Anim. Reprod., v.3, n.2, p.108-120, April/June. 2006

Cryptorchidism and associated problems in animals[1]

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Abstract

Cryptorchidism is not a single disease; it apparently results from failure of one of at least two functions involving products of Leydig cells. Cryptorchidism is symptomatic of underlying testis dysgenesis. Precise terminology is important to distinguish and understand three phases of testis descent. Abdominal translocation involves holding a testis near the internal inguinal ring plus slight downward migration, via an enlarged gubernaculum (Insl3 obligatory) as the abdominal cavity expands away. Concurrently, a cylindrical evagination of peritoneum invades the gubernaculum, cremaster muscle(s) develop within the gubernaculum, and the cephalic ligament regresses (testosterone not obligatory). Transinguinal migration moves the testis through the abdominal wall, via the inguinal canal distended by the gubernaculum. Inguinoscrotal migration involves subcutaneous movement of gubernaculum, vaginal process, epididymis, and testis to proper positions in the scrotum. Directional guidance is provided by chemoattractant calcitonin gene-related peptide, secreted by the genitofemoral nerve (testosterone dependent). Probably incidence of cryptorchidism is higher in companion animals or pigs than in cattle or sheep. In dogs and horses, a retained testis most commonly is abdominal. In horses, but not other species, retention of testes within the inguinal canal is common. In humans, subcutaneous testes predominate. Overall, cryptorchidism usually is unilateral; scrotal testes in unilateral cryptorchid males often produce fewer than normal numbers of sperm, with an increased percentage of abnormal sperm. Non-cryptorchid siblings might manifest similar testicular dysgenesis. Although risk of tumors is greater for cryptorchid males, non-cryptorchid males also develop testis tumors. Germ cell tumors are most common in dogs and horses. Leydig or Sertoli cell tumors are not unusual in dogs. Testis tumors rarely are reported for cattle, pigs, sheep, and rabbits; in cattle Leydig cell tumors are twice as common as Sertoli cell tumors. Producers and veterinarians should recognize that inadvertent exposure of a pregnant dam to estrogenic or anti-androgenic agents could result in testicular dysgenesis.

Keywords: cryptorchidism, testis descent, testis dysgenesis, testis tumors, terminology.

Introduction

Cryptorchidism is failure of one or both testes to be positioned in the scrotum at the time normal for a species. Typically, cryptorchidism is detected at birth or shortly thereafter. Postpubertal abnormalities associated with cryptorchidism (e.g., testis tumors, atypical concentrations of reproductive hormones, altered spermatogenesis in scrotal testis,) are not caused by elevated temperature of an abdominal location (Veeramachaneni, 2006), but as a delayed manifestation of testis dysgenesis. Such underlying testis dysgenesis of fetal origin might be evident in males who are not cryptorchid. The concept of a generalized testicular dysgenesis syndrome (TDS) is considered by Sharpe in this volume (also Skakkebaek *et al*., 1998, 2001; Rajpert-De Meyts, 2006; Sharpe, 2006).

Early reports on cryptorchidism (e.g., de Graaf, 1668) provided evidence of two or more diseases, because undescended testes are not located at a common non-scrotal site. Nevertheless, the general perception had been that cryptorchidism is 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. However, 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 and Woodhouse, 1977; Rothschild *et al*, 1988) and dogs (Cox *et al*, 1978; Nielen *et al*., 2001); also data for men (Czeizel *et al*., 1981). It is evident that abnormalities in >20 genes are associated with human cryptorchidism (Klonisch *et al*., 2004) and, currently it is accepted that cryptorchidism has many causes including genetic, epigenetic, and environmental components.

Terminology

For reasons detailed elsewhere (Amann and Veeramachaneni, 2007), certain traditional nomenclature does not adequately describe where a testis was found or when in the process of testis descent the normal process went wrong. Hence, this review defines and utilizes precise terms and nomenclature equally applicable to companion, food-producing, or wild animals; rodents or rabbits; and humans.

Testes are found in one of four general locations: abdominal cavity, inguinal canal,

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[1]Conference paper. International Symposium on Animal Biology of Reproduction, Nov. 15-18, 2006, Belo Horizonte, Brazil.
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subcutaneous, or scrotal. The distinction between inguinal and subcutaneous locations is important.

An *abdominal testis* is within the abdominal cavity, typically between the kidney and bladder or near the internal inguinal ring. Distinction between proximal vs near inguinal locations is important because it might point to underlying cause.

An *inguinal testis* is within the space limited by the internal and external inguinal rings. At least for horses (inguinal canal might be 10 cm long), position should be precisely defined (Beltran-Brown and Villegas-Alvarez, 1988).

A *subcutaneous 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. Unfortunately, imprecision in defining 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) often is used to describe location of a testis not within a normal scrotum or within the abdominal cavity.

A *scrotal testis* is found, and remains, in a scrotum located at the site typical for that species. However, in rodents and rabbits testes can move in and out from the lower abdominal cavity throughout life, as the inguinal canal never constricts. Occasionally, a scrotal testis might later be retracted permanently into the inguinal canal (retractile testis) or a testis initially deemed to be inguinal or subcutaneous might later be positioned permanently in the scrotum (late descent). Such migration is more common in horses, humans, or pigs than in cattle or sheep.

To stipulate discrete phases in the overall process of testis descent, three terms are needed since there are four general locations (see above). The following terms are descriptive of the process and are useful with any species.

Abdominal translocation of the testis reflects what happens during the first phase. At the end of this phase, the testis is positioned at the inner inguinal ring (ready to enter), with the cauda epididymidis just within the inguinal canal. The absolute distance between a testis and inguinal area changes little during this phase. Rather, the testes "stay put" as the fetus grows and the distance between locations of testes and kidneys widens by slight "downward" relocation to the developing inner inguinal ring (Wensing, 1968; Shono *et al*., 1994a). Hence, the widely used "abdominal descent" overemphasizes what occurs.

Transinguinal migration of the testis pertains to movement through the abdominal wall, from an abdominal to a subcutaneous location. This is a rapid process.

Inguinoscrotal migration of the testis describes the third phase. It covers the quest of the gubernaculum for the scrotum, which can be rather distant from the external inguinal ring, and consequent movement of the attached cauda epididymidis and testis to proper positions in the scrotum.

What went wrong during testis descent, in a simple sense, can be deduced from observed testis location and knowledge of the process. Three terms are needed, but unfortunately and illogically some authors have combined the latter two, without differentiation.

An *abdominal testis* reflects failure to initiate and complete abdominal translocation of the testis, so the testis is not poised near the internal inguinal ring, but rather near the bladder or part way between the inguinal area and kidney.

An *inguinal testis* reflects failure to initiate and complete transinguinal migration of a testis.

A *subcutaneous testis* reflects failure to initiate and complete inguinoscrotal migration of a cauda epididymidis and testis, from outside the inguinal canal to their final destination in the scrotum.

In horses, many retained testes are within the inguinal canal per se and few are subcutaneous (Cox *et al*., 1979; Rodgerson and Hansen, 1997), but testes which actually are subcutaneous often are classified inappropriately as inguinal (Genetzky, 1984). In humans, however, most undescended testes are subcutaneous in the groin, just outside the external inguinal ring, or near the neck of the scrotum (Hutson *et al*., 1992, 1997). It is imprecise to attribute both inguinal and subcutaneous testes to failure of "inguinoscrotal testis descent"; as discussed below, different regulating mechanisms likely are involved.

Testis descent

To present an overview applicable to common species ranging from mice and rabbits to bulls or horses, this compilation reinterprets some information in older publications. This is done with benefit of recent information and without discounting underlying observations in classic papers based on many dissections. Literature was cited extensively and concepts were discussed in Amann and Veeramachaneni (2007), which should be consulted. Here the aim is ease of comprehension.

Structures involved

The crucial starting point is differentiation of an indifferent gonad to a testis (Rajpert-De Meyts, 2006; Sharpe, 2006; Amann and Veeramachaneni, 2007). In brief, primordial germ cells (PGCs) migrate from the hind gut to the gonadal ridge. Then, in the male, mesenchymal cells move into the developing gonad, proliferate, surround the PGCs, and differentiate into fetal Sertoli cells. Shortly afterwards other cells from the mesonephros arrive (later become peritubular myoid cells) to organize nests of fetal Sertoli cells plus PGCs into seminiferous cords. Finally, other mesenchymal cells migrate into spaces among the seminiferous cords and differentiate into fetal Leydig cells. The interval between entrance of PGCs into the

indifferent gonad and differentiation of the gonad to a functional testis is <14 days. This happens near gestational day 14, 33, 35, 40, and 41 in the mouse, dog, pig, horse and bull. Within 2-3 days after arrival, fetal Leydig cells achieve maximum production of testosterone (as a fetal structure, on a per gram basis), and probably insulin-like peptide 3 (Insl3). Initially, testosterone is produced constitutively, but later GnRH and LH come into play to regulate the process.

As the testis is formed, a thin fold of peritoneum covering the gonad evolves as the mesorchium to suspend the gonad dorsally from the mesonephros. The same fold continues cranially as the cephalic ligament (cranial suspensory ligament) and caudally as the posterior gonadal ligament (epididymal ligament; Fig. 1A). The gubernaculum originates early in development from mesenchymal cells among muscle fibers of the abdominal wall, grows into the peritoneal fold forming the posterior gonadal ligament, and soon dominates the fold caudal to where it fuses to the mesonephric duct. The demarcation between the gubernaculum and posterior gonadal ligament is where the cauda epididymidis later transitions to the deferent duct. Scrotal swellings become evident under the skin, although they are not in the final location for the scrotum.

Hunter (1762) coined the term gubernaculum and described its structure. He slightly modified his description and then (Hunter, 1786) wrote "... 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." We now know that the gubernaculum is rich in hyaluronic acid, glycosaminoglycans, and collagen; it often is described as gelatinous although it has collagen fibers and the cells proliferate during expansion.

Clearly, Hunter stated that the cremaster muscle covers the gubernaculum and, hence, considered them as separate structures (but see van der Schoot, 1996; Amann and Veeramachaneni, 2007 for further details). This distinction between the gubernaculum and cremaster muscle(s) is unequivocal in reports pertaining to non-rodent species, but in most reports on rodents or rabbits the cremaster muscles are considered as part of the gubernaculum. This inclusive usage hampers ascribing observations to mechanistic problems (e.g., development of the gubernaculum per se is affected by lack of Insl3, cremaster muscles are not affected by Insl3, development of the gubernaculum per se is not affected by anti-androgen, cremaster muscles are affected by anti-androgen). Herein, the term "gubernaculum" excludes the cremaster muscles or the vaginal process. Formation of the gubernaculum and cremaster muscles, and differences between rodents and rabbits or other species, are detailed in Amann and Veeramachaneni (2007).

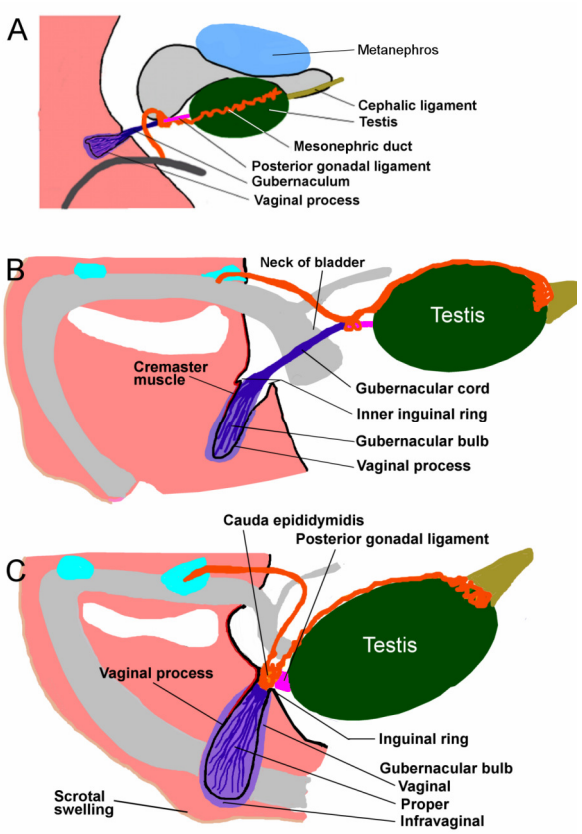

Figure 1. Abdominal translocation of the testes in a typical animal, the horse. The testis is formed at gestational day (GD) ~40 (not shown). **A**, by GD 55 the fetus is developing and abdominal translocation of the testis has started. Note that the gubernaculum extends from within the abdominal wall to the mesonephric duct, fusing with it where the cauda epididymidis later will develop and continue as the defererent duct. The vaginal process has started to invade the gubernaculum. **B**, a view at GD 75 shows that the testis remained near the neck of the bladder although the fetus grew. The inguinal ring is evident, and the stripe-like cremaster muscle is forming against one surface of the cylindrical vaginal process. **C**, at completion of abdominal translocation near GD 175. The gubernacular cord is fully regressed, and the gubernacular bulb has extended far through the inguinal canal, the cauda epididymidis is within the inguinal canal, and the testis is positioned at the inner inguinal ring. Relative size of the testis is much greater in horses than other species. Not to scale. Based on Bergin *et al.* (1970) and other literature.

The vaginal process is formed by the parietal peritoneum invading the underlying gubernaculum within the abdominal wall to form the vaginal process (Fig. 1). In most species, evagination starts early in gestation, shortly after formation of a testis and long before completion of abdominal translocation of testes.

The vaginal process divides the gubernacular bulb into three areas (labeled in Fig. 1C): 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. In rodents and rabbits, however, initial evagination of the vaginal process occurs neonatally, just before completion of abdominal testis translocation discussed below.

A striated cremaster muscle(s) is formed by myoblasts, migrating from the muscles of the abdominal wall and/or differentiating from mesenchymal cells in the gubernaculum. The cremaster muscle is stripe-like in companion and food-producing animals, or humans, and invades the vaginal portion of the gubernaculum on the lateral aspect of the developing vaginal process. In rodents or rabbits, concentric cremaster muscles develop and encompass the proper portion of the gubernacular bulb. This results in formation of a "gubernacular-cremaster complex", one on each side (Fig 2A). The gubernacular-cremaster complex includes the intra-abdominal gubernaculum and both cremaster muscles, but excludes the thin connection (gubernacular cord) extending to the mesonephric duct and the extra-abdominal gubernaculum. We recommend and use the term gubernacular-cremaster complex, rather than "gubernacular cones" commonly used in literature on rodents and favored by van der Schoot (1993, 1996), because this makes clear that the complex has two elements and recognizes that they have different roles during testis descent and later in adults. The sexually dimorphic genitofemoral nerve (not shown) is carried downward with the gubernaculum and innervates the cremaster muscle.

Process of testis descent

There is limited information on regulation of testis descent in common animals, although there are good descriptions on changes in morphology. Hence, this summary is based on what is known from model animals, augmented with data for companion or food-producing animals.

Abdominal translocation results in positioning a testis near the developing internal inguinal ring. The extra-abdominal portion of the gubernaculum becomes longer and wider, but there is little change in the distance between a testis and the inguinal area. In a bull fetus, distance from a testis to the internal inguinal ring decreases by 4 mm whereas the distance from testis to kidney increases by 13 mm, during the time frame required for abdominal translocation; distance from the internal inguinal ring to kidney becomes >25 mm (Edwards *et al*., 2003). In rats, distance from a testis to the internal inguinal ring decreases 0.3 mm whereas testis to a kidney distance increases 4.4 mm (Shono *et al*., 1994a).

The testis is suspended by the cephalic ligament cranially and the posterior gonadal ligament plus gubernaculum caudally (Fig. 1A). Initially, both the posterior gonadal ligament and gubernaculum are short and thin. Leydig cells in the rapidly evolving testis secrete Insl3 and testosterone. Under stimulation of Insl3, the extra-abdominal gubernaculum expands and invades deep into the abdominal musculature (Fig. 1B), to anchor the testis. The vaginal process invades the gubernaculum (especially conspicuous in Fig. 1C), and grows downward as the gubernaculum increases in size. Testosterone might facilitate gradual dissolution of the cephalic ligament, which elongates as the abdominal cavity expands. Consequently, the testis is retained in the inguinal region and the distance between the testis and other structures in the abdomen (e.g., kidney) widens. At final abdominal positioning (Fig. 1C), the cauda epididymidis is just within the inguinal canal and the testis is near the internal inguinal ring. At this time, relative length of the posterior gonadal ligament is variable, so a testis might not be hard against the inguinal ring. Although the testis grows in all species, in horses the testis becomes very large (primarily growth of interstitial tissue) by the time it is positioned near the inner inguinal ring.

In rodents and rabbits, as in other species, formation of the vaginal process is a feature of abdominal translocation of a testis. Hence, it should be included in the first phase of testis descent. By late gestation, the gubernacular-cremaster complex has formed and assumed a conical or cylindrical shape protruding into the abdominal cavity (Fig. 2A) in the femoral triangle area. Then the base of the gubernacular-cremaster complex "sinks" slightly below the plane of the abdominal wall, accommodated by slight cylindrical down-growth of the peritoneal lining (Fig. 2B). This is the first evidence of the forming inguinal canal. In other species, this phenomenon happens much earlier. By this time, the gubernacular cord has fully regressed, so that the cauda epididymidis is positioned against the abdominal face of the future vaginal process and, hence, in close proximity to the intra-abdominal gubernaculum per se or the muscle of the gubernacular-cremaster complex. In any case, the testis is positioned near the entrance to the still forming inguinal canal. Abdominal translocation of testes is completed (Fig. 2B) around gestational day 28 in rabbits, postnatal day 1-2 in mice, or postnatal day 4-5 in rats (Elder *et al*., 1982; Shono *et al*., 1994b, 1996; Lam *et al*., 1998).

In large animals such as horses, at the end of abdominal testis translocation the gubernaculum and vaginal process extend far below the newly formed inguinal canal and the testis is positioned against the internal inguinal ring, with the cauda epididymidis within the inguinal canal (Fig. 1C). In most species, this position is maintained for some time, like a "pause" between 2 separate processes. During the pause before actual transinguinal migration, the gubernacular bulb enlarges greatly (see Fig. 7 in Gier and Marion, 1970),

due to stimulation by Insl3, and the gubernaculum dilates the inguinal canal to allow passage of the testis during the next phase of descent. The pause also might allow maturation of certain nerve tracts, and the fetus is growing.

In rodents and rabbits, at the end of abdominal testis translocation the gubernacular bulb and vaginal process still are relatively short (Fig. 2B). Regression of the gubernacular cord has brought or attached the cauda epididymidis (connected by regressed posterior gonadal ligament to testis) to the apex of the vaginal process covering the gubernacular-cremaster complex, which is recessed into the abdominal wall but still protrudes into the abdominal cavity. In contrast to other species, the cauda epididymidis is not within an inguinal canal, although evagination of the vaginal process is ongoing.

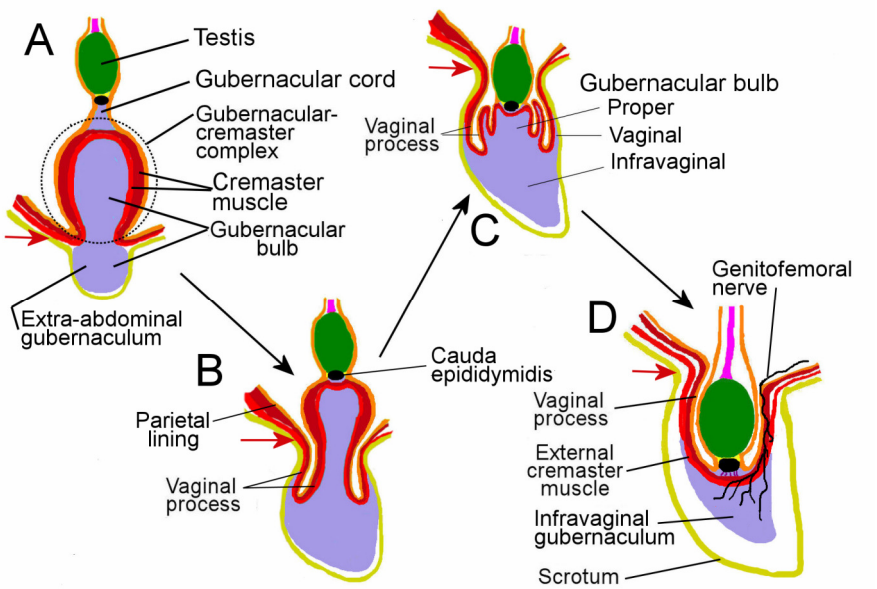

Figure 2. Late abdominal translocation and transinguinal migration of the testis in a rodent or rabbit. Unlabeled red arrows designate level of abdominal wall. **A**, in these species, during abdominal testis translocation the intra-abdominal portion of the gubernacular bulb becomes covered by concentric layers of cremaster muscle forming what might be termed a "gubernacular-cremaster complex". The gubernacular cord already has shortened in this view. The gubernacular bulb has an extra-abdominal portion as in other species (e.g., Fig.1). **B**, at the end of abdominal translocation, early evagination of the vaginal process is evident, the gubernacular cord is fully regressed, and the testis is poised to pass through the inguinal canal as it forms. **C**, transinguinal migration is initiated by intussusception of the cylindrical vaginal process and cremaster muscles concurrent with diminution and migration of the infravaginal portion of the gubernaculum. **D**, transinguinal migration is completed by straightening of the vaginal process and cremaster muscles. Not to scale. Evolved from Rajfer (1980), Elder *et al*. (1982), Wensing (1986, 1988), van der Shoot and Elger (1993), Shono *et al*. (1994a, 1996), and other literature.

The relative points in fetal development when abdominal testis translocation is initiated and completed differ greatly among species (Fig. 3). In cattle, deer, rabbits, and rodents the process is relatively short (<20% of the length of gestation) whereas in horses or pigs it requires >50% of gestation. Reasons for the wide range in time required for abdominal translocation, on either a relative or absolute scale, are not evident.

Transinguinal migration in most species requires reduced absolute size of the testis and distension of the inguinal canal by the gubernaculum sufficient to allow the testis to enter and rapidly move through (Fig. 4). Incompatibility between testis size and diameter of the inguinal canal is considered by some to be a contributing factor to abdominal cryptorchidism in stallions. Force of abdominal pressure on the testis might drive its migration through the inguinal canal.

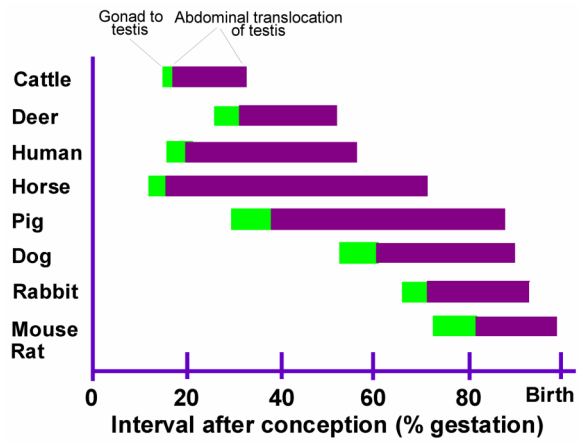

Figure 3. Approximate timing of testis formation and abdominal translocation of the testes in common mammals. For each species, consensus gestational age when events were detectable in fetuses were scaled relative to gestation length. The exactitude of demarcations mask variation and uncertainty in when 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. Despite limitations, this approach facilitates species comparisons. Testis differentiation occurs at the start of the green bar for each species, and that "opens a window" when an endocrine disruptor agent could irrevocably affect a testis. Species differ in when testes differentiate. Time required for abdominal testis translocation ranges from ~16% in cattle to ~57% horses. 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) presented a somewhat different comparative summary, likely reflecting different interpretations of published descriptions of fetal dissections in respect to when testes are poised at the inner inguinal ring.

Transinguinal testis migration in rodents and rabbits (Fig. 2C, 2D), as redistilled from the literature by Amann and Veeramachaneni (2007), is similar to that in other species if one includes initial formation of the vaginal process under abdominal translocation (e.g., Fig. 2B). However, in rodents or rabbits the cauda epididymidis already is attached to the developing vaginal process surmounting the infravaginal gubernaculum. Near postnatal day 2-8, the cremasteric component of the gubernacular-cremaster complex develops a serpentine or ill-defined appearance (Fig. 2C; Elder *et al*., 1982; Lam *et al*., 1998), with a diameter (across the proper portion of the gubernaculum) greater than that of the testis. With a relatively large infravaginal gubernaculum extending along the external face of the abdominal wall, reduction of the proper portion of gubernaculum and growth of the pup, serpentine intussusception of the cylinder of cremaster muscle occurs and, as it unfolds, it assumes an U-shape around the cauda epididymidis and testis. This brings the cauda epididymidis and testis through the newly formed inguinal canal and outside the abdominal wall (Fig. 2D), with the cauda epididymidis still associated with the infravaginal portion of the gubernaculum. At this point, transinguinal testis migration has been completed, but the infravaginal gubernaculum, cauda epididymidis, and testis remain some distance from the tip of the developing scrotum.

Actual transinguinal migration of the testis 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 and Colenbrander, 1986) and anchoring the cauda epididymidis with attached testis as the fetus or newborn grows. The main force moving the testis through the inguinal canal is thought to be downward pressure of viscera and peritoneal fluid enhanced by anchorage of the gubernacular bulb, expansion of the vaginal process, and growth of the abdomen. The gubernaculum might shorten slightly.

Inguinoscrotal migration of a testis, from below the external inguinal ring (Fig 4B) to the final scrotal location, requires extension of the gubernaculum and enclosed vaginal process to the tip of the scrotum. In some species, the extra-abdominal gubernaculum might extend partway into the scrotal folds well before transinguinal testis migration, but because of fetal growth both the gubernaculum and vaginal process must grow in the proper direction to reach the tip of the scrotum. In rodents and rabbits the extra-abdominal gubernaculum, with vaginal process, extend subcutaneously a relatively short distance when transinguinal testis migration is completed (Fig. 2D), but 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 hence the testis, to a proper location.

Scrotal swellings or folds develop early in fetal development (Fig. 1C). However, in some species (e.g., bull, horse, human) they must migrate a considerable distance to the final location of the scrotum. This means that the vaginal process, gubernacular bulb, and epididymis plus testis must follow.

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 the cell body) descending down with the developing gubernaculum and cremaster muscle. Testosterone stimulates release of CGRP, which is chemoattractant and induces the developing tip of the gubernaculum to

grow towards the source of CGRP (Hutson *et al*., 1998; Huston and Hasthorpe, 2005; Ng *et al*., 2005). Assuming this occurs in all common mammals, factors controlling outgrowth and direction of the genitofemoral nerve would have a critical role in final positioning of the testis. Also, lack of testosterone at this time could result in malpositioned subcutaneous testes.

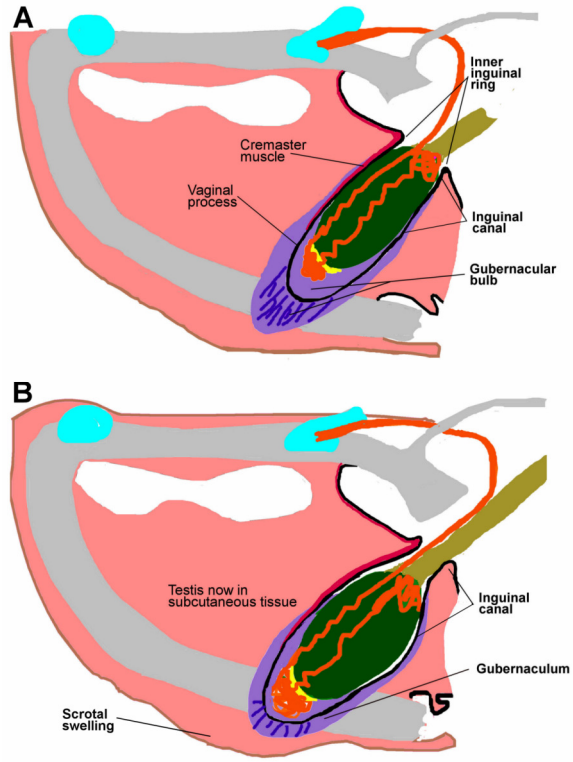

Figure 4. Transinguinal migration of the testes in a typical animal, the horse. The transition from A to B requires a few days and in horses typically occurs between GD 290 and GD 300. **A,** the testis has become smaller in both relative and absolute size (critical in the horse) than in Fig. 1C, and the gubernaculum has distended the inguinal canal and extended within subcutaneous tissue towards the scrotal folds. **B**, the testis is almost entirely through the inguinal canal. Not to scale. Based on Bergin *et al*. (1970) and other literature.

Sequential control of testis descent

There is a large number of genes and gene products involved in regulation of testis descent (Klonisch *et al*., 2004; Basrur and Basrur, 2004; Huhtaniemi and Poutanen, 2004). It is obvious that, at a minimum, products of *Insl3*, *Great*, *androgen receptor*, and *CGRP* genes plus testosterone must be available during critical points in development (Fig. 5; Amann and Veeramachaneni, 2007), and various molecules plus transcription factors probably are obligatory for a normal differentiation of the testis and testicular descent. Studies using estrogenic and anti-androgenic molecules in cattle and pigs, as well as rabbits, rodents and humans, establish that expression of Insl3 and testosterone must occur in different critical time windows. During abdominal testis translocation, Insl3 and testosterone probably are provided by Leydig cells to nearby tissues in a paracrine fashion. By the time of inguinoscrotal migration, GnRH and LH are involved in regulation of testosterone production by Leydig cells; the hypothalamic-pituitary-gonadal axis apparently is operational.

Abdominal testis translocation is blocked by elimination of *Insl3* or *Great* genes in mice, or inactivation of their products (Klonisch *et al*., 2004). Expression of *Insl3* gene can be blocked by estrogenic molecules (Nef *et al*., 2000), because they bind to estradiol receptor present in Leydig cells and suppresses transcription of the *Insl3* gene. The gubernaculum remains under-developed, can not retain the testis near the neck of the bladder, and the testis is moved cranially. An anti-androgen with high affinity for androgen receptor (e.g., flutamide) can compete with testosterone for sites on androgen receptors in the cephalic ligament. However, in both pigs and rats, administration of flutamide at appropriate times during gestation did not affect abdominal translocation of a testis in most fetuses or litters (McMahon *et al*., 1995; Mylchreest *et al*., 1999; Spencer *et al*., 1991), although differentiation of mesonephric duct usually was blocked.

In respect to transinguinal migration of testes, blocking action of testosterone with flutamide usually has no effect. In rat studies cited above, 82 and 87% of testes were found outside the abdominal cavity in pups from pregnant rats administered flutamide. Similarly in the pig study, 95% of testes were outside the abdominal cavity; 42% were subcutaneous and 53% were in the scrotum. Clearly, testosterone is not obligatory for transinguinal migration of a testis.

Inguinoscrotal migration of testes requires availability of testosterone, but not Insl3. Inguinoscrotal testis migration is blocked in null-mice lacking *GnRH-promoter*, *GnRH*, *LH*, or *LH-receptor* genes; hence, Leydig cells no longer have constitutive capacity to secrete testosterone (Hutson *et al*., 1997; Klonisch *et al*., 2004; Huhtaniemi and Poutanen, 2004). Similarly, in boys displaying failure of inguinoscrotal migration (testes below the external inguinal ring) the main etiological factor was impairment of the hypothalamic-pituitary-testis axis (Hadziselimovic *et al*., 1984). Prior to or early during migration of a testis from the external inguinal ring to deep in the scrotum, it is likely that testosterone masculinizes the genitofemoral nerve and induces it to secrete CRGP which bind to its receptors in the tip of the gubernaculum to help provide migratory direction. Concurrently, it is likely that testosterone exerts negative actions to regress the gubernacular bulb in terms of volume and molecular composition. Also,

testosterone might induce closure of the inguinal canal (except in rodents and rabbits) and final regression of the cephalic ligament.

Studies with exogenous agents, or null-mice, might lead one to conclude defective gene expression was an important cause of cryptorchidism. Appropriate studies apparently have not been undertaken with food or companion animals. However, comprehensive analyses of gene sequences in cryptorchid men revealed that *Insl3* or *Great* genes were aberrant in 3-5% of such individuals (Ferlin *et al*., 2003; Klonisch *et al*., 2004; Roh *et al*., 2003) and aberrant *androgen receptor* or *estrogen receptor* genes in <16% of cryptorchid men (Yoshida *et al*., 2005; Garolla *et al*., 2005)

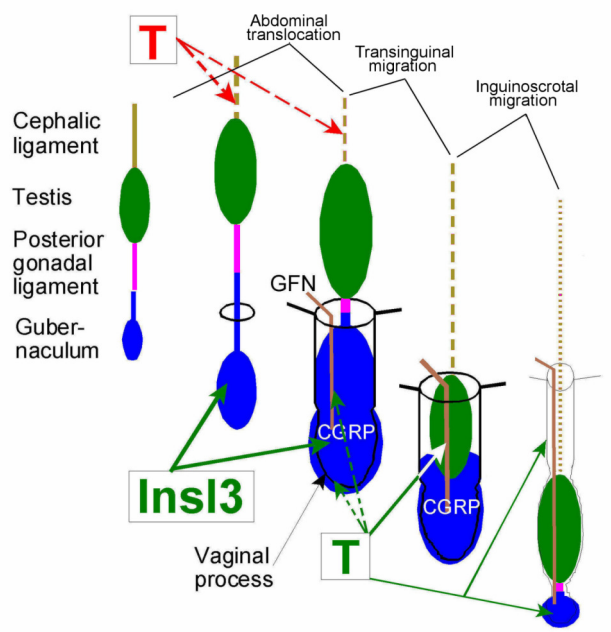

Figure 5. Regulation of testis descent. Abdominal translocation of the testes involves positive action (green) of Insl3 to stimulate growth and expansion of the gubernaculum. Concurrently, testosterone exerts a negative action (red) on the cephalic ligament facilitating its gradual regression. Both hormones are produced constitutively by fetal Leydig cells and bind to receptors in target tissue. Completion of the process requires testosterone from differentiating Leydig cells (under stimulation of LH) to masculinize the genitofemoral nerve (GFN) and stimulate production and secretion of calcium gene-related peptide (CGRP). Testosterone also might stimulate growth of the vaginal process. It is likely that these testosterone-mediated actions have a greater role in inguinoscrotal testis migration than in transinguinal testis migration. In all species, CGRP apparently contributes to directional control as the gubernaculum seeks the scrotum during inguinoscrotal migration.

Prevalence of cryptorchidism

For almost all populations, unilateral cryptorchidism is far more common than bilateral cryptorchidism. Among cryptorchid males, a unilateral phenotype represented 78, 90, 45-70, 81-93, 66-89, 59, 100, and 62% for cats, cattle, dogs, horses, humans, pigs, rabbits and sheep (Amann and Veeramachaneni, 2007). The only known exception to this is for a unique population of Sitka Black-Tailed Deer on the Aliulik Peninsula of Kodiak Island, Alaska. There, among 134 hunter-killed males examined carefully, 69% were bilateral cryptorchids and another 7% were unilateral cryptorchids (unpublished update of Veeramachaneni *et al*., 2006a). In these deer, all undescended testes were in the abdominal cavity, typically part way between the kidney and internal inguinal ring and displaying a thin, underdeveloped gubernaculum.

Location of undescended testes differs greatly among species. For cats, dogs and horses, 50, 92 and 47-60% of retained testes were in the abdominal cavity, although 1 report for horses gave 33% abdominal (see Table 1 in Amann and Veeramachaneni, 2007). There are no reliable data for pigs, but subcutaneous locations might predominate. For humans, abdominal retention was less common (~8%). Because of imprecise descriptions in some reports it is possible that 40-50% of undescended testes were within the inguinal canal. However, a better conclusion probably is that almost 90% of undescended human testes were subcutaneous, just outside the external inguinal ring or near the neck of the scrotum. In humans, perhaps two-thirds of cases self-correct within 3 mo, with descent after 3 mo unlikely (Hutson *et al*., 1997; Barthold and Gonzalez, 2003). A similar phenomenon is reported with dogs and horses.

Eradication of cryptorchidism

Reduction in occurrence of cryptorchidism should include trying to eliminate exposure of pregnant females to environmental agents likely to be estrogenic or anti-androgenic, or toxic via other mechanisms. This is not easy, as most exposures are unintentional and unknown. However, there is strong evidence that altered fetal development can occur at concentrations of estrogenic or anti-androgenic agents not causing detectable damage to the dam or other adults (Gore *et al*., 2006; Sharpe, 2006; Damgaard *et al*., 2006). Concurrent exposure to low concentrations of several agents of the same type (e.g., estrogenic) might impose a damaging combined concentration on fetal tissues at a critical point in development.

Ideally, unilateral cryptorchid males would not be used for breeding because there is risk that the trait will be transmitted to progeny. Determination of actual risk from breeding to unilateral cryptorchid males, or

the sire or siblings of cryptorchids, would be difficult and costly. However, special brother-sister matings of dogs or pigs over several generations increases the incidence of cryptorchidism (Cox *et al*., 1978; Mikami and Fredeen, 1979; McPhee and Buckley, 1984). There is anecdotal opinion that, for horses or pigs, there is familial prevalence of cryptorchidism in some sire lines.

Breeders of race horses or dogs, simply remove an undescended testis from a valuable unilateral cryptorchid and then continue to use him for breeding. They do not eliminate parents or siblings from breeding stock. Most cryptorchid bulls are slaughtered and unilateral cryptorchid bulls usually are not used for breeding. Cryptorchid boars typically are killed neonatally as they are deemed unsuitable for breeding and rearing to market weight would result in a carcass with greatly reduced value [problem is with "boar odor" resulting from 5-androst-16ene-3one produced by remaining testis tissue]. To cull non-cryptorchid male or female littermates, much less the dam, would impose an unacceptable economic penalty.

Other abnormalities associated with cryptorchidism

For reasons evident in Fig. 6, fetal testis dysgenesis resulting in cryptorchidism also might be manifested in a diversity of other reproductive abnormalities later in life. Importantly, non-cryptorchid males might display abnormalities in testis function or behavior consequent to fetal testis dysgenesis. It is known from breeding studies and pedigree analysis that cryptorchidism is among many genetic conditions displaying "incomplete penetrance"; not all animals with abnormal DNA express the undesired phenotype. Also, it is not evident that an aberrant genome accounts for most reproductive abnormalities.

Environmental agents can produce a spectrum of detected (and undetected) alterations in reproductive development (Sharpe and Irvine, 2004; Sharpe, 2006). A variety of animals including cattle (Veeramachaneni *et al*., 1986), horses (Veeramachaneni and Sawyer, 1996, 1998), and deer (Veeramachaneni *et al*., 2006a) "spontaneously" (i.e., in non-experimental situations) manifest TDS. Rabbits (Higuchi *et al*., 2003; Veeramachaneni *et al*., 2006b, 2007) and rats (Gray *et al*., 2000, 2001) can be experimentally manipulated to produce multiple lesions associated with TDS. Most important to individuals working with companion or food-producing animals might be reduced production of sperm and/or increased proportion of abnormal sperm (Veeramachaneni, 2000, 2006).

Testis tumors occur in non-cryptorchid males as well as unilateral or bilateral cryptorchid males. Abdominal location per se does not induce cell transformations (Veeramachaneni, 2006). However, it is likely that testis tumors occur 4-11 times more frequently in cryptorchid than non-cryptorchid males. Classification of testis tumors in animals (Kennedy *et al*., 1998; Amann and Veeramachaneni, in preparation) groups them as: germ cell tumors including carcinoma in situ (CIS), gonocytic seminoma, and spermatocytic seminoma; non-germ cell tumors including Leydig cell tumors, Sertoli cell tumors, stromal cell tumors, and adenoma of the rete testis; mixed tumors; and tumor-like lesions including Leydig cell hyperplasia and microlithiasis.

Based primarily on data for humans, it is thought that CIS cells are formed, from primordial germ cells, early in testis development and later give rise to other types of germ cell tumors (Fig. 6; Almstrup *et al*., 2004; Rajpert-De Meyts *et al*., 2004). CIS cells are found in both abdominal and scrotal testes, without or with overt evidence of other abnormalities (Giwercman, 1992, Hoei-Hansen *et al*., 2003; Veeramachaneni *et al*., 2001, 2006a, 1996b, 2007). Failure of testicular descent per se does not explain the transformation of primordial germ cells into CIS cells (Veeramachaneni, 2006).

Tabulations on occurrence of testis tumors in animals are inadequate to allow any meaningful estimation of prevalence within a species, because of under reporting and disparity in age when most males of different species are castrated or killed/die. However, there are important species differences in most commonly reported testis tumors (Kennedy *et al*., 1998; Amann and Veeramachaneni, in preparation). Germ cell tumors are rare in cattle, but there are approximately 64% Leydig cell tumors and 30% Sertoli cell tumors. Although testis tumors are not common in horses, gonocytic seminoma is the most frequent testis tumor in horses, followed by teratoma or Leydig cell tumors. Spermatocytic seminoma accounts for 32-48% of testis tumors in dogs (some of these might be gonocytic seminomas), with 27-42 % Leydig cell tumors and 20-40% Sertoli cell tumors. Gonocytic seminomas are more likely to be invasive than spermatocytic seminomas (Maiolino *et al*., 2004). In pigs, sheep, and rabbits, testis tumors are rarely reported. However, in the first two species gonocytic seminomas are most common whereas in rabbits Leydig cell tumors apparently are most common.

For rodents, incidence of spontaneous testis tumors is dependent on strain, but not inordinate at 24 mo of age. It might be higher in older rats than older mice (Clegg *et al*., 1997; Cook *et al*., 1999; Biegel, 2001). In both species Leydig cell tumors are by far the most common. In rats, incidence of Leydig cell tumors at 24 mo is 0.1-7.0%, except for Fisher 344 males or cross-breed animals with 76-94% of males developing tumors. In 24-mo old mice, incidence of Leydig cell tumors at 0.4-2.5% and Sertoli cell tumors at 0.1% were tabulated on the web site of one vendor.

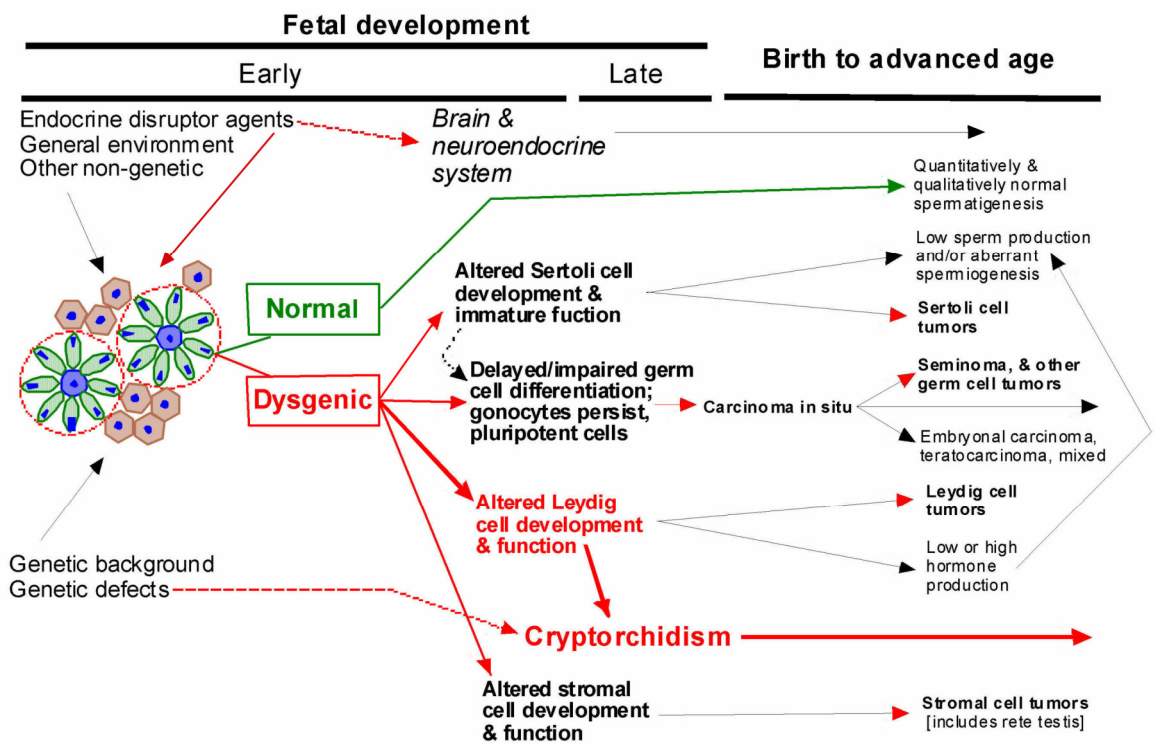

Figure 6. Schematic showing how testis dysgenesis during fetal development could result in an array of problems including cryptorchidism, abnormal sexual behavior, abnormal spermatogenesis, and a variety of testis tumors. The pivotal event is testis formation when progenitor cells proliferate and are programmed to provide fetal cell types. These undergo further programming and proliferation, followed by further differentiation and proliferation or cessation of cell division. For example, primordial germ cells (PGCs) to gonocytes to A-spermatogonia; mesenchymal cells to fetal Sertoli cells with sequentially changing capabilities; or a different wave of mesenchymal cells to fetal Leydig cells (not responsive to LH) to adult-type Leydig cells (responsive to LH) perhaps with further changes in capabilities. Retention of primordial or not fully differentiated cell types in adults might contribute to tumor formation. Both unilateral cryptorchid and non-cryptorchid males can produce abnormal germ cells consequent to fetal testis dysgenesis. Based primarily on extrapolations from published data for rabbits, rodents, and humans.

Deducing plausible cause of cryptorchidism

The spectrum of lesions detected can provide clues concerning the possible cause(s) of cryptorchidism. For example, for Sitka Black-Tailed Deer on the Aliulik Peninsula with 76% cryptorchid males, Veeramachaneni *et al*. (2006a) speculated that an estrogenic environmental agent(s) was the most plausible cause, but did not rule out a long-standing epigenetic alteration transmitted via germ cells, or a classical gene mutation concentrated by inbreeding. Their favored speculation was based on location of undescended testes within the abdominal cavity, underdeveloped gubernaculum associated with each testis, knowledge that if an estrogenic agent blocked timely production of Insl3 during fetal development the observed phenotype would be expected, and prevalence of testis tumors including rete adenocarcinoma that could be induced experimentally by DES, an estrogenic agent (Newbold *et al*., 1985). As discussed above, a typical anti-androgen usually would not result in abdominal testes.

However, it is easy to overlook the possibility that environmental pollutants with a hormone-like action might cause changes in certain cells independent of mechanisms involving classical hormone receptors or even their hormone-like action. For example, some chemicals categorized as anti-androgens apparently have other impacts via still uncertain mechanisms. Phthalate esters do not have high affinity for androgen or estrogen receptors, yet administration to pregnant rats induces proliferation of Leydig cells rather than timely differentiation (Wilson *et al*., 2004) and normal function. As another example, estrogenic molecules are far more likely to induce proliferative testicular tumors than are androgenic or anti-androgenic molecules. Action of an estrogenic agent (e.g., DES, octylphenol, or estradiol) during critical periods of fetal development induced, in some animals: transformation of Sertoli, peritubular, or Leydig cells and neoplastic lesions of

rete testis and/or cystic lesions of excurrent ducts (Newbold *et al*., 1985, 2000); and transformation of primordial germ cells into CIS cells and/or cryptorchidism (Veeramachaneni, 2000, 2006; Veeramachaneni *et al*., 2007). Tumors in the rete testis occurred transgenerationally without further exposure of descendents (Newbold *et al*., 2000). Mechanism of tumor-induction in fetal testes is uncertain.

Acknowledgment

Access to historic publications by John Hunter was graciously provided by Simon Chaplin, Senior Curator, Museums of the Royal College of Surgeons of England, London, UK. Partial support provided by NIH Grant 1R21-ES014607-01. The authors have nothing to declare in respect to conflicting financial interests or relationships with any commercial product or entity.

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