Cryptorchidism in common eutherian mammals

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Abstract

Cryptorchidism is failure of one or both testes to descend into the scrotum. Primary fault lies in the testis. We provide a unifying cross-species interpretation of testis descent and urge the use of precise terminology. After differentiation, a testis is relocated to the scrotum in three sequential phases: abdominal translocation, holding a testis near the internal inguinal ring as the abdominal cavity expands away, along with slight downward migration; transinguinal migration, moving a cauda epididymidis and testis through the abdominal wall; and inguinoscrotal migration, moving a s.c. cauda epididymidis and testis to the bottom of the scrotum. The gubernaculum enlarges under stimulation of insulin-like peptide 3, to anchor the testis in place during gradual abdominal translocation. Concurrently, testosterone masculinizes the genitofemoral nerve. Cylindrical downward growth of the peritoneal lining into the gubernaculum forms the vaginal process, cremaster muscle(s) develop within the gubernaculum, and the cranial suspensory ligament regresses (testosterone not obligatory for latter). Transinguinal migration of a testis is rapid, apparently mediated by intra-abdominal pressure. Testosterone is not obligatory for correct inguinoscrotal migration of testes. However, normally testosterone stimulates growth of the vaginal process, secretion of calcitonin gene-related peptide by the genitofemoral nerve to provide directional guidance to the gubernaculum, and then regression of the gubernaculum and constriction of the inguinal canal. Cryptorchidism is more common in companion animals, pigs, or humans (2–12%) than in cattle or sheep (<1%). Laboratory animals rarely are cryptorchid. In respect to non-scrotal locations, abdominal testes predominate in cats, dogs, and horses. Inguinal testes predominate in rabbits, are common in horses, and occasionally are found in cats and dogs. S.c. testes are found in cattle, cats and dogs, but are most common in humans.

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Introduction

Cryptorchidism is failure of one or both testes to ‘descend’ into the scrotum at the time normal for the species of interest, before or shortly after birth. Obviously, detection is postnatal. This problem was described by de Graaf in 1668 (Jocelyn & Setchell 1972), in respect to humans, dogs and rams. De Graaf cited publications from 1582 and 1618, and presented personal observations of testicles located in the abdominal cavity, near the kidneys, or in the groin area rather than in the scrotum. Both uni- and bilateral cryptorchidism were recognized. These early reports could have been interpreted (but were not) as evidence that cryptorchidism is phenotypic evidence of two or more diseases, because undescended testes are not always located at the same non-scrotal site.

Until recently, the general perception was that cryptorchidism was a single disease with moderate heritability, incomplete penetrance, expressed only in males (sex-specific expression), and concentrated by inbreeding or minimized by culling affected males and all siblings. This is too simplistic. Approximately 25 years ago, the notion of a single locus gene problem gave way to acceptance of a polygenic recessive model, based on relatively small studies with pigs (Sittmann & Woodhouse 1977, Rothschild et al. 1988); dogs (Cox et al. 1978, Nielen et al. 2001); and also for men (Czeizel et al. 1981). Unfortunately, techniques of modern molecular genetics have not been applied to sub-human species with a sufficient incidence of cryptorchidism to justify a study of gene abnormalities (e.g. cryptorchid deer (from an unusual locale; Veeramachaneni et al. 2006), dogs, horses, or pigs). Nevertheless, it is unlikely that sequence changes in 1–4 genes account for most cases of cryptorchidism in common animals. This conclusion is based on comprehensive analyses for hundreds of men; no single gene, considered to be involved in regulation of testicular descent, is aberrant in >10% of cryptorchid men (Ferlin et al. 2003, Roh et al. 2003, Klonisch et al. 2004, Garolla et al. 2005, Yoshida
et al. 2005). It is now accepted that there is a multiplicity of causes for cryptorchidism (Hutson et al. 1997, Klonisch et al. 2004, Hutson & Hasthorpe 2005), including genetic, epigenetic, and environmental components.

Cryptorchidism should be viewed as the ‘tip of an iceberg’, providing early and facile phenotypic detection of testicular disease, which after puberty might be evidenced as other phenotypic defects. These include quantitative and/or qualitative defects in spermatogenesis, or tumors found long after abnormal differentiation of anlage for germ, Sertoli, Leydig, or stromal cells. Although all of these defects might not be detected in a given individual, each is considered to be part of a testicular dysgenesis syndrome (TDS), and possibly all have a common underlying cause from improper development of fetal testes. This topic has received much attention in the past 10–15 years (Skakkebaek et al. 1998, 2001, Rajpert-De Meyts 2006, Sharpe 2006).

The concept of a TDS does not exclude other causative mechanisms for cryptorchidism, such as pituitary failure. Importantly, non-cryptorchidism does not guarantee freedom from other elements of TDS.

A need to better understand the process of testicular descent on a comparative basis arose as we explored an unusually high incidence of cryptorchidism in a unique population of deer (Veeramachaneni et al. 2006; summarized later herein), while we separately observed disparate manifestations of cryptorchidism in rabbits exposed to chemicals or environmental pollutants (Veeramachaneni 2006). We found no recent unifying review of testis descent and pathophysiology of both cryptorchid and non-cryptorchid testes covering domesticated, companion, and laboratory animals together with humans. Hence, we undertook preparation of a comprehensive review. The evolving manuscript was unwieldy, so it was split. Herein, we summarize comparative information on early testis differentiation, structures, and processes involved in testicular descent, timing of testicular descent, incidence and nature of cryptorchidism, and why the problem probably will not be eliminated. A separate review (in preparation) will consider how exposure to certain environmental agents might result in cryptorchidism and, for a subset of agents, tumors of the testis or male reproductive tract.

Formation of a testis

Brief review of testis formation is essential to understand how dysgenesis at this time could lead to seemingly diverse abnormalities, such as cryptorchidism, abnormal spermatogenesis, tumors of the testis or excurrent duct system, or aplasia of male ducts. We augment excellent reviews focused on humans (Rajpert-De Meyts 2006) and rodents (Sharpe 2006), with information for other common animals (Patten 1948, Gier & Marion 1969, 1970, Bergin et al. 1970, Wensing & Colenbrander 1986).

Early in development, a thin fold of peritoneum, the mesonephric sheath, supports the mesonephros and provides the cranial suspensory ligament, which connects the cranial tip of the future gonad to the dorsocranial abdominal cavity (Fig. 1, top). Assume a XY male with genes necessary to drive male development, rather than default female development. Early in embryogenesis, primordial germ cells (PGCs) migrate from the hind gut to the gonadal ridge, on the ventromedial aspect of the mesonephros. Then mesenchymal cells, probably from the neighboring coelomic epithelium, move into the developing gonad, proliferate, and surround the PGCs; in the male they differentiate into fetal Sertoli cells and secrete anti-Müllerian hormone (AMH), which induces demise of the paramesonephric ( Müllerian) duct. The AMH also might affect Leydig cell function. An early consequence of the interaction of fetal Sertoli cells with PGCs is that the latter are prevented from entering meiosis, although proliferation and differentiation can occur. At least in mice, PGCs do not enter meiosis because retinoic acid is not available within the seminiferous cords of a fetal testis (Koubova et al. 2006).

Shortly after arrival of future fetal Sertoli cells, other cells from the mesonephros follow to stimulate proliferation of fetal Sertoli cells, organize nests of fetal Sertoli cells along with PGCs into seminiferous cords (Fig. 1, center; Sharpe 2006), cooperate with fetal Sertoli cells to produce a basal lamina, and apparently give rise to the peritubular myoid cells of an adult seminiferous tubule. More or less concurrently, mesenchymal cells (probably from coelomic epithelium, but mesonephric origin not excluded) migrate into spaces among the seminiferous cords and differentiate into fetal Leydig cells. The interval between entrance of PGCs into the indifferent gonad (not shown) through formation of seminiferous cords requires ≤ 7 days in non-rodent species, and differentiation of the gonad to a functional testis (Fig. 1, bottom) is completed in < 14 days after PGCs arrived in the gonad. Although much growth and refinement of function occurs later, differentiation of a gonad to a testis (Fig. 1, center) producing hormones and growth factors is completed at approximately gestational day (GD): 14, mouse; 16, rat; 22, rabbit; 34, dog; 35, horse; 36, pig; 42, bull; and 56, human.

Within 2–3 days after arrival, fetal Leydig cells achieve maximum production of testosterone, and probably insulin-like peptide 3 (Ins3). Initially, testosterone is produced constitutively or under autocrine/paracrine control in rodents (El-Gehani et al. 1998, Pakarinen et al. 2002), or possibly with stimulation of human chorionic gonadotropin (hCG) entering from maternal blood in humans (Themmen & Huhtaniemi 2000), but later luteinizing hormone (LH) and gonadotrophin-releasing hormone (GnRH) come into play to regulate the process. As the testis continues to differentiate and grow, adult Leydig cells continue to produce Ins3 and testosterone.
Figure 1 Structures involved in testis descent in a typical mammal; upper and lower sketches in lateral view and none in true scale. **Upper, indifferent gonad:** The gonadal ridge forms on ventromedial surface of mesonephros, suspended in folds of peritoneum leading to near the diaphragm (cranial suspensory ligament) and the inguinal area (gubernaculum). The gubernaculum grows out from mesenchymal cells within the abdominal musculature. The area of fusion between the gubernaculum and the external aspect of the mesonephric duct becomes the site of the future cauda epididymis. **Center, testis formation:** After primordial germ cells (PGCs) arrive in the gonad, and assuming genetic drive from a Y-chromosome, the PGCs are surrounded by fetal Sertoli cells and formed into nests, which then are organized into cords by fetal peritubular cells; fetal Leydig cells occupy inter-cord spaces. This is an early fetal testis. Because the 4 cell types intercommunicate via paracrine factors and hormones, abnormal function of 1 cell type early in fetal development likely affects the others and might irreversibly change gonocytes and eventually spermatogonia, and/or adult Sertoli, Leydig, and peritubular cells. Similarly, an exogenous agent affecting differentiation or programming of one cell type could permanently affect the others. **Lower:** The testis usurps the suspensory ligaments as the mesonephros degenerates, the peritoneal lining starts to evert as a vaginal process within the outer limits of the portion of the gubernaculum within the abdominal wall (this happens later in rodents and rabbits), fetal AMH drives regression of the paramesonephric duct (Müllerian duct; not shown), and fetal testosterone drives the mesonephric duct to differentiate as the epididymal plus deferent duct and masculinization of the genitofemoral nerve (not shown). A little later, the accessory sex glands (not shown) also will develop from the mesonephric duct, but this requires dihydrotestosterone rather than testosterone.
Describing cryptorchidism

In preparing this review, it became obvious that the topic suffered from inappropriate and/or imprecise nomenclature, which hindered cross-species comparisons, understanding of regulatory mechanisms, and interpreting actions of exogenous agents. Hence, we used standard anatomical nomenclature (Schaller 1992, International Committee 2005), and defined, and suggested adoption of ‘process terms’ universally applicable to companion, food-producing, or wild animals; rodents or rabbits; and humans. We have taken the liberty of reinterpreting some conclusions in older publications with the benefit of recent information, without discounting the underlying observations. Central in our review were publications describing numerous dissections of fetuses, including those of: cattle (Gier & Marion 1969, 1970, Edwards et al. 2003); dog (Wensing 1968, Gier & Marion 1969); horse (Bergin et al. 1970); human (Hutson et al. 1990, 1997); pigs (Backhouse & Butler 1960, Backhouse 1964, Wensing & Colenbrander 1986, Wensing 1988); rabbit (Rajfer 1980, Elder et al. 1982, van der Schoot 1993, van der Schoot & Elger 1993); and mouse/rat (Wensing 1986, Wensing & Colenbrander 1986, van der Schoot & Elger 1993, van der Schoot 1996, Shono et al. 1994a, 1994b; Hutson et al. 1997, Lam et al. 1998, Hrabovszky et al. 2002).

By definition, cryptorchidism refers to a postnatal phenotype. If one or both testes are not in the scrotum, where are they? Usually non-scrotal testes are in one of three general locations: abdominal cavity, inguinal canal, or s.c. (outside the abdominal wall). Many tabulations combine inguinal and s.c. locations under a single descriptor. However, there is no doubt that testes are found within the inguinal canal in humans (Beltran-Brown & Villegas-Alvarez 1988, Rozanski & Bloom 1995), horses (Rodgerson & Hansen 1997), and rabbits (Veeramachaneni, unpublished).

Since cryptorchidism is failure of testis descent, location of a testis and not size, development, or molecular biology of associated structures (e.g. gubernaculum) should be the prime consideration in deciding if the process involves two, three, or more phases. Non-scrotal testes are found in one of three general locations (abdominal, inguinal, or s.c.), so it is logical that three phases are involved in the process of testis descent. These are: a) abdominal testis translocation, specifically retention near the neck of the developing bladder as the abdominal cavity enlarges following by slight testis relocation to the future internal inguinal ring; b) transinguinal migration of a testis, moving a cauda epididymidis and testis through the abdominal wall; and c) inguinoscrotal migration of a testis, from a s.c. location outside the inguinal canal to correct final position in the bottom of the scrotum. Most authors have combined movement of a testis through the abdominal wall and final migration to the scrotum as ‘inguinoscrotal testis descent’, and consider testis descent to involve two phases rather than three phases as proposed herein.

Accepting that there are three general locations for non-scrotal testes, and that testis descent involves three phases, it follows that cryptorchidism reflects manifestation of at least three prenatal diseases. These are: a) failure to initiate and complete abdominal testis translocation; b) failure to initiate and complete transinguinal migration of a testis; and c) failure to initiate and complete inguinoscrotal migration of a testis. Causation of one of these three phenotypes is complex. We will consider only the most likely causes of terminal failure, namely insufficiency and timeliness of: Insl3, intra-abdominal pressure or reduction of testis size, or testosterone.

The term ‘testicular descent’ is typically used, but ‘translocation’ probably is more descriptive of what happens during the first phase; the absolute distance between a testis and scrotal area changes little (see below); the fetus grows away from the inguinal area, and the testes ‘stay put’ as the kidney is repositioned (Wensing 1968, Shono et al. 1994a). The term ‘migration’ describes both movement of the testis through the abdominal wall and also the separate quest of the testis for the bottom of the scrotum, which can be rather distant from the external inguinal ring.

With complete abdominal retention, both the testes and cauda epididymidis remained in the abdominal cavity, with the testis near the kidney or part-way to the internal inguinal ring and with the cauda epididymidis not juxtaposed to the testis yet within the abdominal cavity; the vaginal process had started evagination from the abdominal wall. With incomplete abdominal cryptorchidism (Stickle & Fessler 1978, Genetzky 1984), the cauda epididymidis had entered the inguinal canal, but the testis remained within the abdominal cavity, relatively close to the internal inguinal ring.

An inguinal testis is within the canicular space limited by the internal and external inguinal rings. Ideally, position would be precisely defined (Beltran-Brown & Villegas-Alvarez 1988), and this would seem especially important for horses since the inguinal canal might be 10 cm long. A s.c. testis usually is found in the femoral triangle, but ectopia of the vaginal process might place the testis at some distance or near a malformed scrotum. Imprecision in describing testis location typifies literature on mice or rats administered an agent, which might affect testis descent, and the uninformative ‘ectopic testis’ (i.e. abnormal location of testis), which is often used to describe location of a testis not within a normal scrotum or abdominal cavity. Wolf et al. (2000) provide an example of an adequate description.

Categorizing s.c. testes as inguinal, vice versa, or inguinoscrotal is common in cat, dog, horse and human literature. This is inappropriate, and for stallions the separate classification of inguinal and s.c. testes had...
been advocated by Genetzky (1984). Regardless, stallion testes rarely are s.c. (Cox et al., 1979, Rodgerson & Hanson 1997), but rather are within the inguinal canal per se. In humans, however, the majority of undescended testes apparently are ‘located in the groin’ or ‘near the neck of the scrotum or just outside the external inguinal ring’ (Hutson et al., 1992, 1997); i.e. s.c. Since it is imprecise to attribute both conditions to failures of ‘inguinoscrotal testis descent’ and different regulatory mechanisms are apparently involved, we use the term ‘transinguinal migration’ for the former and restrict the term ‘inguinoscrotal migration’ to the latter.

Since, there are two testes and at least three non-scrotal locations, a given cryptorchid male should be placed in one of six, if not 8–10, categories defined by a $2 \times 3–4$ matrix (2 sides, 3–4 combinations of testis and cauda epididymidis locations). Such complete information is rare. We have not found a data base pertaining to domestic, companion, or laboratory animals that provides information in adequate detail. The situation is further complicated because the age at examination can affect what is found. This is especially important in species where testes typically reach a scrotal location between 10 days before birth and 14 days after birth (horse, human, and pig; only then does the inguinal canal constrict) or 3–20 days after birth (mouse, rat, and rabbit; inguinal canal never constricts). Further, testes in an inguinal location at first examination might later be positioned permanently in the scrotum (late descent), and occasionally a scrotal testis might later be retracted permanently into the inguinal canal (‘retractile testis’ in human literature). Such migration is more common in horses, humans, or pigs than in cattle or sheep.

Structures involved in testis descent

The same structures are involved in testicular descent in common mammals (Fig. 1, bottom). Most important are the testis formed from an undifferentiated gonad; epididymal and deferent ducts formed from the mesonephric duct; mesonephros which degenerates; metanephros which becomes the kidney; cranial suspensory ligament of testis (cephalic ligament); gubernaculum (see below); vaginal process an evagination of the peritoneum (membrane lining abdominal cavity); cremaster muscle(s), which differs in shape among species; genitofemoral sensory nerve, with L1 to L2 ganglia; and scrotum. There are marked species differences with respect to relative timing of testis descent, but the only substantial difference in structure or process between most animals and rodents or rabbits is with respect to transinguinal testis migration. Because there are far more similarities than differences, we think a unitary approach has merit.

The mesonephros, mesonephric duct leading to the cloaca, and later the gonad, are within thin folds of peritoneum. As the gonad evolves, the fold of peritoneum covering the gonad evolves to a mesorchium suspending the gonad dorsally from the mesonephros, and as the cranial suspensory ligament (Fig. 1), which blends into the diaphragmatic ligament supporting the mesonephros. The cranial suspensory ligament is sexually dimorphic, becoming substantial in females, but not in male fetuses because it regresses during a critical time window (Gier & Marion 1970, van der Schoot & Emmen 1996, Hutson et al. 1997). Caudally, the peritoneum around the gonad continues as the thin caudal mesonephric sheath, which extends to the extreme caudal end of the coelom (abdominal cavity).

Early in development, the gubernaculum originates from mesenchymal cells among muscle fibers of the abdominal wall, grows under the peritoneal lining, and soon dominates the caudal mesonephric sheath. Thus, the gubernaculum extends from within the abdominal wall, under (ventral to) the mesonephric duct with which it fuses (where the cauda epididymidis later transitions to the deferent duct), and connects to the testis.

What is the gubernaculum?

The Latin word gubernaculum pertains to a ‘helm’ or a structure, which guides and was first applied to a reproductive structure by Hunter (1762) because he thought that it guided the testis to the scrotum. Later, he slightly modified his original description and wrote (Hunter 1786) ‘... which at present I shall call the ligament, or gubernaculum testis, because it connects the testis with the scrotum, and seems to direct its course through the rings of the abdominal muscles ... it is certainly vascular and fibrous, and the fibers run in the direction of the ligament itself, which is covered by the fibers of the cremaster or musculus testis, placed immediately behind the peritoneum.’ Clearly, Hunter stated that the cremaster muscle covers the gubernaculum and, hence, he considered them as separate structures.

Hunter (1762, 1786; cited text available on-line) recognized that the morphology of the cremaster muscle differed among species, and considered it to originate from the internal oblique muscle of the abdominal wall. The cremaster muscles are striated, and innervated by the genitofemoral nerve. We now know that the gubernaculum has collagen fibers, is rich in hyaluronic acid and glycosaminoglycans, and its cells proliferate during expansion and include some myoblasts. Hunter described (1762, 1786) what now is termed the vaginal process as a U-shaped evagination of peritoneum into, and later through, the abdominal wall around the gubernaculum. Hence, he probably considered the vaginal process and gubernaculum as separate structures.

As summarized previously, Hunter (1762, 1786) considered the gubernaculum as a ligament and distinguished it from the cremaster muscle and vaginal...
process. However, van der Schoot (1996) argued that gubernaculum be used as an encompassing term to include the gubernaculum per se and also the vaginal process and cremaster muscles; in our opinion contrary to Hunter. In most reports on rodents or rabbits, the cremaster muscles, but not the vaginal process, are considered part of the ‘gubernaculum cone’, sometimes referred to as the gubernaculum without distinction between mesenchymal and muscular elements. In reports pertaining to non-rodent species, distinction between the gubernaculum and cremaster muscle(s) is typical.

Distinction between the gubernaculum and cremaster muscle(s) is not a mere semantic problem. Failure to make the distinction prevents proper description of species differences in embryology or anatomy (e.g. mouse or rat versus bull, horse, human, or pig) or association of observed defects to possible etiological factors. We propose universal adoption of the term ‘gubernaculum’ as excluding the cremaster muscles or the vaginal process, although both penetrate the gubernaculum as it enlarges during the process of testis descent. The term ‘gubernacular–cremaster complex’ is proposed because it is more descriptive than ‘gubernacular cone’ favored by van der Schoot (1993, 1996). Distinct use of the term ‘gubernacular–cremaster complex’ facilitates consideration of structure–function relationships and cross-species comparisons. We hope others will be precise, use clearly defined terms, and adopt this terminology if they are not already using it.

The gubernaculum originates, in the inguinal area, as mesenchymal cells among fibers of the oblique muscles of the abdominal wall (Backhouse & Butler 1960, Gier & Marion 1969, 1970, Wensing 1986, 1988, Wensing & Colenbrander 1986, van der Schoot 1993, 1996). Soon the gubernaculum is evident as a broad-based bulge in the lower abdomen with a papilla invading the caudal mesonephric sheath (which is continuous with the lining of the abdominal cavity). A narrow portion of the gubernaculum soon dominates the remainder of the caudal mesonephric sheath and contacts the mesonephric duct and testis (Fig. 2A and D). These often are described as a gubernacular cord plus a gubernacular bulb, and they have functional differences. The portion of the gubernaculum initially within the abdominal wall is knob-like and gelatinous with collagen fibers. The upward-bulging papilla of gubernaculum is transitory in many animals (compare images for dog and bull in Fig. 2); the major (proper) portion of the gubernacular bulb seems to ‘settle’ into the abdominal wall, accompanied by the peritoneal covering. In rodents and rabbits, the major portion of the gubernacular bulb retains a conspicuous elongated-cone shape (Fig. 2E and F) until just before transinguinal testis migration. In any case, the gubernacular bulb grows and extends through the abdominal wall into the s.c. tissue. This is the extra-abdominal portion of the gubernaculum.

In most animals, the vaginal process is formed by the parietal peritoneum invading the underlying gubernaculum within the abdominal wall (Figs 2B and 3, top). The evagination starts shortly after formation of a testis, and takes the shape of an incomplete cylinder (incomplete because of a reflection continuous with the mesonephric sheath ultimately forming the mesorchium supporting the testis and deferent duct). The vaginal process divides the gubernacular bulb into three areas: proper, central to the cylindrical vaginal process and continuous with the gubernacular cord; vaginal, concentric and outside the vaginal process; and infravaginal, cup-shaped and between the invading peritoneum and distal tip. Downward invasion of the vaginal process, from the developing internal inguinal ring, through the gubernacular bulb continues after transinguinal testis migration, and extends into the developing extra-abdominal gubernaculum. The genitofemoral nerve (not shown) is carried downward with the gubernaculum and innervates the cremaster muscle. In rodents and rabbits, initial evagination of the vaginal process is apparent just before reshaping of the gubernacular–cremaster complex; the latter is central to transinguinal testis migration. This difference among species in transinguinal testis migration is discussed later.

A striated cremaster muscle(s) is formed by myoblasts, migrating from the muscles of the abdominal wall and/or differentiating from mesenchymal cells of the gubernaculum. In any case, the cremaster muscle(s) invades the vaginal portion of the gubernaculum (refer Fig. 2 in Backhouse & Butler 1960). The cremaster muscle is strip-like in companion and food-producing animals or humans, located on the lateral aspect of the developing vaginal process.

In rodents or rabbits, two cremaster muscles develop as concentric and conspicuous layers encompassing the proper portion of the gubernacular bulb (Fig. 2F). They are continuous with the inner oblique and transverse muscles of the abdominal wall (Wensing 1986, van der Schoot 1993, 1996, van der Schoot & Elger 1993, Shono et al. 1994b). This results in paired ‘gubernacular–cremaster complexes’ (Fig. 3, bottom). The gubernacular–cremaster complex includes the intra-abdominal gubernaculum and both cremaster muscles, but excludes the thin connection (gubernacular cord) extending to the testis, apparently devoid of muscle cells, and the extra-abdominal gubernaculum. The gubernacular bulb and cremaster muscle(s) have different roles during testis descent and later in adults.

Process of testis descent
Although there are good descriptions of morphologic changes during testis descent in common animals, there is a paucity of information on regulation of testis descent, or agents disrupting the process, other than experiments
with rodents and observations of humans. In our synthesis, we summarize what is known from model species, augmented with observations on larger animals. We urge study of detailed reviews (Hutson et al. 1992, 1997, Heyns & Hutson 1995, Ong et al. 2005) and especially Klonisch et al. (2004). Also refer Jost (1953) and van der Schoot & Emmen (1996).

**Abdominal testis translocation**

The endpoint is a testis positioned near the internal inguinal ring, often with the cauda epididymidis just within the inguinal canal. The process of abdominal testis translocation is one avoiding cranial displacement rather than substantial movement. The testis is anchored by the cranial suspensory ligament and the gubernaculum (Fig. 1). Initially, the gubernaculum is short and thin. The gubernaculum gradually expands and invades deeper into the abdominal musculature (Fig. 4). The extra-abdominal gubernaculum increases substantially in size, by both cell division and swelling, to provide an anchor (Gier & Marion 1970, Edwards et al. 2003). Presumably the above changes along with fetal growth exert continuous tension on the testis, via the gubernacular cord, while the cranial suspensory ligament weakens. Consequently, the testis is retained in the inguinal region during migration of other structures (e.g. kidney) cranially.

In cattle, the gubernaculum has developed sufficiently by GD 50 so that evagination of the peritoneum has started to form the vaginal process. Then between GD 62–65 and 90–96, the processes described previously are completed (Fig. 4). As abdominal testis translocation proceeds, the testis increases in size (especially in the stallion), accompanied by increased secretion of regulatory molecules. In most species (e.g. cattle, and horse), the vaginal process is carried downward as the gubernaculum grows.

Abdominal testis translocation is accomplished with little change in the distance between a testis and the scrotal area, although the extra-abdominal portion of the gubernaculum becomes longer (Fig. 5A; other data for...
cattle and pigs in Wensing & Colenbrander 1986), and the fetus grows away from this area. For cattle, the distance between the internal inguinal ring and testis remains approximately 1 cm until transinguinal migration (e.g. after GD 90 in Fig. 5A), whereas the distance between the internal inguinal ring and kidney becomes >2.5 cm by GD 95. Maximum distance between a testis and the future scrotum is at initiation of transinguinal migration, at GD 95–100 in.

Similarly in rodents and rabbits, the testis is held near the neck of the bladder, by the gubernaculum, as the abdominal cavity enlarges (Fig. 5B; Shono et al. 1994a).

Figure 3 Comparison of testis descent in a typical mammal, rodent, or rabbit (upper, pig; lower, rat). Left-most drawings illustrate status early during abdominal translocation of testes. Other views depict intermediate stages continuing through transinguinal migration of the testis. In all animals, the gubernaculum grows out from the abdominal wall as well as downward, and the vaginal process is formed by invasion of the peritoneal lining. The gubernaculum has a gubernacular cord and bulb; the latter has 2 regions, shown for the rat as: d, intra-abdominal; and e, extra-abdominal. In all species, the gubernacular bulb later is divided by the invading vaginal process into 3 portions: a, proper; b, vaginal; and c, intravaginal. In most animals, the gubernacular bulb and vaginal process extend deep into the abdominal wall early during abdominal translocation of a testis (e.g. GD 60 pig), and the cremaster muscle grows downward through the extra-abdominal gubernaculum, as a relatively narrow strip on 1 side of the vaginal process. Abdominal testis translocation is completed around GD 100 in the pig. In a rodent or rabbit, starting around GD 16 in the rat, the cremaster muscle envelops the intra-abdominal portion of the gubernacular bulb, forming an extensive dome-like “gubernacular-cremaster complex”. In these species, down growth of the vaginal process does not occur until just before the end of abdominal translocation, around day 21–22. In pigs, transinguinal migration of testes occurs around GD 103–108, by movement through an inguinal canal distended by the proper portion (a) of the gubernaculum. In rodents and rabbits, intermediate stages depict intussusception of the cremaster muscles around the proper portion of the gubernaculum (e.g. day 23–24 rat, equals PND 2–4), which occurs postnatally in rodents, followed by straightening of the muscles to complete transinguinal testis migration as the vaginal process grows. See text for details. For each species, the lower right drawing illustrates status at end of transinguinal migration (rat) or late during inguinoscrotal migration (pig) of testes. Drawings depict structures, and are not to scale within or among drawings. Drawings evolved from Elder et al. (1982), Wensing (1986, 1988), and Shono et al. (1994a), modified on basis of published plates and a consensus of literature.
By GD 16–17 in rats, GD 17 in mice, or GD 18–21 in rabbits, the gubernacula–cremaster complex has formed and assumed a conical or cylindrical shape protruding into the abdominal cavity, from the femoral triangle (Figs 2 and 3; Elder et al. 1982, van der Schoot 1993, van der Schoot & Elger 1993, Shono et al. 1996).

The gubernacular cord has shortened and the testes soon are below the neck of the bladder (Fig. 5B). By the end of abdominal testis translocation, the gubernacular cord has regressed, bringing the cauda epididymidis (already attached to the future vaginal process by virtue of attachment to the mesonephric sheath) against the cremaster muscle or the intra-abdominal gubernaculum per se (Elder et al. 1982, Shono et al. 1996).

Although rarely mentioned, inguinal canals apparently develop on postnatal day (PND) 1–2 in mice (Shono et al. 1996), prior to PND 5 in rats (Shono et al. 1994b), and by GD 28 in rabbits (van der Schoot 1993). Given that an inguinal canal must be formed by cylindrical downward growth of the peritoneal lining, this means that the base of the gubernacular–cremaster complex had been repositioned slightly below the plane of the abdominal wall as the rodent’s abdomen expanded, much like what had happened in a dog or bull by GD 32–34 or 45–52. Further, the developing vaginal process would initiate segregation of the vaginal and proper portions of the gubernacular bulb and demarcate the upper limit of the infravaginal gubernaculum (Fig. 3, bottom). In both rodents and rabbits, weight of the intra-abdominal gubernacular–cremaster...
complex increases five-fold from PND 0–6. Length of gestation ranges over several days (10–15%), and in many rat studies pups are removed surgically on GD 20 so that PND 1 or 2 might be prenatal in another study.

Taking the end of abdominal translocation as positioning of the testis close to the inguinal ring, and not change in the gubernaculum or birth as the endpoint, it is evident that the first phase of testis descent is completed around PND 0–1 in mice (Shono et al. 1994a, 1996) or PND 4–5 in rats (Wensing 1986, Shono et al. 1994b). Transinguinal migration of testes could not begin before these ages.

In all species, including rodents and rabbits, at the end of abdominal testis translocation, the testis is positioned near the internal inguinal ring, the cauda epididymis is within the inguinal canal (or poised to enter), the gubernaculum and vaginal process extend below the newly formed/forming inguinal canal (relatively short distances in rodents and rabbits), and the gubernaculum has both intra- and extra-abdominal regions (Fig. 3). In many species, this situation is maintained for some time, like a ‘pause’ between two separate processes. The genitofemoral nerve has been masculinized by the action of testosterone (Goh et al. 1994). The main force holding the testis low in the abdominal cavity is a considerably expanded gubernaculum.

Transinguinal testis migration

The endpoint is a testis, and epididymis, located just external to the inguinal canal or plane of the abdominal wall. During the pause before actual transinguinal migration, the gubernaculum bulb enlarges greatly (refer Fig. 7 in Gier & Marion 1970; also Figs 3 and 5) and dilates the inguinal canal to allow passage of the testis preceded by the cauda epididymis. To accommodate transinguinal migration of the testis, the cranial suspensory ligament remains only as a thin sheet, and structures contributing to the future spermatic cord lengthen substantially. In due course, the testis decreases in absolute size and as the gubernaculum bulb distends the inguinal canal sufficiently and the testis rapidly moves through (Figs 3, 6 and 7). The reduction in testis size is substantial, especially in horse fetuses, so it can pass through the inguinal canal. Incompatibility between testis size and diameter of the canal is considered by some, to be a contributing factor to abdominal cryptorchidism in stallions.

Transinguinal testis migration is slightly different in rodents and rabbits, for reasons presumably related to substantial development of the dome-shaped cremaster muscle. Dogma is that the gubernaculum–cremaster complex ‘everts’ as a herniation through the abdominal wall. We present a redistilled interpretation (Fig. 3, bottom) of what happens (Elder et al. 1982, Wensing 1986, van der Schoot & Elger 1993, Shono et al. 1994b, 2004, Lam et al. 1998). Central is the feature that the fetus or pup is growing far more rapidly than the gubernaculum–cremaster complex, which does not need to ‘evert’ to relocate the testis (Lam et al. 1998). As noted previously, the extra-abdominal gubernaculum extends into the inguinal canal or through the canal into s.c. tissue (Fig. 7D, also refer to Fig. 8 in Elder et al. 1982) (Rajfer 1980, Elder et al. 1982, Shono et al. 1996, 2004).

Near PND 2 in rabbits (Rajfer 1980, Elder et al. 1982) or PND 4–8 in rats (Lam et al. 1998), the cremaster muscle assumes a serpentine or ill-defined appearance, with a diameter (across the intra-abdominal gubernaculum) greater than that of the testis. With a relatively large portion of gubernaculum protruding along the external surface of the abdominal wall, reduction of the proper
intra-abdominal portion of gubernaculum, and growth of the pup, intussusception of the cylinder of cremaster muscle occurs (Elder et al. 1982) and, as it unfolds, it assumes a U-shape around the cauda epididymidis and testis (Fig. 3, bottom). This brings the cauda epididymidis and testis through the newly formed inguinal canal and outside the abdominal wall, with the cauda epididymidis still associated with the infravaginal portion of the gubernaculum. At this point, transinguinal testis migration has been completed, but the cauda epididymidis and testis remain at some distance from the bottom of the developing scrotum.

Actual passage of testes through the inguinal canal is thought to be rapid; a few days at most even in a large mammal. The gubernaculum *per se* probably has a passive role other than dilation of the inguinal canal (Wensing & Colenbrander 1986) and anchoring the cauda epididymidis with attached testis. The gubernacular cord might shorten slightly. The main forces moving a testis through the inguinal canal are thought to be downward pressure of viscera and peritoneal fluid on the testis (harbored within the gubernacular bulb), expansion of the vaginal process, and growth of the abdomen.

**Inguinoscrotal testis migration**

The endpoint is a testis, and epididymis, positioned normally in a scrotum typical of the species. Inguinoscrotal migration of a testis, from below the external inguinal ring to the final scrotal location, requires extension of the gubernacular bulb and enclosed vaginal process to the bottom of the scrotum, while the gubernacular cord does not elongate. In some species, the extra-abdominal gubernaculum might extend partway into the scrotal folds well before transinguinal testis migration (see above), but because of fetal growth both gubernaculum and vaginal process must grow in the proper direction to reach the bottom of the scrotal sac. In rodents and rabbits the extra-abdominal gubernaculum, with vaginal process, extend a relatively short distance subcutaneously when transinguinal testis migration is completed; they are not into the scrotum. In all species, extension of the vaginal process and gubernaculum over a substantial distance from the external inguinal ring is required to allow the gubernaculum to bring the cauda epididymidis and testis to their proper locations.

Inguinoscrotal testis migration involves growth of the gubernacular bulb and enclosed vaginal process in the proper direction to the bottom of the scrotum. Over time, the gubernaculum regresses to attach the external surface of the vaginal process, as the parietal vaginal tunic, to the scrotal wall (via formation of scrotal ligament) and the internal face to the cauda epididymidis (via formation of ligament of tail of epididymis). A remnant of the gubernacular cord attaches the cauda epididymidis to the testis (proper ligament of testis). Except in rodents and rabbits, the inguinal canal usually constricts to a very narrow passageway precluding herniation of viscera. As detailed below, the primary stimulus for inguinoscrotal migration of a testis is chemoattractant signals received by the tip of the gubernacular bulb.

**Scrotal development and directional guidance**

Scrotal folds develop early in fetal development. However, in some species (e.g. bull, horse, and human) they migrate a considerable distance to the
final location of the scrotum. This means that the vaginal process, gubernacular bulb, and epididymis along with testis must follow. The gubernacular bulb is not attached to the tissue within the scrotum, until final regression.

Directional guidance crucial for inguinoscrotal testis migration is important in all species. This apparently is provided by calcitonin gene-related peptide (CGRP) released from the genitofemoral nerve (sexually dimorphic, with androgen receptors in cell body) descending down with the developing gubernaculum and cremaster muscle. Testosterone stimulates production or release of CGRP, which is the chemoattractant that products of Insl3, Great, androgen receptor, and CGRP genes, and those involved in testosterone production, must be available during critical points in development; it was 20 years until Insl3 was identified. It is now known, based on data for multiple species, that products of Insl3, Great, androgen receptor, and CGRP genes, and those involved in testosterone production, must be available during critical points in development. We will focus on the roles of these molecules in enabling testis descent, but other factors, including intra-abdominal pressure, undoubtedly are involved.

Sequential control of testis descent: mechanisms and what might go wrong

There are a large number of genes and gene products involved in regulation of testis descent (Basrur & Basrur 2004, Klonisch et al. 2004). Baumans et al. (1983) hypothesized that a low molecular weight molecule in aqueous extracts of pig testes stimulated gubernaculum development; it was 20 years until Insl3 was identified. It is now known, based on data for multiple species, that products of Insl3, Great, androgen receptor, and CGRP genes, and those involved in testosterone production, must be available during critical points in development. We will focus on the roles of these molecules in enabling testis descent, but other factors, including intra-abdominal pressure, undoubtedly are involved.

Challenges in interpreting data

When considering regulation of testis descent, it is important to distinguish between what signal molecules impinge on target cells and, which actually are obligatory for normal development and function of a structure. How much of a given molecule actually is necessary, versus the amount typically available, is a second-level question only indirectly addressed in multi-dose studies. There are also strain differences in the response of a given tissue to administered hormone agonists or antagonists.

Understanding regulation of testis descent is challenging because of experimental need to provide or eliminate signals at specific points in the process, almost always, while targeting each of several male fetuses in a litter. In most experiments, after administration of an agent to a pregnant female, there is a non-uniform response among litters when evaluated after birth. Usually this is not commented on or is attributed to ‘unexplained variation’, and causes are not sought. Certainly, male members of a litter of rodents might differ by up to 2 days (11% in late gestation) in stage of development; hence, some might not be within the targeted window of exposure. It is possible that microenvironment provided by neighboring male or female fetuses, and position relative to the cervix, might affect development (Even & vom Saal 1991, Nonneman et al. 1992). Regardless of cause, complete reporting would include: tabulation of all phenotypes; gross appearance of each important structure, not just testes locations; recognition that litter and not male fetus is the experimental unit; and statistical consideration of the non-uniform responses of litters and pups within litters. Given the nature of published data, we have considered status of the majority of testes as evidence for or against the need for a given enabling molecule or factor.

Overview of regulatory mechanisms

Abdominal translocation of testes is dependent on Insl3 to stimulate growth of the gubernaculum to form an anchoring structure; testosterone is not required for completion of this phase. However, testosterone brings about masculinization of the genitofemoral nerve and starts to stimulate growth of the vaginal process and cremaster muscle, to allow completion of the third phase.

Transinguinal migration of testes is dependent on an inguinal canal expanded by the gubernaculum, during the first phase, and movement of an appropriately-sized testis through the inguinal canal by intra-abdominal pressure. Neither Insl3 nor testosterone is required for this phase.

Inguinoscrotal migration of testes is dependent on proper directional growth of the genitofemoral nerve. Normally, testosterone enhances secretion of CGRP from the genitofemoral nerve to provide direction to gubernaculum growth, expansion of the vaginal process.
concomitant with limited growth in the inguinal canal region to constrict the passageway, growth of the cremaster muscle, and regression of the gubernaculum. Testosterone and AMH apparently are not obligatory for thinning and elongation of the cranial suspensory ligament, as the abdominal cavity expands, or for expansion of the gubernaculum. The crucial function of testosterone is to masculinize the genitofemoral nerve early in embryogenesis, well before completion of abdominal translocation of testes or initiation of the last two phases of testis descent.

Studies using estrogenic and anti-androgenic molecules in cattle and pigs, as well as rabbits, rodents and humans, establish that there are different time windows when Insl3 or testosterone must be available to specific developing tissues (references provided below). During abdominal testis translocation, Insl3 and testosterone apparently are provided by Leydig cells to nearby tissues (possibly including nerve bodies of the genitofemoral nerve) via pathways not involving the general circulation. Initially, both hormones are produced under paracrine control or constitutively (Colenbrander et al. 1979, El-Gehani et al. 1998, Pakarinen et al. 2002, Zhang et al. 2004), although in humans stimulation of fetal Leydig cells by hCG from maternal blood (Themmen & Huhtaniemi 2000) might have a role. In rats there is a surge in intra-testicular concentration of testosterone around GD 19, which occurs before LH appearance in fetal blood after GD 20 (El-Gehani et al. 1998).

**Role of Insl3**

Initially, the gubernaculum is short and thin. In mice, Insl3 transcripts are abundant in testes from GD 13.5 through PND 6 (Nef et al. 2000). The gubernaculum (especially bulb) is rich in the Insl3-receptor Great, but other structures involved in testis descent lack the receptor. In all species studied, Insl3 is obligatory to bring about gradual expansion of the gubernaculum as it invades deeper into the abdominal musculature. This expansion is necessary to provide an anchor for normal abdominal translocation of testes. Thereafter, Insl3 probably has no further role in testis descent. Abdominal testis translocation is blocked by elimination of Insl3 or Great genes in mice (Klonisch et al. 2004), or administration of estrogenic molecules, to pregnant females, which bind to α-estradiol receptors present in fetal Leydig cells and suppress transcription of the Insl3 gene (Nef et al. 2000). In resulting young, testes are positioned in the abdominal cavity well above the internal inguinal ring, and gubernacular development is nil.

Over-expression of the aromatase gene can cause cryptorchidism (Klonisch et al. 2004), probably because it raises intra-testicular estradiol. The role of Hoxa10 gene products in testis descent is uncertain, but male null mice have a long, thin gubernaculum and abdominal testes positioned similarly to those in animals deprived of Insl3 stimulation (Rijli et al. 1995); lack of androgen probably is not involved because accessory sex glands and epididymis develop.

**Role of testosterone**

Assignment of a proper role to testosterone requires careful reading of primary literature, and going beyond the fact that ‘testis descent was blocked’ in a knockout animal, by anti-androgen with high affinity for androgen receptor (e.g. flutamide), or some other spontaneous or induced manipulation. Heynes & Hutson (1995) reviewed early literature. In respect to transinguinal migration of testes, studies with mouse or rat pups exposed to anti-androgen in utero must be interpreted with knowledge that birth typically occurs before initiation of transinguinal migration of testes in any male within a litter, and the endpoint in many studies is the anticipated day of birth (e.g. GD 19 for mice, GD 20 for rats). In studies where male mice or rats are reared after natural birth, administration of flutamide usually does not continue (e.g. Shono et al. 1994b, 1996). Hence, by PND 2 or 3, flutamide-induced blockage of tissue responses to endogenous androgen probably is minimal. If there is plasticity in when the developmental signal is received, target tissues might ‘make up’ for previous lack of testosterone stimulation. Recall that, at least in rats, the hypothalamic–pituitary–gonadal axis is operational by PND 1 (El-Gehani et al. 1998).

We separately examined if testosterone was obligatory for completion of each phase of testis descent. For reports where needed data were available, we calculated the percentage of testes completing a given phase of testis descent, as: abdominal translocation – (number of testes below bladder neck, near internal inguinal ring, or further in process)/(2 testes per animal studied); transinguinal migration – (number of testes below external inguinal ring)/(number of abdominal testes below the bladder neck or by internal inguinal ring); and inguinoscrotal migration – (number of testes properly in scrotum)/(number of testes below the external inguinal ring). Results are in Table 1. Importantly, for several studies summarized in Table 1, authors specifically state that flutamide affected differentiation of the mesonephric duct in most animals. There was no report for mice or rabbits with data allowing calculations.

**Abdominal translocation** was completed by > 89% of testes in nine trials with rats or pigs (Table 1) despite prenatal or early postnatal exposure to flutamide (typically injection of pregnant rats with 50 or 100 mg/kg per day). In respect to mice, there is evidence that completion of abdominal translocation of testes is delayed on GD 19 (birth), but not blocked, after exposure to flutamide on GD 14–16. Neonatal location of the testes in mice exposed to flutamide in utero (refer Fig. 6 in Shono et al. 1996) is similar to location of testes

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in mice with genetic agenesis of the pituitary gland (Pakarinen et al. 2002), Tim mice (Hutson 1986, Griffiths et al. 1993), or androgen-receptor knockout mice (Yeh et al. 2002, De Gendt et al. 2004, Notini et al. 2005; photos viewed via web). In all these studies, testes were found low in the abdominal cavity close to the internal inguinal ring, essentially as would be expected at completion of abdominal translocation. Some papers noted that development of the gubernaculum and/or vaginal process was subnormal. In two compilations of humans with severe androgen insensitivity syndrome, 90% of 32 testes were below the internal inguinal ring (Hutson 1986) and 46% of 176 testes were outside the abdominal cavity (Barthold et al. 2000). Thus, in mice exposed to flutamide or mice and humans not expressing normal androgen receptor, abdominal translocation is completed by the majority of testes.

Assuming that flutamide blocked binding of testosterone to androgen receptors in all rat or pig fetuses, testosterone-induced mechanisms are not obligatory for abdominal translocation of testes in these species, even though testosterone might be available to tissues containing androgen receptor. Hence, testosterone is not necessary to regress the cranial suspensory ligament so that testes can remain near the neck of the bladder. These conclusions are consistent with Hutson et al. (1997).

Transinguinal migration was completed by >95% of testes in rat pups or pig fetuses exposed to flutamide (Table 1). Clearly, exposure to flutamide during late pregnancy did not block this phase of testis descent. Most of these studies did not directly address the question whether testosterone is necessary during transinguinal migration of testes. However, data were available for rats exposed to flutamide from GD 12 to PND 27 or administered flutamide by injection on PND 1–14 (listed under inguinoscrotal migration in Table 1); all testes completed transinguinal migration and were found in the scrotum at 16–20 week of age. Hence, transinguinal migration of testes occurs in rat pups, at the normal time or with slight delay, with testosterone-

**Table 1** Impact of prenatal or postnatal exposure to flutamide on completion rate through each phase of testis descent.

<table>
<thead>
<tr>
<th>Time of flutamide exposure species (Referencea)</th>
<th>Age examined</th>
<th>Completion rate (%)</th>
<th>Number testes entering</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal translocation of testes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat 5 GD paradigms (6)</td>
<td>PND 28</td>
<td>97</td>
<td>186</td>
</tr>
<tr>
<td>Rat GD 10–20 (2)</td>
<td>Adult</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Rat GD 12–21 (4)</td>
<td>Adult</td>
<td>98</td>
<td>44</td>
</tr>
<tr>
<td>Rat GD 16–19 (5)</td>
<td>GD 20</td>
<td>100</td>
<td>56</td>
</tr>
<tr>
<td>Rat GD 16–19 (5)</td>
<td>PND 5</td>
<td>100</td>
<td>46</td>
</tr>
<tr>
<td>Rat GD 16–19 (5)</td>
<td>PND 30–35</td>
<td>89</td>
<td>46</td>
</tr>
<tr>
<td>Rat GD 16–22 (1)</td>
<td>PND 30</td>
<td>89</td>
<td>18</td>
</tr>
<tr>
<td>Pig GD 65–113 (1)</td>
<td>GD 114</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>Pig 5 PND paradigms (2)</td>
<td>GD 110</td>
<td>95</td>
<td>180</td>
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<td>Transinguinal migration of testes</td>
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<td></td>
<td></td>
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<tr>
<td>Rat 5 GD paradigms (6)</td>
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<td>180</td>
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<tr>
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<td>100</td>
<td>43</td>
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<tr>
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<td>12</td>
</tr>
<tr>
<td>Pig GD 65–113 (1)</td>
<td>GD 114</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>Pig 5 PND paradigms (3)</td>
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<td>Inguinoscrotal migration of testes</td>
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<td>Rat GD 12–16 (6)</td>
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</tr>
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<td>Rat GD 21 – PND 7 (6)</td>
<td>PND 21</td>
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<td>26</td>
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<tr>
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<td>Adult</td>
<td>100</td>
<td>12</td>
</tr>
<tr>
<td>Rat PND 1–27 (7)</td>
<td>PND 28</td>
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<td>60</td>
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<tr>
<td>Pig GD 35–60 (3)</td>
<td>GD 110</td>
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<td>40</td>
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<td>66</td>
<td>35</td>
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<tr>
<td>Pig GD 65–113 (1)</td>
<td>GD 114</td>
<td>45</td>
<td>20</td>
</tr>
<tr>
<td>Pig GD 75–84 (3)</td>
<td>GD 110</td>
<td>30</td>
<td>43</td>
</tr>
<tr>
<td>Pig GD 85–100 (3)</td>
<td>GD 110</td>
<td>33</td>
<td>30</td>
</tr>
</tbody>
</table>

Flutamide blocks binding of testosterone to androgen receptor.

References: (1) Barthold et al. (1996); (2) Kassim et al. (1997); (3) McMahon et al. (1995); (4) Mylchreest et al. (1999); (5) Shono et al. (1994b); (6) Spencer et al. (1991); (7) Spencer et al. (1993).
binding blocked during gestation or testosterone-binding blocked during the postnatal interval when transinguinal migration actually occurs.

In pigs, testis descent usually is completed by birth on GD 110–114. For pig fetuses exposed in utero to flutamide using six paradigms (Table 1), 100% of testes had passed through the external inguinal ring when examined on GD 110 or GD 114. Further, fetal decapitation on GD 42 did not affect testis descent in 10 of 12 piglets examined on GD 90–113 (Colenbrander et al. 1979). Pituitary input was not necessary to provide sufficient testosterone secretion to enable testis descent. This is consistent with the conclusion that impairment of the hypothalamic–pituitary–testis axis (i.e. insufficient testosterone) does not preclude descent of human testes through the inguinal canal (Hadžiselimović et al. 1984). Collectively, except for postnatally castrated rabbits (see below), cited studies and other data show that testosterone must have minimal if any importance for transinguinal migration of testes.

Elder et al. (1982) studied rabbits orchiectomized on PND 1 and, at examination on PND 14, found that striated muscle of the cremaster muscle was poorly developed and mesenchyme of the gubernaculum had undergone ‘fatty replacement’. Injection of dihydrotestosterone prevented this regression and enabled intussusception of the cremaster muscles, a pivotal step in transinguinal migration that had there been testes.

What changes to bring about transinguinal migration of testes? We have minimized the role of testosterone as a factor facilitating transinguinal migration of a testis (Table 1), via actions such as stimulating reduction in size of the gubernaculum, and attributed action of Insl3 to the abdominal translocation phase. We assume, as have others (Wensing 1988, Hutson et al. 1997), that intra-abdominal pressure exerts sufficient force on a testis, provided it is sufficiently reduced in size, to push it against the gubernaculum pre-positioned in, and dilating, the inguinal canal. This pressure rapidly moves the testis into a s.c. location immediately below the external inguinal ring.

**Inguinoscrotal migration** was completed by 100% of testes in rats exposed to flutamide just before or during the time when inguinoscrotal migration actually occurs postnatally (Table 1). However, when flutamide was administered during a window spanning GD 15.5–18, in five out of seven studies only 53–69% of testes completed inguinoscrotal migration (83 and 87% completion in two studies); the most dramatic effect of flutamide with rats anywhere in Table 1. This is strong evidence that inguinoscrotal migration of testes requires availability of testosterone before, but not during, this phase of testis descent.

In pigs, inguinoscrotal migration of testes starts near GD 100–110, and is completed by or shortly after birth (Gier & Marion 1970). McMahon et al. (1995) found blockage in most piglets exposed prenatally to flutamide on GD 75–84 or GD 85–100 (Table 1); only 30–33% of testes completed inguinoscrotal migration. This is in contrast to a 66–78% completion rate in piglets exposed to flutamide on GD 35–60 or 61–74. Unfortunately, McMahon et al. (1995) did not target GD 95–100 in their trials. Apparently, testosterone is very important to enable inguinoscrotal migration of testes in pigs, but as in rats must be available to target tissues before initiation of the event after GD 100; i.e. during GD 80–100. Since exposure of pig fetuses in the GD 65–113 group encompassed the critical GD 80–100 period, blockage during the latter interval apparently was effective for 55% of testes (based on 45% completion rate, Table 1). Interestingly, on GD 80–100 concentrations of testosterone in serum from fetal pigs apparently is low (<0.4 ng/ml; Wensing & Colenbrander 1986). The rat and pig data lead to the same conclusion; testosterone is not necessary during inguinoscrotal testis migration, but must be available before initiation of the event.

Inguinoscrotal testis migration is blocked in null-mice lacking GnrH-promoter, GnrH, LH-receptor, or Tfm genes and, hence, the drive for testosterone synthesis in Leydig cells is presumed to have minimal constitutive secretory capacity (Hutson et al. 1997, El-Gehani et al. 1998, Klonisch et al. 2004). In 80% of cryptorchid boys with failure of inguinoscrotal testis migration (testis found below the external inguinal ring), the main etiological factor was impairment of the hypothalamic–pituitary–testis axis (Hadžiselimović et al. 1984). In such individuals, the problem likely was not caused by lack of testosterone exclusively during inguinoscrotal testis migration. Rather, the problem likely was caused by lack of testosterone coincident with the initial phase of testis descent, when it very rarely impacts abdominal translocation of testes, just as in rats and pigs.

The crucial role of testosterone apparently is masculinization of the genitofemoral nerve, during the window in fetal development when flutamide was most effective (Table 1). Actions of testosterone on other target tissues cannot be excluded, but any such action(s) is not crucial for completion of testis descent in rats or pigs, and probably humans. The genitofemoral nerve secretes CGRP, which binds to receptors in the growing tip of the gubernaculum bulb to stimulate cell proliferation and provide directional guidance for expansion (Hutson & Hasthorpe 2005, Ng et al. 2005). Idiopathic failure or flutamide-blockage of early masculinization of the genitofemoral nerve would reduce secretion of CGRP even if the nerve later was stimulated by testosterone (if available). This is consistent with the observed separation in time of when flutamide exerts an effect (GD 16–18 in rat) on testis descent and when the effect manifests (after PND 4 in rat). As discussed previously, similar separation in time was evident in pig data. Although masculinization of the genitofemoral nerve apparently is the ‘choke point’ for testosterone in testis descent, this does not mean that testosterone does not

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stimulate other changes; only that the other changes usually can be overcome.

Testosterone also targets the vaginal process to stimulate changes needed to constrict/close the inguinal canal (Barthold et al. 2000; except in rodents and rabbits) and the cremaster muscles to stimulate their growth (at least in rodents or rabbits). The most important regressive effect of testosterone is to drive changes in molecular structure of the gubernaculum bulb and the structure’s virtual elimination leaving a short ligament (McMahon et al. 1995, Barthold et al. 2000). In dogs, orchioctomy on PND 1, after full development of the gubernaculum, prevented regression of the gubernaculum; however, gubernacular regression occurred in castrated pups administered testosterone (Baumans et al. 1983). A non-obligatory action to induce final regression of the cranial suspensory ligament cannot be excluded for testosterone.

What might cause cryptorchidism?

From the previous discussion, it is logical to conclude that the primary defect causing failure of testis descent lies within the testis per se. It failed to produce adequate amounts of Insl3 and/or testosterone when needed. The testis controls its own fate, although defects in other structures or processes involved in testis descent can occur. Aberrant expression of Insl3 and testosterone receptors could cause defects with a similar phenotype. One might be tempted to conclude that cryptorchidism frequently was associated with a detectable change in sequence for one of the ‘important’ genes, or in factors regulating their expression in the testis. However, comprehensive analyses of gene sequences in cryptorchid men revealed that Insl3 or Great genes were aberrant in only 3–5% of such individuals (Ferlin et al. 2003, Roh et al. 2003, Klonisch et al. 2004) and aberrant androgen receptor or estrogen receptor genes in <16% of cryptorchid men (Garolla et al. 2005, Yoshida et al. 2005). Given that most cryptorchid human testes are in a s.c. location (Hutson et al. 1992, 1997), defects in the pathway for testosterone synthesis should be scrutinized. Gene sequence studies apparently have not been undertaken with food-producing or companion animals.

Comparison of timing of testis descent

Comparisons of testis descent among species are facilitated by expressing timing of events as a percentage of gestation length for a given species. Commitment of indifferent gonads to be testes is a triggering event (e.g. programming of testicular cells and secretion of Insl3 and testosterone). In many species this occurs during the first third of gestation, but it occurs later in dogs and especially rodents and rabbits (Fig. 8). Abdominal translocation of the testis is initiated as fetal testicular cells provide tropic factors and extends over an interval differing greatly among species. In all species, abdominal translocation is the longest phase of testis descent, except for dogs in which inguinoscrotal migration occurs over an interval of similar length. Generally, there is a ‘pause’ during which testes remain at the internal inguinal ring. The time-point when transinguinal testis migration is initiated ranges widely among species, but in all species transit of testes through the inguinal canal is rapid, requiring <2–4 days. Inguinoscrotal migration of a testis is a relatively short process in many species, but can be prolonged for >2 months in horses or humans. Typically, final positioning of testes into the scrotum occurs before 50% of gestation in cattle; late in gestation in humans, horses, or pigs; or 3–14 days after birth in rodents and rabbits.

Incidence and nature of cryptorchidism

Unfortunately, there is a tendency for researchers and clinicians to be imprecise in recording and reporting exactly where the testis(es) of a cryptorchid animal was located. Presumably this results from lack of awareness, rather than a sloppy approach to research or clinical medicine. Regardless of the reason, imprecision in data often precludes meaningful deductions on possible causes of the problem in a given male.

Published data on prevalence of cryptorchidism are summarized in Table 2. Prevalence apparently is <5% in most species and breeds/lines; cryptorchidism might be more common in pigs. Anecdotal impressions from cattle and pig breeders suggest that incidence of cryptorchidism has not changed substantially over the past 30 years, but there are no valid estimates of current prevalence. It is evident (Table 2) that for all species unilateral cryptorchidism is far more common than bilateral cryptorchidism, except for one report for dogs and a unique situation in deer (next paragraph). However, the location of undescended testes apparently differs among species. For horses, in most reports a majority of retained testes are stated to be in the abdominal cavity. For humans, abdominal retention is unusual and most testes are just outside the external inguinal ring or near the neck of the scrotum; i.e. s.c. In humans, perhaps two-thirds of cases self-correct within 3 months, with descent after 3 months unlikely (Hutson et al. 1997, Barthold & Gonzalez 2003). This also is reported with dogs and horses.

Sitka Black-Tailed Deer (SBTD) were placed on/near Kodiak Island, Alaska, in 1924–1934, when 25 founder animals were transplanted from several areas in southeast Alaska. Their range now covers the entire Kodiak Archipelago. Recently, we reported (Veeramachaneni et al. 2006; values below include recent unpublished data) that on the low-lying Aliulik Peninsula (~14X 50 km) of Kodiak Island, 109 out of 161 SBTD shot by hunters, and carefully examined, were bilateral
cryptorchid (BCO) and 12 were unilateral cryptorchid (UCO). All retained testes were in the abdominal cavity, typically part way between the kidney and internal inguinal ring and associated with a thin, underdeveloped gubernaculum. This 75% incidence of cryptorchidism is extraordinary as is the 90% predominance of bilateral cryptorchidism (Table 2). We are unaware of any other non-experimental population containing 75% cryptorchid animals. A high proportion of these animals had testicular neoplasia and abnormal antlers. Elsewhere on the Kodiak Archipelago, 12% of 78 SBTD examined were cryptorchid (three UCO and six BCO).

With evidence then available, Veeramachaneni et al. (2006) considered alternative potential causes for the extraordinary prevalence of cryptorchidism among SBTD on the Aliulik Peninsula. On theoretical grounds they discounted the plausibility of a classic mutation in a gene(s) essential for testes descent with marked concentration via inbreeding. Veeramachaneni et al. (2006) hypothesized that it was most likely that this testis-antler dysgenesis resulted from continuing exposure of pregnant females to an estrogenic endocrine disruptor agent, thereby blocking transabdominal descent of fetal testes, transforming testicular cells, and affecting development of the primordial antler pedicles in some males. They recognized that, alternatively, the phenomenon might be a residual epigenetic effect altering/blocking expression of Insl3, Great, and/or other genes, consequent to historic exposure of a founder(s) to an estrogenic EDA. Additional research in both areas is commencing.

Elaboration of cryptorchidism

Dogma is that cryptorchidism is a heritable condition, despite the lack of a strong basis for this thought. It is also possible that at least some cases of cryptorchidism in common animals result from fetal exposure to endocrine disruptor agent. Despite limited size of studies, there is no doubt that brother–sister matings of dogs or pigs over several generations increases incidence of cryptorchidism (Cox et al. 1978, Mikami & Fredeen 1979, McPhee & Buckley 1984). There also is anecdotal opinion that cryptorchidism is familial in some sire lines of horses and pigs.

There has been no recent breeding study to establish if cryptorchidism is a heritable condition. There probably

**Figure 8** Approximate timing of events involved in testis descent in common mammals. For each species, consensus gestational age when events were detectable in fetuses or newborn were scaled relative to gestation length. The exactitude of demarcations in this figure mask the variation and uncertainty in when an event starts or is completed due to differences in development among individual fetuses, error in establishing gestational age, the dissection process, investigator interpretation, and how data are presented in publication. Although imperfect, this approach provides far better comparisons across species than use of absolute time for each species. Testis formation and differentiation (Fig. 1) occurs during 2–10 days immediately proximate to the bars for each species, and would be when an endocrine disruptor agent could irrevocably affect a testis. Time required for abdominal translocation of testes ranges from ~16% in cattle to ~57% horses, and appears to be independent of the relative length of the “pause” (orange) between the abdominal translocation and transingual migration phases of testis descent. The relatively long interval shown for transingunal testis migration for humans and horses probably reflect subject/examiner variation rather than a truly long process in a given individual, although the horse inguinal canal is longer than in other species shown. In rabbits and rodents, changes in the structures during transingunal migration of testes are slightly different than in other species, and requires intussusception of the cylindrical cremaster muscle around the proper portion of the gubernaculum (Fig. 3). Length of gestation, in days, was assumed to be: cattle, 281; mule deer, 203; human, 268; horse, 337; pig, 114; dog, 60; rabbit, 31; and mouse, 20. Compiled from the literature. Note: Klonisch et al. (2004) present a comparative view of testis descent in their Fig. 6, but relative timing of abdominal testis translocation (and also transingunal plus inguinoscrotal migration) for some species is quite different from that herein. Presumably, this reflects different interpretations of published descriptions of fetal dissections in respect to our definitions of abdominal translocation, transingunal migration, and inguinoscrotal migration of testes vs their definitions of transabdominal and inguinoscrotal testis descent, plus deciding when testes arrive in their final position.
are two reasons. First, mode of inheritance and penetrance are difficult to establish in planned studies, and essentially impossible to deduce accurately from retrospective analysis. Sire of a cryptorchid male can be assumed to be heterozygous for the genes causing the disease, but many matings would be needed to determine if a given dam was homozygous or heterozygous for each gene involved. Establishing that an animal is a non-carrier for each gene is even more difficult. Rehfeld (1971) estimated that >40 male offspring would have to be studied at >6 months of age to establish that a dam probably was a non-carrier. Second is economic importance; knowledge of the genetics of cryptorchidism would not alter conventional medical practice for humans.

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