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# Testicular descent and cryptorchidism: the state of the art in 2004

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## Abstract

The understanding of testicular descent has changed much in the 20 years since the authors' laboratory began studying the mechanism. The process is now known to occur in 2 steps with different anatomy and hormonal regulation but with many still unresolved controversies. Recent advances include the recognition of acquired cryptorchidism of critical early postnatal germ cell development and the recommendation for surgery at 6 months of age. The authors still await long-term outcome studies.

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This brief summary describes 20 years of research on the normal mechanism of testicular descent and the result of its derangement in cryptorchidism. The scene opened in 1984 when published literature was reviewed to find out what was known about testicular descent at that time. It was widely believed that human chorionic gonadotrophin and androgens controlled testicular descent as supported by extensive studies on postnatal rodents [1,2]. In addition, there were detailed anatomical studies on pigs showing that the gubernaculum (otherwise known as the genitoinguinal ligament) underwent a "swelling reaction" in the male pigs and may evert through the inguinal canal to create the processus vaginalis (PV) to allow descent [3,4]. Importantly, the hormonal control of the swelling reaction was thought to

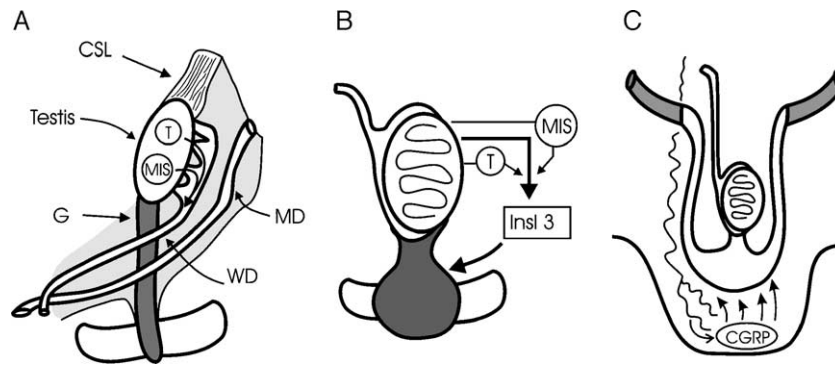
be independent of androgens, leading to an unresolved controversy. Most authors, however, assumed that there was a single hormonal and possibly anatomical mechanism [5-7]. In addition, surgery was recommended when patients reached 2 years because of electron microscopic changes in testicular tubules in cryptorchidism [1].

## 1. Two-stage hypothesis

To resolve this controversy, it was proposed that testicular descent occurred in 2 sequential phases with different anatomical mechanisms and different hormonal controls [8]. The first phase was suggested to be controlled by müllerian inhibiting substance (MIS) (also known as antimüllerian hormone [MIS/AMH]) because mice treated with estrogen or diethylstilboestrol had both cryptorchidism and retention of the müllerian ducts. The second phase was proposed to be dependent on androgens because the testis

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**Fig. 1** Schema showing the different gonadal positions during the 2-stage descent of the testis. A, Initial gonadal position showing testis in urogenital ridge with wollfian ducts (WD), müllerian ducts (MD), and the supporting ligaments, CSL and genitoinguinal ligament or gubernaculum. Testosterone (T) and MIS are secreted down the WD. B, During the transabdominal phase, the testis is held by the gubernaculum near the inguinal region as the fetus enlarges. Insulin 3 stimulates gubernacular enlargement, augmented by MIS and T, which also causes regression of CSL. C, In the inguinoscrotal phase, the gubernaculum migrates and elongates toward the scrotum under androgenic action, which is indirect, via the GFN and release of CGRP.

was descended to the bladder neck but not all the way to the scrotum in mice with complete androgen insensitivity [9].

The 2-stage hypothesis triggered significant controversy, with arguments about whether the early transabdominal phase exists or whether the testis was actually stationary. Other issues were the role of the gubernaculum and hormonal control.

## 2. Transabdominal phase

We and others were able to show that the transabdominal phase clearly did exist, with different positions of the testis compared with the ovary during fetal development in rats [10]. In addition, as can be clearly seen from the measurement of the distance between the gonad and the inguinal region, the testis actually remains in the same distance from the future inguinal canal whereas the ovary ascends with growth of the abdominal cavity (Fig. 1).

A key structure in the transabdominal phase is the gubernaculum (genitoinguinal ligament). It was named by the famous Scottish surgeon-scientist, John Hunter, in 1762 and 1786, because the term “gubernaculum” means helm or rudder [11]. Hunter chose this name because his observations suggested that the gubernaculum steered the testis to the scrotum. This has turned out to be a prescient observation, as will be seen below.

In the early fetus, the gubernaculum is seen as a short, thin ligament connecting the ambisexual gonad and the urogenital ridge (containing both wollfian and müllerian ducts) to the inguinal region. The inguinal abdominal wall muscles form around the mesenchymal end of the gubernaculum, setting the stage for the future inguinal canal. In the male fetus, the caudal end of the gubernaculum enlarges (known as the “swelling reaction”) by mitosis and deposition of hyaluronic acid [3,4]. This prevents the gubernaculum from elongating as the embryo enlarges, as occurs in

the female fetus. The swelling reaction of the gubernaculum holds the testis very close to the future internal inguinal ring, which begins to develop with the peritoneum growing into the swollen “bulb” of the gubernaculum.

At the cranial end of the urogenital ridge, the cranial suspensory ligament (CSL) regresses in male fetuses but not in female fetuses, which enables the gonadal position to be the vector sum of traction by the CSL vs the gubernaculum [12]. In female fetuses, the CSL holds the ovary high in the pelvis or abdominal cavity, depending on the species, whereas in male fetuses, the gubernaculum holds the testis near the inguinal canal [13].

When the 2-stage hypothesis was proposed, MIS/AMH was proposed as the key nonandrogenic hormone because both human beings with persistent müllerian duct syndrome and estrogen-treated mice had intraabdominal testes as well as retained müllerian ducts [14]. However, there was a lot of other evidence that suggested that MIS/AMH was not the major factor, at least in rabbits and rodents [15,16]. In the late 1990s, a new testicular hormone was discovered, known as insulin-like hormone 3 (Insl3), which is related to insulin and relaxin and is also known as relaxin-like factor. The Insl3 is produced by the Leydig cells in the fetal testis, and a gene knockout of Insl3 produced a mouse with intraabdominal testes and a feminized gubernaculum with no swelling reaction [17,18]. This suggested that Insl3 was the missing factor causing the swelling reaction.

To prove that Insl3 was the hormonal regulator, we had rat and human Insl3 synthesized and their effects on the fetal rat gubernaculum in organ culture were tested. This showed that Insl3, augmented by either MIS/AMH or dihydrotestosterone, caused similar levels of mitosis as the testis control [19-21]. In addition, we were able to show that the fetal gubernaculum contained the putative Insl3 receptor as well as the MIS/AMH type II receptor [22,23].

These studies have now shown that the transabdominal phase of testicular descent is regulated anatomically by the

vector sum of traction by the CSL and the gubernaculum [24]. Androgen causes CSL regression and *Ins13* causes the gubernacular swelling reaction, augmented by MIS/AMH and androgen. The feminized gubernaculum in human beings with persistent müllerian duct syndrome suggests that there may be some species differences in the role of MIS/AMH.

### 3. Inguinoscrotal phase

For the inguinoscrotal phase of descent, the 2-stage hypothesis triggered many controversies, including whether it actually existed and, if so, the nature of the mechanism. Further issues included the hormonal control and its mechanism and the possible role of the genitofemoral nerve (GFN) and the autonomic nerves. Finally, there were questions about the difference between what was seen in rodents and in human beings.

Human beings and mice with complete androgen resistance both showed that the testis was half-way descended to the inguinal region and bladder neck, respectively [9]. As the gubernaculum initially ends in the groin and the size of the swollen distal bulb was significantly smaller than the distance to the scrotum, it was clear that simple eversion of the gubernaculum was insufficient and that active migration to the scrotum was necessary [25,26]. In a series of elegant dissections, Heyns [27] showed that the bulb of the gubernaculum was the same size as the testis and that the caudal end was loose under Scarpa's fascia during the migration phase. Most importantly, Heyns showed that the distance to the scrotum was several-fold longer than the size of the gubernaculum itself.

Histological examination of the neonatal rat gubernaculum *in vivo* showed that cell division was occurring most rapidly right at the tip of both the gubernacular bulb and its contained PV, consistent with active elongation rather than passive eversion [28,29]. The cremaster muscle developed in the outer rim of the proximal gubernaculum, outside the PV. Quantitative analysis of bromodeoxyuridine incorporation showed that the gubernacular tip grew fastest between birth and 4 days in rats and that pretreatment of the fetus with both flutamide (competitive blockade of the androgen receptor) and capsaicin (sensory nerve toxin) caused inhibition of cell division at this time [30]. Recently, in organ culture, we tested directly whether exogenous calcitonin gene-related peptide (CGRP), a neurotransmitter known to be present in the GFN, could cause mitosis in the gubernacular tip. Addition of CGRP caused a significant increase in bromodeoxyuridine incorporation in the tip, consistent with a direct trophic action (Huynh et al, unpublished).

### 4. Genitofemoral nerve hypothesis

We had originally proposed the GFN hypothesis in 1987 [31] after repeating a study by Lewis in 1948 [32].

He showed that cutting the GFN in neonatal rats caused undescended testes (UDT), which were interpreted at that time as showing that the cremaster muscle, when denervated, could not pull the testis to the scrotum. To explain how cutting a nerve could completely block the effect of a circulating hormone, we proposed that the GFN acted as a "second messenger" for androgen by release of CGRP to control descent. We suggested that androgens have a limited direct role on the gubernaculum, as it was known that androgen receptors were absent at the time of migration [24,31]. Studies in our laboratory showed that GFN transection caused cryptorchidism in rats [26,33] and that the GFN fibers contained CGRP [34]. In addition, exogenous CGRP caused rapid rhythmic contractions of the gubernaculum (>100 beats/min) *in vivo* [35]. The speed of contractility suggested that the gubernaculum contained an embryonic cardiac muscle because the contractions were too rapid for a smooth muscle and a skeletal muscle does not contract rhythmically after denervation. The origin of these embryonic myotubes remains unresolved.

Receptors for CGRP were found in the rat gubernaculum [36], with localization over the developing cremaster muscle. Initially, our studies suggested that the CGRP was in the motor fibers [34], but subsequent studies refuted this [37]. We went back and repeated these studies and were able to show that the CGRP was localized in the sensory cell bodies of the GFN in the L1-2 dorsal root ganglia [38,39]. The altered effects on the gubernaculum after capsaicin treatment confirmed that the CGRP was reaching the gubernaculum via the sensory nerves [30,40].

To see if the CGRP provided a chemotactic signal for the gubernaculum, we looked at gubernacular cells *in vivo* with equivocal results [41]. Recently, we have been able to show definitive evidence for chemotaxis by using agarose beads soaked in CGRP—these caused deviation (toward the beads) of the gubernacular tip *in vitro* (Yong et al, unpublished).

Animal models of androgen blockade (TFM mouse and flutamide-treated rat) showed clear evidence supporting the GFN hypothesis [42]. The testes were undescended and CGRP was decreased in the GFN, whereas the gubernaculum showed upregulation of CGRP receptors. By contrast, in the mutant transscrotal rat with congenital cryptorchidism, we found excess fibers in the GFN and excess CGRP content in the nerve, whereas the gubernaculum had downregulated CGRP receptors, consistent with the cryptorchidism caused by excess CGRP interfering with the normal chemotactic signals during gubernacular migration to the scrotum.

Recently, Tanyel [43] has suggested that sympathetic autonomic nerves may be more important in testicular descent than the GFN. We have tested this hypothesis in our neonatal rat organ culture model and found no significant effect of  $\beta$ -adrenergic agonists or antagonists [22].

Although the evidence for the GFN hypothesis in rodents is very strong, its relevance to human beings has been questioned because the structure of the human gubernaculum is much different [44,45]. We have addressed this indirectly in human beings by a study of inguinal hernia, on the premise that the primary function of the PV is to allow the intraabdominal fetal testis to exit the peritoneal cavity to the scrotum and, hence, it was likely to be controlled by the GFN, the same as for gubernacular migration [46]. Inguinal hernial sacs removed at herniotomy were placed in organ culture, and we were able to show that CGRP was able to cause fusion of the hernia and obliteration of the peritoneal surface within 48 hours, consistent with the view that the GFN and CGRP control not only gubernacular migration but also obliteration of the PV after testicular descent [46].

To test whether CGRP could treat UDT, we placed mini-osmotic pumps containing CGRP into the scrotum of neonatal piglets with congenital cryptorchidism. We found that slow release of CGRP stimulated migration of the UDT toward the pump [47]. However, whether the studies can be translated into treatments for cryptorchidism or inguinal hernia in children remains unknown.

## 5. Cryptorchidism A: congenital

Our studies suggest that congenital UDT will be caused by any abnormality of the complex anatomical and hormonal mechanisms described above. Impalpable UDT are predicted to be uncommon because the transabdominal phase has such a simple mechanism with only relative movement of the testis as compared with the ovary [24]. By contrast, palpable UDT are predicted to be common because the second phase of descent has such a mechanically complex migration phase [24]. Unilateral UDT are likely to occur because androgens act independently on each side via the ipsilateral GFN and defects in neuronal development or CGRP action could lead to unilateral UDT. We predict that a perineal testis, for example, is caused by mislocation of the ipsilateral GFN, causing the gubernaculum to migrate to the wrong site because the chemotactic signals are arising from the wrong place.

## 6. Cryptorchidism B: acquired

In recent years, there has been growing evidence to support the concept of acquired UDT [48,49]. Our studies suggest that not only are they common [50] but also that they are caused by failure of postnatal elongation of the spermatic cord [51]. At birth, the spermatic cord is 4 to 5 cm in length, but by the 10th year, it is 8 to 10 cm; this doubling in length is inhibited if there is a residual fibrous remnant of the PV, which may be caused by deficient CGRP release from the GFN postnatally [52].

## 7. Germ cells

After birth, the primitive germ cell (gonocyte) transforms into a type A spermatogonium by 4 to 12 months [53,54]. It is now well documented that this transformation is inhibited in UDT [53], leading to a later deficiency of the postnatal germ cell pool and postpubertal oligospermia. We predict that the increased risk of malignancy in UDT may be caused by persistence of a few neonatal gonocytes and their subsequent mutation (caused by abnormally high temperature) into carcinoma *in situ* (CIS) cells and subsequent frank malignancy [24].

In addition to *in vivo* studies of spermatogenesis, germ cell development needs to be studied in an *in vitro* environment. Our approach has been to grow normal prospermatogonia (gonocytes) and spermatogonia *in vitro* without immortalizing manipulations. We have demonstrated extensive proliferation (>7 cell divisions) of prospermatogonial mouse germ cells *in vitro* using a unique male germ cell cloning strategy developed in our laboratory [55,56]. This quantitative assay has been extended to include normal postnatal spermatogonia [57]. These cloning experiments have shown that prospermatogonia and spermatogonia will proliferate freely, provided that the testis somatic cells are absent. In fact, the presence of adherent somatic cell underlays significantly inhibits colony formation with the key regulators being the inhibin/activin growth factor family [56,57]. Studies have been directed to establishing germ cell lines by immortalization with an SV-40 large T antigen [58-60]; however, limited differentiation potential restricts the usefulness of these cell lines. Recently, generation of male gametes from embryonic stem cells has been reported [61,62]. Although this will be a valuable model to study, imprinting a drawback in the temporal sequence of germ cell development will be lost in embryoid body derivation of germ cells.

## 8. Hormone treatment

Hormone treatment with human chorionic gonadotrophin or luteinizing hormone-releasing hormone was proposed to not only stimulate testicular descent but also stimulate germ cell development [63]. Unfortunately, hormone therapy has had poor results on descent [64,65], probably because the inguinoscrotal phase requires GFN differentiation between 15 and 25 weeks of gestation and this does not occur postnatally. In addition, recent studies suggest that, at least in rodents, gonocyte transformation to type A spermatogonium does not require androgens, as it occurs normally in TFM mice (Zhou et al, *in press*). Furthermore, there is some evidence to suggest that MIS/AMH may be involved [66]. Finally, human chorionic gonadotrophin treatment in mice causes precocious stimulation of primary spermatocytes, the resting germ cells before puberty, to enter premature spermatogenesis, which may not be helpful and might even be dangerous for subsequent fertility [67].



## 9. Surgical treatment

All these studies suggest that the optimal timing for treatment of human cryptorchidism is when patients have reached about 6 months so that postnatal germ cell development can proceed normally. However, whether it actually occurs after early surgery remains to be confirmed by follow-up studies, which do not yet reflect the outcome of such early intervention. By contrast with congenital UDT, acquired UDT are best treated once the spermatic cord is too short to allow the testis to reside naturally in the scrotum. In these testes, early germ cell transformation should be normal; hence, there should be no residual gonocytes present later in childhood to form CIS cells and subsequent cancer, which concurs with known risks for acquired UDT [68]. By contrast, the high extrascrotal temperature may deplete the spermatocyte pool, leading to decreased fertility and, hence, the rationale for treatment.

We are progressing toward a better understanding of normal and abnormal testicular descents, which is one of the most obvious anatomical features of sexual dimorphism. Its importance was appreciated by the church in the middle ages when a female pope was elected, leading to a scandal when she gave birth to a baby during a papal procession through Rome. Following this episode, the porphyry chair was produced as a way of determining definitively whether any future pope was a man and hence could become the Holy Father. The chair has a cut out in the seat such that the elected cardinal could sit on the chair, suitably robed, but a junior cardinal could reach in from behind and palpate the scrotum. If the scrotum contained 2 testes, they would chant “*duo testes bene pendulum*” (he has 2 testes and they hang well), confirming masculinity and eligibility for the papacy [69]. To this day, a fused scrotum containing 2 testes is still the best test for manhood and is still better than any genetic or hormonal test for a “man” [70].

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