In patients with non-obstructive azoospermia, testicular sperm extraction (TESE) is a method of choice to recover spermatozoa as a male therapeutic approach in intracytoplasmic sperm injection (ICSI) programmes. However, the efficacy of TESE in this indication is burdened by a frequent failure of sperm recovery, which renders useless both the invasive testicular intervention and ovarian stimulation of the patient’s spouse. One of the most frequent pathological pictures characterizing complete absence of spermatozoa is germinal aplasia (Sertoli cell-only syndrome or SCOS). Two different histological patterns of SCOS have been already described during the past five decades. These two patterns can be characterized as the congenital (pure) and the secondary (mixed) forms. Both patterns, with different prognosis to retrieve spermatozoa by therapeutic testicular biopsy, are frequently confused when TESE is performed during ICSI programmes. Useful criteria to predict the absence of spermatozoa can be obtained by a definite recognition of the two typical histological patterns during the diagnostic testicular biopsy. The diagnosis of congenital or acquired SCOS can be refined by endocrine, chemical, immunohistochemical and molecular biology aids. Reduction of both sperm retrieval failure and unnecessary ovarian stimulation can be achieved by combination of these methods.

Key words: non-obstructive azoospermia/Sertoli cell-only syndrome/testicular histology

Introduction

Diagnostic testicular biopsy (DTB) is one of the most important diagnostic procedures in the study of male infertility. Testicular aspiration was first advocated as a diagnostic method for azoospermia by Huhner (Huhner, 1913, 1928), and since then it has been used only for the diagnosis and prognosis in cases of male infertility such as azoospermia and unexplained severe oligozoospermia (Charny, 1940, 1968; Ragab et al., 1961; Dubin and Hotchkiss, 1969; Amelar and Dubin, 1973; Wong et al., 1973; Shirren, 1974; de Kretser and Holstein, 1976). Clermont (Clermont, 1963), in describing the cycle of human tubular epithelium, proposed a quantitative method for the most precise evaluation of tubules population. Other authors suggested major advantages in this procedure, expressing germ cell counts per unit length of seminiferous tubule (Steinberger and Tjoe, 1968) or cell counts per Sertoli cells (Rowley and Heller, 1971; Skakkebaek and Heller, 1973) to reach the best diagnosis possible. During the 1980s, the interest in DTB decreased.

During the 1990s, the introduction of intracytoplasmic sperm injection (ICSI) procedures in patients with severe oligozoospermia (Palermo et al., 1992; Van Steirteghem et al., 1993) dramatically increased the interest in DTB again as fertilization and pregnancies were first reported after using testicular spermatozoa, with ICSI, from men with obstructive azoospermia (Craft et al., 1993; Schoysman et al., 1993). Testicular biopsy became for the first time a therapeutic procedure and, together with the ICSI technique, was considered an effective fertility treatment also for patients with non-obstructive azoospermia, who were offered the possibility of fathering their own genetic children (Devroey et al., 1994, 1995).

Primary testicular failure affects ~1% of all men, and 10% of those seeking fertility evaluation (Schlegel, 1991). The most common histological patterns of these patients are: hypospermatogenesis, maturation arrest and Sertoli cell-only syndrome (SCOS), with or without focal spermatogenesis. Despite severe defects in spermatogenesis, good pregnancy and implantation rates were reported when ICSI was used with spermatozoa recovered from non-obstructed patients, although these figures were lower than those reported using spermatozoa from patients with obstructive azoospermia (Kahraman et al., 1996; Aboulghar et al., 1997; Mansour et al., 1997; Ghazzawi et al., 1998; Ubaldi et al., 1999). However, failure to find spermatozoa in testicular sperm extraction (TESE) programmes may occur in up to 57% of non-obstructed patients, resulting in an unnecessary ovarian stimulation cycle for the partner (Devroey et al., 1995; Kahraman et al., 1996; Schlegel et al., 1997; Silber et al., 1997; Tournaye et al., 1997; Ezeh et al., 1998; Su et al., 1999; Ubaldi et al., 1999).
Among prognostic indicators of sperm retrieval success with TESE, some authors (Tournaye et al., 1997; Su et al., 1999; Ubaldi et al., 1999) have found the histology in DTB to be the most accurate. Spermatozoa can be retrieved after TESE in almost all patients with a histological diagnosis of hypospermatogenesis. More disappointing results are reported in patients with maturation arrest or with SCOS in DTB. With regard to the latter histological pattern, a successful sperm retrieval rate of 86% was reported when focal spermatogenesis is present, and 19% when a pure SCOS pattern is present in the histological diagnosis (Tournaye et al., 1997). Although these results have been confirmed more recently by other authors (Su et al., 1999), a more detailed histological classification is necessary in patients defined with pure SCOS. Two distinct patterns of SCOS, with different pathogenesis and prognosis, have been already described (Charny and Meranze, 1942; Wong et al., 1973; Chemes et al., 1977; Nistal and Paniagua, 1984; Schlutze, 1984a,b). Two histological patterns with substantial differences in the tubular wall histology, in the germ cells (GC) tubular composition and in the appearance and cellularity of the interstitial tissue can be distinguished between the pure SCOS pattern, as first described in the late 1940s (del Castillo et al., 1947), and the mixed or secondary SCOS described by other authors differentiating noxae patognenae (Girgis and Hafez, 1977; Nistal and Paniagua, 1984; Schlutze, 1984a,b). This distinction is very important, since it is impossible to find spermatozoa in the pure SCOS pattern, and it is also impossible or extremely difficult to find them in some cases of most advanced mixed SCOS patterns, where testicular tissue did not survive the noxa patogena that had damaged the testicle. The better understanding of the histological characteristics of these two distinct patterns will lead to a reduction of unnecessary multiple testicular tissue sample removals and useless ovarian stimulation. In this way, the successful sperm retrieval rate will be increased. Although the histological characteristics are of major importance, it is also useful to use specific laboratory tools to help differentiate between these two SCOS patterns, especially all in particularly difficult cases.

The aim of this study was to make a review of the SCOS and to describe the two different histological patterns in order to distinguish those patients in whom it is not possible to retrieve spermatozoa.

The Sertoli cell
Named after its discoverer, Enrico Sertoli (Sertoli, 1865), the Sertoli cell is also known, among many other eponyms, as the ‘bridge cell’ (because it acts as a bridge between the lymphatic/vascular external compartment and the inner tubular environment) and as the ‘nurse cell’ (all the spermatogenic processes take place within the cell itself). The Sertoli cell sustains spermatogenic activity throughout the human lifespan, and provides several functions for the physiological health of both the seminiferous epithelium and the tubular wall.

Embryology
The first characteristic to appear in a male embryo is the seminiferous cord. Its presence makes possible the earliest differentiation between male and female embryos. The seminiferous cord is filled with a number of cells that are the Sertoli cell embryonic precursors, of mesenchymal origin. The primordial GC, which will be the future gonocytes and later on pre-spermatogonia, have been documented to originate in a different location which is distant from the seminiferous cord. They are of epithelial origin and emerge in the yolk sac (Witschi, 1948; McKay et al., 1953), from which they have to migrate through the mesenteric pathways to the area where the seminiferous cord is located, in order to reach their final destination. Embryonic gonocytes, during their migration phase, depend on other cells in their environment for metabolic sustenance (Zamboni and Merchant, 1973), and this behaviour is typical of GC in the fetal, postnatal, prepubertal and adult life. As they reach their final seminiferous cord position, the GC are encompassed by the Sertoli cell precursors (Jost et al., 1974), which sustain them throughout their lifespan. So far, the GC are dedicated both to the unique process of mitotic/meiotic cellular multiplication as well as to the final maturation of the male gamete.

Pathophysiology
The Sertoli cell controls most processes that are involved in spermatogenesis, while it represents the main target cell for many others. The Sertoli cell is controlled by pituitary and local paracrine factors secreted from Leydig cells and the GC themselves (Jegou, 1992; Russell and Griswold, 1993; Spiteri and Nieschlag, 1993) and also from peritubular myoid cells (Skinner, 1991, 1993).

Abnormalities in the paracrine regulation of sperm production may contribute to the pathophysiology of idiopathic oligozoospermia or azoospermia (Baker et al., 1986; Matsumoto, 1991). Germinal cell loss and disruption of the Sertoli cell function, induced by irradiation, can cause a marked alteration in the spermatogenic cell as well as in the peritubular cell morphology, confirming the reciprocal paracrine regulation of both peritubular cells and GC on the Sertoli cell (Terada and Hatekeyama, 1991; Jegou, 1992).

Any alteration of the morphology of the Sertoli cell is of enormous interest when considering tubular pathology. Due to its numerous attachments to the basal membrane, the Sertoli cell destiny is connected to that of the tubular wall. Any thickening of this wall will cause modification of the obligatory anatomical pathway for the metabolic interchange between GC and the blood stream. Therefore, both sufferance and disruption of the GC will arise. The Sertoli cell must preserve its morphological and functional integrity in order to ensure the healthy function of the tubule (Jegou, 1992; Schlatt et al., 1997).

From the outset, it was believed that testicular damage could be due to acquired injuries (Charny and Meranze, 1942; Wong et al., 1973; Chemes et al., 1977; Rothman et al., 1982; Nistal and Paniagua, 1984). More recently, however, morphological studies have been conducted by others (Nistal et al., 1990; Terada and Hatekeyama, 1991; Nistal et al., 1998) in an attempt to relate most of the spermatological tubular disorders to the morphological appearance of the Sertoli cell. In these
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papers, spermatogonial arrest was associated with a particular type of Sertoli cell morphology (Nistal et al., 1998) and evidence of two types of Sertoli cell in idiopathic SCOS histology was presented (Terada and Hatekeyama, 1991).

SCOS patterns and their aetiopathogenesis
In DTB, two histological patterns of SCOS can be distinguished. The pure or primitive form is due to a disturbance of the migration of the primordial GC from the yolk sac to the seminiferous cord (Jost et al., 1974; Wartenberg, 1989). In this condition, primordial GC have not reached their final destination in the primitive testicle. A histological examination during the postnatal period reveals an intact basal membrane with numerous Sertoli cells in a very good morphological shape, evenly arranged on top. The tubule:interstitial ratio appears to be normal, and the tubules are all of the same diameter but do not present any histological alteration (Figure 1) when compared with the normal testicular tissue (apart from the absence of spermatogonial cells). Interstitial cells appear to be normal in number and shape. Due to this congenital disorder, it is impossible to find spermatozoa in the testicles of these patients.

In the secondary or acquired or mixed form, a noxa patogena injured the healthy testicular tissue postnatally, and in these testicles irreversible and typical histological alterations can be observed. In this form the tissue is made up of tubules of different diameters, though all smaller than normal. Some are extremely tiny since they are undergoing a process of reabsorption (Figures 2 and 3). Most of the tubules reveal only Sertoli cells arranged on the membrane, while others may be filled with a hyaline substance. In all the tubules, the basal membrane appears altered: it may be either thickened (Figures 3 and 4), with their bonds shrunk and loosened (Figure 5), or oedematous (Figures 6 and 7). Unlike the congenital disorder, in this form the Sertoli cells appear to be morphologically damaged and functionally compromised. Only a few tubules may have diameters of just less than or even normal size, with a slightly compromised basal membrane on which a residual spermatogonial process can be seen and sometimes round, elongated or mature spermatids or even spermatozoa can be found. These tubules represent those which have survived the noxa patogena that damaged the testicle, and continue their function, albeit partially. All the above pathological features can be found in the same DTB sample close to each other (Figure 8). This secondary form has been called mixed (Girgis and Hafez, 1977), and may permit the recovery of few spermatozoa that can be successfully used for ICSI. However, in cases in which the mixed form is long-established, the alterations of the membrane are so consistent that no single tubule may appear still functional. The testicular tissue may appear fibrosclerotic, and tubules are devoid of cells and are being reabsorbed. In such cases no spermatozoa can be found.

Attempts to distinguish different aetiologies that could lead to the SCOS pictures have been elaborated. They are mostly based on the morphological appearances of the Sertoli cell, as seen in some syndromes, e.g. hypogonadotrophic hypogonadism, cryptorchidism and in those histological pictures that are usually observed during oestrogenic therapy (Schulze, 1988) or chemotherapy (Nistal et al., 1990). The Sertoli cells are known to show different morphology during embryonic, fetal, pre-pubertal and adult life. In some pathological conditions, Sertoli cells can display morphological characteristics that are normally expressed in earlier periods of life. However, no relationship between these findings and the aetiology of the pure SCOS has yet been found. Nuclear changes in the Sertoli cells, detected by measuring the differences of the nuclear surface, volume and morphology by using elegant computer-assisted three-dimensional reconstructions and mathematical calculations (Bruning et al., 1993) have also been proposed to better understand the alterations of Sertoli cell morphology in pathological conditions. Nevertheless, although remaining brilliant tools in the evaluation of Sertoli cell cellular and nuclear morphometry, further studies are needed to interpret these data to explain a new aetiology for SCOS. A detailed discussion of these methods is beyond the scope of this review.

Histology
Sertoli cells in normal testicular tissue
The seminiferous tubule is supported by a basal peripheral structure called the ‘limiting membrane’ (Clermont, 1958). This membrane is constituted by an inner layer or ‘lamina propria’, with a lamellar organization (that contains fibrils) and an outer layer (sometimes known as the basal membrane) that contains collagen fibres (Burgos et al., 1970). Myoid and fibroblast-like cells surround all the external part of the basal membrane.

The basal portion of the Sertoli cell lies upon the inner lamella, while the adluminal portion points towards the tubular lumen in a radial orientation. The Sertoli cell has a very elaborate shape because of its close association with the GC that are hosted in its cytoplasm in very specialized invaginations. The contacts between the Sertoli cell and the GC are made of typical junctions (tight junctions and ectoplasmic specialization). Other junctions exist between the basal portions of the Sertoli cell, making the Sertoli cell–Sertoli cell union so tightly joined as to make the so-called blood–testis barrier.

The cytoskeleton of the Sertoli cell is very complex because of its multiple functions (support, communication and nutrition of the GC). As well as these functions, the cytoskeleton has to provide the self architecture-maintaining processes. They consist mainly of actin filaments, intermediate filaments and microtubules which lie among all the other organelles. Actin filaments (forming the tubulobulbar complexes) control the internal movement of the spermatogonial cells and the communication with the neighbouring Sertoli cell (ectoplasmic specializations). These Sertoli cell functions are involved in the process of the spermatogenetic wave. The microtubules should be involved in translocating spermatids into the cytoplasm and in maintaining the columnar shape of the Sertoli cell. Intermediate filaments radiate from the perinuclear region to the periphery, where they join up with the plasma membrane making contact with adjacent Sertoli cell. These intermediate filaments contain vimentin-type proteins (Franke et al., 1979;
Mali et al., 1987; Aumuller et al., 1988) that are typical of mesenchymal origin cells.

The term Sertoli cell currently refers to the mature cell as seen in adulthood, but as its morphological (and functional) importance is different in fetal, pre-pubertal, pubertal periods (Hadziselimovic, 1977) as well as in adult pathological conditions, it is necessary to clarify the developmental stage or pathological condition to which we are referring. It should be borne in mind that the behaviour, morphology, appearance and immunoreactivity of Sertoli cell are linked to its developmental stage.

It should also be remembered that: (i) the embryonic Sertoli cell is primarily important in starting up the developmental process of the undifferentiated gonad in a genetically male testis (Alberts et al., 1989; Small, 1989; Bennett, 1990). Its cytoskeleton is made up of intermediate filaments, as in the adult testis, and contains not only the same vimentin-type protein but also protein of cytokeratin type (18 and 28) which is known to be pertinent to the epithelial cells. This implies that the Sertoli cell may become functionally different during its maturation, as the cytokeratin will gradually disappear in the period between birth and puberty. (ii) The Sertoli cell

Figures 1–8. For legends, see facing page.
structure and function undergo many changes during the postnatal period. For example, the shape of the cell changes from round to elongated, and point from the basal lamina on which they lie, towards the tubule’s lumen. (iii) During the pre-pubertal period the pre-spermatogenetic GC and the Sertoli cell maintain more or less equal size and number ratio, though with a minor preponderance of Sertoli cells, which are randomly arranged. Immunolabelling makes both the vimentin intermediate filaments and the cytokeratin protein filaments evident in the Sertoli cell cytoplasm. (iv) At the onset of puberty this picture changes dramatically, with an extensive proliferation of GC and a reversal of the GC:Sertoli cell ratio. This brings about an enlargement of Sertoli cell cytoplasm due to the acceleration and to the increase of its function. This cytoplasm enlargement provides adequate support for the more actively proliferating spermatogenetic cells. Vimentin filaments are still present, while cytokeratin filaments disappear. Cytokeratin is thought to be co-expressed only in the fetal and pre-pubertal phases. Some authors suggest that the presence of cytokeratin in the adult Sertoli cell cytoplasm is an expression of its functional impairment (Bergmann and Kliesh, 1994). The Sertoli cell in these conditions is able neither to initiate nor to continue the spermatogenetic process. Others authors suggest that cytokeratin is a marker of GC degeneration (Romeo et al., 1995).

**Sertoli cells in pathological testicular tissue**

In pathological conditions, the tubular wall undergoes remarkable modifications, e.g. oedema, thickening and sclerosis. Many testicular disorders are associated with a thickened aspect of the tubular wall of the seminiferous tubules, which impairs the relationship between the inner tubular population and the interstitium. During the pathological thickening processes, Sertoli cell functions are progressively altered and eventually suppressed. As the disturbance progresses, the GC slough off into the tubular lumen without maturing, and it is possible to observe a progressive arrest of the spermatogenetic process. Subsequently, the lamina propria begins to separate due to the oedema, then thickens and finally shrinks. At the end of this process the wall is absorbed, and leaves the so-called ghost tubules in the tissue (see Figures 2, 3 and 8).

Lipid and glycogen inclusions are observed in the Sertoli cell cytoplasm following a massive degeneration of GC (e.g. iatrogenic damage after oestrogen therapies or combined radio/chemotherapies) (Schulze, 1988). Sertoli cells phagocytose the degenerated cells and appear filled up with their lipid residues.

In SCOS, accumulation of intermediate filaments is observed around the nuclei and in the apical region. They are composed only by vimentin-type protein (Mali et al., 1987). Cytokeratin-type protein filaments are present only in the immature Sertoli cell, together with vimentin filaments, but are absent in the mature Sertoli cell (Stosiek et al., 1990) and therefore in the pure SCOS pattern.

After long-term oestrogen therapies, concentrations of testosterone and gonadotrophins are reduced in the blood (Rodriquez-Rigau et al., 1977), and it is presumed that even mature Sertoli cells, deprived of their normal stimulation, are also transformed into undifferentiated Sertoli cells similar to the Sertoli cell precursors observed before puberty.

In many pathological conditions, the Sertoli cell nuclei do not appear adjacent to the basal lamina, but stay in the centre of the cell; their volume and shape are not homogeneous. All these histological features are typical of the mixed form of SCOS in which it is possible to observe almost all the regressive histological alterations described (see Figures 6–8). In case of doubt, one (or more) test could be used to identify better the type of SCOS.

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**Figure 1.** Typical pure Sertoli cell-only syndrome (SCOS) picture. The tubules are of normal diameter, the membrane appears normal and fine, and healthy Sertoli cells (SC) can be seen attached to it. The nucleus of the SC is large and located at the basal compartment of the cell. The cytoplasm is copious but devoid of germ cells (GC), and numerous intermediate filaments can be seen around the nucleus and at the adluminal compartment. No histological alteration can be seen, even in the interstitium where the Leydig cells appear normal in number and shape. Compared with the picture of a normal seminiferous tubule, the absence of GC in the epithelium is the only distinctive characteristic (magnification ×225, low-power field).

**Figures 2, 3 and 4.** Mixed or secondary Sertoli cell-only syndrome (SCOS). The diameters of the tubules are variable, ranging from almost normal to very tiny ones. Some tubules appear to be completely reabsorbed (‘ghost tubules’). All basement membranes are thickened, some are oedematous and have lost their internal borders. The Sertoli cells are diminished in number and highly altered in shape; in many tubules they are lost and replaced with a hyaline substance (magnification ×225, low-power field).

**Figure 5.** Mixed Sertoli cell-only syndrome (SCOS). Altered tubular membranes showing typical ‘tearing’. They are thickened, and the layers—which appear increased in number and corrugated—have loosened their bonds. A vacuolized hyaline substance can be seen inside the tubules, and the Sertoli cells appear rounded and to be suffering, although they are still well arranged on the membranes. A few Leydig cells, many fibroblast-like cells and many infiltrating cells can be seen around the tubules, in the interstitial tissue. Many peritubular alterations are likely to have arisen from the extracellular matrix (magnification ×225, low-power field).

**Figure 6.** Mixed Sertoli cell-only syndrome (SCOS). Tubules with diminished diameters. Oedematous membranes caused alteration in their exchanging functions with the interstitium: the Sertoli cells still appear numerous, but no longer include germ cells. The oedema does not allow the lamina propria and other basal membrane layers to be distinguished (magnification ×225, low-power field).

**Figure 7.** Mixed Sertoli cell-only syndrome (SCOS). The same situation as described in Figure 6. Some Sertoli cells also look sodden and oedematous. Ghost tubules with oedematous membranes can also be seen (magnification ×400, high-power field).

**Figure 8.** Mixed SCOS. Close to tubules with altered membrane, which is devoid of germ cells (GC) and Sertoli cells, tubules with almost normal membranes can be found. Residual spermatogenesis with final production of some spermatids and spermatocytes can be seen among the GC. These tubules are those which had survived the *诺萨派格尼娜* that damaged the testicle (magnification ×225, low-power field).
Laboratory tools

Several laboratory tools can be helpful in the prediction of the presence of spermatozoa in the testes of men with non-obstructive azoospermia. Some of these are based on the characteristic distribution of specific cell components in testicular tissues obtained by testicular biopsies. Other tests are less invasive, and can be performed using blood, serum or semen samples.

Vimentin and cytokeratin immunoreactive assays

The Sertoli cell contains intermediate filaments that make a fibrillar meshwork across all of its cytoplasm. Intermediate filaments are present in the juxta-nuclear and in the apical region, where spermatids are usually located in the pre-spermiation stage. Intermediate filaments are of the vimentin type (Franke et al., 1979; Mali et al., 1987; Aumuller et al., 1988), and this confirms their mesenchymal origin.

During adulthood, cytokeratin-type filaments are absent in Sertoli cell cytoplasm, while they are present in the fetal and pre-pubertal period as well as in pathological conditions. The GC epithelium, of epithelial origin, lacks vimentin-type filaments but differs from other true epithelia because it also lacks cytokeratin-type filaments.

Sections of testicular biopsy can be used in immunofluorescence microscopy with vimentin antibodies to make a confirmatory diagnosis of SCOS, where Sertoli cells are the only cells showing this protein in its intermediate filaments. Both vimentin and cytokeratin immunoreactive tests can provide more detailed conclusions.

Telomerase assay

Telomerase is required for cell proliferation because of its ability to reintegrate the telomeric sequences at the end of chromosomes that are usually lost in the continuous process to produce cells. The telomerase maintenance mechanism is reported to be present for cellular immortalization processes (Herbert et al., 1999), and telomerase activity is reported to be present in the testis and is likely to be pertinent only to GC. Telomerase activity has been detected in male GC of mice (Prowse et al., 1995; Yamamoto et al., 1999a), rats (Eisenhauer et al., 1997) and humans (Wright et al., 1996; Yamamoto et al., 1999b). The activity of this enzyme in spermatogenetic cells has been shown to decrease during the progression of meiotic and post-meiotic differentiation, with the highest levels being measured in spermatogonia and primary spermatocytes and the lowest in round spermatids. No telomerase activity has been detected in testicular and epididymal spermatooza (Prowse et al., 1995; Eisenhauer et al., 1997; Yamamoto et al., 1999a). The first study relating telomerase activity with the histology of the human testicle was conducted recently (Fujisawa et al., 1998). These authors did not detect any differences between testicular biopsy samples originating from men with obstructive azoospermia and from men with maturation arrest. Patients with SCOS showed no telomerase activity, whereas activity was shown in patients with normal spermatogenesis, hypospermatogenesis and maturation arrest. More recently, a more sensitive quantitative telomerase assay was used to demonstrate a relationship between telomerase activity per unit weight of protein from minced testicular tissue and the quality of spermatogenesis (Yamamoto et al., 1999b). In this study, higher telomerase activity was found in those men where haploid germ cells (spermatozoa and/or spermatids) were detected by DTB (Yamamoto et al., 1999b).

Inhibin B and FSH in serum

FSH regulates testicular function mainly by augmenting or inhibiting the production of different protein factors, such as hormones, growth factors and cytokines by Sertoli cells (Sharpe, 1994). Among these factors, inhibin B is of particular interest because it forms part of a negative feedback loop regulating serum FSH concentrations by suppressing FSH release from pituitary gonadotrophic cells (Illingworth et al., 1996). The secretion of inhibin B by Sertoli cells is stimulated by FSH, but it is also modulated by Sertoli cell interactions with neighbouring GC (Pineau et al., 1990). In the rat testis, the secretion of inhibin B by seminiferous tubules is mainly dependent on the presence of spermatids (Allenby et al., 1991).

Serum FSH is widely used in the diagnostic work-up of the infertile male, but does not reveal either the nature of the azoospermia or the possible presence of spermatozoa in the DTB. Inhibin B reflects more accurately the azoospermic picture, especially that of SCOS where serum concentrations reach their lowest level (Bohring and Krause, 1999; von Eckardstein et al., 1999), probably due to the damage of the Sertoli cell.

Serum inhibin B concentration is negatively correlated with the patient’s age and serum LH and oestradiol concentrations, but is positively correlated with thyroid stimulating hormone and sperm concentration in the ejaculate, and with testicular volume (Mahmoud et al., 1998). However, notable exceptions to these general rules do exist. Recently, a combination of elevated FSH and normal inhibin B serum concentrations was reported in seven out of 25 oligozoospermic men (Foresta et al., 1999a). The physiological significance of such discrepancies is not known. However, neither very high serum FSH concentrations nor very low serum inhibin B concentrations exclude the possibility of recovering (by therapeutic testicular biopsy) a few spermatozoa which can then be used for ICSI (Jezek et al., 1998; Foresta et al., 1999b).

In human testicular pathologies, the serum FSH and inhibin B concentrations are negatively correlated with each other (Anawalt et al., 1996; Nachtigall et al., 1996; Anderson et al., 1997; Leifke et al., 1997). An inverse ratio between the two serum concentrations has been observed in azoospermic patients (Foresta et al., 1999a; von Eckardstein et al., 1999). In SCOS patients, the inhibin B concentration was significantly lower than in the control group (Foresta et al., 1999b). Higher diagnostic sensitivity and specificity, even predicting the presence of elongated spermatids in DTB, is expressed by the combination of both inhibin B and FSH assays (Pierik et al., 1998; von Eckardstein et al., 1999). A similar relationship has been found with regard to the inhibin B concentration in seminal plasma (Anderson et al., 1998).
**Search for lipids in the Sertoli cell**

The cytoplasm of Sertoli cell in the normal testicle contains huge quantities of lipid granules which originate from the digestion of cytoplasmic residue of spermatids after the process of spermiation. In the mixed form, the Sertoli cell contains many lipid granules due to reabsorption of the degenerated GC. The Sertoli cell of the pure SCOS contains only a very small amount of lipids and glycogen because it has never been in contact with GC.

**Examination of ejaculated GC**

In non-obstructive azoospermia, immature GC are often released prematurely from their association with the Sertoli cell, and may be found in the ejaculate. Examination of the amount and viability of different stages of GC in the ejaculate can offer an indirect insight into the testicular function, without performing invasive diagnostic procedures. Identification of GC in the ejaculate, and their distinction from other types of round cells, is a difficult task. However, specific markers can be used to distinguish between GC and non-germ round cells.

Recently, it was suggested that the detection of spermatids in the ejaculate predicted the probability of recovering spermatozoa from the testis (Ezeh et al., 1998), but this was not confirmed (Tesarik et al., 1998). The latter authors found a relationship between the detection of spermatids in the ejaculate and in the testicular biopsy, although sometimes spermatids could not be detected in the biopsy in spite of their previous presence in the ejaculate. This latter observation may be explained by the focal character of residual spermatogenesis in the testis, so that even a multiple-site testicular biopsy may miss a small active zone that is responsible for the production of spermatids detected in the ejaculate.

**Differences between the tests and their use**

The absence of cytokeratin, together with the presence of vimentin in the proteins of the intermediate filaments of the Sertoli cell in the DTB slices, provides an immunochemical confirmation of a pure SCOS syndrome (Franke et al., 1979; Aumuller et al., 1988; Bergman and Kliesch, 1994; Romeo et al., 1995).

The identification of telomerase in the DTB slices provides confirmation of the presence of GC (Fujisawa et al., 1998). The quantitative telomerase assay is considered capable of predicting the presence of haploid cells (Yamamoto et al., 1999b), and its determination in DTB slices can help to identify a mixed SCOS in which haploid cell foci could be present. The presence of haploid cell foci means that the patient with mixed SCOS should be included in the ICSI programmes. On the other hand, the lack of haploid cell foci can confirm a case of pure SCOS (Fujisawa et al., 1998) where no spermatozoa could be found.

The scarcity of cytoplasmic lipids is evidence that there has been no metabolism of cytoplasmic residues, which means that no spermatids should be present.

In cases of SCOS, each of these three tests, when performed at the moment of the DTB, may provide help in correct diagnosis of the type of SCOS. The use of all three tests can lead to more sophisticated conclusions and, as a result, to greater certainty. The serum concentrations of inhibin B and FSH do not have such specific functions, but can also be used in this respect.

Analysis of azoospermic semen samples may also predict the presence of GC, since specific markers can be used to distinguish between GC and non-germ round cells.

**Conclusions and suggestions**

In the absence of a previous DTB, or if the DTB is only aimed at detecting spermatozoa without a detailed histological examination, it is impossible to distinguish between pure SCOS (in which it is impossible to find spermatozoa) and secondary SCOS (in which spermatozoa may sometimes be found). Consequently, attempts at sperm recovery by therapeutic testicular biopsy fail in cases of pure SCOS which has not been diagnosed correctly. Under these circumstances, women undergo unnecessary ovarian stimulation and the male partners are exposed to possible hazards due to multiple testicular extractions (Schlegel and Su, 1997). Moreover, no reasonable conclusions can be drawn from the failed treatment attempt for future therapeutic strategies. Therefore, performing extensive therapeutic testicular biopsies without complete previous diagnosis is ethically questionable.

Carrying out a DTB with a correct histological examination in all cases of non-obstructive azoospermia before starting stimulation of the partner should generally avoid unnecessary ovarian stimulation and multiple testicular biopsies. In cases of histological patterns of SCOS, the histological features of mixed SCOS should be accurately searched for, and immunohistochemical tests should be used in other tissue sections. If the typical picture of a mixed SCOS is recognized, and the immunohistochemical tests confirm the presence of haploid cells, then the TESE procedure can be justified. In contrast, if the idiopathic form is recognized, such invasive surgical procedure can be avoided since spermatozoa cannot be found. Similarly, any surgical approach should be avoided if the presence of a mixed form is recognized in which the histological picture is so compromised and disrupted that the spermatogenic process has disappeared.

In conclusion, the DTB enables us to recognize the mixed SCOS picture, and its histological features are decisive. If SCOS is diagnosed, other slices of the same DTB can be processed for immunolabelling tests, and determinations of serum inhibit B and FSH concentrations may be performed. These procedures may reduce the risk of testicular sperm retrieval failure, may avoid unnecessary ovarian stimulation and, in agreement with the principles of good ethical behaviour, can offer the correct diagnosis to couples, without exposing them to unnecessary hazards and inconveniences.

**References**


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