

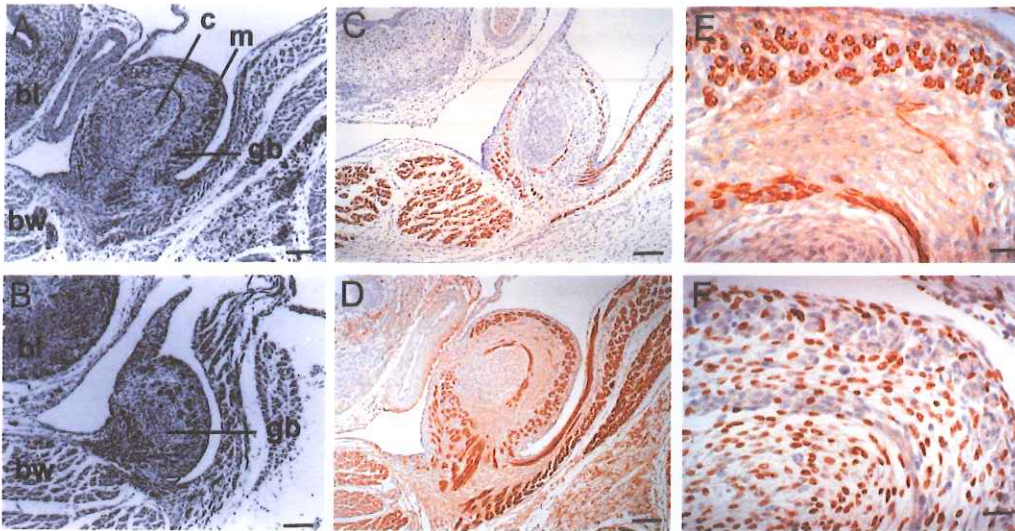
**Figure 3.3 - Testicular position in *Ins13*<sup>-/-</sup>, *Ar/Y* and *Ar/Y Ins13*<sup>-/-</sup> male mice.**

(A) Dissected abdominal region of a 4-week-old wild-type mouse shows the testes (t), which were already descended into the scrotal sac, adjacent to the bladder (b). (B) Genital tract of a 4-week-old wild-type female shows the position of ovaries (o) adjacent to the kidneys (k), uterine horns (u) and ovarian arteries (oa). (C) Free moving genital tract in the abdomen of 4-week-old *Ins13*-deficient male. The Wollfian duct derivatives are normally differentiated into epididymis (e), vas deferens (v) and accessory glands (not shown). Note the torsion (arrowhead) of the vas deferens and testicular artery (ta). (D) Testes of 3-week-old *Ar/Y* mouse located above the bladder and attached to the abdominal wall with cranial suspensory ligament and gubernaculum. (E) Testes of 4-week-old *Ar/Y Ins13*<sup>-/-</sup> male situated adjacent to the kidneys in a comparable position as ovaries in wild-type mouse (B).



# Chapter 6

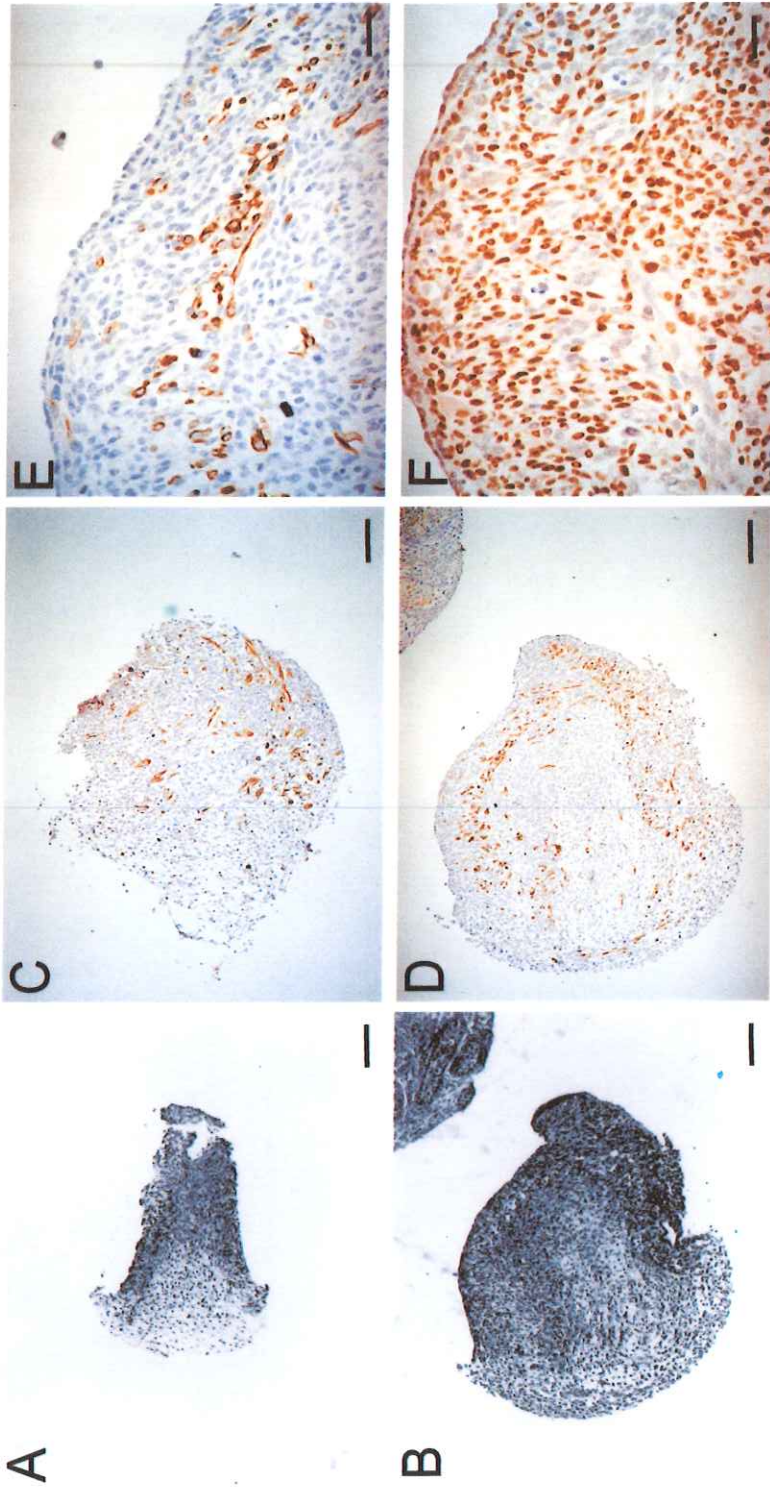
*Hormonal control of gubernaculum development  
during testis descent*



**Figure 6.2 - Histology and immunohistochemistry of gubernaculum development in the rat fetus *in vivo*.**

(A-B) Histological analysis of the gubernaculum at E19 in the male (A) and female rat fetus (B). In the male gubernacular bulb (gb), the muscular layer (m) and mesenchymal core (c) can be discriminated. Although the gubernacular bulb of the female fetus also consists of myoblasts and mesenchymal cells, it is smaller and less well-organized when compared to the male bulb. bl, bladder; bw, body wall. (C-F) Immunohistochemical localization of myosin (C-E) and androgen receptor (F) in the developing male gubernaculum of the rat. At E17, (C) the muscular layer of the gubernaculum bulb can be clearly distinguished by myosin immunostaining. At E19, (D) the muscular layer is more pronounced when compared to E17, as demonstrated by a strong anti-myosin immunoreaction. Detail (E) of the muscular layer from section (D), showing differentiating myoblasts which are myosin-positive. (F) is a section from the same male rat fetus (D, E), but stained with antibodies against the androgen receptor (AR), showing that the mesenchymal cells are AR-positive (F). Scale bar = (A, B, C, D) 100  $\mu\text{m}$ , (E, F) 25  $\mu\text{m}$ .





**Figure 6.4 - Histological and immunohistochemical features of gubernacular explants, prior to and after 5 days of culture.** (A-B) Morphology of gubernacular explant prior to culture (A) and after 5 days of culture in presence of mouse testicular tissue (B). Note the pronounced increase in size of the gubernacular bulb after culturing. (C-E) Immunolocalization of myosin (C-E) and androgen receptor (AR) (F) after culturing in the presence of R1881 ( $10^{-8}M$ ) (C) or testis (D-F). Both gubernacular explants (C,D) demonstrate immunostaining with myosin. In the gubernacular explant cultured in presence of R1881 (C), myosin-positive cells can be seen across the whole explant. In contrast, the gubernacular explant cultured in presence of a testis is showing a myosine-positive outerlayer. Detail (E) of the muscular layer from section (D), showing myoblasts which are myosine-positive. (F) is a section from the same explant (D, E), but stained with antibodies against the AR, showing that the mesenchymal cells are AR-positive (F). Scale bar = (A, B, C, D) 100  $\mu m$ , (E, F) 25  $\mu m$ .





