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***Bmp7* expression and null phenotype in the urogenital system suggest a role in re-organization of the urethral epithelium**

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Abstract

Signaling by Bone morphogenetic proteins (Bmps) has multiple and diverse roles in patterning and morphogenesis of the kidney, eye, limbs and the neural tube. Here, we employed the *Bmp7^{lacZ}* strain to perform a detailed analysis of *Bmp7* expression and the null phenotype during development of the mouse urogenital system. The urethral compartment originates in mid-embryogenesis from the ventral part of the cloaca, a transient cavity at the caudal end of the hindgut. At mid-gestation, *Bmp7* expression was detected within several specific domains in the cloacal epithelium and mesenchyme. In late embryogenesis, *Bmp7* expression was present in the urethra, rectum, the urethral glands, corpus cavernosum, and in the male and female genital ducts. Importantly, loss of *Bmp7* resulted in arrest in cloacal septation, and severe defects in morphogenesis of the genital urethra and mesenchyme. Together, our analysis of *Bmp7* expression and the null phenotype, indicates that *Bmp7* may play an important role in re-organization of the epithelium during cloacal septation and morphogenesis of the genital tubercle.

Keywords

Bone morphogenetic protein 7 (Bmp7); urogenital; anorectal; cloaca; rectourethral fistula; genital tubercle; hypospadias

1. Results and discussion

The mammalian urogenital system, the bladder, urethra and external genitalia, develop from the epithelium and mesenchyme of the cloaca, a transient embryonic cavity at the caudal end of the hindgut (Fig. 1A). Urogenital malformations occur at high frequency in humans, and often combine malformations of the genital tubercle (GT), the penis in males and clitoris in females, and defects in separation of the urethral and rectal compartments (Hendren, 1992; 1996; 1998; Kurzock et al., 1999; Baskin et al., 2001; Mo et al., 2001). Morphogenetic mechanisms that direct development of the urogenital system are not well understood. The caudal homeobox genes, *Hoxa13* and *Hoxd13*, are expressed in the cloacal epithelium and mesenchyme, and are essential for morphogenesis of all cloacal derivatives (Warot et al., 1997; Morgan et al., 2003). Expression of *p63* (Cheng et al., 2006) and *Sonic hedgehog (Shh)* (Haraguchi et al., 2001; 2007; Mo et al., 2001; Seifert et al., 2008) in the cloacal and urethral epithelium is also important both for septation of the cloaca, and development of the bladder

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and external genitalia. In addition, *Fibroblast Growth Factors 8 and 10*, *Bmp4* and *7*, and *Wnt5a* have been implicated in development of the GT (Yamada et al., 2006), but their role in morphogenesis of the cloaca has not been explored. In this study, we employed the *Bmp7^{lacZ}* reporter strain (Godin et al., 1998) to perform a detailed analysis of *Bmp7* expression and the null phenotype during development of the urogenital system in males and females.

1.1 LacZ activity accurately reflects expression of *Bmp7* in the urogenital system

Comparative analysis of *Bmp7* expression by in situ hybridization with an antisense *Bmp7* RNA probe (Lyons et al., 1995), and by X-gal staining of tissues in *Bmp7^{lacZ/+}* background has shown that LacZ activity accurately reflects the expression of the wild type *Bmp7* allele in many embryonic tissues, including the kidney (Godin et al., 1998; Dudley et al., 1999) and the urethra (Fig. 4C, D). In addition, our analysis of *Bmp7^{lacZ/+}* GT (Fig. 1–3) showed LacZ activity in the urethral plate and genital mesenchyme in complete agreement with previous reports on *Bmp7* expression (Morgan et al., 2003; Suzuki et al., 2003). Thus, we conclude that LacZ activity in *Bmp7^{lacZ}* strain is an accurate readout of the activity *Bmp7* promoter in the lower urogenital system and the hindgut.

1.2 *Bmp7* expression and null phenotype in the cloacal area at E11.5–E13.5

The cloacal cavity is defined as the caudal end of the hindgut when, at embryonic day 9.5 (E9.5) in mice, it comes in contact with the ectoderm just anterior to the tail at the site of the future anal opening (Perriton et al., 2002; Hynes and Fraher, 2004a,b,c; Sasaki et al., 2004). Starting at E10, the ventral end of the cloaca grows anteriorly giving rise to the embryonic urogenital sinus (UGS), the primordium of the pelvic urethra and the bladder (Fig. 1). Studies in mouse models indicate that septation of the cloaca is driven by the caudal growth of the lateral mesenchyme known as the urorectal septum at E10.5 – E13.5, and is completed with disintegration of the cloacal membrane to uncover the anal and the caudal urethral orifices at E13.5 – E14.0 (Kluth et al., 1995; Hynes and Fraher, 2004a,b,c; Sasaki et al., 2004; Seifert et al., 2008). Interestingly, analysis of *Bmp7^{lacZ/+}* heterozygous embryos showed that *Bmp7* is expressed specifically in the urorectal septum (URS) at E11.5 (Fig. 1C) and E12.5 (Fig. 2F), and expression is dramatically reduced at E13.5 (Fig. 3C, D). We further asked whether *Bmp7* function may be required for cloacal septation. From E11.5 to E13.5, heterozygous embryos showed normal descent of the URS (Fig. 1C; 2E; 3C; Hynes and Fraher, 2004a-c; Sasaki et al., 2004), and progressive separation of the urethral and hindgut compartments from anterior to caudal (Fig. 2G–I; 3E–G; Hynes and Fraher, 2004a-c; Sasaki et al., 2004). *Bmp7* null embryos showed normal invagination of the URS into dorsal cloaca at E11.5 (Fig. 1D). However, further septation was delayed at E12.5 (Fig. 2K, L compare to G, H) and E13.5 (Fig. 3I, J compare to E, F). At E12.5 and E13.5, transverse sections of *Bmp7* null GT showed persistent cloaca, whereas corresponding sections of heterozygous animals showed separate urethral and hindgut compartments (Fig. 2, 3).

1.3 *Bmp7* expression and null phenotype in the GT at E11.5–E13.5

The initial stages of development of the GT at E11 – E15 are gender-independent, and begin with the appearance of genital swellings laterally to the cloaca at E10.5 (Perriton et al., 2002). At E11.5, *Bmp7* expression was detected in the mesenchyme of the genital swellings, and the ectoderm of the ventral body wall and the tail (Fig. 1C). Distal outgrowth of the genital swellings is accompanied by the extension of the urethral epithelium and lumen (Kuzrock et al., 1999; Yamada et al., 2003; 2006; Seifert et al., 2008). At E12, the distal urethral walls adhere to form a solid urethral plate (Fig. 2H, I; Kuzrock et al., 1999; Yamada et al., 2003; 2006; Seifert et al., 2008). Analysis at E12.5 showed *Bmp7* expression in the urethral plate (Fig. 2G, H), the adjacent mesenchyme (Fig. 2H), and the ventral ectoderm (Fig. 2E). In the null, LacZ activity marked the urethral plate, and was present in a broad domain in the GT

mesenchyme (Fig. 2K, L). Distal extension of the urethral plate appeared 50% shorter in the null (Fig. 2D, H, L), most likely due to unzipping of the urethral folds at the ventral side of the GT (Fig. 2C; and K, L, inserts) and formation of a large urethral groove (Fig. 2C).

At E13.5, *Bmp7* expression appeared in the dorsal GT mesenchyme (Fig. 3C, D) which may contribute to the corpus cavernosum (Yamada et al., 2003). *Bmp7* expression was maintained in the ventral mesenchyme, but decreased in the URS (Fig. 3C, D). In the genital urethra, *Bmp7* was expressed in a gradient with the strongest expression in the distal urethral epithelium (DUE) at the tip of the GT, which also expresses *Fgf8* (Haraguchi et al., 2000), and the weakest at the urorectal septum (Fig. 3F, G). In the null, LacZ activity was lost in the medial and proximal parts of the urethral plate, but maintained in the DUE (Fig. 3K, L). In the ventral GT mesenchyme, *Bmp7* expression was restricted to the urethral plate in heterozygous (Fig. 2H; 3G, H). In contrast, in the null, LacZ-positive mesenchyme spread laterally (Fig. 2K, L; 3K, L).

1.4 *Bmp7* expression and null phenotype in the male urogenital system at E17.5 and P0

Starting at E15.5 in mice, the external genitalia undergo sex-specific differentiation largely controlled by androgens in the male (Drews et al., 2002; Kim et al., 2002; Yucel et al., 2003, 2004; Buckley et al., 2006). In E17.5 male embryos, *Bmp7* expression was detected in the urethra (Fig. 4D, 5B, D, H), the bulbourethral and preputial glands (Fig. 4D, 5H), the vas deferens (Fig. 4D, insert) and the corpus cavernosum (Fig. 4D, 5E, F, H). LacZ activity was not detected in the bladder, nor in the testes (Fig. 4D, and insert). To precisely localize LacZ activity to the epithelial and mesenchymal compartments of the GT, we embedded X-gal stained tissues in paraffin and carried out analysis of histological sections (Fig. 5). Some of the sections were co-immunostained for cytokeratin 14 (CK14) which marked the squamous epithelium in the urethra and the rectum (Fig. 5). *Bmp7* expression was detected in the dorsal and ventral epithelium of the urethra (Fig. 5B, H), in the preputial glands (Fig. 5D, H), and in the crypts of the intestine (Fig. 5H). *Bmp7* was also present in the mesenchyme of the urethra (Fig. 5D, K), the rectum (Fig. 5K), and in the corpus cavernosum (Fig. 4D; 5E, F, H). Normal morphogenesis of the penile urethra involves closure of the original caudal orifice (Perriton et al., 2002; Yamada et al., 2003), fusion of the ventral urethral groove (Fig. 5C; D; Yamada et al., 2003), and displacement of the ventral urethral seam by the genital mesenchyme (Fig. 5A; Kuzrock et al., 1999; Yamada et al., 2003; Seifert et al., 2008). In heterozygous GT, *Bmp7* was expressed in the mesenchyme surrounding the ventral epithelial fusion (Fig. 5D). LacZ-positive mesenchyme also localized to the abnormal genital urethra in *Bmp7* null (Fig. 5P-T, below). Further analysis showed that *Bmp7* null males developed complex rectourethral malformations, including rectourethral fistula and severe hypospadias (Fig. 4E, F; 5P-T; 8B). In the null, the hindgut lacked a separate anal opening (Fig. 4E, F; 8B), and a narrowed rectum opened directly into the urethra (Fig. 4E, F; 5P-S), consistent with the arrest in cloacal septation in mid-embryogenesis (Fig. 2, 3). Strong LacZ activity was detected in the mesenchyme of the fistula and the cloaca (Fig. 4E, F; 5O, R, S). LacZ activity was also detected in the basal epithelium of the urethra and hindgut (Fig. 5 N, O). In *Bmp7* null males, the caudal urethral opening was maintained (Fig. 4E; 8B), and the tubular penile urethra did not form (Fig. 5T compare to 5A, C). Instead, the urethral epithelium lined the ventral surface of the GT adjacent to the cloacal opening (Fig. 5T). Strong LacZ-activity was present in the mesenchyme adjacent to the urethral epithelium (Fig. 4E, F; 5R, S). Other urogenital abnormalities in *Bmp7* null males included loss of bulbourethral glands (Fig. 4E compare to D) and enlarged os penis (Fig. 5M, and S compare to I, K).

1.6 *Bmp7* expression and null phenotype in the female urogenital system

In the female urogenital system (Fig. 6–8), the pattern of *Bmp7* expression and the *Bmp7* null phenotype were very similar to what was observed in the male (Fig. 4, 5 and 8). In females,

Bmp7 was expressed in the urethra (Fig. 6C; 7B, E), the rectum (Fig. 6C; 7E), the intestinal crypts (Fig. 7B), the preputial glands (Fig. 6C; 7B), and the corpus cavernosum (Fig. 7E). In addition, LacZ activity was detected in all Mullerian duct derivatives, the oviducts, uterus and developing vagina (Fig. 6C–E). LacZ activity was not present in the ovaries, nor in the bladder (Fig. 6C, D). Analysis of *Bmp7* null females showed rectourethral fistula (Fig. 7I, J), enlarged caudal urethral/cloacal opening (Fig. 7L, 8C), and abnormalities in the topology of the genital epithelium (Fig. 7L) and mesenchyme very similar to those observed in the null males (Fig. 4, 5, 8B). Strong LacZ activity was detected in the mesenchyme of the fistula and the ventral mesenchyme of the GT (Fig. 6E; 7G–L). *Bmp7* null females also showed enlarged os clitoris (Fig. 7M, and I, L compare to C, F), and excessive branching of the genital vein (Fig. 7I and C) similar to what was reported in the *Hoxa13*^{−/−} GT (Morgan et al., 2003).

Bmp signaling mediated by Smad1/5/8 transcriptional mediators has multiple and diverse roles in organogenesis, including regulation of programmed cell death (Morgan et al., 2003), cell survival (Dudley et al., 1999), neural and limb patterning (Chesnutt et al., 2004; Pizette et al., 2001) and cell adhesion (Linecum et al., 1998). Although many details in morphogenesis of the anus and external genitalia are still unclear, our analysis of *Bmp7* expression and the null phenotype indicates that Bmp7 signaling may play an important role in reorganization of the urethral epithelium and mesenchyme during cloacal septation and formation of the penile urethra. *Bmp7* targets in the GT include the homeobox genes *Msx1,2* (Morgan et al., 2003) which can regulate cadherin-dependent cell adhesion (Linecum et al., 1998). Thus, further studies are needed to determine whether *Bmp7* may regulate cell adhesion in the urethral epithelium, and contribute to the migration and differentiation of the genital mesenchyme.

2. Experimental procedures

Animal work was conducted under NYUSM IACUC approved animal protocol. *Bmp7*^{lacZ} strain has been described previously (Godin et al., 1998; Dudley et al., 1999). Genotyping, whole mount X-gal staining, and preparation of histological sections have been performed as described previously (Grishina et al., 2005). Some of the sections were counterstained with hematoxylin and eosin. At each stage from E11.5 to E13.5, we analyzed six to ten animals of the same genotype. At E17.5 and P0, we analyzed six to ten animals of the same sex and genotype. Measurements of the genital structures was performed on transverse sections of the null and heterozygous embryos, and Student's t-test was used to determine the significance of differences. For immunofluorescent analysis, paraffin sections were incubated with antibodies for cytokeratin 14 (CK14, Covance, PRB-155P) at 1:100 dilution over night at 4°C, followed by 546 Alexa Fluor secondary antibodies (Invitrogen) diluted at 1:1000 for 1 hour at room temperature. In situ hybridization analysis was performed in whole mount as previously described (Grishina et al., 2005) using 0.9 kb digoxigenin-labeled antisense RNA probe for *Bmp7* (Lyons et al., 1995).

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Fig. 1. *Bmp7* expression in the cloacal area at E11.5. (A) Schematic depiction of the cloacal. The genital swellings (GS), cloaca (cl), urorectal septum (urs), urogenital sinus (ugs), hindgut (hg), tail gut (tg), peritoneal cavity (pc), body wall (bw) and tail (tl) are indicated. (B) Whole mount X-gal stained *Bmp7^{lacZ/+}* and *Bmp7^{lacZ/lacZ}* embryos at E11.5. Areas of sections in (C, D) are indicated with frames. (C, D) Sagittal sections of *Bmp7^{lacZ/+}* (C) and *Bmp7^{lacZ/lacZ}* (D) embryos show strong LacZ-activity in the urorectal septum (urs), the mesenchyme of the genital swellings (GS) and the ventral ectoderm of the body wall and the tail. Scale bar: 50 μ m.

Fig. 2.

Bmp7 expression and null phenotype in the urogenital system at E12.5. (A) *Bmp7*^{lacZ/+} embryo stained with X-gal, left hindlimb is removed, area of sections is shown in a frame. (B) Schematic depiction of the cloacal area at E12.5 showing the genital tubercle (GT), cloaca (cl), urorectal septum (urs), urogenital sinus (ugs), hindgut (hg), peritoneal cavity (pc), and the tail (tl). Arrows indicate planes of transverse sections in (F-H, J-L). (C) Ventral view of X-gal stained *Bmp7*^{lacZ/+} and *Bmp7*^{lacZ/lacZ} GT. In heterozygous, *Bmp7* is expressed in the urethral plate (up) and adjacent mesenchyme. In the null, the urethral plate unzips ventrally to form a large groove. LacZ activity marks the urethral epithelium (ue) at the surface of the groove. (D) Ventral extension of the urethral plate was measured on transverse sections (red arrows in H, L) in heterozygous (n=8) and null (n=6), and presented relative to the length of the GT. Standard deviation values are indicated as error bars. $P < 0.01$. (E, I) Sagittal sections of heterozygous (E) and null (I) embryos. LacZ activity was detected in the urorectal septum, the urethral plate and adjacent mesenchyme, ventral ectoderm (ve) and the kidney (k). Transverse sections of heterozygous (F-H) and null (J-L) embryos. *Bmp7* is detected in urorectal septum (F, G), the urethral plate (G, H) and adjacent genital mesenchyme (H). In the null, LacZ-positive genital

mesenchyme (gm) spreads laterally from the urethral plate (K, L), which makes two (*) or more contacts with the ventral ectoderm (K, L, inserts). Scale bar: 100 μ m.

Fig. 3.

Bmp7 expression and null phenotype in the urogenital system at E13.5. (A) *Bmp7*^{lacZ/+} embryo at E13.5 stained with X-gal, left hindlimb is removed, the area of the sections is shown in a frame. (B) Schematic depiction of the urogenital area at E13.5. The genital tubercle (GT), cloaca (cl), urorectal septum (urs), bladder (bl), urethra (ur), hindgut (hg), peritoneal cavity (pc), body wall (bw) and tail (tl) are indicated. Arrows indicate planes of transverse sections in (E-L). (C, D) Sagittal sections of a *Bmp7*^{lacZ/+} embryo show *Bmp7* expression in the ventral (vGM) and dorsal (dGM) genital mesenchyme. (E-H) Transverse sections of *Bmp7*^{lacZ/+} embryo show *Bmp7* expression in the lateral mesenchyme of the body wall (E), in the epithelium of the urethra (ur) and the urethral plate (up) (F-H), and the adjacent genital mesenchyme (F-H). Strong LacZ activity was observed in the distal urethral epithelium (DUE) at the tip of the GT (F, G). (I-L) In the null, LacZ activity was lost in the medial (L) and proximal (K) parts of the urethral plate, but maintained in the DUE (L and G). In the null, LacZ-positive genital mesenchyme occupied a broad lateral domain (L). Scale bar: 100 μ m.

Fig. 4.

Bmp7 expression and null phenotype in the male urogenital system at E17.5 (I). (A, B) Schematic representation of a normal male urogenital system (A), and in *Bmp7* null (B). Bladder (bl), vas deferens (vd), urethra (ur), genital tubercle (GT), corpus cavernosum (cc), intestine (in), rectum (r), anus (an), and the cloaca (cl) in the null (B), are indicated. Dashed lines indicate the planes of sections in Fig. 5. (C) Wild type male urogenital tissues were hybridized in whole mount with digoxigenin labeled antisense *Bmp7* RNA probe (Lyons et al., 1995) which detects both *Bmp7-1* and *Bmp7-2* transcripts (Vega Mouse Database). *Bmp7-1* corresponds to the complete *Bmp7* mRNA. *Bmp7-2* is a short non-translatable RNA specific for the testes (Ross et al., 2007; Steve Munger, personal communication). *Bmp7* expression was detected in the urethra, bulbourethral gland (bg), genital tubercle, vas deferens, body wall (bw) and the testes (t). Negative control with sense probe produced no color (not shown). (D) X-gal stained E17.5 *Bmp7*^{lacZ/+} male urogenital system, lateral view. LacZ activity was detected in the urethra, urogenital sinus (ugs), vas deferens (vd), bulbourethral and preputial glands (pg), corpus cavernosum, the rectum and the body wall. LacZ activity was not detected in the bladder and the testes (insert). (E, F) X-gal stained *Bmp7*^{lacZ/lacZ} null male, lateral view (E), and ventro-caudal view (F). In the null, the rectum adjoins the urethra (E). Bulbourethral glands were not detected (E). Strong LacZ activity was detected in the rectum (E, F), urethra (E), cloaca (E, F), preputial gland (E), body wall (E), and the mesenchyme of the corpus cavernosum at the ventral side of the GT (F).

Fig. 5.

Bmp7 expression and null phenotype in the male urogenital system at E17.5 (II). (A-F) Coronal sections of *Bmp7*^{lacZ/+} penis, dorsal side of the penis is up: (A, B) proximal area, (C, D) mid-penis, (E, F) distal glans. (G-L, N-T) Transverse sections of *Bmp7*^{lacZ/+} (G-L) and *Bmp7*^{lacZ/lacZ} (N-T) embryos, ventral side of the trunk is up. Planes of sections are shown in Figs. 4A, B. (A, C, G, J, N, P, T) Immunolabeling for cytokeratin 14 (CK14, yellow) marks the squamous epithelium in the urethra (ur), the ventral urethral seam (s) (C), the rectum (r) (J, N, P), the preputial glands (pg) (C), the mesothelial membrane of the corpus cavernosum (cc) (C, J, T), and the ectoderm (ec) (J). In the male, *Bmp7* is expressed in the urethral epithelium (ur) (B, D, H) and mesenchyme (um) (D, K). During formation of the penile urethra, LacZ-positive mesenchyme is concentrated to the secondary epithelial fusion (ef) (D). *Bmp7* is also expressed in the mesenchyme of rectum (r) (K) and corpus cavernosum (cc) (E, F, H), and in the epithelium of the preputial glands (pg) (D, H) and the intestinal crypts (in). (N-T) In *Bmp7*^{lacZ/lacZ} null, LacZ activity was detected in the mesenchyme of the urethra, cloaca (cl) and the rectum (N, P, R). In E17.5 null males, the urethral epithelium (ue) (T) and LacZ-positive mesenchyme (um) (R) localized to the ventral surface of the GT (T). (M) *Bmp7* null males showed wider cartilage condensations in the proximal and distal segments of the os penis (M, and S compare to I, K). The width of the proximal (ps) and distal (ds) segments of the os penis (op) was measured on sections of heterozygous (n=10) and null (n=8) males and presented in relation to the width of the GT. Standard deviation values are indicated as error bars. $P < 0.01$. Scale bar 100 μ m in (A) is same for all panels.

Fig. 6.

Bmp7 expression and null phenotype in the female urogenital system at E17.5. (I) Whole mount. (A, B) Schematic depiction of normal (A) and *Bmp7* null (B) female urogenital systems. The bladder (bl), Mullerian ducts (MD), urethra (ur), genital tubercle (GT), intestine (in), rectum (r), anus (an), and the cloaca (cl) in the null, are indicated. Dashed lines indicate planes of sections in Fig. 7. (C) X-gal stained *Bmp7*^{lacZ/+} female urogenital system, lateral view, and caudal view (insert). *Bmp7* expression was present in the Mullerian ducts, the urethra, corpus cavernosum (cc), preputial gland (pg), and the rectum. *Bmp7* expression was detected in the oviducts (ov), but not in the ovaries (o). (D, E) *Bmp7*^{lacZ/LacZ} null female, lateral view (D), caudal view (E). In the null, the rectum adjoins the urethra (E). Cloacal opening is visible beneath the genital tubercle (D, E). LacZ activity was detected in the urethra, rectum, Mullerian ducts, body wall (bw), the mesenchyme of the genital tubercle, and the kidney (k).

Fig. 7.

Bmp7 expression and null phenotype in the female urogenital system at E17.5. (II) Transverse sections of *Bmp7^{lacZ/+}* (A-F) and *Bmp7^{lacZ/lacZ}* (G-L) females. Planes of sections are indicated in Fig. 6A, B. (A, D, K) Immunolabeling for CK14 marks the squamous epithelium of the urethra (ur) (A, D), rectum (r) (D), and the cloaca (cl) in the null (K). Heterozygous female shows *Bmp7* expression in the epithelium and mesenchyme (um) of the urethra (B, E), in the intestine (in) (B), the mesenchyme of the rectum (E, F), in the preputial glands (pg) (B), and the corpus cavernosum (cc) (E, F). (G-L) *Bmp7* null female shows rectourethral fistula (G-J) and enlarged urethral/cloacal opening (L). In *Bmp7* null, LacZ activity was detected in the epithelium and mesenchyme of the urethra (G-I) and the cloaca (J), and in the mesenchyme of the rectum (G-H), corpus cavernosum (cc) and glans clitoridis (gs) (L). *Bmp7* null female shows increased branching of the genital vein (I compare to C), and a wider proximal (ps) segment of the os clitoridis (M, and I compare to C). (M) The width of the proximal (ps) and distal (ds) segments of the os clitoris (oc) was measured on sections of heterozygous (n=8) and null (n=8) females and presented in relation to the width of the GT. Standard deviation values are indicated as error bars. $P_{ps} < 0.01$, $P_{ds} < 0.05$. Scale bar in (A) same for all sections: 250 μ m.

Fig. 8.

External genitalia in a normal *Bmp7^{lacZ/+}* male (A), and in a *Bmp7^{lacZ/lacZ}* null male (B) and female (C) at P0. (A) In a normal newborn male, the genital tubercle (GT) is located ventrally to the anus (an). LacZ activity is present in the urethral meatus (m). (B, C) In the null male (B) and female (C), strong LacZ activity is detected in the cloacal area. Tail (tl) is removed in (B).