African Journal of Urology

REVIEW Open Access

The curious case of testicular descent: factors controlling testicular descent with a note on cryptorchidism

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Abstract

Background The testicular descent is a uniquely complex process depending upon multiple factors like growth and reorganisation of the specific gonadal ligaments, hormones, etc., which interplay with each other. Though an unambiguous event, it is still laced with incredulity since the data interpretation were intermingled between different species creating more ambiguity in certain aspects of this process. In order to understand the aetiopathology of cryptorchidism the extensive study of the factors controlling the descent is necessitous.

Main body Though testes originate in the abdomen, they migrate to an extra abdominal site the scrotum, which makes it vulnerable to pathological conditions associated with the descent. The hormones that play vital role in the first phase of descent are insulin-like hormone 3 (INSL3), Anti-müllerian hormone as well as testosterone, whereas androgens, genitofemoral nerve and its neurotransmitter calcitonin gene-related peptide (CGRP) influence the second phase. Despite the vast research regarding the complex nexus of events involving the descent there are disparities among the cross species studies. However all these discrepancies make testicular descent yet again fascinating and perplexing. Our aim is to provide a comprehensive review including recent advances which provides thorough coverage of anatomical and hormonal factors in the descent as well as cryptorchidism.

Conclusion Though our understanding on testicular descent has evolved over the decades there still has obscurity surrounding it and the studies on the factors responsible for descent are becoming more intense with the time. Our knowledge on many factors such as INSL3 and CGRP is more established now; however, on the other hand the role of androgens still remains speculative. As the knowledge and understanding of the biological process of testicular descent increases it will pave ways to new treatment plans to treat cryptorchidism more effectively.

Keywords Testicular development, Descent, Anatomical factors, Hormonal factors, Cryptorchidism

1 Background

Testicular descent is a very unique and obscure sexually dimorphic event that occurs under the influence of anatomical and hormonal factors. In most mammals testes migrates from abdomen to scrotum except in some species where they are situated within the abdominal cavity [1]. The coherent explanation for why testes attain an extracorporeal location could be the low ambient temperature provided by the scrotum necessary for the physiological functioning of the male gonads [2, 3]. The scrotal temperature is believed to be 2–4 °C lower than the core body temperature [4]. The significance of hypothermic environment provided by the scrotum has been associated with normal testicular spermatogenesis [2]. To understand the process of testicular descent and cryptorchidism, in this review we focus on the two significant

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factors, i.e. anatomical and hormonal and how they interplay in this intricate process of descent.

2 Main text

Testicular descent is a complex multi-staged event. The theories behind the testicular descent dates back to centuries, nevertheless it continues to intrigue the researchers around the world. The most popular theory with regard to testicular descent is the 2 staged model [5, 6]. Multiple factors are suggested to influence this enigmatic interdependent event. Though this theory is the most accepted one, it is not with exception of controversies. The uncertainty still remains regarding the exact time of the testicular descent as the timings of the phases vary between the species.

2.1 The two phased model

The biphasic model still garners debate despite being the most accepted model explaining the testicular descent. It comprises of trans-abdominal phase, which involves the positioning of the testes near the inguinal ring and inguinoscrotal phase which involves the movement of testis through the inguinal canal to its final destination the scrotum (Fig. 1).

2.2 Trans-abdominal phase

It is of no doubt that the both the phases are regulated by distinct anatomical and hormonal factors. Between 6 and 9 weeks the metanephros enlarges and ascends from sacral to lumbar region below the suprarenal gland. The lateral displacement of gonad is believed to be caused by this movement [7, 8]. In the early development, the anti-Müllerian hormone (AMH) produced by sertoli cells causes regression of müllerian duct and its mutation results in persistent müllerian duct syndrome. Meanwhile the testosterone produced by leydig cell results in the masculinization of the external genitalia and persistence of Wolffian duct [9]. The two anatomical ligaments that are highlighted in this phase are the cranial suspensory ligament (CSL) and the gubernaculum. The upper pole of testis is attached to the diaphragm by the cranial suspensory ligament which under the influence of testosterone degenerates in the male foetus, resulting in the release of its cranial end [10]. On the contrary the cranial suspensory ligament persists in the female thus allowing the ovary to retain its position in the pelvis [11]. In this phase, the thickening of gubernaculum is a noticeable anatomical change which helps in positioning of the testes near the inguinal canal [9, 12–14]. This process of gubernacular thickening appears to be controlled by INSL3 a hormone produced by leydig cells [13, 15–17]. The studies in rodent model showed that the mutation in this gene or its receptor LGR8 lead to abdominal cryptorchidism [18, 19]. Despite these findings only a minority of cryptorchid patients showed mutation in the gene [16]. The first phase of descent is believed to occur between 8 - 15 weeks of gestation [10, 20].

2.3 Inguinoscrotal phase

It is quite fascinating that after the first phase of descent there is a pause before the commencement of the second phase, i.e. the Inguinoscrotal phase, which happens in humans around 25–35 weeks of gestation and from birth to postnatal 3–4 weeks in rodents [21]. Reny et al. [14] reported second phase of descent occurs between 26 weeks to full term in human foetuses. The thickening of the gubernaculum and the increased abdominal pressure by the growth of the viscera results in the subsequent dilatation of the inguinal canal which is an important requisite for this phase [9, 17]. This ensures the smooth passage of the testes and epididymis through the canal engulfed by the processus vaginalis to the scrotum. Thus in this phase the gubernaculum grows and migrates across the pubic region towards the scrotum along with the tip of processus vaginalis which provides a peritoneal diverticulum to leave the abdomen [9, 22].

However it was suggested by Attah and Hutson [23] that as the gubernaculum contains the abdominal peritoneal diverticulum, the processus vaginalis, the role of intra-abdominal pressure might be supplementary [22]. Once the descent is complete the processus vaginalis closes and later involutes. As the testes passes to the scrotum the collagen content of the gubernaculum increases implying its gradual involution [16]. It is now widely believed that androgens control the second phase of descent indirectly and not directly [9, 13, 16]. There are studies suggesting that androgens acts as paracrine factors and may control the inguinoscrotal phase via the sensory branch of genitofemoral nerve its neurotransmitter calcitonin gene-related peptide (CGRP) [20, 22, 24]. Androgen induces the gubernaculum regression by increasing the collagen content and decreasing its extra cellular matrix [13]. Studies have shown that this phase was affected in the androgen resistant or androgen deficient rodent models [25, 26].

2.4 The three phased model

As we are progressing towards more evolved understanding on testicular descent and cryptorchidism, new theories and hypothesis are also proposed by many researchers for easier comprehension of the same. One such new theory is the three phased testicular descent suggested by Amann and Veeramachaneni [15] in their study. The study proposes that since in cryptorchidism the non-scrotal testes are found in one of three general locations (abdominal, inguinal, or s.c.), the testicular

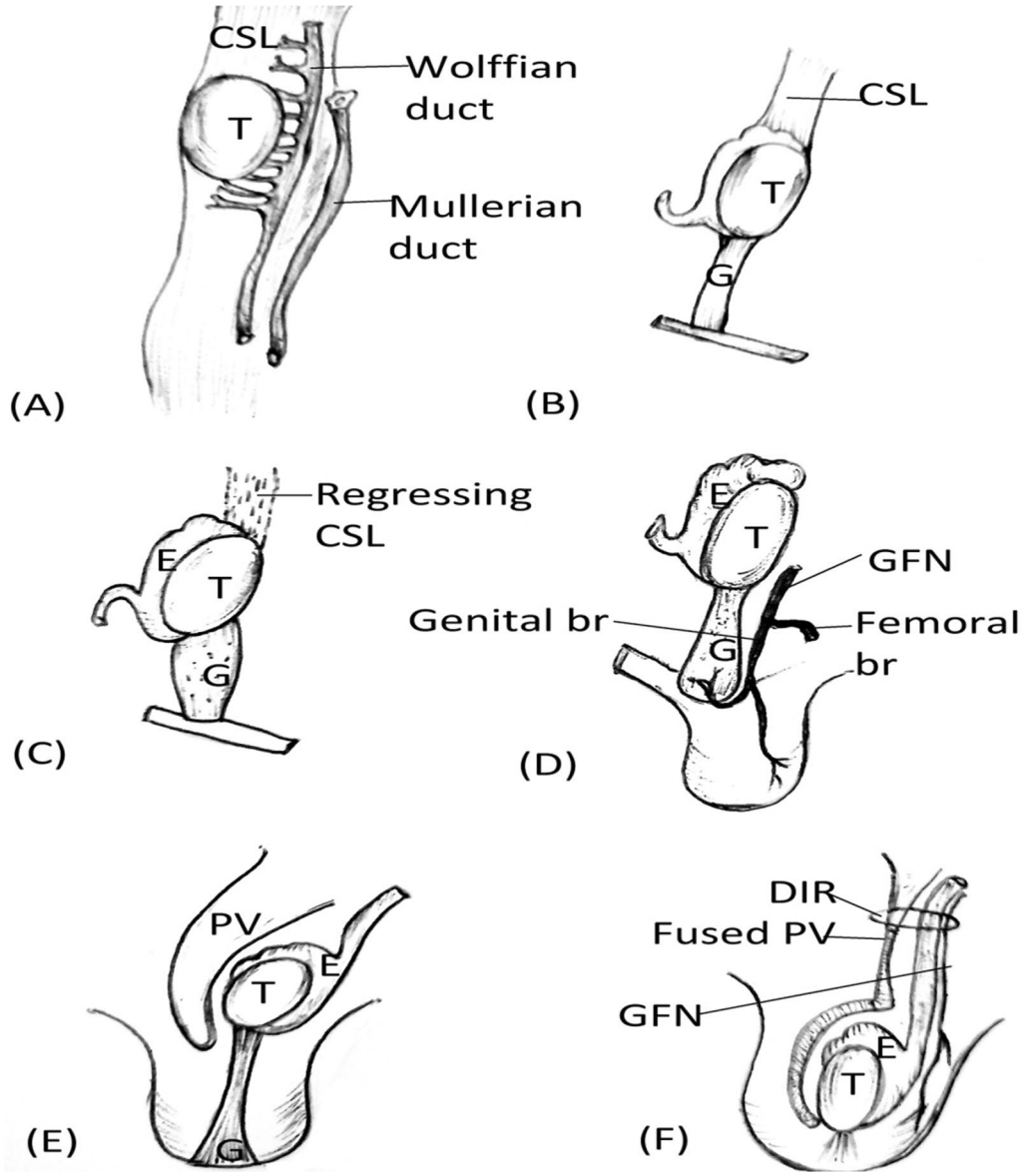

Fig. 1 Diagrammatic schema showing testicular descent, **A**–**C** shows the trans-abdominal phase and **D**–**F** shows the inguinoscrotal phase. **A** and **B** Testis (T) in the urogenital ridge with Müllerian duct, Wolffian duct, CSL (cranial suspensory ligament) and Gubernaculum (G). **C** The CSL regression occurs during the trans-abdominal phase. **D** The gubernacular enlargement occurs under influence of various factors like INSL-3, supported by MIH. The genital branch of genitofemoral nerve (GFN) innervates the gubernaculum and scrotal wall. **E** and **F** in the inguinoscrotal phase gubernaculum migrates and elongates along with processus vaginalis (PV) through the deep inguinal ring (DIR) to the scrotum

descent should logically be consisting of three phases instead of classical two phased model. The three phases suggested by the study comprises of the following steps. (a) Abdominal testis translocation, this happens as the abdominal cavity enlarges, and a slight testicular relocation takes place to the future internal inguinal ring; (b) transinguinal migration of a testis, this comprises of movement of cauda epididymis and testis through the abdominal wall; and (c) inguinoscrotal migration of a testis, which involves testicular movement from the inguinal canal to the scrotum. The study also proposes the term translocation should be used instead of descent as it is more descriptive with regard to the first phase.

2.5 Anatomical factors

2.5.1 Gubernaculum

The protagonist attracting much attention in the process of descent is gubernaculum. Nevertheless it has also

been suggested that gubernaculum is not required for the descent [27]. It was first described by Scottish Surgeon John Hunter in 1762. The gubernacular morphology appears to be similar in eutherian species but differs among mammals and small rodents [28]. Gubernaculum develops as a mesenchymatous cord which connects the distal portion of the ambisexual gonad and mesonephros to ventro-lateral abdominal wall [29]. However it was also suggested that the gubernaculum is attached to epididymis and only indirectly to testis [27, 30]. Reny et al. [14] refutes this observation that gubernaculum is indirectly attached to testis. The gelatinous and bulky nature of gubernaculum is due to the hydrophilic nature of hyaluronic acid [17]. During 10–15 weeks in male foetuses the caudal end of the gubernaculum undergoes thickening and anchors the developing testis to the inguinal region [31]. This thickening known as gubernaculum/swelling reaction is believed to be caused by the increase in glycosaminoglycans and hyaluronic acid. On the contrary no such phenomenon takes place in female foetuses [32]. Many genes are reported to regulate the first phase by their action on gubernaculum. It was reported that Wnt-5a and its receptor Ror2 were strongly expressed within the mesenchyme of the rodent gubernaculum early during its development, and their mutation resulted in undescended testes [21].

During the process of descent, the proximal end of gubernaculum appears short [28]. The shortening of the gubernacular cord with subsequent regression of cranial suspensory ligament, facilitates the positioning of testes over the inguinal ring [12, 33–35]. This also results in the dilation of inguinal canal and scrotum allowing testicular movement to its destination in its second phase of descent. An important factor influencing the gubernacular function in testicular descent is insulin-like hormone 3 (INSL3) which causes increase in gubernaculum volume [19, 36, 37]. Interestingly the role of the hormone estradiol has been proposed with regard to its action on gubernacular growth. The estradiol was proposed to have an inhibitory role in gubernacular growth by causing the reduction in volume and size of extracellular matrix [13, 33, 34]. The gubernaculum also shows characteristic morphological changes in the second phase of descent from growing and elongating to regression.

2.5.2 Cranial suspensory ligament

Much of the attention was given to the gubernaculum with regard to gonadal descent. The role of cranial suspensory ligament which is a fibromuscular structure connecting the bipotential gonad to the posterior abdominal wall remains widely unexplored. Van der Schoot and Elger [38] studied the development of the cranial suspensory ligament and claimed that this ligament has a significant role in the gonadal descent. The persistence of cranial suspensory ligament aids ovarian ascent whilst its regression under the influence of androgen facilitates testicular descent, thus making it a very significant gonadal ligament contributing in the final position of gonad [11, 35, 38]. In male rats, the prenatal inhibition of androgen resulted in well-developed cranial suspensory ligament and a high intra-abdominal position of testis at birth. In female rats the prenatal exposure of testosterone showed a failure of development of cranial suspensory ligament with lower intra-abdominally placed ovaries [39, 40]. The question still remains whether it is the gubernaculum or the cranial suspensory ligament that plays as the key anatomical structure in the process, especially in the first phase of descent. Though the gubernaculum is favoured in this aspect, more studies are needed to understand the function and role of cranial suspensory ligament with regard to descent.

2.5.3 Abdominal pressure

Many studies have implied the supplementary role of intra-abdominal pressure in testicular descent [12, 23, 41]. It is suggested that the intraabdominal pressure aids in the inguinoscrotal descent of testis from the inguinal canal to the scrotum [41, 42]. However, Heyns [12] stated in his study that an elevated intra-abdominal pressure would have exerted an equal force on both testes, which does not account for the 17% of asymmetrical descent noted in his study. During inguinoscrotal phase, the gubernaculum takes a longer route considering its size for aiding the testicular descent and the intra-abdominal pressure might assist by providing the required force of movement [17].

2.5.4 Genitofemoral nerve

It was believed that androgens acted directly to control inguinoscrotal phase. But later many studies with regard to genitofemoral nerve and cremaster muscle suggested that the role of the androgen may be indirect. The hypothesis of indirect action of androgen via genitofemoral nerve, and cremaster providing the necessary traction became popular after the study by Lewis [43]. The study by Lewis [43] stated that cremaster muscle when denervated could not aid in the testicular descent to scrotum. Moreover the nerve transection also caused undescended testis in the neonatal rats [24, 44]. The study by Hutson and Hasthorpe [24] emphasises that the CGRP could be the pivotal factor for the gubernaculum development in the second phase of descent. On the contrary the studies has reported that accurate division of genitofemoral nerve did not result in undescended testis [45].

A sexually dimorphic neuropeptide transmitter, calcitonin gene-related peptide (CGRP) is present in the genitofemoral nerve. It was proposed that the CGRP receptors have also been localised to the developing cremaster muscle in rodent gubernaculum [46]. The androgens indirectly control the migration of gubernaculum during the inguinoscrotal phase by means of CGRP released from the sensory branch rather than the motor nerve of genitofemoral nerve [47, 48]. The CGRP induces rhythmic contractions in gubernaculum which were more pronounced by the increased intra-abdominal pressure [49, 50]. Interestingly the contractions observed were highly vigorous which neither a smooth muscle nor a skeletal muscle could produce under the given conditions; hence, these studies suggested the possibility of an embryonic cardiac muscle in gubernaculum [24, 50]. In addition to these properties, it is suggested that CGRP provides a chemotactic signal to the gubernaculum thus facilitating the descent [51]. Equitably important, are other contradicting studies that points out that CGRP may not be aiding the testicular descent at all. The study by Husmann and Levy [34] suggests that CGRP may have a role in rodent models, but may not be applicable in humans. This study also proposes a novel androgen-independent factor, descending from a normal testis that could be responsible for the descent by causing selective growth of gubernacular cells [34]. Whilst Houle and Gagne [52] claimed that CGRP had no effect on postnatal mice testicular descent. Although all these studies lays strong foundation for the genitofemoral nerve hypothesis, disputes still arise with the question of whether the rodent models are significant in case of humans as the gubernacular anatomy differs.

2.6 Hormonal factors

The renowned French endocrinologist Alfred Jost's experiments 60 years ago directly or indirectly became the harbinger of revolution in reproductive developmental biology. His studies contributed the detailed models of hormone action on gonadal and genital development. Three potential hormones which has a specific role in the testicular descent are discussed here, (1) AMH (2) INSL3 and (3) Androgens (Fig. 2).

2.6.1 Müllerian-inhibiting substance/anti-Müllerian hormone (MIS/AMH)

Since its discovery the role of AMH has evolved from regulation in biological processes to the routine clinical uses. Our ideology on the role of AMH has also undergone burgeoning with recent scientific advancements. AMH is a dimeric glycoprotein consisting of two identical 70-kDa subunits. It belongs to transforming growth factor-beta (TGF-β) superfamily, encoded by the AMH gene, which contains 5 exons and is produced by immature sertoli cells [53, 54]. TGF-β superfamily with multifunctional factors are found both in vertebrates and invertebrates. The members of this superfamily play significant role in the regulation of cellular proliferation, apoptosis and biological process such as spermatogenesis and folliculogenesis [55, 56]. The regulation of

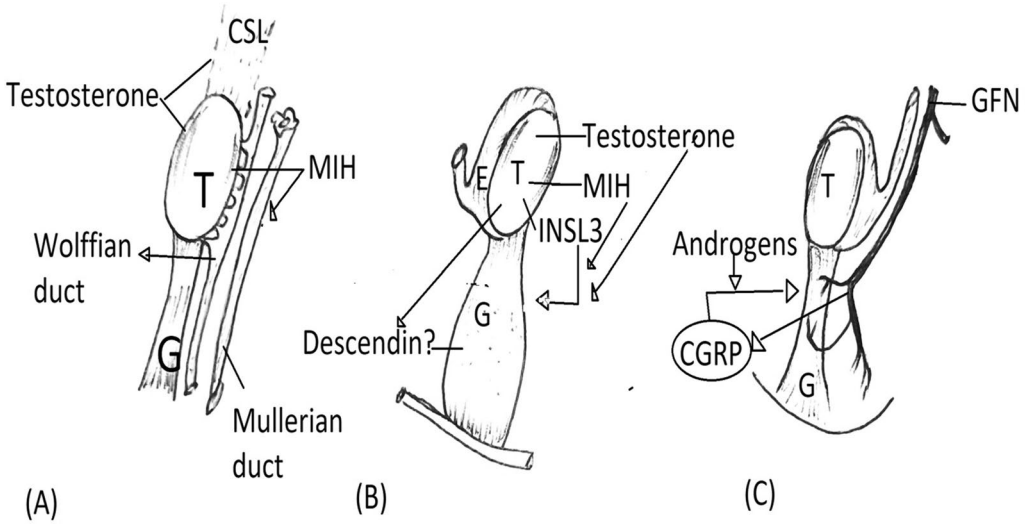

Fig. 2 Diagrammatic schema showing the hormonal factors influencing the testicular descent. **A** Shows early gonadal position in gonadal ridge. In the male foetus, MIH and testosterone causes the regression of the embryonic müllerian duct and regression of CSL, respectively. **B** MIH or Testosterone amplifies the action of INSL3 which stimulates gubernaculum enlargement. Descending is proposed to cause selective growth of gubernacular mesenchymal cells which might aid in testicular descent. **C** Androgens via CGRP released by the GFN influences the gubernacular migration and elongation in the inguinoscrotal phase

AMH production from the sertoli cells is controlled by several genes. In the male foetus, the SRY gene is essential for the activation of SOX9. The SOX9 is considered as the triggering factor for the AMH expression along with other regulating factors such as WT1, SF1, GATA4, and FSH [57]. AMH binds to type II receptor, AMHR-II. This receptor is expressed on the mesenchymal cells surrounding the müllerian duct. It is also expressed in sertoli cells, granulosa cells as well as leydig cells [58, 59]. AMH secreted by sertoli cells plays a significant role in the phase I of testicular descent by inducing the regression of the embryonic müllerian ducts in male foetuses [60]. However the study by Bartlett et al. [61] states that MIS affected the cremaster muscle development but does not appear to influence gubernaculum development and the descent in the mouse model. In addition, there are studies which observed that AMH receptor–deficient mice had normal testicular descent [13, 62]. Interestingly a novel non-androgenic factor from the Sertoli or germ cells was also suggested to be responsible for controlling gubernacular outgrowth [63].

The persistence of müllerian duct by disrupted or abnormal of secretion of AMH, or any genetic abnormality of AMH or its receptors results in persistent müllerian duct syndrome (PMDS), rare autosomal recessive condition. This condition results in highly mobile testis, intra-abdominal testis in ovarian position or para renal testis together with persistent uterus and fallopian tubes, in phenotypically normal 46 XY male infant or adult [10, 54]. It is suggested that the mutation interferes with the normal gubernacular morphological features such as shortening and swelling, instead a long and thin cord resembling to round ligament is observed [21]. It is believed that in PMDS there is a failure in gubernacular swelling reaction which eventually leads to cryptorchidism [64]. Currently there is a vast body of the literature existing on the role of AMH, but nevertheless disparity still remains between the theories proposed and our knowledge on the exact regulation of the AMH still remains unknown.

2.6.2 Insulin-like factor 3

INSL3 initially designated Leydig insulin-like (LeyI-L) [65] and relaxin-like factor (RLF) [66] belongs to insulin-like superfamily. This is expressed in pre- and post-natal leydig cells of testis and post-natal thecal cells of ovary and is supposedly controlled by hCG and LH [37, 65]. Though studies imply its regulation by LH, it is still not clear whether it is directly or via the intratesticular testosterone [67]. The receptor for INSL3 is relaxin family peptide receptor 2 (RXFP2) which was formerly known as leucine-rich repeat-containing G-protein coupled receptor 8 (LGR8). G-protein-coupled receptor affecting testis descent (GREAT) is an alternative name for this protein with regard to its role in testicular descent [9].

The testicular leydig cells produce INSL3 in all mammalian species including the humans [65, 67]. In humans three types of leydig cell population are observed (1) foetal (2) perinatal and (3) adult. INSL3 is expressed in all these three populations and testosterone follows the same pattern [67]. This implies that INSL3 demonstrates the phase of leydig cell differentiation. Study by Bay et al. [37] reported that measurable INSL3 levels in amniotic fluid from male foetuses were reported around 15–21 weeks were their levels may peak at or before 15 weeks of gestation. Interestingly, this time frame was the INSL3 were detected coincides with that of gubernacular outgrowth and subsequent processus vaginalis development. This study also reported the hormone was absent in case of all female foetuses [37].

The study on foetal rat testis showed that INSL3 and DHT have pronounced effect on the gubernacular growth [18]. The action of INSL3 is amplified either by the MIH or DHT [24]. The disruption of this hormone or its receptor results in bilateral intra-abdominal perirenal testes and impaired gubernaculum development [18]. These observations indicate a role of INSL3 in the growth and differentiation of the gubernaculum in male mice [36]. Moreover in the female mice the disruption of the Insl3 gene resulted in impaired fertility associated with deregulation of the oestrus cycle [19]. In addition their over-expression in transgenic female mice-induced gubernaculum morphology similar to male and their descent near to the bladder neck [68]. The study by Johnson et al. [69] states that INSL3 mediates gubernacular development by inducing production of extracellular ligands and glycoprotein. The study also demonstrated INSL3 signalling in the foetal gubernaculum both in vitro and in vivo comprises growth factor or morphogenetic signalling pathways, including HGF, WNT, and BMP [69]. Though all these studies strongly indicate the significant role of INSL3, there are other equally important studies which questions the role of INSL3. The mutation analysis of human Great gene in 60 cryptorchid patients showed a unique missense mutation in the ectodomain of the GREAT receptor, in only one patient [70]. In addition the study by Gorlov [70] suggested INSL3 may play a role by regulating trophic interactions between muscle cells and developing axons implying their role may be indirect in gubernacular outgrowth.

There are many supporting studies on the role of INSL3 and its receptor, GREAT/LGR8 in the testicular descent and cryptorchidism however its exact regulation is unclear so is the various other regulatory factors. It has to be acknowledged that the studies showing mutations in INSL3 or RXFP2 possibly causing the cryptorchidism

are reported in isolated human cryptorchidism cases. Another conflict is whether the INSL3 action is androgen dependant or independent as the literature provide studies supporting the both models. Hence additional studies are required to understand its role in the aetiology of human cryptorchidism. Assessing the literature it appears that for testicular descent both INSL3 and androgen are required and neither one of them alone is not sufficient for the process.

2.6.3 Androgens

It is generally believed the most important mechanism for the descent of the testes is the hormonal stimulation which induces gubernacular reaction. The two crucial hormones produced by the foetal leydig cells are testosterone and insulin-like peptide 3 (INSL3) whose role in testicular descent is intriguing. Bouin and Ancel (1903) first suggested that interstitial leydig cells produce androgens [71]. Leydig cells begin to produce testosterone as early as 6–7 weeks of gestation [72]. Testosterone is necessary for the virilisation of male reproductive tract and in differentiation of the Wolffian duct in to epididymis, ductus deferens, and seminal vesicle [13]. It is also important for the differentiation of the male external genitalia and the prostrate. The androgen secretion by the foetal leydig cell is first influenced by the human chorionic gonadotropin (hCG) and later by the luteinizing hormone (LH) produced by the anterior pituitary. It was during 1980s the hypothesis suggesting the role androgens in the second phase of descent became stronger. Yet the relative physiological role of testosterone versus Dihydrotestosterone still remains poorly understood [21].

In order to understand what makes role of androgen still debatable understanding both the supporting and contradicting theories in the literature is important. The androgen effects are mediated via by the ligand binding to the intracellular nuclear androgen receptor (AR). The study by Hutson [25] suggested the role of androgen in the inguinoscrotal phase of testicular descent. The study reported that in mice with androgen sensitivity, by the time of birth the testes descended normally to the inguinal ring but not to the scrotum suggesting that the second phase is androgen dependent. The prenatal treatment of mice with flutamide (antiandrogen) resulted in cryptorchidism also implies role of androgen in the testicular descent [26]. The gubernaculum failed to elongate, evert or migrate in to the scrotum as it does normally and remained at or just beyond the external inguinal ring in the androgen resistant mice [73]. Although all these studies allude the significance of androgens on the gubernaculum and consequently on its role on the second phase of descent they lacked in elaborating the exact mechanism involved.

Later came the hypothesis that androgen acts not directly but indirectly on the gubernaculum during the second phase of testicular descent. The theory was based on the assumption that the androgen may act indirectly via the genitofemoral nerve (GFN) to regulate the descent and involves masculinization of the nerve. The masculinization results in the increased release of CGRP. The study by Beasley and Hutson [44] stated that the GFN upon the stimulation from the androgen releases the neurotransmitter CGRP, which plays a crucial role in inguinoscrotal descent by acting as a second messenger, because the transection of the nerve in neonatal rat showed failure of gubernacular descent to the scrotum [45]. The CGRP causes rhythmic contractions of the gubernaculum thus assisting in the descent. The study by Shenker et al. [74] also showed the significance of androgen stimulation on the genitofemoral nerve. Their study reported that there was no increase in gubernacular cell proliferation when exogenous CGRP added to gubernaculum from flutamide-treated rats, suggesting that the androgens are necessary to pre-programme the proliferative response of the gubernaculum to CGRP. All these studies indicate the possible role of androgens in the testicular descent. However the study by Van der Schoot [35] refutes this hypothesis as they have reported that there was no effect of flutamide on either phase of descent implying that androgens are not necessary for the descent.

Despite the strong evidences by many studies suggesting the vital role of androgens in testicular descent, the conflict that still persists in the literature is, whether androgens initiate the gubernaculum regression or its outgrowth in the second phase of descent? Do they act indirectly or directly on the gubernaculum to cause the descent? The answer to these questions however still remains unclear. The reason could be that the second phase of testicular descent is a very intricate phase with vast number of factors interplaying to bring about the complex process of testicular descent.

2.7 Cryptorchidism

Given the route testes has to travel to reach its final destination, the scrotum and the various factors interplaying in aiding the process it's understandable why cryptorchidism happens to be the most common birth defect in male children. An undescended testis or cryptorchid testis may be situated along its normal route of descent or in an ectopic position. Though the exact cause of cryptorchidism is not known the studies prove that it could be the disruption of any one anatomical or hormonal factor or the combination of both. The significant anatomical remodelling happens in the inguinoscrotal phase with contribution from many hormonal factors and therefore it appears to be the most affected of the two phases.

Apart from these factors, environmental factors may also aid to this pathological condition. It is believed that there are many factors like pesticides, phthalates and bisphenol that can interfere with normal androgen synthesis and function as they have estrogenic effects [18]. Abnormal action of the hypothalamic–pituitary–testicular axis, disruption in normal testicular differentiation, low production of placental gonadotropin, disruption of synthesis and action of androgens, INSL3 factor and AMH or their receptors are few factors causing cryptorchidism. In addition conditions like low birth weight, maternal exposure to oestrogens during the first trimester, inherited X-chromosome-linked anomalies, aberrations of ipsilateral GFN regulation, increased GGN repeat length of the androgen receptor gene causing decreased androgen receptor function are all associated with high incidence of undescended testes [24, 29]. Although we know lot about the undescended testes and their possible causes, the exact regulation is still not known as well as there are conflicts in the choice of first line of treatment and the most advantageous time for implementing the same. While many suggest surgical intervention as first line treatment others still believe hormonal therapy shows results or it could be the combination of both yet it all depends upon the location of testes. It cannot be denied that the hormonal therapy has controversies surrounding it. Cryptorchidism is one of the factors under the testicular dysgenesis syndrome which also comprises hypospadias, reduced semen quality, and testicular cancer. All these conditions are thought to have common origin in prenatal testicular maldevelopment which affects leydig, sertoli and germ cell development [19]. Studies have also reported maldevelopment to some extend in seminiferous tubules including sertoli cell only tubules and spermatogenic arrest associated with undescended testis [75]. It is estimated that in men 5–10% risk of testicular cancer is associated with history of persistent cryptorchidism [25].

UDT (undescended testes) occurs in up to 4–5% of males at birth and if left untreated, cryptorchidism may lead to torsion, impaired fertility caused by the disrupted spermatogenesis, and a higher incidence of testicular cancer in later life [24, 25]. Unilateral undescended testis is four times more common than bilateral [19]. UDT can be classified in to (a) unilateral or bilateral (b) congenital and acquired (c) palpable and non-palpable. The classification of the UDT on the basis of testicular location, may be anywhere along the normal course of descent (Fig. 3), i.e. abdominal, inguinal canal, external inguinal ring, prescrotal and suprascrotal or ectopic, when testes takes unusual way than the normal pathway of descent [19, 76].

There is a promising wealth of the literature suggesting environmental factors or endocrine disrupters being the potent risk factors of cryptorchidism. The studies in male animals exposed in utero or perinatally to exogenous oestrogens (diethylstilboestrol, ethinyl oestradiol, bisphenol A) and anti-androgens [flutamide,1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene (DDE), 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT)] developed hypospadias, undescended testis, low sperm counts teratomas and leydig cell tumours [39, 75]. The other factors linked to cryptorchidism are disruption in leydig cell hormones which includes testosterone and INSL3. Bilateral cryptorchidism was observed in INSL3−/− mice because of small undifferentiated gubernaculum without a central core of mesenchyme. This results in testes remaining high in abdomen near to kidneys [27, 45]. Factors such as 17α- and β-estradiol, diethylstilbestrol (DES) and maternal exposure to oestrogens have been suggested to cause down regulation of INSL3 [39] interestingly though many studies points out significance of INSL3 in testicular descent, only a minority of patients with cryptorchidism show mutations in the INSL3 gene [24].

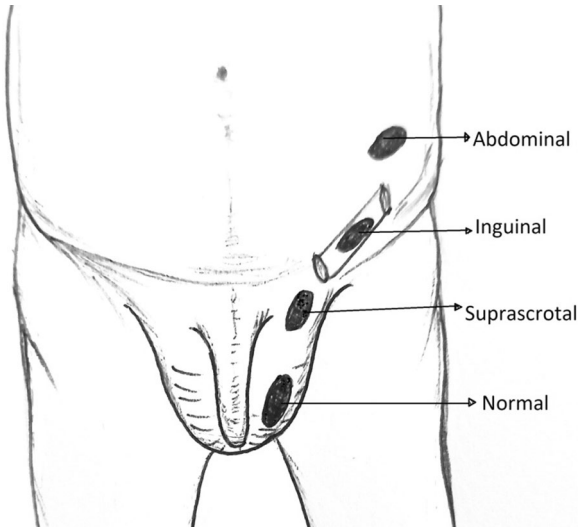

Fig. 3 Diagrammatic schema showing the locations of cryptorchid testis along the normal course of descent

The failure of complete disappearance of the processus vaginalis was suggested as a common cause of acquired UDT which would also result in inguinal hernia (widely patent processus vaginalis), hydrocele (communicating or non-communicating where narrow processus vaginalis persists), and ascending/retractile testes (partially obliterated lumen but persistence of processus vaginalis). Cryptorchidism is also commonly observed in boys with malformations of brain such as microcephaly because of disruption in the hypothalamic–pituitary–testicular axis which could be due to impaired androgen production and function [25]. Studies in the rodent model had reported

that the excess oestrogens can inhibit trans-abdominal descent and reduce the amount of extracellular matrix and size of the gubernaculum [24]. HOXA gene has been reported to play a role in inguinoscrotal descent by acting on the caudal end of gubernaculum. Disruption of Hoxa10 genes in the mouse model caused cryptorchidism; however, their role in human model is unclear [18]. The genetic defect in AMH gene or its receptor resulted in PMDS with long gubernaculum and undescended testes [19, 65]. However dispute still remains regarding its action on gubernaculum. Testicular migration may be disrupted by androgen deficiency resulting in the undescended testes [25]. The mutations of Wnt-5a or Ror2 are also associated with undescended testes [29]. It was also observed that boys with severe hypospadias are also at risk of having cryptorchidism, which is likely caused by prenatal androgen disruption.

3 Conclusion

In conclusion testicular descent with no doubt is a very fascinating and complex process with many coexisting factors playing their roles harmoniously to bring about such an intricate process. In the recent times, the role of INSL3 and CGRP in the testicular descend has been widely explored and documented however the androgenic influence in the second phase of descend still remains controversial. The aetiology of cryptorchidism still is elusive, though some studies propose the cause is related to the genetic mutation of the genes RXFP2 or INSL3. Recent advances in understanding the testicular descent might also pave way to in-depth comprehension of causes and possible novel treatment plans of cryptorchidism. Though a vast wealth of the literature is available for the factors influencing this event, disparity among the studies still persists. It cannot be overlooked that cross species differences might also add to the discrepancies. However, they do fuel in rethinking of our ideologies of testicular descent and make a way for more exciting studies in future.

Abbreviations

AMH	Anti-müllerian hormone
MIS	Müllerian-inhibiting substance
DHT	Dihydrotestosterone
CSL	Cranial suspensory ligament
INSL3	Insulin like factor 3
Leyl	L-Leydig insulin-like
RLF	Relaxin-like factor
RXFP2	Relaxin family peptide receptor 2
LGR8	Leucine-rich repeat-containing G-protein coupled receptor 8
CGRP	Calcitonin gene-related peptide
TGF	β-Transforming growth factor-beta
AMHR-II	Anti-müllerian hormone receptor-II
PMDS	Persistent müllerian duct syndrome
LH	Luteinizing hormone
hCG	Human chorionic gonadotropin
DES	Diethylstilbestrol
BMP	Bone morphogenetic protein
HGF	Hepatocyte growth factor
WNT	Wingless related integration site
HPT	Hypothalamic–pituitary–testicular
AR	Androgen receptor
GFN	Genitofemoral nerve
UDT	Undescended testes
DDE	Flutamide,1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene
DDT	1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane
LHRH	Luteinising hormone releasing hormone
GnRH	Gonadotropin releasing hormone

Acknowledgements

We thank Dr. Deepak N Modi, *FNASc*, Professor and Head Department of Molecular and Cellular Biology laboratory, National Institute of Research in Reproductive Health India, for his insights and elaborative discussions on the interesting aspects of testicular descent.

Author contributions

All the authors contributed significantly to this paper and met the authorship criteria. Concept, design, literature search, data acquisition and analysis, manuscript preparation, manuscript editing were done by SER. Concept, design, data analysis, manuscript editing, and manuscript review were done by AM. Data analysis, manuscript editing, and manuscript review were done by MMP. All authors read and approved the final manuscript.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 27 March 2021 Accepted: 19 February 2023
Published online: 02 March 2023

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