SUMMARY

Congenital lower urinary tract disorders are a family of diseases affecting both urinary storage and voiding as well as upstream kidney function. Current treatments include surgical reconstruction but many children still fail to achieve urethral continence or progress to chronic kidney disease. New therapies can only be achieved through undertaking research studies to enhance our understanding of congenital lower urinary tract disorders. Animal models form a critical component of this research, a corner of the triangle composed of human *in-vitro* studies and clinical research. We describe the current animal models for two rare congenital bladder disorders, posterior urethral valves (PUV) and bladder exstrophy (BE). We highlight important areas for researchers to consider when deciding which animal model to use to address particular research questions and outline the strengths and weaknesses of current models available for PUV and BE. Finally, we present ideas for refining animal models for PUV and BE in the future to stimulate future researchers and help them formulate their thinking when working in this field.

INTRODUCTION

Congenital lower urinary tract disorders are rare childhood diseases that present unique challenges to the researcher due to limited access to clinical subjects as well as the logistical and ethical barriers of acquiring biological tissue from children for study. An additional challenge is that their key pathogenic steps occurs in the womb, a barrier to convenient observation. Thus, antenatal *in vivo* animal models are a key component in the study of these disorders, bridging the gap between *in vitro* research and human clinical studies. Best visualised as a triangle of interconnected research (**Figure 1**), each of these research modalities provide information to facilitate discoveries in the other two. In this article, we will first categorise the different types of animal model available for researchers. We will then outline the development of several important animal models which have advanced our understanding of two rare congenital bladder disorders; namely posterior urethral valves (PUV) and bladder exstrophy (BE). For both, incontinence remains a stubborn challenge for the urologist and paediatrician. Furthermore, with regards to PUV, the additional high incidence of chronic kidney disease means it is one of the most frequent indications for renal replacement therapy in children [1]. For each condition, we describe how animal models have contributed to our understanding of human disease alongside their limitations. Finally, we will discuss several unanswered questions in this field of research, with suggestions on how animal models of congenital lower urinary tract disorders could be refined and used in the future.
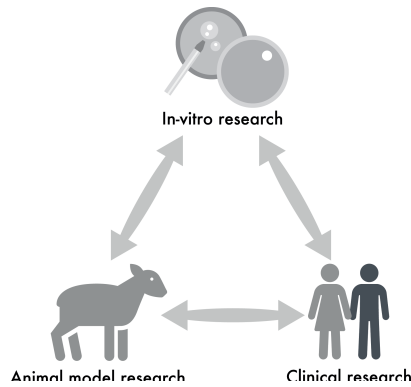
Figure 1: Key components of biomedical research

CATEGORISATION OF MODELS

There are several important considerations (**Table 1**) when designing an animal model aiming to replicate the features of a human congenital anomaly depending on the research question being asked. Species differences can limit the translation of findings in animals to humans, for example, bladder-voiding patterns are different between rodents and humans. Additionally, sex differences in disease progression need to be considered alongside changes in organ function between infants and adults.

Table 1: Considerations in animal model design

IMPORTANT CONSIDERATIONS IN ANIMAL MODEL DESIGN
● Inadequate understanding of the human disease pathogenesis limits the potential for genetic and teratogenic model development, e.g. Lack of understanding of pathogenic steps limit some animal models to those of anatomical recreation.
● Differences between species can limit translatability of findings between the animal model and human, e.g. significant differences in rodent bladder voiding patterns mean that cystometric experiments in rodents have little translatable value to human bladder function.
● Differences between sex, e.g. conditions that only one, such as posterior urethral valves demand one selection in animal experiments.
● Age of animal, e.g. bladder function changes with age, thus experiments conducted on adult animals may not extrapolate to the infant.

Animal models can be categorised depending on how faithfully they replicate the human condition and the method by which they are created (**Table 2**). Spontaneous equivalent congenital disorders that are homologous to human ones are ideal, as they are likely to replicate many facets of aetiology and pathology. These are sometimes encountered in the study of acquired human diseases such as dementia and various cancers [2,3]. These types of models are rare for congenital disorders, as the disease often affects survival or maternal care. Instead, we must model components of the analogous human condition, either by (i) creating the final phenotype through *in utero* surgery , (ii) examining teratogenic effects that recreate disease appearance or (iii) targeted genomic modifications based on studies of human mutations . Surgical strategies still present challenges to the researcher as our understanding of the aetiology of PUV and exstrophy remain theoretical [4,5]. Additionally, both PUV and exstrophy are presumed to result from polygenetic inheritance with several environmental influences [6,7] adding to the difficulty of creating genetic or teratogenic models.

Table 2: Categorisation of different animal model types, by form or by manner of creation

CATEGORISATION BY FORM	DESCRIPTION
Homologous	Symptoms, aetiology or consequences of the model duplicate as faithfully as possible, the human disorder.
Isomorphic	Resembles the human condition but not generated through presumed pathophysiologic processes – allowing the study of underlying mechanisms of dysfunction.
Predictive	Does not resemble the human condition but has predictive value where the system may be manipulated to produce functional outcomes of interest.

CATEGORISATION BY CREATION METHOD	
Experimental/induced	
- Surgical/anatomic	Surgery used to create the model. These can be immediate or progressive (where the surgery puts into place the necessary lesion but requires time or growth to complete).
- Genetic/teratogenic	Created through specific and known genetic manipulations or found via discovery of toxin-related teratogenic effects.
Spontaneous/natural	Disease states that are similar to their human equivalents. The main drawback is unreliability in occurrence.
Negative models	A model where a disease phenotype does not develop after an experimental exposure that would otherwise cause it in humans. Common in infectious disease research.
Orphan models	A term for a disorder found only in that species, but yet to be seen in humans.

ANIMAL WELFARE

A poorly designed animal model not only limits its translatability to patients, but also exposes animals to extraneous suffering. Adhering to high standards of animal welfare is essential, with the '3R' principles a practical framework for study design. The 3Rs guide investigators to consider replacing animal models where techniques such as cell culture studies of human tissue or organoid research may be able to replicate the biological processes being examined. Secondly, experiments should be designed to reduce animal numbers by using accurate power calculations with biological meaningful end-points and minimising treatment arms. Finally, techniques should be refined by 'good experimental design and surgical technique to minimise variability and maximise scientific validity' [8]. Applying these principles helps scientists arrive at a core of research needs where animal models are particularly important

– that is assessing a disease process or putative treatment where a whole organism and its concomitant physiology and circulating factors must be present.

POSTERIOR URETHRAL VALVES

Posterior urethral valves (PUV) is a congenital condition affecting 1 in 4,000 liveborn males [9], although the true incidence may be higher as there is a significant chance of fetal demise *in utero*, with selective termination of pregnancy also a factor obscuring its incidence [10]. PUV is characterised by a gestational partial obstruction of the urethra; there is a wide range of variability in the clinical phenotype and this partial obstruction may result in bladder distension, oligohydramnios and often kidney damage. Cystoscopic valve ablation is accomplished at birth, but continence and renal function outcomes remain poor due to pathologic bladder remodelling from the high-pressure environment *in utero* [11]. Epidemiological studies suggest that incontinence is a factor in virtually all patients lives, with one in four requiring bladder reconstruction and one in five requiring renal replacement therapy and kidney transplant [12].

Antenatal surgical models have relied on replicating the partial obstruction generated by valves within the urethral lumen with external constriction near the vesico-urethral junction. The first antenatal models of this kind, utilising the sheep did not create an obstructive phenotype as the urachus was left un-ligated, acting as an outlet for urine [13]. Subsequently, Harrison successfully created fetal obstruction in sheep with an ameroid constrictor and balloon cuff [14], a technique further refined by Peters evolving into a staple method of gradual constriction with open metal rings (**Figure 2**) [15].

These studies found that antenatal obstruction causes bladder wall thickening with muscle hypertrophy and hydronephrosis in the kidneys. Variable degrees of fibrosis (increased connective tissue content) were also observed in both the bladder and kidney [15, 16] (**Table 3**). Fibrosis is generally the final common pathway for chronic kidney disease [17], but increasingly there is the recognition that it is also an index of disease for congenital bladder disorders [18]. Increased bladder fibrosis results in the replacement of functioning detrusor with non-functioning scar tissue. The variability of fibrosis has been explored in several studies with Farrugia and colleagues finding that fibrosis and functional hypocontractility in the bladder were absent after short periods of obstruction (9 days) and only emerged with prolonged obstruction (30 days) [19,20]. Recent research using a complete urethral obstruction model has shown a fibrotic phenotype within five days [21]. These studies suggest bladder fibrosis is a result of both length of time spent obstructed and its severity, though the relative weighting of each is yet to be elucidated. Interestingly, historical human autopsy studies of PUV have increased bladder thickness primarily from muscle hypertrophy and moderate or no increase in the percentage of fibrotic tissue present in the bladder wall compared to healthy controls [22,23]. These discrepancies between surgical models and human pathology reports may reflect the mid-gestation creation of the partial obstruction in the animal model versus the first-trimester development of the human disorder, providing the bladder different lengths of time to remodel.

A true genetic animal model of PUV does not exist, but one that has similarities to the disorder is the Megabladder (Mgb) mouse [24], the result of a random transgene insertion into chromosome 16, which is then translocated into chromosome 11. Homozygote Mgb mice present with deficient detrusor and functional voiding impairment with bladder distension,

hydronephrosis and renal failure. Its most striking similarities to PUV are the preferential unilateral hydronephrosis, reminiscent of a 'pop-off' mechanism seen in children, secondary vesicoureteric reflux, the variable clinical course and progressive kidney disease despite vesicostomy [25]. However, unlike PUV, which only affects males, a milder phenotype is also seen in female Mgb mice without an actual obstructive lesion such as an overt urethral valve. This model may be a closer analogue of Prune-Belly syndrome (PBS) due to the pattern of bladder distension over bladder wall thickening. Recent research suggests that loss of myocardin may mediate the pathophysiological effects seen in this model [26]. Myocardin is a key transcriptional co-activator of smooth muscle differentiation and development,with human cases of PBS being found with defects in myocardin expression. When the function of myocardin in Mgb mice is further genetically manipulated by crossing heterozygote mice with mice that are heterozygous for constitutive knockout of myocardin, they also manifest a heart defect – patent ductus arteriosus [25]. Myocardin-variants have been discovered in several index families exhibiting heart defects and antenatally-detected megabladders, suggesting myocardin as a potential mechanistic regulator of megabladder as well as other smooth-muscle disorders in human disease.

Fetal PUV animal models

1. Partial fetal delivery through maternal hysterotomy and amniotic capsulotomy.

- Range of gestational age described between 55 -95 days

2. Exposure of bladder and penis in male fetuses only, although some studies have described similar procedures on female fetuses.

3. Urachal ligation must be accompanied with both techniques.

4. Partial or complete urethral constriction techniques have been described.

5. Various delivery techniques described ranging from 105 day caesarean-section to term delivery.
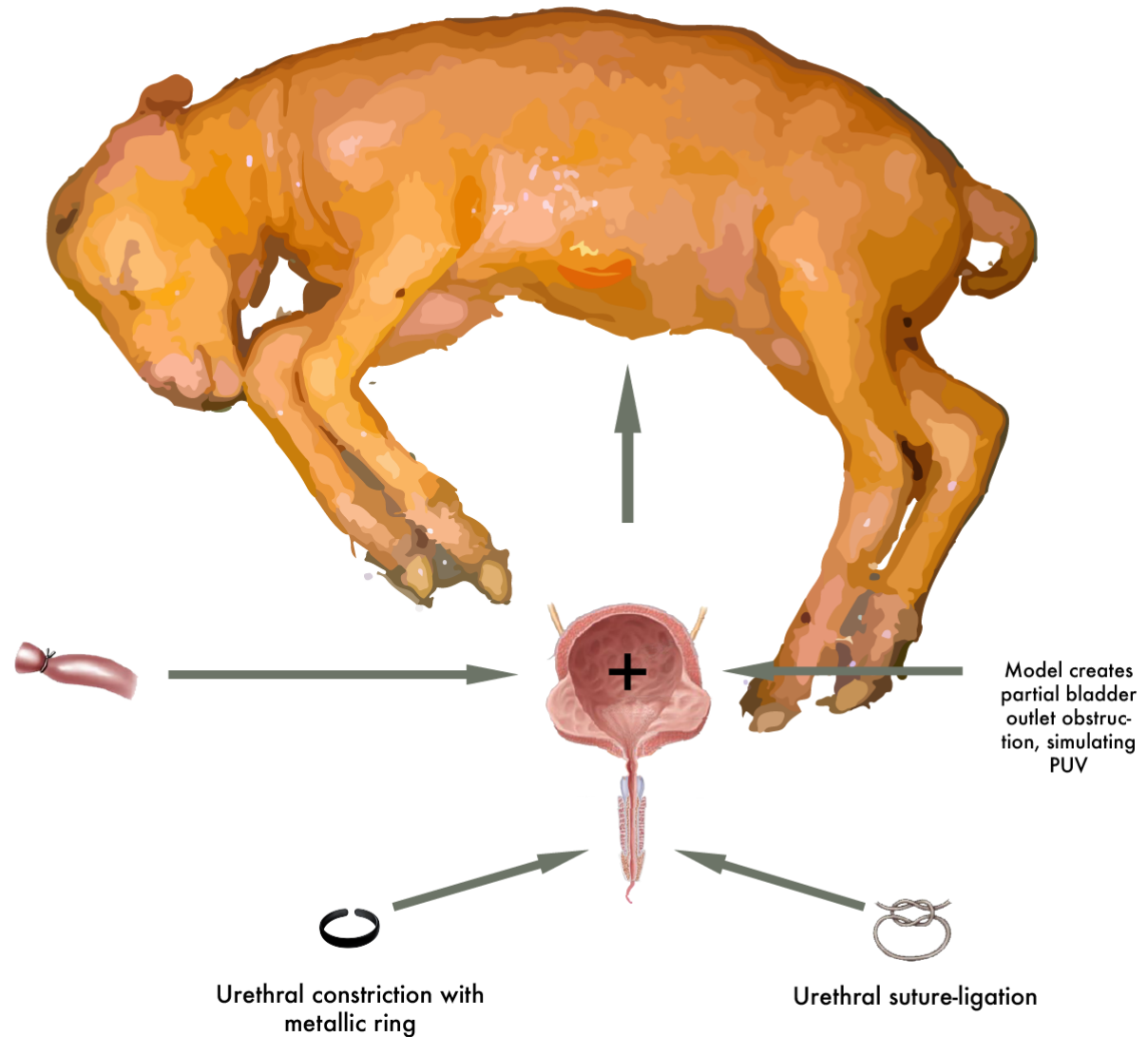
Model creates partial bladder outlet obstruction, simulating PUV

Urethral constriction with metallic ring

Urethral suture-ligation

Figure 2: Surgical PUV models

Table 3: Fetal animal models of PUV

PAPER	ANIMAL	METHOD	KEY FINDINGS
Harrison et al., 1983 [14]	Sheep	Complete urethral ligation at 62-84 days gestation	Noted the urachus remains patent if not ligated.
Peters et al., 1992 [15]	Sheep	Complete urethral clip ligation at 55-60 days.	Noted increase in upper and lower tract size and mass after fetal obstruction.
Cendron et al., 1994 [27]	Sheep	Partial obstruction with jump ring (5-6mm) around bladder neck, 90 days gestation.	Observed increase in bladder capacity and weight with obstruction.
Rohrmann et al. 1997 [28]	Rabbit	Partial obstruction with loose urethral ligation on day 23 (full term 31-32 days).	Hypertrophy and increased connective tissue content noted.
Dalmose et al., 2000 [29]	Pig	Partial obstruction at 111-115 days of gestation	First model in pigs with subsequent in-utero urodynamics.
Thiruchelvam et al. 2003. [30]	Sheep	Partial obstruction with 2mm omega-shaped ring at 75 days.	Noted atropine resistance and bladder hypocontractility likely due to denervation.
De Tayrac et al. 2003 [31]	Sheep	Partial obstruction with 3mm ring and urachal catheterisation at 87 days.	First antenatal urodynamic in sheep, noted biphasic fill-void patterns with end-fill overactivity in obstruction.
Farrugia et al., 2006 [20]	Sheep	Partial obstruction with 3mm Omega ring at 75-82 days.	Shorter-length obstruction (9 days), noting milder morphological changes to bladder and kidney. Urachal ligation alone produced similar effects, suggesting ovine urachus is the main conduit for fetal voiding in mid-gestation.
Singh et al. 2007 [24]	Mouse	Genetic: insertion of transgene resulting in translocation from chromosome 16 to chromosome 11	Heritable genetic defect leading to a lower urinary tract obstruction phenotype – though without a true obstructive lesion. Reduced detrusor smooth muscle causes the defect, protective response in females.

BLADDER EXSTROPHY

Bladder exstrophy (BE), part of the Bladder-exstrophy-epispadias cloaca complex (BEEC) is a condition that affects both genders with global incidence of one per 50,000 births. It is characterised by an open and exposed bladder plate; many variant types exist, including some that have a skin covering [32]. In classic bladder exstrophy (CBE), the most common form, the bladder defect occurs alongside an abdominal muscle defect, an unfused pubic symphysis and a penile epispadias – a dorsal urethral opening. Other members of this complex of disorders include isolated epispadias and cloaca exstrophy (where the hindgut is also exposed). Different pathogenic theories exist, entailing either a failure of cellular migration in the abdominal wall during development; problems with bladder, hindgut and urethral separation with abnormal cloaca membrane development or membrane rupture also a possible culprit [33,34].

Whilst canine and avian surgical models exist [35], the majority of antenatal models have used sheep (**Table 4**). First described by Slaughenhoupt, an exstrophy phenotype is created through an abdominal wall defect, opening and trimming the bladder to size and suturing the edges to the abdominal defect and completed by urethral division to create epispadias [36] (**Figure 3**). Histologically, bladders from this model show an increase in the ratio of collagen-to-smooth muscle, mirroring research into human exstrophy tissue [37]. In addition, the model has been used as a testbed to evaluate the regenerative capacity of bladder augmentation, demonstrating equivalence to normal bladders when patched with collagen scaffolds [38].

The antibiotic nigericin and mould-toxin ochratoxin A have been reported as teratogens capable of creating cloacal exstrophy in avians [39,40]. Injection of the dye trypan blue or the anti-trypanosomal agent suramin also creates cloacal exstrophy in chickens [41,42], although only in 7-20% of exposures alongside an array of other abnormalities such as aortic aneurysms. Observations of thinning of the ventral body wall in this model contributes to the hypothesis that abdominal wall rupture underlies exstrophy. The issues with these models are manifold, including a lack of reproducibility in mammals [43] and the low reproducibility even in avians – suggesting these teratogens may be interfering with broader developmental signalling.

Genetic mouse models have been used to highlight signalling pathways underlying bladder exstrophy pathogenesis. Alx4 is a homeobox gene important in body patterning. $Alx4^{Lst/Lst}$ mice bear a naturally occurring 16bp deletion to the homeobox-binding domain of Alx4. These mice exhibit an exomphalos, thin body wall and bladder wall hypoplasia [44], a phenotype associated with elevated expression of sonic hedgehog, a molecular pathway with important roles in the generation of morphogenic gradients determining body patterning [45]. In $Alx4^{Lst/Lst}$ mice, sonic hedgehog expression and its downstream target Ptc1, are increased in the cloacal epithelium and mesenchyme respectively. Additionally, when $Alx4^{Lst/Lst}$ or $Alx4^{Lst/+}$ mice are bred with mice with a naturally occurring deletion in Gli3,a negative regulator of hedgehog signalling ($Gli^{Xt/Xt}$), they exhibit genital tubercle hypoplasia, which is extremely severe in the case of the compound homozygotes.

The protein p63 is involved in epithelial stratification and development. It can be expressed in multiple isoforms, the most predominant in the ventral bladder being ΔNp63 [46]. In $p63^{-}$

$^{/-}$ mice, the ventral bladder and abdominal walls are absent, the bladder epithelium is not stratified and it exhibits increased apoptosis. Restoration of ΔNp63, specifically either isoform β or γ, reduces the levels of apoptosis present, partially rescuing the phenotype.

Bladder exstrophy models

1. Partial fetal delivery through maternal hysterotomy and amniotic capsulotomy.

- Range of gestational age described between 55 -95 days.

2. Exposure and evisceration of bladder in male and female fetuses. Bladders incised and trimmed in several ways. Bladder trigone and 'plate' sutured to laparotomy wound.

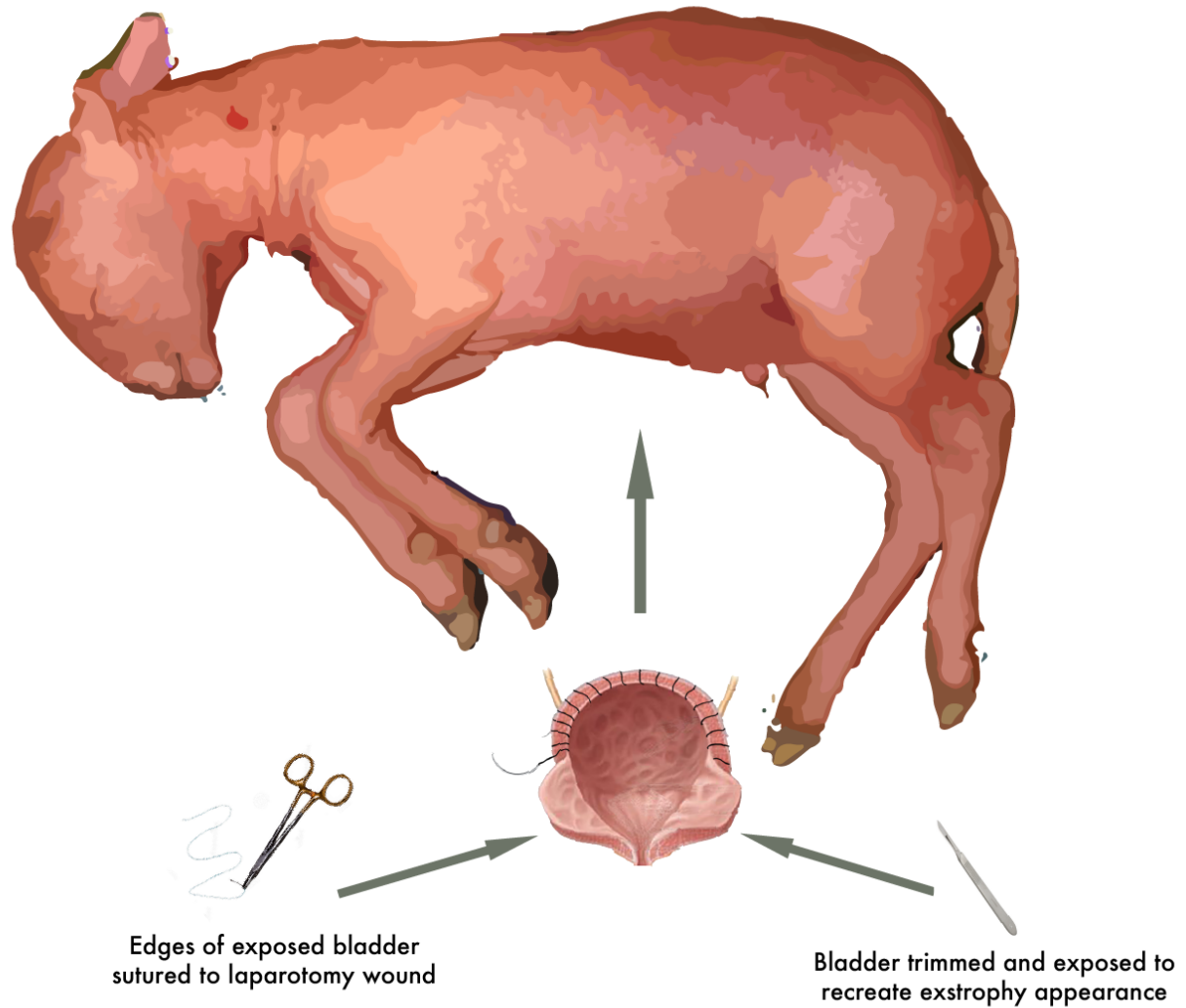3. Various delivery methods and timing described.

Edges of exposed bladder sutured to laparotomy wound

Bladder trimmed and exposed to recreate exstrophy appearance

Figure 3: Surgical exstrophy models

Table 4: Animal models of fetal bladder exstrophy

PAPER	ANIMAL	METHOD	KEY FINDINGS
Marshall & Muecke, 1964 [47]	Chicken	Surgical interference with cloacal membrane.	Earliest exstrophy model.
Thomalla et al., 1985 [48]	Chicken	CO_2 laser injury to caudal omphalomesenteric vessels at 68-76 hours of development.	Speculated that cloacal exstrophy is caused by early disruption of the cloacal membrane.
Wei et al., 1996 [39]	Chicken	Injection of fungal toxin Ochratoxin A into air sac of incubated eggs.	Primarily a model for caudal dysgenesis but exstrophy also noted.
Slaughenhoupt et al., 1996 [36]	Sheep	First large-mammal model of exstrophy.	Noted an increase in connective tissue to smooth muscle ratio in animals with exstrophy phenotype created.
Manner & Kluth, 2005 [49]	Chicken	Suramin and Trypan blue injection leading to 7% and 20% rates of exstrophy	Proposed that the exstrophy caused by these agents may be related to a relative thinning of the developing ventral body wall leading to rupture and exposure of the cloaca.
Cheng et al., 2006 [48]	Mouse	Complete knock-out of Delta isoform of *p63*	Spectrum of BE defects produced: ventral wall abnormality, absence of pubic symphysis, genital anomalies, umbilical hernia and ventral location of anus.

OTHER ANTENATAL MODELS OF CONGENITAL BLADDER DISORDERS

There are several models for congenital neurogenic bladder (NB), a result of neural tube defects. According to the 'two-hit model' [50], the first pathogenic step is fetal cord extrusion whilst the second damaging step results from cord damage from trauma and amniotic irritation. This process results in disruption of pelvic innervation leading to limb paralysis and bladder obstruction through detrusor-sphincter dyssynergia. Surgical models for NB have existed for many decades, but the lamb model for myelomingocoele is most prominently described. This is achieved via laminectomy at L1 to L5 level and excision of the dura mater with or without myelotomy to produce a cerebrospinal leak [51]. There are over 80 murine models for neural tube defects mainly created with teratogens such as valproic acid, fumonisins and retinoic acid. These models demonstrate high homology with human disease and are treatable with folic acid. Research with these models has implicated several genes such as *Cart1, Cited2, Crooked tail* and others [52]. While the research in this area is plentiful, research focused on bladder outcomes represents only a small fraction with findings that pathologic changes mirror those seen in human disease, but disagreement as to the extent of functional restoration with repair [53,54].

Models exist for several other congenital lower urinary tract disorders. Homozygous mutations in Heparanase-2 creates a partial analogue for Urofacial syndrome in mice [55]. The chemotherapeutic agent doxorubicin has been associated with common urogenital sinus, although it has also been associated with high rates of bladder agenesis and ano-rectal malformations [56].

OUTSTANDING QUESTIONS IN ANTENATAL ANIMAL MODEL RESEARCH

Specificity of genetic models

Genetic models for some congenital bladder disorders have benefitted from having a well-established teratogenic association in humans (e.g. folate deprivation and neural tube defects). However, this is not the case for conditions such as bladder exstrophy or PUV, where consistent or high-homology genetic models have yet to emerge. Case to case, these disorders have highly variable phenotype and often do not share a genetic aetiology. High throughput sequencing technologies will be extremely useful in finding new candidate genes from human cases which can then be used to design novel mouse models. GWAS and projects like the 100,000 genomes project may help to find candidate genes [57,58]. However, due to the diverse aetiology of both PUV and bladder exstrophy, the likelihood that most cases are polygenic in aetiology, mouse models generated from these approaches still face difficulties in being relevant to the human disorder.

Timing of surgical model creation

Most PUV and BE animal models have relied on in-utero surgery to recreate the phenotypes of these two disorders. The extent of our understanding of fetal bladder remodelling and wound healing is limited and thus two challenges naturally arise, the first being our current technical inability to recreate these conditions prior to mid-gestation, whereas both conditions arise during the first trimester in humans. This limits the homology of these models until techniques enable earlier intervention. A more species-relevant question is of the urachus, which closes in mid-gestation in humans but has species-variable outflow resistance and remains patent for much longer in animals such as sheep. Closure with fetal model creation is necessary or it will act as a pressure 'pop-off' mechanism. However, it is unclear

whether its continued patency is an important feature of the animal's gestational bladder development.

Correlation with postnatal models and reduction of animal research

It is highly probable that *in utero* and postnatal pathological processes such as the bladder response to obstruction share similarities. Determining this fact through correlation of antenatal and postnatal research may yield the benefit of reducing the technical difficulty of engaging in this field of research by not having to create antenatal models. Refining surgical technique or knowing when *in vitro* research with human tissue may be utilised can offer the hope of reducing the use of animals in biological models.

Another way of reducing the number of animal models required is to use cell culture models. Bladder organoid cultures, essentially organs-in-miniature where some of the cellular architecture (urothelium, mesenchyme) have been recapitulated on a sphere, have been used to model tumour development and potential therapies [59,60]. This is an exciting area that may provide the ability to model and test therapies for obstructive uropathy phenotypes without the need for whole organisms.

It is also important to recognise that congenital bladder disorders are rare, often with orphan aetiology, and affect children. The acquisition of human tissue for explant or organoid culture is invariably a challenge, creating a prime need for animal research work. This must be undertaken with utmost respect for the welfare of test animals and studies designed to maximise humane outcomes.

What are the implications of these animal models to therapy?

Animal models have also provided a beneficial platform for testing and advancing fetal interventions. An area where animal models may be of benefit is determining the optimal method of fetal vesico-amniotic shunting (VAS). Analysis of the results of clinical trials such as PLUTO (percutaneous shunting in lower urinary tract obstruction) showed improvement in fetal survival but weighed against a lack of demonstrable benefit to the urinary tract and the risk of pregnancy loss [61]. Recent animal model research has shown that fetal shunting creates a deleterious fibrotic response in the shunted bladder [62]. What would be of value is to determine the threshold of outflow resistance and length of obstructed time that leads to this fibrotic effect, to help guide further efforts at human VAS and discover the optimal method and timing of this procedure.

CONCLUSION

Antenatal animal model research helps to improve understanding of biological systems and diseases processes in rare disorders, this is particularly true for PUV and exstrophy, rare conditions that continue to provoke debate within communities of clinicians managing these conditions. At the root of the problem is the rarity of these anomalies and difficulty in undertaking laboratory and clinical research with human patients. It helps to expand our conceptual ideas of disease pathogenesis and provides new avenues for laboratory and clinical research. It is important for the researcher to both understand the methods by which models are selected, along with the established techniques for creating various fetal models in order to yield improvements in modelling as well as new scientific discoveries to benefit patients.

REFERENCES

[1]   UK Renal Registry. 21st Annual Report - UK Renal Registry. [Online]. Available from: https://www.renalreg.org/reports/data_to_end_2017/ [Accessed 16th July 2020].

[2]   Pinho SS, Carvalho S, Cabral J, Reis CA, Gärtner F. Canine tumors: a spontaneous animal model of human carcinogenesis. *Transl Res*. 2012 Mar 1;159(3):165-72.

[3]   Kaushal A, Wani WY, Anand R, Gill KD. Spontaneous and induced nontransgenic animal models of AD: modeling AD using combinatorial approach. *Am J Alzheimers Dis Other Demen*. 2013 Jun;28(4):318-26.

[4]   Dewan PA. Congenital posterior urethral obstruction: the historical perspective. *Pediatr Surg Int*. 1997 Feb;12(2-3):86-94. PMID: 9156879.

[5]   Patten BM, Barry A. The genesis of exstrophy of the bladder and epispadias. *Am J Anat*. 1952 Jan;90(1):35-57. doi: 10.1002/aja.1000900103. PMID: 14902688.

[6]   Trembath DG, Rijhsinghani A. Possible maternal inheritance of a common obstructive urinary tract anomaly. Report of a case of a woman with multiple urinary tract infections and two sons with posterior urethral valves. *J Reprod Med*. 2002 Nov;47(11):962-4. PMID: 12497693.

[7]   Ebert AK, Reutter H, Ludwig M, Rösch WH. The exstrophy-epispadias complex. *Orphanet J Rare Dis*. 2009 Oct 30;4:23. doi: 10.1186/1750-1172-4-23. PMID: 19878548; PMCID: PMC2777855.

[8]   Sneddon LU, Halsey LG, Bury NR. Considering aspects of the 3Rs principles within experimental animal biology. *J Exp Biol*. 2017;220(Pt 17):3007-16.

[9]   Brownlee E, Wragg R, Robb A, Chandran H, Knight M, McCarthy L. Current epidemiology and antenatal presentation of posterior urethral valves: Outcome of BAPS CASS National Audit. *J Pediatr Surg*. 2019 Feb 1;54(2):318-21.

[10]   Cromie WJ. Implications of antenatal ultrasound screening in the incidence of major genitourinary malformations. *Semin Pediatr Surg*. 2001 Nov;10(4):204-11. doi: 10.1053/spsu.2001.26843. PMID: 11689994.

[11]   Matsell DG, Yu S, Morrison SJ. Antenatal Determinants of Long-Term Kidney Outcome in Boys with Posterior Urethral Valves. *Fetal Diagn Ther*. 2016;39(3):214-21.

[12]   Boghossian NS, Sicko RJ, Giannakou A, Dimopoulos A, Caggana M, Tsai MY, et al, Rare copy number variants identified in prune belly syndrome. *Eur J Med Genet*. 2018 Mar;61(3):145-151

[13]   Tanagho EA. Surgically induced partial urinary obstruction in the fetal lamb. II. Urethral obstruction. *Invest Urol*. 1972;10(1):25-34.

[14]   Harrison MR, Ross N, Noall R, de Lorimier AA. Correction of congenital hydronephrosis in utero. I. The model: fetal urethral obstruction produces hydronephrosis and pulmonary hypoplasia in fetal lambs. *J Pediatr Surg*. 1983;18(3):247-56.

[15]   Peters CA, Vasavada S, Dator D, Carr M, Shapiro E, Lepor H, et al. The effect of obstruction on the developing bladder. *J Urol*. 1992;148(2 Pt 2):491-6.

[16]   Gobet R, Cisek LJ, Chang B, Barnewolt CE, Retik AB, Peters CA. Experimental fetal vesicoureteral reflux induces renal tubular and glomerular damage, and is associated with persistent bladder instability. *J Urol*. 1999;162(3 Pt 2):1090-5.

[17]   Hewitson TD. Fibrosis in the kidney: is a problem shared a problem halved? *Fibrogenesis & tissue repair*. 2012 Dec 1 (Vol. 5, No. S1, p. S14). BioMed Central.

[18]   Lu YT, Tingskov SJ, Djurhuus JC, Nørregaard R, Olsen LH. Can bladder fibrosis in congenital urinary tract obstruction be reversed? *J Pediatr Urol*. 2017 Dec 1;13(6):574-80.

[19]   Farrugia MK, Long DA, Godley ML, Peebles DM, Fry CH, Cuckow PM, et al. Experimental short-term fetal bladder outflow obstruction: I. Morphology and cell biology associated with urinary flow impairment. *J Pediatr Urol*. 2006;2(4):243-53.

[20]   Farrugia MK, Godley ML, Woolf AS, Peebles DM, Cuckow PM, Fry CH. Experimental short-term partial fetal bladder outflow obstruction: II. Compliance and contractility associated with urinary flow impairment. *J Pediatr Urol*. 2006;2(4):254-60.

[21]   Kitajima K, Aoba T, Pringle KC, Seki Y, Zuccollo J, Koike J, et al. Bladder development following bladder outlet obstruction in fetal lambs: optimal timing of fetal therapy. *J Pediatr Surg*. 2010;45(12):2423-30.

[22]   Freedman AL, Qureshi F, Shapiro E, Lepor H, Jacques SM, Evans MI, et al. Smooth muscle development in the obstructed fetal bladder. *Urology*. 1997;49(1):104-7.

[23]   Workman SJ, Kogan BA. Fetal bladder histology in posterior urethral valves and the prune belly syndrome. *J Urol*. 1990;144(2 Pt 1):337-9.

[24]   Singh S, Robinson M, Nahi F, Coley B, Robinson ML, Bates CM, et al. Identification of a unique transgenic mouse line that develops megabladder, obstructive uropathy, and renal dysfunction. *J Am Soc Nephrol*. 2007;18(2):461-71.

[25]   McHugh KM. Megabladder mouse model of congenital obstructive nephropathy: genetic etiology and renal adaptation. *Pediatr Nephrol*. 2014;29(4):645-50.

[26]   Houweling AC, Beaman GM, Postma AV, Gainous TB, Lichtenbelt KD, Brancati F, et al. Loss-of-function variants in myocardin cause congenital megabladder in humans and mice. *J Clin Invest*. 2019;129(12):5374-80.

[27]   Cendron M, Horton CE, Karim OM, Takishima H, Haberlik A, Mostwin JL, et al. A fetal lamb model of partial urethral obstruction: experimental protocol and results. *J Pediatr Surg*. 1994;29(1):77-80.

[28]   Rohrmann D, Monson FC, Damaser MS, Levin RM, Duckett JW, Jr., Zderic SA. Partial bladder outlet obstruction in the fetal rabbit. *J Urol*. 1997;158(3 Pt 2):1071-4.

[29]   Dalmose AL, Hvistendahl JJ, Olsen LH, Eskild-Jensen A, Djurhuus JC, Swindle MM. Surgically induced urologic models in swine. *J Invest Surg*. 2000;13(3):133-45.

[30]   Thiruchelvam N, Nyirady P, Peebles DM, Fry CH, Cuckow PM, Woolf AS. Urinary outflow obstruction increases apoptosis and deregulates Bcl-2 and Bax expression in the fetal ovine bladder. *Am J Pathol*. 2003;162(4):1271-82.

[31]   de Tayrac R, Cuckow PM, Devlieger R, Deprest J, Bogaert G, Ville Y. Antenatal urodynamic studies in the fetal lamb: experimental protocol and preliminary results. *Prenat Diagn*. 2003;23(3):187-92.

[32]   Maruf M, Benz K, Jayman J, Kasprenski M, Michaud J, Di Carlo HN, et al. Variant Presentations of the Exstrophy-Epispadias Complex: A 40-Year Experience. *Urology*. 2019 Mar;125:184-190. doi: 10.1016/j.urology.2018.10.049. Epub 2018 Dec 18. PMID: 30576745.

[33]   Marshall VF, Muecke EC. Variations in exstrophy of the bladder. *J Urol*. 1962;88(6):766-96.

[34]   Mildenberger H, Kluth D, Dziuba M. Embryology of bladder exstrophy. *J Pediatr Surg*. 1988;23(2):166-70.

[35]   Fein RL. Artificial exstrophy in the dog for separated renal function studies. *J Surg Res*. 1969;9(4):235-9.

[36]   Slaughenhoupt BL, Chen CJ, Gearhart JP. Creation of a model of bladder exstrophy in the fetal lamb. *J Urol*. 1996;156(2 Pt 2):816-8.

[37]   Johal NS, Arthurs C, Cuckow P, Cao K, Wood DN, Ahmed A, et al. Functional, histological and molecular characteristics of human exstrophy detrusor. *J Pediatr Urol*. 2019;15(2):154 e1- e9.

[38]   Roelofs LA, Kortmann BB, Oosterwijk E, Eggink AJ, Tiemessen DM, Crevels AJ, et al. Tissue engineering of diseased bladder using a collagen scaffold in a bladder exstrophy model. *BJU Int*. 2014;114(3):447-57.

[39]   Wei X, Sulik KK. Pathogenesis of caudal dysgenesis/sirenomelia induced by ochratoxin A in chick embryos. *Teratology*. 1996;53(6):378-91.

[40]   Vedel-Macrander GC, Hood RD. Teratogenic effects of nigericin, a carboxylic ionophore. *Teratology*. 1986;33(1):47-51.

[41]   Manner J, Kluth D. A chicken model to study the embryology of cloacal exstrophy. *J Pediatr Surg*. 2003;38(5):678-81.

[42]   Manner J, Kluth D. The morphogenesis of the exstrophy-epispadias complex: a new concept based on observations made in early embryonic cases of cloacal exstrophy. *Anat Embryol (Berl)*. 2005;210(1):51-7.

[43]   Lendon RG, Forbes M. Deformities of the renal tract associated with spina bifida in trypan blue treated rat embryos. *J Anat*. 1971;110(Pt 3):383-91.

[44]   Matsumaru D, Haraguchi R, Moon AM, Satoh Y, Nakagata N, Yamamura K, et al. Genetic analysis of the role of Alx4 in the coordination of lower body and external genitalia formation. *Eur J Hum Genet*. 2014;22(3):350-7.

[45]   Andrews MG, Kong J, Novitch BG, Butler SJ. New perspectives on the mechanisms establishing the dorsal-ventral axis of the spinal cord. *Curr Top Dev Biol*. 2019;132:417-450. doi: 10.1016/bs.ctdb.2018.12.010. Epub 2018 Dec 26. PMID: 30797516.

[46]   Cheng W, Jacobs WB, Zhang JJ, Moro A, Park JH, Kushida M, et al. DeltaNp63 plays an anti-apoptotic role in ventral bladder development. *Development*. 2006;133(23):4783-92.

[47]   Muecke EC. The Role of the Cloacal Membrane in Exstrophy: The First Successful Experimental Study. *J Urol*. 1964;92:659-67.

[48]   Thomalla JV, Rudolph RA, Rink RC, Mitchell ME. Induction of cloacal exstrophy in the chick embryo using the CO_2 laser. *J Urol*. 1985;134(5):991-5.

[49]   Manner J, Seidl W, Heinicke F, Hesse H. Teratogenic effects of suramin on the chick embryo. *Anat Embryol (Berl)*. 2003;206(3):229-37.

[50]   Hutchins GM, Meuli M, Meuli-Simmen C, Jordan MA, Heffez DS, Blakemore KJ. Acquired spinal cord injury in human fetuses with myelomeningocele. *Pediatr Pathol Lab Med*. 1996 Sep-Oct;16(5):701-12. PMID: 9025869.

[51]   Meuli M, Meuli-Simmen C, Yingling CD, Hutchins GM, Hoffman KM, Harrison MR, et al. Creation of myelomeningocele in utero: a model of functional damage from spinal cord exposure in fetal sheep. *J Pediatr Surg*. 1995;30(7):1028-32; discussion 32-3.

[52]   Greene ND, Copp AJ. Mouse models of neural tube defects: investigating preventive mechanisms. *Am J Med Genet C Semin Med Genet*. 2005;135C(1):31-41.

[53]   Burgos L, Encinas JL, Garcia-Cabezas MA, Peiro JL, Lopez-Santamaria M, Jaureguizar E. Bladder changes after several coverage modalities in the surgically induced model of myelomeningocele in lambs. *Actas Urol Esp*. 2014;38(1):55-61.

[54]   Tang L, Zhong H, Chen H, Shen J, Bi Y, Xiao X. In utero repair of fetal rat myelomeningocele affects neuromuscular development in the bladder. *Exp Ther Med*. 2017;14(4):3681-7.

[55]   Roberts NA, Hilton EN, Lopes FM, Singh S, Randles MJ, Gardiner NJ, et al. Lrig2 and Hpse2, mutated in urofacial syndrome, pattern nerves in the urinary bladder. *Kidney Int*. 2019;95(5):1138-52.

[56]   Liu MI, Hutson JM. Ontogeny of bladder agenesis in rats induced by adriamycin. *BJU Int*. 2001;87(6):556-61.

[57]   Turnbull C, Scott RH, Thomas E, Jones L, Murugaesu N, Pretty FB, et al. 100 000 Genomes Project., The 100 000 Genomes Project: bringing whole genome sequencing to the NHS., *BMJ*. 2018 Apr 24;361:k1687.

[58]   Verbitsky M, Westland R, Perez A, Kiryluk K, Liu Q, Krithivasan P, et al. The copy number variation landscape of congenital anomalies of the kidney and urinary tract., *Nat Genet*. 2019 Jan;51(1):117-127

[59]   Vasyutin I, Zerihun L, Ivan C, Atala A. Bladder Organoids and Spheroids: Potential Tools for Normal and Diseased Tissue Modelling. *Anticancer Res*. 2019 Mar;39(3):1105-1118

[60]   Wang S, Gao D, Chen Y. The potential of organoids in urological cancer research. *Nat Rev Urol*. 2017 Jul;14(7):401-414

[61]   Nassr AA, Shazly SAM, Abdelmagied AM, Araujo Junior E, Tonni G, Kilby MD, et al. Effectiveness of vesicoamniotic shunt in fetuses with congenital lower urinary tract obstruction: an updated systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2017;49(6):696-703.

[62]   Kitagawa H, Pringle KC, Koike J, Nagae H, Zuccollo J, Aoba T, et al. Is a vesicoamniotic shunt intrinsically bad? Shunting a normal fetal bladder. *J Pediatr Surg*. 2007;42(12):2002-6.