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Article

Ovarian tissue cryopreservation in female-to-male transgender people: insights into ovarian histology and physiology after prolonged androgen treatment

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KEY MESSAGE
This study describes a surprisingly normal distribution of cortical follicles in the ovaries of transsexual men after more than a year of testosterone treatment. This work confirms the presence and in-vitro maturation potential of cumulus-oocyte complexes obtained during the tissue processing of ovaries procured in transsexual men.

A B S T R A C T

Female-to-male transsexual people (transsexual men) are faced with the risk of losing their reproductive potential owing to gender-affirming hormone treatment and genital reconstructive surgery. This observational, prospective cohort study investigates the effect of prolonged androgen therapy on their ovarian histology and fertility preservation perspectives. Serum levels, ovarian histology and cumulus-oocyte complexes (COC) of 40 transsexual men were analysed at the moment of hysterectomy with bilateral oophorectomy in the context of genital reconstructive surgery after testosterone treatment (58.18 ± 26.57 weeks). In the cortex, most follicles were primordial (68.52% total follicle count) compared with 20.26% intermediate and 10.74% primary follicles. Few secondary follicles (0.46%) and a single antral follicle were found in the sections analysed. In total, 1313 COC were retrieved from the medulla of 35 patients (37.51 ± 33.58 COC per patient). Serum anti-Müllerian hormone was significantly correlated with number of COC (R_s

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0.787, $P < 0.001$). After 48 h in-vitro maturation, 34.30% metaphase II oocytes were obtained, with 87.10% having a normal spindle structure. In conclusion, the cortical follicle distribution in transsexual men, after more than a year of testosterone treatment, seems to be surprisingly normal. This work confirms the presence and in-vitro maturation potential of cumulus-oocyte complexes.

Introduction

Female-to-male transsexual people (transsexual men), are faced with the risk of losing their reproductive potential owing to gender affirming hormone treatment and genital reconstructive surgery. Transgender people also tend to start sex reassignment treatment at a young age, when reproductive wishes are not yet clearly defined nor fulfilled (De Sutter, 2001). About one-half of transsexual men, however, express the desire to have children (Wierckx et al., 2012). It is, therefore, recommended by the most recent Standards of Care of the World Professional Association for Transgender Health to clearly discuss fertility and fertility preservation before any treatment (Coleman et al., 2012). Hence, transgender people represent a new group of patients consulting fertility centres. Their specific needs certainly require more insight into the effects of gender-affirming hormone therapy on fertility and an adjusted approach in fertility centres.

If taking steps towards preservation of fertility, cryopreservation of ovarian tissue at the time of hysterectomy and oophorectomy is a possibility (De Roo et al., 2016; De Sutter, 2001). No additional surgical procedure is then needed, nor ovarian stimulation in combination with frequent vaginal ultrasound monitoring as would be the case if oocyte cryopreservation is chosen. The latter is perceived as both a physical and a psychological burden by transsexual men (De Roo et al., 2016). Future use of the frozen tissue could either be transplantation of thawed cortex or in-vitro activation, growth and maturation of the cortical immature follicles. Although surgical challenges can be overcome in time, making future use of banked ovarian cortex of transsexual men a reality, transplantation of ovarian cortical strips in transsexual men can include unwanted side-effects by restoring female hormone activity. In this perspective, in-vitro activation, growth and maturation of these immature follicles would broaden reproductive perspectives for transsexual men. The developmental potential of the residing follicles in the ovary, having been exposed for a prolonged period to supraphysiological doses of testosterone, remains to be determined.

Primordial follicles are not depleted from the ovarian cortex of transsexual men as a result of the gender-affirming hormone treatment (Van den Broecke et al., 2001a). Furthermore, it has been shown that the cortical-residing follicles actually can resume growth and maturation after xenotransplantation (Van den Broecke et al., 2001b).

The standard procedure for banking of ovarian tissue, as described elsewhere (Donnez et al., 2004; Segers et al., 2015; Wilken-Jensen et al., 2014) still implies in many centres, that the cortical region composed of primordial follicles is cryopreserved and the medulla discarded. This could lead to discarding potentially important gametes as growing antral follicles have been found in the medulla during the manipulation of the ovarian cortex for cryopreservation in oncologic fertility preservation programmes (Fasano et al., 2011; Huang et al., 2008). It has been shown, in an oncologic patient cohort, that these cumulus–oocyte complexes (COC) can be recovered from the antral follicles and subsequently matured and cryopreserved *in vitro* (Fasano et al., 2011; Huang et al., 2008; Segers et al., 2015; Wilken-Jensen et al., 2014). The presence of these COC, nor their in-vitro maturation potential, has so far been confirmed during the processing of ovaries originating from transsexual men.

The primary aims of this study were to investigate ovarian histology in transsexual men and the possibility of ex-vivo harvesting of COC in the medulla during the processing of ovarian tissue cryopreservation after a prolonged period of testosterone use. These biological observations will be plotted against the hormonal status of the transsexual men at the time of ovarian tissue cryopreservation, aiming to find associations between ovarian cortical follicle count or medulla derived COC content and clinical or biochemical markers.

Materials and methods

Study design

This study was approved by the Ethical Committee of Ghent University Hospital (UZ Ghent Reference: 2012/780, Belgian registration number B670201 21 5468) on 13 November, 2012. A total of 40 people were included between April 2013 and May 2015. Of these 40 people, cortical histology and anti-Müllerian hormone (AMH) serum levels were analysed. Additional hormone serum level sampling (FSH, LH, oestrogen, progesterone, testosterone, total and free fraction and sex hormone binding globulin (SHBG)) was introduced in the study protocol after inclusion of the first 10 people ($n = 30$). Information on the number of COC was included in 35 people. After a period of optimization (Lierman et al., 2014), COC of 27 individuals could be in-vitro matured following our established research protocol (as described below). In 16 individuals, 124 of the matured second metaphase (MII) oocytes were subsequently used for detailed spindle analysis. The remaining matured MII oocytes (two MII oocytes were lost during fixation/staining procedure) were used for other research projects.

Hormone serum levels

A preoperative blood sample, at the time of hysterectomy with bilateral oophorectomy, was taken to define the hormone serum levels. An overview of the patient characteristics, including hormonal status and reference values, is given in **Table 1**. Hormone levels were determined at the Department of Clinical Biology of Ghent University Hospital as part of the standard patient follow-up using a E170 Modular® (Roche Diagnostics, Mannheim, Germany), except for AMH serum levels (A73818, Elisa Immunotech, Beckman Coulter, Woerden, Nederland). Because of changes in the Department of Clinical Biology of Ghent University Hospital, AMH serum levels of five patients were determined using the AMH Roche E170 (Roche Diagnostics, Mannheim, Germany), and were therefore excluded from our analysis as it is not recommended to compare absolute AMH values from

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