OVOTESTIS

Background

Ovotestis refers to the histology of a gonad that contains both ovarian follicles and testicular tubular elements. Such gonads are found exclusively in people with ovotesticular disorder of sexual development (OT-DSD), formerly known as true hermaphroditism. Those diagnosed with this rare condition represent a small fraction of patients within the diagnostic category of the disorders of sexual development (DSD), formerly known as intersex. Within the spectrum of DSD, there are varying degrees of discordant genitalia to sex chromosomes. A diagnosis of OT-DSD is based solely on the presence of ovarian and testicular tissue in the gonad and not on the characteristics of the internal and external genitalia, even if ambiguous.

Pathophysiology

People with ovotesticular disorder of sexual development are individuals who have both ovarian and testicular tissue. This diagnostic nomenclature is applied regardless of the peripheral karyotype. The gonads may be ovotestis, or they may be a combination of an ovary on one side and a testis or ovotestis on the other. Ovotestes are usually compartmentalized, with connective tissue separating the ovarian components from the testicular components. However, on rare occasions, an intermixture of these elements may occur. Additionally, testicular and ovarian tissue may develop on the same side of the pelvis as a separate ovary and testis.

Ovotestes are the most frequent gonad present (60%), followed by the ovary and then the testis (9%). The ovotestis tends to be anatomically located in an ovarian position, in the labioscrotal fold, in the inguinal canal, or at the internal inguinal ring. Ovaries, when found, can occupy the normal abdominal position, although they may occasionally be found at the internal inguinal ring. Interestingly, ovaries occur more commonly on the left side than the right. The reason for this predilection is unknown. Testes are usually found in the scrotum, although they can be found at any level along the path of embryonic descent from abdomen to scrotum, frequently presenting as inguinal hernias.

Ovaries and ovarian portions of ovotestes appear normal and demonstrate follicular growth with estradiol production. Approximately 50% of ovotestes show evidence of ovulation. The presence of estradiol in developing ovarian follicles usually inhibits spermatogonia development in adjacent or contralateral seminiferous tubules. Degeneration and hyalinization of the seminiferous tubules with poor germ cell development is frequently observed. In all documented biopsied cases, there is a significant decline in germ cell development and an increase in tubular sclerosis by puberty. Leydig cell hyperplasia may also occur with aging. Spermatogenesis in testis and ovotestis is rare.

Internal duct development usually corresponds to the adjacent gonad. Many patients with ovotesticular disorder of sexual development have a uterus. Müllerian duct structures typically develop on the gonad side(s) not containing testicular tissue. Wolffian
duct structures tend to be observed on the gonad side(s) containing functioning testicular tissue.

People with OT-DSD are born with ambiguous genitalia. Most affected individuals are reared as males due to the size of the phallus. Most have varying degrees of labioscrotal fusion and/or hypospadias. However, because of functioning normal ovarian tissue, most people experience breast development at puberty, and approximately two-thirds of those with a 46,XX peripheral karyotype menstruate.

**Frequency**

**International**

Ovotesticular disorder of sexual development is a rare condition. Most cases have a sporadic distribution, although there are a few documented cases of familial recurrence. Genital ambiguity occurs in 1 in 4,500 births, and ovotesticular disorder of sexual development occurs in fewer than 10% of all disorders of sexual development.

**Mortality/Morbidity**

Aside from the physical and emotional consequences associated with genital ambiguity, patients with true hermaphroditism usually do not possess other developmental malformations. These individuals usually possess average intelligence and in general have a normal life expectancy.

- **Neoplasia**
  - Gonadal tumors with malignant potential occur in 2.6% of all cases of ovotesticular disorder of sexual development. The testis or testicular component of an ovotestis is likely to be dysgenetic; dysgerminomas, seminomas, gonadoblastomas, and yolk sac carcinomas have all been reported.
  - Those with the 46,XY karyotype are at the greatest risk of developing a gonadal malignancy. Benign tumors, including mucinous cystadenomas, benign teratomas, and Brenner tumors, have also been reported.
  - If a testis is located in the scrotum, maintaining rigorous follow-up with sonography and/or pelvic MRI is prudent, and a biopsy after puberty is indicated to detect early premalignant or malignant transformation.
  - One case report of a 47-year-old 46,XX/46,XY woman with a malignant phyllodes tumor in the right breast and an invasive lobular carcinoma in the left breast suggests a modified breast cancer risk similar to that observed in Klinefelter syndrome.¹
  - Another case reported an invasive squamous cell carcinoma of the vagina, serving as a reminder that malignant changes can occur in residual müllerian tissue.²
- **Obstructed genital tract:** Cryptomenorrhea, hematometra, and lower abdominal pain associated with endometriosis may occur in individuals with cervical atresia or other forms of müllerian duct anomalies.
- **Hernias and cryptorchism:** Because of malposition of the gonads, gonadal torsion, and associated duct structures, a variety of organs have been encountered within
the inguinal canal, and inguinal hernias are a common occurrence. Complications associated with undescended or partial testicular descent also may be encountered.

Race

Geographic variation has been noted, with the highest incidence occurring in the black population of southern Africa.

Age

Despite the fact that most people with true hermaphroditism present with genital ambiguity, less than 20% are diagnosed before age 5 years. Seventy-five percent are diagnosed by age 20 years.

Clinical History

The presence of both testicular and ovarian tissue within one individual gives rise to varying degrees of ambisexual development. The first contact with a patient with ovotesticular disorder of sexual development is in the newborn period. A newborn usually presents with ambiguous genitalia and discussions must be had with the parents to rule out immediately life-threatening diseases. Although some cases of OT-DSD are diagnosed in the newborn period, only 20% are diagnosed prior to age 5 years. Most cases of OT-DSD are diagnosed in the pubertal period when the young male begins to experience feminization. By puberty, most of the life-threatening conditions associated with genital ambiguity have been ruled out.

Physical

A thorough physical examination is mandatory.

Newborn period: When faced with the delivery of an infant with genital ambiguity, the physician must determine if the newborn is a virilized female, an undermasculinized male, or a rare person with ovotesticular disorder of sexual development. Physical examination should focus on the following considerations:

- Genetic stigmata: A general assessment of the infant's body habitus should be made, giving special attention to the presence of any genetic stigmata.
- Skin pigmentation: Skin pigmentation pattern should be noted as areolar, and scrotal hyperpigmentation may be a manifestation of high serum adrenocorticotropic hormone (ACTH) levels associated with congenital adrenal hyperplasia (CAH). Skin mottling and heterochromia of the iris may be present in the rare person with chimeric hermaphroditism.
- Genital examination: Determine the penile length and the location of the urethral opening, inspect the frenulum, determine the labioscrotal condition, document the number and location of perineal orifices, and identify the position of the gonads. One important clinical finding that may be present is a palpable gonad in one of the labioscrotal folds. If the newborn has OT-DSD, the palpated gonad is most
likely an ovotestis or testis and usually is located on the right side. Discerning a difference between the ovarian (firmer) and testicular (softer) components of an ovotestis by palpation may be possible.

**Pubertal period:** Most cases of OT-DSD are diagnosed in the pubertal period when the young male begins to experience feminization. By puberty, most of the life-threatening conditions associated with genital ambiguity have been ruled out. Aside from the physical examination findings discussed in the newborn section, pay special attention to the following:

- **Sexual hair distribution:** Adrenarche is a normal part of both male and female puberty. The absence of pubic and axillary hair suggests androgen insensitivity. Alternatively, a feminine sexual hair pattern in a pubescent male should trigger further investigation.

- **Uterus:** In ovotesticular disorder of sexual development, the presence or absence of a uterus is variable. Anomalies are common when the uterus is present. A hemiuterus, uterine hypoplasia, and cervical atresia are the most frequent uterine anomalies noted. Approximately 60% of those individuals with a 46,XX peripheral karyotype menstruate or present with signs of obstructed genital outflow tract at puberty.

- **Vagina:** The vagina, when present, is normal in only 9% of cases of ovotesticular disorder of sexual development. The vagina generally shares the urogenital sinus with the urethra as the common external orifice.

- **Phallus:** The phallus, if present, is almost always in chordee. Phallic length varies greatly. The urethra most commonly opens as a urogenital sinus. Hypospadias, often associated with bifid scrotal folds, is the most common anomaly of the external genitalia encountered.

- **Labia/scrotum:** The presence or absence of labioscrotal fusion is variable. In one review, 7% of people with true hermaphroditism had normal labia majora, 13% had hemiscrotum, 17% had a normal scrotum, and 63% had labioscrotal folds. Gonads were found frequently on the right side of the labioscrotal folds.

- **Breast development:** Most people with OT-DSD are given a male sex assignment at birth but develop breasts at puberty or later. Thelarche occurs in more than 90% of people with ovotesticular disorder of sexual development.

**Causes**

Normal sexual differentiation is based on genetic sex (XX or XY), which is established at conception. Until 7 weeks of gestation, the fetus is sexually indifferent, internally developing both wolffian and mullerian ducts. Expression of sex-determining genes on the early bipotential gonad promotes development of the testis or ovary.

Various genes expressed by the Y chromosome at very specific times during development are responsible for the differentiation of the testes. A 35-kilobase (kb) gene determinant located on the distal short arm of the Y chromosome, known as the **SRY** (sex determining region of the Y chromosome) is responsible for initiating testes formation. **SRY** codes for a transcription factor that acts in the somatic cells of the genital ridge. The
transient expression of this gene triggers a cascade of events that leads to the development of testicular Sertoli and Leydig cells. SRY expression directs testicular morphogenesis, characterized by the production of MIS (müllerian-inhibiting substance), and, later, testosterone.

Surprisingly, more than half of the patients with XX ovotesticular disorder of sexual development lack SRY, despite the presence of testicular differentiation. This suggests that this gene codes for a product that reacts with other genes on Y, X, and/or autosomes to complete testicular differentiation. Research has looked at SRY-related high mobility box 9 (SOX9) gene, located on autosomal chromosome 17, as a contributor to Sertoli cell differentiation. Increased expression of SOX9 is being studied as a cause for female-to-male sex reversal in 46,XX SRY negative people with ovotesticular disorder of sexual development. Recently, an inactivating mutation of SOX9 was shown to be associated with autosomal sex reversal and camptomelic dysplasia.

The DAZ (deleted in azoospermia) gene family consists of a cluster of genes on the Y chromosome that give rise to proteins that influence male germ cell differentiation. In humans, deletion of any 1 of 3 DAZ regions (ie, AZFa, AZFb, AZFc) disrupts spermatogenesis. Today, deletion of the AZFc region of the Y chromosome is the most frequent molecularly defined cause of spermatogenic failure.

In 46,XY males, the Sertoli cells of the testes are responsible for the production of müllerian-inhibiting substance, which causes regression of the müllerian ducts. The Leydig cells then produce testosterone, which promotes the development of the epididymis, vas deferens, and seminal vesicles.

For the fetus exposed to only the X chromosome, female gonadal development ensues. Ovarian differentiation appears to rely on a mechanism that is triggered mostly, but not solely by the absence of the testicular determinant. Female development is no longer viewed as only a default pathway for reproductive differentiation. In humans, a complete 46,XX chromosomal complement is necessary for normal ovarian differentiation. Autosomal genes also appear to be involved in ovarian maintenance. Properties of the X-linked gene DAX1 (Dose sensitive sex reversal locus on X chromosome, gene 1) suggest that this gene is important in ovarian determination. Investigators have postulated that the DAX1 gene product may actually be an anti-testes factor and may be antagonistic to the action of SRY. An additional signaling molecule, Wnt4, is found in müllerian ducts and contributes to the development of female internal genitalia.

Internal genitalia of the female fetus develop if there is no exposure to the SRY gene and its signaling molecules. The wolffian duct regresses and the müllerian duct then matures into the oviduct, uterus, cervix, and upper vagina.

Hormone expression during the 9th week of gestation, from the testes or ovary, stimulates external genitalia development. By the 14th week of gestation the external genitalia have been formed. During the developmental process, there are multiple opportunities for errors in differentiation, all of which have been theorized as possible causes of the ovotesticular disorder of sexual development.
In humans, genetic sex has traditionally been evaluated through establishing the karyotype of peripheral lymphocytes. However, the peripheral karyotypes of patients with OT-DSD show marked variation. Approximately 60% are 46,XX; 15% are 46,XY; and 25% show various forms of mosaicism. Less than 1% show 46,XX/46,XY chimerism or the existence of 2 or more cell lines, each of which has a different genetic origin.

Therefore, ovotesticular disorder of sexual development is a genetically heterogeneous condition. Phenotypic, gonadal, and molecular studies have led to several causation theories:

- **Genetic chimerism:** Fewer than 1% of people with OT-DSD have 46,XX/46,XY chimerism or the existence of 2 or more cell lines, each of which has a different genetic origin. Chimerism can result from several events.
  - Dispermic chimerism (double fertilization) can arise from fertilization of the secondary oocyte and first polar body, fertilization of the ovum and the first polar body, or fertilization of the ovum and the second polar body.
  - Chimerism can also arise as an exchange of cells between dizygous twins of different sex (ie, fusion of 2 embryos).
- **Nondisjunction:** Postzygotic mitotic errors arising from anaphase lag may occur in 45,X/46,XY or 45,X/46,XY/47,XYY mosaicism. Note, however, that most 45,X/46,XY individuals have mixed gonadal dysgenesis as opposed to true hermaphroditism.
- **X-Y translocation:** Paternal meiotic exchange between the pseudoautosomal regions of chromosomes X and Y could provide a mechanism for the translocation Y-chromosomal sequences, including SRY onto an X chromosome in some forms of 46,XX testicular differentiation.
- **Mutation:** A mutation of a gene on the X chromosome or alternatively on an autosome that allows testis determination without the SRY gene could explain some forms of 46,XX testicular differentiation. In addition, some 46,XX with OT-DSD have been observed to have a translocation of SRY onto the X chromosome. However, most individuals with ovotesticular disorder of sexual development with 46,XX are SRY negative.
- **Occult mosaicism:** Although most people with OT-DSD have a 46,XX peripheral karyotype, recent case reports have documented the detection of occult mosaicism in the gonads of some of these individuals through molecular techniques. Polymerase chain reaction (PCR) has identified SRY -positive tissue in gonads from several, but not all, people with 46,XX ovotesticular disorder of sexual development.

Mutation of downstream autosomal genes involved with testicular differentiation and mutation/duplication or deletion of an X-linked locus may explain SRY –negative ovotesticular disorder of sexual development.
**Treatment**

**Medical Care**

Sex hormone replacement might be required for those with pubertal delay.

**Surgical Care**

With the exception of 46,XX individuals with CAH or documented maternal androgen excess, most patients with genital ambiguity require surgical exploration for diagnostic confirmation and removal of contradictory gonadal tissue. This should be performed to allow for maximal gender-specific development and to increase fertility potential. Ovotesticular disorder of sexual development can only be confirmed with gonadal biopsy results.

Indications for surgery include biopsy of the gonads for histologic confirmation, removal of intra-abdominal testis or streak gonads with Y chromosome-DNA (because of their increased malignant potential), removal of wolffian structures and testicular tissue if the patient has been given a female gender assignment, removal of müllerian structures and ovarian tissue if the patient has been given a male gender assignment, and orchiopexy to reposition a maldescended testis in a patient with male gender assignment.

Ovotestes frequently can be separated into ovarian and testicular components using frozen sections to define clear margins. Conservative gonadal surgery is the procedure of choice. This approach entails partial resection of ovotestes guided by intraoperative histologic examination. It allows preservation of gonadal tissue concordant with sex of rearing, and removal of all discordant tissue. An ovarian segment can be preserved in people who are given a female sex assignment. Frequently, these segments demonstrate normal ovarian function and confer potential for reproduction.

Clitoral recession, vaginoplasty, and labioscrotal reduction are necessary for people with ovotesticular disorder of sexual development who are given a female sex assignment. Ideally, this should occur at age 3-6 months. For feminizing procedures, clitoral reduction and perineoplasty should be performed as early as possible as a 1-stage procedure. Follow-up procedures, such as reconstruction of the clitoral hood, repositioning of the urethral meatus, and widening of the vagina with vaginal dilators, are common and are often performed after puberty. Masculinizing genitoplasty consists of correction of the penile curvature and repositioning of the urethral meatus, also in a 1-stage procedure. Follow-up surgery may be required if fistulas form or if grafts are used.

**Consultations**

Because of the complexities surrounding gender assignment/reassignment, use of an experienced team is the best approach. The decision of when and if to perform surgery is controversial. Many patients have expressed the sentiment that decisions concerning surgery on the external genitalia should not be made without the understanding and
consent of the affected person. Because of the complexity and ramifications of this decision, the clinician must approach the issue in concert with the family in a compassionate and thoughtful manner.

Members should represent expertise in gynecologic and urologic surgery, reproductive endocrinology, pediatrics, and psychiatry. All should be comfortable with the gender assignment decision, which depends on the diagnostic evaluation results. Gender assignment in the newborn period should be based on the individual's potential for normal sexual function and the potential for future reproductive function. Because most cases of ovotesticular disorder of development are diagnosed during the adolescent period, gender is usually not reassigned. Rather, surgery is performed to conform to the patient's preestablished gender. Most maintain a male sexual assignment, although male reproductive potential in these individuals is rare. Identity and self-esteem issues in this age group make psychological support of utmost importance.