Ambiguous Genitalia and Intersex

Introduction

Intersex disorders are rare congenital malformations and the reported incidences of the different malformations are mostly based on small series. Bertrand et al. [1] and David et al. [2] reported about their 25 years’ experience of 325 infants with intersexuality. Altogether 167 patients were diagnosed with female pseudohermaphroditism, of which 80% were found to have congenital adrenal hyperplasia (CAH). Hyperandrogenism due to overproduction by the mother was found in 3% only. Abnormal male sex differentiation was diagnosed in 150 children, 27% of those with either gonadal dysgenesis or male pseudohermaphroditism. Hormonal problems like testicular deficiencies were found in 13% and androgen insensitivity syndrome in 16% [1, 2]. It can be challenging to determine the correct gender at birth and even more difficult to establish the right diagnosis inside the various intersex disorders.

In order to understand the wide variety of genital ambiguity it is crucial to be aware of the regular sexual differentiation process. Appreciating the embryologic development and the hormonal influence on the development of the fetus in utero is essential to counsel the parents appropriately about sex assignment, necessary treatments and the surgical possibilities [3]. Sexual differentiation of the embryo starts after the 7th week of gestation. Before that time point the urogenital ridge in both sexes holds...
mesonephric (Wolfian) and paramesonephric (Mullerian) duct structures. The differentiation into each gender is dependent on the exact sequence of events occurring at specific time points, dependent on chromosomal, hormonal and hormonal receptor function. While the genetic sex and the gonadal differentiation are determined by the sex chromosomes, the phenotype is dependent on the influence of the hormonal secretions from the testes. The sex-determining region on the Y chromosome (SRY) is the main gene required to differentiate the bipotential gonad into a testis [4, 5] which than give raise to the Sertoli cells. In the absence of male Mullerian-inhibiting substance (MIS) from the Sertoli cells and testosterone from the Leydig cells a female phenotype will develop. In males the Wolfian ducts develop under testosterone production while the Mullerian ducts regress under the influence of MIS. In females on the other hand the lack of testosterone and MIS cause the Wolfian ducts to regress and the Mullerian ducts to develop, respectively. The differentiation of the external genitalia is under the control of dihydrotestosterone (DHT), a metabolite of testosterone under the enzymatic control of 5α-reductase. Much like the gonads, the external genitalia have bipotential in the fetus and develop into a male phenotype only under the influence of DHT, while a lack of DHT will irreversibly lead to a female external phenotype in both chromosomal sexes [6]. The complexity of the physiologic sexual differentiation explains the wide range of intersex disorders and the variety of presentation among the subgroups. The most definite and undisputable factor is the genetic sex, determined by the karyotype. Therefore, it seems logic to classify the intersex disorders into female pseudohermaphrodites (FPH) with a female karyotype (46,XX) and masculine features caused by an excess amount of androgens in utero (e.g. CAH) and male pseudohermaphrodites (MPH) with a male karyotype (46,XY) and female features due to a lack of testosterone or androgen receptors [7]. Some patients cannot be placed into these two categories, e.g. those with chromosomal abnormalities like Klinefelter’s (47,XXX) or Turner syndrome (45,X0). Additional groups, which are not considered to be ambiguous, include phenotypical females with vaginal atresia (Mayer-Rokitansky-Küster-Hauser syndrome), phenotypic males with additional female internal structures due to lack of MIS, or males with a micropenis.

The birth of a child with intersex is a true emergency situation. Acute life-threatening situations can arise in children with CAH from electrolyte disturbances caused by salt-wasting 21-hydroxylase deficiencies. But besides the medical emergencies, it is of utmost importance to identify the correct diagnosis as early as possible in order to counsel the anxious parents appropriately towards the sex of rearing.

Evaluation and Diagnostic Pathway of the Newborn with Ambiguous Genitalia

The birth of a newborn with ambiguous genitalia often comes as a surprise for the parents, treating physicians and nursing staff. Although some authors report that 60% of affected children are diagnosed prenatally [8], many parents are faced with the situation at birth.

The child should immediately be transferred to a medical center familiar with the diagnosis and management of intersex conditions. Not only life-threatening situation as in salt-wasting CAH have to be addressed immediately, but also the psychological devastation of the parents.

For the referring inexperienced physician, it is often best not to confuse the situation by discussing the issue with the parents but rather transfer the child to a center. Until a diagnosis is made and a sex of rearing has been decided, the parents should be encouraged not to name the child or register the birth to prevent later changes and explanations to their social surroundings [9].

The evaluation should begin with a detailed history from the parents towards ambiguity, hirsutism or unexplained sudden infant death in the family. Additionally the maternal exposure to hormonal medications or cortisone during the pregnancy has to be evaluated.

Serum studies should be initiated, including electrolytes to rule out salt-wasting CAH, testosterone, DHT, 17-hydroxyprogesterone and certainly a karyotype to determine the chromosomal sex. The testosterone level can help to determine if the intersex condition is due to a lack of androgen or cortisone synthesis or rather due to a receptor defect. Key to the physical examination is the palpation of the groin and scrotal or labial folds to determine the presence of the gonads. The outer genitals are inspected and presence and length of the phallus noted. A rectal exam can confirm the presence of a uterus and cervix and should always be performed. The examination should be done in the presence of the parents to familiarize them with the situation.

Further evaluations include a pelvic and abdominal ultrasound for the evaluation of female internal organs and to rule out possible accompanying urological or adrenal anomalies. The urogenital sinus can be anatomically defined by a retrograde genitogram, localizing the position and entrance of the urethra and vagina into the
sinus. It is important that the surgeon planning to do the reconstructive procedure is present to assist the radiologist with the genitogramm. In rare cases where a definite diagnosis cannot be determined an exploratory laparoscopy with possible biopsy of the gonads can become necessary.

**Male Pseudohermaphroditism**

If gonads are palpable, the presence of a Y chromosome is almost certain, since ovotestes or ovaries usually do not descend. Consequently FPH can be excluded and the differential diagnosis shifts towards inadequate production of testosterone or androgen receptor deficiencies in a genetically male. Almost all MPH lack Mullerian structures due to MIS production from the Sertoli cells, but the insufficient testosterone stimulation leads to an inadequate male phenotype. Testosterone is responsible for the formation of the epididymis, vas and seminal vesicles while the external masculinization is dependent on DHT metabolized from testosterone via 5α-reductase. Therefore patients with 5α-reductase deficiency present with female external genitalia but regular male internal genital structures, while individuals with complete androgen receptor defects block the external and internal masculinization completely. These patients are also described as a complete form of androgen insensitivity syndrome (CAIS) and have normal or elevated levels of testosterone, a regular female phenotype with normal breast development and a short blind-ending vagina. But they have no internal female genitalia due to intact MIS production from the Sertoli cells and they usually do not become apparent until puberty when they are evaluated for primary amenorrhea.

Incomplete forms of AIS (PAIS) on the other hand rather present with an incomplete masculinization than a female phenotype and can have a varying range of internal male structures. Frequent features include phenotypic males with a penoscrotal hypospadias and a bifid scrotum, often containing small testes. Additionally a small, blind-ending vaginal pouch without evidence of other female structures can be found.

A very rare condition, persistent Mullerian duct syndrome, is found in genetically and phenotypically normal males. They have a deficiency of MIS and form complete internal female genitalia but usually are not detected until undergoing surgery for a hernia or an undescended testis.

Children with complete gonadal dysgenesis (Swyers syndrome) [10] can only be identified by their XY karyotype, since the complete absence of Sertoli and Leydig cells in the bilateral streak gonads leads to a complete female phenotype with normal internal and external female genitalia. But since they lack ovaries they do not produce sufficient estrogens and therefore become apparent at puberty due to primary amenorrhea, missing breast development and elevated serum gonadotropins [11]. Interestingly, pregnancies are possible after transfer of fertilized eggs in the functioning uterus. The most important fact in complete gonadal dysgenesis is the 30% risk of developing germ cell tumors, mostly gonadoblastomas [12].

**Female Pseudohermaphroditism**

The most common form of FPH in over 80% is caused by the various enzymatic defects of CAH. The majority of CAH cases (>90%) are caused by 21-hydroxylase deficiency and can clinically impress with a wide spectrum of clinical variants. The enzymatic defect results in the inability to produce cortisol, leading to an increase in ACTH, stimulating the adrenal glands. The enzymatic block together with the glandular stimulation results in an increase of 17-hydroxyprogesterone, progestins and androgen precursors. While the androgen excess is responsible for the masculinization of the fetus, the progestins are accountable for the salt loss of the kidneys. In the mild forms the loss can be compensated by increased production of aldosterone, while the severe forms are commonly known as salt-wasting CAH, which can be fatal if not compensated after birth. Less common are the 11β-hydroxylase (≤5%) and the 3β-hydroxysteroid deficiencies [7].

The clinical manifestation of all CAH forms is characterized by the virilization of the outer genitalia. It can be mild with a clitoral hypertrophy or a fusion of the posterior labial folds only, but also as severe as a male phenotype with bilateral undescended testes. On further examination, all patients with a regular karyotype of 46,XX have regular ovaries, Mullerian structures and regressed Wolffian ducts, since the missing SRY prevented the development of Sertoli cells and MIS, respectively.

Pure gonadal dysgenesis in genetic females is characterized by bilateral streak gonads but regular female external and internal genitalia. However, affected females usually present with sexual infantilism consisting of hypergonadotropic hypogonadism, primary amenorrhea and lack of breast development. They are closely related
in their sexual development to patients with Turner syndrome (45,X0) but lack the syndrome-typical features [13].

**True Hermaphroditism**

The unique feature in true hermaphrodites is the coexistence of regular testicular and ovarian tissue in the same individual. The chromosomal distribution in about 65% of individuals is 46,XX, although 46,XY and mosaic forms like 45,XO/46,XY are possible. The patients can either have a testis on one and an ovary on the other side or they have a combination of both tissues in the same gonad (ovotestis). The external phenotype is dependent on the amount of androgens produced by the gonads, but most patients are masculinized and about 75% are raised as males [14]. Almost all patients have a uterus and a urogenital sinus. On the side of the ovary, usually a fallopian tube is found while the testis side always has a vas. If an ovotestis is present, more than 65% will have a fallopian tube only, while the rest either have only a vas or both structures [15]. A cunning and interesting fact is the existence of a testis in 46,XX patients, however the testis part of the ovotestis is usually dysgenetic and even pregnancies are possible from follicles of the ovarian part of the ovotestis [16].

**Disorders of Gonadal Differentiation**

Conditions in this category include seminiferous tubule dysgenesis, like in Klinefelter’s syndrome (47,XXY), with small firm testes, gynecomastia, azoospermia, eunuchoid stature and increased serum gonadotropins. Other chromosomal aberrations are seen in Turner syndrome (45,X0) with short stature, lack of secondary sexual characteristics, webbed neck, and coarctation of the aorta.

Mixed gonadal dysgenesis in most cases is characterized by a chromosomal distribution of 45,X0/46,XY. They usually present with an intraabdominal testis on one and a streak gonad on the other side [17]. Equivalent to PAIS, patients present with a wide range of incomplete masculinization, dependent on the degree of the gonadal mal-development and the amount of androgens and MIS secreted [15]. The Mullerian structures on the testis side are usually regressed but can be in place on the streak gonad side.

**Management of Intersex Disorders and Sex Assignment**

The wide range of chromosomal, hormonal and clinical presentations along with the expectations and cultural circumstances of the family require an individualized treatment of each patient. After the clinical diagnosis is determined a careful evaluation of the situation with the help of psychiatrists, endocrinologists and pediatric urologists has to be provided to the parents who ultimately have to decide the appropriate gender for their child. Since 70% of patients have FPH, the actual need for assigning a different sex of rearing from that of the karyotypical gonadal sex is quiet small.

Lately there has been an active discussion in the literature concerning sex assignment of children born with intersex disorders or other anomalies involving the outer genitalia like cloacal extrophy or traumatic penile loss. In the past it was believed that children without adequate male genitals should be assigned to the female sex of rearing [18, 19]. This common view was challenged by reports of chromosomally male patients raised in the female sex who, although they did not know about their previous sex assignment, converted back to their chromosomal sex later in life [20–22]. Schober [23] reported about 10 intersexuals who for the most part preferred sex partners that had the same sex as their own assigned ones at birth. Additionally the author concludes that the individuals are successful in partnering and are satisfied with their sexual function and that hormonal factors may influence the behavior more than the correct anatonical situation. Migeon et al. [24] followed 39 46,XY patients with sexual ambiguity. Individuals assigned to the male gender had significantly more surgeries and a worse surgical appearance than females, but the majority was satisfied with their body image in both groups. Although 23% were dissatisfied with their sex of rearing determined by their parents, the majority of patients was exclusively heterosexual and viewed themselves according to their assigned sex.

Adding to the controversy whether feminizing genitoplasties should be performed is a recent study by Crouch et al. [25]. Using questionnaires and functional assessments, 6 women who underwent genitoplasties in the past were found to have severely impaired sensory and sexual function. But it has to be considered that most of these patients were operated upon years ago by children’s surgeons. These observations along with pressure from support groups question the practice of sex reassignment and pose the question whether children with ambiguous genitalia should be raised in an intersex gender until they can
make a decision themselves which gender they choose [26]. Nevertheless, in Migeon’s study [24], with many years of follow-up, none of the affected individuals wanted a third gender and all agreed that surgery should be completed in infancy or early childhood. Other groups are concerned that the pressure and confusion of the intersex condition could traumatize the child and the parents more than the risk of choosing a gender for the child [8, 27]. Until more data are available it will remain a controversy and irreversible surgical procedures like removal of the gonads or an underdeveloped phallus are currently rarely considered.

The Committee of Genetics and the sections of Endocrinology and Urology of the American Academy of Pediatrics set up a number of considerations when deciding the appropriate sex of rearing. According to the Committee, children with CAH or exposure to maternal androgens should be raised in the female sex of rearing since they are potentially fertile. The Committee acknowledges the potential of testosterone imprinting in utero and the well-known fact of more male-typical behavior of CAH girls, but also points out that they generally do not demonstrate sexual identity problems [9]. Other considerations are the capacity for normal sexual and endocrine function as well as the potential for malignant change of the gonads.

More data and studies are needed before general guidelines and recommendations can be made. Until then the parents have to be individually counseled and if desired, a contact can be established with support groups or other affected parents.

However, once a sex has been assigned, the treating physicians should respect and support the decision and carry out the necessary surgical and medical steps.

**Surgical Management of Ambiguous Genitalia**

The timing of the surgical procedures and the hormonal replacement is important from a surgical and social point of view [28]. Surgical modifications usually concern the gonads and the outer genitalia and often the presence of a urogenital sinus. If a sex reassignment of a chromosomal male is decided, the gonads should be removed in the first weeks of life. If a male sex of rearing is chosen, the gonads and the outer genitalia and often the presence of a urogenital sinus can be separated in the first 18 months of life.

In CAH girls glucocorticoid and, if necessary, mineralcorticoid replacement is started after diagnosis and the effectiveness controlled by routine serum 17-hydroxyprogesterone and androstenedione measurements [29]. Lately the use of adrenalectomy as a treatment for CAH has been proposed but has to be evaluated on higher numbers and with longer follow-up to evaluate the long-term consequences of removing the adrenal glands [30].

**Feminizing Genitoplasty**

**Clitoroplasty.** In the past clitoroplasties were performed when the child was around 1 year of age [31]. However, advances in pediatric anesthesia and surgical techniques cause most surgeons today to do the procedure in the newborn period [32]. Formerly, the clitoris used to be simply amputated if the surgery was performed by an adult surgeon operating on an infant. Today, the surgery is performed by pediatric surgeons and reconstructive nerve sparing procedures are favored with the subtunical excision of the erectile tissue [33]. Gearhart et al. [34] described a nerve sparing procedure that protects the neuromuscular bundle and allows regular nerve conduction and sensation and preserves later orgasmic function.

**Vaginoplasty.** Some authors prefer to correct the external genitalia in a single stage procedure in the newborn period to take advantage of all native genital tissue and avoid scarring [35]. Other groups advocate waiting with the vaginal reconstruction until puberty when vaginal dilatations are more feasible to prevent vaginal stenosis [36].

The technique used is dependent on the anatomic findings. A simple cut-back vaginoplasty can be performed when the vaginal orifice is separated from the urethra and already reaches the perineum but is covered by fused labia. If the vagina enters the urogenital sinus in a low position, skin flaps can be created and placed into the posteriorly opened urogenital sinus [37]. However, if this procedure is performed in a high confluence sinus, problems with hypospadias of the urethra and subsequent vaginal voiding and incontinence can occur [38]. In vaginas with a high take off from the sinus, a pull-through vaginoplasty [39] with complete separation of the vagina from the urogenital sinus or a complete urogenital mobilization without separation of the vagina can be performed [40].

In patients with absent or rudimentary vaginas a complete replacement is necessary. Progressive dilation using dilators is a noninvasive method but requires continuous dilatation and is not commonly used anymore [41]. Later McIndoe [42] introduced a split-thickness skin graft sewn over a vaginal mold to fit in the dissected rectovesical space [43]. Initial results were favorable, but continuous dilatations and stenosis remain problematic. The inter-
position of a sigmoid bowel loop as vaginal replacement is commonly used today since it eliminates the need for dilatation while the intestinal mucus provides lubrication of the neovagina and allows excellent results [44, 45].

**Labioplasty.** The labioplasty is performed at the time of the vaginoplasty and gives the external genitalia a normal female appearance. The dorsal hood of the foreskin is incised and brought down to create labia minora, the labia majora are moved inferiorly using a YV plasty [28].

**Phalloplasty.** Testosterone stimulation in puberty can increase the size of the phallus and should be substituted. Radical mobilization at the time of hypospadias repair can increase phallic length.

Phalloplasty using vascularized skin flaps with later insertion of penile prosthesis can be applied after puberty in patients with complete absence of penis.

However, currently there is no tissue available to increase the size of an underdeveloped phallus in humans. But several groups are investigating the possibilities in tissue engineering to replace or create lost or mal-developed tissue or organs. Kropp et al. [46] used porcine intestinal submucosa for the repair of corporal deviations. Kim et al. [47] seeded chondrocytes isolated from human ears on rod-shaped biodegradable polymer scaffolds and implanted them subcutaneously into athymic rats. The same group successfully seeded human cavernosal smooth muscle and endothelial cells on three-dimensional acellular collagen matrices derived from donor corpora. They concluded that this might be useful techniques in the future to replace lost erectile tissue for penile reconstruction [48]. Currently human embryonic stem cells are under investigation to regenerate the bladder in a rat model. Therefore, with further research in tissue engineering it might be possible in the future to replace and enhance underdeveloped phalluses and vaginas [49].

**Conclusion**

The evaluation and diagnosis of the patient with genital ambiguity is challenging. Affected children should be immediately transferred to a medical center familiar with the management of these complex disorders. Accurate diagnosis, close psychological support and information are necessary to help the parents to deal with the situation. Surgical correction techniques have developed and can provide satisfactory cosmetic and functional results.

The discussion of the management of patients with intersex disorders continues. Current data challenge the past practice of sex reassignment. Further data are necessary to formulate guidelines and recommendations fitting for the individual situation of each patient. Until then the parents have to be supplied with the current data and outcome studies to make the correct choice for their child.
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