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Review Article

Current concepts in hypospadias surgery

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Abstract: Anatomical anomalies in hypospadias are an abnormal ventral opening of the urethral meatus, abnormal ventral curvature of the penis and abnormal distribution of the foreskin around the glans with a ventrally deficient hooded foreskin. The techniques of hypospadias surgery continue to evolve. The current standard of care for hypospadias repair includes not only a functional penis adequate for sexual intercourse and urethral reconstruction offering the ability to stand to urinate, but also a satisfactory cosmetic result. Tubularized incised plate repair has been the mainstay for distal hypospadias. In cases of proximal hypospadias, one-stage repairs such as the Duckett repair or the Koyanagi repair have been well established, while two-stage repairs remain important alternatives. Whether dorsal plication or ventral lengthening should be used to correct penile curvature is still controversial, and long-term results are required. Efforts have been made in this decade to improve cosmetic appearance, constructing a slit-like meatus or performing foreskin reconstruction, and to prevent onerous complications.

Key words: hypospadias, penis, surgery, urethra.

Introduction

The techniques of hypospadias surgery continue to evolve. Today, the standard of care for hypospadias repair includes a functional penis adequate for sexual intercourse, urethral reconstruction offering the ability to stand to urinate, and a satisfactory cosmetic result.

Three periods of irregular development mark the history of hypospadias repair; the nineteenth century, during which the principles of this surgery were remarkably good but the technical facilities were insufficient;[1,2] the first two thirds of the twentieth century, during which many procedures were published, often advocating multi-stage reconstruction, the use of inadequate tissues for urethroplasty, and accepting imperfect results; and finally, the 1980s, during which the modern principles were standardized,[3,4] offering better and more functional results, and the 1990s, during which sophisticated and simplified principles were the mainstay of hypospadias repair,[5,6] using some of the nineteenth century ideas which were revived successfully.[1,2]

Preoperative evaluation

The essential step in patient evaluation is to identify the meatal position, the presence or absence of penile curvature, adequacy of skin for repair, and penile size.

Hypospadias is generally an isolated anomaly, but it may present as one of the features of over 200 different syndromes.[7] Because associated malformations of the urinary tract are common in proximal or complex hypospadias, they require a thorough evaluation. If one or both testes are impalpable, it may signify the presence of disorders of sex development, such as mixed gonadal dysgenesis or adrenogenital syndrome caused by congenital adrenal hyperplasia.[8] In this instance, there should always be a karyotype study and radiographic examination, such as ultrasonography and magnetic resonance imaging of the urinary tract and internal genital organs.[9]

Fluoroscopic imaging, such as retrograde urethrography or voiding cystourethrography, is rarely carried out in patients with distal hypospadias; however, it is useful to evaluate the presence or absence, the position and the size of the prostatic utricle and to identify possible future problems in regard to urinary stasis, infection, stone formation, and difficulty with catheterization in patients with proximal hypospadias in advance of the operation.[10,11]

Furthermore, endoscopic examination of the urethra at the time of surgery is useful in patients with proximal hypospadias to clarify the presence of a prostatic utricle and guide the catheter into the bladder.[12]

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Timing of surgery

Factors that can influence the timing of hypospadias repair include the environment in which the patient will be managed, anesthetic risk, penile dimensions and the psychological effect of genital surgery. After the age of 6 months, the risk of anesthesia is no greater than when older,[13] provided the anesthesia is administered by a specialist anesthetist and the patient is cared for in a designated pediatric facility.

It has become well accepted that early hypospadias repair is desirable. Manley and Epstein reported reduced patient anxiety when repairs were carried out before 18 months of age.[14] Belman and Kass found no increased incidence of surgical complications when surgery was carried out between 2 and 11 months of age.[15] Based on the consideration of emotional, cognitive, and psychosexual factors, Schultz advocated that hypospadias surgery should be completed by 15 months.[16] A report from the American Academy of Pediatrics (AAP) in 1996 suggests that the optimal time for elective male genital surgery is between 6 and 12 months.[17]

Methods of surgical treatment

Trends in the construction of a neourethra

Surgical procedure for distal hypospadias without penile curvature

According to the investigation by Cook *et al.*, 92 % of pediatric urologists prefer using a tubularized incised urethral plate (TIP) to repair distal hypospadias without penile curvature, while 4% use the flip-flap (Mathieu) technique (Fig. 1), 3% prefer the onlay island flap, and 2% carry out meatal advancement and glanuloplasty or urethral advancement.[18]

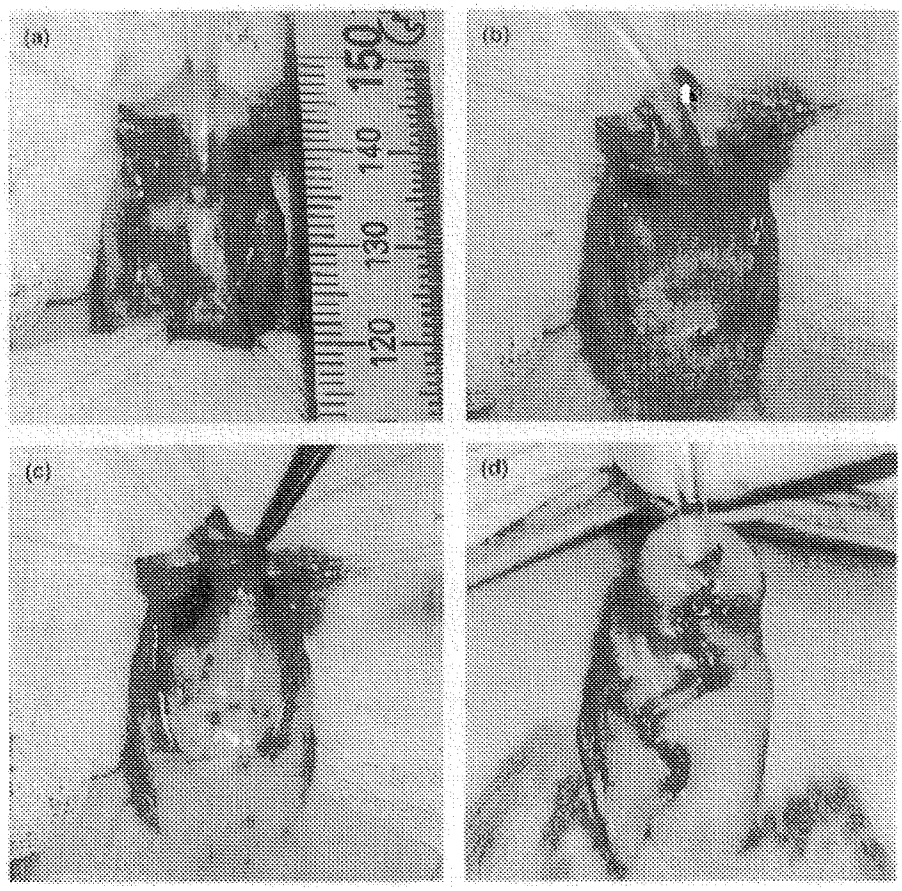

Fig. 1 Flip-flap Mathieu urethroplasty. (a) Ventral meatal-based skin flap is developed. Glans wings are dissected sufficiently after two paramedian incisions are made along the urethral plate and extended to the tip of the glans. (b,c) Ventral flap is flipped and anastomosed to the plate. (d) Glans wings are re-approximated over the neourethra.

Surgical procedure for mid-shaft hypospadias without penile curvature

Similar to the repair of distal hypospadias, the majority of pediatric urologists (83%) prefer TIP to repair mid-shaft hypospadias without penile curvature, while 16% prefer an onlay island flap.[18]

Surgical procedure for proximal hypospadias without penile curvature

For proximal hypospadias without penile curvature, 43% prefer TIP repair (Fig. 2) and 43% prefer onlay island flap repair (Fig. 3), respectively.[18]

Transverse onlay island flap repair was developed two decades ago by Elder *et al.*,[4] and subsequently, the indication for the onlay island flap has been extended to more proximal hypospadias.[19]

History is now starting to repeat itself. After TIP urethroplasty was initially indicated for distal hypospadias,[5] Snodgrass *et al.* broadened the indication to proximal hypospadias.[6] They reported few complications in a series of proximal hypospadias repair, which reflects the good vascularity of the urethral plate and tension-free closure of the neourethra.[6]

Surgical procedure for proximal hypospadias with moderate penile curvature

For proximal hypospadias with moderate penile curvature, 35% of pediatric urologists prefer the onlay island flap and 24% use the TIP.[18]

Braga *et al.*[20] conducted a comparative analysis of 35 patients repaired with TIP versus 40 patients repaired with onlay island flap urethroplasty for penoscrotal hypospadias. As a result, complications occurred in 60% of the TIP group and 45% of the onlay island flap group.

Surgical procedure for proximal hypospadias with severe penile curvature

For proximal hypospadias with severe penile curvature, 40% of pediatric urologists prefer the transverse tube island flap, called Duckett repair, transecting the urethral plate[3] (Fig. 4); however, 3% use TIP repair and 11% onlay island flap, preserving the urethral plate. On the other hand, nearly half carry out staged repair.[21,22] Some pediatric urologists recently converted from the Duckett repair to the Koyanagi repair or its modification for proximal hypospadias with severe penile curvature,[23] because in tube island flap repair, a stricture often occurred at the circumferential end-to-end anastomosis between the native meatus and the neourethra, and diverticular change of the neourethra tended to occur. The Koyanagi repair is urethroplasty with a parameatally based and fully extended circumferential foreskin flap and does not require end-to-end anastomosis, and one-stage repair is available even for scrotal or perineal hypospadias.[24] However, because of the incidence of complications, which seem to be caused by insufficient blood supply to the neourethra, some modifications were developed with the preservation of vascularity to the peripheral portion of the neourethra[25–27] (Fig. 5).

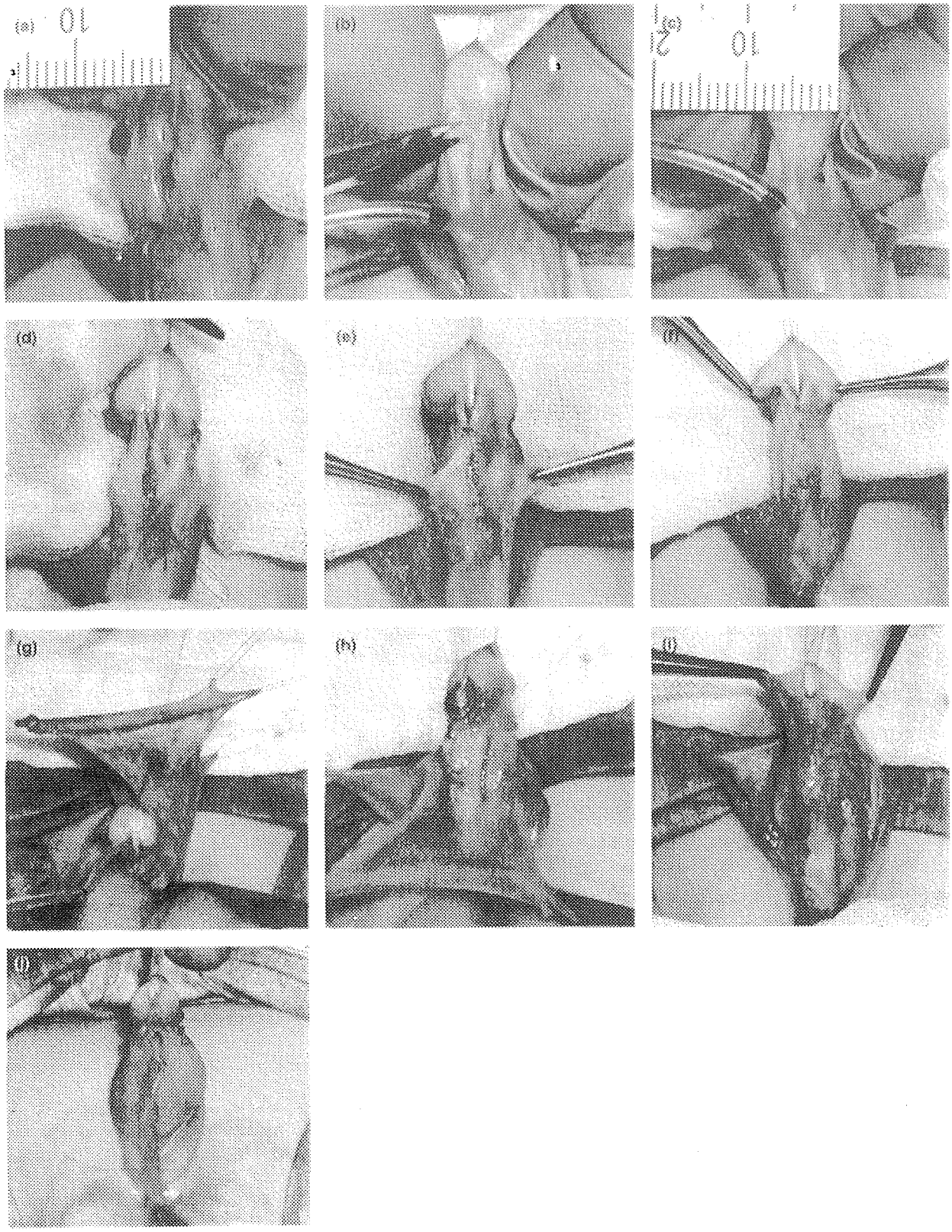

Fig. 2 Tubularized incised plate urethroplasty (TIP repair) (a) A U-shaped skin incision is made surrounding the meatus and the width of the urethral plate is 4 mm. (b,c) Uethral plate is incised in the midline and widened to 8 mm. (d) Urethral plate is tubularized over a 6F stent. (e) Periurethral tissue is closed over the first suture line and diverted spongiosa are taken off the tunica albuginea on both sides of the urethral plate. (f) Spongiosa are wrapped over the neourethra; spongioplasty. (g–i) Dartos pedicle flap obtained from dorsal prepuce and shaft skin is button-holed and transposed ventrally to cover the neourethra. (j) Completed repair.

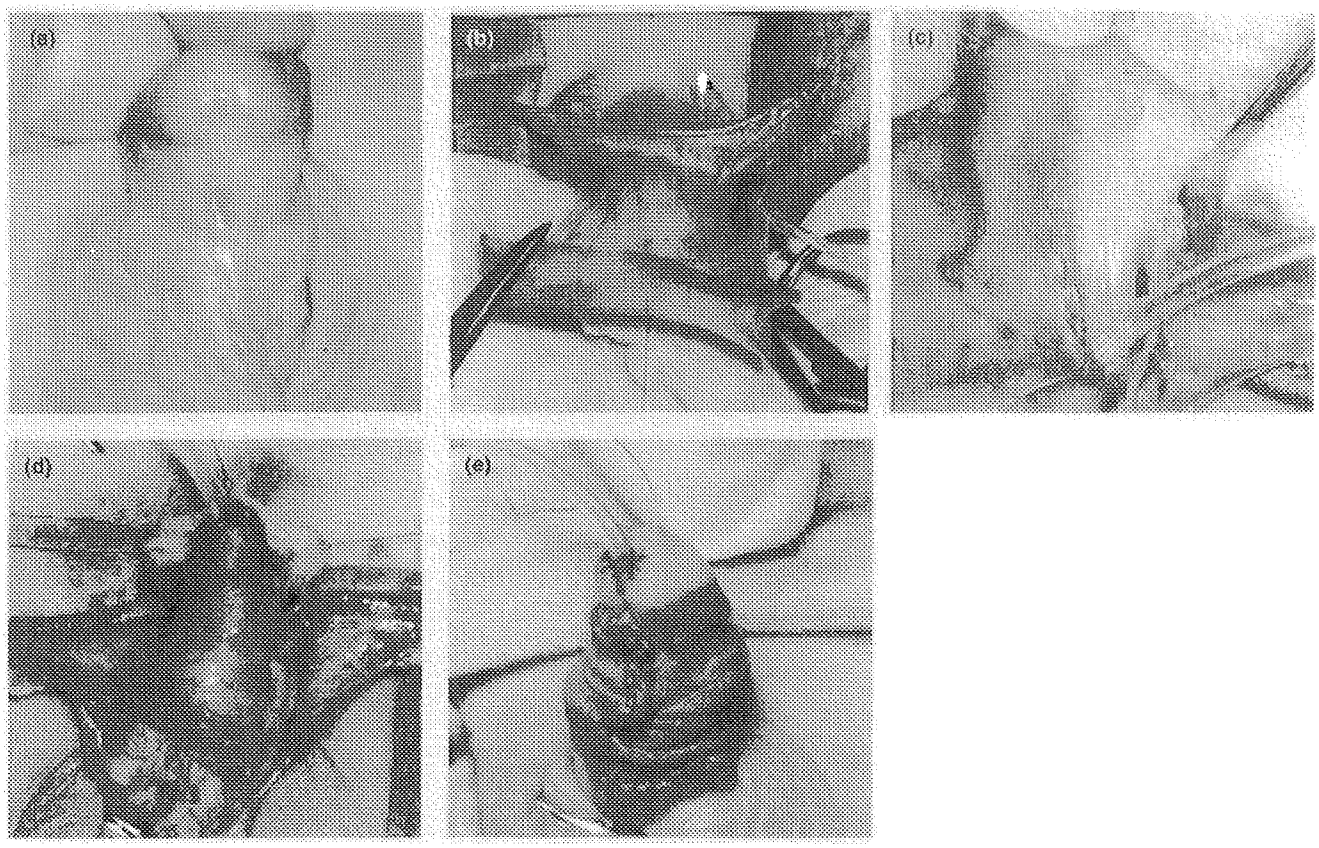

Fig. 3 Transverse preputial onlay island flap repair. (a) Preoperative appearance of proximal hypospadias. (b) The inner preputial onlay flap with its pedicle is developed and separated from the dorsal penile skin. (c) The onlay island is sutured to the urethral plate. (d) After the completion of neourethral reconstruction, the inferolateral border of the onlay pedicle is advanced as second layer coverage of proximal and longitudinal suture lines. (e) Glans closure and skin closure are completed.

Trends in the correction of penile curvature

Severe penile curvature is challenging for pediatric urologists. Recently, there has been increasing support for the hypothesis that curvature is related to primary corporeal disproportion.[28-30] Severe penile curvature is usually secondary to corporeal disproportion with a shorter ventral surface of the corpora cavernosa.[31]

Unfortunately, during the 20th century, the published reports did not provide any objective guidelines as to the degree of congenital penile curvature, in an infant or child, which inhibits sexual intercourse in adulthood. Additionally, published reports lack long-term follow-up of correction of penile curvature with or without hypospadias.

With this background, in 1999, a survey of members of the American Academy of Pediatrics Section on Urology attempted to establish a consensus on the management of penile curvature, including determining the severity that mandates intervention, as well as the optimal technique for correction.[32] Bologna *et al.* found that 99% of pediatric urologists would intervene and most would perform some form of dorsal plication for the treatment of moderate chordee (30 to 40 degrees) associated with hypospadias. Severe chordee (greater than 50 degrees) was approached ventrally by 54% of pediatric urologists. An international cohort conducted by Cook *et al.* was concordant with these results:[18] 83% of pediatric urologists would repair moderate curvature (30 to 40 degrees) via a dorsal approach while 69% would correct severe curvature (greater than 50 degrees) using a ventral approach.

Dorsal plication

A useful approach for penile curvature is to shorten the dorsal surface of the corpora cavernosa.

Since Nesbit[33] first used dorsal plication sutures to straighten a bending penis after removing elliptical segments of the tunica albuginea from the lateral aspect of each corpus cavernosum in 1965, the Nesbit procedure has become one of the most useful maneuvers for correcting congenital or acquired chordee.[34] In 1994, Baskin *et al.*[35] modified the Nesbit procedure, avoiding elliptical excisions in favor of plicating the incised tunica albuginea, fixing the outer edges with permanent sutures. Initially, Baskin *et al.* recommended that dorsal plications of the tunica albuginea on each side of the penis at the 2 o'clock and 10 o'clock positions be taken at the point of maximal bend and Buck's fascia with the neurovascular bundles be elevated on each side.[35] In 2000, however, after histological experiments, Baskin *et al.* recommended that penile plication be conducted at the 12 o'clock position without incision of the tunica albuginea.[36] Baskin *et al.* made no attempt to mobilize the dorsal veins or incise the tunica albuginea, but made the stitches and tied them off.[36] On the other hand, Hayashi *et al.* cut Buck's fascia longitudinally at 12 o'clock, made tiny parallel incisions on the tunica albuginea after dividing the dorsal veins bilaterally and approximated the outer edges of the incisions[37] (Figs 6,7).

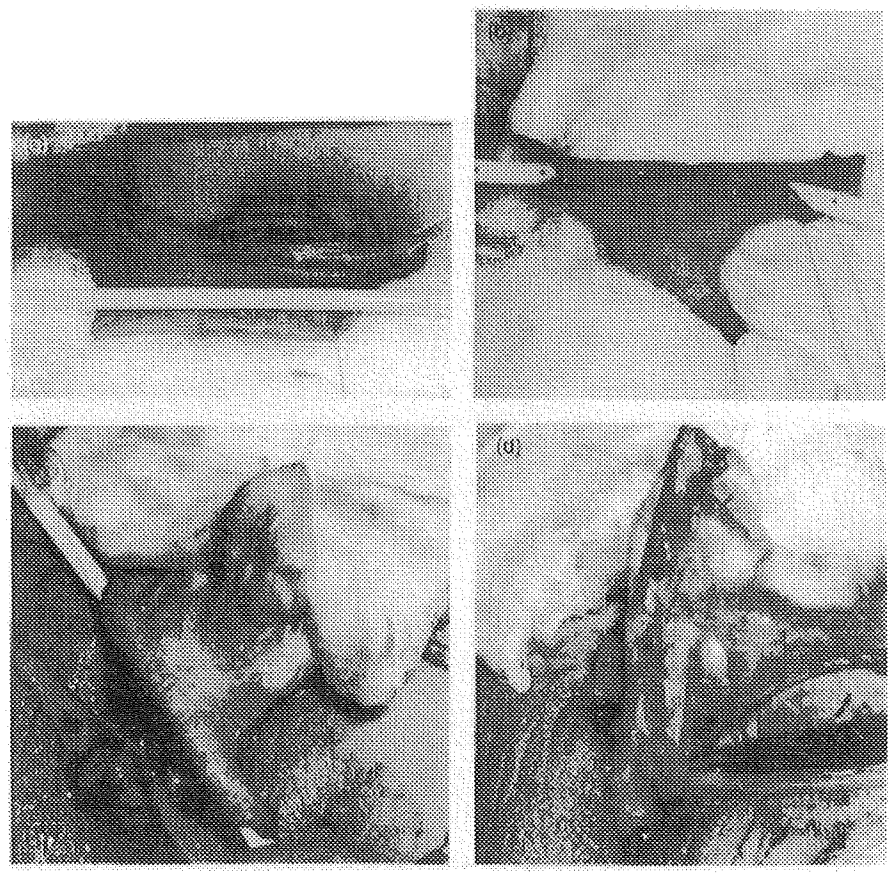

Fig. 4 Transverse preputial tube island flap repair (Duckett repair) (a) Rectangular flap is prepared from dorsal prepuce. (b) Running subcuticular suture tubularization is carried out over an appropriately sized tube. (c,d) Pedicle tube is transposed ventrally through a buttonhole and anastomosed to the proximal end of the native urethra.

Ventral graft or flap

Conversely, Devine *et al.* reported that it is illogical to intervene on the dorsal radius of the penis, as hypospadias involves only the ventral radius.[38] Another approach for penile curvature is to lengthen the ventral surface by patching the tunica albuginea with synthetic or natural materials.

The concept of correcting penile curvature by adding tissue to the tunica albuginea is not new. Devine and Horton initially reported using dermal grafts in 1975 to correct penile curvature associated with secondary cases of hypospadias and epispadias[39] based on their earlier experience with dermal grafts and Peyronie's disease.[40] In 1983, Kogan *et al.* reported their experience of using dermal grafts to correct extraordinary penile curvature.[41] In 1993, Horton *et al.* used small dermal grafts to augment the ventral tunica albuginea in 24 of 182 pediatric hypospadias patients, resulting in a completely straight, normal appearing erection in all patients.[42]

However, dermal grafting involves special instruments and expertise in procurement and handling of the graft as well as additional postoperative care and complications at the donor site. Because the tunica vaginalis can be easily procured from the scrotum by a urologist, grafting with a tunica vaginalis from the testes was clinically established for Peyronie's disease as a free graft by Amin *et al.*[43] and Das *et al.*[44] in 1980.

Ritchey and Ribbeck used a tunica vaginalis free graft in 19 pediatric patients with severe penile curvature and reported a successful outcome, except in one patient.[45] Kajbafzadeh *et al.* reported that the majority of patients with proximal hypospadias and severe penile curvature were successfully treated with single stage repair using a tunica vaginalis free graft to correct curvature without recurrence.[46]

However, Vandersteen and Husmann observed recurrent curvature in cases augmented with the tunica vaginalis free graft.[47] They believed that fibrosis and graft contracture probably resulted from inadequate vascularization. Furthermore, Caesar and Caldamone reported that penile curvature recurred in 60% of cases managed by a tunica vaginalis free graft.[48]

Postulating that a tunica vaginalis flap may not be associated with the same degree of contracture, Hafez *et al.* compared tunica vaginalis placed as a flap and a graft to cover defects in the ventral surface of the tunica albuginea in a rabbit model.[49] As a result, they reported that tunica vaginalis grafts were associated with early flap necrosis and delayed tunica albuginea regeneration with subsequent marked fibrosis and contracture. On the other hand, in the flap group, early regeneration of the tunica albuginea at the edges of the flap was evident at 2 weeks and complete by 12 weeks, and flap vascularity was maintained, with the mesothelial layer of the tunica vaginalis seeming to act as a scaffold for normal regeneration of the tunica albuginea.

Clinical application of tunica vaginalis flap patching on the ventrum to correct severe penile curvature was initially reported in the literature in 2005[50] (Fig. 8). Braga *et al.*[51] reported in 2007 that the short-term outcome of ventral penile lengthening using a tuniva vaginalis flap to correct severe penile curvature was favorable, with a 95% success rate.

Surgical trends to improve cosmetic outcomes

Meatal appearance

Although Mathieu repair has been used for distal hypospadias because it is applicable for all distal hypospadias as long as there is sufficient penile skin proximal to the meatus to carry out the flip-flap,

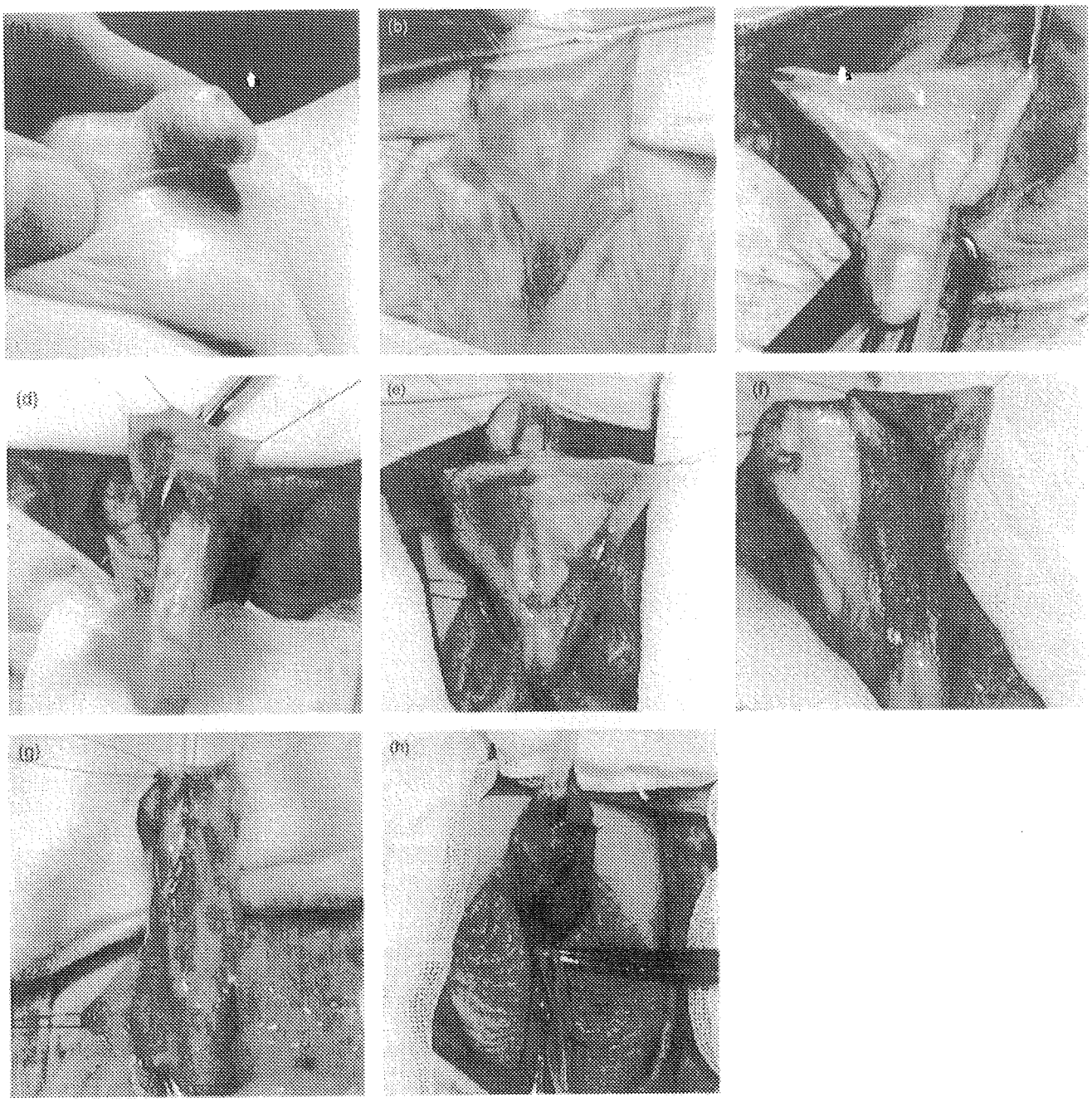

Fig. 5 Modified Koyanagi repair (a) Preoperative appearance in scrotal hypospadias. (b) A U-shaped skin incision is made surrounding the meatus and extended laterally and dorsally to the dorsal prepuce. (c) A circumferential incision is made along the corona and the portion between the dartos fascia and Buck's fascia is dissected on the dorsal side. (d) A buttonhole is made through the dartos pedicle. (e) Parameatal skin flap and vascular pedicle are transposed ventrally through a buttonhole. (f) Internal side of the loop is closed. (g) External side is approximated to create a neourethra. (h) Meatus is created by splitting the glans, and Byar's skin flaps are approximated.

the opening is often horizontal, rounded or irregular, in contrast to the slit-like appearance of a normal meatus. Although a deeply grooved plate could heal with a vertical orientation, a flat plate yielded a horizontal meatus.

Boddy and Samuel developed a method of V-incision sutured meatoplasty as a modification of the Mathieu procedure (MAVIS) to form a cosmetically acceptable natural vertical slit glandular meatus.[52]

Rich *et al.*[53] described 'hinging' the flat plate by incising its distal aspect to improve meatal cosmesis when using flip-flaps or onlay island flaps.

Unfortunately, neither of these techniques has become widely established, which is why, we suspect, a more simplified and effective procedure was developed to achieve a slit-like meatus for distal hypospadias repair by Snodgrass in 1994.[5] The TIP procedure has become

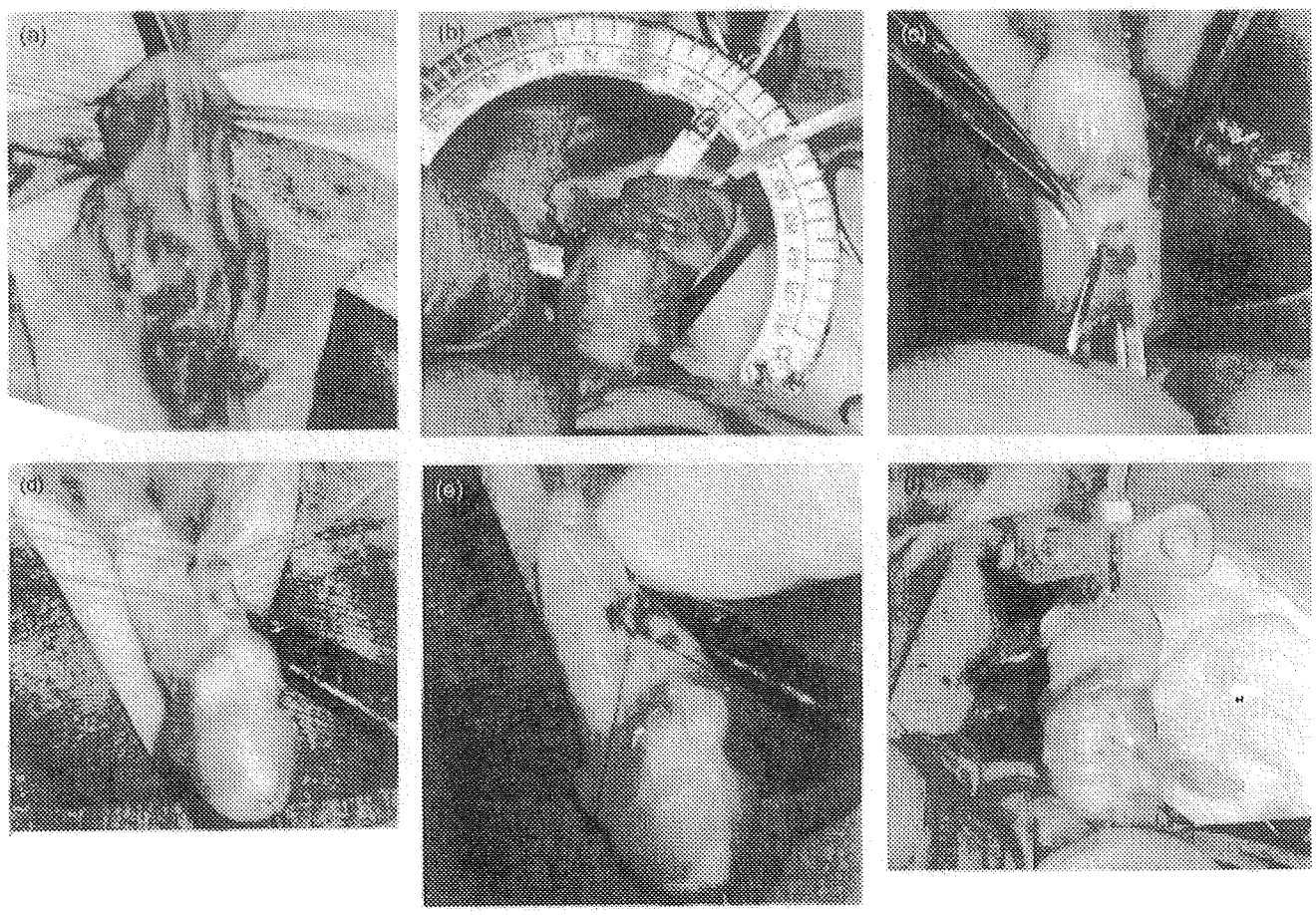

Fig. 6 Dorsal plication preserving urethral plate. (a) Skin degloving without dividing the plate. (b) Artificial erection demonstrates 30-degree curvature. (c) Buck's fascia is cut longitudinally at the 12 o'clock position and two-point incisions are made through the tunica vaginalis. (d) Permanent sutures (5–0 polypropylene) are used for plication. (e) Outer edges of incisions approximated in a way that buries the knots. (f) Straight penis is verified by repeated artificial erection.

popular because it claims to produce a vertically orientated, normal-looking meatus, which is cosmetically superior to other techniques with a high success rate. Ververidis *et al.*[54] carried out an objective assessment study of the penile appearance after hypospadias repair and reported that TIP repair was more effective in producing a vertically oriented meatus (87.5%) than the Mathieu repair (37.5%).

Appearance of ventrum proximal to meatus

For those in whom the foreskin is used or removed, creation of a collar of skin on the ventral surface proximal to the coronal sulcus, as described by Firlit,[55] helps to normalize the appearance of the circumcised penis. A cosmetically normal circumcised phallus was achieved in more than 500 patients undergoing reconstruction of the mucosal collar[56] (Fig. 9).

Preputial appearance

Because circumcision is unusual in continental Europe, most parents prefer to avoid circumcision. Several reports on foreskin reconstruction concomitantly carried out with hypospadias repair have been published in the past two decades.[57–61] Foreskin reconstruction with hypospadias repair produces an excellent postoperative appearance, similar to the penis of normal uncircumcised boys; however, it sometimes introduces an extra risk of postoperative complications, such as stenosis and phimosis of the reconstructed foreskin, foreskin dehiscence and foreskin fistula.

Frey and Cohen[58] performed foreskin reconstruction in combination with the MAGPI procedure in 46 patients with distal hypospadias, and experienced foreskin dehiscence in 10 patients (21.7%).

Although Klijn *et al.*[61] carried out foreskin reconstruction with hypospadias repair in 77 patients and instructed the parents to begin retraction at 10 days after foreskin reconstruction to prevent secondary phimosis and other complications, they encountered complications in 25 patients (32.5%).

Most recently, foreskin reconstruction was carried out during TIP urethroplasty[62] (Fig. 9). Snodgrass *et al.*[62] carried out foreskin reconstruction in 58 patients during penile surgery, including the TIP procedure in 47 patients, and reported foreskin dehiscence in two patients (3.5%) and an unacceptable cosmetic result requiring secondary circumcision in two patients (3.5%).

In many Asian countries, as in continental European countries, routine circumcision is rarely carried out in newborns because the religion does not require circumcision. Because an uncircumcised penis is considered normal in many Asian countries, parents of boys with hypospadias prefer their sons to have a normal-looking penis with

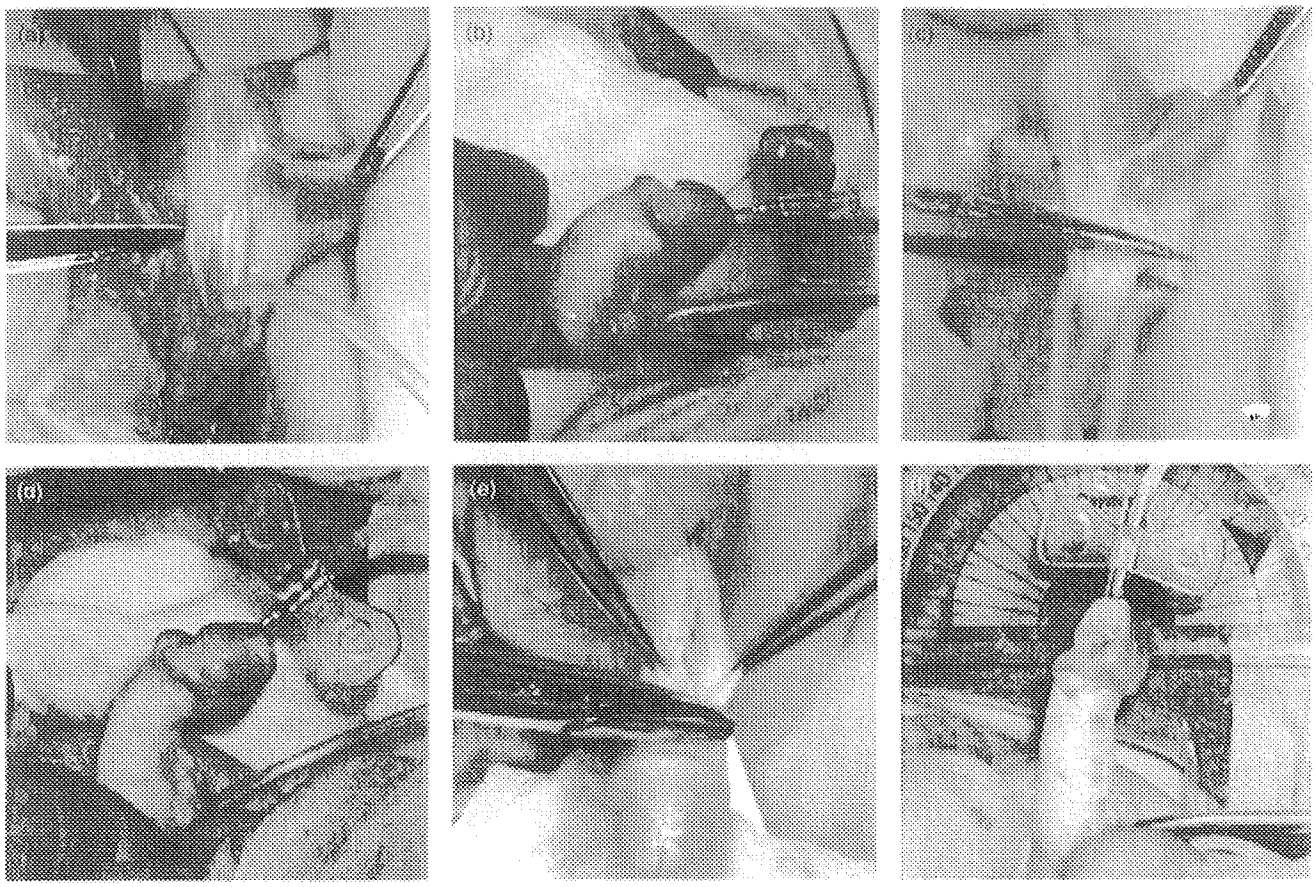

Fig. 7 Dorsal plication after transecting urethral plate. (a) Skin degloving without dividing the plate. (b) Artificial erection demonstrates 60-degree curvature. (c) Urethral plate is transected because of severe curvature. (d) Penile curvature reduces to 30 degrees. (e) Dorsal plication is carried out at the 12 o'clock position. (f) Repeated artificial erection shows 5-degree curvature, which is acceptable.

a foreskin if they are told that foreskin reconstruction is available during hypospadias surgery; but they prefer a penis with an easily retracted foreskin without severe phimosis. Modification of foreskin reconstruction should be considered to suit parents' preferences in Asian countries.[63]

Trends in the management of complications

Urethrocutaneous fistula

Urethrocutaneous fistula is a most common complication, the rate of which is reported to range from 5–44%.[64,65]

Prevention. Second layer coverage of the neourethra has been shown to significantly reduce the fistula rate, and various procedures have been developed to avoid the occurrence of fistulas.

Since Retik *et al.* used the dartos flap as a covering layer between the neourethra and penile skin,[66] it has been the mainstay to avoid urethrocutaneous fistula in hypospadias repair. Most reports involve mobilizing a vascularized pedicle flap from the dorsal aspect and rotating this for use on the ventrum[5,67,68].

Because this may cause penile torsion, the dorsal dartos flap is buttonholed and brought ventrally to lie over the neourethra[69–71] (Fig. 2); however, the dorsal prepuce tends to suffer blood supply deterioration when the dorsal dartos is aggressively dissected from the skin to provide an abundant dartos flap and to avoid penile rotation.

Belman[72] wrapped the neourethra with a de-epithelialized preputial skin flap, which was rotated to the ventrum from the dorsal aspect and the incidence of fistula after hypospadias repair was only 3.5%.

Tunica vaginalis provides an abundant large flap to wrap the urethroplasty or fistula closure with a very high success rate.[73] However, it might be difficult to obtain a long pedicle because the proximal extent of the tunica vaginalis is sometimes limited anatomically. A back-up system with external spermatic fascia was developed because it lies more superficial than the tunica vaginalis and it is long, wide, and easily mobilized with no risk of injury to spermatic vessels[74] (Fig. 10).

The scrotal dartos flap could also supply a more abundant blood flow than the tunica vaginalis or external spermatic fascia;[75] however, it requires more time to obtain dartos tissue from the scrotum and to wrap the neourethra compared with other material.[76]

The Y to I spongiosum wrap (spongioplasty) was developed by Yerkes *et al.*,[77] who mentioned that it is more effective than dartos tissue because of the much thicker, vascular nature of erectile tissue, although their small series did not definitively prove that the fistula rate is lower with this technique. (Fig. 2) Unfortunately, since this spongiosal tissue is not always mobilized and placed over the neourethral suture line, Cooper *et al.*[78] left the spongiosal tissue *in situ* and buried the suture line of the onlay flap with the excellent vascularized spongiosal tissue without aggressive dissection of the spongiosal tissue.

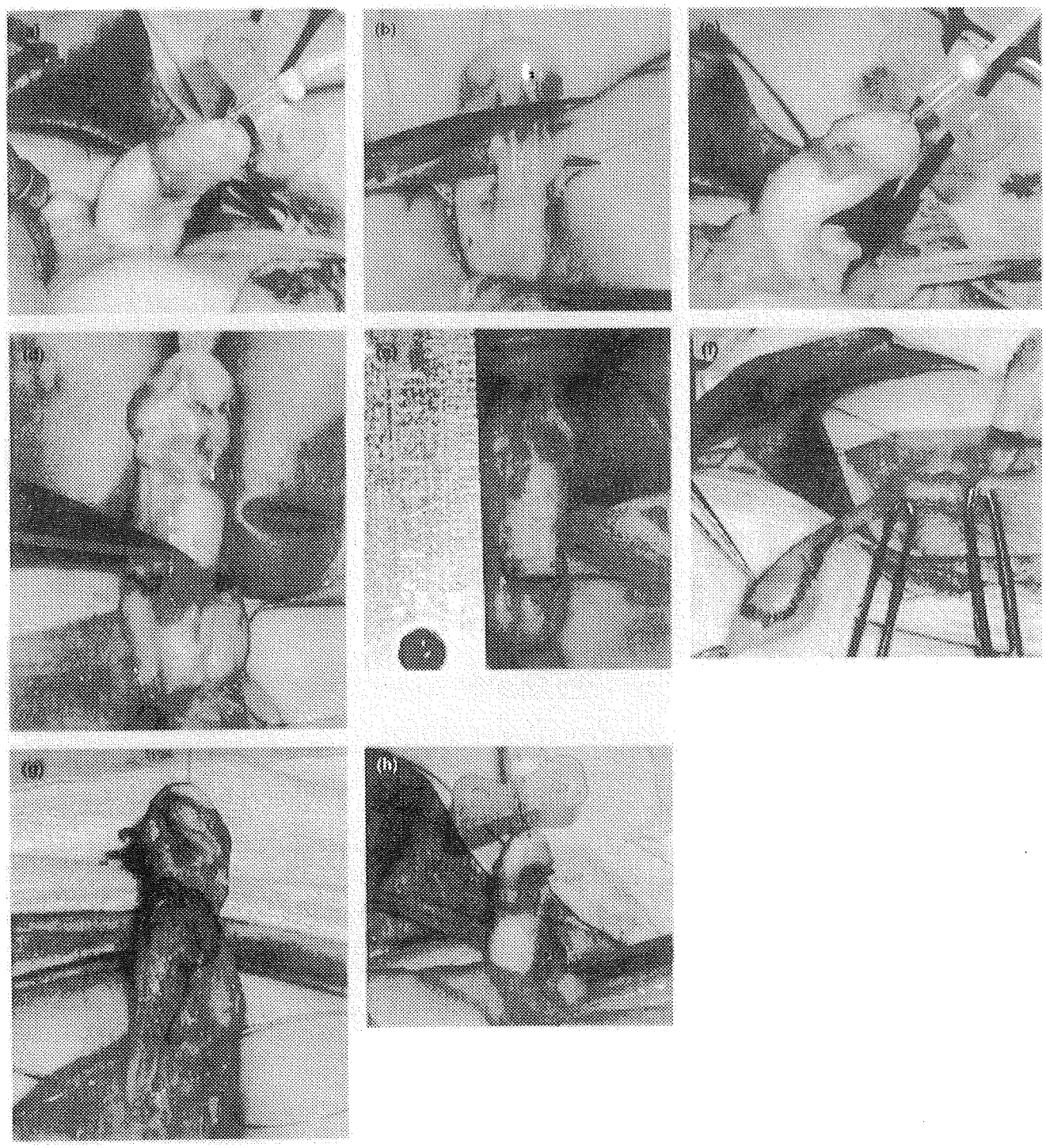

Fig. 8 Tunica vaginalis flap patching for ventral lengthening. (a) Artificial erection demonstrates 60-degree curvature. (b) Urethral plate is transected because of severe curvature. (c) Penile curvature remains 60 degrees. (d) Transverse incision through the tunica albuginea is made at the point of maximal curvature. (e) Tunica albuginea is dissected free from underlying erectile tissue. (f) Tunica vaginalis is incised longitudinally, preserving vascular supply from the cremasteric artery. (g) Surface of mesothelial layer is placed facing erectile tissue and the edge of the superficial mesothelial layer is anastomosed to the tunica albuginea. (h) Repeated artificial erection ensures complete correction of curvature.

Smith[79] carried out tubularized incised plate urethroplasty in 64 patients and used the ventral based pedicle flap to cover the neourethra in 56 of the repairs (85.5%) without complications. Soygur *et al.*[80] and Hayashi *et al.*[81] adopted the ventral-based dartos flap and found it much easier to construct with little time added to surgery and no harvesting-related complications. They also obtained satisfactory results with fistula rates of 8.3%[80] and 8.8%,[81] respectively.

Treatment. Location of the fistula site is identified by retrograde injection of a dilute povidone-iodine solution while the bulbous urethra is being compressed to prevent instillation into the bladder. The fistu-

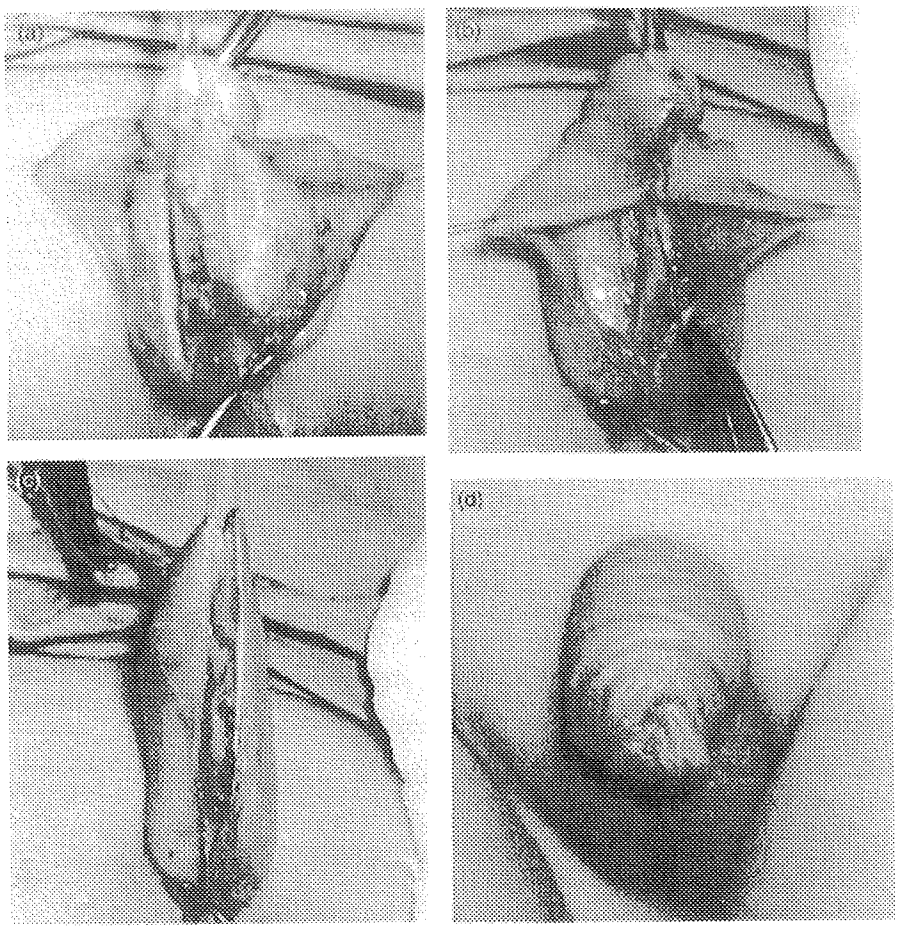

Fig. 9 Foreskin reconstruction (preputioplasty). (a) tubularized incised urethral plate (TIP) urethroplasty is carried out and a V-shaped incision made in the ventrum between the distal corners of the hooded prepuce. (b) The inner layer is approximated in the midline. (c) The outer layer is approximated in the midline. (d) Postoperative appearance.

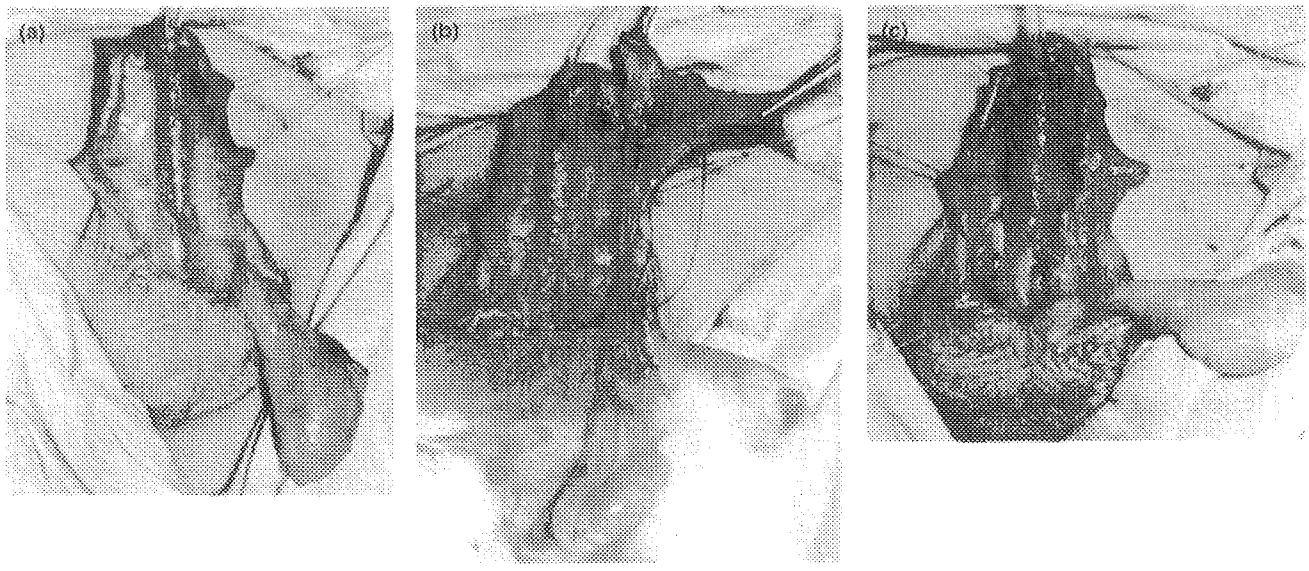

Fig. 10 External spermatic fascia flap. (a) After urethroplasty for severe hypospadias, left scrotal contents are brought out. (b) An external spermatic fascia is dissected from the testis and the cord, up to the level of the external ring. (c) The entire suture line of the neourethra is covered with the flap.

lous tract is traced to the urethra and excised. An inverting closure is preferred over an intraurethral catheter to prevent urethral narrowing.

Small fistulas are successfully closed with a multi-layer simple closure in over 90% of cases.[82]

Urethral strictures

Urethral strictures are generally more problematic than fistula because they tend to cause urinary retention, requiring immediate intervention.

Although strictures can be seen at any location following surgery, the proximal anastomotic site of a tubularized repair appears to be particularly at risk (Fig. 11).

Prevention. In order to prevent stricture formation, the use of a spatulated anastomosis and an adequate amount of tissue to give a satisfactory initial caliber of the neourethra are needed.

Treatment. Minor urethral strictures can often be managed successfully with gentle urethral dilation. If the stricture is symptomatic, endoscopic cold knife urethrotomy might be successful;[83] however, internal urethrotomy should be reserved for a short, filmy stricture because Hsiao *et al.* reported a success rate of only 50% in 20 patients who underwent direct vision internal urethrotomy for urethral stricture after hypospadias repair.[84] For recurrent or extensive strictures, open urethroplasty is necessary. Additional tissue must be brought to the strictured area to increase the diameter of the urethra. Occasionally, complete reconstruction of the neourethra is required.

Meatal stenosis

The complication of meatal stenosis is perhaps most commonly due to technical issues at the time of repair, such as fashioning the urethral meatus with too narrow a lumen or too tight glanuloplasty.

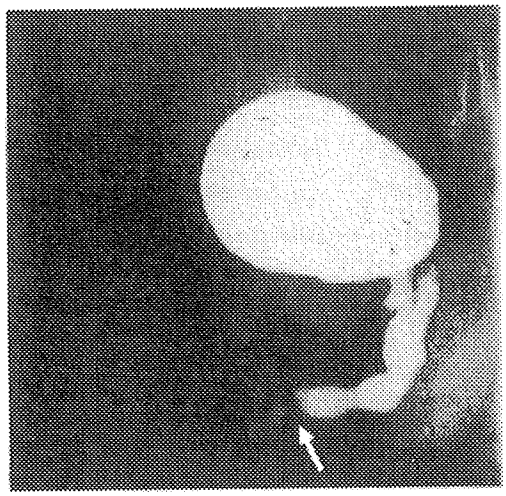

Fig. 11 Urethral stricture. Voiding cystourethrography shows urethral stricture (arrow) at the anastomotic site between the hypospadiac meatus and the tubularized repair.

Prevention. Meatal stenosis can be prevented by generously incising ventrally and laterally along both sides of the site proposed for neourethral placement when glanular wings are constructed. Adequate development of the plane between the tips of the corpora and glans tissue is necessary.

Treatment. The scarred meatus should be incised ventrally and the skin then sutured to the spatulated neourethra. Furthermore, a more complex meatal stenosis involving distal urethral stricture would require a more extensive procedure, such as onlay island flap, flip-flap or TIP urethroplasty.

Urethral diverticulum

Diverticulum may be caused by distal obstruction or poor support and distensibility of the reconstructed urethra. Physical examination may demonstrate ballooning of the urethra when voiding or urinary dribbling when palpating the urethra. A retrograde urethrogram is helpful in delineating the severity and extent of the diverticulum (Fig. 12).

Prevention. A significantly decreased incidence of diverticulum was reported by Weiner *et al.*[85] after onlays rather than tube island flaps, probably reflecting the use of less genital skin; however, Patel *et al.*[86] modified the tube island flap procedure by anchoring the medial edge of the flap to the corpora and discarding the redundant flap skin triangularly to construct an approximately sized neourethra with satisfactory results of no diverticulum.

Treatment. Treatment consists first of alleviation of any distal obstruction. Meatotomy will occasionally allow decompression with improvement of symptoms. If symptoms persist, excision of redundant tissue with urethral tapering is necessary.

Persistent penile curvature

The most important question is whether the straightening achieved in childhood is maintained during puberty and, to date, no reports have documented such outcomes for dorsal plication or the various forms of ventral corporeal lengthening.

Prevention. Penile straightening must be confirmed after degloving the skin and dartos layer, preserving the urethral plate, using the artificial erection test.[87] Under secure placement of an elastic band at the base of the penis, the injection of normal saline with a small-gauge butterfly needle placed in the corporeal body will fill the entire organ. Multiple tests may be necessary to ensure complete correction or acceptable slight curvature.

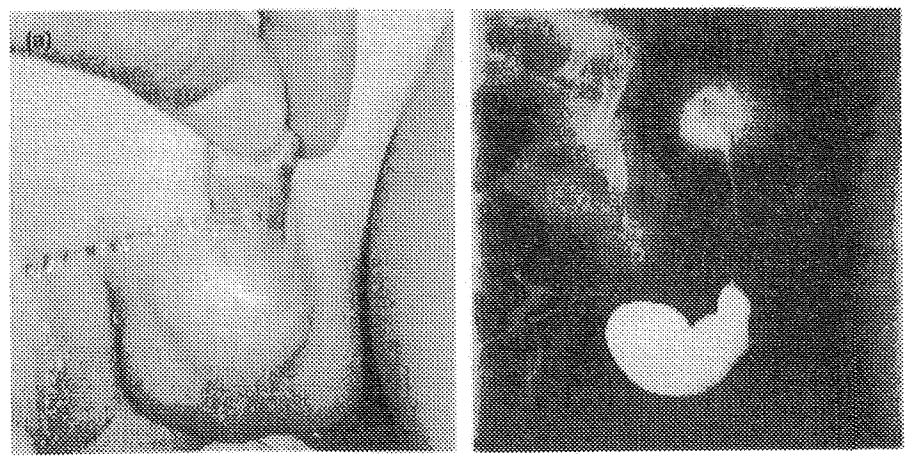

Fig. 12 Urethral diverticulum. (a) Physical examination shows ballooning of the neourethra after tubularized island flap repair. (b) A retrograde urethrogram reveals diverticular dilatation of the neourethra.

Treatment. It is important to preserve the neourethra if possible so that further urethroplasty is not needed. Once the point of maximum curvature is found in cases of mild curvature, repeat plication could be carried out. Otherwise, mid-urethral transection and simultaneous add-on urethroplasty using a pedicle flap or free graft should be considered.

Hairy urethra and stone formation

Hairy urethras are usually seen in older patients who have had multiple-stage procedures in which hair-bearing skin was used for urethral reconstruction. Furthermore, stone formation in the neourethra associated with hair growth, which can occur when hair-bearing scrotal skin is included in the neourethra, is the most onerous complication.

Prevention. In order to prevent hair growth inside the neourethra, non-hair-bearing skin is desirable for the neourethra; in particular, the inner prepuce is most feasible because it closely resembles the natural urethra.

Treatment. Finkelstein and Blatstein[88] used epilation with transurethral neodymium: YAG laser to achieve effective epilation of hair in their four patients. Crain *et al.*[89] reported transcutaneous laser hair ablation for cosmetic hair removal. Kukreja *et al.* instilled depilatory thioglycolate cream into the neourethra and accomplished complete epilation in 16 patients successfully.[90]

Giordano *et al.*[91] reported their experience of urethral calculus in a hypospadic boy successfully treated with shock wave lithotripsy (SWL) after various unsuccessful attempts at urological extraction. Hayashi *et al.*[92] carried out endoscopic urethrolithotripsy with Lithoclast for two hypospadiac patients with urethral calculus associated with hair growth.

Long-term follow-up

Postoperative functional evaluation

Uroflowmetry is a simple, non-invasive and important tool for the evaluation of functional long-term outcome after hypospadias repair and the detection of asymptomatic stricture.

The functional results of hypospadias repair based on uroflowmetry appear to be satisfactory. Garibay *et al.*[93] reported normal flow rates in 82% of boys with flip-flap Mathieu repair, 100% of boys with onlay island flap repair, and 73% of boys with tube island flap repair.

Reporting uroflow data in 48 boys after primary TIP repair, Hammouda *et al.*[94] noted that that although 69% had normal peak flow rates, 31% had lower rates than age- and volume-related nomogram values.

Postoperative psychological evaluation

The long-term psychological aspects of hypospadias appear to be positive. Gender development, identification, and behavior have not been found to be abnormal in most men with previous hypospadias repair.[95] They may have a more negative genital self-concept, but this does not appear to interfere with sexual activity. In societies that do not practice circumcision, a lack of foreskin may impact negatively upon self-image.[96] Those who have had the fewest postoperative complications appeared to have the best self-esteem.[97]

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REGULAR ARTICLE

Exposure assessment of phthalate esters in Japanese pregnant women by using urinary metabolite analysis

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Abstract
Objectives Our objectives were (1) to evaluate whether single spot urine is suitable media for longer-term phthalate esters exposure assessment, and (2) to estimate intake level of phthalate esters of Japanese pregnant women using urinary metabolites as an indicator of prenatal exposure level in their offspring.
Methods We analyzed nine metabolites (MMP, MEP, MnBP, MBzP, MEHP, MEOHP, MEHHP, MINP, MnOP) of seven phthalate esters in spot urine samples from 50 pregnant women by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Using four urine samples collected from each of 12 subjects from 50 pregnant women within 5–12 weeks, we compared intra- and interindividual variation in urinary metabolites by calculation of intraclass correlation coefficient (ICC). We estimated daily intakes of 50 pregnant women from their urinary metabolite concentrations.
Results ICCs for seven phthalate metabolite concentrations in single spot urine samples were: MMP (0.57), MEP (0.47), MnBP (0.69), MBzP (0.28), MEHP (0.51), MEHHP (0.43), and MEOHP (0.41) in 12 pregnant women. Phthalate ester metabolites had high detection rates in 50 subjects. The mean daily intake ranged from 0.01 to 2 μg/kg per day. The daily intake levels in all subjects were lower than corresponding tolerable daily intake (TDI) set by the European Food Safety Authority (EFSA), though maximum value for DnBP of 6.91 μg/kg per day accounted for 70% of TDI value.
Conclusions Higher ICCs indicated that phthalate metabolite levels in single spot urine could reflect longer-term exposure to the corresponding diesters of subjects. Although the current exposure level was less than TDIs, further studies and exposure monitoring are needed to reveal the toxicity of phthalate esters to sensitive subpopulation.

Keywords Phthalate esters · Biomarkers · Urinary metabolites · Intraclass correlation coefficient · Daily intake

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Abbreviations

MMP	Monomethyl phthalate
MEP	Monoethyl phthalate
MnBP	Mono-*n*-butyl phthalate
MBzP	Monobenzyl phthalate
MEHP	Mono-2-ethylhexyl phthalate
MEHHP	Mono-(2-ethyl-5-hydroxyhexyl)phthalate
MEOHP	Mono-(2-ethyl-5-oxohexyl)phthalate
MINP	Mono-iso-nonyl phthalate
MnOP	Mono-*n*-octyl phthalate
MOE	The Ministry of the Environment in Japan
EFSA	European Food Safety Authority

Introduction

Phthalate esters are produced in abundance all over the world because of their multiple uses. For instance, di(2-ethylhexyl)phthalate (DEHP) has been produced as one of the major plasticizers for many years [1]. Other phthalate esters, such as di-*n*-butyl phthalate (DnBP) and diethyl phthalate (DEP), are used in paints and consumer products, and as plasticizers [2, 3]. Taking the abundance of plastics and other phthalate-ester-containing materials in our immediate environment into consideration, it is highly probable that humans are exposed to various phthalate esters in daily life.

The recognition that some phthalate esters have adverse effects on male reproductive system in experimental animals, especially via in utero exposure, has been increasing [4]. Testosterone production of fetal male rodent is reduced following in utero phthalate ester exposure, which results in malformation of male reproductive tract, such as reduced anogenital distance (AGD), retained nipples, hypospadias, cleft phallus, undescended testis epididymal agenesis, and testicular atrophy [5, 6].

Moreover, several epidemiological studies have suggested adverse health effects that were similar to reproductive toxicities observed in experimental animals [7–9]; for example, Swan et al. reported the relationship between mother's phthalate ester exposure levels in gestational period and their male newborn's anogenital distance index (AGI). This report suggested that prenatal phthalate ester exposure could affect human reproductive health. They also speculated that humans might be more sensitive than experimental animals to reproductive effect of phthalate ester because the exposure levels of the human subjects in that report were very low compared with those administered to animals in the in vivo experiments [8]. Therefore, it has become much more important to evaluate phthalate ester exposure levels in human population of sensitive period (prenatal, newborn, and infant), and also to assess human health effects caused by exposure.

Once phthalate esters are taken into human body, they are rapidly metabolized to monoesters by hydrolysis and oxidation and then are conjugated with glucuronide; subsequently most of the phthalate esters are excreted in the urine within 24 h [10, 11]. This metabolic process can to a certain extent differ due to the length of alkyl chain of the phthalate esters; for example, phthalate esters with shorter alkyl chain are mainly excreted as monoesters, while those with longer alkyl chain are further metabolized to more hydrophilic oxidative form [11].

Since Blount et al. first reported a sensitive analytical method for phthalate ester metabolites in human urine using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) [12], exposure assessment of phthalate esters has become routine. Although single spot urine sample was usually used for exposure assessment [7, 13–17], concern has been raised over whether monoester concentration in single spot urine represents the subject's long-term exposure to phthalate esters because biological half-life in human body is short. Hoppin et al. [18], Hauser et al. [19], and Teitelbaum et al. [20] reported utility of single spot urine for phthalate esters exposure assessment; however, Fromme et al. [21] did not. Hence it is still unclear whether spot urine is usable for human exposure assessment of phthalate esters.

The main purposes of the present study are (1) to evaluate whether single spot urine is suitable media for the assessment of longer-term phthalate ester exposure, and (2) to estimate intake level of phthalate esters of Japanese pregnant women using urinary metabolite concentrations as an indicator of prenatal exposure level of their offspring.

Materials and methods

Participants

Fifty healthy Japanese pregnant women, who were outpatients of a department of obstetrics and gynecology of a hospital in Tokyo, participated in this study during 2005–2006. They were randomly chosen from outpatients who had no pathological symptoms as diagnosed by a gynecologist. The subjects agreed to participate after being explained the purpose and the procedure of this study by the gynecologist. Ethical Committee of the hospital approved this study.

Sampling

We collected single spot urine samples from the subjects at one of their regular maternal health check-ups. Sampling was done at 25–40 gestational weeks. The urine samples were collected in polypropylene (PP) bottle that was washed with ultrapure water and methanol prior to use, and stored at −20°C until analysis. One spot urine sample was collected from all of the 50 subjects to assess phthalate exposure during pregnancy. Additional three urine samples were collected with 1–6 weeks interval from 12 of the 50 subjects to evaluate intra- and interindividual variation of urinary phthalate metabolite concentrations.

Chemicals

Standard solutions (100 μg/ml) of nine phthalate monoesters (monomethyl, monoethyl, mono-*n*-butyl, monobenzyl,

monoisononyl, mono-*n*-octyl, mono-2-ethylhexyl, mono-2-ethyl-5-hydroxyhexyl, mono-2-ethyl-5-oxohexyl) (>98% purity) were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). $^{13}C_4$-labeled phthalate monoesters were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA) for an internal standard. Acetonitrile, formic acid, and acetic acid were purchased from Nacalai Tesque Co Ltd. (Kyoto, Japan). Ammonium acetate and ammonia water were purchased from Wako Pure Chemical Industries Co Ltd. (Osaka, Japan). β-Glucuronidase (E. coli K12 origin; 200 units/ml) was obtained from Roche Diagnostics (Mannheim, Germany). Creatinine test Wako for Jaffe reaction was purchased from Wako Pure Chemical Industries Co Ltd. (Osaka, Japan).

Materials

All of the glassware used in this study was washed by sonication, rinsed with ultrapure water, dried, and heated at 400°C. Volumetric glassware was washed similarly except for the 400°C heating, which was replaced by methanol rinse.

Analytical procedure

One milliliter of urine was taken to a glass centrifuge tube to which was then added 300 µl mixed internal standard solution (100 ng/ml), 250 µl 1 mol/l ammonium acetate buffer (pH 6.5), and β-glucuronidase (5 µl). Subsequently, urine sample was vortex-mixed, and incubated at 37°C for 60 min for the hydrolysis of glucuronide. After incubation, 3 ml ammonium water (pH 8.0) was added to urine samples, and then loaded onto solid-phase extraction cartridge (OASIS-MAX 150 mg/6 cc, Nihon Waters Co Ltd. Tokyo, Japan), which had been conditioned with acetonitrile (10 ml) and ultrapure water (5 ml). After washing the cartridge with ultrapure water (5 ml) and acetonitrile (5 ml), target monoesters were eluted with 5 ml acetonitrile containing 1% formic acid to a new glass centrifuge tube. The eluent was evaporated to dryness with a gentle stream of nitrogen gas; the residue was redissolved in 200 µl ultrapure water and transferred to an autosampler vial. The nine phthalate monoesters were determined by HPLC-MS/MS. The HPLC was from Agilent technologies (Agilent 1100, CA, USA) and the MS/MS was from Micromass (Micromass Quattro Ultima, Manchester, UK).

Creatinine concentrations in urine samples were determined by Jaffe reaction.

Daily intake estimates

We used Eq. 1 for the estimation of daily intake of seven phthalate diesters from urinary monoester concentrations, as employed by David [22] and Koch et al. [14].

$$\text{Daily intake } (\mu g/kg/day) = \frac{\text{ME}(\mu g/g) \times \text{CE } (mg/kg/day)}{F_{UE} \times 1{,}000\ (mg/g)} \times \frac{MW_d}{MW_m} \quad (1)$$

ME is creatinine-adjusted monoester concentration, CE is creatinine excretion rate normalized by body weight as 18 mg/kg per day for women [14]. F_{UE} is the molar fraction of the urinary-excreted monoester related to the parent diesters. F_{UE} values were as follows: for MnBP, 0.69 was used based on report of Anderson et al. [23]. F_{UE} for MMP and MEP were unknown and substituted with that for MnBP. For MEHP, MEHHP, and MEOHP, 0.059, 0.233, and 0.154 were used, respectively [24]. F_{UE} value of 0.0215 was used for MINP based on report of Koch et al. [25]. For MnOP, 0.043 was used from the animal data of Albro and Moore [26]. MW_d and MW_m are the molecular weight of phthalate diesters and phthalate monoesters, respectively.

Statistical analyses

Phthalate metabolite concentrations were corrected for urine volume by urinary creatinine concentration (µg/g cre). In the following statistical analyses, we used log-transformed phthalate metabolite concentrations adjusted creatinine concentrations because the seven phthalate monoester concentrations in urine samples from the pregnant women were log-normally distributed. To calculate inter- and intraindividual variance in urinary metabolite concentrations through 5–12 weeks, we employed mixed models by using SAS proc mixed version 9.1 (SAS Institute inc., Cary, NC). Intraclass correlation coefficient (ICC) was defined as the proportion of interindividual variance to total variance.

Results

Analysis of urinary phthalate metabolites

Quantitative recoveries of added metabolites were obtained: 71% (MINP) to 117% (MMP) for 3 ng/ml added level and 96% (MEP, MINP, MnOP) to 112% (MMP) for 30 ng/ml added level. Those values were means of four replicates. The reproducibility of analysis expressed as relative standard deviation ranged from 3.4% for MEOHP

to 27.8% for MEHP. These results indicated that our monoesters analysis was accurate in terms of adequate precision and recoveries. No phthalate metabolites were detected in procedural or travel blanks.

Intra- and interindividual variation in urinary metabolite concentrations

Four spot urine samples were taken from each of 12 subjects within 5–12 weeks in late pregnancy. Creatinine-adjusted phthalate ester metabolite concentrations in the 48 urine samples were analyzed for inter- and intraindividual variance. Since MINP and MnOP were hardly detected in the urines, these metabolites were not included in the statistical analyses. Table 1 shows the results of inter- and intraindividual variance and ICCs in log-transformed creatinine-adjusted urinary metabolite concentrations. The ICCs for seven urinary metabolites were MMP (0.57), MEP (0.47), MnBP (0.69), MBzP (0.28), MEHP (0.51), MEHHP (0.43), and MEOHP (0.41).

Table 1 Inter- and intraindividual variance and intraclass correlation coefficient (ICC) of each urinary phthalate metabolite concentrations

Urinary metabolites	Interindividual	Intraindividual	ICC[a]
MMP	0.07215	0.05477	0.569
MEP	0.06827	0.07789	0.467
MnBP	0.06876	0.03022	0.695
MBzP	0.06605	0.1739	0.275
MEHP	0.0462	0.04371	0.514
MEHHP	0.04404	0.05944	0.426
MEOHP	0.04009	0.05736	0.411

$n = 48$, four spot urine samples from 12 pregnant women, within 1–6 weeks intervals during 5–12 sampling period

Log-transformed urinary metabolite concentrations were used in this statistical analysis because the seven phthalate monoester concentrations in 48 single spot urine samples from 12 pregnant women were log-normally distributed

[a] ICC = Interindividual variance/(interindividual variance + intraindividual variance)

Phthalate metabolites levels in Japanese pregnant women

Table 2 shows limit of detection, percentage of subjects with detectable concentration in urine, and median concentrations of urinary metabolites with min–max ranges for the 50 Japanese pregnant women. Limit of detections were 0.008–0.07 ng/ml as urinary level for the nine phthalate ester metabolites. All of the nine creatinine-unadjusted and creatinine-adjusted urinary metabolite concentrations showed log-normal distribution and, therefore, geometric mean, geometric standard deviation, and median concentration were shown in this table. MMP, MEP, MnBP, MBzP, MEOHP, and MEHHP were detected in 100% of the subjects. Urinary MnBP concentration (median 66.6 μg/g cre) was highest among the nine monoesters analyzed while those of MINP and MnOP were low.

Comparison of the estimated daily intake level of phthalate esters based on urinary metabolite levels with other estimates

Table 3 shows estimated daily intake of seven phthalate esters based on urinary metabolite concentrations of the 50 subjects of the present study. The mean estimated intakes of the seven phthalate esters ranged 0.1 to 2 ng/kg per day. None of the estimated intake levels of the present subjects exceeded the tolerable daily intake (TDI) for DEHP, DnBP, and BBzP (Table 3). The TDIs were based on the reproductive and developmental toxicity of phthalate esters

Table 2 Urinary phthalate metabolite concentrations in 50 pregnant women

Phthalate monoesters	Limit of detection (ng/ml)	Detection rate (%)	Monoester concentrations							
			Unadjusted (ng/ml)				Adjusted (μg/g cre)			
			Min	Median	Max	GM (SD)	Min	Median	Max	GM (SD)
MEHP	0.022	98	<LOD	3.96	70.3	3.63 (3.89)	<LOD	5.15	67.8	4.63 (3.32)
MEHHP	0.008	100	1.23	10.6	89.7	10.2 (2.57)	3.90	10.7	164	12.9 (1.98)
MEOHP	0.015	100	0.91	11.0	132	11.0 (2.75)	3.46	11.9	174	13.7 (2.06)
MMP	0.024	100	0.57	6.61	464	8.13 (3.72)	2.16	8.15	714	10.1 (3.18)
MEP	0.02	100	0.33	7.83	1,067	8.71 (4.07)	1.25	9.4	1430	10.9 (3.59)
MnBP	0.066	100	2.92	57.9	504	52.5 (2.75)	11.1	66.6	211	66.9 (1.79)
MBzP	0.03	100	0.26	3.74	74.5	4.57 (3.31)	0.69	4.37	106	5.74 (2.65)
MINP	0.035	14	<LOD	<LOD	2.53	<LOD	<LOD	<LOD	3.65	<LOD
MnOP	0.017	28	<LOD	<LOD	0.22	<LOD	<LOD	<LOD	0.23	<LOD

All urinary metabolite concentrations were log-normally distributed in 50 pregnant women

Table 3 Daily phthalate intake levels in 50 pregnant women and comparison with TDI of EFSA and the daily intake levels in pregnant women of Swan et al. [8]

Phthalate diesters	Urinary metabolite	Daily intake (μg/kg per day)				TDI (μg/kg per day)	Swan et al. [8]
		Min	Median	Max	GM		Median (μg/kg per day)
DEHP	MEHP	0.005	2.20	29.0	1.99	50	2.37
DEHP	MEOHP	0.400	1.10	16.8	1.33	50	2.00
DEHP	MEHHP	0.554	1.90	27.9	2.20	50	1.33
DEHP[a]	3 metabolites	0.62	1.73	24.6	1.94	50	1.70
DMP	MMP	0.061	0.229	20.1	0.285	–	–
DEP	MEP	0.037	0.280	42.6	0.324	–	5.32[b]
DnBP	MnBP	0.362	2.18	6.91	2.19	10	0.67[b]
BBzP	MBzP	0.021	0.132	3.20	0.173	500	0.35[b]
DINP	MINP	0.018	0.056	4.38	0.071	–	–
DnOP	MnOP	0.007	0.026	0.135	0.011	–	–

DMP dimethyl phthalate, *DEP* diethyl phthalate, *DnBP* di-*n*-butyl phthalate, *BBzP* butylbenzyl phthalate, *DEHP* di(2-ethylhexyl)phthalate, *DINP* di-isononyl phthalate, *DnOP* di-*n*-octyl phthalate

[a] The value was based on mean values of DEHP intake from the three DEHP metabolite concentrations for each subject

[b] These diesters were statistically significant with reduced AGI in the Swan et al. [8] study and calculated by Marsee et al. [41]

References: TDI for DBP [28], DEHP [29], and BBzP [30]

[28–30]. Maximum DEHP and DnBP intake levels of the present subjects accounted for 50% and 70% of the TDIs, respectively.

Discussions

Intra- and interindividual variations in urinary metabolite concentrations

Intra- and interindividual variations in urinary metabolite concentrations were compared based on calculation of ICCs. Except for MBzP, the ICCs were greater than 0.4, which indicated that urine metabolite data were reproducible over the sampling period (5–12 weeks) for most of the phthalates. According to Rosner [34], ICC of 0.4–0.75 is considered "fair to good" reproducibility. Based on the statistical analysis and higher ICCs, we concluded that the urinary metabolite levels in single spot urine could reflect longer-term (approximately 2-month) exposure level of the corresponding diester of the subject.

To date, a couple of studies have been carried out to evaluate the utility of spot urine for the exposure assessment of phthalate esters by analyzing inter- and intraindividual variance of urinary excretion of metabolites. Our results of ICCs, 0.28 to 0.69, in urinary metabolite concentrations over 5–12 weeks were at the same levels or relatively higher than those reported. Hauser et al. [19] reported ICCs for MMP (0.27), MEP (0.43), MnBP (0.71), MBzP (0.55), and MEHP (0.54) in 3 months. Teitelbaum et al. [20] reported MEP (0.26), MnBP (0.35), MBzP (0.62), MEHP (0.29), MEHHP (0.24), and MEOHP (0.23) in 6 months. Hoppin et al. [18], Hauser et al. [19], and Teitelbaum et al. [20] concluded that spot urine was usable for longer-term (up to a couple of months) exposure assessment. On the contrary, the conclusion of Fromme et al. [21] was not similar to ours. They measured concentrations of ten urinary metabolites in 50 subjects during eight consecutive days and analyzed for within-subject variance to find substantial day-to-day variation in urinary monoester levels of the subjects. ICCs were moderate and they concluded that spot urine was not suitable for exposure assessment.

Differences in exposure patterns, including exposure pathway, exposure sources, and exposure timing, might result in such inconsistency among studies on reproducibility of phthalate metabolite concentration in spot urine samples.

Theoretically, utility of spot urine would be limited for long-term exposure assessment of a chemical with short biological half-life, such as phthalate esters, if exposure level varied randomly either interindividually or intraindividually. In spite of this limitation, higher ICCs found in this study and other previous ones suggested that phthalate ester exposure level of an individual was primarily determined by a relatively steady habit (e.g., food and personal care products) of the individual. Until the utility of single spot urine for exposure assessment of phthalate esters is established, however, multiple urine samples may ideally be collected from subjects in epidemiologic studies, as

Hauser et al. [19] pointed out. This obviously requires cost and effort of both subjects and examiners.

Urinary phthalate metabolite levels in 50 pregnant women

MEHP, MINP, and MnOP were detected in 98%, 14%, and 28% of subjects, respectively. Frequent detection of urinary metabolites shows that subjects were exposed to various phthalate esters. This may allow us to assume that the general Japanese population is also exposed to them on a daily basis. In the case of MINP, several studies have shown that MINP is not main metabolite of DINP, and is further metabolized to oxidative forms [25, 27]. This may be related to the lower MINP concentrations in this study.

For MMP, MEP, MnBP, MBzP, and MEHP, we compared median phthalate monoester concentrations in the present Japanese subjects with those reported in the previous studies, although the sample size and characteristics of the subject population was not comparable. There were greater differences between our results and literature values for MnBP and MEP (Fig. 1) than those for other phthalate metabolites. Urinary MEP concentration in this study (9.4 μg/g cre) was lower than those in other countries/regions by one order of magnitude (Fig. 1). This may be related to the much lower production of DEP in Japan (700 tons in 2002 [35]) than in the USA (11,700 tons in 1988 [2]). On the contrary, urinary MnBP concentration was higher in the present study and in European countries [14, 36] than in the USA, except for Hoppin et al.'s [18] study. However, production of DBP was 4,135 tons in Japan [35] and 7,752 tons in USA [3], thus it is unlikely that the difference in MnBP concentration between countries was related to production amount of DBP. Rather the difference in urinary MnBP concentration between the two countries may reflect the difference in the usage of products containing DBP in the proximate environment of general population.

In contrast, MEHP level in this study was similar to in other studies. Fujimaki et al. [17] measured MEHP, MEOHP, and MEHHP concentrations in spot urine samples from pregnant women in Tokyo and obtained median values of 9.83, 10.4, and 10.9 μg/g cre, respectively, which were of similar order to the present results. The level of MBzP was slightly higher in this study than in others (data not shown). Thus exposure levels of some but not all phthalate esters are different among countries or regions, probably due to differences in use and application of phthalate esters among countries/regions.

Daily intake levels of phthalate diesters and exposure sources

Figure 2 shows comparison of daily exposure level of selected phthalates estimated in this study and those by the Ministry of the Environment (MOE), Japan. The MOE estimate was the sum of the diester intakes from food, indoor air, and drinking water, which were based on the reported mean concentrations in each media in Japan [31–33]. Estimated mean daily intake of DMP, DEP, and DnBP in this study was from two to four times greater than the MOE values, suggesting the presence of exposure sources of these phthalate esters other than food, air, and drinking water for the present subjects (Fig. 2). The difference in the estimation based on urine analysis and environmental monitoring has also been pointed out recently by Itoh et al. [37]. One possible exposure sources that the MOE failed to include was personal care products

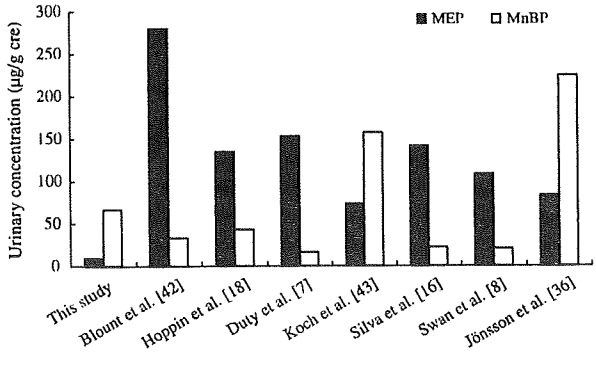

Fig. 1 Comparison of urinary monoester concentrations among recent phthalate exposure assessments with this study. These urinary concentrations were from median values in each study. We compared MEP, MnBP, MMP, MBzP, and MEHP. Comparison results for MEP and MnBP are shown because of their variations in urinary monoester concentration among regions

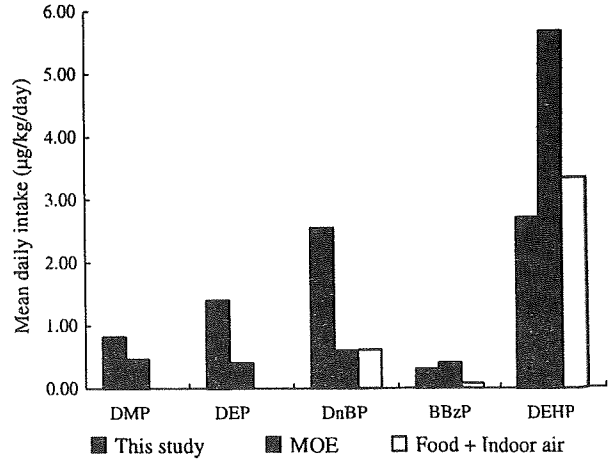

Fig. 2 Mean daily phthalate-intake level in this study, MOE, and estimated intake from food [39] and indoor air [40] for DnBP, BBzP, and DEHP