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Original Article

Uterine and ovarian changes during testosterone administration in young female-to-male transsexuals

Giuseppe Loverro [a], Leonardo Resta [b], Miriam Dellino [a], Di Naro Edoardo [a], Maria Arcangela Cascarano [b], Matteo Loverro [a], Salvatore Andrea Mastrolia [a, *]

[a] Department of Obstetrics and Gynecology, Azienda Ospedaliera Universitaria Policlinico di Bari, School of Medicine, University of Bari Aldo Moro, Bari, Italy
[b] Department of Pathology, Azienda Ospedaliera Universitaria Policlinico di Bari, School of Medicine, University of Bari Aldo Moro, Bari, Italy

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ABSTRACT

Objective: Female-to-male transition remains a specific clinical indication for long-term testosterone administration. There is a limited number of studies dealing with the effect of androgen treatment on their female receptive targets (mainly breast and uterus) and the knowledge in this field is scarce and, sometimes, contradictory.
Materials and Methods: We performed a prospective study including 12 patients aged between 20 years and 32 years, with a diagnosis of gender dysphoria, treated with parenteral testosterone administration before sexual reassignment surgery.
Results: Endometrial histology revealed the presence of active endometrium in 10 cases and secretive endometrium in two cases. Multifollicular ovaries were observed in all cases of active endometrium, while corpus luteum was present in the two cases of secretory endometrium. Fibroids or hypertrophic myometrium were observed in 58% of the patients. Estrogen receptor was very high (59%) in the endometrial epithelial cells and low (17%) in the myometrium. Androgen receptor expression was modest in endometrial epithelial cells (24%) and sustained in myometrium (69%). Ki67 expression is steadily present in all uterine compartments, varying from 8% in epithelial endometrium to 2% in the myometrium.
Conclusion: Our data suggest that long-term testosterone administration to female-to-male patients during reproductive age induces a low proliferative active endometrium, associated with some hypertrophic myometrial changes.

Introduction

In recent years, a number of therapeutic protocols have included androgens as part of the traditional postmenopausal hormonal therapy [1,2], although there are few efficacy data.

Female-to-male (FtM) transition remains a specific clinical indication for testosterone administration. There is a limited number of studies dealing with long-term treatments and the effect of androgens on their targets (mainly breast and uterus) and the knowledge in this field is scarce and, sometimes, contradictory.

Theoretically, after androgen treatment, the uterine changes of postmenopausal women may be different from those occurring in women taking long-term androgen therapy during reproductive age [3]. A point that still needs to be adequately addressed regards the uterine changes induced by long-term testosterone administration during the reproductive age.

Therefore, the purpose of this study was to determine the type of histological and steroid receptor changes in the endometrium and myometrium of FtM transgender individuals undergoing long-term testosterone therapy, prior to hysterectomy during the process of sexual reassignment surgery.

Materials and methods

We performed a prospective study including 12 patients aged between 20 years and 32 years, with a diagnosis of gender

* Corresponding author. Department of Obstetrics and Gynecology, Azienda Ospedaliera Universitaria Policlinico di Bari, School of Medicine, University of Bari Aldo Moro, Piazza Giulio Cesare 11, Bari 70124, Italy.
E-mail address: mastroliasa@gmail.com (S.A. Mastrolia).

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dysphoria, performed according to the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) [4]. All patients were recruited at our Department of Obstetrics and Gynecology between 2011 and 2013 and were eligible for total hysterectomy with bilateral salpingo-oophorectomy during sexual reassignment surgery.

Patients underwent colposcopy with cytologic examination, and pelvic ultrasound in order to exclude genital tract pathologies contraindicating hormonal treatment.

Serum concentrations of free testosterone, total testosterone, 17-β estradiol, sex hormone binding globulin (SHBG), as well as hematocrit and Ferriman and Gallwey score, were evaluated before treatment, after the initiation of hormonal treatment but before surgery, and after the surgical sex reassignment procedure.

Cross hormonal therapy started preliminarily with parenteral administration of 100 mg testosterone enanthate along a 5.8 ± 3.8-month period every 2 weeks and was then implemented with intramuscular administration of testosterone enanthate at a dose of 200–250 mg every 2–3 weeks [5].

Recruited patients received androgen treatment during a period of 31.9 ± 14.3 months—the time needed to obtain the permission of the Court to proceed to hysterectomy with bilateral salpingo-oophorectomy—which was performed by laparoscopic approach in all patients.

Testosterone concentrations were measured every 3 months for the 1st year and then every 6 months in subsequent years. The goal was to maintain serum testosterone concentration < 55 ng/dL.

A mean time lapse of 12 months of testosterone treatment was needed for whole phenotypic masculinization. After oophorectomy or after masculinization has been reached, interval of testosterone administration was reduced to 3–4 weeks, maintaining the levels of serum testosterone within half the normal masculine levels.

The modulation of testosterone administration was based on levels of circulating androgens, the degree of satisfaction of masculinizing changes and absence of overdose symptoms such as hypertension and restlessness.

Follow-up consisted mainly of the following: (1) anamnestic surveys (menstrual history before and after the initiation of treatment with androgens, occurrence of amenorrhea, recurrence of bleeding); (2) measuring the degree of androgenization (Ferriman–Gallwey score, changes in tone of voice, appearance of alopecia and seborrhea); (3) arterial blood pressure measurement; (4) waist circumference and hip ratio; (5) evaluation of changes in body weight (body mass index calculation); and (6) work up with interest on the metabolic balance and hormonal changes (follicle-stimulating hormone, luteinizing hormone, total and free testosterone, SHBG, 17-β estradiol, insulin, thyroid-stimulating hormone, and erythropoietin).

Hormonal concentrations, hematocrit values, and Ferriman–Gallwey score taken into account in the present study are those obtained at the last evaluation just before performing the hysterectomy.

After surgery, every uterus was embedded in 10% buffered formalin and sent for pathological examination. The pathologist was asked to focus on the endometrial (epithelial and stromal) and myometrial histologic characteristics as well as to perform an immunohistochemical study of receptors for estrogens, progesterone, and androgens, but also for the proliferation marker Ki67.

The following immunohistochemical probes were used to study the above mentioned molecules: a monoclonal antibody clone 6F11 (Novocastra; Leica Biosystems, Newcastle-Upon-Tyne, UK), was used for estrogen receptors, with 1:50 dilution, and antigenic unmasking in citrate; a monoclonal antibody clone 1A6 (Novocastra), was used for progesterone receptors, with 1:40 dilution, and antigenic unmasking in citrate; a monoclonal antibody clone AR441 (Dako, Ely, UK), was used for androgen receptors, with 1:10 dilution, antigen unmasking in EDTA; a monoclonal antibody clone Mib-1 (Dako), was used for Ki67, with 1:25 dilution, antigen unmasking in citrate [6,7]. The immunohistochemical evaluation was based on the percentage of positive cells, similarly to breast cancer evaluation of steroidal receptors.

Definitions

The description of the endometrium also included cyclical changes, if present, as described by Noyes [8].

Endometrium was classified as inactive when tubular glands lined with cuboidal epithelium and low nucleus–cytoplasm ratio were present, in the absence of secretory or proliferative activity, separated by compact stroma. Active or proliferative endometrium was defined by presence of compact cellular stroma and tubular endometrial glands lined by column epithelium, with some of the glands showing pseudostratification and occasional mitosis.

The histological definition of ovary micropolycystic-like was made in the presence of numerous cystic follicles with a diameter between 5 mm and 10 mm, delimited by the granulosa cells and below a thickened ovarian cortex, associated with stromal hyperplasia.

Statistical analysis

The data on continuous variables with normal distribution were presented as mean ± SD, and compared between study groups using Student *t* test. Categorical data were assessed by Chi-square. A two-sided p value < 0.05 was considered significant. Statistical analysis was done using SPSS version 20 (SPSS Inc., Chicago, IL, USA).

Results

Amenorrhea occurred 8–12 months after starting of the therapy and had a mean duration of 21.8 ± 8.7 months before the hysterectomy.

Free testosterone and total testosterone concentrations progressively increased from basal value, reaching a peak at 6 months ($p < 0.001$) after testosterone administration. Concentrations of 17-β estradiol were reduced after testosterone administration due to inhibition of folliculogenesis, reaching postmenopausal concentrations at 6 months after initiation of androgen therapy (116.5 ± 62.3 pg/mL vs. 51.6 ± 18.6 pg/mL, respectively). Basal hematocrit values were significantly lower than those after 6 months of androgen treatment as well after year of therapy (39.9 ± 2.3 vs. 43.7 ± 2.8 vs. 44.6 ± 2.2, respectively; $p = 0.01$ for all comparisons). Ferriman–Gallwey score progressively increased from pretreatment value until 1 year after surgery (4.5 ± 3.7 vs. 9.9 ± 4.2 vs. 14.2 ± 4.4 vs. 16.6 ± 8.65 vs. 17.3 ± 8.96, respectively; $p < 0.001$ for all comparisons). Remarkably, Ferriman–Gallwey score increased from 4.5 ± 3.7 before treatment to 16.6 ± 8.65 soon after the surgery. As a result of the direct inhibition exercised by androgens on its synthesis in the liver, SHBG concentrations underwent a slight but significant decrease (43.8 ± 4.6 vs. 21.9 ± 5.6; $p = 0.001$; Table 1).

Table 2 summarizes the results of the histological examinations of uterus and ovaries removed obtained from the 12 patients after surgery. Endometrial histology revealed the presence of active endometrium in 10 cases and secretive endometrium in two cases. Multifollicular ovaries were observed in all cases of active endometrium, while corpus luteum was present in the two cases of secretory endometrium. Fibroids or hypertrophic myometrium has been observed in 58% (7/12) of the patients.

Table 1
Serum concentrations of total testosterone, free testosterone, 17-β estradiol, sex hormone binding globulin, as well as hematocrit and Ferriman–Gallwey score values.

	Before therapy	After 6 mo of therapy	After 12 mo of therapy	6 mo after surgery	12 mo after surgery	*p*
Total testosterone (ng/mL)	0.6 ± 0.18	7.7 ± 2.3	6.6 ± 2.02	4.7 ± 2.4	7.8 ± 3.62	< 0.001
Free testosterone (ng/dL)	2.06 ± 0.8	28.4 ± 13.6	20.7 ± 7.9	15.5 ± 8.9	16.9 ± 7.5	< 0.001
Hematocrit (%)	39.9 ± 2.3	43.7 ± 2.8	44.6 ± 2.2	43.5 ± 1.6	44.3 ± 1.3	0.01
Ferriman–Gallwey score	4.5 ± 3.7	9.9 ± 4.2	14.2 ± 4.4	16.6 ± 8.65	17.3 ± 8.96	< 0.001
17-β estradiol (pg/mL)	116.5 ± 62.0	80.9 ± 35.0	96.2 ± 38.7	17.6 ± 7.6	51.6 ± 18.6	0.22
SHBG (nM)	43.8 ± 4.6	21.9 ± 5.6	20.9 ± 8.1	24.8 ± 9.2	20.7 ± 6.2	0.001

Data are presented as mean ± SD.
SHBG = sex hormone binding globulin.

Table 2
Results of the pathologic examination of endometrium, myometrium, and ovaries in the 12 female-to-male patients.

Patient no.	Age (y)	Endometrium	Myometrium	Ovary
1	26	Secretive	Fibrosis	Corpus luteum
2	37	Active	Normal	Multifollicular
3	22	Active	Fibrosis	Multifollicular
4	26	Active	Fibrosis	Multifollicular
5	37	Active	Normal	Multifollicular
6	20	Active	Normal	Multifollicular
7	31	Active	Fibrosis	Multifollicular
8	34	Active	Fibrosis	Multifollicular
9	31	Secretive	Hypertrophy	Corpus luteum
10	33	Active	Normal	Multifollicular
11	20	Active; squamous metaplasia	Normal	Multifollicular
12	20	Active	Hypertrophy	Multifollicular

The immunohistochemical study of steroid receptors showed, in the endometrial epithelial cells, a sustained mean expression of estrogen receptors (54%) and progesterone receptors (59%), while the intensity of androgen receptors expression was modest (24%) and ki67 (8%) expression very low. In the stroma, the mean expression of estrogen and progesterone receptors was lower if compared to the epithelium (40%), while that of androgen receptors was higher, reaching a mean value of 39%. Lastly, myometrium had the lowest expression of estrogen receptors (17%), but the highest expression of progesterone (68%) and androgen receptors (69%). Figure 1 shows the different expression of the androgen receptors in the endometrium (epithelium and stroma) and the myometrium. Ki67 expression was constantly low in all uterine compartments, declining from 8% in endometrial epithelium to 2% in myometrium (Table 3), as shown in Figure 2.

Table 3
Intensity of expression of hormonal receptors and Ki67 receptor.

		ER	PR	AR	Ki67
Endometrium	Epithelium	54%	59%	24%	8%
	Stroma	40%	40%	39%	5%
Myometrium		17%	68%	69%	2%

Data are presented as mean value.
AR = androgen receptor; ER = estrogen receptor; PR = progesterone receptor.

Discussion

The long-term effect of testosterone on human endometrium is a subject for debate [9]. Randomized controlled trials have been conducted and suggest that in women with surgical and natural menopause, testosterone alone [10] or in combination with estradiol [11–13] has a positive impact on sexual function and is well tolerated [9,14].

In vitro studies have suggested that high dose androgen treatment could induce endometrial atrophy, through an inhibitory effect on cells proliferation, as observed with an administration of intramuscular injection of 100 mg Testoviron Depot/10 days for at least 1 year [15].

Androgen receptor expression in postmenopausal women versus. women in reproductive age undergoing androgen treatment

Results of clinical studies on menopausal women have indirectly confirmed those findings, since androgens administered in association with estrogen apparently blunt the development of hyperplasia [16,17]. Moreover, adding the testosterone to treatment with

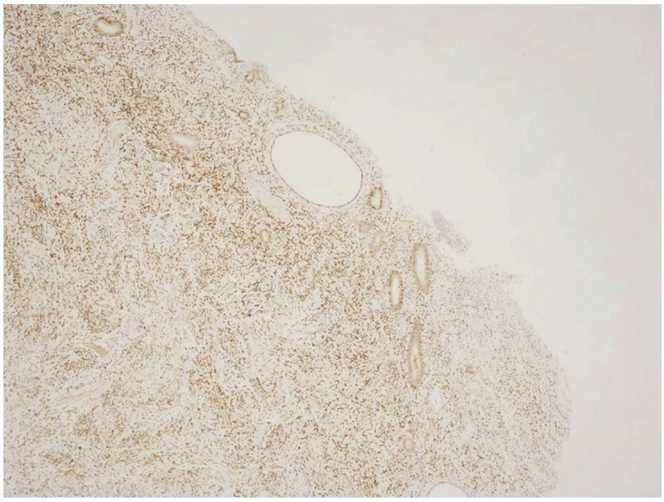

Figure 1. Androgen receptor expression in the endometrium (epithelium+, stroma ++) and myometrium (+++) magnification (40×).

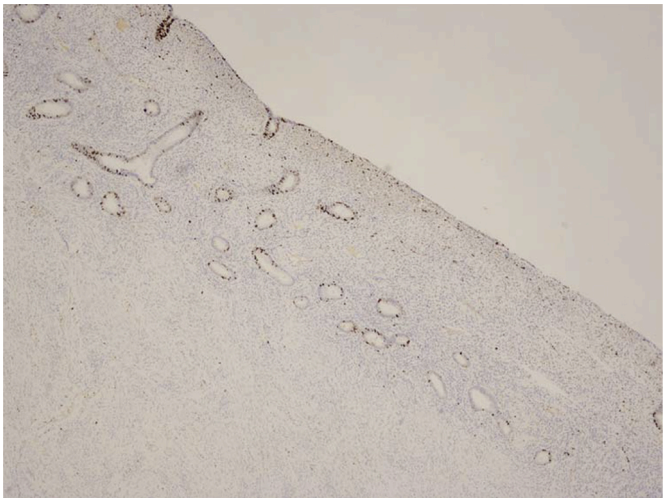

Figure 2. Expression of Ki67 proliferative marker magnification (40×).

estrogen/progesterone does not increase endometrial proliferation, as evidenced by the absence of histological changes [18].

Therefore, in postmenopausal women, a 3-month treatment with undecanoate testosterone does not induce an increase in endometrial proliferation but, if associated to estrogen, induces a modest antagonistic effect on estrogen-induced proliferation [19].

The absence of stimulatory effect of testosterone on endometrium is moreover confirmed by various experimental studies in rodents *in vivo* and cell cultures *in vitro* [15,20].

Nevertheless, many of the aforementioned studies have been performed in postmenopausal women, in which testosterone administration occurred along with estrogen or estrogen/progestin association, and usually for a short period of time [17,21,22].

By contrast, recent studies have found that long-term testosterone administration can induce a proliferative effect on the endometrium in young women during reproductive age, as a consequence of aromatization of androgens into estrogens. Futterweit and Deligdisch [23] studied 19 transsexual FtM individuals undergoing long-term treatment with a low dose of testosterone enanthate (parenteral 400 mg every 3–4 weeks) confirming the possibility of an endometrial stimulatory effect of testosterone administered during reproductive age. In that study, 63.2% of FtM had a proliferative endometrium, 18% had an endometrial glandular cystic mild degree hyperplasia, and 36.8% had an inactive endometrium [23], suggesting an aromatization of testosterone executed by an endometrial aromatase.

The relationship between androgen administration and endometrial biology in reproductive age

However, normal human endometrium is not able to aromatize androgens to estrogen [24,25], since aromatase is not expressed in physiological conditions by epithelial and stromal endometrial cells [26,27], but only by cancerous endometrial cells [20]. In order to better understand the relationship between androgen administration and endometrial biology during reproductive age, we examined uterine histological patterns, receptor status, and Ki67 in endometrial epithelium and stroma, and myometrium, after hysterectomy performed during sex reassignment surgery in young women who desire FtM reassignment.

Histologically, we have found a high rate of active endometrium, sometimes evolving in secretory patterns, without cases of atrophic endometrial involution.

Our findings suggest that high dose of testosterone administered for a long period of time in young women does not induce an endometrial atrophy, but could be associated with a persistent although modest proliferative activity, according to the presence of multiple follicular cysts in both ovaries resembling, from a functional point of view, the picture of polycystic ovaries. This is confirmed in the literature through the association of serum testosterone levels and polycystic ovaries according to antral follicle count and ovarian volume in reproductive-aged women [28].

By contrast, the finding of sporadic secretory endometrium, observed in 16.6% of the cases, in the presence of corpus luteum during prolonged androgen treatment, is in agreement with a minimal proliferative activity [29] and the unexpected high level of expression of estrogen and progesterone receptors (Figures 3 and 4).

Androgen receptors' endometrial pattern of expression

Androgen receptors have cyclical expression patterns in human endometrium and are considered to have an important role in female reproductive function [30]. Estrogens induce an upregulation of mRNA and protein expression of androgen receptors while progesterone downregulates this expression [31], according to

Figure 3. Endometrial and myometrial expression of estrogen receptors magnification (40×).

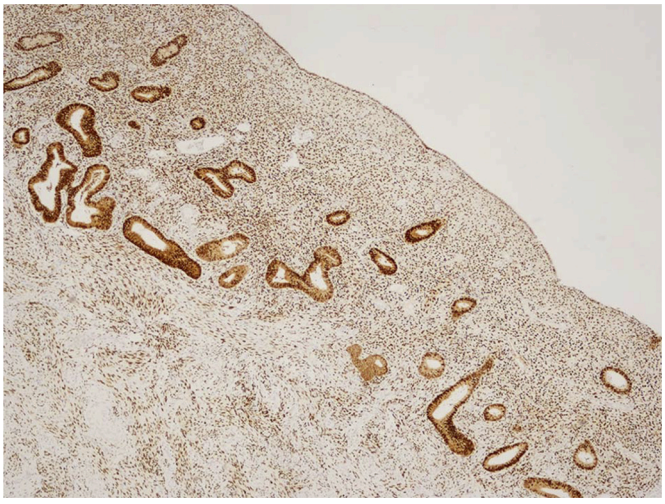

Figure 4. Immunohistochemical study of the expression of progesterone receptors in the endometrium layers and the myometrium magnification (40×).

increased androgen receptor expression induced by progesterone antagonists in the rhesus macaque and human endometrium [32]. Lastly, androgen administration increases the expression of androgen receptors in endometrial adenocarcinoma cells [33].

As for the site and intensity of expression of androgen receptors in various uterine compartments, their expression, evaluated by immunohistochemical techniques appears more intense in endometrial stromal and myometrial cells than in the endometrial epithelial compartment [34] (Figure 1).

These findings may suggest an active role of androgens in myometrial hypertrophy, as seen in the present study.

Moreover, in order to better understand the activity status of the endometrium in FtM transgender during androgen treatment, we have studied the level of Ki67 expression, as a marker of mitotic activity.

A previous study showed that Ki67 has higher expression in endometrial epithelium during the proliferative phase, but is absent during the secretive phase [35]. In postmenopausal women treated with estrogen/progestin associated with testosterone, the expression of Ki67 appears to be downregulated, if compared with its expression in women treated with estrogen alone. By contrast, treatment of postmenopausal women with the testosterone alone

does not induce any changes in Ki67 endometrial expression in the endometrial epithelium and stroma [19].

In our experience, Ki67 expression was very low in all endometrial compartments (Figure 2), suggesting that the high incidence of active proliferative-like endometrium is related to the low but prolonged estrogen levels produced by multiple small ovarian follicles.

Androgen receptor expression in the myometrial compartment

As concerning myometrial compartment, the high incidence of fibroids, myometrial hypertrophy and fibrosis in FtM could be strictly related to an intense androgen receptor expression after prolonged testosterone administration. Recent experimental data suggest that testosterone administration is associated with an increase of satellite cells and myometrial cell hypertrophy. Indeed, testosterone upregulates expression of follistatin and downregulates the expression of genes involved in transforming growth factor-β signaling activity [36].

Ovarian findings related with androgen administration in women in reproductive age

Lastly the findings of enlarged multifollicular ovaries, appear as a consequence of direct or indirect effect of androgen on proliferation and growth of stromal ovarian cells inducing an increased ovarian volume and fibrous collagen content [37]. The multifollicular morphologic changes of ovaries due to direct effect of androgen has been observed in an animal study in which exogenous supplementation with androgens directly led to polycystic ovaries and reduced menstrual cycles [38]. In addition, multifollicular morphological changes during prolonged testosterone administration in genetically normal women could be attributed to increased stimuli of growth factors exerted by androgens [39].

Conclusions

A large body of literature suggests an atrophic effect of long-term androgen therapy in FtM individuals treated with testosterone. Our data provide evidence that testosterone administration produces active endometrial and myometrial changes in women of reproductive age undergoing androgen therapy during the course of the process leading to sex reassignment surgery. This can be the result of the association of ovarian changes, responsible for a continuous, albeit at low concentration, estrogen production, leading to androgen upregulation in the myometrial expression of androgen receptors. The correlation between long-term androgen administration and uterine histological and receptor changes is scarce, and more studies are needed in order to establish the effect of such therapy on uterine tissues. This is extremely important in those patients requiring cross-sex hormone therapy for gender dysphoria without sex reassignment surgery, who may deserve surveillance due to the risk of endometrial degenerative changes due the apparent proliferative activity induced by androgen therapy.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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