DISORDERED SEXUAL DIFFERENTIATION
(AMBIGUOUS GENITALIA):AN APPROACH TO DIAGNOSIS AND MANAGEMENT

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The underlying causes of ambiguous genitalia (disordered sexual differentiation) constitute a rare group of disorders that present a diagnostic, investigative and management challenge to any health care provider. To the family they present many psychosocial challenges as they are faced with feeling of disappointment, guilt and confusion. These infants need expedient, accurate and individualised care to appropriately institute their medical treatment, assign a gender, allay parental anxiety and minimise psychological and social complications. The complexity of these conditions necessitates referral to a tertiary centre where there is a team experienced in the management of these infants. The purpose of this review is to outline the development of the reproductive systems with regards to sex determination and differentiation, leading to an understanding of the various causes of sexual ambiguity. This forms the basis for deciding on a rationale investigative and management plan for each patient..

DEVELOPMENT OF THE REPRODUCTIVE SYSTEM

Between 5 and 6 weeks gestation the undifferentiated embryo develops bipotential gonads and both pairs of genital ducts (Mullerian and Wolffian).

Differentiation begins at 6 to 7 weeks gestation and by the end of the first trimester formation of both the internal tracts and external genitalia is complete.

The process of sex determination and differentiation is not a simple one. It requires the precise temporal and dose dependent interaction of numerous genes present on the sex chromosomes and autosomes to ensure complete sexual development.

Figure 1: Ambiguous genitalia - a dilemma

Sex determination *(Diagram 1)* involves transcription and translation of the genetic code to direct the formation of either an ovary or a testis within the urogenital ridge. Simply put, the presence of the sex-determining region of the Y chromosome (SRY) instructs the bipotential gonad to develop into a testis. If there is no Y chromosome but 2 X chromosomes, the bipotential gonad forms an ovary. Both copies of the X chromosome are required for ovarian survival. Streak gonads are the fibrous remnants of ovaries that undergo involution due to the lack of a second X chromosome. In actual fact numerous genes not on the sex chromosomes are involved in sex determination.

The testis and ovary consist of supporting cells (sertoli, granulosa), hormone producing cells (leydig, theca) and germ cells (spermatozoa and ova). The primordial germ cells migrate from the wall of the yolk sac and enter the primary sex cords to develop into ova and sperm.

Sex differentiation follows sex determination, which ended with the formation of the gonad. Sex differentiation is dependent upon the stimulation, production and recognition of hormones from the gonads to complete development of the internal and external genital structures.

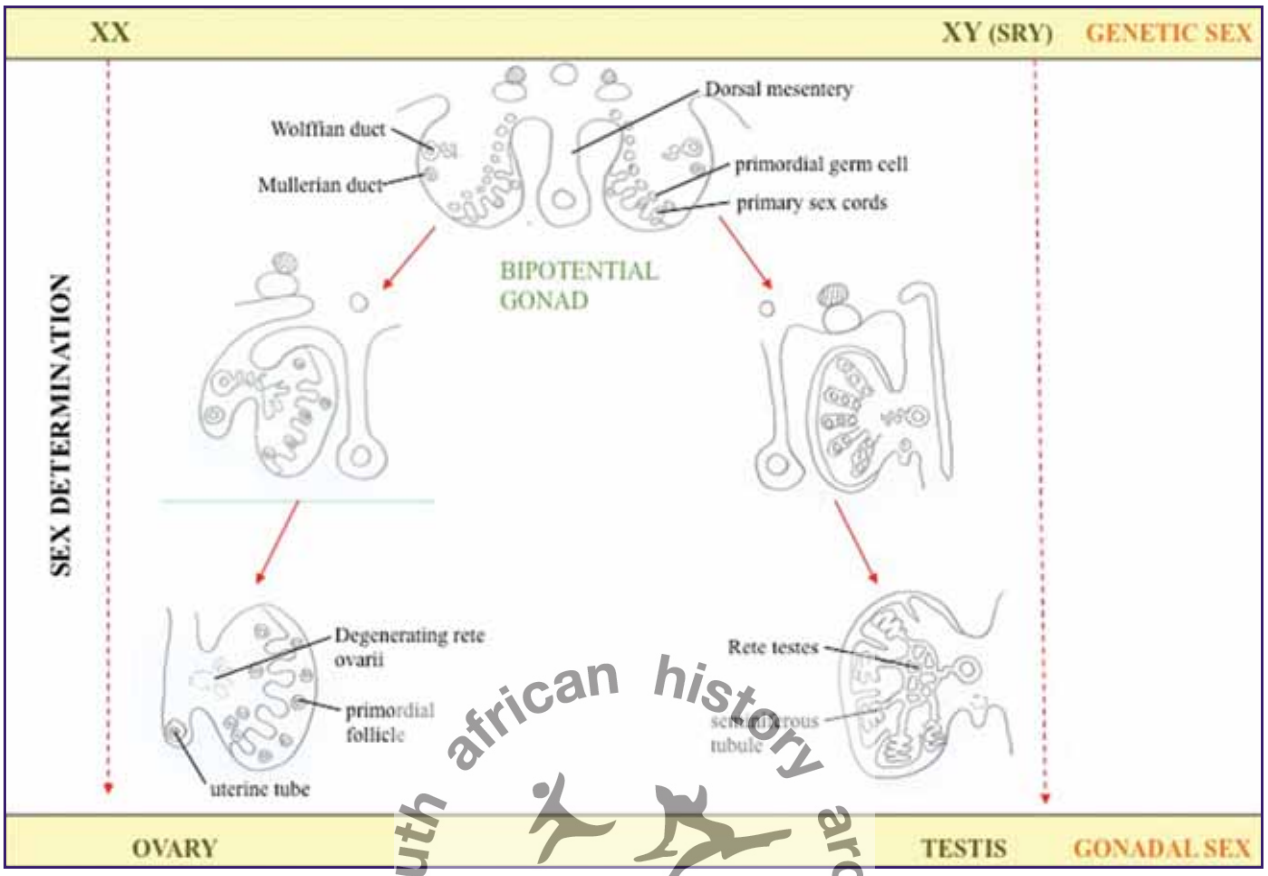

Diagram 1: Sex Determination

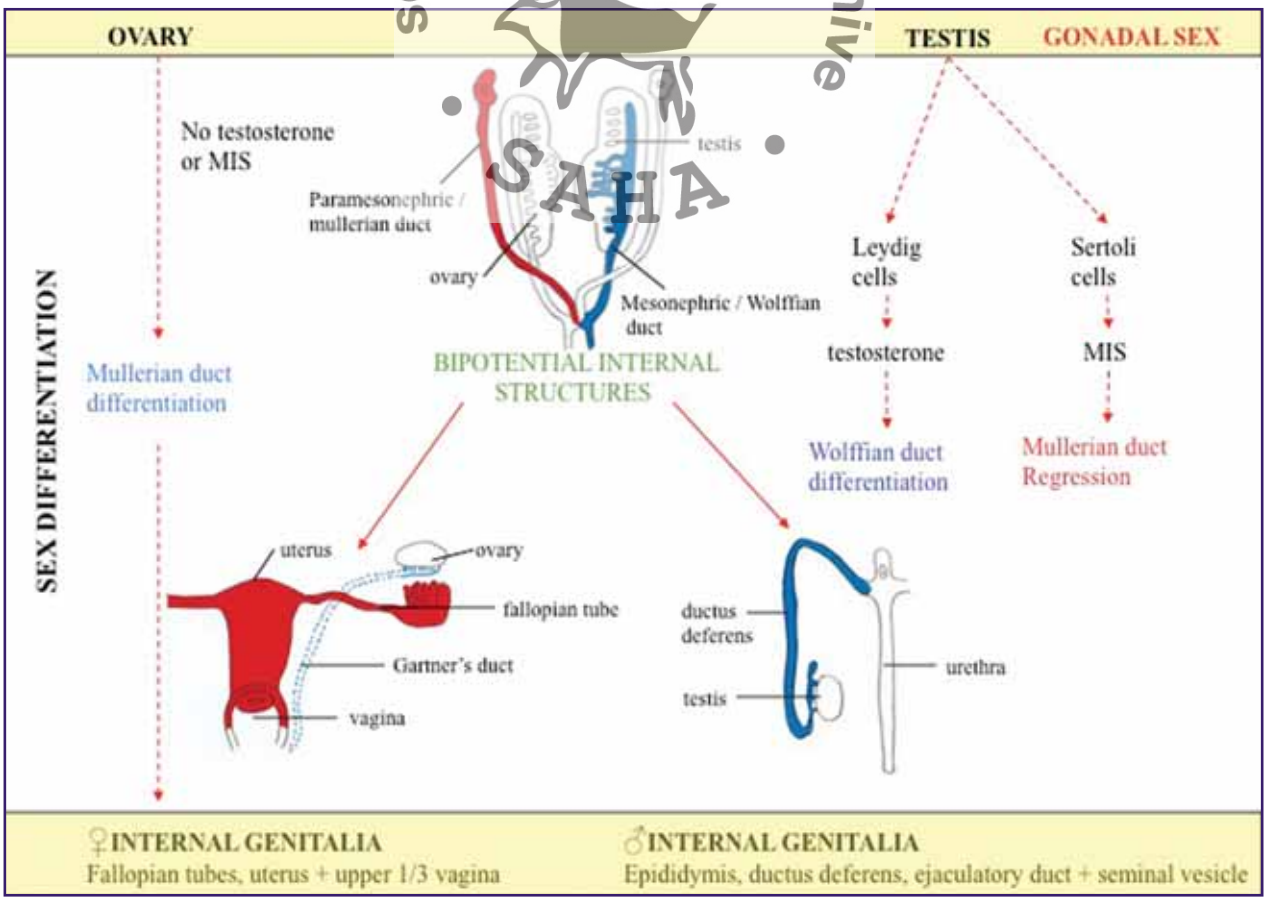

Diagram 2: Sex Differentiation

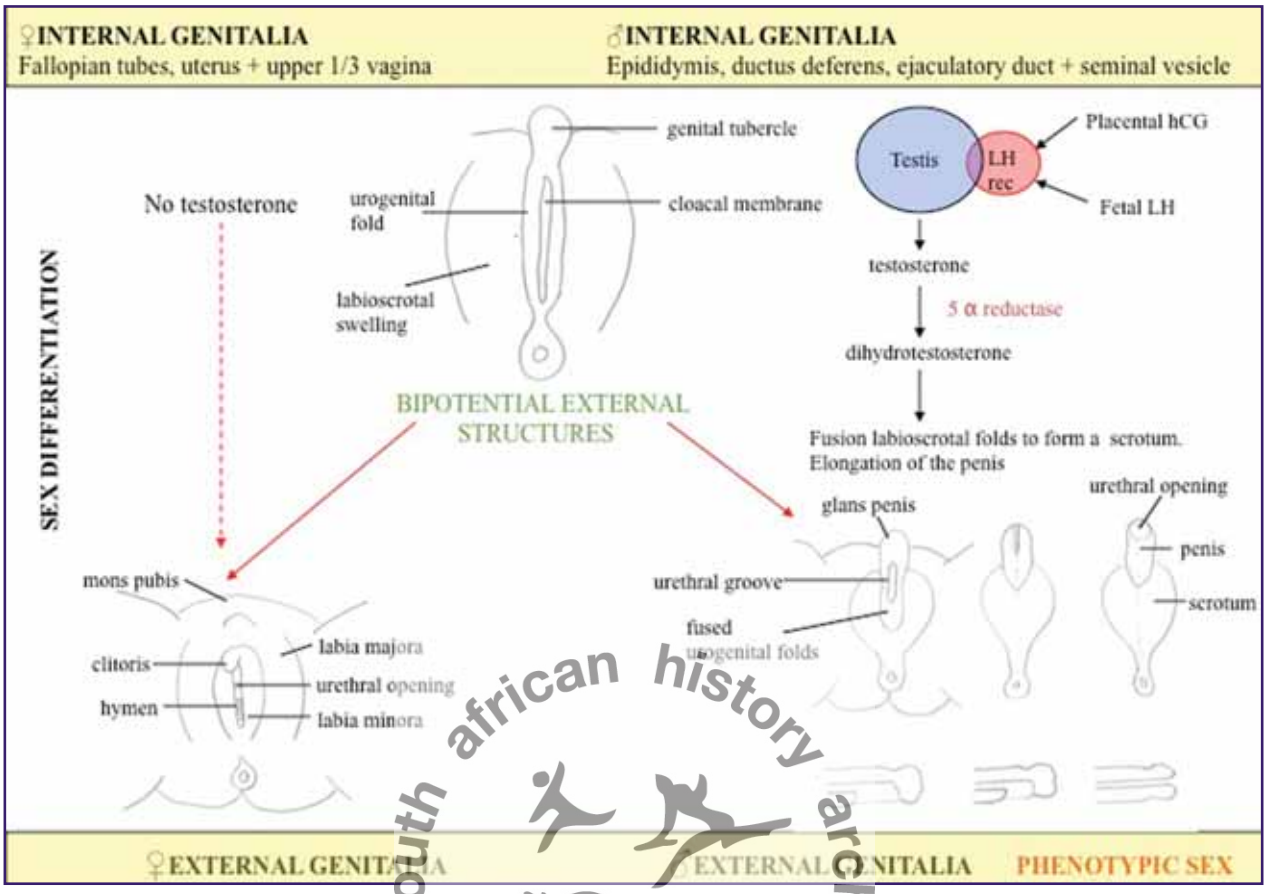

Diagram 3: Sex Differentiation continued

The development of the internal ducts results from a paracrine effect from the ipsilateral gonad. Testosterone secreted by the leydig cells of the testis is responsible for ipsilateral Wolffian duct stabilisation. Mullerian inhibiting substance (MIS) secreted by the Sertoli cells causes regression of the ipsilateral mullerian structures. In the absence of testosterone and mullerian inhibiting substance the default pathway is that of female differentiation with the formation of an ovary and persistence of mullerian structures.

High levels of local testosterone are needed for Wolffian duct stabilisation. For this reason high systemic levels of androgen seen in female fetuses with congenital adrenal hyperplasia (CAH) and maternal androgen exposure do not result in male internal genitalia.

During the first trimester the testes produce testosterone under the stimulation of placental human chorionic gonadotropin (hCG) and later under the stimulation of fetal pituitary derived luitenizing hormone (LH). The 5α reductase enzyme present in the cytoplasm of cells of the external genitalia and urogenital sinus convert testosterone to dihydrotestosterone (DHT), a much more potent steroid than testosterone, which then acts through the androgen receptor to complete the process of male external genital differentiation. The urogenital folds fuse to form the spongy urethra and the labioscrotal swellings fuse to form the scrotum. The genital tubercle elongates to form the penis. This development is complete by 15 weeks. Thereafter testosterone exposure results in elongation of the phallus.

In the absence of this androgen effect, female external genitalia develop. The urethra and vagina open into the urogenital sinus that becomes the vestibule of the vagina. The urogenital folds become the labia minora and the labioscrotal swellings the labia majora. The genital tubercle becomes the clitoris.

CLASSIFICATION OF DISORDERS OF SEXUAL DIFFERENTIATION.

It is easiest to think of the disorders of sexual differentiation (DSD) in 4 groups.

1. Overvirilisation of a female fetus (or 46XX DSD)
2. Undervirilisation of a male fetus (or 46XY DSD)
3. True hermaphrodite (or ovotesticular DSD)
4. Gonadal dysgenesis

1. Virilisation of a female fetus

(See diagram 4)

Virilisation of a female may range from mild clitoromegaly to labial fusion with a urogenital sinus to a normal looking penis with a well-developed but empty scrotum. Virilisation is

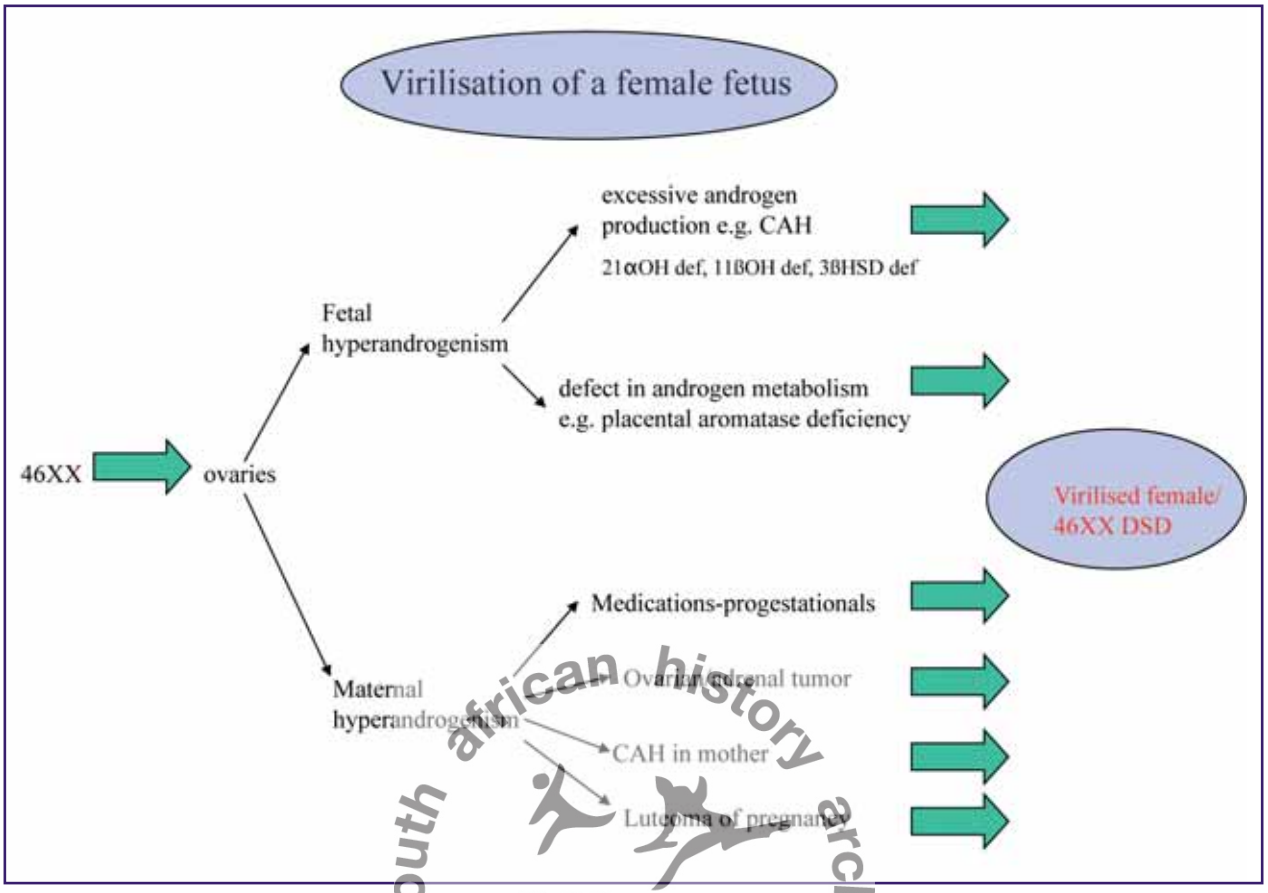

Diagram 4: Overvirilisation of a Female Fetus

dependant on the timing, duration and level of testosterone exposure. If this exposure occurs during the first trimester, labial fusion and phallic enlargement will occur; later exposure will only result in clitoral enlargement

CAH is the most common cause of virilisation in a female fetus.

CAH is an autosomal recessive disorder with a high carrier frequency in the Ashkenazi Jewish population. The genetic mutation leads to a deficiency in enzyme function in the cortisol and aldosterone pathways in the adrenal gland. Reduced negative feedback from cortisol on the pituitary gland causes an increase in adrenocorticotropin hormone (ACTH), which leads to adrenal hypertrophy (hyperplasia). The enzyme blockage results in

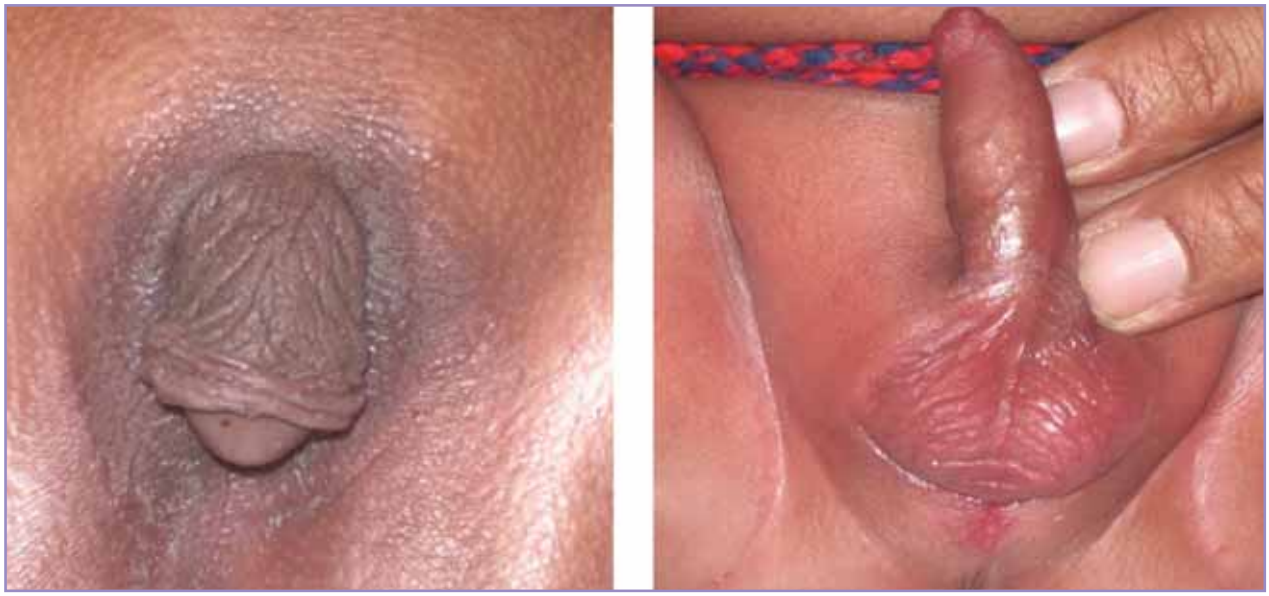

Figure 2: Spectrum of virilisation of a female. (46 XX with CAH)

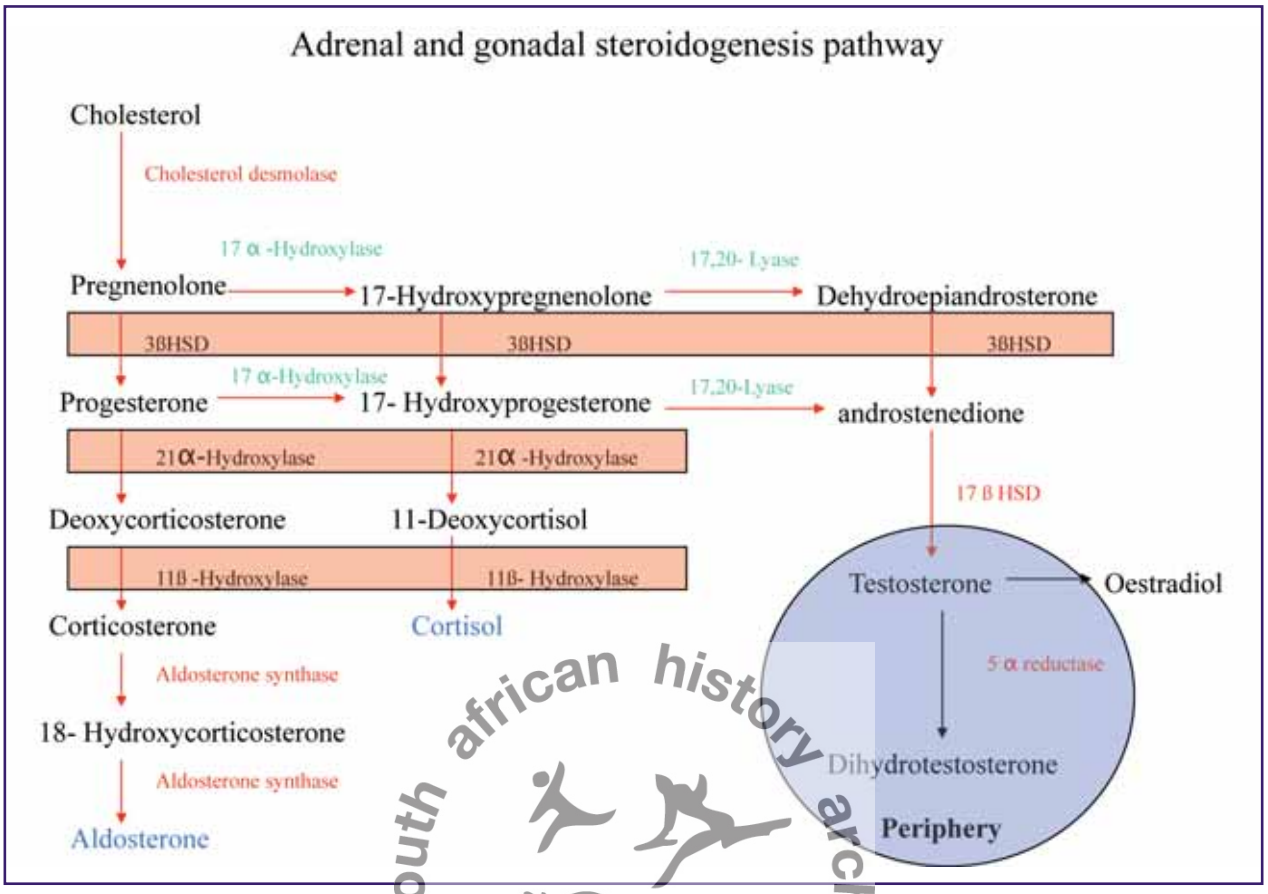

Diagram 5: Adrenal and gonadal pathway of steroid synthesis

deficient production of adrenal hormones in the affected pathways with overproduction along remaining pathways. Deficient aldosterone production causes salt-wasting and deficient cortisol production an adrenal crisis. The overproduction of adrenal androgens: DHEAS, androstendione and testosterone lead to virilisation.

21 hydroxylase (21OH) deficiency accounts for more than 90% of cases. There is a spectrum of 21OH deficiency from mild to complete deficiency. These infants have decreased mineralocorticoids and glucocorticoids with increased androgens. This leads to salt wasting (decreased Na and increased K) and vascular collapse at 1-3 weeks after birth. Female infants are virilized with normal internal genital tracts (no MIS). Male infants have no genital ambiguity and hence go unrecognised and often demise from a salt wasting crisis. They present with vomiting, lethargy and poor feeding and are often misdiagnosed as a gastroenteritis or pneumonia. The clue to the diagnosis is the low serum Na and high K. The increased androgens cause accelerated childhood growth with early epiphyseal fusion.

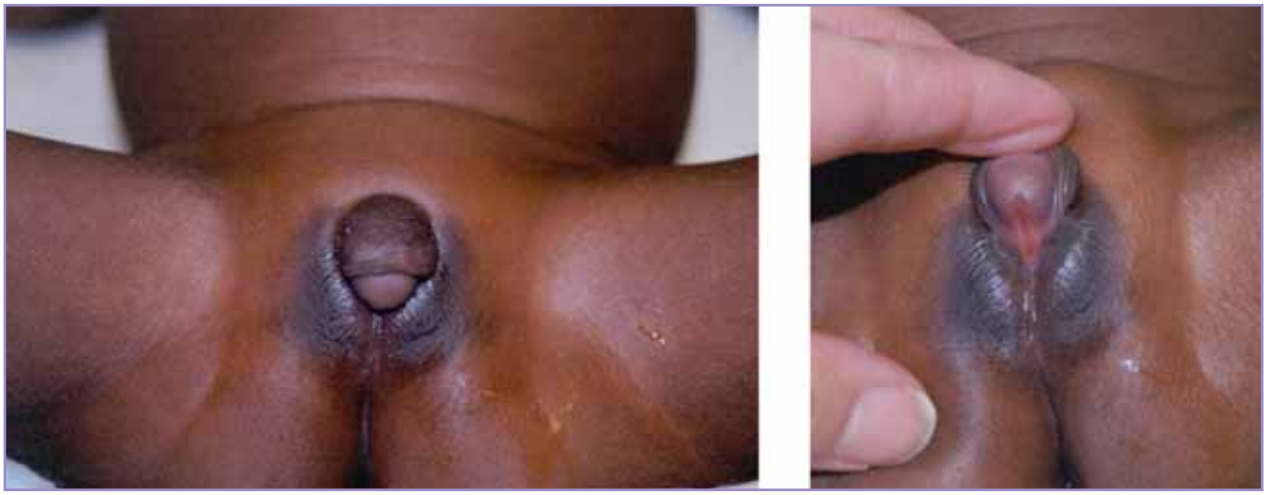

Figure 3: 21 alpha hydroxylase deficiency in a female infant

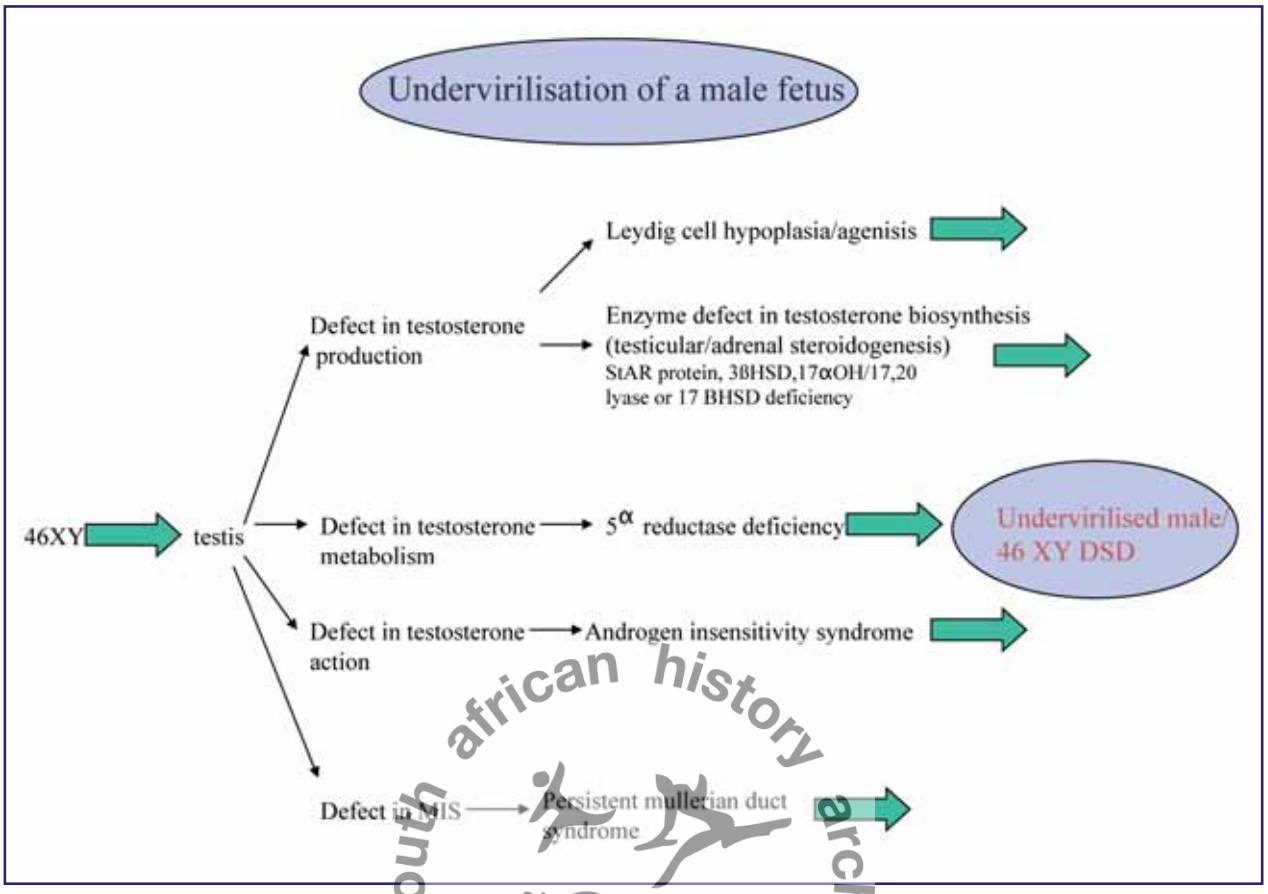

Diagram 6: Undervirilisation of a male fetus

11αOH deficiency accounts for less than 10% of cases. There is accumulation of deoxycorticosterone (DOC) proximal to the deficient enzyme. At high levels DOC exerts a mineralocorticoid effect leading to salt retention and hypertension with decreased plasma renin activity.

3βHSD deficiencies is rare. The increase in the weak androgen DHEA is sufficient to virilise a female fetus but insufficient to completely develop a male fetus, hence both males and females will have ambiguous genitalia.

2. Undervirilisation of a male fetus

(See Diagram 6)

During the first trimester beta-human chorionic gonadotrophin(≤hCG) stimulates testosterone production by the Leydig cells. This is converted by five alpha(5±) reductase to dihydroxytestosterone(DHT), which is responsible for differentiation of the external genitalia. During the second trimester luteinising hormone (LH) from the fetal pituitary stimulates testosterone production, which causes phallic enlargement and testicular descent. Because external genital development is complete prior to 15 weeks hypopituitarism with LH deficiency does not cause genital ambiguity but may result in a micropenis.

Defects in testicular development as well as defects in testosterone production, metabolism and action lead to a wide spectrum of undervirilized phenotypes. Isolated urogenital defects such as micropenis, undescended testes and hypospadius need to be differentiated from hormonal disorders and require further investigation.

Androgen insensitivity syndrome (AIS) is an x-linked recessive disorder caused by mutations of the androgen receptor on the long arm of the X-chromosome. Mild defects (partial AIS) may present as boys with pubertal delay, moderate defects with genital ambiguity whilst the most severe (null) mutations result in a female phenotype (complete AIS). These infants have female external genitalia with a blind vaginal pouch. Testes are usually located intra-abdominally but may present as inguinal masses. There are normal Wolffian duct derivatives and absent Mullerian structures owing to normal testosterone and MIS production. At puberty they develop a female body habbitus with spontaneous breast development due to aromatisation of androgens to estrogens. They have scant sexual hair (lack of dermal androgen receptors) and primary amenorrhoea as they have no uterus. Gender identity is unequivocally female despite the high circulating androgen levels as testosterone receptors in the brain are also affected.

3. True hermaphrodite / ovotesticular DSD

This condition is common in central and southern Africa. By definition there is both ovarian and testicular tissue present, either in the form of a testis and an ovary, a testis and an ovotestis, an ovary and an ovotestis or two ovotestes. The external genitalia can be normal or ambiguous. Ovaries are intra-abdominal but testes and ovotestes may descend. In Africa 46XX is the most common karyotype while 46XX/46XY is more common in North America and Europe. In some cases an autosomal recessive and rarely autosomal dominant pattern of inheritance can be supported. Endocrine function parallels histological findings with testosterone levels normal to decreased and FSH and LH normal to decreased. Ovotesticular DSD should be suspected when there is biochemical evidence of testicular tissue in the presence of Mullerian structures. A definitive diagnosis is made on histology..

4. Gonadal dysgenesis

This is a spectrum of disorders, which lead to the maldevelopment of the gonads and subsequently varying degrees of DSD.

46XX gonadal dysgenesis
46XY complete and partial gonadal dysgenesis
45XO streak ovaries = Turner syndrome
45X/46XY mixed gonadal dysgenesis
47XXY seminiferous tubular dysgenesis = Klinefelter syndrome

DIAGNOSIS

The diagnosis of DSD needs to be efficient and accurate to expediently treat the infant and to counsel the parents appropriately. These infants should be referred to centres experienced in the management of DSD.

a) History

As many of these disorders are autosomal recessive a good family history is necessary including any consanguinity, infant deaths, abnormal genitalia and infertility.
Medications taken during pregnancy and virilization of the mother will give clues in certain cases.

b) Examination

A good general examination needs to be carried out looking for dysmorphic features, e.g. Turner syndrome. Abnormal pigmentation suggests raised ACTH in patients with CAH.

Examination of the external genitalia
Inspect the external genitalia under good lighting.

Figure 4: Examination of External Genitalia

Phallus
Measure phallic length - the phallus must be stretched with a ruler pressed against the pubic ramus and the prepubic fat pushed away. Measure along the dorsum to the tip of the glans. A phallus less than 2, 5 cm long in a term male is considered a micropenis. A clitoris more than 1 cm long is abnormal.

Measure phallic breadth - roll the corporeal bodies between your fingers to assess girth. An enlarged clitoris is greater than 6mm in breadth. Preterm infants have a prominent clitoris because clitoral size is fully developed by 27 weeks and there is less fat in the labia majora.

Orifices
Look for the position of the urethral meatus and a vaginal orifice.

Labioscrotal folds
The labioscrotal folds must be assessed for asymmetry and degree of rugosity of the skin. In virilised females look for the degree of labial fusion and formation of a urogenital sinus.

Gonads
Place the infant in the frog-leg position.
Palpate along the inguinal canal to the scrotum/labial area sliding 2-3 fingers with varying pressure along the groin. Use the opposite hand to hold onto any gonad palpated. Note the size and consistency of any gonad found.

General rules of thumb
- If a gonad is palpated, an overvirilised female can normally be excluded.
- A well-developed phallus indicates that there was significant testosterone exposure in-utero, but does not predict future testosterone production.
- An asymmetric scrotum is due to asymmetric gonads.
- A palpable gonad is almost always a testis or an ovotestis

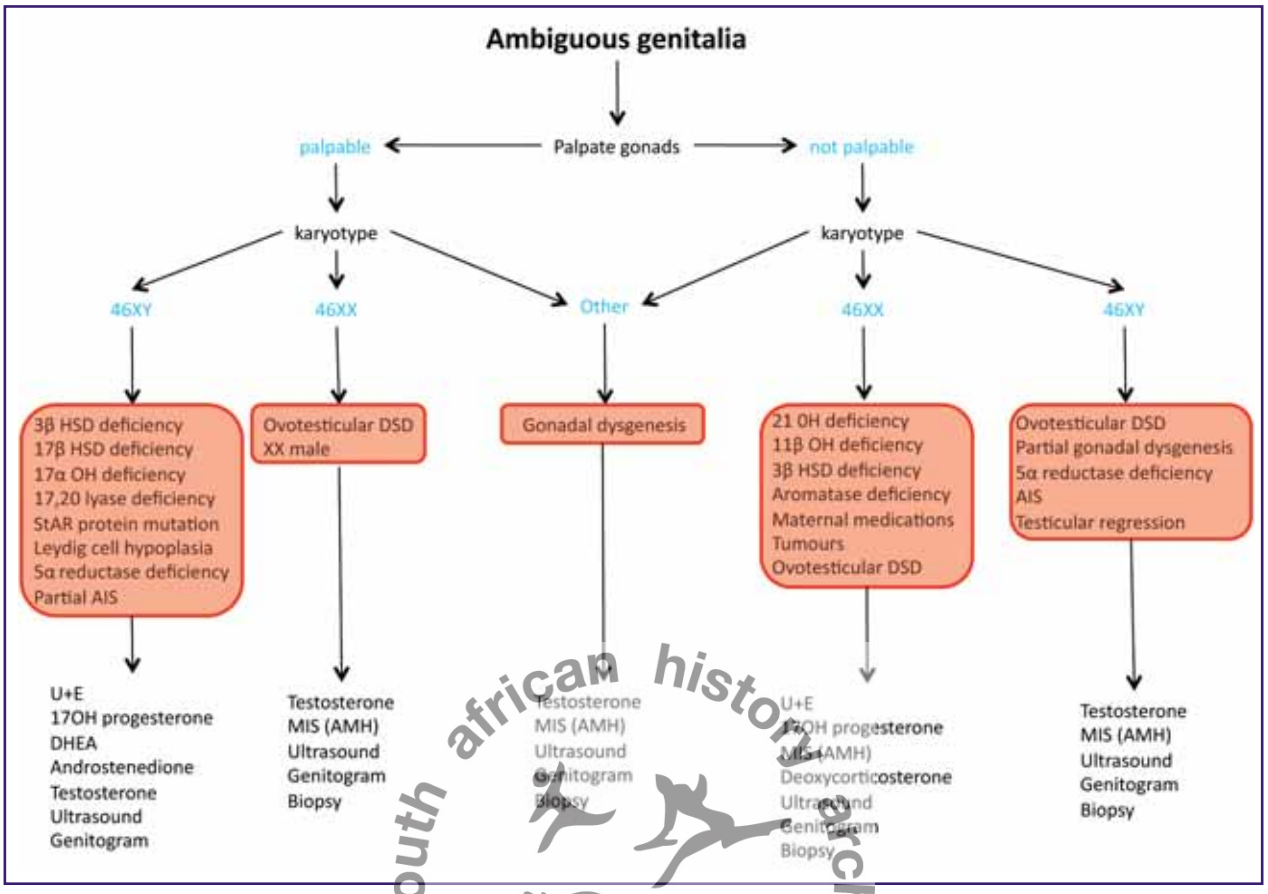

Diagram 7: Investigative approach to ambiguous genitalia

c) Investigations)

Who should be investigated?

1) all infants with ambiguous genitalia
2) females with clitoral hypertrophy, fused labia, a palpable gonad or an inguinal hernia
3) males with impalpable testes, severe hypospadius or hypospadius with undescended testes
4) infants with a family history of a DSD

A small number of infants will still be missed and present during adolescence with amenorrhoea, virilisation, inappropriate breast development or cyclical hematuria. *(ref. CPP 2001)*

BLOOD TESTS

All infants need a karyotype within 24 hours of delivery and results should be available within 72 hours. There is a spectrum from 46XX males to 46XY females with anything in between. The karyotype gives clues to the underlying etiology but does not determine the sex of rearing.

Clues:

- 46XX is most likely an overvirilised female or a true hermaphrodite
- 46XY an undervirilised male, a true hermaphrodite or a testicular dysgenesis
- 46XY/46XX a true hermaphrodite or gonadal dysgenesis
- 46XY/46XO a gonadal dysgenesis

Gene probe studies are becoming available as most genes in the pathway of normal sexual differentiation have been identified and cloned, but are not usually commercially available.

A 17OH progesterone, dehyrdroepiandrosterone (DHEA), androstenedione and a testosterone level form a good baseline screening profile that will guide the differential diagnosis and indicate what further investigations should take place. With these results CAH can be diagnosed as well as a defect in testosterone biosynthesis, metabolism or action. Testosterone levels can be done at birth and again between 6 weeks and 4 months due to the physiological surge in LH. At other times an hCG stimulation test is needed to test the androgen production axis.

Urgent electrolytes should be sent after 24 hours to determine if the neonate is salt wasting.

At this stage referral should be made to a specialist centre for further investigations and management.

IMAGING

The purpose of imaging is to gain more information about the internal genital structures, primarily the presence or absence of a uterus. Localization and identification of gonads is difficult with all modes of imaging and requires an experienced radiographer.

Ultrasound is the preferred mode of imaging. Although MRI and CT give good definition, the long acquisition time means that heavy sedation or general anaesthetic is required. Ultrasound also prevents unnecessary ionising radiation. Palpable gonads should be viewed with ultrasound because testes, dysgenetic testes and ovotestes can sometimes be differentiated. Calcification in a gonad suggests gonadoblastoma transformation.

Genitograms are done is specialist centres and are tailored to the specific patient. In the case of CAH it is important to determine the level at which the vagina opens into the urogenital sinus for the planning of surgery. Genitograms are useful in determining if there is a vagina and to outline the uterine canal and fallopian tubes. If a fallopian tube is found it can be deduced that the ipsilateral gonad did not produce adequate MIS, or an MIS receptor mutation exists.

Most cases will have a definitive diagnosis by this stage, however further investigations, which include laparoscopic visualisation and biopsy of gonads may be required.

MANAGEMENT

The management of ambiguous genitalia in an infant requires a team approach. The team includes a family doctor, a paediatric endocrinologist, a surgeon, a geneticist and a social worker, together with the child and their parents or caregivers.

Sex assignment

All infants must be assigned a sex before discharge. When considering sex assignment there are a few important factors to consider:

1) **The fertility potential**
   All virilised females due to CAH or maternal androgens are potentially fertile and are normally raised as females.

2) **Adult sexual function**
   The phallus size and functional potential is important when considering male sex of rearing.

3) **Gender identity**
   The development of gender identity is a gradual process and has considerable plasticity. It is now apparent that testosterone imprinting on the fetal brain may play a significant role in determining male gender identity and male typical behaviours. Nature appears to be more powerful than nurture. Long-term gender identity in testosterone-exposed infants however cannot be predicted; so feminising genitoplasty should be delayed until gender identity is clearly established.

4) **Endocrine function**
   The ability of gonads to produce hormones in line with the chosen sex of rearing needs to be considered, although hormone replacement is possible.

5) **Parental wishes**
   Education of the parents is paramount for them to have the ability to contribute to the decision making process with any degree of informed consent.

Sex assignment should only take place once ALL the data is available and the team is in agreement. In the minority of cases it may still be difficult to assign a gender e.g. in partial androgen insensitivity. The parents need to be informed that gender assignment is provisional and that the final “true” gender may only become apparent at a later stage..

Surgery

Surgery, if required, should be performed at specialist centres that have the necessary expertise and experience. Genitoplasty in virilized females remains controversial. New surgical techniques in clitoroplasty allow for preservation of erectile function and innervation. Vaginoplasty and labioplasty can be performed in the newborn period or at puberty. Males may need chordee and hypospadius repair, which is normally undertaken at 6 to 18 months of age. Orchidopexy of undescended testes is best done at the time of initial gonadal biopsy. Gonads that are histologically abnormal on biopsy or dysgenetic gonads with a Y-chromosomal component should be removed because of the increased risk of gonadoblastoma transformation.

Sex steroid replacement

Sex steroids are often needed to initiate puberty, induce secondary sexual characteristics, ensure a pubertal growth spurt and optimise bone mineral accretion.

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DISORDERED SEXUAL DIFFERENTIATION (AMBIGUOUS GENITALIA)
Which of the following statements is/are true?

1) Sexual differentiation begins at 6-7 weeks gestation. Of the following, what causes the Wolffian (Mesonephric) duct to differentiate into male internal genitalia?
a) Mullerian inhibiting substance
b) Testosterone
c) 5α reductase
d) Dihydrotestosterone
e) Sex determining region Y chromosome

2) In congenital adrenal hyperplasia caused by 21 hydroxylase deficiency which of the following are true?
a) Plasma ACTH levels are raised
b) Female infants may have virilisation
c) Male infants may present with hyponatraemia and hyperkalaemia
d) Male infants may have ambiguous genitalia
e) Is associated with high plasma cortisol levels

3) A term newborn presents with ambiguous genitalia characterised by micropenis and bilateral undescended testes. Which of the following diagnosis could fit?
a) Fetal pituitary LH deficiency
b) Fetal pituitary GH deficiency
c) XX CAH 21 hydroxylase deficiency
d) XY CAH 21 hydroxylase deficiency
e) E Turner syndrome

4) Which of the following statements is true?
a) A palpable gonad is usually a testis
b) 3 HSD deficiency causes sexual ambiguity in males and females
c) In the absence of ovaries the female genital system does not develop
d) In undervirilised males (46XY), the infant should always be raised as male
e) CAH is an autosomal dominant disorder

5) When considering sex of rearing the following factors play a role
a) The future fertility potential
b) The gender identity
c) The parent's wishes
d) The surgeon's ability with regards to genitoplasty
e) None of the above