Normal and abnormal sex development:

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Fetal development of the gonads, external genitalia, Mullerian ducts and Wolffian ducts can be disrupted at a variety of points, leading to a wide range of spectrum of clinical presentations. Disorders of sex development (DSD) occur when there is a disruption of either gonadal differentiation or fetal sex steroid production or action. Mullerian anomalies and Wolffian duct remnants occur when there is disruption of the embryological development of the systems. An understanding of the embryology, as well as molecular genetics, helps us determine the biological basis of these conditions. Many of these cases may present in infancy, with initial investigations and treatment performed by pediatric endocrinologist and fertility subspecialist (if presented with primary amenorrhea or infertility)). Pediatric and urological surgeons may have initially treated others. Patients’ with DSD may have coexisting medical problems and requires thorough evaluation. As well as anatomical and fertility concerns for these patients, there are often many psychological issues, therefore management in a multidisciplinary team (MDT) is essential for the management of more complex cases. In some conditions, the optimal operative management is still uncertain, and there is currently debate regarding the optimal timing or need for genital surgery in patients with DSD conditions who present in childhood.

Normal Embryological Development of the internal & external genitalia:

Genetic sex is determined at the moment of conception by the presence or absence of the Y chromosome, and after week 6 should guide the subsequent development of the fetus down one of two standard pathways- male or female. Until this time, development is the same in all fetuses. Primordial germ cells (the precursor of gametes) can be seen at 3 weeks in the endoderm of the yolk sac wall. During week 5 and 6, they migrate by amoeboid movement to the genital ridge (future gonad) an area of mesenchyme medial to the developing mesonephrons and Wolffian (or mesonephric) ducts. During week 6, primitive sex cords form around the germ cells in the indifferent gonad. The two Mullerian (or paramesonephric) ducts also appear lateral to the Wolffian ducts. At the same time, at the caudal end of the fetus, the cloacal membrane folds and it separated into the anterior urogenital and posterior anal parts. The urogenital section with the genital tubercle will become the future external genitalia, and by week 7, the urogenital membrane has degenerated and the urogenital sinus freely communicate with amniotic fluid.

The first noticeable divergence in male and female fetuses is the differentiation of gonadal structure. The indifferent gonad has the potential for testicular or ovarian development.

The presence of intact germ cells seem necessary for the ovary to develop, but this is not required for testicular development. The gonad remains undifferentiated until around 42 days. After gonadal differentiation has occurred, the presence or absence of hormone production and other fetal factors then guides the development of the Mullerian ducts, Wolffian ducts and external genitalia. Sertoli cells in the fetal testis secrete anti-Mullerian hormone (AMH-also called Mullerian inhibiting substance, MIS), leading to active degeneration of the Mullerian ducts. Each ledig cells commence production of testosterone which acts through the androgen receptor on the Wolffian ducts, leading to the development of the vas deference, seminal vesicles, and epididymis. Testosterone is converted to its active metabolite dihydrotestosterone (DHT). The fetal ovaries do not secrete androgen or AMH, and therefore there is female external genital development, growth of the Mullerian ducts and spontaneous regression of the Wolffian ducts.

**Standard male development:**

In an XY fetus, activation of the SRY (sex-determining region of the Y chromosome) gene at the end of week 6 guides the indifferent gonad to commence development into a testis. The medullary sex cord cells become Sertoli cells, surrounding the primitive germ cells. At puberty, these will become the seminiferous tubules surrounding the spermatozoa. Sertoli cells produce AMH, which acts locally to cause apoptotic regression of the adjacent Mullerian ducts from 7 weeks. The appendix testis and prostatic utricle are usually all that remain of the Mullerian ducts in the male.

At around week 8-10, Ledig cells appear in the testis and start to secrete testosterone. The control of testosterone production may be independent initially, then under the control of placental human chorionic gonadotrophin (hCG) through the shared leutinizing hormone/hCG receptor (LHCGR), and subsequently by 20 weeks the hypothalamic pituitary (gonadotrope) axis becomes active and fetal leutinizing hormone (LH) production control steroidogenesis. Testosterone acts through the androgen receptor and causes development of the Wolffian ducts into the vasa deferentia, and later the seminal vesicles and epididymis. Testosterone is also released into the circulation and undergoes peripheral conversion to its more active metabolite DHT by the enzyme 5 α-reductase type 2, and acts on the target tissues of the perineum resulting in development and growth of genital tubercle, urogenital sinus, urogenital folds and labioscrotal swellings into the glans penis, penile shaft, urethral tube and the scrotum, respectively. The penis is similar in size to the clitoris at 14 weeks and under the influence of DHT, continues growing until birth. Testicular descent is mediated by the Ledigcells under the influence of the hypothalamic pituitary (gonadotrope) axis, commences at 12 weeks, and is usually complete by week 34.

**Standard female pathway:**

Traditionally, ovarian development in an XX fetus has been considered a “default pathway”, however, emerging research indicates that a distinct set of genes are expressed in the developing ovary and required for the maintaining ovarian integrity and active opposing testicular development, termed “anti-testis” genes. These genes in combination with the absence of SRY gene, cause the indifferent gonad to commence ovarian differentiation at around week 7. The sex cord cells degenerate and secondary sex cords form and surround the primordial germ cells. Between 5 and 24 weeks, rapid mitotic expansion of the primordial oogonia occurs, followed by first meiotic division (8-36 weeks), and subsequently meiotic arrest as primordial follicles. The presence of ovaries are not required for regression of the Wolffian ducts, and it is the absence of local testosterone that cause their regression at 10 weeks. The paroophoron, epoophoron and Gartner’s cysts are all that may remain of the Wolffian ducts in the female. The absence of circulating testosterone also leads to an absence of peripheral DHT and directs the genital tubercle, urogenital sinus, urogenital folds and labioscrotal swellings to develop into the clitoris, lower vagina, labia minora and labia majora respectively.

As AMH is not produced by the fetal ovary, the Mullerian ducts continue to develop. These paired mesodermal ducts originate in week 5, lateral to the Wolffian ducts at the third to thoracic segment. They are thought to be associated with the basement membrane of the Wolffian ducts and grow caudally guided by them. The cranial end of the Mullerian ducts are independent on the Wolffian ducts and remain separate as the fallopian tubes. At the pelvis, the Mullerian ducts cross the Wolffian ducts anteriorly to lie medially next to each other. At weeks 8-10, the pelvic Mullerian ducts have fused and subsequent breakdown of their medial walls leads to single tube, which will become the upper vagina, cervix and the uterine epithelium and glands. Surrounding mesenchymal tissue will become the myometrium and stroma. At their caudal end, the fused Mullerian tubercle, which connects with a thickened area of the urogenital sinus that develops into the paired sinovaginal bulbs. This connection of the endodermal urogenital sinus and mesodermal Mullerian ducts forms the vaginal plate-a column of squamous tissue. In weeks 10-16, the vaginal plate enlarges and develops a cavity, which is separated from the urogenital sinus by an endodermal membrane.

Gradual change of the lower Mullerian ducts epithelium from columnar to stratified squamous epithelium occurs, ending at the future external cervical os. By month 5, the urethra and vagina are separated by a septum, and the endodermal membrane between the vagina and urogenital sinus breaks down to form the hymen. The urogenital sinus forms the vaginal vestibule.

***Human sex development:*** it can be divided into three main parts:

1. Chromosomal sex (presence of X and/or Y chromosome).
2. Gonadal sex (development of the gonad into either testis or ovary).
3. Phenotypic or anatomic sex (appearance of the internal or external genitalia).

This differs from the concept of gender or “brain sex”, which encompasses gender identity (which is one’s self-representation, gender role behavior and sexual orientation. Occasionally, there is discordance between sex and gender, as well as the elements of gender.it is important to note that no single category dictates someone sex when managing the patient with DSD.

***Abnormal embryological development: Disorders Of Sex Development (DSD)***

***Definition and classification:*** over the last 20 years, there has been a significant increase in the understanding of the underlying etiology of many abnormal embryological development. Clinicians, were becoming increasingly aware that traditional terms, such as intersex, true hermaphroditism, female pseduhermaphroditism and male pseudohermaphroditism, were confusing, inaccurate and often considered negative by the patient involved. Therefore a new consensus meeting was held in 2005, and one of the main elements to emerge was a new nomenclature system for this group of disorders, which is more generic, diagnostically accurate and less derogatory.

The term “disorder of sex development” or DSD was established to describe congenital conditions with a typical development of chromosomal, gonadal or anatomical sex. This encompasses a blend of physically defining features associated with male or females, i.e. karyotype, gonadal structure, internal genitalia and external genitalia, and covers a diverse range of conditions including individuals with standard male or female genitalia who may have variety of internal genital organs and karyotype, and also those with ambiguous external genitalia. In addition, an update classification system was proposed which divided conditions into:

* Sex chromosome DSD.
* 46XY DSD.
* 46XX DSD.

Updated nomenclature for DSD:

|  |  |
| --- | --- |
| **Terminology used previously** | **Proposed new terminology** |
| **intersex** | **DSD** |
| male pseudohermaphroditism |  |
| Undervirilization XY male |  |
| Undermasculinisation XY male | 46XY DSD |
| female pseudohermaphroditism |  |
| Overvirilization of an XX female |  |
| virilization of an XX female | 46XX DSD |
| True hermaphrodite | Oveotesticular DSD |
| XX male or XX sex reversal | 46XX testicular DSD |
| XY sex reversal | 46XY complete gonadal dysgenesis |

This new classification system also embodies a wider range of disorders, for instance sex chromosome DSD, includes Turner’s syndrome (45XO) & Kleinefelter’s syndrome (47XXY), which were not previously considered “intersex” conditions. Similarly, female patients with congenital adrenal hyperplasia (CAH), who were traditionally considered to have an adrenal disease, and now included as 46XX DSD due to the surgical and reproductive consequences associated with this disorder.

With new knowledge concerning fetal sexual differentiation and development, greater awareness and understanding of female sexual function and a more patient-centered emphasis condition management, it is hoped that patient care will be improved in this group of individuals.

***Incidence:***

The incidence of DSD conditions in the UK is unknown. An estimate of prevalence is one in 2000. Conditions with autosomal recessive inheritance are more prevalent in communities in which consanguinity is common.

***Aetiology:***

Most DSD conditions occur due to genetic or environmental disruption to the pathway of fetal sexual development. This disruption can be of gonadal differentiation or development, sex steroid conversion or tissue utilization of sex steroids.

***Presentation & investigations:***

Every DSD has a spectrum of severity and therefore may present in a variety of ways:

* Ambiguous genitalia at birth.
* Mismatch of fetal chromosomal results, such as amniocentesis or chorionic villous sample with phenotype at birth.
* Salt-losing crisis in neonatal life (CAH).
* Sibling history of intersex.
* Ambiguity of the genitalia developing in childhood or puberty.
* Inguinal hernia with unexpected gonad.
* Pelvic mass with gonadal tumor.
* Primary amenorrhea or pubertal delay.
* Infertility.
* Sexual dysfunction.
* Part of syndrome with other anomalies (e.g. renal anomalies in Denys-Drash syndrome).

Initial investigation will depend on presentation, but should include karyotype, testosterone, LH, FSH, 17-hydroxyprogesterone and pelvic U/S. Further investigation will depend on initial findings, external genital appearance & clinical presentation, and may include androstendione, DHT, oestradiol, 24-hour urinary collection of steroid metabolites, hCG stimulation test, renal U/S scan, MRI and DNA for genetic testing.

***Management:***

The areas to consider in intersex management are:

* Accurate diagnosis.
* Need for hormone replacement therapy (HRT).
* Screening for associated medical conditions.
* Providing information on the condition.
* Psychological treatment.
* Disclosure of diagnosis.
* Genetic counselling for other family members.
* Sex assignment for children.
* Gonadal malignancy risk.
* Fertility option.
* Genital surgery options for ambiguous genitalia.
* Vaginal enlargement options.
* Access to peer support.

Accurate diagnosis at presentation is essential and referral to an appropriate pediatric or adult multidisciplinary DSD service (endocrinology, gynecology, surgery, and psychology expertise), is ideal. Individuals with different DSD conditions may require specific medical and surgical treatment. However, all should have access to experienced clinical psychological support. It is no longer considered good practice to withhold condition details from the patient. There have been no long-term psychological outcomes with concealed or revealed diagnosis information; however, it is considered that disclosure should be planned with the opportunity for ongoing dialogue, and in general disclosure is associated with enhanced psychological adaptation. Gonadal malignancy and fertility options vary with the different DSD conditions.

Gynecologist are more often involved in the care of older child developing ambiguous genitalia at puberty, or in follow-up of adults who underwent feminizing genital surgery as children. In many subjects born with ambiguous genitalia, there will be vaginal hypoplasia or agenesis, and the gynecologist will need to discuss the treatment options at appropriate time. Where childhood surgery has been performed, there is a strong possibility that repeat surgery will be required for vaginal stenosis, hypoplasia or genital cosmesis. The treatment is indicated to improve psychological and sexual outcomes.

***46XX DSD – abnormal embryological development of Mullerian ducts & persistence of Wolffian ducts:***

Abnormal development of the Mullerian ducts can lead to a wide range of conditions. Many are subtle variations of normal Mullerian anatomy, and often remain a symptomatic or require no treatment. Others are transverse or longitudinal structural abnormalities or a genesis of parts of the Mullerian ducts and may present to the gynecologist in a variety of ways.

Occasionally, Mullerian anomalies may be associated with other conditions such as renal or spinal abnormalities, or more rarely, developmental defects of the cloaca such as bladder exstrophy, cloaal anomalies or anorectal anomalies. Ovarian development is independent of Mullerian ducts development.

***Mullerian anomalies:***

There have been many attempts to classify Mullerian abnormalities, and the American Fertility Society classification is the most widely used:

1. Hypoplasia/agenesis:
2. Vaginal
3. Cervical
4. Fundal
5. Tubal
6. Combined.
7. Unicornuate:
8. Communicating.
9. Non communicating
10. No cavity.
11. No horn.
12. Didelphus.
13. Bicornuate:
14. Complete.
15. Partial.
16. Septate.
17. Complete.
18. Partial.
19. Arcuate.
20. DES drug related.

***Incidence:***

The prevalence is thought to be 0.5% in the female population. The incidence in women with infertility is substantially higher. The most common is septate and bicornuate anomalies.

***Aetiology:***

The cause of Mullerian anomalies is unknown; they may be due to genetic errors, teratogenic events or a combination of these. Only a minority of cases appear to have family history. It is assumed that there has been failure of fusion of the two Mullerian ducts, failure of one or both ducts to develop, or failure of resorption of the areas of Mullerian duct fusion. The causes of transverse vaginal septae are unknown.

***Presentation & investigation:***

The spectrum of anomalies is wide and around 75% of these women will remain asymptomatic. The remaining 25% will present in a variety of ways. Secondary sexual development is normal as ovarian development and function are independent of Mullerian duct and urogenital sinus growth.

***Presentation of Mullerian anomalies:***

* Primary amenorrhea.
* Cyclical abdominal pain (obstruction to menstruation).
* Severe dysmenorrhea (obstruction to menstrual drainage from one Mullerian duct, e.g. the non-communicating rudimentary horn associated with unicornuate uterus).
* Pelvic mass-hematocolpos (vagina distended with menstrual blood) or hematometra (uterus distended with menstrual blood).
* Menorrhagia.
* Dyspareunia (transverse or longitudinal septae).
* Infertility & recurrent miscarriage.
* Ectopic pregnancy.
* Obstetric complications, e.g. preterm birth, abnormal lie & uterine rupture.

***Investigation of Mullerian anomalies:***

* U/S.
* MRI.
* HSG.
* Sometimes associated with laparoscopy or hysteroscopy.
* Imaging of renal tract is indicated.

***Management:***

Management of these anomalies depend on the type of the anomaly and the presenting features. Symptomatic uterine and longitudinal vaginal septae can be resected hysteroscopically. The horns of the bicornuate uterus can be joined together into one cavity by an abdominal metroplasty. Any form of obstruction to menstrual flow requires surgery to relieve the obstruction & prevent pain and endometriosis. The didelphic uterus is often associated with vaginal septae that can lead to unilateral obstruction and requires careful vaginal surgery to remove the septum. Transverse vaginal septum can be of various thickness, and complete removal is essential to try to prevent a stenotic ring at the site of surgery. For thick transverse septa, a combined abdomino-perineal procedure is often required.

The hymen usually opens after the fifth month of fetal life. An imperforate hymen presents either in neonatal life with a mucocolpos or at puberty with hematocolpos. A purple-blue bulge at the interoitus associated with primary amenorrhea is diagnostic. Surgery to create an adequate window for vaginal drainage cures the problem.

***Rokitansky syndrome (Mayer-Rokitansky-kuster-hauser”MRKH” syndrome):***

***Definition& incidence:***

This condition is agenesis or hypoplasia of the vagina & the uterus. The uterus is either absent or consist of a small central rudimentary uterine bud or bilateral uterine buds on the pelvic side walls. The incidence in UK is estimated as between one in 4000 and one in 6000 females.

The etiology remains unknown. The control mechanism leading to Mullerian duct regression in males and Mullerian duct survival in females are not well defined.

***Presentation & management:***

The usual presentation is primary amenorrhea with normal secondary sexual characteristics. Occasionally, the condition is identifies in childhood. Investigation is as standard for primary amenorrhea, and should exclude intersex conditions and include renal tract imaging due to the 30-40% incidence of associated renal anomalies.

Management needs to encompass both psychological intervention, such as accepting the diagnosis, and live with it, forming relationships and improving sexual function and quality of life outcomes, with the interventions that can be used to enlarge or create vagina (either by surgical vaginoplasty or self-applied vaginal dilatation).

***Incomplete regression of the Wolffian ducts:***

It presents as cysts lateral to the Mullerian duct. Usually asymptomatic, although they can be grow to be large. The epoophoron and paraoophoron can be found beside the ovary in the mesosalpinx. Gartner’s duct (the lower part of the Wolffian duct) cysts can occur anywhere from the broad ligament down to the vagina, and may present as vulval or vaginal masses.

***46XX DSD – Congenital adrenal hyperplasia:***

This condition occurs in XX female due to enzyme deficiency (usually 21 –hydroxylase) in the adrenal gland. The XX fetus proceed down the female development pathway, with ovarian formation and development of Mullerian ducts into uterus, cervix and upper vagina. Owing to the adrenal enzyme deficiency undergoes hyperplasia to try to produce sufficient cortisol. A by-product of this survival mechanism is the production of large quantities of androgens. These high circulating androgen levels lead to masculinizing effects at the external genitalia, and ambiguous genitalia or normal-looking male genitalia at birth.

This is one of the most common DSD conditions, with a UK population prevalence estimated at one in 10000 of whom half are female. It is the only DSD condition that can be life threatening, as unrecognized cortisol deficiency can lead to salt-wasting crisis in the neonate. Management aims to correct the cortisol deficiency and excess androgen production. Gender assignment at birth is usually female due to the presence of ovaries and uterus. Genital surgery to cosmetically feminize the appearance by reducing the clitoral size. At puberty, a review of the vagina is necessary to identify obstruction, stenosis or hypoplasia.

***Other causes of XX fetal virilization***:

In a manner similar to that of CAH, other exogenous causes of androgen (e.g. maternal androgen-secreting tumors or the use of virilising drugs such as Danazole in pregnancy) may rarely lead to masculinization of the external genitalia in an XX fetus.

***Karyotypic abnormalities:***

There are number of karyotypic abnormalities that may present to the gynecologist with an initial complaint of primary amenorrhea. Others will have been diagnosed in childhood but are referred on for further management by pediatricians and endocrinologist.

***TURNER’S SYNDROME (45X AND MOSAICS):***

This is probably the commonest abnormality in females involving the sex chromosomes. Although 1 in 2500 live-born girls are affected, most pregnancies with this abnormality miscarry, probably secondary to major cardiac defects. It is estimated that 10% of all miscarriages have a 45X karyotype. The incidence does not increase with advanced maternal age, but screening early pregnancy for increased nuchal thickness has led to more cases been diagnosed antenatally, as cystic hygroma and non- immune hydrops are frequently features of Turner’s syndrome. Over half of these girls will have some form of mosaicism.

***Physical abnormalities associated with Turner’s syndrome:***

* Growth failure: low birth weight and short stature.
* Ovarian failure: no secondary sexual characteristics in most cases, occasionally secondary amenorrhea in mosaicism.
* Inverted, widely spaced nipples and shielded chest.
* Webbed neck.
* Puffy hands and feet in babies due to lymphedema.
* Low hairline.
* Cubitus valgus.
* Short fourth metacarpal.
* Renal dysgenesis.
* Left-sided cardiac malformations, coaractation of the aorta.
* Nail dysplasia.
* Eye deformities.

Intelligence is normal, but there is an increased risk of impairment of non-verbal skills, e.g. math. Usually diagnosed in infancy and childhood. The girls are usually referred to the gynecologist after optimal growth potential has been achieved. Using growth hormone, for advice about long –term HRT. In most girls ovarian failure will have been occurred early in life, although they have uterus and vagina, they will not develop any secondary sexual characteristics without hormonal supplements. A low dose of estrogen is given initially to encourage steady growth of breasts, this is usually started after the age of 12 years, as the administration of estrogen promotes epiphyseal fusion, which stops further growth. The dose of estrogen is gradually increased over 2 years. The transdermal route is preferred as it has better effect on bone density. The uterus will respond to estrogen therapy, so after 2 years it is necessary to add progesterone cyclically to produce regular endometrial shedding, or in continuous combined regime to suppress endometrial development. HRT should be continued until at least the age of 50 years.

There are numbers of long-term health issues which affect women with Turner’s syndrome:

* Hypertension.
* Coaractation of aorta-11%, bicuspid aortic valve-16%, dissecting aortic aneurysm.
* Diabetes.
* Hypothyroidism.
* Coeliac disease.
* Renal disease.
* Eye problems- red-green color blindness in 8%.
* Osteoporosis.

Ideally women with Turner’s syndrome should have annual check of blood pressure, thyroid function and liver function tests, lipid and glucose. Every 3-5 years they should have ECG, bone densitometry and audiogram.

***47XXX:***

* Occurring in about 1 in 1000 live-born females.
* Normal or tall height.
* They may have genitourinary abnormalities.
* Sexual development is normal.
* Academic performance is below average.
* Premature ovarian failure, so present with secondary amenorrhea needs HRT.
* Give birth to chromosomally normal children.

***46XY:***

The diagnosis is made when a phenotypically normal girl presents with primary amenorrhea or delayed puberty. There are variety of conditions that are associated with this karyotype.

***In androgen insensitivity syndrome,*** the problem lies with the end-organ response to testosterone. It is an X-linked recessive disease due to a mutation in AR gene. The testes are functional and Mullerian inhibition factor is produced, so the uterus and vagina do not develop. Breast development usually take place, as circulating testosterone is peripherally converted to estrogen, but there is absent pubic hair due to abnormal androgen receptors.

***In Sawyer’s syndrome,*** or pure gonadal dysgenesis, there is a lack of functional gonadal tissue and as a consequence, Mullerian structures persist and a normal uterus and vagina are found. Breast development is not normally occur and typically girls present above average height with delayed puberty. Pubic and axillary hair may be present due to the effect of peripherally produced androgens. Treatment is similar to that of Turner’s syndrome, with estrogen to develop the breasts and the addition of progesterone to cause withdrawal bleeding. Donor egg and embryo pregnancies have been recorded.

Streak gonads may be present, as there is a 30% risk of malignancy, gonadectomy is advised.