50.
33. Wallace WH, Thomson AB, Kelsey TW. The radiosensitivity of the human oocyte. HumReprod. 2003;18:117-21.
34. Wallace WH, Thomson AB, Saran F, Kelsey TW. Predicting age of ovarian failure after radiation to a field that includes the ovaries. IntJ RadiatOncolBiolPhys. 2005;62:738-44.

35. Hawkins MM, Smith RA. Pregnancy outcomes in childhood cancer survivors: probable effects of abdominal irradiation. IntJCancer. 1989;43:399-402.
36. Signorello LB, Mulvihill JJ, Green DM, Munro HM, Stovall M, Weathers RE, et al. Stillbirth and neonatal death in relation to radiation exposure before conception: a retrospective cohort study. Lancet. 2010;376:624-30.
37. Wo JY, Viswanathan AN. Impact of radiotherapy on fertility, pregnancy, and neonatal outcomes in female cancer patients. IntJ RadiatOncolBiolPhys. 2009;73:1304-12.
38. Teh WT, Stern C, Chander S, Hickey M. The impact of uterine radiation on subsequent fertility and pregnancy outcomes. BioMed research international. 2014;2014:482968.
39. Blumenfeld Z, von WM. GnRH-analogues and oral contraceptives for fertility preservation in women during chemotherapy. HumReprodUpdate. 2008;14:543-52.
40. Moore HC, Unger JM, Phillips KA, Boyle F, Hitre E, Porter D, et al. Goserelin for ovarian protection during breast-cancer adjuvant chemotherapy. NEnglJMed. 2015;372:923-32.
41. Hovatta O. Methods for cryopreservation of human ovarian tissue. ReprodBiomedOnline. 2005;10:729-34.
42. Sonmezer M, Oktay K. Orthotopic and heterotopic ovarian tissue transplantation. BestPractResClinObstetGynaecol. 2010;24:113-26.
43. Oktay K, Buyuk E, Rosenwaks Z, Rucinski J. A technique for transplantation of ovarian cortical strips to the forearm. Fertility and Sterility. 2003;80:193-8.
44. Rosendahl M, Greve T, Andersen CY. The safety of transplanting cryopreserved ovarian tissue in cancer patients: a review of the literature 2. JAssistReprodGenet. 2013;30:11-24.
45. Gosden RG, Baird DT, Wade JC, Webb R. Restoration of fertility to oophorectomized sheep by ovarian autografts stored at -196 degrees C. HumReprod. 1994;9:597-603.
46. Jensen AK, Macklon KT, Fedder J, Ernst E, Humaidan P, Andersen CY. 86 successful births and 9 ongoing pregnancies worldwide in women transplanted with frozen-thawed ovarian tissue: focus on birth and perinatal outcome in 40 of these children. Journal of assisted reproduction and genetics. 2017;34:325-36.
47. Gellert SE, Pors SE, Kristensen SG, Bay-Bjorn AM, Ernst E, Yding Andersen C. Transplantation of frozen-thawed ovarian tissue: an update on worldwide activity published in peer-reviewed papers and on the Danish cohort. Journal of assisted reproduction and genetics. 2018;35:561-70.
48. Dolmans MM, Marinescu C, Saussoy P, Van LA, Amorim C, Donnez J. Reimplantation of cryopreserved ovarian tissue from patients with acute lymphoblastic leukemia is potentially unsafe. Blood. 2010;116:2908-14.
49. Clough KB, Goffinet F, Labib A, Renolleau C, Campana F, de la Rochefordiere A, et al. Laparoscopic unilateral ovarian transposition prior to irradiation: prospective study of 20 cases. Cancer. 1996;77:2638-45.
50. Batteb R, Brown DE. Protection of ovaries from radiation. Lancet. 1956;270(6929):939-40.
51. Morice P, Haie-Meder C, Pautier P, Lhomme C, Castaigne D. Ovarian metastasis on transposed ovary in patients treated for squamous cell carcinoma of the uterine cervix: report of two cases and surgical implications. GynecolOncol. 2001;83:605-7.

52. Cil AP, Bang H, Oktay K. Age-specific probability of live birth with oocyte cryopreservation: an individual patient data meta-analysis. Fertil Steril. 2013;100:492-9.e3.
53. Doyle JO, Richter KS, Lim J, Stillman RJ, Graham JR, Tucker MJ. Successful elective and medically indicated oocyte vitrification and warming for autologous in vitro fertilization, with predicted birth probabilities for fertility preservation according to number of cryopreserved oocytes and age at retrieval. Fertil Steril. 2016;105:459-66.e2.
54. Cai H, Niringiyumukiza JD, Li Y, Lai Q, Jia Y, Su P, et al. Open versus closed vitrification system of human oocytes and embryos: a systematic review and meta-analysis of embryologic and clinical outcomes. Reproductive biology and endocrinology: RB&E. 2018;16:123.