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Original Article

Histopathological Variation on Testis of Sex Reassignment Surgery (SRS) Transgenders on Hormonal Therapy

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ABSTRACT

Background: The purpose of this study was to investigate whether the histopathology of the male transsexual's testis could provide insight into the causes of male transsexualism. Hence, the present study is to planned to investigate significant tissue changes in the male transsexual's testis via histopathological analysis. **Materials and Methods:** Light microscopy was used to examine histological sections of testicular tissues taken during sex reassignment surgery from phenotypic male transsexuals (*n*=50, average age range: 39 years) with XY sex chromosomal constitution. The patients had been receiving feminizing hormone (oestrogen) therapy for two to three years when their diagnoses were made based on clinical and psychological data. **Results:** The fifty participants' notable histological findings included: (i) Spermatozoa with maturation arrest; (ii) Hypo-spermatozoa; (iii) Rete testis hyperplasia; (iv) Epithelial hyperplasia; (v) thicker basement membranes and tubular atrophy; and (vi) seminiferous tubules; primarily displaying sertoli cells with cytoplasmic vacuolization and maturation arrest. **Conclusion:** The current study, propose that refractoriness to oestrogen and the iatrogenic effects of oestrogen superimposed on normal or altered hypoathalamo-pituitary function are the causes of the observed histological characteristics.

Keywords: Histology, Hormone therapy, Transsexual's testis, Sex reassignment surgery, Seminiferous tubules.

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INTRODUCTION

In India, the number of people who identify as gender non-binary and transgender is rising. The most recent estimates put the number of Americans who identify as transgender at up to 2%. As a result, more of these people are obtaining medical attention. In particular, there has been a noticeable rise in the number of people looking to physically change from male to female. For some patients, a methodical route to treatment is offered, involving hormone medication and then surgery for gender reassignment. Surgery for those who want to physically change from being a man to a woman involves both genital and non-genital operations. Testis ectomy is a part of genital operations. Given that most people seeking male to female physical adaptation are young adults, testis specimens rank among the most significant from a pathologic perspective.

Transgender hormonal therapy, also known as hormonal reassignment, allows a person to develop secondary sexual characteristics that match their desired gender identity.[1] Hormones from the other sex are substituted for the ones that are ordinarily present in the bodies of transgender individuals undergoing hormonal.[2] Man-to-woman hormonal therapy is intended to promote feminine characteristics and reduce masculine ones, such as breast development and body hair removal.[3] Feminine hormone therapy is a common method used by transgender men to mimic the physical changes in their bodies brought on by female hormones throughout adolescence.[4] These changes are known as secondary sex characteristics. A person's body-gender identity alignment can be improved with hormone therapy.[5] Feminist hormone therapy is also known as gender-affirming hormone therapy. Feminine hormone treatment involves taking medicine to block the effects of the hormone testosterone. It also entails consuming the hormone oestrogen. When oestrogen is present, the body generates less testosterone. Additionally, it starts the emergence of characteristics linked to feminine secondary sex. Hormone therapy and feminine surgery can be used in tandem or apart. In the present study, we reported the alterations was identified as the range of histological changes that patients undergoing feminizing hormone (estrogen) therapy and sex reassignment surgery.

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MATERIALS AND METHODS

This study was conducted at Mahatma Gandhi Medical College and Research Institute (MGMCRI), Pondicherry, India, a rural tertiary care hospital. Annually, the hospital caters the needs of over one lakh patients living in and around the region of Pondicherry. The present study comprises the sample received from September 2021 to December 2023 in the Department of Pathology, MGMCRI, Pondicherry.

Tissue Processing and Histopathological Slide Preparation

Testis will be removed from Male to Female transgender undergoing sex reassignment surgery and the testis will be sent to histopathology department. The testis is undergoing various processing after grossing done by the pathologist which includes Fixation, Tissue processing, impregnation, embedding, block making and section and section cutting. Then the slide with testis section is stained with Haematoxylin and Eosin stain and mounted. Which will be done by the pathologist. Principal investigator will observe the stained slide under the microscope to see the histopathological variation of the testis. The tissue samples dissected from individuals were fixed in 10% formalin and processed further for paraffin section preparation. For histopathology study, a 5 µm thicknesses tissue sections were cut using a microtome (Leica RM 2025, Germany) and mounted on glass slides and stained with haematoxylin and eosin for general morphology examination.

The study was approved by Institutional Human Ethical Committee, Mahatma Gandhi Medical College and Research Institute (MGMCRI), Pondicherry, India. Participants were recruited from Mahatma Gandhi Medical College and Research Institute (MGMCRI), Pondicherry, India, who undergoing Sex Reassignment Surgery, resulting in a sample size of 50 Transexual' individuals with varying levels of education.

The seven control subjects with rete testis hyperplasia underwent an orchiectomy for a germ cell Tumour. The group determined that 43 patients (n=43) at Pondicherry's Mahatma Gandhi Medical College and Research Institute (MGMCRI) had orchiesthemies, or surgeries to change a patient's sex from male to female. 39 was the average age of the patients. For a mean of two to three years, each had undergone hormone therapy and sex reassignment surgery. Seven control specimens in all were taken from patients (n=7) who underwent orchiectomy for germ cell tumors.

RESULTS

Gender confirmation surgery is increasingly common in persons with gender dysphoria. We observed changes seen in gonads from individuals seeking male-to-female physical adaptation.

The following were the primary histological features of the 50 orchiectomy specimens used in Sex Reassignment Surgery (Table 1). A 76% of spermatozoa with maturation arrest (controls: 0%) (Figure 1A); 18% of hypo-spermatozoa (controls: 0%) (Figure 1B); and 10% of normal spermatozoa (controls: 100%). The patients who provided the seven normal sex reassignment surgery orchiectomy specimens had an average duration of 2 to 3 years on hormone therapy. A 32% (controls, 0%) had rete testis hyperplasia (Figure 1C) and 52% (controls, 0%) had epithelial hyperplasia (Figure 1D). A 78% of thicker basement membranes and tubular atrophy (Figure 2A) and 58% of Sertoli cells with cytoplasmic vacuolization and maturation arrest. The presence of oestrogen receptors in both tissue types was likely the cause of the hyperplasia observed in specimens from sex reassignment surgery orchiectomy. For a germ cell Tumour, the seven control participants with rete testis hyperplasia got an orchiectomy.

DISCUSSION

In every field of medicine, transgender women face inequities in their health. There are knowledge gaps in surgical pathology about the principles of transgender care. Hormone therapy and/or surgery to feminize the body may be part of the medical transition for transgender women and trans-feminine people. Surgical pathologists will be better able to care for this particular patient population if they are aware of the prevalent histological abnormalities seen in specimens from feminizing procedures and other specimens from patients receiving feminizing hormone therapy. The majority of the research on transgender women's histology findings that has been published so far comes from

Table 1: Testicular histopathology in male transsexuals receiving estrogen therapy.

Sl. No.	Histological Part	Histological Observations	No. of Patients
1	Spermatozoa with maturation arrest	76%	36
2	Hypo-spermatozoa	18%	9
3	Normal spermatozoa	10%	5
4	Rete testis hyperplasia	32%	16
5	Epithelial hyperplasia	52%	26
6	Thicker basement membranes and tubular atrophy.	78%	39
7	Sertoli cells with maturation arrest and cytoplasmic vacuolization.	58%	29

the analysis of genitourinary materials removed following feminizing operations. Common benign abnormalities associated with feminizing hormone therapy include developing acini and lobules in the breast, testicular tubular changes and squamous metaplasia of the prostate and urethra. Neoplastic examples include meningiomas, pituitary adenomas, anal squamous cell carcinoma, testicular germ cell tumors, prostatic adenocarcinoma and breast adenocarcinoma. It is necessary to conduct more research evaluating the results in various organ systems and population-based studies evaluating neoplasia rates. To get the best outcomes, though, future research has to collaborate with patients and physicians as well as interact with the surgical pathology community.

Patients on feminizing hormone therapy who identify as transgender or gender nonconforming may not self-identify to their healthcare providers due to a history of discrimination and a lack of understanding of their requirements.[6] The pathologist may not have access to this information, even in situations when the patient-provider interaction clearly demonstrates gender non-conformity. Having data on both sex and gender recorded globally could make it easier for pathologists and clinicians to obtain gender histories. Nonetheless, based on histological features, a pathologist might be able to recognize specimens as originating from individuals receiving feminizing hormone therapy even if the patient has a history of hormone therapy. Patients on feminizing hormones exhibit the expected alterations in the testes (Figures 1 and 2). The figures displayed the alterations in the testes.

The alterations mentioned above are not common. For instance, some patients using feminizing hormones do not have any significant histological changes in their testes. According to Thiagaraj *et al.*, (1987), certain patients' testes could not respond well to hormone therapy.[7] Schneider *et al.*, (2015) speculate that patient variability in therapy and individual reactions to anti-androgen regimens are to blame.[9] In addition, Jindarak *et al.*, (2018) point out that hormone therapy is frequently stopped

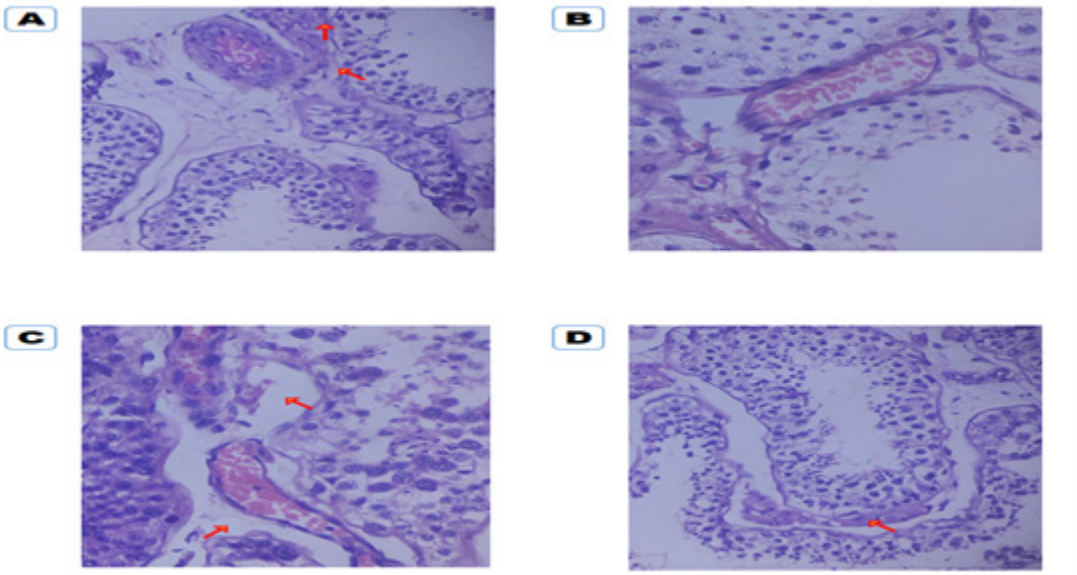

Figure 1: (A) Spermatozoa with maturation arrest. (B) Hypo-spermatozoa. (C) Rete testis hyperplasia. (D) Epithelial hyperplasia.

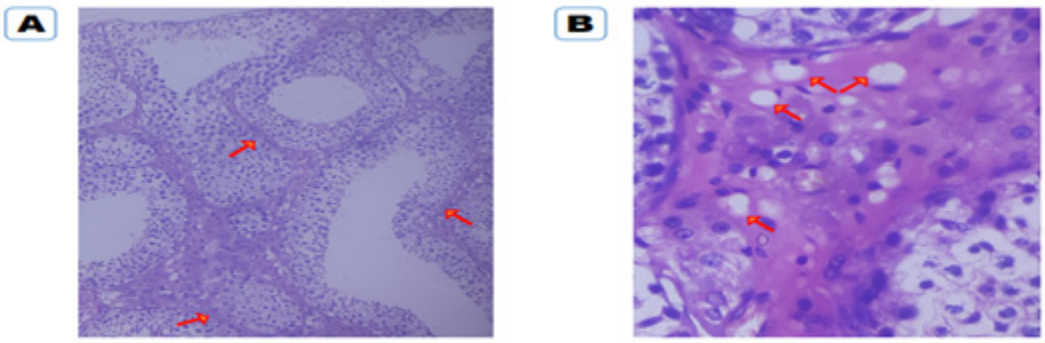

Figure 2: (A) Thicker basement membranes and tubular atrophy. (B) Sertoli cells that have stopped maturing and have cytoplasmic vacuolization.

prior to gender-affirming surgery and they speculate that this could allow for the resolution of alterations brought on by the injection of exogenous hormones.[10]

In a study involving 99 testes from transgender women, the seminiferous tubules were shown to have considerably reduced diameters (0.137±0.002 versus 0.237±0.007 mm; $p<.001$).[11] The length of therapy has no bearing on the degree of maturation arrest.[8,18] The most frequent observations in the stroma were decreased Leydig cell counts and increased fibrosis, along with Leydig cell vacuolization. A subset of treated cases in certain trials (5 of the 18) displayed normal spermatogenesis and no histologic alterations.[7-10,12] Two patients in different research had ectopic adrenal tissue in their testes. In all patients and controls in previous investigations where immunohistochemical staining was carried out, the head of the epididymis showed strong and diffuse positive ER immunohistochemistry staining, while the rete testis showed focal positive staining.[11-13]

There were 18 researches that documented the histologic alterations in the testes of people receiving feminizing hormones. The atrophy was the most frequent finding.[9-11,13,14] Rather than medication time, the degree of atrophy corresponded with serum hormone concentration.[9,15] Testicular size and both free and total testosterone showed significant relationships (reported as $p<.005$) in one research of 108 patients receiving feminizing hormone treatment and having sex reassignment.[9] The most frequent histological observation was maturation arrest together with a smaller diameter of the seminiferous tubules and a thicker basement membrane.[7,11,16,17-21] The cytoplasmic vacuolation and a corresponding increase in Sertoli cells within tubules were also noted.[22,23]

The sex assigned at birth and the gender identity that transgender women and other trans-feminine people identify with are inconsistent.[24] A person who identifies as female, woman, male to female, trans-female, trans woman, transgender woman, or any of the many culturally or contextually relevant gender identities is referred to as "trans-feminine." Medical gender change includes feminizing hormonal and/or surgical therapies. Estradiol (trans-dermal, oral, or intramuscular) is commonly administered with or without an anti-androgen to promote breast growth, testicular atrophy and redistribution of body fat.[24] Additionally, feminizing hormones affect how fat and bone are metabolised, among other metabolic systems. It has been noted that transgender women on feminizing hormones have higher or maintained bone mineral density as well as higher fat mass.[25] Patients on feminizing hormone therapy also frequently exhibit changes in a number of biochemical indicators, including increased HDL, decreased haemoglobin and red blood cell count.[26] Breast/chest (augmentation mammoplasty), nongenital/nonbreast (facial feminization surgery, liposuction, lipofilling, voice surgery, thyroid cartilage reduction, hair reconstruction and other aesthetic procedures) and genital (penectomy, orchiectomy, vaginoplasty, clitoroplasty, vulvoplasty) surgeries are common feminizing surgical procedures.

As part of their gender transition, a person may choose to have hormonal treatment, surgical treatment, both, or none at all. Additionally, feminizing hormones affect how fat and bone are metabolised, among other metabolic systems. It has been noted that transgender women on femininizing hormones have higher or maintained bone mineral density as well as higher fat mass.[25] Patients receiving feminizing hormone therapy also often show changes in several biochemical markers, such as reduced hemoglobin, increased HDL and red blood cell count. Breast/chest (augmentation mammoplasty), nongenital/nonbreast (facial feminization surgery, liposuction, lipofilling, voice surgery, thyroid cartilage reduction, hair reconstruction and other aesthetic procedures) and genital (penectomy, orchiectomy, vaginoplasty, clitoroplasty, vulvoplasty) surgeries are common feminizing surgical procedures. As part of their gender transition, a person may choose to have hormonal treatment, surgical treatment, both, or none at all.

Patients undergoing feminizing hormone therapy with the aim of transitioning to a different gender constitute a distinct patient group. Over the past few decades, the medical community has become more and more conscious of the need for specialized care for patients who identify as transgender or gender non-conforming. Nonetheless, there are still knowledge gaps in the pathology community about gender transition, the effects of hormone therapy and proper terminology to use in reports and casual conversations. Ahmad *et al.*, (2015) have proposed the use of transition-affirming terms in anatomic pathology reports as a means of addressing this issue.[27] While using gender-specific language correctly is crucial to providing better care for patients who identify as gender non-conforming, it ignores potential pathological or histological differences between this population and their transgender counterparts.

CONCLUSION

The histologic results in the test is linked to cross-hormone treatment in patients having sex reassignment surgery are presented in this study. Not much research has been done on the histological changes brought on by hormone therapy in surgical pathology specimens from patients undergoing sex reassignment surgery and feminizing hormone therapy. The purpose of this work is to present a summary of the literature on histological changes associated with hormone therapy for feminization. This article is meant to be a reference for daily work and highlight topics that require further investigation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

SRS: Sex Reassignment Surgery; **MTF:** Male to Female; **H&E:** Haematoxylin and Eosin; **HRT:** Hormone Replacement Therapy; **ER:** Estrogen Receptor; **HDL:** High-Density Lipoprotein.

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