The biopsy of the undescended testis: pros and cons

Abstract

Undescended testis is the most common disorder of the urogenital system. It has been shown that spermatogenesis is affected in cryptorchid boys after the 9 months of age when spermatogenic index decreases rapidly. There are many studies about possible consequences of this condition such as infertility or testicular cancer. Although orchidopexy before puberty decreases the risk of testicular cancer it does not completely eliminate it. If the child underwent bilateral orchidopexy it will carry 6-fold greater risk of being infertile when compared with unilaterally cryptorchid men and general population. There is still a debate if the biopsy should be taken during orchidopexy. The result of biopsy should give us an idea of the number of germ cells and AdS cells which hold a high predictive value of future spermatogenesis. Scientists still have many questions that require answers such as if there is any less invasive way to find out about the level of AdS at the time of orchidopexy such as measurement of testicular volume. Although testicular biopsy is not a standard part of the protocol for orchidopexy, detecting low AdS at the histopathological examination could point out the patients for postorchidopexy hormonal therapy.

1. Introduction: 1. 1. Incidence and aethiology: Undescended testis or cryptorchidism is the most common disorder of the urogenital system that predominately affects preterm infants. Up to one third of preterm infants have undescended testis at the time of birth [1]. The reason for a high number of cryptorchid preterm boys is explained by the mechanism of the testicular descent which occurs in two phases. The first phase that occurs between 8 and 15 weeks of gestation is abdominal phase, and the second one is inguinoscrotal phase. In the second phase testis migrates from the inguinal area to the scrotum and this phase is usually completed by the time of birth [2]. In preterm infants this phase is interrupted, which explains the high incidence of undescended testis in preterm infants. In a large population-based study of 819111 non-syndromic boys in Denmark, Jensen and colleagues analyzed associations between birth weight, prematurity and cryptorchidism, which occurred in 14.1 cases out of 1000 boys [3]. The incidence of cryptorchidism in a full term male infants is between 1.8-8.4% [2]. Although there are many risk factors for cryptorchidism the exact aetiology is still unknown. As the proces of descent is under the regulation of hormons it was suggested that primary or secondary hormonal malfunction plays an important role in cryptorchidism. Recent studies have shown rapid increase in the incidence of cryptorchidism and the reason for this could be explained by environmental rather then genetic factors [4]. This is the reason why today endocrine disruptors are blamed among the other for this condition too [5]. But the role of causative factors in aetiology of cryptorchidism requires further research.

It is well known that the possibility of spontaneous descent of testis after first six months of life is poor [6]. This fact together with the fact that spermatogenesis decreases over time led to the new suggestion that the therapy of undescended testes should start at 4 months of age in a full-term baby and at 6 months of age in a pre-term baby [6].

1.2. Spermatogenesis:

The process of spermatogenesis in an undescended testis is interrupted. The testis contains three types of cells: germ cells, Sertoli and Leydig cells. Spermatogenesis takes place in the seminiferous tubules. The transformation of gonocytes into adult dark spermatogonia (AdS) occurs at around 3 months of age and it appears to be under control of a surge of gonadotropins and androgens in a so-called “mini-puberty” phase [7]. By the 5 years of age, the AdS willfurther differentiate into primary spermatocytes as they will remain until puberty and the onset of spermatogenesis [8-11]. For the physiological functioning of the process of spermatogenesis it is essential that gonocytes transform into AdS as they represent future stem cells for spermatogenesis (diagram 1).

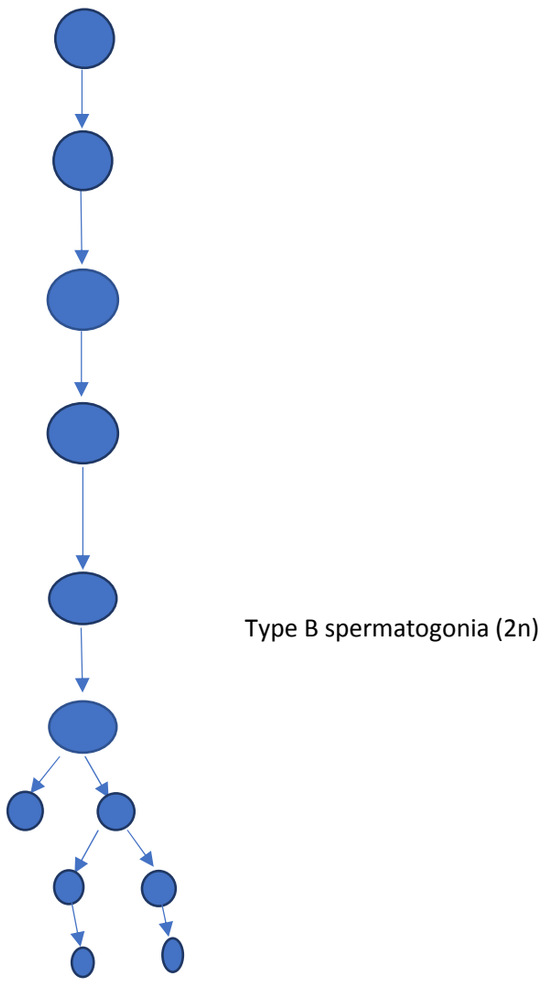

Diagram 1. The process of spermatogenesis

This particular process is affected in children with undescended testis [12], and it implies that they might have future problem with lower sperm counts and subsequent infertility (diagram 2).

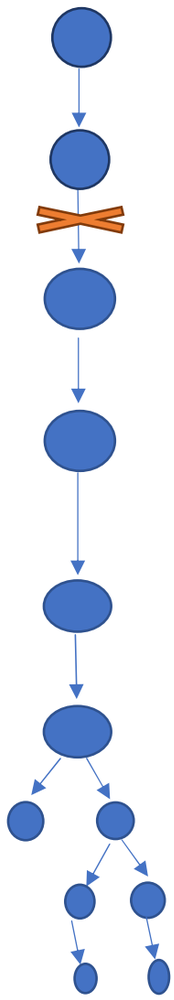

Diagram 2. Interruption of the process of spermatogenesis in cryptorchid boys

2. Discussion:

The results of the biopsies of the undescended testis in adult men showed a high rate of Sertoli cell-only (SCO) syndrome in one study recently conducted in Turkey [13]. SCO syndrome, also called germ cell aplasia, describes a condition of the testes in which only Sertoli cells line the seminiferous tubules. Typically, men with SCO syndrome are found to be azoospermic. As an example, Klinefelter syndrome is characterized by SCO and Leydig cell hyperplasia. Apart from SCO syndrome a high rate of maturation arrest on testicular biopsies were noted where numerous spermatogonia, few spermatocytes and no spermatides were reported. Hypospermatogenesis was defined as presence of all cell types (Sertoli cells, spermatogonia, spermatocytes and spermatides) with a low level of cellularity within the seminiferous tubules and was noted in 5 out of 244 patients [13]. The risk for azoospermia in cryptorchid boys is 10 times more likely than in general population and it raises up to 98% in untreated bilateral cryptorchid adult men.

2.1. Association with testicular cancer

It is clear that the biopsy should be done in adult patients with cryptorchidism as the incidence of testicular cancer is higher than in general population. Men with a history of undescended testis have an increased risk of developing testicular cancer compared with men in the general population in whom the age adjusted incidence is approximately 5.7 per 100000 [14]. It shows an increasing trend over the years [14]. However, the magnitude of increased risk is poorly quantified [15]. In a 2013 meta-analysis of nine case-control studies (including a total of 2281 cases) and three cohort studies (including more than 2 million boys), the pooled relative risk (RR) of testicular cancer in men with isolated cryptorchidism was 2.9 (95% CI 2.2-3.8) but there was significant heterogeneity between studies (with individual RRs ranging from 3 to 50), risk of publication bias, and overall lack of high-quality evidence [16]. The risk of developing testicular cancer is further increased in men with bilateral cryptorchidism (which may be

associated with endocrinologic abnormalities or abnormal karyotype) and intra-abdominal undescended testes [17-19].

A history of cryptorchidism is also a risk factor for developing germ cell neoplasia in situ (formerly called intratubular germ cell neoplasia of unclassified type [20]), a premalignant condition. The most recent study published in 2017 by Osterballe et al. included a cohort of 1403 boys operated on cryptorchidism between 1971 and 2003. The standardized incidence ratio of testicular cancer in this study was 2.7 (95% CI, 1.5-4.3). For bilateral cryptorchidism this ratio was 4.1 (95% CI, 1.8-8.1), compared to 2.0 (95% CI, 0.8-3.9) in unilateral cases [21]. Intratubular germ cell neoplasia is predominantly observed prepubertally in boys who had associated congenital anomalies, chromosomal and/or genetic disorders and syndromes [22]. It has been recommended that testicular biopsy should be performed at time of orchidopexy in intraabdominal testes, abnormal external genitalia or known abnormal karyotype [23]. The biopsy that shows Oct3/4 or D2-40 immunopositive germ cells in seminiferous tubules of prepubertal cryptorchid testes may predict development of later testicular cancer especially if cryptorchidism is associated with other congenital anomalies, chromosomal and/or genetic disorders and syndromes [22]. Surgical repositioning of the testis (orchiopexy) before puberty appears to decrease the risk of testicular cancer but does not completely eliminate it [24, 25]. In a cohort of 16983 men who underwent orchiopexy between 1964 and 1999 and were followed for a total of 209984 person-years, 56 cases of testicular cancer were identified [24]. Among those who were treated before 13 years of age, the RR of testicular cancer compared with the general population was 2.2 (95% CI 1.6-3.1). Among those who were treated at ≥13 years of age, the RR was 5.4 (95% CI 3.2-8.6). These findings support those of several smaller case-control and cohort studies [17, 26-28].

2.2. Association with infertility

About 10% of infertile men have a history of cryptorchidism and orchidopexy. If the child underwent bilateral orchidopexy it will carry 6-fold greater risk of being infertile when compared with unilaterally cryptorchid men and general population [29]. It has been shown that spermatogenesis is highly affected in cryptorchid boys after the 9 months of age when spermatogenic index decreases rapidly. If the descent of testis is stopped in the abdomen this testis would be affected with a greater germ cell loss than testis located in inguinal canal. As already mentioned earlier, the time for the orchidopexy had moved to 6 months of age for pre-term and 4 months of age for full-term baby. The study of Hadziselimovic and Herzog published in Lancet 2001 had demonstrated that biopsies taken from the undescended testis at the time of surgery performed before 6 months of age showed normal number of germ cells per tubule compared to boys having surgery after 6 months of age [30]. Unfortunately a long term follow up showed that there was no association between germ cell counts on biopsy and the total sperm count on semen analysis, but this association was found for the number of AdS and future fertility. Hadziselimovic suggests that the biopsy should be performed during orhidopexy in boys with undesended testes who had no response to LHRH therapy [31]. The result of such biopsy should give us a clear idea of the number of germ cells and AdS cells which hold a high predictive value of future spermatogenesis.

Unlike undescended testes, truly retractile testis requires no therapy as the fertility potential of boys with retractile testes is usually considered to be normal [32]. However, this condition should be followed up as studies have shown that during 3 year median follow-up this testis could become cryptorchid or decreased in size [33] when it requires surgery. On the other hand, ectopic and undescended testes are considered by Hutchenson et al. the variant of a single congenital anomaly and therefore should be treated the same [34].Mechlin et al. in their review article from 2014 concluded that biopsy specimens from cryptorchid testes should be analyzed for total germ cell concentration, presence of AdS, AdS/tubule ratio, presence of gonocytes (placental alkaline phosphatase positive cells), presence of

primary spermatocytes (in boys older than 3 years of age) and the degree of fibrosis [35]. These findings have significant prognostic value for future fertility potential in cryptorchid boys.

Literature debates about the possible effect of neoadjuvant hormonal therapy before orchidopexy. In a randomized, double-blinded, placebo-controlled study buserelin (GnRH agonist) 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 [36].

Accepting the above mentioned correlation between low number of AdS at the time of orchidopexy and future problems with fertility we should focus on children with low AdS after orchidopexy at the age of 6 months to prevent future fertility problems. Hadziselimovic observed that GnRH administration after orchidopexy might result in improved fertility [37].

As the major concern is the invasiveness of testicular biopsy when detecting the level of AdS at the time of orchidopexy, researchers are trying to find any less invasive way to detect patients who might have future fertility problems.

2.3. Possibilities of less invasive replacement for testicular biopsy

If 80-90% of the testicular mass consists of seminiferous tubules containing germinal and Sertoli's cell [38] it means that testicular volume is largely a reflection of spermatogenesis [39]. Apart of the higher possibilities of the decrease of spermatogenesis in cryptorchid boys with intraabdominal and nonpalpable testis with low testicular volume, there are no high evidence studies that suggest this association with only testicular volume. Recent studies have discussed measuring inhibin B comming from working Sertoli cells as potential substitution for testicular biopsy for early identification of testicular dysfunction after orchidopexy [40]. Thorup et al. proposed measuring inhibin B and gonadotropins and combining these results with histopathology to identify patients with high risk of infertility [41]. 2.4. Risks of testicular biopsy

Performing testicular biopsy during orchidopexy carries some risks as additional intervention. Although anaesthesia has improved during the last decades and is now considered safe for infants with low rate of serious complications [42] it is true that performing one more procedure (testicular biopsy) during orchidopexy may prolong time required to finish surgery. But in hands of a skilled pediatric surgeon or urologist this should not be of concern as the procedure might just take several of minutes. However, it is important to be performed in a major centers with specialized paediatric anaesthesia services. Another risk in performing testicular biopsy is the risk of haemorrhage. This risk exists during orchidopexy, but is higher if testicular biopsy is performed during the same procedure. All patients and their parents should be informed about this risk. Furthermore, the risk of breaching the blood-testis barrier depends on the time of orchidopexy and testicular biopsy. As orchidopexy is usually performed during first year of life and the junctional specializations between Sertoli cells are absent until about 8 years of age this risk is minimal [43]. Of course when orchidopexy and biopsy are performed around puberty this risk increases.

3. Conclusion:

For now, apart from biopsy there are no other ways of measuring AdS and detecting future fertility problems in boys with undescended testis that might benefit from postorchidopexy hormonal therapy and prevent future fertility problems. Although testicular biopsy is not a standard part of the protocol for orchidopexy, it is possible that detecting low AdS at the histopathological examination can point out the patients for postorchidopexy hormonal therapy. Thorup et al. in their pilot study from 2018 suggest that cryopreservation could be an option in case of treatment failure of adjuvant LHRH. They even suggest cryopreservation during bilateral orchidopexy to avoid repeated surgery with biopsy with note that this procedure may be indicated in 22 to 36% of cases [44]. It is possible that this could be a way to stop this increasing trend of fertility problems.

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