RESEARCH PROTOCOL

Turner Fertility

Preservation of ovarian cortex tissue in girls with Turner syndrome

A national exploratory intervention study

Version 7.7 – June 2020

This study protocol was developed with input and feedback of:
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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)
AE	Adverse Event
AMH	Anti-Müllerian hormone
BMI	Body Mass Index
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CV	Curriculum Vitae
DSD	Disorders of Sexual Differentiation
DSMB	Data Safety Monitoring Board
E2	Estradiol
e-CRF	Electronic case report forms
EDC	Electronic Data Capture
FFPE	Formalin Fixated and Paraffin Embedded
FISH	Fluorescence *in situ* hybridization
FP	Fertility Preservation
FSH	Follicle-stimulating hormone
GCP	Good Clinical Practice
HIV	Human Immuno-deficiency Virus
IBM SPSS	International Business Machines Corporation Statistical Package for the Social Sciences
IC	Informed Consent
ICH	international Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use
IQR	Inter-quartile ranges
LBR	Live birth rate
LH	Luteinizing hormone
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
OTC	Ovarian tissue cryopreservation
PhD	Doctor (of Philosophy), Academic title
POI	Premature ovarian insufficiency
POF	Premature ovarian failure
(S)AE	(Serious) Adverse Event
SD	Standard deviation
SPC	Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
TS	Turner syndrome
Wbp	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale: Infertility due to premature ovarian insufficiency is a major concern for girls with Turner syndrome (TS) and their parents. Physicians are often asked about possible options to preserve their fertility. However, despite some experimental case reports, clear evidence for fertility preservation in these girls is lacking and many questions remain. Without evidence on the effectiveness of fertility preservation it cannot routinely be offered to girls with ovarian failure due to Turner syndrome.

Objective: To investigate the occurrence of live birth in women with TS after ovarian tissue cryopreservation in childhood followed by auto transplantation in adulthood.

Study design: A national multicentre exploratory intervention study

Study population: Girls diagnosed with Turner Syndrome, aged 2-18 years.

Intervention: Ovarian tissue cryopreservation in childhood followed by auto transplantation in adulthood. In order to obtain the ovarian tissue for cryopreservation, all girls must undergo a laparoscopy under general anaesthesia which will be performed in academic/university clinics with paediatric surgery. During the laparoscopic intervention, a unilateral oophorectomy will be performed, thereby leaving the other ovary intact for hormone production, ovulation, spontaneous pregnancies and as an auto transplantation site for cryopreserved-thawed ovarian cortical tissue later on. Furthermore, a small sample of the ovarian cortex will be used to assess the oocyte quality and genetics (e.g. the presence of germ line mosaicism). Oocytes will be karyotyped by using Fluorescence *in situ* hybridization (FISH). Karyotypic and hormonal data will be collected once at the yearly clinical visit at the paediatric-endocrinologist. Therefore, a buccal swab, one portion of urine, and one extra blood sample of 3.5mL will be taken and evaluated during the routine laboratory evaluation.
In the future, auto transplantation of frozen-thawed ovarian cortex strips will be performed.

Main study parameters/endpoints: The main study endpoint is live birth after auto transplantation of cryopreserved-thawed ovarian cortical tissue in the future.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:
The primary objective remains to preserve the fertility of the respective (minor) patient, facing a very high risk of POI of 95-98%. Disadvantages of participating in this study are the potential risk of complications related to the laparoscopic unilateral oophorectomy and/or the unknown effect on future fertility of these girls. Moreover,

the procedure might raise false hope in patients (and/or parents) about the chance of getting pregnant after auto transplantation of cryopreserved-thawed ovarian tissue in the future. However, we attempt to overcome this by extensive and objective information provision by both written materials and face to face counseling.

1. INTRODUCTION AND RATIONALE

Turner syndrome (TS) is a condition in which a female is partly or completely missing an X chromosome. The complete or partial loss of an X chromosome leads to dysmorphic signs and a constellation of physical findings that often includes short stature, premature ovarian insufficiency (POI) or failure (POF), and cardiac and renal abnormalities. Signs and symptoms vary among girls with TS, and intelligence is generally normal.
TS is the most common chromosomal abnormality in females, affecting 1 on 2,500 live born girls [1-3]. In some cases, TS is diagnosed prenatally by chorionic villus sampling or amniocentesis in case of cystic hygroma or intra-uterine growth retardation. The majority of girls receive the diagnosis TS before the age of 5 because of growth retardation. Occasionally, the diagnosis TS is concluded in adolescence because of delayed puberty.
Several interview studies [4, 5] show that, regardless of age, the possible or probable infertility due to POI is the main concern for girls/women with TS and also for their parents. Due to an accelerated loss of germ cells, most girls with TS undergo ovarian failure at a very early age, starting as early as 18 weeks fetal age [6, 7]. The timeline at which this occurs is less clear, and may be different for each patient with TS [7]. Spontaneous pregnancies are rare, and occur in approximately 2-5% of women with TS [8-10]. Up to 30% of females with TS have some pubertal development and up to 10% experience spontaneous menarche [11]. Hence, 90% of girls with TS will suffer from POI during childhood, and thus do not enter puberty.
A Swedish research group reported that primordial follicles can be found in the ovaries of both mosaic and non-mosaic girls with TS up to 17 years of age [12]. Due to this new insight, physicians are often asked by girls with TS and their parents about the options of fertility preservation [13]. Fertility preservation includes the cryopreservation of the patient's own gametes, either by preserving mature oocytes or ovarian tissue containing primordial follicles. Cryopreservation of mature oocytes is a proven fertility preservation approach but can be performed only in post pubertal females, since oocyte maturation requires ovarian stimulation with exogenous FSH administration followed by transvaginal ultrasound-guided oocyte retrieval [14]. Ovarian tissue cryopreservation, however, appears to be a promising technique to preserve the fertility of younger girls with TS as it provides the possibility to store a larger number of primordial follicles before their disappearance [15].
Cryopreservation of ovarian tissue is accepted and established in girls and young women undergoing gonadotoxic cancer treatments with a risk of POI ≥ 50% [16-19]. Over the past decades several clinical guidelines [20-29] and decision tools [30-35] for ovarian tissue cryopreservation in cancer patients have been developed and evaluated. Auto transplantation of cryopreserved-thawed ovarian cortical tissue in cancer survivors, has resulted in restoration of ovarian function (63-67% of cases), pregnancies (18-35% of cases) and live births (10-25% of cases) [19, 36-42].

Despite the fact that fertility preservation in girls facing fertility threatening cancer treatment with a risk of POI ≥ 50% is accepted and established [16-18], there are currently no recommendations on fertility preservation in girls with TS facing a very risk of POI of 90%. Still, preservation procedures have been performed experimentally in girls and adolescents with TS [12, 15, 43, 44]. The promise of fertility preservation is at present hypothetical, given that no girl with TS who has undertaken these approaches thus far has returned for auto transplantation or IVF with embryo transfer. Further research is needed to provide supporting evidence for the efficiency of fertility preservation techniques in this specific group of patients. With regard to the occurrence of spontaneous pregnancies (2-5%), it is fundamental to identify which girls with TS might benefit from fertility preservation. Hence, there is a need for reliable markers to estimate the prognosis of the ovarian function for each individual patient as early in life as possible, to more precisely pinpoint the urgency of fertility preservation. Herewith, proper counseling is possible and if wished, treatment options can be chosen for each individual patient (i.e. cryopreservation of ovarian tissue, cryopreservation of mature oocytes or awaiting spontaneous pregnancy). Furthermore, a decision aid based on the information needs of girls with TS and their parents is needed to help them to make a deliberate decision.

2. OBJECTIVE

To investigate the occurrence of live birth in women with TS after ovarian tissue cryopreservation in childhood followed by auto transplantation in adulthood.

3. STUDY DESIGN

A multicenter exploratory intervention study.

The participating centres are:

- Radboud university medical centre, Nijmegen, *The Netherlands* (Principal Investigator): 75 patients
- Maastricht University Medical Centre, Maastricht, *The Netherlands*: 25 patients

Patients will be included between 2017 – 2020.

4. STUDY POPULATION

4.1 Population (base)
The study population includes girls with TS.

4.2 Inclusion criteria
In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Girls and young females with classic Turner (i.e. 45X monosomy) or Turner variants (e.g. 45X/46XX mosaicism, ring X mosaicism, isochromosome X),
- Aged 2 through 18 years,
- who completed the diagnostic work up phase of TS including routine cardiac screening*,
- whose agreement to participate in this study has been signed by the parents (girls aged 2-11 years old),
- whose agreement to participate in this study has been signed by the patient and her parents (girls aged 12-15 years old),
- whose agreement to participate in this study has been signed by the patient (girls aged 16-18 years old).

4.3 Exclusion criteria
A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Contra-indications for laparoscopic unilateral oophorectomy under general anaesthia (e.g. severe cardiovascular comorbidity and/or BMI >40 kg/m^2)*,
- Contra-indications for cryopreservation (i.e. active HIV, hepatitis-B or hepatitis-C infection)

*Based on the international Cincinatti Turner Guideline Concensus Meeting, July 2016[1] and consultation of Dutch cardiologists, paediatric-cardiologists and anaesthesists between 2016-2017 there are no absolute cardiovascular contra-indications for surgical intervention and/or pregnancy. Advice against surgical intervention and/or pregnancy should be based on the patient-specific cardiovascular risk profile. The 2% mortality risk due to acute aortic dissection is based on one survey and literature review study [45] that reported the outcomes of 101 pregnancies in patients with TS after oocyte donation. Only 50% of the patients were screened by a cardiologist before entering the oocyte donation programme. Therefore, all girls who want to participate in this study should have completed the diagnostic work up phase of TS including routine cardiac screening and will be

[1] *Claus H. Gravholt, N.H.A., Gerard S. Conway, Olaf M. Dekkers, Mitchell E. Geffner, Karen O. Klein, Angela E. Lin, Nelly Mauras, Charmian A. Quigley, Karen Rubin, David E. Sandberg, Theo C.J. Sas, Michael Silberbach, Viveca Söderström-Anttila, Kirstine Stochholm, Janielle A. van Alfen-van der Velden, Joachim Woelfle, and Philippe F. Backeljauw. On behalf of the International Turner Syndrome Consensus Group., Clinical Practice Guidelines for the Care of Girls and Women with Turner Syndrome. Eur J Endocrinology, 2017.*

screened by a paediatric anaesthesist. Exclusion will be based on the patient specific risk profile.

4.4 Sample size calculation

As this is an exploratory study, the estimated LBR for girls with TS is currently unknown. Hence, the sample size calculation should be based on data obtained from performing OTC in other patient groups (e.g. girls undergoing gonadotoxic treatments).
The LBR per transplantation of earlier cryopreserved ovarian tissue in cancer survivors is about 25% [19].

In order to describe the dichotomous outcome live birth rate (LBR), we used the sample size calculation of Hulley et al. 2013 [46] via http://www.sample-size.net/sample-size-conf-interval-proportion:

Confidence level (CI) =95%
Expected proportion (P) = 25% (LBR)
Total width of confidence interval (W) =20% (5% - 45%)
$\alpha = (1\text{-}CL)/2 = 0.025$
Standard normal deviate for $\alpha = Z_\alpha = 1.960$

Sample size (N) = $4Z_\alpha^2P(1\text{-}P)/(W^2) = 72$
Expected positive results in sample (x) = 18

One should consider that auto transplantation will not be performed in all girls who are participating in this study, due to several reasons (e.g. in the case that no follicles are found, in the case of *de novo* contra-indications for auto transplantation in the future and/or on patient's preference). Furthermore, the number of miscarriages in patients with TS is known to be higher i.e. 30% *versus* 15% in healthy women [10]. However, in our opinion, performing an exploratory intervention study in >200 minors, is unrealistic and inappropriate.
Therefore, we aim to include primarily a total number of 100 girls with TS in this 'proof of concept' study.

5. TREATMENT OF SUBJECTS

Recruitment of participants and informed consent
Girls with TS and their parents will be informed about this study by their paediatrician in the Dutch hospitals and/or by the Dutch TS patient support group (Turner Contact Nederland, or TCN). Girls with TS who meet the inclusion criteria and are potentially interested, will receive the study information for patients and/or parents during their yearly visit at their paediatric endocrinologist. Needless to say, the girls and their parents are given time as individually requested to consider participation in this study. If the girl and the parents are interested in the study, they will be counseled by a gynaecologist. For the counseling, an additional document can be used where the existing options (i.e. awaiting spontaneous pregnancy, vitrification of oocytes, oocyte donation, high technological surrogacy, adoption and/or fostership) are compared with ovarian tissue cryopreservation. Furthermore, we will further develop a decision aid based on appendix E4b and E4c and input of (parents of) patients and professionals, to help girls with TS and their parents to make a deliberate decision to participate in this study or not. The current decision aid includes a flowchart and background information on the existing options (i.e. awaiting spontaneous pregnancy, vitrification of oocytes, oocyte donation, high technological surrogacy, adoption and/or fostership) and ovarian tissue cryopreservation (see attachment E4b and E4c). Written consent must be given by the (parents of) patients before study participation. Study consent forms and the cryopreservation contract will be collected by one of the research nurses.
Every year, the participant (and her parents) are asked for reconsent via the cryopreservation contract. When a paediatric participant becomes 16 years old, she will receive a new cryopreservation contract for reconsent.

Data collection
Castor Electronic Data Capture (EDC) will be used for Good Clinical Practice (GCP)-compliant data collection. All participant data will be reported in electronic case report forms (e-CRF). Medical record data will be collected only by the members of the research group. Missing data will be supplemented by the paediatric endocrinologists.

Procedures
If not previously performed, a buccal swab will be taken during the yearly visit at the paediatric-endocrinologist to minimize the burden of travelling. Also, one portion of urine will be taken during one of the planned hospitals visits. Furthermore, one extra blood sample of 3.5mL will be taken for hormonal analysis and Hepatitis B/Hepatitis C/ HIV screening during the routine laboratory evaluation to minimize the number of vena punctures.
In some cases, girls with a minimum age of 10 and 45,X karyotype will take an additional blood sample of 3,5mL during counseling (e.g. when certain hormones are missing or when the last hormonal analysis was carried out more than a year ago). In this way this specific group, will be informed properly about their chances of preserving fertility with cryopreservation of ovarian tissue.

All participating girls with TS will undergo preoperative screening and risk assessment by a paediatric anaesthetist, and on indication by a paediatric-

cardiologist. High-risk patients and their parents will be informed about their risks and excluded from this study in order to ensure patient safety. In low-risk participants the surgical procedure, followed by a hospital stay of one night, will be planned. Ovarian cortical tissue will be harvested by performing a unilateral oophorectomy via laparoscopic approach, thereby leaving the other ovary intact. A collaborating team of well-trained paediatric surgeons, gynaecologists specialized in reproductive medicine, and laboratory workers specialized in human ovarian cortical tissue cryopreservation, will ensure safe and efficient cryopreservation of the ovarian tissue. Cryopreservation of the ovarian tissue fragments will be performed according to the Dutch protocol 'Cryopreservation and transplantation of ovarian tissue' (Dutch Network Fertility preservation, September 2012). A safe storage of the cryopreserved ovarian tissue will be provided at the registered cryobank Radboud university medical centre. Our cryobank is ISO-accredited (accreditation number M101, ISO 15189) and registered by the Dutch Ministry of Health, Welfare and Sport (VWS) (registration number 5515 L/EO). A biopt of 5x5mm of the ovarian cortex will be used to assess the oocyte quality and genetics (e.g. the presence of germ line mosaicism). The DXZ1 probe will be used to detect the X chromosome in the isolated primordial follicles and denuded primordial follicles, the isolated primordial follicle derived granulosa cells and the ovarian stromal cells, by using fluorescence *in situ* hybridization (FISH). FISH analyses will be performed in close collaboration with the department of Genetics (Radboudumc).
In the future, all women with TS who are interested in auto transplantation of earlier cryopreserved ovarian tissue, will be counseled by the gynaecologist. Afterwards, all interested participants will again undergo preoperative screening and risk assessment by a specialized cardiologist and anaesthetist. During a multidisciplinary meeting, the individual risk profile of each interested participant is discussed to see if there are any contra-indications for the laparoscopic procedure and/or future pregnancy. In low-risk participants the surgical procedure, followed by a hospital stay of one night, will be planned by the gynaecologist. Orthotopic transplantation of the autograft is performed via laparoscopic approach [19, 36, 38, 42]. High-risk patients will be informed about their risks by the gynaecologist, and excluded from this study in order to ensure patient safety.

Hospital stay and follow-up
Cryopreservation of ovarian tissue:
Participants will be discharged after a hospital stay of 1 night. A follow-up visit with the gynaecologist is planned 6 weeks after surgery to inform patients about their results. When primordial follicles are found, an additional blood sample of 3,5ml blood will be taken three to six months after surgery to gain insight into the hormonal status (FSH, LH, AMH, E2, inhibin B) and to start hormonal therapy when needed. The blood collection will take place during a routine follow-up visit with the paediatric-endocrinologist or paediatrician. Thereafter the hormonal status will be monitored during the yearly visit at the paediatric-endocrinologist or paediatrician by collecting 3,5ml extra blood (FSH, LH, AMH, E2, inhibin B) during the yearly blood collection within their usual care. All blood samples will be sent to the Radboudumc in Nijmegen for hormonal analysis.
In addition, girls with TS who are having a menstruation will use a period calendar to monitor their cycle. This is possible by using an App or they can write it down on paper.

Xenotransplantation
Part of the ovarian cortex fragment that is available for research purposes is used to determine the density of the ovarian follicles and, when follicles are present, FISH analysis is performed on the follicular cells and the stromal cells (see procedures). Our recent results of these FISH analyses [70,71] has shown that in most patients with numerical X chromosomal aberrations the follicular granulosa cells are all 45,X. To study the effect of these 45,X granulosa cells on folliculogenesis we will perform xenotransplantation in mice with the remaining part of the ovarian cortex research fragment of selected patients (approximately 20 samples). This xenotransplantation model is used frequently in research directed at ovarian cortex cryopreservation and will provide us with important information on the functionality of follicles in TS girls. These experiments will be performed in collaboration with the group of Prof. M.M.Dolmans at the Université Catholique de Louvain (Brussels-Belgium). This group has extensive experience with xenotransplantations of human ovarian cortex tissue [72,73] and all the necessary local permissions.

Auto transplantation of cryopreserved-thawed ovarian tissue:
Participants will be discharged after a hospital stay of 1 night. A follow-up visit with the gynaecologist is planned 6 weeks after surgery. No other hospital visits are foreseen.

Temporarily results
Participants and their parents will be informed about their individual results (e.g. blood test results) during the foreseen hospitals visits. The number of follicles found and the results of the FISH-analysis will be reported to the participant and/or her parents during the follow-up visit with the gynecologist 6 weeks after surgery. Ovarian tissue cryopreservation will be performed in all participants, regardless the number of primordial follicles or oocyte genetics.

Financial aspects
Fertility preservation care in girls under 18 years old in the Netherlands is currently organized as following: the costs of cryopreservation and storage of ovarian tissue is funded by the Department of Obstetrics and Gynaecology of the hospital where the cryopreservation is performed. The costs for the laparoscopic removal of 1 ovary under general anaesthesia followed by a 1-night hospital stay are declared to the health insurance. Since there is no deductible excess for minors, no costs should be declared to the participating girls and/or their parents.

6. METHODS

6.1 Study parameters/endpoints

6.1.1 Main study parameter/endpoint

- Live birth after auto transplantation of cryopreserved-thawed ovarian cortical tissue (i.e. live birth rate or LBR)

6.1.2 Proximate

- The number of primordial follicles found in the ovarian tissue

6.1.3 Secondary study parameters/endpoints

- The association between patient's age at cryopreservation and LBR
- The association between patient's genotype and LBR
- The association between patient's AMH level at cryopreservation and LBR
- The association between patient's FSH level at cryopreservation and LBR

6.1.4 Tertiary study parameters/endpoints

- The willingness of girls with TS to perform a unilateral oophorectomy for fertility preservation (i.e. the study participation rate)
- The number of eligible participants
- The age of the participant
- The incidence of somatic mosaicism in buccal cells, peripheral lymphocytes and urine cells
- The incidence of germ cell mosaicism (i.e. oocytes versus somatic cells)
- The developmental capacity of small follicles
- Serum hormone levels (i.e. FSH, LH, AMH, E2, inhibin B)
- The number of complications related to the laparoscopic procedure
- The incidence of spontaneous puberty and/or spontaneous menarche after laparoscopic oophorectomy
- The incidence of spontaneous pregnancies after laparoscopic oophorectomy
- The incidence of menstruation cycle recovery after auto transplantation of cryopreserved-thawed ovarian tissue in the future
- The incidence of pregnancies after auto transplantation of cryopreserved-thawed ovarian tissue in the future
- The number of ongoing pregnancies after auto transplantation of cryopreserved-thawed ovarian tissue in the future
- The number of miscarriages after auto transplantation of cryopreserved-thawed ovarian tissue in the future
- Time to pregnancy after auto transplantation of cryopreserved-thawed ovarian tissue in the future
- Time to live birth after auto transplantation of cryopreserved-thawed ovarian tissue in the future

6.2 Randomisation, blinding and treatment allocation

Laparoscopic removal of one of both ovaries followed by cryopreservation of the ovarian cortical tissue and auto transplantation in the future will be performed without randomization, blinding or treatment allocation.

6.3 Study procedures

A non-invasive imaging technique will be used to assess the number of follicles in the ovarian tissue fragments. A small biopsy of 5x5mm of the ovarian cortex will be used to assess the oocyte quality and genetics (i.e. germ cell mosaicism). During embryogenesis the primordial follicle is assembled by the association of granulosa cells and a single oocyte. Both cell types are crucial for follicular development but have different embryonic origins. In case of mosaicism the karyotype of the oocytes might therefore be different from that of the granulosa cells and/or the stromal cells. To investigate the mosaicism at the level of the follicle and ovary, oocytes, granulosa cells and ovarian stromal cells will be karyotyped by using fluorescence in situ hybridization analysis (FISH analysis) using an X-chromosome specific probe. The remaining part of the ovarian tissue will be cryopreserved for fertility preservation purposes. The 3.5mL blood sample will be used for hormonal analysis (i.e. FSH, LH, AMH, E2 and inhibin B) and screening on Hepatitis B/ Hepatitis C / HIV. The cells isolated from the buccal swab and the urine sample will be karyotyped by using the FISH analysis described above. In all subjects, classic karyotyping on lymphocytes (white blood cells) has been performed before diagnosis of TS.

6.4 Withdrawal of individual subjects

Subjects may withdraw from the trial at any time. Subjects do not need to state a reason for withdrawal. The investigator may withdraw subjects who do not comply with the study requirements.

6.5 Replacement of individual subjects after withdrawal

Subjects who withdraw from the study during recruitment will be replaced.

6.6 Follow-up of subjects withdrawn from treatment

Subsequent treatment options for withdrawn subjects will be subject to the clinician's judgment. Participation in this study will not negatively impact the subject's standard of care.

6.7 Premature termination of the study

Safety reviews are planned primarily to guard against the unfavourable results in this study. Adverse events and complications related to the laparoscopic unilateral oophorectomy and/or general anaesthesia will be closely monitored in order to pick up any (unexpected) trends. Furthermore, the number of primordial follicles will be monitored as a proximate. A data and safety monitoring board (DSMB) at the initiating centre Radboudumc will be formed to control this trial (see 7.4 for more information about this DSMB). Only the DSMB may temporarily or permanently

discontinue the trial at a single site or at all sites for safety, ethical, compliance or other reasons. If this is necessary, the DSMB will endeavour to provide advance notification to the site. If the site or trial is suspended or discontinued, the principal investigator will be responsible for ensuring prompt notification to the CCMO. Where required by local regulations, the principal investigator will be responsible for informing the CCMO of trial or site discontinuation.
A safely storage of the ovarian tissue will be provided till the participant has reached the maximum age for autotransplantation of ovarian tissue (cryocontract). All coded patient data will be safely stored till the endpoint of this study has been reached, i.e. the last livebirth has been registered. In case of an early study termination due to safety reasons, each individual patient and/or her parents may freely decide about the continuation of the storage of the ovarian strips at the Radboud university medical center, transportation of the ovarian strips to another hospital or the elimination of tissue.

7. SAFETY REPORTING

7.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardize subject health or safety. The sponsor will notify the CCMO without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the CCMO. The investigator will take care that all subjects are kept informed.

7.2 AEs and SAEs

7.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study period, whether or not considered related to the investigational product/ trial procedure/ the experimental intervention. All adverse events reported or observed by the investigator or his staff during the first study period (i.e. from informed consent till the follow-up visit at the gynaecologist 6 weeks post-surgery) and the second study period (i.e. from ART-request in the future till end of study) will be recorded.

7.2.2. Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalization or prolongation of existing in patients' hospitalization;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgment by the investigator.

Any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention, but could have been based upon appropriate medical judgment. An elective hospital admission will not be considered as a serious adverse event. SAE´s should be reported to the Sponsor, registered by the Coordinating Investigator and reported to the CCMO when section 10, subsection 4 of the WMO is applicable. The local investigator will report all SAEs to the main principal investigator without undue delay after obtaining knowledge of the events.
The principal investigator will report the SAEs through the web portal *ToetsingOnline* (*https://toetsingonline.nl)* to the CCMO that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the principal investigator has first knowledge of the serious adverse events.

7.3. Annual safety report

In addition to the expedited reporting of SAEs, the main principal investigator will submit, once a year throughout the clinical trial, a safety report to the CCMO and competent authority.
This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the intervention.

Follow-up of adverse events
All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

7.4. Data and Safety Monitoring Board (DSMB) / Safety Committee

An Independent Data and Safety Monitoring Board (DSMB) is established comprising of independent experts who have no conflict of interest and agree with the outline of the protocol. Members of the DSMB are M.C. de Vries, M.D., PhD, paediatric endocrinologist and medical ethicist at the Leiden University Medical Center (NL) (Chair), T.C.J. Sas, M.D., PhD, paediatric endocrinologist at the Albert Schweitzer Hospital and Sophia Children's Hospital (NL) (clinician), and M.M.H.J. van Gelder, PhD, assistant professor in Epidemiology at the Radboud university medical center (NL) (methodologist). The committee will meet at least once in 6 months during the inclusion period to evaluate the results of the interim analysis. During the study, the committee may decide to change the frequency of discussion. All data presented at this meeting will be considered confidential. Following this meeting, the DSMB will report to the principal investigator their advice on whether or not to continue the study. The committee may recommend changes in the conduct of the study. The advice(s) of the DSMB will be sent to the principal investigator of the study. Should the principal investigator decide not to fully implement the advice of the DSMB, the principal investigator will send the advice to the CCMO, including a note to substantiate why (part of) the advice of the DSMB will not be followed.

8. STATISTICAL ANALYSIS

All data in this pilot study will be analyzed on both intention-to-treat and per-protocol analysis. Data of girls who are lost to follow-up will be included as far as possible. Missing data will be reported along with the reason. Baseline data will be described quantitatively. Continuous variables will be summarized as means with standard deviations (SDs) or as medians with inter-quartile ranges (IQRs), depending on their distribution. Dichotomous and ordinal data will be summarized as percentages. For all analyses, IBM SPSS will be used.

8.1 Descriptive analysis

Descriptive statistics will be used to analyze the number of primordial follicles in the ovarian tissue, and the number of (ongoing) pregnancies, miscarriages and live born children after auto transplantation of earlier cryopreserved ovarian tissue.
The primary outcome (dichotomous) 'live birth after auto transplantation of cryopreserved-thawed ovarian tissue' will be assessed and reported as a percentage. The number of primordial follicles (continuous) will be reported as a mean with standard deviations (SDs). The number of pregnancies, ongoing pregnancies and miscarriages (dichotomous) will be reported as a percentage
The time to pregnancy and the time to live birth after auto transplantation will be reported as a mean with standard deviation (SD).
Furthermore, the relationship between the age of the participant when ovarian tissue cryopreservation (OTC) is performed (years), serum FSH level (IU/L), and serum AMH level (ng/ml), *versus* the number of primordial follicles, the incidence of (ongoing) pregnancy, and live birth will be described using Spearman's correlation coefficient. In addition the number of primordial follicles, the incidence of (ongoing) pregnancy, and live birth will be described by the patient's karyotype (i.e. 45,X monosomy or mosaicism).

8.2 Interim analyses

Interim analyses on safety and futility are planned every 6 months until the last participant (n=100) has undergone the laparoscopic unilateral oophorectomy followed by ovarian tissue cryopreservation. The first interim analysis will be performed after the inclusion of the first 10 patients. Each interim analysis will be reported to the independent DSMB (D*ata and Safety Monitoring Board*) by the coordinating investigator on a structured way by using the interim analysis framework (table 1 - Interim analysis framework).
The percentage of participants with follicles in their ovary will be used as a futility indicator. Unfortunately, there is not much information on the expected success probability of ovarian tissue cryopreservation in girls with Turner syndrome.
According to the findings of Hovatta et al. follicles could be found in 26% of girls with Turner syndrome. Primordial follicles were found in girls with a 45X, 46XX mosaicism genotype in 86% of cases, and in 11% of cases in girls with a 45X monosomy genotype. However, their findings are based on the examinations of one small formalin-fixated and paraffin-embedded ovarian tissue sample instead of evaluating

the whole ovary. The question remains whether this ovarian tissue sample correctly represents the presence and number of primordial follicles the whole ovary. It is known that primordial follicles are unevenly distributed throughout the cortex with a variation up to 20,000.
Therefore, we will not implement a formal futility stopping rule but the DSMB will at each interim analysis evaluate the number of primordial follicles that has been retrieved per girl.
The percentage of participants who had one or more complications related to the laparoscopic oophorectomy and/or anaesthesia will be used as a safety indicator. The DSMB will evaluate the number and type of complications at each interim analysis.
Missing data will be reported along with the reason for missingness. Baseline data will be described quantitatively. The number of primordial follicles (continuous) will be reported as a median with a range. Descriptive statistics will be used to analyze the number of complications related to anaesthesia and/or the laparoscopic procedure. In addition, the percentage of participants with follicles in their ovary and the percentage of patients with one or more complications will be categorized by genotype and age-group. For all analyses, IBM SPSS will be used.
The DSMB will discuss the results of each interim analysis and advice the principal investigator on the continuation of the trial based on the balance of futility and safety. The principal investigator will report each DSMB advice to the CCMO. Should the principal investigator decide not to fully implement the advice of the DSMB, the principal investigator will send the advice to the CCMO, including a note to substantiate why (part of) the advice of the DSMB will not be followed.

Table 1 - Interim analysis framework:

Interim analysis number:
Study period:
Date:

1. Results of the current study period

1.1 Inclusion Radboudumc

Number of patients included:
Number of patients excluded:
Number of patients who have undergone the laparoscopic oophorectomy:

1.2 Inclusion MUMC

Number of patients included:
Number of patients excluded:
Number of patients who have undergone the laparoscopic oophorectomy:

1.3 Participants in the current study period (n=):

Hospital (*Radboudumc=1, MUMC=2*)				
Research number				
Age (*years*)				*Mean age (years) and standard deviation*
Genotype (*45,X monosomy or mosaicism*)				*% 45, X monosomy and % 45,X mosaicism*
FSH level (*IU/l*)				*Median FSH level (IU/l) and range*
AMH level (ug/l)				*Median AMH level (ug/l) and range*
Primordial follicles found in the ovarian tissue (*yes/no*)				*% of participants with primordial follicles in the ovarian tissue*

The number of primordial follicles found in the ovarian tissue				*Median number of primordial follicles found in the ovarian tissue with range*
Complication related to the laparoscopic oophorectomy (*yes/no and a description of complication and management*)				*% participants with one or more complications related to the laparoscopic oophorectomy*

1.4 Futility indicators:

The percentage of participants in the current study period with primordial follicles in the ovarian tissue: %

Relationship with age:
Age 2-11 years old: %
Age 12-18 years old: %

Relationship with genotype:
45x monosomy genotype: %
45x/46xx mosaicism genotype: %
other genotypes: %

1.5 Safety indicators:

The percentage of participants in the current study period who had one or more complications related to the laparoscopic oophorectomy: %

Relationship with age:
Age 2-11 years old: %
Age 12-18 years old: %

Relationship with genotype:
45x monosomy genotype: %
45x/46xx mosaicism genotype: %
other genotypes: %

2. Overall study results (all periods)

2.1 Inclusion Radboudumc

Overall number of patients included:

Overall number of patients excluded:

Overall number of patients who have undergone the laparoscopic oophorectomy:

2.2 Inclusion MUMC

Overall number of patients included:

Overall number of patients excluded:

Overall number of patients who have undergone the laparoscopic oophorectomy:

2.3 Futility indicators:

The overall percentage of participants with primordial follicles in the ovarian tissue: %

Relationship with age:
Age 2-11 years old: %
Age 12-18 years old: %

Relationship with genotype:
45x monosomy genotype: %
45x/46xx mosaicism genotype: %
other genotypes: %

2.4 Safety indicators:

The overall percentage of participants who had one or more complications related to the laparoscopic oophorectomy: %

Relationship with age:
Age 2-11 years old: %
Age 12-18 years old: %

Relationship with genotype:
45x monosomy genotype: %
45x/46xx mosaicism genotype: %
other genotypes: %

9. ETHICAL CONSIDERATIONS

9.1 Equality

The WHO states that all individuals have the right to decide freely the number, spacing and timing of their children and that patients should have access to all medical treatments [47]. The right to access fertility preservation services, however, is not legally stated.

Over the last years, ovarian tissue cryopreservation (OTC) has been experimentally performed in hundreds of girls that already underwent or should undergo gonadotoxic cancer treatments. Auto transplantation of cryopreserved-thawed ovarian tissue in cancer survivors has resulted in restoration of ovarian function, multiple pregnancies and >90 live births [19, 36-42].

Some people believe that fertility preservation services should be available for everybody, without discrimination. This has led to a new debate to expand OTC as a fertility preservation options to other patient groups than girls undergoing gonadotoxic cancer treatments (e.g. transgenders and girls with TS).

The promise of fertility preservation for these patient groups is at present hypothetical, as it is for each individual oncofertility patient. However, the Oncofertility Consortium[2] states that OTC is a promising approach to provide fertility options to girls with TS [48]. In some countries, OTC is already routinely offered to girls with TS [12, 15, 44, 48, 49]. We believe that such an intervention should not be offered to these girls as common clinical practice but in a safe and controlled research setting. Recently, ethical approval was given to perform OTC in Dutch transgenders in a research setting. We believe it is time to expand these options to girls with TS too. Qualitative research shows a strong need for new options like fertility preservation services (citation of a girl with TS: “I wish I could have kids. I wish I had the choice”). In daily practice, physicians are often asked by girls with TS and/or their parents about fertility preservation services. This is also stated by Grynberg et al. in a recently published review on fertility preservation in girls with TS [13], in the patient support letter that is attached to this research proposal (see attachment A3), and in the following citations from our patient meeting:

“Should we then accept what nature has decided?” (adolescent with TS)

“At the moment you have no choice” (adolescent with TS)

"One thing I know, her wish is very strong" (parent of a girl with TS, aged 12 years old)

[2] *The Oncofertility Consortium is a multi-institutional group that assessed the impact of cancer and its treatment on reproductive health. The term oncofertility refers to an interdisciplinary field that bridges oncology, reproduction, and women’s health research for the purpose of exploring and expanding options for the reproductive future of cancer patients.*

9.2 Regulation statement

This study will be performed in accordance with this study protocol, the Declaration of Helsinki, the Medical Research Involving Human Subjects Act (WMO), the ICH Harmonized Tripartite Guideline for Good Clinical Practices, and all other applicable regulatory requirements.

9.3 Recruitment and consent

As stated in *The Medical Research Involving Human Subjects Act* (WMO) states, all participating girls (and their parents) will be fully informed about the nature of this study, its purpose, procedures, expected duration and the potential risks and benefits involved in study participation along with any discomfort it may entail, in order to make a deliberated decision and to prevent false hope. Information will be age-specific, easy-to-read and complete. Furthermore, each subject will be informed that participation in the study is voluntary and that withdrawal of consent will not affect the right to the most appropriate treatment, neither the relationship with their doctor relationship. This informed consent will be given by means of a standard written statement. Each subject and/or her parents will be given enough time to read and understate the statement before signing consent and dating the document. Each girl and/or her parents will receive a copy of the written statement once signed (see appendix).

A safely storage of the ovarian tissue will be provided till the participant has reached the maximum age for autotransplantation of ovarian tissue (cryocontract). All coded patient data will be safely stored till the endpoint of this study has been reached, i.e. the last livebirth has been registeredIn case of a study drop out or an early study termination due to safety reasons, each individual subject and/or her parents may freely decide about the continuation of the storage of the ovarian strips at the Radboud university medical center, transportation of the ovarian strips to another hospital or the elimination of tissue.

9.4 Objection by minors

The WMO states that minors aged 12 or older are able to understand the possibilities and limitations of medical investigations. In minors <12 years old, parents are deciding whether their daughter will undergo laparoscopy for future fertility insurance. In minors aged 12 - 15 years old, both parents and the participant have to give consent. This means that the procedure can only be done if they agree and their parents consent, but not if they object.

Participants <12 years old may be legally not competent to make their own decision, requiring an approved consent from both parents. However, interviews with parents of girls with TS [4], showed that girls aged <12 years old already have major concerns regarding their future fertility and are distressed upon learning of their possible inability to become biological mothers. Tailored information can help to inform minors on this study in order to promote the autonomy of the minor and her parents. Therefore, we developed an illustrated brochure.

Moreover, all girls involved in this study are free to decide whether they want to make use of auto transplantation of cryopreserved ovarian tissue in the future, or not. In case of a minor's resistance regarding participation or continuation in this study, none of the study procedures will be performed. Not only capacity-based but also distressed-based child's objections [50] regarding participation or continuation in this study will be respected.

9.5 Benefits

The Medical Research Involving Human Subjects Act (WMO) states that experimental medical research in children or minors is forbidden, unless the research is deemed of a net benefit (i.e. therapeutic research). Since this intervention study aims to preserve the fertility of the respective (minor) participant, facing a very high risk of POI of 95-98%, it relates to therapeutic research, and therefore, in principal permissible scientific research.

9.5.1 Success rates of fertility preservation in other patient groups

Recently, the success rate of cryopreservation of ovarian tissue in advance of cytotoxic therapies and later transplantation(s) of the tissue was reported [19]. The FertiPROTEKT study group found a restoration of ovarian function in 67% per transplantation. Pregnancy and live birth rates were 33 and 25% per transplantation, respectively [19]. The number of auto transplantation sessions is depending on the number of ovarian strips. However, we cannot automatically project this data on women with TS, neither is it possible to estimate the adjusted pregnancy and live birth rates for this patient group.

9.5.2 The number of primordial follicles

The occurrence of pregnancy and live birth after auto transplantation of frozen cortex strips is correlated with the number of primordial follicles found in the ovarian tissue [51]. Borgström et al.[15] reported the number of follicles found in the ovarian biopsies of both 45,X mosaic patients as patients with 45,X monosomy aged 8.0 - 19.8 years old. However, the number of follicles in the frozen ovarian strips could not be determined since there was no non-invasive imaging technique available. Instead, the study group performed a standard histological analysis of an adjacent formalin fixated and paraffin embedded (FFPE) ovarian tissue fragment. The number of follicles found in the FFPE-fragment varied between 0 and 1200. The question remains if their results are representative for the frozen cortex strips, since it is known that primordial follicles are unevenly distributed throughout the ovarian cortex with a variation up to 20,000 [52, 53].

9.5.3 The functionality of the primordial follicles

Furthermore, the functionality of the primordial follicles should be taken into account. Women with TS are known to have a higher risk of having a miscarriage (30.8% compared to 15% for healthy women [10]) and a slightly higher risk of having a child with a congenital disorder (the probability of a child with Down Syndrome is estimated to be 4% compared to 0.4% for healthy women, the

probability of a child with TS is estimated to be 11 % compared to 0.5% for healthy women)[10, 54]. However, we do not know if their increased risks are related to functionality and/or the chromosome profile of their follicles.
In order to fully inform the participant and her parents about her chances, a small sample of the ovarian tissue will be karyotyped. The number of follicles found and the results of the FISH-analysis will be reported to the participant and/or her parents.

9.5.4 Reduction of the psychosocial burden

This study aims to reduce the burden related to the uncertainty about fertility in girls with TS hereby to improve their psychosocial wellbeing. Qualitative research [4] showed the impact of TS on the life of patients with TS aged 7-59 years. Regardless of age, infertility was the most frequently cited concern in this interview study.
From girls undergoing gonadotoxic treatments, we know that uncertainty about fertility leads to a reduction of quality of life and can even lead to serious psychosocial disorders [55]. Girls who were not offered/counseled about fertility preservation services, experienced a worse quality of life [56]. About 70% of the girls who survived their cancer preferred to have genetically concordant children above any other form of parenting [57].
Decisions surrounding fertility preservation (FP) in children, adolescents, and adults can be difficult due to the distress of a cancer diagnosis and the time constraints for decision-making [58-61]. The decision-making process is promoted when counseling has been done by a specialized gynaecologist, when there was enough opportunity for patients to ask questions, and when a decision-aid was available [58-61].
In girls with TS, we will be able to provide tailored counseling by the time that the child has completed the diagnostic work-up phase. In this patient group, we do not experience the time pressure as in oncological children. Proper counseling will be done by a specialized gynaecologist. Therefore, our concept decision aid will be further developed.

9.6 Risks

The laparoscopic removal of ovarian tissue may be in <1% complicated by injuries to the bladder, ureters or bowel, and the risk of infection and bleeding [62]. To minimize the risk of the laparoscopic procedure and to ensure safe and efficient cryopreservation of the ovarian tissue, the procedure will be performed by a collaborating team of well-trained paediatric surgeons, gynaecologists specialized in reproductive medicine, and laboratory workers specialized in human ovarian cortical tissue preservation. All children will be preoperatively screened for co morbidities. A follow-up visit will be done 6 weeks after surgery. Moreover, participants and their parents will be well-informed about all aspects of this procedure including the benefits and risks.
Like with any other new intervention or treatment, there might be a chance of raising false hope in the participating girls (and/or parents). However, we attempt to overcome this by extensive and objective information provision by both written

materials and face to face counselling. Therefore, all girls with TS and their parents will be fully-informed and properly counseled about all aspects of this procedure including the benefits and risks, and their other options (i.e. awaiting spontaneous pregnancy, vitrification of oocytes, oocyte donation, high technological surrogacy, adoption and/or fostership) [63].
The possible effects of the removal of one ovary on future fertility of girls with TS are unknown. Recent studies [64-67] show that the surgical removal of one ovary in a healthy child does not affect the menstrual cycle and / or the chances of getting pregnant in the future. The surgical removal of one ovary in a healthy adult can lead to an earlier menopause up to 3 years in comparison to a woman who still has both ovaries [68]. It has been suggested that one intact ovary may compensate for the loss of the other [69].

9.7 Benefits and risks assessment

Due to a lack of evidence, it is not possible to predict the outcome for each individual participant. However, there are 3 possible scenarios:

1) The participant belongs to the largest group of girls (95-98%) who are infertile at the end of adolescence. OTC could be successfully performed (e.g. follicles where found in the cortex strips).
Due to early menopause, this girl does not have any spontaneous chances of getting pregnant. The removal of one ovary will not reduce her spontaneous pregnancy chances (0% → 0%). Her fertility might be saved for later.

2) The participant belongs to the largest group of girls (95-98%) who are infertile at the end of adolescence. OTC was not successful (e.g. no follicles where found in the cortex strips).
Due to early menopause, this girl does not have any spontaneous chances of getting pregnant. The removal of one ovary will not reduce her spontaneous pregnancy chances (0% → 0%). Unfortunately, her fertility could not be saved.
The intervention does not have a personal benefit for this participant.

3) The participant belongs to the smallest group of girls (2-5%) who may become spontaneously pregnant in the future. OTC could be successfully performed (e.g. follicles where found in the cortex strips).
Due to a solid ovarian reserve in her remaining ovary, this participant still has a chance of spontaneously becoming pregnant. Her fertility might be saved for later if needed.
All girls and their parents will be counseled about the 3 possible scenario's before participating in this study.

9.8 Are there other options?

Needless to say, it would be preferable to offer fertility preservation services to girls

that are competent to make their own decision. However, the majority of girls is infertile by the end of adolescence due to the accelerated loss of germ cells. Cryopreservation of mature oocytes is a proven fertility preservation approach but can be performed only in a small group of girls with TS. Since oocyte maturation requires ovarian stimulation with exogenous FSH administration followed by transvaginal ultrasound-guided oocyte retrieval [17], this procedure can only be performed in post pubertal girls. Unfortunately, 90% of girls with TS will not have a spontaneous menstruation onset [11]. Furthermore, transvaginal ultrasound, the ovum pick up and the administration of exogenous FSH over a longer period are not appropriate in young (virgo) girls.

9.9 Compensation for injury

The principal investigator has a liability insurance which is in accordance with article 7 of the WMO. The principal investigator (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

10. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

10.1 Handling and storage of data and documents

Data will be collected using Castor. Data monitoring will be done by the principal investigator and local investigators in each of the participating centres. Data handling will be done confidentially and coded, with the participant code only available to the investigator working in the local centre.

10.2 Annual progress report

The main principal investigator will submit a summary of the progress of the trial to the CCMO once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

10.3 Temporary halt and (prematurely) end of study report

The main principal investigator will notify the CCMO of the end of the study within a period of 8 weeks. The end of the study is defined as the last participant's last visit. The main principal investigator will notify the CCMO immediately of a temporary halt of the study, including the reason of such an action. In case the study is ended prematurely, the main principal investigator will notify the CCMO within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the main principal investigator will submit a final study report with the results of the study to the CCMO.

10.4 Public disclosure and publication policy

The trial will be registered at clinicaltrials.gov. The principal investigator will publish the results of the study as soon as appropriate.

11. BIOBANK GOVERNANCE

In this study, all ovarian tissue will be handled and stored according to the World Medical Association (WMA) Declaration of Taipei on ethical considerations regarding health databases and biobanks. 67th WMA General Assembly. Taipei, Taiwan. October 2016, with consideration of the following governance arrangements.

1. The purpose of the biobank
Cryopreservation and storage of ovarian tissue for both clinical use, i.e. fertility preservation, and to expand scientific knowledge.

2. The nature of health data and biological material that will be contained in the biobank
This cryobank will contain cryopreserved ovarian tissue and patient data, including consent forms. All clinical data will be recorded in EPIC an electronic medical record system.
Castor Electronic Data capture (EDC) will be used for Good Clinical Practice (GCP)-compliant research data collection. Patient data, with exclusion of the cryotubes and the consent forms, is coded and will be reported in electronic case report forms (e-CRF). Medical record data will be collected only by the members of the research group.

3. Arrangements for the length of time for which the data or material will be stored
A safely storage of the ovarian tissue will be provided till the participant has reached the maximum age for autotransplantation of ovarian tissue (cryocontract). All coded patient data will be safely stored till the endpoint of this study has been reached, i.e. the last livebirth has been registered

4. Arrangements for regulations of the disposal and destruction of data and material
Once a year all subjects will be asked if they want to continue the storage of their ovarian tissue. Each individual patient and/or her parents may freely decide about the continuation of the storage of the ovarian tissue at our cryobank, transportation of the ovarian tissue to another cryobank, donation for research, or the elimination of their cryopreserved ovarian tissue. Storage, transportation, destruction or donation of ovarian tissue can be performed only with the signed consent of the person (s) legally entitled to decide what should happen with the tissue. Destruction of ovarian tissue is performed according to the guidelines of the Radboud university medical center in an anonymous and respectful manner. In case of disposal or destruction of ovarian tissue upon patient's consent, the contract for the storage of ovarian tissue will be closed and discarded. In case of transportation, a new contract should be signed by the patient and the person(s) who are responsible for the governance of the recieving cryobank.
Patients who are interested in auto transplantation of the cryopreserved ovarian tissue, will be counselled by the gynaecologist. Afterwards, all patients will again undergo preoperative screening and risk assessment by an anaesthesist and cardiologist. During a multidisciplinary meeting, the individual risk profile of each participant is discussed to see if there are any contra-indications for the laparoscopic procedure and/or future pregnancy, In low-risk patients the surgical procedure, followed by a hospital stay of one night, will be planned by the gynaecologist.

Orthopic transplantation of the autograft is performed via a laparoscopic procedure. All patients will be informed about the number of ovarian strips used for auto transplantation and the number of strips which are still remaining.
High-risk patients will be informed about their risks by the gynaecologist. in such cases, auto transplantation is delayed or will be discouraged. However, patients can decide freely to transport their tissue to another hospital and ask for a second opinion.

5. Arrangement for how the data and material will be documented and traceable in accordance with the consent of the concerned persons
All research data and material will be documented and traceable in accordance with the consent of the patients involved.

6. Arrangement for how the data and material will be dealt with in the event of change of ownership or closure
In case of an early study termination or change of cryobank governance, all patients and/or their parents will be informed. Each individual patient and/or her parents may freely decide about the continuation of the storage of the ovarian tissue at our cryobank, transportation of the ovarian tissue to another cryobank, donation for research, or the elimination of their cryopreserved ovarian tissue.

7. Arrangement for obtaining appropriate consent or other legal basis for data or material collection
The collection, storage and use of data and ovarian tissue from the participating patients must be voluntary. Therefore, specific informed consent of the participants and/or their parents is obtained in accordance with the Declaration of Helsinki. In this study the following consent forms are used:

1) Informed consent form for participation in this study for patients
2) Informed consent form for participation in this study for parents of patients
3) Informed consent form for the storage of ovarian tissue

Research on ovarian tissue is carried out upon patient's consent only, and in accordance with this research protocol on which a competent medical ethical committee has given a positive judgment.
When a participant is interested in donating her tissue for research, she will be informed about the research purposes, the chance of unintended findings and how she will be informed about these findings.

8. Arrangements for protecting dignity, autonomy, privacy and preventing discrimination
The dignity, autonomy and privacy of the patients will be respected by the duty of confidentiality of all who are involved in handling data and biological material. There will be no discrimination.

9. Criteria and procedures concerning the access to and the sharing of the health data or biological material
A safe storage of all biological material and data, including the consent forms, is provided at our cryobank. Our cryobank is located at a restricted area in the Radboud university medical center. Access is permitted by electronic authorization only. Our cryobank is ISO-accredited (accreditation number M101, ISO 15189), and registered

by the Dutch Ministry of Health, Welfare and Sport (VWS) (registration number 5515 L/EO). All data will be coded with exception of the cryotubes and the consent form for storage of ovarian tissue. Members of the research team, the Data and Safety Monitoring Board, and the Health Care Inspectorate are the only persons who have access to the key to the code. This key of code is stored in Castor Electronic Data capture (EDC).
There will be no sharing of health data and/or biological material.

10. The person or persons who are responsible for the governance
The persons who are responsible for the governance of the Radboudumc ovarian tissue cryobank are L. Ramos (embryologist, head of the IVF laboratory) and C.C.M. Beerendonk (gynaecologist).

11. The security measures to prevent unauthorized access or inappropriate sharing
As mentioned under 9.

12. The procedures for re-contacting participants where relevant
All subjects will be asked for the continuation of the storage of the ovarian tissue yearly. When the participant turns 16 years old, she will be asked for re-consent to participate in this study and the continuation of storage of her ovarian tissue by mail.

13. The procedures for receiving and addressing enquiries and complaints
Enquiries and complaints can be addressed anonymously via the complaints committee of the Radboud university medical center. In case of any questions, patients and their parents can contact the independent physician dr. Timmers who has no further involvement in the investigation.

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