Original Research

Seeding cell approach for tissue-engineered urethral reconstruction in animal study: A systematic review and meta- analysis

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Abstract

We systematically reviewed published preclinical studies to evaluate the effectiveness of cell-seeded tissue engineering approach for urethral reconstruction in an animal model. The outcomes were summarized by success factors in the animal experiments, which evaluate the possibility and feasibility of a clinical application in the future. Preclinical studies of tissue engineering approaches for urethral reconstruction were identified through a systematic search in PubMed, Embase, and Biosis Previews (web of science SP) databases for studies published from 1 January 1980 to 23 November 2014. Primary studies were included if urethral reconstruction was performed using a tissue-engineered biomaterial in any animal species (with the experiment group being a cell-seeded scaffold and the control group being a cell-free scaffold) with histology and urethrography as the outcome measure. A total of 15 preclinical studies were included in our meta-analysis. The histology and urethrography outcome between the experimental and control groups were considered to be the most clinically relevant. Through this systematic approach, our outcomes suggested that applying the cell-seeded biomaterial in creating a neo-urethra was stable and effective. And multi-type cells including epithelial cells as well as smooth muscle cells or fibroblasts seemed to be a better strategy. Stem cells, especially after epithelial differentiation, could be a promising choice for future researches.

Keywords: Tissue engineering, urethral reconstruction, animal model, cell-seeded/unseeded biomaterial, urethral defect, meta-analysis

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Introduction

Urethral defects stemming from congenital malformations, trauma, inflammation, or carcinoma have always been one of the hot topics in urological research field. Hypospadias, a malformation of the penis due to an incomplete development of the ventral part of the penis, is one of the most common congenital abnormality in western countries with high incidence of 0.4%–0.6%.[1] Meanwhile, the incidence of urethral strictures secondary to trauma and iatrogenic injuries is high in developing countries.[2] The treatments for urethral defects remain problems in urology. Due to the shortage of autologous urethral tissue available for urethral reconstruction, especially for anterior urethral strictures and hypospadia, autologous tissue from genital and extragenital skin flaps or grafts,[3] bladder or buccal mucosal grafts[4] have been used in urethral reconstruction. However, complications such as oral discomfort, nerve damage, bleeding, and hematoma usually occur at the donor site after harvesting.[5] Moreover, donor tissue resource is so limited that it hardly provides a sufficient graft for surgery when the case suffers with panurethral or reoccurring strictures and grafts or flaps have been harvested previously. Currently, this treatment mode of sacrificing healthy tissue for repairing a lesion is controversial.

However, tissue engineering and regenerative medicine (TERM) technology presented an approach for urethral reconstruction.[6,7] This approach includes a novel method that avoids the complications arising from tissue harvesting and reduces the patients' injuries. Many researchers have focused on the optimum decellularized biological scaffolds, which are applied with and without autologous cells. In the field of urethral reconstruction, acellular matrices and decellularized scaffolds have been used in clinic.[8,9] Nevertheless, to date, there

has been no large-scale application of tissue-engineered urethra in clinical practice.[10] Several investigators demonstrated that cell seeded scaffolds were not indispensable for the urethral remodeling,[11] whereas some scholars demonstrated that tissue regeneration and vascular organization would benefit by cell seeding in the unseeded matrices. To our knowledge, the differences between seeding or not seeding autologous cells in scaffolds for tissue engineering in urethral reconstruction have not been identified.

In this evidenced-based systematic review and meta-analysis, we presented a comprehensive overview that focuses on the basic research concerning preclinical application of tissue engineering approaches for urethral reconstruction in animal models. We also compared case groups with and without cell-seeded material. To assess the effect of this tissue engineering approach, we adopted histological results and urethrography as the outcome measures.

Materials and methods

Search strategy

We conducted a systematic search in the PubMed, Embase, and Biosis Previews (web of science SP) databases through 1 January 1980 to 23 November 2014. We followed the search strategy as previously described.[12,13] Briefly, we united synonyms for tissue engineering (tissue-engineered regenerative medicine, tissue culture techniques, etc.) and synonyms for urethral reconstruction (neo-urethra, urethroplasty, urethral repair, etc.). Mesh terms or EMTREE terms and free text words ([tiab] or/ti, ab.) were used together for the entire search. Subsequently, the results of the PubMed and Embase searches were filtered through preciously designed animal filter.[14,15] As a complementary database, we used the built-in filters if they were specific to the animal. The Supplementary File 1 shows the entire strategy used (Figure 1).

Study inclusion

First, EndNote X7 (Thomson Reuter) was employed to remove the duplicates and triplicates gleaned from the searches in the above-mentioned databases. Next, two independent reviewers (JD Xue and J Gao) deleted the articles that had no relationship with our subjects according to the titles and abstracts. If the reviewers had conflicting results, the article was included and more information was used to determine its ultimate inclusion in the next iteration. Third, the two reviewers read the full text of the remaining articles and used the following inclusion criteria: (1) primary paper, (2) animal model, (3) urethral reconstruction after urethral defect, and (4) experimental group with a cell-seeded biomaterial and control group with a biomaterial alone. During this procedure, reference lists of included articles were screened for missed studies. If the reviewers' reference lists were not in an agreement, another reviewer (H Xie) would be consulted to make a final decision for inclusion. Thus, we included a total of 15 articles for our meta-analysis.

Data extraction

The following characteristics were extracted from each eligible study: the first author and year of publication of the study, the animal model information, details about the biomaterial and cell type, the methods or procedure and outcome measures. We defined a successful study as satisfied histology and urethrogram outcome. Histological outcome displayed intact epithelium formation, and urethrogram outcome demonstrated a wide urethral caliber without any sign of stricture, fistula, and diverticulum. If there were no urethrogram results due to complications, the cases were considered to be failure.

Quality assessment

The methodological quality of each study was evaluated, based on a checklist modified from the collaborative approach to meta-analysis and review of animal data

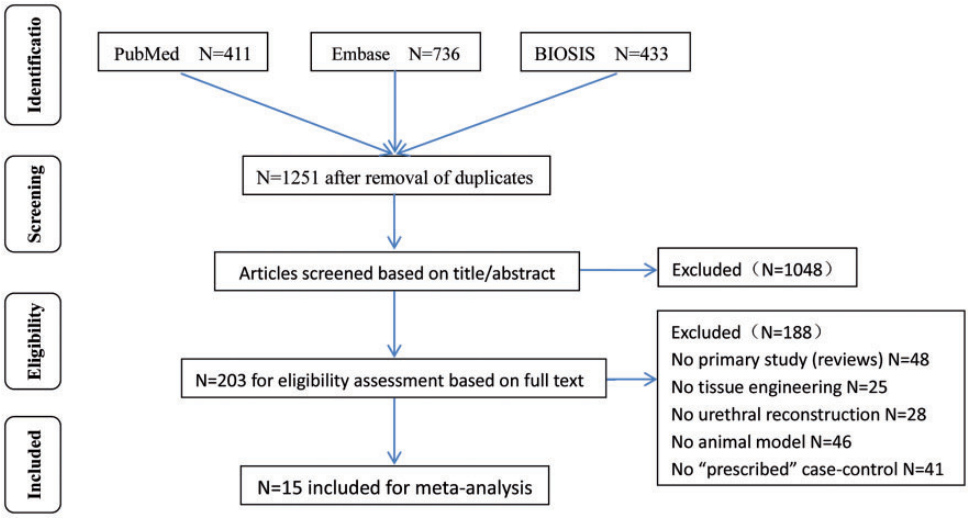

Figure 1 Flow chart of the study selection process. (A color version of this figure is available in the online journal.)

from experimental studies (CAMARADES)[16,17] with minor modifications. The checklist was composed of 10 items. Among them, two items were modified according to our animal model inclusion criteria, which were acceptable because of their initial aim to STROKR status. On the aspect of animal modeling, ''blinded induction of ischemia'' was converted to ''blinded modeling of the urethral defect,'' and the point of ''use of anesthetic without significant intrinsic neuroprotective activity'' was changed to ''surgeries performed by the same surgeons,'' in terms of external intervention. One point was given for each quality criterion.

Statistics analysis

We performed our meta-analysis in line with the PRISMA checklists.[18] All statistical analyses were performed with Review Manager 5 software and STATA 12.0 software. The meta-analysis statistical significance level was determined by a Z-test with a P value of less than 0.05. Between-study statistical heterogeneity was assessed by the I_2 statistic, where index values of 25, 50, and 75% indicated the presence of low, moderate, and high between-trial heterogeneity, respectively.[19] Dichotomous outcomes were expressed as relative risk (RR). For all analyses performed, if no significant heterogeneity was noted, fixed effect model (FEM) analysis using the Mantel–Haenszel method was used. Otherwise, results of the random effects model (REM) analysis were presented and sensitive analysis was also performed.[20] Finally, the Begg's rank correlation method[21] and the Egger's weighted regression method[22] were carried out to detect the publication bias ($P < 0.05$ was considered statistically significant).

Results

Description of studies

A total of 1580 studies were acquired from the PubMed (N = 411), Embase (N = 736), and Biosis Previews (web of science SP) (N = 433) databases. The literature selection process used is illustrated in Figure 1. A total of 1251 studies were included after removal of any duplicates. Next, 1048 studies were excluded according to the titles and abstracts. The remaining 203 studies were full text reviewed, and 188 studies were excluded according to the inclusion criteria as follows: 48 were not primary studies, 25 had no relation to tissue engineering, 28 did not concern about urethral reconstruction, 46 included no animal models, and 41 did not meet our case or control criteria. Finally, in the current study, 15 eligible case control studies[11,23–36] that satisfied the inclusion criteria were included in our meta-analysis.

Characteristics of the included studies

The characteristics of included studies are detailed in Table 1 as follows: (i) animal models: the included studies used rabbits (*New Zealand*) and canines (*Beagles*) as the preferred animal models for urethral reconstruction. Only one study[34] used female animals, while the others used male animals in their experiments. It was disappointing that six of the studies failed to describe the animals' weight or ages. (ii) Biomaterials: we found that a majority of the studies[11,23,25–30,32,35] would rather implant biologically derived, especially autologous, scaffold materials including bladder acellular matrix (BAM). Some new materials were also used, such as a high-density collagen gel,[31] skeletal muscle fragments,[33] silk fibroin matrices,[34] and insoluble type I collagen.[36] (iii) Cell types: a wide variety of cell types were used to seed the biomaterials, illustrated in Table 1. Bladder epithelial cells and smooth muscle cells (SMCs) were used most often. Four studies[24,27,29,34] also employed oral keratinocytes. In addition, there was a rising trend of using stem cells, such as adipose-derived stem cells (ADSCs),[30] bone marrow mesenchymal stem cells,[29] and umbilical cord mesenchymal stem cells.[33] There were two types of surgical approaches (tube and patch) used equally in the 15 included studies. The type of approach may depend on the biomaterial and urethral defect size. The defects were mainly located in the anterior urethral region, and only one study[33] reconstructed a defect in the posterior urethral region.

The follow-up, complications, and measure outcomes are listed in Table 2. Most of the included studies collected data at multiple time points to trace the experimental progress. All of the studies compared the success rate of urethral reconstruction in an animal model between cellular and acellular biomaterials groups, investigating whether (pre-) seeding of the biomaterial was advantageous. Finally, the histological outcome measures reflected the reconstruction diversity, which was classified into two conditions: one indicating improvement, consisting of organized epithelial layers, organized muscle fiber bundles, and vascularization, and the other indicating the opposite, consisting of fibrosis, and the infiltration of inflammatory cells.

Methodological quality of the included studies

In general, the quality of 15 included studies was unsatisfactory. The mean score was approximately 3.87 (ranging from 2 to 6), which was disappointing (Table 3). Specifically, no study monitored physiological parameters during the animal experiments or calculated the sample sizes before conducting the animal experiments. Moreover, we were sorry to discover that only one study established a blinded model of the urethral defect, and no statement of blinded assessment of outcomes and thus avoiding manual subject intervention was included. One study reported that the surgeries were performed by the same surgeons. As a result, an urgent call for the standardization of animal experimental methods was proposed since the guidelines used had been reported,[37] so that valid comparison and full use of the animal results can be made.

Comparison results

Comparison1: Cell-seeded versus cell-unseeded – Overall efficacy. With the 15 studies, we conducted a meta-analysis to compare the efficacy of cell-seeded and cell-free biomaterials for use in urethra reconstruction. It is unequivocal that the purpose of the meta-analysis was to acquire the trends of the outcomes, and not a precise point estimate, between the studies.[38] Due to the high

Table 1 The basic characteristics of the included studies

	Species	Sex					Cell type		Location	
Reference	Strain	*Weight/age*	Group Size	Study design	Bio-material	Scaffold size	Amount	Surgical	Length	Outcome measure
DE Filippo et al.[35]	*Rabbits* *New Zealand*	*Male* [a]	Gr1:12 Gr2:12	Gr1:bladder EC + SMC[b] + BCM Gr2:BCM (acellularity)	BCM[c]	l = 3 cm d = 5.3 mm	bladder EC/SMC each 5×10^{-7}	Tubular	Anterior 1 cm	1Retrograde urethrography 2organ bath studies 3Histological and immunohistochemical analyses
Fu Q et al.[25]	*Rabbits* *New Zealand*	*Male* 10 weeks	Gr1:9 Gr2:9	Gr1:foreskin EC + BCM Gr2: BCM(acellularity)	BCM	l = 1.5 cm w = 1 cm	bladder EC $1.5–3 \times 10^{-6}$	Tubular	Anterior 1.5 cm	1Retrograde urethrography 2Histology
Li C et al.[27]	*Rabbits* *New Zealand*	*Male* 10 weeks	Gr1:12 Gr2:12	Gr1:oral keratinocytes + BCM Gr2: BCM(acellularity)	BCM	l = 2.2 cm w = 1 cm	Keratinocytes 6.6×10^{-6}	Patch	Anterior 2 cm	1Retrograde urethrography 2Histology
Feng et al.[24]	*Rabbits* *New Zealand*	*Male* 10 weeks	Gr1:6 Gr2:6 Gr3:6	Gr1:ACSM(acellularity) Gr2: oral keratinocytes + ACSM[d] Gr3: keratinocytes + CSMC[e] + ACSM	ACSM	l = 2 cm d = 1 cm	Keratinocytes $2.2–4 \times 10^{-6}$ CSMC:4×10^{-8}	Patch	Anterior 1.5 cm	1Retrograde urethrography 2Histology
Gu et al.[26]	*Rabbits* *New Zealand*	*Male* [a]	Gr1:9 Gr2:9	Gr1:mesothelial cells + BCM Gr2: BCM(acellularity)	BCM	l = 1.5 cm w = 1 cm	mesothelial cell 3×10^{-6}	Tubular	Anterior 1.5 cm	1Retrograde urethrography 2Histology
DE Filippo et al.[35]	*Rabbits* *New Zealand*	*Male* [a]	Gr1:9 Gr2:6	Gr1:bladder EC + SMC + BCM Gr2:BCM (acellularity)	BCM	l = 3 cm d = 5.3 mm	bladder EC/SMC each 1×10^{-7}	Tubular	Anterior 3 cm	1Retrograde urethrography 2 molecular studies 3Histological and immunohistochemical analyses
Micol et al.[31]	*Rabbits* *New Zealand*	*Male* 2.5-3.5 kg	Gr1:8 Gr2:8	Gr1:bladder SMC + hdCGT[f] Gr2:hdCGT (acellularity)	hdCGT	l = 2 cm d = 3 mm	bladder SMC 3×10^{-6}	Tubular	Anterior 1 cm	1Retrograde urethrography 2Histology
Orabi et al.[32]	*Canines* *Beagle*	*Male* [a]	*Gr1:15* *Gr2:6*	Gr1:bladder EC + SMC + BCM Gr2:BCM (acellularity)	BCM	l = 7 cm d = 4.7 mm	bladder EC/SMC $32/3.2 \times 10^{-7}$	Tubular	Anterior 6 cm	1Retrograde urethrography 2Histology
Li et al.[29]	*Rabbits* *New Zealand*	*Male* *2.0-2.5 kg*	Gr1:24 Gr2:6	Gr1:BMSC[g] + SMC + BAM Gr2: BAM (acellularity)	*BAM*	l = 3 cm w = 1.5 cm	BMSC/SMC each 1×10^{-6}	Tubular	Anterior 3 cm	1Retrograde urethrography 2Histological and immunohistochemical analyses
Sayeg et al.[11]	*Rabbits* *New Zealand*	*Male* 3 kg/6–8 mos	Gr1:9 Gr2:9	Gr1: bladder SMC + BCM Gr2: BCM(acellularity)	BCM	[a]	bladder SMC [a]	Patch	Anterior 3.5 cm	1Cystourethrography and urethroscopy 2Histology

(continued)

Table 1 Continued

Reference	Species / Strain	Sex / Weight/age	Group Size	Study design	Bio-material	Scaffold size	Cell type / Amount	Surgical	Location / Length	Outcome measure
Li et al.[29]	*Rabbits* *New Zealand*	*Male* 10 weeks	Gr1:9 Gr2:9 Gr3:9	Gr1:keratinocyte + fibroblast + BCM Gr2:oral keratinocytes + BCM Gr3:BCM(acellularity)	BCM	l = 2.2 cm w = 1 cm	Keratinocytes fibroblast 6.6×10^{-6}/[a]	Patch	Anterior 2 cm	1Retrograde urethrography 2Histology
Sun et al.[33]	*Rabbits* *New Zealand*	*Male* [a]	Gr1:21 Gr2:7	Gr1:hUCMSCs[h] + SKFs[i] Gr2:SKFs(acellularity)	SKFs	l = 0.5 cm w = 0.5 cm	hUCMSCs 1×10^{-6}	Patch	Posterior 0.5 cm	1Retrograde urethrography and urethroscope 2Histology
Li et al.[30]	*Rabbits* *New Zealand*	*Male* [a]	Gr1:12 Gr2:12 Gr3:12	Gr1: BCM(acellularity) Gr2: Und-rASC[j] + BCM Gr3:Epith-rASC[k] + BCM	BCM	l = 2.0 cm w = 0.8 cm	Und-rASC Epith-rASC 8×10^{-6}	Patch	Anterior 2 cm	1*Retrograde urethrography* 2*Electron microscopy* 3*Multicolor* immunofluorescent and Western *blot*
Xie et al.[34]	*Canines* *Beagle*	*Female* 11.5 kg/1.3 y	Gr1:5 Gr2:5	Gr1:keratinocyte + fibroblast + SF[l] Gr2: SF(acellularity)	SF	l = 5.0 cm w = 1.5 cm	Keratinocyte Fibroblast $22.5/0{,}75 \times 10^{-6}$	Patch	/ 5 cm	1*Retrograde urethrography and urethroscope* 2Histology
Silva et al.[36]	*Rabbits* *New Zealand*	*Male* 2.5–3.45 kg	Gr1:8 Gr2:8	Gr1:bladder SMC + CBS[m] Gr2: CBS (acellularity)	CBS	l = 2 cm d = 3 mm	bladder SMC $8–14 \times 10^{-6}$	Tubular	Anterior 1 cm	1Retrograde urethrography 2Histological and immunohistochemical analyses

[a]Not mentioned.
[b]Smooth muscle cell EC = epithelial cell.
[c]Bladder acellular matrix.
[d]Acellular corpus spongiosum matrices.
[e]Corporal smooth muscle cell.
[f]hdCGT = high-density collagen gel tube.
[g]Bone marrow mesenchymal stem cell.
[h]Hypoxia-activated human umbilical cord mesenchymal stem cells.
[i]SKFs = skeletal muscle fragments.
[j]Undifferentiated adipose-derived stem cell.
[k]Epithelial-differentiated rabbit adipose-derived stem cell.
[l]Silk protein.
[m]CBS = collagen-based scaffold.

Table 2 The outcomes of the included studies

Reference	Follow-up	Sub-group	Complications	Urethrography outcomes		Histological outcomes				
				wide caliber	Strictures	①[a]	②[b]	③[c]	④[d]	⑤[e]
DE Filippo et al.[35]	1,2,3,6 mos	Gr1	None	12	0	Δ[f]	Δ			Δ
		Gr2	Fistulas or collapse(12/12)	0	12	Δ		Δ		
Fu et al.[25]	1, 2, 6 mos	Gr1	None	9	0	Δ				Δ
		Gr2	Fistulae(1/9)[g]	0	8	Δ		Δ		
Li et al.[27]	1, 2, 6 mos	Gr1	None	12	0	Δ				
		Gr2	Die of infection(2/12)[g] Fistulae(2/12)[g]	0	8				Δ	
Feng et al.[24]	1, 2, 6 mos	Gr1	None	0	6				Δ	
		Gr2	None	0	6	Δ			Δ	
		Gr3	None	6	0	Δ	Δ		Δ	
Gu et al.[26]	1, 2, 6 mos	Gr1	None	9	0	Δ	Δ			
		Gr2	Shrink and fibrosis(9/9)	0	9	Δ		Δ		
DE Filippo et al.[35]	1, 2, 3, 6 mos	Gr1	none	9	0	Δ	Δ			Δ
		Gr2	Fistulas or collapse(6/6)	0	6	Δ		Δ		
Micol et al.[31]	1, 3 mos	Gr1	fistula(3/8)	3	5	Δ	Δ			
		Gr2	Fistula(4/8)	2	6	Δ	Δ			
Orabi et al.[32]	1, 3, 6, 12 mos	Gr1	Urethral stents removal (4/15)	15	0	Δ	Δ			
		Gr2	Urine leakage(6/6)	0	6			Δ		
Li et al.[29]	2, 4, 8, 16wks	Gr1	None	24	0	Δ	Δ			Δ
		Gr2	Die of urethras obstruction(3/6),	6[h]	0[h]			Δ		
Sayeg et al.[11]	1, 2, 4, 12wks	Gr1	None	9	0	Δ			Δ	
		Gr2	None	9	0	Δ			Δ	
Li et al.[29]	1, 2, 6 mos	Gr1	None	9	0	Δ			Δ	
		Gr2	None	9	0	Δ				
		Gr3	Fibrosis and shrink(9/9)	0	9				Δ	
Sun et al.[33]	2, 4, 12wks	Gr1	None	21	0	Δ	Δ			Δ
		Gr2	None	2	5	Δ				
Li et al.[30]	2wks	Gr1	Scarring and contracture(#/12)	0	12			Δ		
	1, 2, 6 mos	Gr2	Fistula(1/12) and contracture(#/12)	0	12			Δ		
		Gr3	None	12	0	Δ	Δ			
Xie et al.[34]	6 mos	Gr1	None	5	0	Δ				
		Gr2	Dysuria and bladder distension(5/5)	0	5			Δ	Δ	
Silva et al.[36]	1, 3 mos	Gr1	Fistulas (1/8)	7	1	Δ	Δ			Δ
		Gr2	None	4	4	Δ			Δ	

[a]Organized epithelial layers.
[b]Organized muscle fiber bundles.
[c]Fibrosis.
[d]Inflammatory cells.
[e]Vascularization.
[f]Mentioned in this article.
[g]No urethrography outcomes due to this complication.
[h]The outcomes conflicted with the necropsy.

heterogeneity (Figure 2(a), $Chi^2 = 45.71$, $df = 14$, $I^2 = 69\%$), we had to adopt the REM. As shown in Figure 2(a), the global estimated RR was 5.40 (95%CI = 2.60–11.20), but with unacceptable statistical heterogeneity. Exclusion of the study by Sayeg et al.[11] from the analysis significantly improved heterogeneity ($I^2 = 27\%$), resulting in no significant change in the final result (Figure 2(a), RR = 5.67; 95%CI = 3.03–10.62).

Comparison2: One cell type versus two cell types. In all our included studies, the postoperative outcomes involving one cell-seeded and two cells-seeded were compared only in two studies Feng et al.[24] and Li et al.[28] From the comparisons in every aspects between these two groups (Table 4), we concluded that two types seemed to be better than one type in terms of successful events (one type vs. two types: 0% vs.100%) and cellular layers (one type vs. two types: 2–3 vs. 5–7) according to Feng et al. However, in Li et al.'s study, the outcome of two types seemed similar to one type in terms of successful events (one type vs. two types: 100% vs.100%) and cellular layers (one type vs. two types: 5–7 vs. 5–7). But Li et al. discovered that there was difference in the formation of capillary between two groups (formation of capillary in one type vs. two types: none vs. yes).

Table 3 Quality characteristics of included studies.

References	1	2	3	4*	5	6*	7	8	9	10	Score
DE Filippo et al.[23]	√		√				√				3
Li C et al.[27]	√		√	√			√		√		5
Fu et al.[25]	√		√				√			√	4
Feng et al.[24]	√						√			√	3
Gu et al.[26]	√		√				√				3
DE Filippo et al.[35]	√		√				√			√	4
Micol et al.[31]	√						√		√		3
Orabi et al.[32]	√						√		√	√	4
Li CL et al.[29]	√						√				2
Sayeg et al.[11]	√		√		√		√		√	√	6
Li C et al.[28]	√		√				√		√		4
Sun et al.[33]	√						√		√	√	4
Li H et al.[30]	√						√			√	3
Xie et al.[34]	√		√			√	√		√		5
Silva et al.[36]	√	√					√		√	√	5

Studies fulfilling the criteria of (1) peer reviewed publication; (2) monitoring of physiological parameters such as body temperature; (3) randomization; (4)* blinded modeling of the urethral defect (modification of the CAMARADES criteria.); (5) blinded assessment of the outcome; (6)* surgeries performed by the same surgeons (modification of the CAMARADES criteria.); (7) use of a suitable animal model; (8) sample size calculation; (9) compliance with animal welfare regulations; and (10) statement of potential conflict of interests.

Then, we performed a meta-analysis between two cell types and one cell type again. Results displayed that there was evidence for the advantage of two type cells seeding (Figure 3: two cell types vs. one cell type: RR = 2.51, 95%CI = 0.23–27.99, $I^2 = 71\%$).

Comparison3: Undifferentiated and epithelial-differentiated stem cells. From the animal experiments including Li et al., Sun et al., and Li et al., we discover that the stem cells-seeded approach has been a novel choice for urethral reconstruction in tissue engineering. However, only Li et al. made a comparison of undifferentiated and epithelial-differentiated stem cells in a unique aspect. The difference was established in Table 5. It is obvious that epithelial-differentiated adipose-derived stem cells (Epith-rASCs) were remarkably superior to undifferentiated adipose-derived stem cells (Und-rASCs). Specially, Epith-rASCs still kept the ability of SMCs differentiation potential although those stem cells have epithelial differentiated.

Sensitivity analysis and publication bias. To assess the effect of each individual study on the overall meta-analysis estimate, we excluded one study at a time, and the exclusion of any single report did not alter the significance of the final decision, suggesting that the outcomes were stable (Table 6).

Funnel plots were made to evaluate publication bias. However, asymmetry in the plots indicated the presence of publication bias. (Figure 4(a), Begg's test: $P = 0.077$, Figure 4(b), Egger's test: $P = 0.000$).

Discussion

Together with the advancement of preclinical experiments, some classical tissue engineering materials such as small intestine submucosa (SIS),[39,40] BAM,[41,42] and tissue-engineered buccal mucosa[43–45] have been applied in several clinical trials. Though achieving certain curative outcomes in the short-term, urethral reconstruction by tissue engineering nevertheless remains in the preclinical phase due to ethical issues and the potential immune rejection. First, every biomaterial prepared in vitro is tested for biocompatibility, mechanical properties, biodegradation, and cell growth. Then, the biomaterial is assessed further in vivo in an animal model in which the surgeon performs reconstructive surgery using an animal urethral defect model with the tissue-engineered material. Postoperative follow-up evaluation is then conducted using an urethrograph or urethroscopy, pathological assessment, and voiding functional summary. To determine whether tissue-engineered neo-urethra could be a new potential treatment for urethral reconstruction, we systematically searched the literature and separated all of the basic research regarding tissue engineering for urethral reconstruction in an animal model. Using predefined criteria, 15 studies were included in our study. Subcategories of cell-seeded scaffolds groups and scaffolds alone groups were compared by postoperative urethrography, complications, and histological changes including epithelium, vascularization, and inflammation.

Treatment strategies can usually be divided into two categories in tissue engineering. One strategy is using an acellular matrix graft (ACMG) to create a favorable condition for new tissue regeneration. This technology allows the natural process of regeneration and was successfully applied experimentally in animal models and clinically in patients. Acellular collagen matrices derived from the bladder have already been successfully applied as an onlay graft for urethral repair in both experimental and clinical practice.[46,47]

The other strategy is using a cell-seeded matrix for repairing the pathologic urethra, which was considered to be

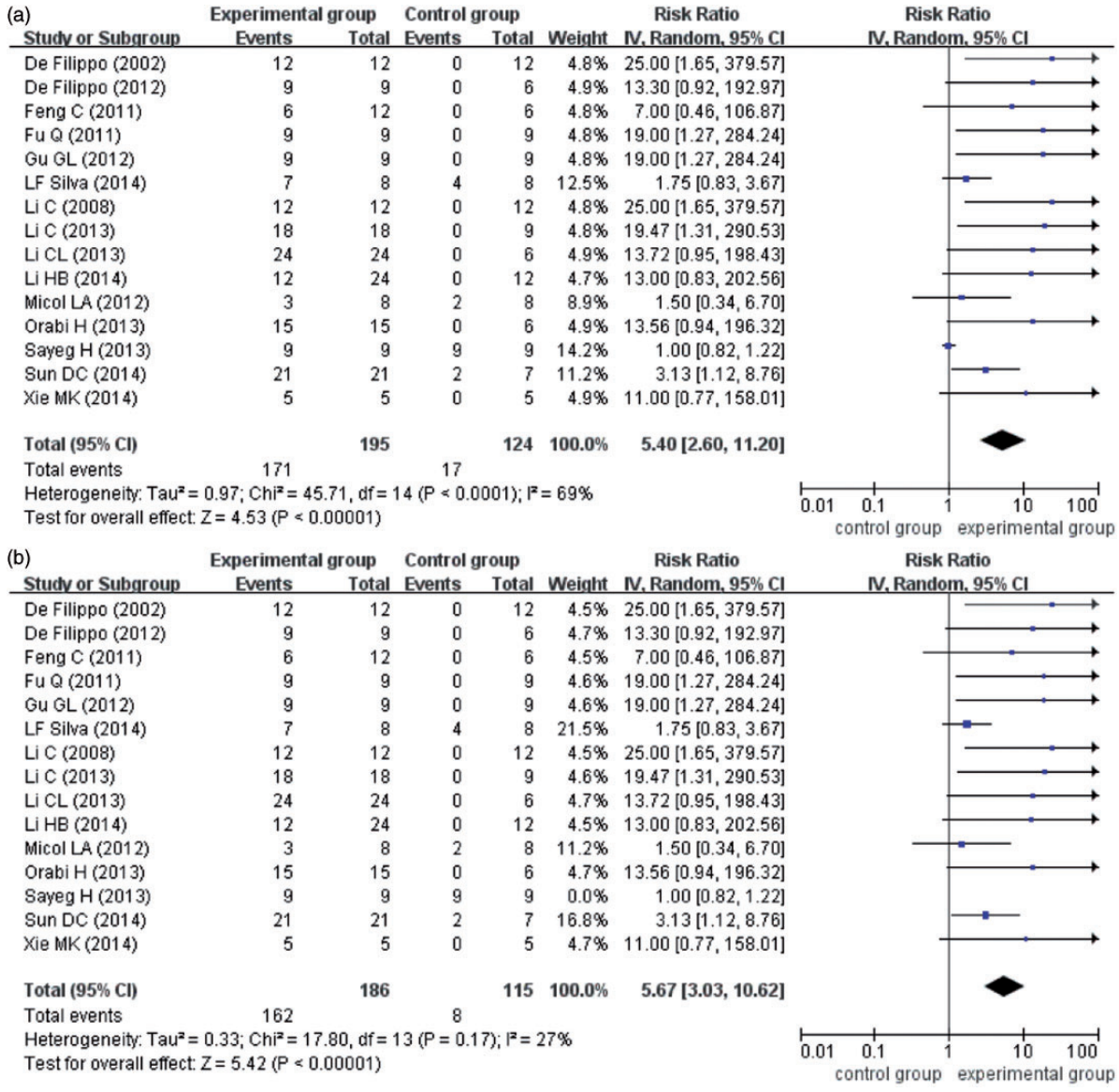

Figure 2 Forest plot of RRs with 95% CIs for cell-seeded biomaterials and their effect on urethral reconstruction. The center of each square represents the RRs, the area of the square is the number of samples and thus the weight used in the meta-analysis and the horizontal line indicates the 95%CI

Table 4 The comparison of one cell type and two cell types

		References			
		Feng et al.[24]		Li et al.[29]	
Type		One type	Two types	One type	Two types
Cell types		Keratinocytes	Keratinocytes; SMCs	Keratinocytes	Keratinocyte; fibroblasts
Successful event/total		0/6	6/6	9/9	9/9
urethrogram		(±)	(−)	(−)	(−)
Histology	Intact epidermal layer	YES	YES	YES	YES
	Cellular layers	2–3	5–7	5–7	5–7
	Muscle fiber bundles (Observed time-point)	Unorganized (1–6 mos)	organized(2 mos) normal-like(6 mos)		
	Formation of capillary			NO	YES

SMC: smooth muscle cells.

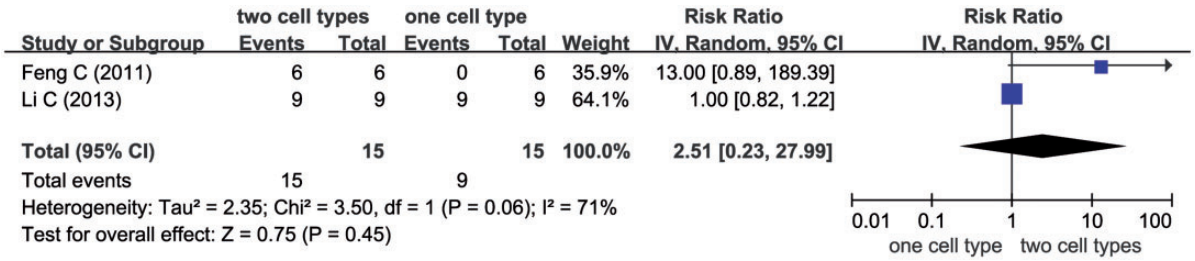

Figure 3 Forest plot of RRs with 95% CIs for the effect of two cell types and one cell type. (A color version of this figure is available in the online journal.)

Table 5 The comparison of undifferentiated and epithelial-differentiated stem cells

Reference	Li et al.[30]	
Cell types	Und-rASCs	Epith-rASCs
Successful event/total	0/12	12/12
electron microscopy	Und-rASCs organized loosely	Epith-rASCs organized compactly
Urethrogram	Stricture	Close to normal
Immunofluorescence (observed time-point)	Monolayer epithelial (1–2 mos)	Continuous epithelial layer(2 weeks) multilayer epitheliums (1–2mos)
SMC differentiation potential	YES	YES

Table 6 The results of sensitivity analysis

No.	Omitting study	RR (95% CI)
1	DE Filippo et al.[35]	4.86 (2.35, 10.06)
2	Li et al.[27]	4.86 (2.35, 10.06)
3	Fu et al.[25]	4.96 (2.38, 10.33)
4	Feng et al.[24]	5.34 (2.52, 11.31)
5	Gu et al.[26]	4.96 (2.38, 10.33)
6	DE Filippo et al.[35]	5.09 (2.43, 10.68)
7	Micol et al.[31]	6.35 (2.88, 14.03)
8	Orabi et al.[32]	5.09 (2.43, 10.66)
9	Li et al.[29]	5.08 (2.42, 10.65)
10	Sayeg et al.[11]	5.67 (3.03, 10.62)
11	Li et al.[29]	4.95 (2.38, 10.31)
12	Sun DC et al.[33]	6.02 (2.69, 13.46)
13	Li et al.[30]	5.12 (2.44, 10.74)
14	Xie et al.[34]	5.17 (2.46, 10.87)
15	Sliva et al.[36]	7.10 (2.93, 17.20)

the better choice by the present analysis. In this meta-analysis, there was a significant difference between cellular and acellular groups in repairing the urethra stricture (RR = 5.67), which indicates that the use of cell-seeded biomaterials for urethral reconstruction is approximately 5.67 times better than using unseeded biomaterials (see Figure 2(b), Table 6). It is noted that this result was based on the exclusion of the Sayeg et.al.[11] which carried the greatest weight and made the result unacceptable due to the huge heterogeneity. In contrast to the other studies included in this meta-analysis, Sayeg et al.[11] compared the urethroplasty in an animal model with ventral urethral defect measuring 3.5 cm long by 0.5 cm wide by using smooth muscle cell-seeded biomaterials and cell-free biomaterials via an onlay approach. Surprisingly, those results showed that the two groups had a similar postoperative outcome (cell-seeded group 9/9, cell-free group 9/9). Subsequently, we analyzed the possible mechanisms for this similarity and determined that it might be due to the well supported dorsal urethral bed in the animal model. Cell-free matrix was often insufficient for long defects (>1 cm) due to graft shrinkage or restriction after chronic immune reactions, fibrosis formation, and calcification.[48] It is likely that the maximum defect distance, which is suitable for normal tissue formation, appears to be 0.5 cm.[49]

Urothelial cells, fibroblasts, endothelial cells, nerve cells, and SMCs, which all exist in the normal urethra, each undertake a task in the construction of a tissue-engineered urethra. A continuous layer of epithelial cells may constitute a barrier that prevents a corrosion effect and urinary fistula because of the urine alkaline composition in the urethra, which would reduce the inflammatory response. Vascularization may also ensure better blood supply, providing the neo-urethra an environment that promotes high survival. SMCs may enhance the mechanical properties of the scaffold. These cell-specific mechanisms would not be present in the unseeded scaffolds. The implanted cells can be categorized into three parts. The favorite choices, including epitheliogenic cells, urothelial cells and particularly bladder epithelial cell,[23,32,35] are congeneric with uroepithelium. But researchers are having difficulties in harvesting and culturing them. Moreover, those cells are not always available, such as in cases of transitional cell carcinoma. Epidermal cells seem to overcome those disadvantages mentioned above. For example, foreskin epithelial cells[25] and oral keratinocytes[24,27,28,34] are abundant, and can be

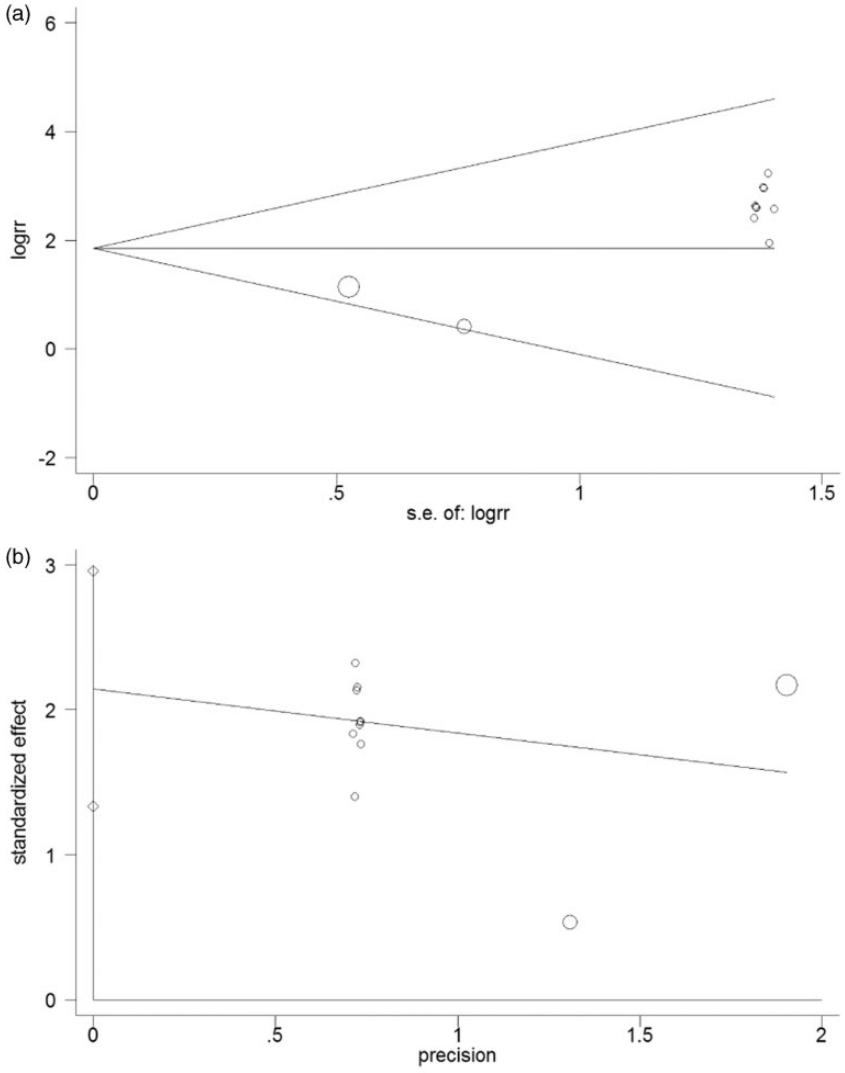

Figure 4 Funnel plots (a: Begg's test; b: Egger's test) Log (OR)/the standard error of log (OR) and MD/the standard error of MD were adopted to yield funnel plots

harvested with only a minor operative wound, and cultured easily. Surprisingly, studies displayed that epidermal cells were similar to normal uroepithelium in morphology and function, surrendered by increasingly well-developed muscle bundle fibers and evolving neovasculature. However, it takes a long time for the epidermal cells to adapt to the urethral environment. Apart from these, mesothelial cells[26] serve as a novel option. Not only are they easy to harvest through a small umbilical incision, but they maintain an epithelial cell growth pattern. The second popular use is SMCs, which can form a corresponding layer on the outer surface of urethral cavity. SMCs could also play a role in proper urothelial differentiation induced by cellular cross-talk. In our manuscript, there were two studies[31,36] that only SMCs were used and not for urothelial cells in seeded constructs. And we found that they all used scaffolds with or without cell seeded for a 1 cm-length circumferential urethral repair. In their control group (without cell seeded), there was successful cases as well. Actually, there was a "1 cm rule" raised by Atala et al.[48] for successful urethral replacement may be more critical mentioned above. In the SMC-seeded group, more SMC expression and neovascularization was observed, and less mononuclear and giant cells could be found. Da Silva LFA et al.[36] hold the view that the implanted SMC-seeded constructs reduced inflammatory response and enhanced smooth muscle regeneration and neovascularization. Better blood supply and less inflammatory response would help better regeneration of native urothelium. Last but not least, stem cells cannot be ignored.[50] Nowadays, stem cells have potential to differentiate into mature effector cells. (ADSCs, embryonic stem cells (ESCs), bone marrow-derived mesenchymal stem cells, urine-derived stem cells (USCs), etc. are used in reconstructive urology. Among them, ADSCs are chosen as the seeded cells mostly because of abundant adipose tissues which caused less trauma, and a high proliferative potential into epithelial lineage.[51]

Inclusion of two cell types, it is likely that using SMC or fibroblasts and epithelial cells was better than using just one cell type as the seeding resource for urethral reconstruction. Moreover, the meta-analysis results showed two cell types seeded group was better than one cell type group

statistically in the section of comparison 2 (Figure 3). Undoubtedly, in Feng et al.'s[24] study, the outcome of two types was found to be better than one type in terms of successful events with intact epithelium formation and wide urethral caliber and more cellular layers. However, according to the definition of successful case, seeding two type cells seemed not to occupy the advantage in Li et al.'s[28] study, but there was difference in the formation of capillary between two groups. No evidence of the formation of capillary was found in the epithelial lower layer in one type cell-seeded group, suggesting that only one type cells (oral keratinocytes) were insufficient for urethral reconstruction. Actually, seeding two type cells was more profitable for urethral reconstruction, including organized muscle fiber bundles and capillary formation. From the literature, we assume that cell-seeded scaffolds including epithelial cells can construct an urothelial barrier to prevent urine leakage into the suburothelial tissue and prevent fibrosis. Another contributing factor to the success of a cell-seeded urethral replacement could be the developing muscle layer that keeps the urethra from collapsing and prevents wall adhesions for the formation of capillary. Moreover, as a primary effector during wound healing, fibroblasts can aid in healing by synthesis and secretion of collagen matrix. In that way, another attempt is to seed three or more kinds of cell on the matrix for a multifunction tissue. Till now, no one has reported yet. Technically, it needs a breakthrough.

Based on previous studies, a lack of epithelial layers might result in the development of narrow urethral caliber after urethral reconstruction. Li et al.[30] conducted a comparison experiment ADSC after epithelial differentiation and undifferentiated ones to confirm that Epith-ASCs were likely to become potential substitutes of urothelium for urethral tissue engineering. In the first place, Epith-ASCs accelerate the differentiation progress of ADSCs relatively, which could prevent inflammation cell infiltration and fibrosis of lumen. In addition, Epith-ASCs still possess the characteristic SMC differentiation, contributing to the reconstruction of organized muscle bundles in the neo-urethra. Before that, Epith-ASCs had been used in laryngeal tissue engineering successfully.[52,53]

To determine the effectiveness of a tissue engineering approach, we aimed to identify an animal model that would adequately predict the behavior of tissue-engineered constructs for urethral reconstruction. After systematic search in our article, we found out the studies that only rabbits and dogs were included. Even more, the female dogs were enrolled in one study. The canine is a more clinically relevant animal model due to its long urethral segment. The urethra in female canine anatomy, between the bladder and the pubic symphysis, was comparatively long (about 7–8 cm) and thick, which was suitable for simulating the posterior urethral defection. But urethra in male canine anatomy with bone structure in its penis was totally different from human, which will increase the difficulty of operation. As far as we know, there was another study with male dogs included in our analysis. They explored the feasibility of engineering clinically relevant long anterior urethras (about 6 cm) for surgical reconstruction in a canine model that is simulation of anterior urethroplasty. Thus, the canine model can simulate the long-segment urethral defect, which often occurs in the clinical patients. Larger animals (dogs) with longer urethral length allow for the evaluation at clinically relevant sites with constructs of a comparable size. Meanwhile, small animal models (such as the rabbit) appear to yield the desired results as well. More studies chose the rabbit model for their experiments because it presents some important advantages: (i) easy to manipulate; (ii) familiar urethra histology and anatomy even more alike to humans, and (iii) inexpensive.[54]

Publication bias is known to be a vital issue, which is a threat to the validity of all systematic reviews. Actually, we had done a lot of work on the extensive search of relevant papers. Despite these efforts, publications bias still existed. In this respect, publication bias often been observed in previous systematic reviews of animal studies.[55–57] It is unlikely that the publication bias reported here is limited to the effect of cell-seeded biomaterial on the urethral reconstruction and is likely to be prevalent in reports of laboratory-based research based on animal models. Small sample size, true heterogeneity, or even the methodological quality all could be regarded as a potential explanation for the funnel plot. Prospective registration of animal experiments might reduce publication bias.

Study limitations and recommendations

This systematic review mainly compared the urethral reconstruction efficiency of cell-seeded scaffolds and cell-free scaffolds in two animal models. Our results showed that cell-seeded biomaterials ensured better outcomes after urethroplasty. However, there are still some limitations in our study. Firstly, animal experiments are not set as random control trials strictly, and the quality of our included studies was actually moderate. Therefore, the reporting of the methodology and representativeness of results was relatively poor in the reviewed studies. Standardization of the animal experimental methods was established in a guideline. Secondly, only published literatures were included in this meta-analysis. Thus, studies with negative results were not well represented in samples due to their presence in publications. Thirdly, large controlled and comparative studies are still inadequate owning to animal welfare and ethical debate. Advancements in this field are urgent for ethical, scientific, and economic causes. Only in this way, could researchers have the chance to repeat studies reliably and make an unbiased decision in the future experiment.

Conclusion

To the best of our knowledge, this is the first meta-analysis of a comprehensive assessment of the advantages of using a cell-seeded scaffold compared to a cell-free neo-urethra in animal studies. The advantages of using a cell-seeded scaffold compared to a cell-free neo-urethra were confirmed in the evaluated animal studies statistically. Through this preclinical evidence, cell-seeded biomaterial strategy for tissue-engineered urethral reconstruction was recommended for the future experimentation. Multi-type cell

methodology, especially epithelial cells and SMCs or fibroblasts, was suggested to be implemented in the future. Additionally, with the improvement of stem cells, epithelial differentiated stem cells, as a new idea, could be a promising choice for researchers to attempt in the days to come. In terms of future animal experiments, animal models including rabbits (*New Zealand*) and canines (*Beagles*) should be standardized, so that valid comparison can be made. And similar studies should be designed more rigorously, particularly in randomization and blinding application, which can make data more reliable. Definitely, animal welfare is not to be overlooked.

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DECLARATION OF CONFLICTING INTERESTS

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