

CASE REPORT

Recognising the Sertoli-cell-only (SCO) syndrome: a case studyR. Anniballo¹, R. Brehm² & K. Steger³

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Summary

Total Sertoli-cell-only (SCO) syndrome is often confused with a focal SCO picture, in which testicular illness caused damage to seminiferous tubules and compromised the Sertoli cell range of maturation and functions, but from which still some spermatozoa can be retrieved for assisted reproductive techniques. Here, a possibly new SCO syndrome phenotype is reported exhibiting complete lack of germ cells despite normal architecture of the seminiferous tubules with presence of mature Sertoli cells and normal Leydig cells in the intertubular tissue. Sertoli cells are immunonegative for the prepubertal differentiation markers cytokeratin-18, anti-Muellerian hormone and M2A antigen, but reveal a positive signal for the gap junctional protein connexin 43 known to be expressed in Sertoli cells with an adult type of differentiation. The complete lack of germ cells in combination with fully differentiated adult-type Sertoli cells in this case is in contradiction with known SCO subtypes and with the current hypothesis of reciprocal regulation of Sertoli and germ cell differentiation.

Introduction

The Sertoli-cell-only (SCO) syndrome is characterised by azoospermia and complete lack of germ cells in testicular biopsies solely exhibiting Sertoli cells (del Castillo *et al.*, 1947). The term SCO, however, is frequently and incorrectly used for a similar morphological picture, focal SCO where some spermatozoa are still present (Anniballo *et al.*, 2000). In the latter cases, an illness affected the testes causing focal damage to spermatogenesis, while according to del Castillo *et al.* (1947), gonocytes lose their way during migration from the yolk sac to the embryonic gonads. Focal SCO reveals areas, where seminiferous tubules with residual spermatogenesis reside close to areas displaying severe spermatogenic impairment associated with immature Sertoli cells (Steger *et al.*, 1996, 1999; Maymon *et al.*, 2000).

In fact, more than half the cases diagnosed as SCO syndrome reveal small foci of seminiferous tubules with at least qualitative normal spermatogenesis, which allow

testicular sperm extraction (TESE) to be carried out (Craft *et al.*, 1993; Devroey *et al.*, 1995; Silber *et al.*, 1997) delivering spermatozoa which, subsequently, may be used for intracytoplasmic sperm injection (ICSI) (Palermo *et al.*, 1992). TESE in combination with ICSI allows even men with severe spermatogenic impairment to father a genetically own child (Kahraman *et al.*, 1996; Silber *et al.*, 1996; Silber, 2000). Diagnostic sperm retrieval followed by cryoconservation for later ICSI should, therefore, represent the procedure of choice for all patients exhibiting nonobstructive azoospermia (Verheyen *et al.*, 2004). It is of pivotal importance to ascertain the real nature of SCO tubules in therapeutic testicular biopsies, as in case of only a focal SCO syndrome, TESE in combination with ICSI may be carried out, while in case of a total SCO syndrome, unnecessary multiple biopsies and TESE-ICSI procedures without a chance of success may be avoided.

To date, predictive markers for successful sperm retrieval are scarce (Tournaye *et al.*, 1997). More data are necessary

to finally decide whether micro-TESE could improve the yield of testicular spermatozoa (Schlegel, 1999). On the molecular biology level, amplification of protamines by reverse transcription-polymerase chain reaction represents a reliable biomarker for the presence of testicular spermatozoa (Steger *et al.*, 2001, 2003). This technique, however, gains best results in cryopreserved material. Although Su *et al.* (1999) reported that testicular histology can predict the success of sperm retrieval, more reliable markers are necessary. We, therefore, performed immunohistochemistry (IHC) against Sertoli cell-specific antigens to characterise the state of Sertoli cell maturation and improve the diagnosis of SCO syndrome.

Materials and methods

Testicular biopsies

Testicular biopsies were fixed by immersion in Bouin's fixative and embedded in paraffin wax using standard techniques. Subsequently, sections (5 μm) were either stained with haematoxylin and eosin (H&E) for histological evaluation or used for IHC.

Immunohistochemistry

After deparaffinisation and rehydration of paraffin sections, IHC was performed for the following antigens:

For vimentin staining, sections were exposed to 20% acetic acid for 15 s, blocked with 5% bovine serum albumin (BSA) for 30 min and incubated with the monoclonal anti-vimentin primary antibody (1 : 50; DAKO, Hamburg, Germany) overnight. Sections were then exposed to the secondary antibody (rabbit anti-mouse IgG, 1 : 50; DAKO) followed by a mouse alkaline phosphatase-anti alkaline phosphatase-antibody complex (1 : 100; DAKO) for 30 min each. Finally, immunoreaction was visualised using HistoMark Red (KPL, Gaitersburg, MD, USA).

For AMH staining, sections were treated with 3% H_2O_2 and blocked with 5% BSA for 30 min each and incubated with the polyclonal anti-AMH antibody (1 : 1500; Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight. Sections were then exposed to the biotinylated secondary antibody (rabbit anti-goat IgG, 1 : 100; DAKO) for 30 min and to the avidin-biotin-peroxidase (ABC) complex (Vectastain Elite ABC Standard Kit; Vector, Burlingame, CA, USA) for 30 min. Immunoreactivity was visualised by diaminobenzidine (DAB).

For cytokeratin-18 (CK18) staining, sections were incubated with the monoclonal anti-CK18 antibody (ready-to-use; Coulter-Immunotech, Brea, CA, USA) applying the ABC method. Sections were digested with proteinase K (10 $\mu\text{g ml}^{-1}$, diluted in Tris-buffered saline, pH 7.4)

for 20 min, treated with 3% H_2O_2 for 30 min and blocked with 5% BSA for 30 min. Overnight incubation with the primary antibody was followed by incubation with biotinylated secondary antibody (goat anti-mouse IgG, 1 : 100; DAKO) for 30 min and ABC complex for 30 min. For colour development, sections were incubated with 3-amino-9-ethylcarbazole (AEC).

For M2A antigen staining, sections were microwaved for 25 min at 1000 watts in sodium citrate buffer (pH 6.0), treated with 3% H_2O_2 for 30 min, blocked with 5% BSA for 30 min and incubated with monoclonal anti-D2-40 antibody (1 : 100; DAKO) for 1 h. Sections were then exposed to biotinylated secondary antibody (goat anti-mouse IgG, 1 : 100; DAKO) for 30 min and ABC complex for 30 min. Immunoreactivity was visualised by DAB.

Finally, for connexin 43 (cx43) immunostaining, sections were microwaved for 25 min at 1000 watts in sodium citrate buffer (pH 6.0), treated with 3% H_2O_2 for 30 min, blocked with 5% BSA for 30 min and incubated with a polyclonal anti-cx43 antibody (1 : 100; New England Biolabs, Frankfurt, Germany) overnight. Sections were then exposed to biotinylated secondary antibody (goat anti-rabbit IgG, 1 : 200; DAKO) for 30 min and ABC complex for 30 min. Immunoreactivity was visualised by AEC.

Clinical history

Our patient (DJF) is a 32-year-old healthy sportsman, who after 2 years of childlessness was found azoospermic after prolonged centrifugation with positive fructose and α -glycosidase reaction. No relevant previous medical illness (e.g. mumps) or genital anomalies were present. He was nonsmoker and nondrug abuser, had normal sexual libido and was sexually very active.

Physical examination

Physical examination revealed normal hairiness with an adult male voice, a height of 182 cm and weight of 84 kg. Both testicles in the scrotum were normal sized (volume 20 ml left, 18 ml right), soft in consistency and not painful. There was no varicocele or family history of vein varicosity.

Laboratory data

Laboratory testing revealed the following: Serum FSH 9.8 (reference scale 5–15), LH 5.5 (reference scale 1–9), inhibin-B 150 (reference scale 100–400), testosterone 20 mg (reference scale 10–20); karyotype: 46,XY; Y-microdeletion: absent.

Follow-up story

DJF was very disappointed in not finding spermatozoa to inject after eight attempts in our centre and was not convinced of our explanation that he could have been affected

by SCO syndrome. He returned 2 years later having undergone multiple biopsies on both testicles with no spermatozoa found and no explanation given. He adopted a child, but then returned to us for one final attempt before adopting a second child. Clinical history, physical examination and testicular biopsy were repeated. Six new fragments were taken for biopsy from three different spots in each testicle. However, these six fragments revealed the same histology as the previous two. This time, in addition to all biopsies, IHC was performed to get more information about the Sertoli cell state of differentiation.

Results

Histology

At the level of testicular biopsy, both testes exhibit characteristics of a typical SCO syndrome with complete absence of germ cells. Sertoli cells display an adult type of differentiation being attached to the basal membrane, forming a tubular lumen and exhibiting a well shaped nucleus with clearly visible nucleoli (Fig. 1).

Immunohistochemistry

The intermediate filament protein vimentin, which is known to be present in all differentiation states of Sertoli cells, revealed positive signals within the cytoplasm of the cells present in the seminiferous tubules identifying these cells as Sertoli cells (Fig. 2a). Sertoli cells were immuno-

negative for the intermediate filament protein CK18 (Fig. 2b), anti-Muellerian hormone (AMH) (Fig. 2c) and the M2A antigen (Fig. 2d). Immunohistochemical staining for the gap junctional protein cx43 exhibited positive signals between adjacent Sertoli cells, as well as in interstitial Leydig cells (Fig. 3).

Discussion

For the clinician, it is of pivotal importance to separate total SCO cases lacking sperm and hence unable to father children from focal SCO cases known to exhibit small foci of at least qualitative spermatogenesis, which allow TESE that may be followed by ICSI to be successfully carried out (Craft *et al.*, 1993; Devroey *et al.*, 1995; Silber *et al.*, 1997). According to del Castillo *et al.* (1947), SCO syndrome is characterised by (i) total absence of germ cells within seminiferous tubules, (ii) normal Sertoli cells with a copious healthy cytoplasm, (iii) lack of histological degeneration of the testicular tissue and (iv) reduced testicular volume.

In our patient (DJF), testicular ultrasonographic volume is within the normal range, an observation that is in contradiction with focal SCO cases, the testes of which are smaller than average. In addition, typical focal SCO testes are generally harder in consistency and sometimes painful at touch. DJF, however, exhibits soft and pain free testes. Serum FSH and inhibin-B levels of DJF reveal normal mid-range values. Most SCO cases reveal elevated serum FSH levels associated with immature Sertoli cells (Bergmann

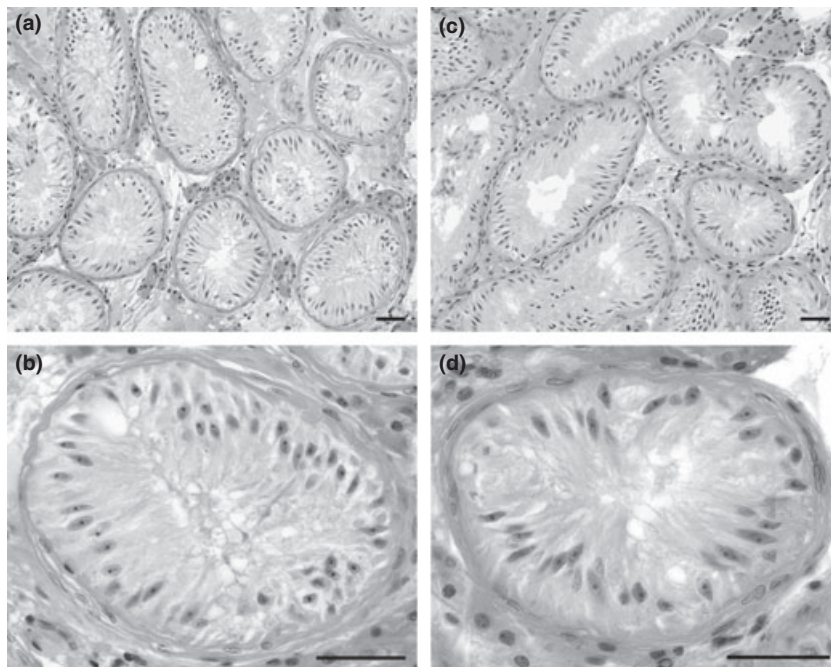


Fig. 1 Paraffin sections showing haematoxylin and eosin (H&E) staining from right (a, b) and left (c, d) testis. Both testes exhibit characteristics of a typical Sertoli-cell-only syndrome with complete absence of germ cells. Sertoli cells display an adult type of differentiation. They are attached to the basal membrane, form a tubular lumen and exhibit a well shaped nucleus with clearly visible nucleoli. Seminiferous tubules reveal normal diameters. Leydig cells are normal in number and shape. Scale bar: 25 μ m.

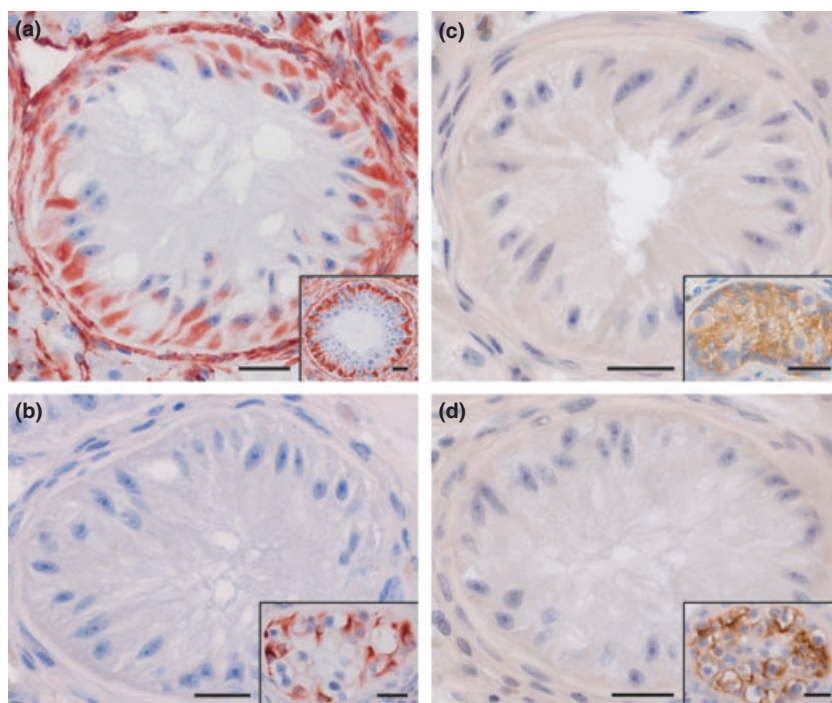


Fig. 2 Paraffin sections showing immunohistochemical staining for vimentin (a), cytokeratin-18 (CK18) (b), AMH (c) and M2A antigen (d). Vimentin serves as a marker for identification of Sertoli cells (a, Inset: positive control showing a seminiferous tubule with normal spermatogenesis). Sertoli cells of this patient are immunonegative for CK18 (b, Inset: positive control showing positive Sertoli cells associated with carcinoma-*in-situ*; CIS germ cells), AMH (c, Inset: positive control showing a prepubertal testis) and M2A antigen (d, Inset: positive control showing neoplastic germ cells in human CIS). Scale bars: 25 μ m.

et al., 1994). The inhibin-B concentration, which appears to be positively correlated with the testicular ultrasonographic value (Bohring & Krause, 1999), indicates that Sertoli cells in DJF are mature and respond well to the solicitation of the pituitary providing the normal feedback mechanism even in the total absence of germ cells.

Nistal *et al.* (1990) reported that testicular biopsies from infertile men exhibiting SCO tubules reveal four types of Sertoli cells: (i) normal adult mature cells showing an intended nucleus, grossly triangular in shape with a prominent tripartite nucleolus, (ii) immature cells with round regularly outlined nuclei and immature cytoplasm, (iii) dysgenetic cells showing immature nuclei and a nearly mature cytoplasm with less developed cytoplasmic organelles and (iv) involuting cells with very irregular outlined nuclei and a mature cytoplasm containing abundant lipid droplets, residual bodies and atypical inter-Sertoli junctional specialisations. To precisely confirm the Sertoli cell state of differentiation, we suggest IHC against Sertoli cell-specific maturation markers. Presence or absence of Sertoli cell-specific proteins is due to certain periods of Sertoli cell maturation (Steger *et al.*, 1996, 1999; Maymon *et al.*, 2000; Brehm *et al.*, 2002, 2006; Sharpe *et al.*, 2003; Franke *et al.*, 2004; Brehm & Steger, 2005).

While vimentin is permanently present in Sertoli cells, CK18, AMH and M2A antigen are not. The loss of M2A antigen expression in Sertoli cells represents a clear sign of transition from prepubertal to adult cells (Steger *et al.*, 1999). In the case of DJF, IHC for vimentin, CK18, AMH

and M2A antigen reveals a staining pattern, which is normal for adult-type Sertoli cells. In addition, synthesis of the gap junctional protein cx43 assures that Sertoli cells in

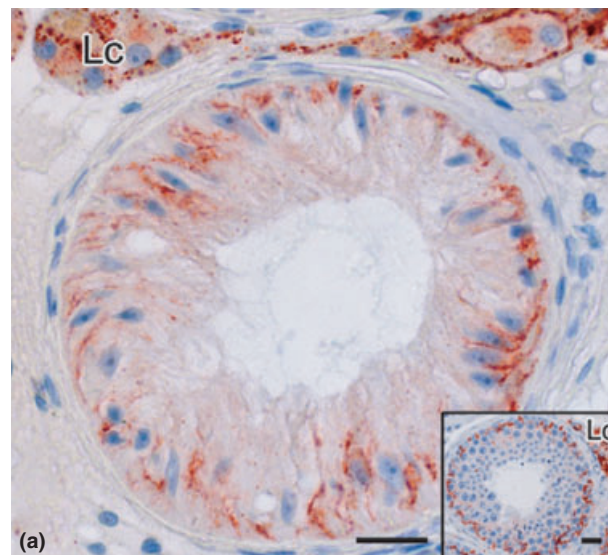


Fig. 3 Paraffin section showing immunohistochemical staining for the gap junctional protein connexin 43 (a, Inset: positive control showing a seminiferous tubule with normal spermatogenesis). During normal spermatogenesis, connexin 43 reveals strong signals between adjacent Sertoli cells occurring apical to spermatogonia and basal to primary spermatocytes and along the Sertoli-Sertoli junctional complexes as well as in interstitial Leydig cells (Lc). In this patient, both Sertoli cells and Lc are immunopositive for connexin 43. Scale bars: 25 μ m.

DJF may communicate with neighbouring cells (Brehm *et al.*, 2002, 2006). Interestingly, our data on cx43 IHC are in line with ultrastructural studies from Chemes *et al.* (1977) reporting extensive junctional complexes between neighbouring Sertoli cells that extend from the basal lamina all the way up to the lumen of SCO tubules. Taking all data into consideration, Sertoli cells in DJF appear to be completely capable of sustaining spermatogenesis. Interestingly, the complete lack of germ cells in combination with fully differentiated adult-type Sertoli cells in DJF is in clear contradiction with the current hypothesis of reciprocal regulation of Sertoli and germ cell differentiation (Steger *et al.*, 1999) and may represent a new type of SCO. Normal Sertoli cell maturation, therefore, may be more impaired by interrelationship with anomalous germ cells rather than by complete lack of germ cells.

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