Towards a rational terminology in the study of the gubernaculum testis: arguments in support of the notion that the cremasteric sac should be considered the gubernaculum in postnatal rats and other mammals

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ABSTRACT

There is need for a consistent definition of structures caudal to the testis that variously are termed 'gubernaculum testis' as a basis for understanding the emergence and sexually dimorphic further growth and differentiation of this specifically mammalian structure. Rodent fetuses undergo a stage of development during which there is almost complete unanimity as to the definition of the 'gubernaculum' as a papilla-like structure (often called conus inguinalis) protruding from the area of the internal inguinal ring. This structure shows at least 3 readily distinguishable components before birth. Postnatally, the papilla-like gubernaculum starts to undergo inversion to become the tunica of the sac-like processus vaginalis. The 3 components of the fetal gubernaculum develop further uninterruptedly, but with a different spatial arrangement, as parts of the wall of a sac rather than as parts of a papilla-like structure. Comparison of this process in rodents with that in other mammals (pig, horse, man) revealed an essentially identical emergence of a papilla-shaped gubernaculum with similar constituents. Initial development of the processus vaginalis also began with the inversion of the gubernacular papilla. Cattle fetuses appeared exceptional as outgrowth of the processus vaginalis occurred without a preceding papilla-shaped gubernaculum in the area of the inner inguinal ring. The findings lead to the following conclusions. (1) In rodents, the whole of the postnatally developing processus vaginalis, including therefore its cremasteric muscles but without the attached genital mesentery, is to be viewed as the male postnatal gubernaculum. Sexual differentiation of the gubernacular primordia includes a sex-specific effect on the morphogenesis of all constituents of the processus vaginalis sac including cremasteric muscle cells. (2) Gubernacular growth and differentiation appear essentially a uniform process throughout the placentalia mammalian class; only quantitative differences occur in the extent of initial development of an intra-abdominal conus inguinalis and later cremaster muscles.

Key words: Testicular descent; genital development; cryptorchidism.

INTRODUCTION

In 1762, Hunter introduced the term gubernaculum to indicate a structure, potentially involved in testis descent, between the caudal pole of the testis and the inner wall of the scrotum. Large numbers of studies have been, and are still being, performed to analyse and understand the mechanism of normal and disturbed descent throughout the mammalian class. In such studies the gubernaculum is frequently included without specific definition or without an examination of its proposed meaning as compared with that in other studies. There was (Weber, 1898; Fig. 1) and still is (Backhouse, 1982; Tables 1, 2) a surprising variation and confusion among those using the term gubernaculum. Not unexpectedly, then, results from different studies are often difficult to compare as obviously different organs are analysed, despite the use of a single term to indicate them. There is need for a consistent rationale and consensus on the

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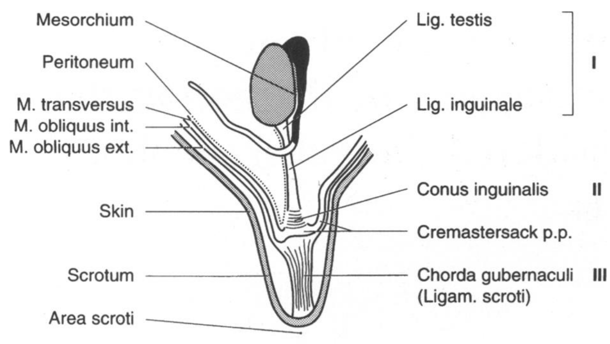

M. Weber, *Studien über Säugetiere*, Fischer, Jena, 1898

Fig. 1 (redrawn from Weber, 1898). Various structures caudal to the testes before descent and which play a role in the definition of the 'gubernaculum' by various contemporary authors and earlier investigators around the turn of this century. In his original definition Hunter proposed as gubernaculum all structures indicated by I, II, and III. Van den Broek (1933) explicitly excluded the conus inguinalis (II). Most investigators of fetal rats and other rodents have taken the conus inguinalis as 'the' gubernaculum prior to birth. Postnatally, they considered as the gubernaculum the caudal part of the derivatives of the so-called ligamentum inguinale (lower part of I). Various authors (e.g. Wensing et al. 1980) have doubted the existence of inguinal cones in larger mammals. The chorda gubernaculi (synonymous with ligamentum scroti) is to be judged a nonexistent entity during early fetal development (for explanation, see text).

Table 1. *The gubernaculum Hunteri: definition and components*

Original definition (Hunter, 1762). From a study of human fetuses: '...a substance which runs down from the lower end of the testis to the scrotum, and which at present I shall call the ligament, or gubernaculum testis, because it connects the testis with the scrotum, and directs its course in its descent.... It is hard to say what the structure or composition of this ligament (= gubernaculum) may be. It is certainly vascular and fibrous, and the fibres run in the direction of the ligament itself. It may be muscular; and I am inclined to believe that it is in part composed of the cremaster muscle turned inwards, and running upwards to join the lower end of the testis....'.

Potential components. The testicular ligamentum proprium (also called ligamentum testis), running between the testis and the epididymis; the so-called genital inguinal ligament (a ligament-like anterior end and the associated mesenteric fold lying between the cauda of the epididymis and the region of the inner inguinal ring); the conus inguinalis (a component of the abdominal wall); and the scrotal ligament (between the base of the conus inguinalis and the scrotal dermis).

Components proposed here. The components of the gubernacular cone and the structures developing from them during later life: (1) the bulge of dense connective tissue developing in the abdominal wall in front of the anterior edge of the obliquus muscles; (2) the cremaster muscles; (3) the covering sheath of the processus vaginalis.

structures to be indicated as 'the gubernaculum' during prenatal and postnatal life throughout the mammalian class.

Table 2. *The gubernaculum Hunteri: variation in meanings of 'gubernaculum' in older and recent publications*

1. The emerging muscular cremasteric sac in bulls and freemartins (Lillie, 1917, 1923).
2. In cattle the term gubernaculum 'will signify the ligament of attachment of the genital cord to the bottom and wall of the saccus vaginalis (= *gubernaculum as defined by Lillie*! if that ligament thickens and lengthens in the abdominal cavity' (Bissonnette, 1924).
3. The conus inguinalis and attached inguinal mesenteric fold in rodents (Radhakrishnan et al. 1979).
4. Only the conus (also called crista) inguinalis and this structure is considered a derivative of the inguinal abdominal wall (e.g. Elder et al. 1982; Husmann & MacPhaul, 1991; Bentvelsen & George, 1993).
5. Only the conus (also called crista) inguinalis and this structure is considered a derivative of the mesonephric mesenchyme; no definition of the gubernaculum in postnatal animals (Bodemer, 1968; Backhouse, 1981; Radhakrishnan & Donahoe, 1981; Hutson & Donahoe, 1986).
6. Only the conus inguinalis without specific consideration of its origin (Dantschakoff, 1941; Rajfer, 1980).
7. The inguinal fold of the genital mesentery and the mesenchymal core, but not the muscular wall, of the conus inguinalis (van den Broek, 1933).
8. Only the inguinal fold of the genital mesentery and not the conus inguinalis in rodents (Hadziselimovic & Herzog, 1980; Hadziselimovic & Girard, 1981; Habenicht & Neumann, 1983; Hadziselimovic, 1995).
9. The rodent conus inguinalis before birth and the remnant of the inguinal fold of the genital mesentery after birth (Shono et al. 1994).
10. 'Mesenchymal structures' (mesonephric derivatives) caudal to the lower pole of the testis (Wensing et al. 1980; Ullmann, 1993) and surrounded by sex-specifically differentiating musculature (Raifer & Walsh, 1977).
11. The 'Thread-like connection between the caudal pole of the testis and the bottom of the muscular cremaster sac' (Starck, 1965; Rajfer, 1980). It is 'generally accepted that the gubernaculum is a maintained mesenchymatous residuum which ultimately differentiates and therefore disappears' (Redman, 1993).

The present study aimed first to design a rational and consistent nomenclature of the gubernaculum in fetal and postnatal rodents. Care was taken to use the term to indicate similar structures in males and females and in successive developmental stages. From such an analysis it might become possible to determine more satisfactorily the potential sites of sexual differentiation of the gubernacular primordia: as yet it is unclear what target cells play a role in this process and what fetal testis factor(s) might be involved (Fentener van Vlissingen & van der Schoot, 1992; Visser & Heyns, 1995).

The second aim of this study was to compare the results obtained in rodents with those in other placental mammals (cattle, pig, horse, man) in order to examine whether, and if so in what respects, gubernacular development follows distinct patterns throughout the placental mammalian class (Gier & Marion, 1970; Wensing & Colenbrander, 1986). Marsupials were not included in this analysis. However, in a recent study it was noticed that their gubernacular development follows a path which is different from that of placental mammals (Griffiths et al. 1993).

MATERIALS AND METHODS

The first part of this study consisted of the analysis of the perinatal changes in the gubernaculum of male and female rats. Male and female fetuses between days (d) 13 and 22 after conception (d 22 = day of birth) were serially sectioned transversely or sagittally (0.01 mm per section; 4 or more fetuses of both sexes on all days) and the sections (mounted 1:1 or 1:10) were stained with haematoxylin and eosin. The results were compared with those from the study of serially sectioned newborn hamsters (n = 5) and 19 to 30-d-old rabbit fetuses (n = 15; slides examined at the Department of Physiology of Development, Collège de France, Paris).

The second part of the study consisted of the analysis of fetuses of cattle, pig, horse, and man. Serial sections (mostly cut transversely) were obtained from the Department of Anatomy and Embryology (University of Leiden, The Netherlands: pig and human fetuses), the Department of Physiology of Development (Collège de France, Paris: cattle) and the Zentrum der Morphologie (Klinikum J. W. Goethe Universität, Frankfurt/Main: horse). Except for the horse (a single male specimen of 48 mm) there were several specimens available with developmental stages spanning the period between the early emergence of the gubernacular primordia until after the onset of the sexually dimorphic differentiation of these structures (cattle: 58 specimens between d 30 and 89 pc; pig: 25 specimens between 6 and 35 mm CR length; human fetuses: 30 specimens between 9.5 and 50 mm CR length).

Part of the study consisted of a re-examination of the effect of exposure of male fetal rats and hamsters to antiandrogens on gubernacular development. Pregnant rats had been injected daily, from d 10 postcoitum (pc) with 10 mg (in 0.1 ml 1,2-propanediol) 4-nitro-3-fluoromethyl-isobutyr-anilide (flutamide, or SCH 13521; Neri et al. 1972). Effectiveness of flutamide to affect male offspring was noticed at birth by the persistence of nipples, and inhibition of penis development (Van der Schoot & Elger, 1993). The gubernacula in male offspring were examined on the day of birth. The male cremaster sacs were examined at the age of 100 d. For comparison, the gubernacula of neonatal male and female rats were analysed. Serially sectioned male hamsters (d 16 pc; n = 10) were re-examined, the mothers of which had been injected daily, on d 8–15 pc, with 10 mg cyproterone acetate to study genital sexual differentiation as described before (Elger et al. 1970). The results were compared with similar sections of newborn untreated male hamsters (n = 5).

RESULTS

The gubernaculum in prenatal and postnatal male rats and other rodents (*Figs 2–5*)

In 14 and 15-d-old male and female rat fetuses a minor bulge of dense connective tissue was present in front of the ventromedial edge of the expanding primordia of the abdominal wall obliquus musculature, and just lateral to the primordium of the abdominal rectus muscle. This bulge appeared to be the first sign of the emerging gubernaculum. On d 13 pc such a structure was not unequivocally distinguishable. On d 16 pc this bulge had become enlarged so that the overlying peritoneal epithelium protruded as a minor elevation in the region of the developing inner inguinal ring (Fig. 2*A–C*). On d 17 pc in male (Fig. 2*D–F*) but not in female fetuses (data not shown) the bulge and surrounding tissues had become further enlarged so as to appear as a distinct papilla (often called the gubernacular or inguinal cone; see Table 2) in the region of the inguinal ring. The tip and dorsal aspect of this papilla served as the sites of attachment of an inguinal extension (the inguinal ligament of many other authors: see, e.g. Habenicht & Neumann, 1983) of the mesonephric mesentery in the inguinal region (Fig. 2). The tip consisted of the earlier bulge of dense connective tissue in the abdominal wall (compare gc in Figs 2A and D). Its further wall consisted for the major part of 2 layers of myoblasts continuous with the developing layers of muscles of the adjacent ventral abdominal wall. Its loose mesenchymal core was confluent with the loose adjacent subdermal mesenchyme (Fig. 3).

On d 4 18–22 pc, the papilla-like structures enlarged further in males (Fig. 3) but not in females. Their tip in males remained prominent through the presence of the dense mesenchymal core. Their wall showed increasingly obviously the 2 distinct layers of myoblasts continuous with the obliquus muscles of the developing abdominal wall. At their base, a loose mesenchymal core developed and by d 21/22 pc, an

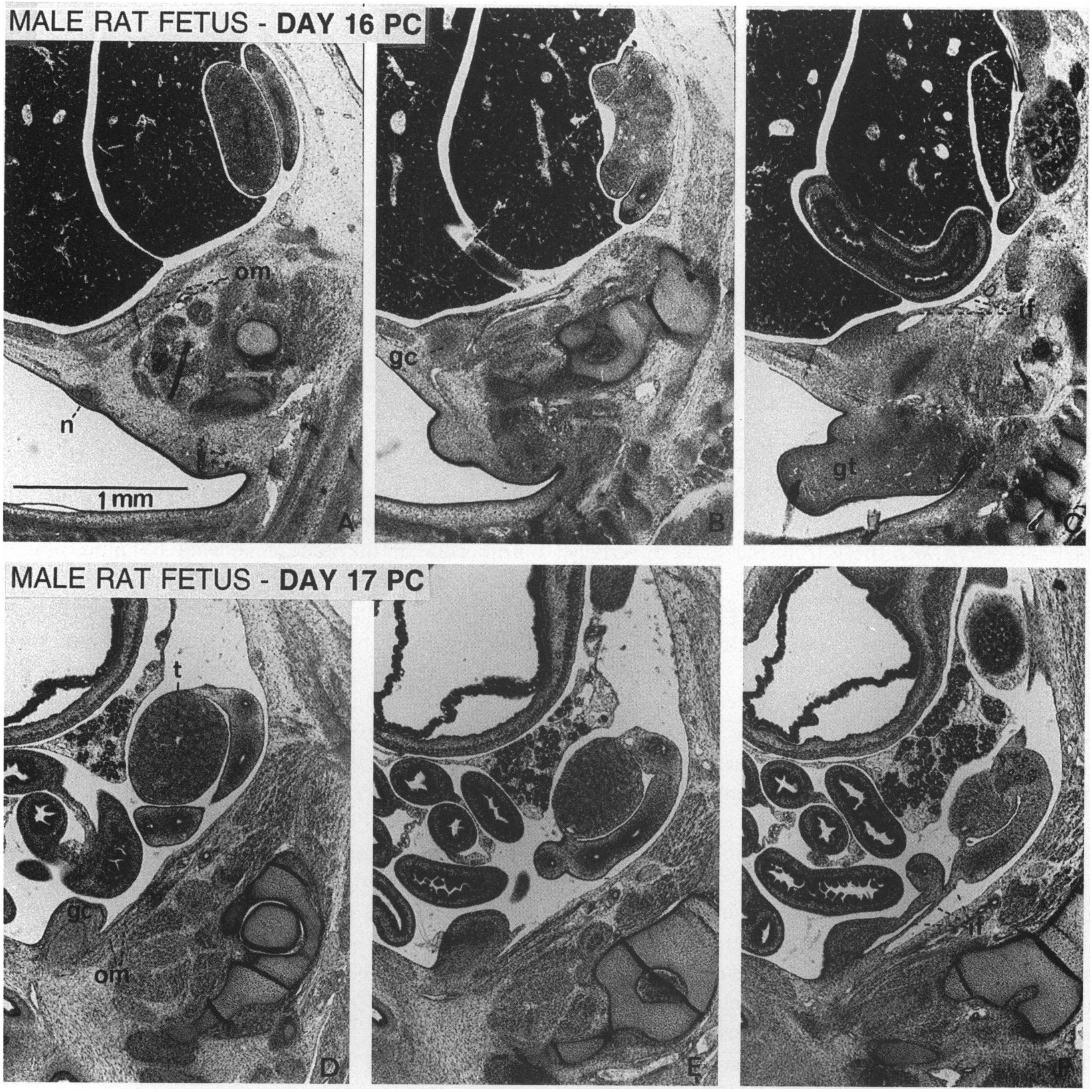

Fig. 2. The gubernacular primordium of 16-d-old (*A–C*) and 17-d-old (*D–F*) male rat fetuses. Left side of panels is ventral, right side dorsal (also in Figs 3–5). Three sagittal sections from each fetus from lateral to medial. (*A*) Anterior border of the developing internal oblique muscle with (*B*) a mass of dense connective tissue visible in the abdominal wall close to this anterior border; the site of insertion of the inguinal fold of the genital mesentery just medial to *B* is present in *C*. (*D–F*) Roughly similar structures in a 17-old male rat fetus. The anterior border of the developing abdominal wall musculature has become a major constituent of the wall of a papilla-like structure emerging from the region of the inner inguinal ring. The mass of dense connective tissue (in *E*) is now at the tip of this papilla. The genital mesentery inserts into the tip of this prominence (in *F*). The papilla-like structure has a core of loose mesenchymal tissue which is confluent with the mesenchyme of the abdominal wall subdermis. t, testis; k, kidney; n, nipple; om, obliquus musculature; gc, dense mesenchyme of gubernacular cone; if, inguinal fold of the genital mesentery; gt, genital tubercle.

open space separating this tissue from the further abdominal wall mesenchyme. The anterior end of the inguinal fold of the mesonephric mesentery remained inserted on the tip, and the fold further attached over the whole length of the posterior wall (Fig. 3*A*). The mesonephric duct became differentiated into the epididymis and remained closely associated with the tip of the papilla (Fig. 3*B*).

Soon after birth this structure started to invert (as for inversion of the finger of a glove) so that the processus vaginalis emerged as a caudal extension of the abdominal lumen. The tissues making up the papilla before birth developed further uninterruptedly, but with a different spatial arrangement (Fig. 3*A–C*). All components of the papilla remained unequivocally identifiable during and after this in-

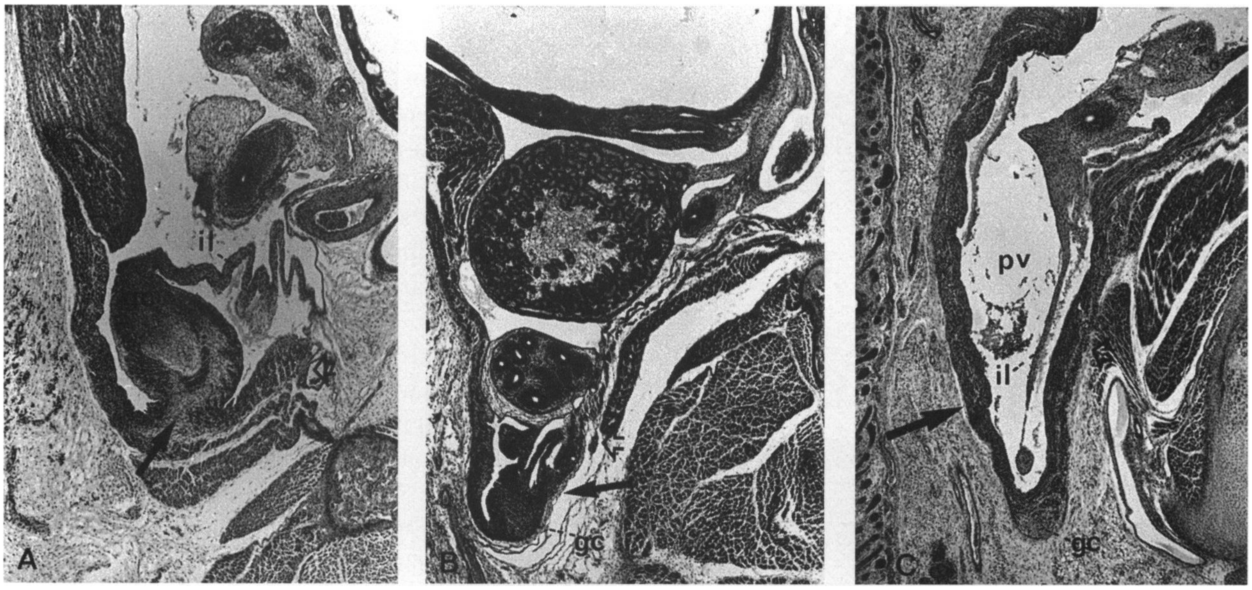

Fig. 3. Inversion of the gubernaculum around birth and formation of a cremasteric sac postnatally in rats. The fully developed gubernaculum (*A*; d 22 pc) projects into the region of the inner inguinal ring. The layers of its muscular wall are confluent with those of the ventral abdominal wall (anteriorly) and inner inguinal ring (posteriorly). The posterior aspect is connected to the posterior body wall through a fold of the genital mesentery (if). This anterior border of this mesentery inserts onto the tip of the gubernacular cone (gc) on the mass of dense connective tissue. The level of the base of the gubernacular cone is marked by nerves and vessels: in particular, the genitofemoral nerve (open arrowhead) runs, for some distance, horizontally along the posterior brim of the inner inguinal ring. Inversion of the gubernaculum (in *B*; day of birth) is facilitated through the development of a tissue space between its base and the surrounding tissues. During the inversion process its earlier mesenchymal core (solid arrow) becomes its connective tissue sheath: this tissue becomes extended below the level of the genitofemoral nerve. In *C* (7 d after birth; testes removed on the day of birth) a section of the anterior end of the inguinal fold of the genital mesentery appears as a bulge just above the base of the processus vaginalis (= inverted gubernaculum cone). To the other side of the base of the processus vaginalis, the remnants of the earlier mass of dense connective tissue are recognisable. The genitofemoral nerve indicates the plane around which inversion of the earlier gubernaculum took place. pv, processus vaginalis. Magnification as in Figure 2*A*.

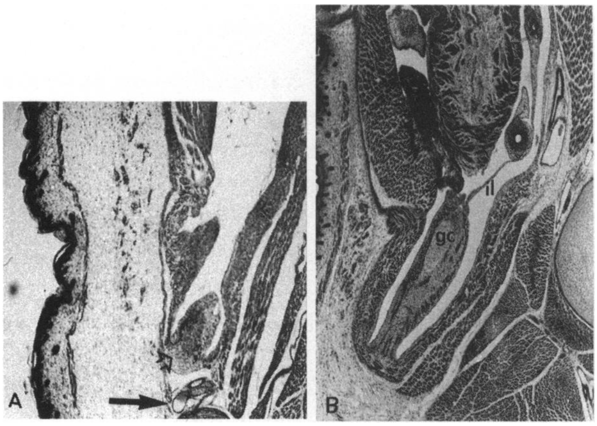

Fig. 4. The maximum size, on the day of birth, of the gubernaculum (gc) of a female rat (*A*) and a male rat exposed to flutamide from d 10 pc (*B*). In the female organ (*A*) similar elements can be identified as in the male but their size is less. Exposure of male fetuses to antiandrogen (*B*) leaves development of the gubernaculum unaltered in all earlier mentioned respects. For explanation of arrows, see legend to Figure 3; gc, gubernaculum cone; if, inguinal fold of genital mesentery. × 18.

version process: (1) the dense connective tissue in the tip became part of the base of the processus vaginalis (Fig. 3*C*); (2) the myoblasts of the wall became the major constituents of the wall of the processus

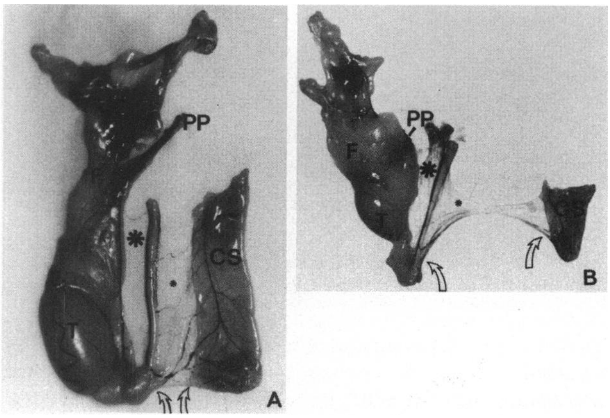

Fig. 5. The testis (T) and adjacent structures emerging after eversion of the cremasteric sac (CS) of an adult male control rat (*A*) and a rat (with an intra-abdominal testis) exposed to flutamide during prenatal life (*B*). There is a peritoneal fold between the testis/epididymis and the vas deferens (large asterisk) and between the vas deferens and the (everted) wall of the cremasteric sac (small asterisk). The base of the cremasteric sac is connected to the caudal pole of the epididymis (in *A*) or epididymal remnant (in *B*) via a minor or long ligament (between the tips of the open arrows) respectively; this ligament is to be considered the remnant of the inguinal fold of the genital mesentery during fetal life. PP, pampiniform plexus; F, epididymal fat pad.

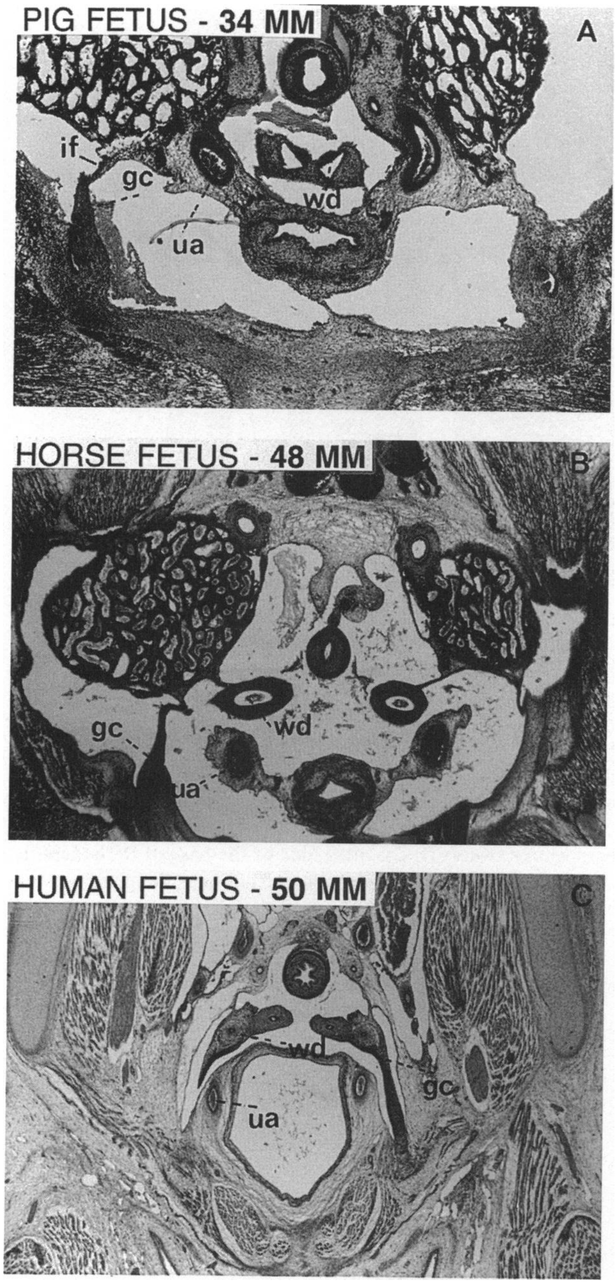

Fig. 6. The gubernaculum during fetal development in pig, horse, and man. *A* and *B* show transverse sections through the mesonephros and its attached, laterally directed, mesenteric fold. *C* is just below the level where mesonephric remnants are visible. The mesenteric fold inserts on top of the gubernaculum which consists, in all 3 sections, of a mass of dense connective tissue with its base in the developing ventral abdominal wall. In other sections of the pig and horse specimen the attachment between this connective tissue mass and the anterior border of the developing internal oblique muscle is visible. This muscle is seen in *C* at the base of the left gubernacular cone (gc). if, inguinal fold of genital mesentery; ua, umbilical artery; wd, mesonephric (wolffian) duct. × 22.

vaginalis (Fig. 3*A*–*C*); (3) the mesenchymal core developed into the outer covering of the processus vaginalis wall; initially, this sheath was without contact with the surrounding mesenchymal tissues (Fig. 3*C*). The plane of inversion of the papilla was recognisable from the position of a horizontally running part of the genitofemoral nerve which remained unaltered in its localisation (Fig. 3*B*, *C*). Throughout this process of inversion there were no specific connections visible between the inverting papilla and its surrounding tissues. The inverting organ, instead, appeared 'bathed' within an enlarging tissue space separating its mesenchyme from that of its surroundings.

As the animals grew larger postnatally, the muscularity of the wall of the processus vaginalis (= tunica vaginalis) increased rapidly (Fig. 3*C*: 7 d after birth). During adulthood, 'cremasteric sacs' had developed (Fig. 5): these were the further enlarged, but qualitatively unaltered, structures which had been the intra-abdominal papillae before birth and the emerging processus vaginalis sacs after birth.

Fetal male rabbits (d 19–30 pc) showed a similar growth and differentiation of structures protruding from the region of the inner inguinal ring. On d 30 pc there was no sign of the imminent inversion of these structures to processus vaginalis sacs. Newborn male hamsters (n = 5) showed papillae which were in the process of inversion towards processus vaginalis sacs.

The perinatal gubernaculum in female and male rodents exposed to antiandrogen during fetal life (*Fig. 4*)*; the gubernaculum during adulthood* (*Fig. 5*)

For the sake of descriptive simplicity and in view of arguments presented later in the discussion, the above described papilla in prenatal rodents will be considered further as the gubernaculum.

Prenatal development of gubernacula in female rats began in a similar fashion on d 14–15 pc as in males (data not shown). On d 22 they showed similar components as in males but they were quantitatively much less developed (Fig. 4*A*). Exposure of fetal male rats to biologically effective doses of the antiandrogen flutamide seemed to have no detrimental effect, qualitatively or quantitatively, on male-like development of the gubernacular cone on d 22 pc (Fig. 3*B*). However, in several of the flutamide-exposed animals there was an elongated inguinal fold of the mesonephric mesentery—with its anterior end running between the tip of the gubernacular cone and the caudal part of the wolffian duct remnants—as compared with that in untreated male rats (compare Fig. 4*B* with Fig. 3*B*). Accordingly, the testes were present occasionally at a higher intra-abdominal position than those of controls. During adulthood, cremasteric sacs had developed in flutamide-exposed rats in-

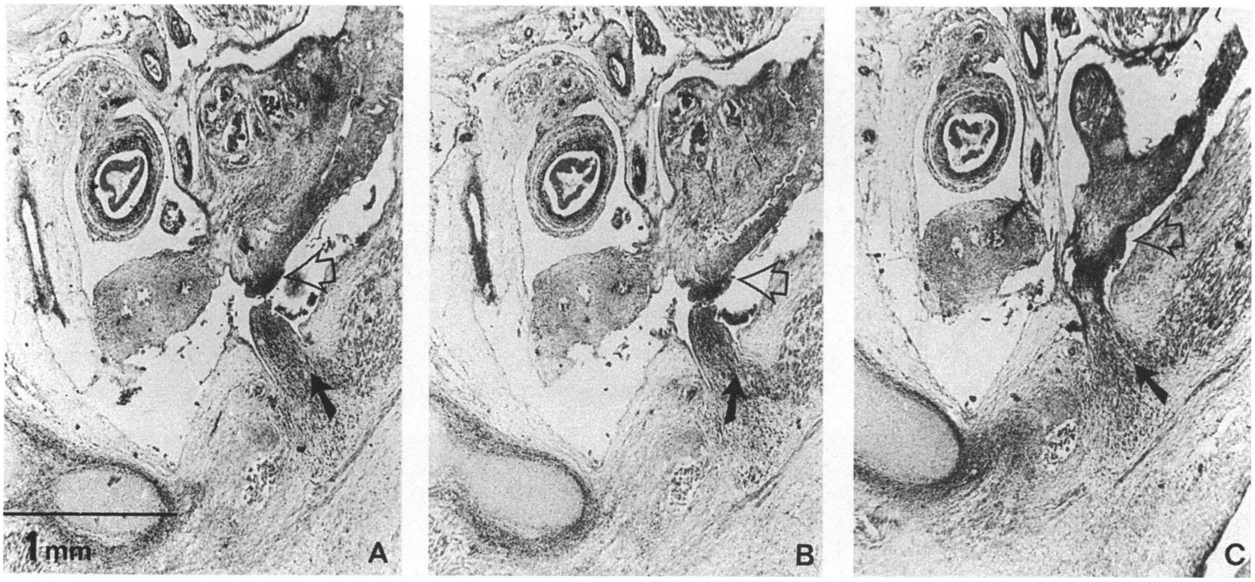

Fig. 7. The gubernaculum during further human fetal development (Leiden, S55, slides 168/169). The developing genital system approaches the developing abdominal wall very closely (open arrows). From this wall the gubernaculum protrudes into the abdominal lumen and is connected directly to the mesonephric derivatives. Not much is visible of the inguinal fold of the mesonephric mesentery. The layers of cells in the developing gubernaculum can be observed to be confluent with the developing layers of muscles in the surrounding body wall (solid arrows).

dependent of whether or not testes had descended into them (Fig. 6). In rats with descended testes the cremasteric sacs were similar to those of untreated control male animals. In rats with intra-abdominally retained testes the cremasteric sacs were of small size. In all these animals there were minor or elongated, respectively, inguinal folds between the base of the cremasteric sacs and the caudal tips of the testes or wolffian duct remnants (Fig. 5*B*).

Near-term male hamster fetuses (d 16 pc; n = 10), which had been exposed to the antiandrogen cyproterone acetate from d 8 pc, confirmed the unaltered and male-type development of the gubernaculum, whereas the development of male-specific mesonephric duct derivatives (epididymis and vas deferens) had become completely suppressed (data not shown).

Early stages of gubernaculum development in pig, horse and man (Figs 6, 7)

Serial sections through the lower abdomen of pig and human fetuses showed the development of essentially similar gubernacula as those observed in rodents: (1) the initial development of dense connective mesenchyme in the abdominal wall in front of the ventromedial end of the emerging obliquus musculature; (2) the insertion of the anterior end of an inguinal fold of the mesonephric mesentery on the tip of the dense mesenchymal mass and further attachment of this fold along the dorsal aspect; (3) temporary growth (in human fetuses for a period lasting beyond attainment of a body length of 50 mm) of a papilla-like structure in the region of the inner inguinal ring with the dense mesenchyme at its tip. Full inversion of the gubernacular cones, with the emergence of the processus vaginalis as its consequence, occurred in human fetuses at an older postconceptional age than the oldest specimens analysed in this study.

Early gubernacular development in cattle (Fig. 8)

Serial sections through the lower abdomen of bovine fetuses showed the initial development of a core of dense connective tissue within the abdominal wall just anteromedial to the developing obliquus internus muscle and with the insertion of an inguinal fold of the mesonephric mesentery on its tip (Fig. 8*A–D*). Different from rats and the other species examined, the dense connective tissue mass never developed into a papilla-like cone protruding from the abdominal floor (period analysed between 36 and 89 d pc). Instead, it appeared, in males and females, that a processus vaginalis developed immediately with the above mass at its base and with partial lining by the developing cremaster muscles (Fig. 8*E–H*, *I–L*).

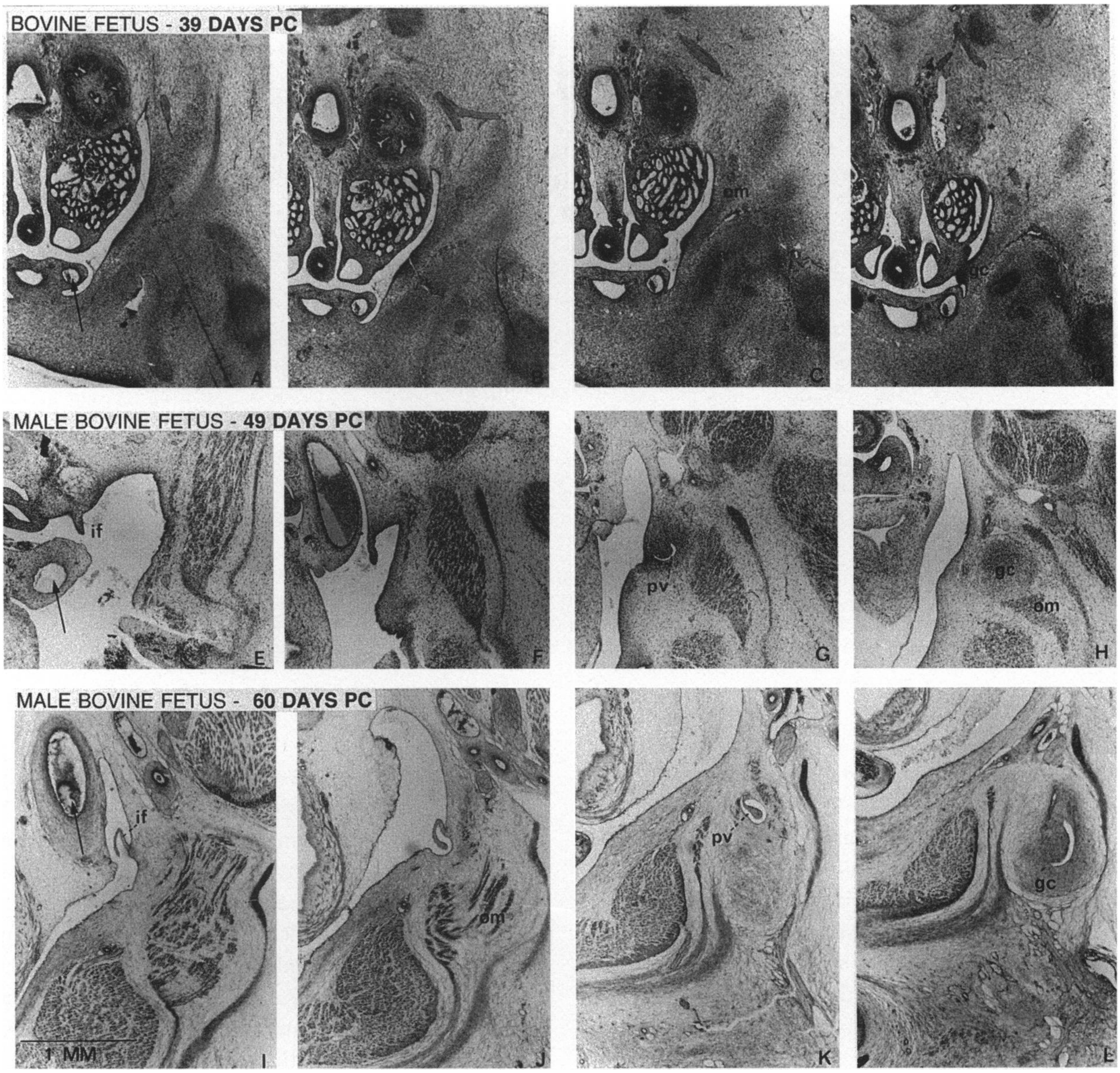

Fig. 8. The development of the gubernaculum in 39–60-d-old bovine fetuses. (39 d: female, Jost VT126 (*A–D*); 49 d: male, Jost VT 69 (*E–H*); 60 d, Jost C27 (*I–L*).) Gubernacular development in bovine fetuses differs from that in the other species through the absence of gubernacular cones. Cores with dense connective are not yet identifiable on d 39. On d 49 they are present at the base of the already identifiable processus vaginalis (pv). The latter structure becomes deeper with advancing fetal age. The inguinal fold (if) of the genital mesentery is attached along its posterior wall and is inserted at its base on the tip of the dense connective tissue mass. gc, gubernacular cone; om, obliquus musculature.

DISCUSSION

More than 200 years ago the term gubernaculum was proposed (Hunter, 1762) to indicate '...a substance which runs down from the lower end of the testis to the scrotum, and which at present I shall call the ligament, or gubernaculum testis, because it connects the testis with the scrotum, and directs its course in its descent...'. Many later authors have adopted the term gubernaculum without considering the wide variation in the definitions which came into use in different investigations, in different mammalian species, and in studies of fetal or postnatal animals. Persistence of inconsistency in the definitions used (Weber, 1898; Backhouse, 1982; Table 2) is to be judged an important determinant of the relatively poor state of the knowledge which has been gained today about the emergence, and control of the sexually dimorphic development of the gubernaculum. Whereas several aspects of sexual differentiation of the mesonephros, its ducts and their derivatives have been analysed into the molecular details (Josso et al. 1993; Quigley et al. 1995) there is not even unanimity about the anatomical definition of the gubernaculum. Ignorance of the facts of its normal development is the more surprising as the gubernaculum is to be regarded, during certain stages of the fetal development of various mammals, as the most prominent sexually dimorphic feature of the internal genital primordia (Lillie, 1917, 1923; Bissonnette, 1924; Rajfer, 1980; van der Schoot & Elger, 1993).

The present study first addressed the growth and development of a structure developing in the rodent inguinal area and which is considered widely (but, admittedly, not unanimously) as 'the' rodent gubernaculum. This structure presents itself as a distinct organ during the final days of fetal life: the analysis of its development during prenatal and postnatal life could provide a template with which to build a consistent and rational definition of the gubernaculum. Studies, which aim to uncover the proposed fetal testis factor(s) effecting male gubernacular development, presuppose an undisputed consensus regarding the nature of the gubernaculum—and this is not available at the present time. The cross-species generality of a proposed definition in a given species should be clear as only a single species, i.e. *Sus domesticus*, has been used until now to isolate this putative factor (Fentener van Vlissingen et al. 1988; Visser & Heyns, 1995).

The present study describes in detail the development of the rat gubernaculum from d 13/14 pc. The results suggest that, in the above held definition, the gubernaculum emerges as a part of the developing ventral/inguinal abdominal wall. The pattern of its development after d 14 pc reveals that the enlarging papilla-shaped structure on d 17 and later is unlikely to be a derivative of the mesonephros. Instead, it seems that this papilla emerges from within the tissues of the abdominal wall and provides, with its tip and posterior aspect, a site of anchorage for a mammalian specific inguinal fold of the mesonephric mesentery. This view of the emerging rodent gubernaculum is in line with descriptions provided earlier this century (Weber, 1898; Lillie, 1917; van den Broek, 1933; Grosser, 1953) and with its appearance in fetuses of nonrodent mammalian species such as pig, horse, and man (Gier & Marion, 1970). This view differs, however, from the widely held contemporary view in which the rat gubernaculum is considered as a structure protruding from the mesonephros (Radhakrishnan et al. 1979; Radhakrishnan & Donahoe, 1981; Baumans et al. 1981; Wensing & Colenbrander, 1986; Hutson & Beasley, 1992; Ullmann, 1993; Hutson & Baker, 1994; see also Table 2). The latter view is difficult to reconcile with uninterrupted development of the gubernacular primordia after their surgical separation from the mesonephric derivatives (Bergh et al. 1978; Radhakrishnan & Donahoe, 1981; Elder et al. 1982; Frey & Rajfer, 1984; Beasley & Hutson, 1988). The view presented here is easy to reconcile with this finding as it implies that this surgical intervention only damages a part of the mesonephric mesentery but leaves the papilla-like abdominal wall derivative intact (see also Rajfer, 1980; Elder et al. 1982). There are no major nervous or vascular connections between the mesonephric mesentery and the gubernacular cone as these connections enter the latter structure from the inguinal abdominal wall (genitofemoral nerve; external pudendal artery and vein).

Observations in postnatal rats stress that all components of the above defined fetal gubernaculum participate in shaping together the rat processus vaginalis sac during neonatal life: no new components are added and perinatal gubernacular inversion includes only spatial rearrangement of these components followed by rapid enlargement of all of them. There is no evidence that fetal gubernacular components disappear during postnatal life. Specifically, contrary to the suggested regression of the mesenchymal gubernacular core around birth (Radhakrishnan et al. 1979; Wensing & Colenbrander, 1986; George, 1989) this structure develops further, during and after inversion of the gubernacular cone, to become the outer sheath of the processus vaginalis wall (Grant & Wright, 1971; Rajfer, 1980; Elder et al. 1982; van der Schoot & Elger, 1993). It seems compelling logic to consider as the postnatal gubernaculum the structure which develops from the prenatal gubernaculum. This logic leads to the conclusion that the whole of the cremasteric sac is to be considered 'the gubernaculum' in postnatal rats, thus including (1) the bulge of dense connective tissue on the dorsomedial aspect of the base of the cremasteric sac; (2) the cremaster muscles; (3) the covering sheath of the processus vaginalis.

In many studies on postnatal rats and males from other species including man, the term gubernaculum applies to the minor strand of tissue between the epididymal cauda and the base of the processus vaginalis (Nelsen, 1953; Hadziselimovic & Herzog, 1980; Hadziselimovic & Girard, 1981; Backhouse, 1982; Platzer, 1989; Larkins & Hutson, 1991; Redman, 1993; Shono et al. 1994). In fetal rats this structure represents the inguinal extension of the mesonephric mesentery and there are only a few reports considering this structure as the gubernaculum (Hadziselimovic & Girard, 1981; Habenicht & Neumann, 1983). There should be no reason to maintain this view which, by necessity, introduces a misleading inconsistency when fetal and postnatal stages of 'the' rodent gubernaculum, the papilla and mesonephric mesentery remnant respectively, are considered in a developmental analysis (Bentvelsen & George, 1993).

Male and female gubernacular development in rats starts similarly at 13–14 d pc when the inguinal extension of the mesonephric mesentery becomes identifiable as well as the primordium of the gubernaculum within the ventral abdominal wall. During the subsequent days, sex differences accumulate rapidly. In males, the papillae show rapid enlargement and they develop layers of myoblasts as extensions of the internal and external oblique (Weber, 1898; Rajfer, 1980; Radhakrishnan & Donahoe, 1981). In females, only minor enlargement occurs and virtually no growth of muscle cells. There can be no doubt that muscle cells are a constitutive element of the fetal male rodent gubernaculum and, therefore, a likely target structure of the proposed fetal testis hormone which controls male gubernacular differentiation (Rajfer, 1980; Bentvelsen & George, 1993; van der Schoot et al. 1995).

The comparison in this study, across various species including man, of the earliest stages of gubernacular development revealed an unexpected similarity of the initial emergence of a core of dense connective tissue in the abdominal wall of all species studied. Except for cattle, there was an initial stage of gubernacular cone development in the region of the inner inguinal ring with this core of dense connective tissue at its tip. Earlier workers had suggested that this prominence was a feature characteristic only for rats and other laboratory rodents. Based on this assumption, distinct models for gubernacular development across species have been proposed (Gier & Marion, 1970). As a consequence of this distinction between different patterns of gubernacular development, it was proposed that rats would not provide a valid model for gaining insight into normal or aberrant gubernacular development in man. The demonstration, in this study, of a stage with gubernacular cones in pig, horse, man and rats asks for reconsideration of this assumption. In view of the similarity of early gubernacular development in man and rats, there seems every reason to suggest that analysis of rat gubernacular development may provide useful information of normal and aberrant development of this structure in man.

Bovine fetuses show a unique feature in their gubernacular development as there is no stage with a recognisable intraabdominal gubernacular cone: the core of dense tissue remains embedded in the abdominal wall throughout. However, absence of such a stage does not need to represent more than a temporary difference as gubernacular cones disappear in all mammals and the subsequent stage with extending processus vaginalis sacs is no longer different between them. There is no easy explanation for failure of gubernacular cone development in cattle. Interestingly, as rediscovered recently, gubernacular cones may appear in cattle freemartins (Lillie, 1917; Bissonnette, 1924) due to 'reversed inversion' of cremasteric sacs in advanced stages of fetal life (van der Schoot et al. 1995). The mere development of gubernacular cones in such a surprising way confirms the basic similarity of the developing gubernaculum of cattle and the other mammals examined.

The above observations and arguments lead to the view that gubernacular development in placental mammals is a unitary process. There is no reason to distinguish between various types such as with and without temporary gubernacular cones (Gier & Marion, 1970) or with strip-like or sac-like cremaster muscles (McMahon et al. 1995). This conclusion is not without significance for directing further research. The old view made it unlikely that laboratory rodents were useful as models for studying features of normal and (frequently occurring) aberrant testis descent in man. Accepting the view presented here may lead to abandoning this older notion. The possibility of a unitary process of gubernacular development throughout the placental mammalian subclass stresses the importance of reanalysing gubernacular development in marsupials. If the pattern of growth differs fundamentally from the pattern described here, further support is adduced for the notion that gubernacular development in the 2 mammalian subclasses represents at least partially independently evolved processes (Griffiths et al. 1993).

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