

## Review Article

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# Undescended testicle: An update on fertility in cryptorchid men

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Cryptorchidism or undescended testis is one of the most common anomalies encountered in paediatric urology and is estimated to affect 1 to 4 per cent of full term and upto 30 per cent of preterm male neonates. The associated problems of sub-fertility or infertility and malignant transformation have been recognized for long. Fertility is impaired after both unilateral and bilateral cryptorchidism. The reported paternity rates in adults are about two-third for unilateral undescended testis and less than one-third for bilateral disease. Over the last five decades, the concepts related to cryptorchidism have changed dramatically as knowledge about its effects has accrued from research conducted worldwide. The recommended age of orchidopexy has fallen progressively from adolescence to less than one year. The realization that the infantile testes are not in a state of 'suspended animation' and the recognition of the defect in the androgen dependent transformation of gonocytes into adult dark spermatogonia in cryptorchidism have been recognized as the primary cause of sub-fertility in these patients. This has paved the way for hormone therapy in an attempt to simulate the 'post-natal gonadotropin surge' or 'mini-puberty'. This review summarizes the current knowledge about the various factors affecting the fertility status in cryptorchidism with a particular focus on the derangements in the development and maturation of the germ cells and the role of surgery, hormone therapy and antioxidants in reversing these changes.

**Key words** Cryptorchidism - fertility - hormone therapy - orchidopexy - reactive oxygen species - undescended testis

## Introduction

Undescended testis or cryptorchidism is a common anomaly encountered in paediatric urology and is estimated to affect 1 to 4 per cent of full term and up to 30 per cent of preterm male neonates<sup>1</sup>. Ever since the first description of this condition by Hunter in 1786<sup>1</sup>, a lot of research has been done to understand the aetiopathogenesis, morphogenesis and the molecular and hormonal milieu associated with undescended testis with an impact on the functional outcomes

and prediction of complications in the long term. Cryptorchidism, especially bilateral, is associated with impaired spermatogenesis, endocrine derangements and increased risk of testicular malignancy<sup>1</sup>.

## Infertility in cryptorchidism

Fertility is impaired after both, unilateral or bilateral cryptorchidism. It has been quoted that around 90 per cent of patients with untreated bilateral cryptorchidism ultimately develop azoospermia as against the reported 0.4 to 0.5 per cent incidence in the general population<sup>2</sup>.

The incidence of azoospermia drops to 32 per cent in medically managed patients and to 46 per cent after bilateral orchidopexy<sup>1,2</sup>. The incidence of azoospermia in unilateral cryptorchidism is 13 per cent regardless of the fact as to whether the condition is corrected. About 10 per cent of infertile men from the general population will have a history of cryptorchidism and orchidopexy<sup>1</sup>.

Several old studies have documented reduced fertility in patients with cryptorchidism using various criteria such as paternity, hormones or semen data<sup>3-7</sup>. Lee *et al*<sup>8,9</sup> have demonstrated that infertility in patients with unilateral cryptorchidism is two times more common than the general population. Furthermore, infertility amongst patients with bilateral cryptorchidism is about 3.5 times more frequent than the unilateral group and more than 6 times as frequent among the general population<sup>8,9</sup>. The reduced fertility has been ascribed to the reduction in the total number of germ cells and to defective pre-pubertal germ cell maturation. There is a spectrum of testicular functions amongst these patients ranging from normal to mildly deranged spermatogenesis to severe dysfunction and the chances of fertility are related to the degree of functional derangement<sup>10</sup>.

### Critical steps in germ cell maturation

Primitive germ cells are present in the testes at the time of birth and are not in 'suspended animation' as thought previously<sup>11</sup>. The testis-specific gene activation leads to a timed sequence of events which include regulated cell proliferation and differentiation of spermatogonia, meiosis and haploid differentiation or spermiogenesis<sup>12</sup>.

*Appearance of primordial germ cells (PGC) or gonocytes:* The embryologic origin of the sperm can be traced back to the PGCs which are formed in the epiblast during the second week and move to the wall of the yolk sac. These migrate towards the developing gonads by the end of the fifth week. Mitosis continues during and after migration resulting in proliferation. PGCs or the gonocytes act as foetal reservoir of stem cells.

*Disappearance of gonocytes (foetal stem cell pool) and appearance of adult dark (Ad) spermatogonia (adult stem cell pool):* This is the first major step in the maturation of the hypothalamic-pituitary-testicular axis and is accompanied by establishment of adult stem cell pool which replaces the foetal stem cell pool and a

dramatic reduction in the total number of germ cells per tubule. The Ad spermatogonia exhibit a characteristic dark (electron-dense) cytoplasm and a bright nuclear spot. The transformation starts at 2-3 months of age and is normally complete by six months. The transformation is believed to be a consequence of a transient surge in the serum hormonal levels (follicular stimulating hormone or FSH, luteinizing hormone or LH and testosterone); this phase has been labelled as 'mini-puberty'. Almost simultaneous with the hormonal surge, there is an increase in the testicular weight and volume. The Ad spermatogonia once formed persist for the rest of life. This process is sensitive to minor genetic aberrations and to adverse environmental conditions; consequently not all neonatal gonocytes are transformed into the Ad spermatogonia and the remaining gonocytes undergo apoptosis<sup>2</sup>.

*Transient appearance of primary spermatocytes and the prophase of first meiotic division:* This is the second crucial step in the maturation of the hypothalamic-pituitary-testicular axis and occurs at 4-5 yr of age. It is characterized by the transient onset of meiosis and histological appearance of primary spermatocytes with a transient rise in both the germ cell count and Ad spermatogonia count. Spermatogenesis arrests at this stage and resumes after the onset of puberty.

*Factors contributing to infertility in patients with cryptorchidism:* Infertility in patients with cryptorchidism may be multifactorial and related to the aetiology of testicular maldescent, age at the time of surgical correction (duration of uncorrected cryptorchidism) and consequences of the treatment of cryptorchidism. It has been proposed that both the steps in the maturation of the hypothalamic-pituitary-testicular axis are abnormal in undescended testis<sup>13</sup>. The contralateral descended testis is also affected by similar changes but to a milder extent.

*Failure of transformation of gonocytes into Ad spermatogonia:* Hadziselimovic and colleagues<sup>14</sup> have suggested that the disappearance of gonocytes (foetal stem cell pool) and appearance of Ad spermatogonia (adult stem cell pool) may be a prerequisite for the normal future spermiogenesis and fertility. This transformation is delayed and ineffective in cryptorchidism and leads to delay in the establishment of the adult stem cell pool and prolonged persistence of the foetal stem cell pool. Besides this, the reduction in the number of germ cells per tubule does not take place during this phase and the germ cell count continues to

be high as late as the beginning of the second year of life giving a 'false' impression of histologically normal cryptorchid testis. Thereafter, the total number of germ cells falls below normal.

A testicular biopsy at the time of orchidopexy in boys with cryptorchidism older than two years showed a lower germ cell count per tubule in 10-40 per cent of the boys<sup>15</sup>. Hadziselimovic *et al*<sup>16</sup> observed that the sperm count was 7-fold higher in unilateral cryptorchid boys who had demonstrated the presence of Ad spermatogonia in the testicular biopsy as compared to the other group. In boys with bilateral cryptorchidism with Ad spermatogonia on biopsy, the median sperm count was 88-fold higher than in boys with absence of Ad spermatogonia. A 3-fold higher sperm count was seen in patients who underwent orchidopexy before the age of three years as compared to those operated after the age of eight years<sup>16</sup>. The same group<sup>17</sup> observed that the sperm counts after puberty correlated with the number of Ad spermatogonia found at the time of orchidopexy. Analysis of 178 testicular biopsies from 89 boys who were subjected to orchidopexy and bilateral testicular biopsy indicated three groups of high, intermediate and low risk of fertility based on the presence of Ad spermatogonia. All males in the high risk of infertility group turned out to be oligospermic (mean:  $8.9 \times 10^6$  sperms/ejaculate) after puberty, and 20 per cent of them were azospermic. These patients had 25 times lower sperm counts as compared to the group with presence of Ad spermatogonia in bilateral testis<sup>17</sup>. Correlation between the testicular histology and post-pubertal hormonal levels confirmed a relative gonadotropin deficiency in most of these patients.

*Failure of hormonal surge:* A surge in luteinizing hormone releasing hormone (LHRH) causes release of LH which stimulates the release of testosterone. The testosterone in turn, triggers the maturation of the germ cells and establishment of an adequate size of adult stem cells. Gendrel *et al*<sup>18</sup> have shown that the normal surge in LH and testosterone at 2-3 months of age is significantly lower in patients remaining cryptorchid, either unilaterally or bilaterally than in infants with delayed spontaneous descent of one or both testes. Testicular biopsy specimens from cryptorchidism patients prone to develop azospermia display histological features of impaired mini-puberty<sup>19</sup>. Hadziselimovic *et al*<sup>17</sup> have demonstrated the presence of Leydig cell hypoplasia in cryptorchid testes and related this finding to the deficient hormonal stimulation of the Leydig cells due to defective hypothalamic-pituitary axis. The under-

stimulated Leydig cells are not capable of bringing about a testosterone surge of magnitude sufficient to effect germ cell maturation. Huff *et al*<sup>13,20</sup> have reported that the blunted neonatal surge of gonadotropin in cryptorchid boys trigger a cascade of hormonal and secondary histological abnormalities which are likely to result in a reduced fertility potential. However, the positive predictive value that bilateral cryptorchidism will have abnormally low testosterone level is only about 23 per cent<sup>21</sup>. In a study by Barthold *et al*<sup>22</sup>, no significant difference could be appreciated in the hormonal levels (testosterone, estradiol, LH and FSH in both plasma and urine, inhibin B, sex hormone-binding globulin and leptin in plasma) between the non-syndromic cryptorchid boys and controls during the activation of the pituitary-testicular axis in early infancy.

*Delayed onset of meiosis and appearance of primary spermatocytes:* This step is delayed or failed in patients with unilateral or bilateral cryptorchidism. Huff *et al*<sup>13</sup> analysed the testicular biopsies in a group of 529 unilaterally cryptorchid boys (2-9 yr old) at orchidopexy. Transient onset of meiosis with appearance of primary spermatocytes was absent in all but one out of 529 undescended testes. That patient was nine years of age and it was likely that he might have already entered puberty. There was no increase in the number of total germ cells or in the number of Ad spermatogonia suggesting reduced maturation and proliferation of germ cells. The total and differential germ cell count was significantly less in the undescended testicle as compared to the contralateral descended testis. Transient onset of meiosis with appearance of primary spermatocytes was observed in 19 per cent (101 out of 529) of the contralateral descended testes.

*Abnormal gene expression in cryptorchid boys at risk of azospermia:* The process of spermatogenesis is regulated by 2000 genes, most of which are present on autosomes. Approximately 30 genes involved in spermatogenesis are present on the Y chromosome and are exclusively involved with reproduction<sup>23</sup>. The early growth response gene (*EGR4*) which regulates the critical genes involved in early stages of meiosis and regulation of LH secretion has been demonstrated to be virtually silent in the high risk for azospermia (HAZR) group<sup>24</sup>. Similarly, *EGR1* which is preferentially expressed in the Leydig cells of the testes is also insignificantly expressed in cryptorchidism. *EGR4* is critical as a redundant transcription factor required for

sustaining male infertility when *EGR1* is mutated in the germline<sup>24,25</sup>.

Hadziselimovic *et al*<sup>19</sup> analyzed whole genome expression signatures of undescended testes at risk of developing azoospermia. They identified 483 genes which were not expressed or under-expressed in the azoospermia risk group as compared to the control group or patients with low risk for azoospermia (LAZR). It was observed that several genes important for the meiotic and post-meiotic stages of spermatogenesis can be detected in Ad spermatogonia positive (Ad+) but not in Ad spermatogonia negative (Ad-) prepubertal testis. The molecular events initiating the testicular expression programme at the onset of puberty and maintaining it during adulthood occur very early in the pre-puberty testis and were impaired in the LAZR group lacking Ad spermatogonia. Transcriptional activity for several genes implicated in spermatogenesis and fertility was demonstrated in Ad+ but not in Ad- testes.

Hadziselimovic *et al*<sup>26</sup> further observed that uncontrolled transposon activity inducing genomic instability and germ cell death may be responsible for the decreased germ cell count in cryptorchid boys with impaired mini-puberty. They observed that five of eight genes that are important for transposon silencing were not expressed in the high azoospermia risk group of cryptorchid boys but were expressed in the low azoospermia risk and control groups.

### **Surgery for undescended testes: Implications for fertility**

The mode of treatment for undescended testes has been debated for long. Recently, a group of specialists in various related disciplines from the Nordic countries summarized the available information from literature, dwelled upon the pros and cons of different treatment modalities and framed a consensus on the management of undescended testes<sup>27,28</sup>. The group suggested that efforts should be made to ensure descent of the retained testis. The small difference of 2-3 degree centigrade between the abdomen and the scrotum is detrimental to normal spermatogenesis<sup>7</sup> and fertility in the long-term. Studies have revealed that the placement of the testis into the scrotum before the age of 13 yr reduces the risk of malignancy significantly<sup>29</sup>. The increased susceptibility of an undescended testis to testicular torsion or injury and the associated psychological stigma are other concerns. The group evaluated the meta analyses of the available randomized controlled studies comparing the 'hormonal therapy' with

orchidopexy<sup>30-32</sup>. The overall efficacy of hormonal treatment was around 20 per cent which dropped to 15 per cent in the follow up due to secondary re-ascent while the overall efficacy of primary orchidopexy was 95 per cent. Considering the poor efficacy of hormonal treatment and its potential adverse effects on spermatogenesis<sup>33,34</sup>, the group preferred orchidopexy over hormonal therapy for testicular descent.

The recommended age of orchidopexy has fallen progressively over the past five decades. In 1986, the American Academy of Pediatrics<sup>28</sup> recommended surgery at 4-6 yr of age. However, with the realization that the number of germ cells per tubule starts to decline below normal at 1-2 yr of age, the recommended age was lowered to one year in 1996<sup>28</sup>. Based on sonographic parameters, Kollin *et al*<sup>35</sup> documented the beneficial effect of orchidopexy at nine months of age on the growth of previously undescended testes. Kollin *et al*<sup>36</sup> further compared the growth of congenital, unilaterally undescended testes following orchidopexy at age nine months or three years. They used the testicular volume as an approximate indirect measure of spermatogenic activity and documented that surgical treatment at nine months resulted in partial catch-up of testicular growth until at least age four years compared to surgery at three years, indicating that early surgery had a beneficial effect on testicular growth. The Nordic group<sup>27</sup> made a consensus that orchidopexy be performed prior to one year of age for maximum preservation of potential for future fertility. If the condition is diagnosed later in life, surgery should be done at the earliest.

### **Hormonal therapy for undescended testis: Implications for fertility**

Cortes<sup>15</sup> observed that 10-40 per cent of cryptorchid boys older than two years of age lacked germ cells on testicular biopsy at the time of orchidopexy. A biopsy without germ cells is associated with 33-100 per cent risk of infertility. The number of spermatozoa per tubule is prognostic for subsequent fertility potential<sup>29</sup>. Furthermore, cryptorchid boys with fewer than 0.2 cells per cross-section of the seminiferous tubules have a relatively higher probability of being infertile after puberty regardless of other factors.

The realization that infertility in cryptorchidism is related to the effacement of the hormonal surge at mini-puberty resulting in impaired transformation of gonocytes into foetal spermatogonia has paved the way for the 'fertility oriented' hormonal therapy in cryptorchidism<sup>14-17</sup>.

*Pre-orchidopexy hormone therapy:* Hadziselimovic *et al*<sup>37</sup> demonstrated that there was a significant increase in the number of germ cells in the testes of patients with both unilateral and bilateral cryptorchidism after treatment with alternate day Buserelin regime (LHRH analogue) for 6 months. Older subjects (>7 yr of age) exhibited a slight though significant rise in testosterone in the first morning-voided urine at the end of treatment.

In a randomized, double-blinded, placebo-controlled study buserelin was shown to be capable of inducing testicular descent in addition to increasing simultaneously the number of germ cells and provoking further development of the epididymis<sup>38</sup>. Foresta *et al*<sup>39</sup> demonstrated that human recombinant-erythropoietin acted directly on the human Leydig cells and influenced testicular steroidogenesis by stimulating testosterone production in man. Cortes *et al*<sup>40</sup> further demonstrated that erythropoietin administration in two cryptorchid boys resulted in a higher number of spermatogonia per tubular cross-section in the testicular biopsies as compared to the control group. There was no carcinoma-*in-situ* pattern. Another study also demonstrated a significant rise in the number of spermatogonia per tubule after administration of buserelin (nasal spray @20µg/day for 28 days) followed by hCG (intramuscular injection @1500 IU once a week for 3 wk) or hCG (intramuscular injection @1500 IU once a week for 3 wk) alone<sup>41</sup>. Salvage of active germinal tissue and a significantly higher mean fertility index after neoadjuvant gonadotropin-releasing hormone (GnRH) therapy (nasal spray @1.2 mg/day for 4 wk) were demonstrated prior to orchidopexy<sup>42</sup>. The best results were seen in boys younger than 24 months. Jallouli *et al*<sup>43</sup> also demonstrated in a prospective randomized control trial that neoadjuvant GnRH treatment improved the fertility index in prepubertal unilateral cryptorchidism and consequently, should improve fertility in adulthood.

However, the efficacy of neoadjuvant hormone therapy on the fertility status in cryptorchid patients has not been reported universally and there are reports that counter the projected beneficial effects. Cortes *et al*<sup>44</sup> reported a higher number of spermatogonia per tubule in patients who underwent direct orchidopexy as compared to those who received pre-operative human chorionic gonadotropin (hCG) or GnRH in an attempt to bring about descent of the testis. They have suggested that in 1 to 3 yr old boys with cryptorchidism GnRH or hCG given for testicular descent may

suppress the number of germ cells. Significantly better sperm counts have been reported in patients who underwent orchidopexy as compared to those who were administered hormones<sup>45</sup>.

*Post orchidopexy hormone therapy:* Hadziselimovic *et al*<sup>46</sup> observed that GnRH administration after orchidopexy might result in improved fertility indices in patients who did not respond to intramuscular hCG therapy directed towards bringing about testicular descent. Testicular biopsy performed at the time of orchidopexy demonstrated <0.2 spermatogonia per tubular cross-section in all cases. Administration of intra-nasal buserelin (10 µg on alternate days for 6 months) resulted in significant increase in the total number of spermatozoa per ejaculate, number of normal forms of spermatozoa and sperm motility as compared to control group which did not receive hormonal treatment after successful orchidopexy<sup>46</sup>. In another study, Hadziselimovic<sup>47</sup> studied 15 unilateral cryptorchid boys who after successful orchidopexy (& testicular biopsy) between the ages of 1-6 yr were administered buserelin (10 µg intranasal spray on alternate days for 6 months). At a mean follow-up of 19 yr of age, they all had Tanner V stage of sexual development, normal erectile function and the average sperm concentration was significantly higher as compared to the controls. There were no adverse effects and no changes in the Tanner stage of pubertal development during the hormonal treatment. Huff *et al*<sup>48</sup> reported similar results with the GnRH analogue Naferelin.

*Effects of neoadjuvant hormone therapy on the contralateral descended testis:* The beneficial effects of hormone therapy in bringing about testicular descent in patients with primary cryptorchidism are well established. There are hardly any studies on the effects of the administered hormones on the contralateral supposedly normal testis in these patients who experience testicular descent in response to hormones administered and an orchidopexy is not subsequently warranted. Zivkovic *et al*<sup>49</sup> have demonstrated the beneficial effects of hormonal therapy aimed at testicular descent on the histology of the contralateral descended testis without any adverse effects on the germ cells. The number of germ cells per tubule in the contralateral descended testis of patients who experienced testicular descent in response to hormone therapy was significantly higher than the count in those who were subjected to direct orchidopexy. Seven weeks of hormonal therapy induced a rise in the number of

germ cells per tubule in the contralateral descended testis. It was also beneficial for the number of adult dark spermatogonia per tubule and the number of primary spermatocytes, although these differences did not reach significance.

*Adverse effects of hormone therapy on future spermatogenesis and fertility potential:* Hjertkvist *et al*<sup>50</sup> demonstrated that a single large dose of hCG resulted in a marked rise in the intra-testicular pressure (approximately 40 mm Hg), interstitial oedema and leukocyte extravasation in cryptorchid rats. This may be related to the increased vascular permeability coupled with insufficient lymph drainage in the cryptorchid testis or to vasomotor inhibition.

Bergh *et al*<sup>51</sup> have demonstrated that hCG treatment in rats results in increased testicular interstitial fluid volume, formation of inter-endothelial cell gaps in post-capillary venules and increased macromolecular permeability in these vascular segments within four hours. Post-hCG treatment, they demonstrated leucocytes adhering to the endothelium in the post-capillary venules and leak of dextran from these venular segments into the interstitium. The post-hCG testicular behaviour was compared to tissue oedema in inflammation.

Chandrasekharam *et al*<sup>52</sup> documented experimentally that varying doses of prepubertal hCG administration in male prepubertal Wistar rats adversely affected both the testosterone levels and the germ cell haploid cell population. hCG induced testicular inflammation in rats via local activation by Leydig cells and production of pro-inflammatory cytokines by resident macrophages have also been demonstrated<sup>53</sup>. The authors speculated the possibility that repeated high pharmacological doses of hCG being used to treat boys with cryptorchidism might result in cytokine-mediated testicular inflammation and could affect the function of the testis adversely. Similar morphology has also been demonstrated in the biopsy obtained at the time of orchidopexy in human beings at the end of unsuccessful hCG treatment<sup>54</sup>. Dermirbilek *et al*<sup>55</sup> also demonstrated a mild, inflammation-like reaction in the cryptorchid testes in the period immediately following the last hCG injections. Six to nine months later, most of the changes regressed except for the volume density of blood vessels, interstitial bleeding and diameter of the seminiferous tubules.

Heiskanen *et al*<sup>56</sup> have demonstrated that hCG (and/or androgen) withdrawal increases germ cell

apoptosis in the human testis. In another study, 25 adult men with a history of cryptorchidism, 15 of whom had a history of hCG therapy were studied for apoptotic DNA fragmentation in testicular biopsy specimens taken during orchidopexy. Only a few scattered apoptotic spermatogonia were seen by end-labelling of biopsies from patients not treated with hCG whereas more extensive labelling of spermatogonia was seen after hCG treatment<sup>34</sup>. The low molecular weight DNA fragmentation correlated negatively with the testis volume and positively with the serum FSH levels 20 years after biopsy. This suggests that the normal development of the testis is disrupted by the hCG treatment, possibly through apoptosis<sup>34</sup>.

### **Role of antioxidants in cryptorchidism: Implications for fertility**

Subfertility in cryptorchidism has also been ascribed to the inguinal heat stress<sup>57</sup> which induces intratesticular generation of reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical, nitric oxide and hydrogen peroxide<sup>58</sup> in association with reduction in endogenous antioxidant enzymes such as the superoxide dismutase and catalase<sup>59</sup>. The high degree of chemical reactivity associated with these ROS could stimulate lipoperoxidation thereby causing deleterious changes in cell membrane lipoprotein complexes in addition to testicular damage, reduced spermatogenesis and subfertility. It was hypothesized that the xanthine oxidase system could be responsible for the generation of these ROS and demonstrated attenuation of experimental cryptorchidism-induced testicular regression with xanthine oxidase inhibitors allopurinol and BOF-4272<sup>60</sup>.

DeFoor *et al*<sup>57</sup> examined the time course of apoptosis in the Hoxa 11 knockout mouse (with bilateral cryptorchidism and uniform sterility) and demonstrated attenuation of apoptosis and improved spermatogenesis with the nitric oxide synthase inhibitor nomega-nitro -L-arginine methyl ester (L-NAME). A significant reduction of lipoperoxidation in rat testis has also been demonstrated in response to  $\alpha$ -tocopherol<sup>61</sup>. Furthermore, in the long-term, use of  $\alpha$ -tocopherol may result in an increase in the area and maturation of the seminiferous epithelium, decrease in apoptosis and histological alterations and an increase in fertility.

Human spermatogonial stem cells from cryptorchid patients can progressively differentiate into meiotic and haploid spermatids by treatment with retinoic acid and stem cell factor<sup>62</sup>. This may provide an invaluable

source of autologous male gametes for treating male infertility in azoospermic patients. Acikgoz *et al*<sup>63</sup> have demonstrated that the number of mast cells are increased in interstitial and sub-tubular locations in rats with unilateral cryptorchidism resulting in fibrosis and deterioration of spermatogenesis. Ketotifen, a mast cell blocker was effective in interruption of this process of inflammation and fibrosis before and after surgical treatment. It has been suggested that the co-existing increase in the number of mast cells in the contralateral descended testis may further be responsible for the decline in fertility potential which may be blocked with administration of a mast cell blocker.

### Conclusions

The concept of 'early orchidopexy' has established itself with a scientific background. However, orchidopexy alone is not enough to completely restore spermatogenesis and there is scope for a germinal epithelial protective substance. Altered germ cell maturation has a role to play in search for the missing link between orchidopexy and subsequent fertility. Hormonal treatment may have some beneficial effect to achieve normal transformation to adult dark spermatogonia. Although there is growing evidence advocating the use of hormone therapy with hCG or GnRH analogues as an adjunct to orchidopexy to improve the fertility prospects of cryptorchid patients, the possibility of the damaging effects of hormones on future spermatogenesis is always there. The exact role of pre- or post-orchidopexy hormone therapy is yet to be defined conclusively. The key role of intratesticular heat and ROS needs further research and has the potential to alter the management of cryptorchidism in a positive direction.

### References

- Chung E, Brock GB. Cryptorchidism and its impact on male fertility: a state of art review of current literature. *Can Urol Assoc J* 2011; 5 : 210-4.
- Hadziselimovic F, Herzog B. Importance of early postnatal germ cell maturation for fertility of cryptorchid males. *Horm Res* 2001; 55 : 6-10.
- Gross RE, Jewett TC Jr. Surgical experiences from 1,222 operations for undescended testis. *J Am Med Assoc* 1956; 160 : 634-41.
- Gilhooly PE, Meyers F, Lattimer JK. Fertility prospects for children with cryptorchidism. *Am J Dis Child* 1984; 138 : 940-3.
- Fallon B, Kennedy TJ. Long-term follow-up of fertility in cryptorchid patients. *Urology* 1985; 25 : 502-4.
- Cendron M, Keating MA, Huff DS, Koop CE, Snyder HM 3<sup>rd</sup>, Duckett JW. Cryptorchidism, orchiopexy and infertility: a critical long-term retrospective analysis. *J Urol* 1989; 142 : 559-62.
- Lee PA, Coughlin MT. Fertility after bilateral cryptorchidism. Evaluation by paternity, hormone and semen data. *Horm Res* 2001; 55 : 29-31.
- Lee PA, O'Leary LA, Songer NJ, Coughlin MT, Bellinger MF, LaPorte RE. Paternity after unilateral cryptorchidism: a controlled study. *Pediatrics* 1996; 98 : 676-9.
- Lee PA, O'Leary LA, Songer NJ, Coughlin MT, Bellinger MF, LaPorte RE. Paternity after bilateral cryptorchidism: a controlled study. *Arch Pediatr Adolesc Med* 1997; 151 : 260-3.
- Lee PA, Coughlin MT, Bellinger MF. Inhibin B: comparison with indexes of fertility among formerly cryptorchid and control men. *J Clin Endocrinol Metab* 2001; 86 : 2576-84.
- Hutson JM, Li R, Southwell BR, Petersen BL, Thorup J, Cortes D. Germ cell development in the postnatal testis: the key to prevent malignancy in cryptorchidism? *Front Endocrinol (Lausanne)* 2013; 3 : 176.
- Yan Q, Wu X, Chen C, Diao R, Lai Y, Huang J, *et al.* Developmental expression and function of DKK:1/Dkk1 in humans and mice. *Reprod Biol Endocrinol* 2012; 10 : 51-9.
- Huff DS, Fenig DM, Canning DA, Carr MG, Zderic SA, Snyder HM 3<sup>rd</sup>. Abnormal germ cell development in cryptorchidism. *Horm Res* 2001; 55 : 11-7.
- Hadziselimovic F, Thommen L, Girard J, Herzog B. The significance of postnatal gonadotropin surge for testicular development in normal and cryptorchid testes. *J Urol* 1986; 136 : 274-6.
- Cortes D. Cryptorchidism-aspects of pathogenesis, histology and treatment. *Scand J Urol Nephrol* 1998; 196 (Suppl) : 1-54.
- Hadziselimovic F, Hocht B, Herzog B, Buser MW. Infertility in cryptorchidism is linked to the stage of germ cell development at orchiopexy. *Horm Res* 2007; 68 : 46-52.
- Hadziselimovic F, Hoecht B. Testicular histology related to fertility outcome and postpubertal hormone status in cryptorchidism. *Klin Padiatr* 2008; 220 : 302-7.
- Gendrel D, Roger M, Job JC. Plasma gonadotropin and testosterone values in infants with cryptorchidism. *J Pediatr* 1980; 97 : 217-20.
- Hadziselimovic F, Hadziselimovic NO, Demougin P, Oakeley EJ. Testicular gene expression in cryptorchid boys at risk of azoospermia. *Sex Dev* 2011; 5 : 49-59.
- Huff DS, Hadziselimovic F, Snyder HM 3<sup>rd</sup>, Blyth B, Duckett JW. Early postnatal testicular maldevelopment in cryptorchidism. *J Urol* 1991; 146 : 624-6.
- Job JC, Toubanc JE, Chaussain JL, Gendrel D, Roger M, Canlorbe P. The pituitary-gonadal axis in cryptorchid infants and children. *Eur J Pediatr* 1987; 146 : S2-5.
- Barthold JS, Manson J, Regan V, Si X, Hassink SG, Coughlin MT, *et al.* Reproductive hormone levels in infants with cryptorchidism during postnatal activation of the pituitary-testicular axis. *J Urol* 2004; 172 : 1736-41.
- Hargreave TB. Genetic basis of male infertility. *Br Med Bull* 2000; 56 : 650-71.

24. Hadziselimovic F, Hadziselimovic NO, Demougin P, Krey G, Hoecht B, Oakeley EJ. *EGR4* is a master gene responsible for fertility in cryptorchidism. *Sex Dev* 2009; 3 : 253-63.
25. Kurpisz M, Havryluk A, Nakonechnyi A, Chopyak V, Kamieniczna M. Cryptorchidism and long-term consequences. *Reprod Biol* 2010; 10 : 19-35.
26. Hadziselimovic F, Hadziselimovic NO, Demougin P, Krey G, Oakeley EJ. Deficient expression of genes involved in the endogenous defense system against transposons in cryptorchid boys with impaired mini-puberty. *Sex Dev* 2011; 5 : 287-93.
27. Ritzen EM, Bergh A, Bjerknes R, Christiansen P, Cortes D, Haugen SE, *et al.* Nordic consensus on treatment of undescended testes. *Acta Paediatr* 2007; 96 : 638-43.
28. Ritzen EM. Undescended testes: a consensus on management. *Eur J Endocrinol* 2008; 159 : S87-90.
29. Pettersson A, Richiardi L, Nordenskjold A, Kaijser M, Akre O. Age at surgery for undescended testis and risk of testicular cancer. *N Engl J Med* 2007; 356 : 1835-41.
30. Pyorala S, Huttunen NP, Uhari M. A review and meta-analysis of hormonal treatment of cryptorchidism. *J Clin Endocrinol Metab* 1995; 80 : 2795-9.
31. Henna MR, Del Nero RG, Sampaio CZ, Atallah AN, Schettini ST, Castro AA, *et al.* Hormonal cryptorchidism therapy: systematic review with metaanalysis of randomized clinical trials. *Pediatr Surg Int* 2004; 20 : 357-9.
32. Ong C, Hasthorpe S, Hutson JM. Germ cell development in the descended and cryptorchid testis and the effects of hormonal manipulation. *Pediatr Surg Int* 2005; 21 : 240-54.
33. Kaleva M, Arsalo A, Louhimo I, Rapola J, Perheentupa J, Henriksen K, *et al.* Treatment with human chorionic gonadotropin for cryptorchidism: clinical and histological effects. *Int J Androl* 1996; 19 : 293-8.
34. Dunkel L, Taskinen S, Hovatta O, Tilly JL, Wikstrom S. Germ cell apoptosis after treatment of cryptorchidism with human chorionic gonadotropin is associated with impaired reproductive function in the adult. *J Clin Invest* 1997; 100 : 2341-6.
35. Kollin C, Hesser U, Ritzen EM, Karpe B. Testicular growth from birth to two years of age, and the effect of orchidopexy at age nine months: a randomized, control study. *Acta Paediatr* 2006; 95 : 318-24.
36. Kollin C, Karpe B, Hesser U, Granholm T, Ritzen EM. Surgical treatment of unilaterally undescended testes: testicular growth after randomization to orchidopexy at age 9 months or 3 years. *J Urol* 2007; 178 : 1589-93.
37. Hadziselimovic F, Huff D, Duckett J, Herzog B, Elder J, Snyder H, *et al.* Long-term effect of luteinizing hormone-releasing hormone analogue (buserelin) on cryptorchid testes. *J Urol* 1987; 138 : 1043-5.
38. Bica DT, Hadziselimovic F. Buserelin treatment of cryptorchidism: a randomized, double-blind, placebo-controlled study. *J Urol* 1992; 148 : 617-21.
39. Foresta C, Mioni R, Bordon P, Miotto D, Montini G, Varotto A. Erythropoietin stimulates testosterone production in man. *J Clin Endocrinol Metab* 1994; 78 : 753-6.
40. Cortes D, Visfeldt J, Thorup JM. Erythropoietin may reduce the risk of germ cell loss in boys with cryptorchidism. *Horm Res* 2001; 55 : 41-5.
41. Zivkovic D, Bica DT, Hadziselimovic F. Relationship between adult dark spermatogonia and secretory capacity of Leydig cells in cryptorchidism. *BJU Int* 2007; 100 : 1147-9.
42. Schwentner C, Oswald J, Kreczy A, Lunacek A, Bartsch G, Deibl M, *et al.* Neoadjuvant gonadotropin-releasing hormone therapy before surgery may improve the fertility index in undescended testes: a prospective randomized trial. *J Urol* 2005; 173 : 974-7.
43. Jallouli M, Rebai T, Abid N, Bendhaou M, Kassis M, Mhiri R. Neoadjuvant gonadotropin-releasing hormone therapy before surgery and effect on fertility index in unilateral undescended testis: a prospective randomized trial. *Urology* 2009; 73 : 1251-4.
44. Cortes D, Thorup J, Visfeldt J. Hormonal treatment may harm the germ cells in 1 to 3-year-old boys with cryptorchidism. *J Urol* 2000; 163 : 1290-2.
45. Vinardi S, Magro P, Manenti M, Lala R, Costantino S, Cortese MG, *et al.* Testicular function in men treated in childhood for undescended testes. *J Pediatr Surg* 2001; 36 : 385-8.
46. Hadziselimovic F, Herzog B. Treatment with a luteinizing hormone-releasing hormone analogue after successful orchiopexy markedly improves the chance of fertility later in life. *J Urol* 1997; 158 : 1193-5.
47. Hadziselimovic F. Successful treatment of unilateral cryptorchid boys risking infertility with LH-RH analogue. *Int Braz J Urol* 2008; 34 : 319-26.
48. Huff DS, Snyder HM 3<sup>rd</sup>, Rusnack SL, Zderic SA, Carr MC, Canning DA. Hormonal therapy for the subfertility of cryptorchidism. *Horm Res* 2001; 55 : 38-40.
49. Zivkovic D, Bica DG, Hadziselimovic F. Effects of hormonal treatment on the contralateral descended testis in unilateral cryptorchidism. *J Pediatr Urol* 2006; 2 : 468-72.
50. Hjertkvist M, Bergh A, Damber JE. hCG treatment increases intratesticular pressure in the abdominal testis of unilaterally cryptorchid rats. *J Androl* 1988; 9 : 116-20.
51. Bergh A, Rooth P, Widmark A, Damber JE. Treatment of rats with hCG induces inflammation-like changes in the testicular microcirculation. *J Reprod Fertil* 1987; 79 : 135-43.
52. Chandrasekharam VV, Srinivas M, Das SN, Jha P, Bajpai M, Chaki SP, *et al.* Prepubertal human chorionic gonadotropin injection affects postpubertal germ cell maturation and androgen production in rat testis. *Urology* 2003; 62 : 571-4.
53. Assmus M, Svechnikov K, von Euler M, Setchell B, Sultana T, Zeetterstrom C, *et al.* Single subcutaneous administration of chorionic gonadotropin to rats induces a rapid and transient increase in testicular expression of pro-inflammatory cytokines. *Pediatr Res* 2005; 57 : 896-901.
54. Hjertkvist M, Lackgren G, Ploen L, Bergh A. Does hCG treatment induce inflammation-like changes in undescended testes in boys? *J Pediatr Surg* 1993; 29 : 254-8.
55. Drmirbilek S, Atayurt HF, Celik N, Aydin G. Does treatment with human chorionic gonadotropin induce reversible changes in undescended testes in boys? *Pediatr Surg Int* 1997; 12 : 591-4.



56. Heiskanen P, Billiq H, Toppari J, Kaleva M, Arsalo A, Rapola J, *et al*. Apoptotic cell death in the normal and cryptorchid human testis: the effect of human chorionic gonadotropin on testicular cell survival. *Pediatr Res* 1996; 40 : 351-6.
57. DeFoor WR, Kuan CY, Pinkerton M, Sheldon CA, Lewis AG. Modulation of germ cell apoptosis with a nitric oxide synthase inhibitor in a murine model of congenital cryptorchidism. *J Urol* 2004; 172 : 1731-5.
58. Ishii T, Matsuki S, Iuchi Y, Okada F, Toyosaki S, Tomita Y, *et al*. Accelerated impairment of spermatogenic cells in SOD1-knockout mice under heat stress. *Free Radic Res* 2005; 39 : 697-705.
59. Ahotupa M, Huhtaniemi I. Impaired detoxification of reactive oxygen and consequent oxidative stress in experimentally cryptorchid rat testis. *Biol Reprod* 1992; 46 : 1114-8
60. Kumagai A, Ko dama H, Kumagai J. Xanthine oxidase inhibitors suppress testicular germ cell apoptosis induced by experimental cryptorchidism. *Mol Hum Reprod* 2002; 8 : 118-23
61. Viquerat-Villasenor RM, Ojeda I, Gutierrez-Perez O, Chavez-Saldana M, Cuevas O, Maria DS, *et al*. Protective effect of  $\alpha$ -tocopherol on damage to rat testis by experimental cryptorchidism. *Int J Exp Pathol* 2011; 92 : 131-9.
62. Yang S, Ping P, Ma M, Li P, Tian R, Yang H, *et al*. Generation of haploid spermatids with fertilization and development capacity from human spermatogonial stem cells of crylptorchid patients. *Stem Cell Rep* 2014; 3 : 663-75.
63. Acikgoz A, Asci R, Aydin O, Cavus H, Donmez G, Buyukalpelli R. The role of ketotifen in the prevention of testicular damage in rats with experimental unilateral descended testes. *Drug Des Devel Ther* 2014; 8 : 2089-97.

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