

Functional status of the Sertoli cell in azoospermic men

To the Editor:

The article by Bar-Shira Maymon et al. (1) is noteworthy in attempting to differentiate the Sertoli cell functional status between normal and pathological biopsies. Once this was a task of diagnostic histology that was based on morphological appearance alone. A noxa patogena, by altering the tubular membrane and Sertoli cell functionality, alters the Sertoli cell germ cells' interrelationship, provoking ailment and impairment of the germ-cell line and finally compromising fertility. This mechanism has not yet been clarified fully, but there is a sufficient body of evidence to indicate that Sertoli cell functionality, and its evaluation, is of crucial importance in azoospermia cases, where the likelihood of finding spermatozoa for testicular sperm extraction–intracytoplasmic sperm injection programs proves low. Therefore, in general, this immunological tool (2) could be of great use in diagnosing pictures when histology on its own cannot (3). However, some observations need to be made on the article to further the understanding and the potential uses of this tool.

1. In the paper, only three groups of azoospermia are considered (mixed atrophy, A; spermatocyte arrest, B; and obstructive azoospermia, C). In each, the rate of chromosome pairing in spermatocytes also has been observed to ensure that group B azoospermia is really due to a germ-line defect (4). Strangely, no cases of fibrosclerosis, germ cell aplasia, Klinefelter syndrome, immature testis, or hypospermatogenesis (5) that could have revealed more convincing results on Sertoli cell functionality were included.
2. In group A, mixed atrophy microphotographs (wherein spermatogenesis is lost and the cellular appearance is of only Sertoli cell syndrome show (Fig. 1, microphoto B) only a little part of a Sertoli cell only syndrome (SCO) tubule. In this small part of the tubule, the cytokeratin 18 (CK18) immunoreactive expression of the Sertoli cell cannot be entirely evaluated. There is no other immunolabeling reactivity shown, for example, of more compromised Sertoli cell syndrome (such as those aligned along the tubular membrane of highly impaired tubules with thickening of the basal membrane, or oedema, and tubular regression into ghost tubules), where the anti-Müllerian (AMH) and CK18 expression should be revealed more clearly.
3. Bar-Shira Maymon et al. imply that AMH or CK18 can be singularly expressed, or coexpressed, with vimentin. This is only true because the process is probably sequential. Indeed it is difficult to interpret the actual status of the Sertoli cell's impairment and the moment at which the Sertoli cell stops secreting vimentin and begins to revive the production of

AMH and CK18. The sole expressions of AMH or CK18 from Sertoli cell syndrome are unable to provide a reliable cutoff point because the level of their impairment, in our experience, varies from one Sertoli cell to another as well as from one tubule to another. Often, on the same slide and perhaps in the same tubule, the morphological appearance of the Sertoli cell and its AMH or CK18 immunolabeling expression is ambiguous; therefore, histological and lab tests are needed (3).

4. A correlation should have been sought between the morphological status of the Sertoli cells (and the tubule to which they belong), and their immunolabeling expression for vimentin, AMH, and CK18.

In any case, this work is noteworthy because it aims to examine and differentiate the Sertoli cell functionality in pathological pictures. Therefore, it is a welcome aid in this field: its future contribution to a better understanding of azoospermia cases, particularly nonobstructive azoospermia cases, seems promising.

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References

1. Bar-Shira Maymon B, Yogev L, Paz G, Kleiman S, Schreiber L, Botchan A, et al. Sertoli cell maturation in men with azoospermia of different etiologies. *Fertil Steril* 2002;77:904–9.
2. Steger K, Rey R, Kleish S, Louis F, Schleiger G, Bergman M. Immunohistochemical detection of immature Sertoli cell markers in testicular tissue of infertile adult men: a preliminary study. *Int J Androl* 1996;19:122–8.

3. Anniballo R, Ubaldi F, Cobellis L, Sorrentino M, Rienzi L, Greco E, et al. Criteria predicting the absence of spermatozoa in the Sertoli cell-only syndrome can be used to improve success rate of sperm retrieval. *Hum Reprod* 2000;15:2269–77.
4. Egozcue J, Templado C, Vidal F, Navarro J, Morer-Fargas F, Marina S. Meiotic studies in a series of 1100 infertile and sterile males. *Hum Genet* 1983;65:85–88.
5. Levin HS. Testicular biopsy in the study of male infertility. Its current usefulness, histologic techniques, and prospects for the future. *Hum Pathol* 1979;10:569–84.

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Reply of the Authors:

We thank Anniballo et al. for their interest in our article, “Sertoli cell maturation in men with azoospermia of different etiologies” (1). This study verifies Sertoli cell differentiation by immunohistochemistry for the immature Sertoli cell markers, anti-Müllerian hormone and cytokeratin 18 (CK-18), within three groups of azoospermic men who differed by testicular histopathology (focal spermatogenesis, A; spermatocytic maturation arrest, B; and normal spermatogenesis, C) and by the presence of testicular spermatozoa in multiple biopsies. These groups had also been previously defined for bivalent formation of chromosomes in spermatocytes by the fluorescence in situ hybridization (FISH) methodology to ensure that group B is due to a germ-line defect (1, 2). This study has the novelty of integrating the results obtained from the immunohistological evaluation with the data obtained from the FISH analysis and, by this approach, contributes to the understanding of the multifactorial mechanism underlying failure of spermatogenesis.

Anniballo et al. had four comments to which we are pleased to reply. The immunohistochemical stainings that were applied in our study provide a diagnostic tool for assessing Sertoli cell differentiation. This tool was used previously in our studies in certain spermatogenic pathologies as Sertoli cell only (3) or in men with deletions in the AZF region of the Y chromosome (3). We use this immunohistological tool for assessing Sertoli cell maturation in dysgenetic immature gonads, and more studies in this field of interest are currently in progress. However, evaluation of testicular biopsies simultaneously, using both methodologies, that is, immunohistochemistry for Sertoli cell maturation and FISH for bivalent formation of chromosomes in spermatocytes, cannot be performed in some of the cases as suggested by Anniballo and colleagues. Sertoli cell-only or immature testis are not suitable because germ cells are absent in the former, whereas spermatocytes have not yet undergone meiosis in the latter.

The microphotographs of group A were chosen for elucidating the phenomenon of mixed atrophy as well as for demonstrating the variable pattern of the immunostaining in focal spermatogenesis or in tubules with a spermatogenic defect. Additional microphotographs for demonstrating the expression of CK-18 in Sertoli cell cytoplasm in various

spermatogenic impairments previously had been published by our group (3).

As for the last comments of Anniballo et al., we would like to emphasize that the vimentin marker is consistently expressed in Sertoli cells’ cytoplasm, regardless of the state of Sertoli cell differentiation (4), whereas only the CK-18 and anti-Müllerian hormone are markers for immature Sertoli cells (5). Expression of anti-Müllerian hormone and/or CK-18 in the adult seminiferous epithelium is regarded as a sign of either maintaining or regaining undifferentiated immature features (5). As we reported in the current study, expression of vimentin was uniformly detected in all of the Sertoli cells, and a correlation between the morphological status of tubules aligned with immature Sertoli cells, immunolabeled by CK-18 and/or anti-Müllerian hormone, is described as well (1).

We hope that our replies have helped to clarify the issues raised by our distinguished colleagues.

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References

1. Bar-Shira Maymon B, Yogev L, Paz G, Kleiman SE, Schreiber L, Botchan A, et al. Sertoli cell maturation in men with azoospermia of different etiologies. *Fertil Steril* 2002;77:904–9.
2. Yogev L, Gamzu R, Kleiman S, Botchan A, Hauser R, Yavetz H. Evaluation of meiotic impairment of azoospermic men by fluorescence in situ hybridization. *Fertil Steril* 2000;74:228–33.
3. Bar-Shira Maymon B, Paz G, Elliott DJ, Hammel I, Kleiman SE, Yogev L, et al. Maturation phenotype of Sertoli cells in testicular biopsies of azoospermic men. *Hum Reprod* 2000;15:1537–42.
4. Franke WW, Grund C, Schmidt E. Intermediate-sized filaments present in Sertoli cells are of the vimentin type. *Eur J Cell Biol* 1979;19:269–75.
5. Steger K, Rey R, Kliesch S, Louis F, Schleicher G, Bergmann M. Immunohistochemical detection of immature Sertoli cell markers in testicular tissue of infertile adult men: a preliminary study. *Int J Androl* 1996;19:122–8.

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Effect of FSH priming on response to hCG in subjects with follicle stimulating hormone receptor (FSHR) mutations

To the Editor:

I have read with great interest the manuscript by Vaskivuo et al. (1) on the lack of effect of FSH and hCG in patients with an inactivating mutation of the FSH receptor. The authors are to be congratulated for their elegant and scholarly workup of their patients bearing this interesting experiment of nature.