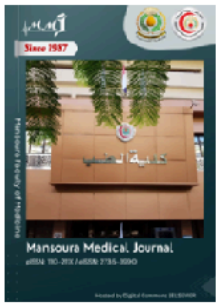
MANSOURA MEDICAL JOURNAL

ISSN - Print: 1110-211X - Online: 2735-3990

journal homepage: mmj.mans.edu.eg



Volume 37 | Issue 2 Article 2

IMMUNOHISTOCHEM1CAL STUDY OF MYOID CELLS AND SERTOLI CELLS OF RAT TESTIS DURING POSTNATAL DEVELOPMENT

Nawal Hasanain

Histology and Cytology Department, Faculty of Medicine, Mansoura University

IMMUNOHISTOCHEMICAL STUDY OF MYOID CELLS AND SERTOLI CELLS OF RAT TESTIS DURING POSTNATAL DEVELOPMENT

By

Nawal A. Hasanain

From

Histology and Cytology Department, Faculty of Medicine,

Mansoura University

ABSTRACT

Microfilaments and intermediate filaments (Ifs) represent an important components of the cytoskeleton. In mammals including rats, peritubular myoid cells (PMCs) contain alpha smooth muscle actin (α-SMA) microfilaments, while sertoli cells contain intermediate filaments vimentin. Previous studies mentioned some aspects about the distribution of these filaments in these cells, however little was mentioned about the changes in their distribution during postnatal (p.n) development.

In the present study, immunohistochemical methods were used to detect the changes in distribution of α-SMA in PMCs and vimentin in sertoli cells during postnatal development of rat testis (one day,7 days,14 days,28 days,42 days and 56 days)

α- SMA immune reaction was seen early from the 1st day of p.n age in few PMCs. On p.n day 7, there was increase in PMCs exhibiting +ve α-SMA immune reaction. This reaction was well established around the whole circumference of the seminiferous tubules on p.n day 14 and the adult distribution pattern was seen on p.n day 28.

Intermediate filaments (Ifs) vimentin were mainly observed at the basal nuclear region of few sertoli cells on p.n day 7 and the first appearance in supranuclear or apical region was on p.n day 14. Vimentin immune reaction was fully established in sertoli cells by

p.n day 28 and the adult distribution pattern was seen on p.n day 42.

Conclusion : during postnatal development of rat testes, sequestral qualitative changes take place in expression of α- SMA of PMCs and in vimentin of sertoli cells suggesting a sequestral functional relationship between myoid cells and sertoli cells.

INTRODUCTION

Sertoli cells and peritubular myoid cells (PMCs) comprise a morphological and functional unit that forms the cytoarchitectural scaffolding of the seminiferous tubule. This cytoarchitecture is believed to be essential for the generation and maintenance of the unique microchemical and physical environment required for germinal cell development (Tung and Fritz, 1990).

It has been reported that, in laboratory rodents like rats, hamsters and mice, only one layer of peritubular myoid cells is present in the testis. Myoid cells contain abundant actin filaments which are distributed in the cells in a specific manner. Peritubular myoid cells (PMCs) maintain the structural integrity of the tubules, take part in the regulation of spermatogenesis and testicular function and have contractile function involved in the tansport of spermatozoa and testicular fluid (Maekawa et al., 1996).

Six actin isoforms have been found in mammalians and constitute a family of closely related proteins expressed in tissue specific way. β- and γ-cytoplasmic actins are ubiquitous. In addition four muscle actins with very similar primary sequences are found in different muscle types; α-skeletal (α-SKA), α- cardiac (α-CAA), α- smooth muscle actin (α-SMA) and γ-smooth muscle actin (γ-SMA) (Clement et al., 2006). Despite their high similarity, many authors have been able to develop specific antibodies for some actin isoforms: α-SMA (Skalli et al., 1986), β-cytoplasmic actin (Gimona et al., 1994), α-SKA (Clement et al., 1999) and α- CAA (Franke et al., 1996 and Clement et al., 2003).

Alpha smooth muscle actin (α-SMA) is an isoform specifically expressed by vascular smooth muscle cells and by peritubular myoid cells

(PMCs) (Franke et al., 1980; Owens et al., 1986; Skalli et al., 1986 and Benzonana et al., 1988). α.- SMA was reported to be readily detectable in peritubular myoid cells in situ and in vitro, but not in other types of cells within the seminiferous tubules (fibroblasts, sertoli cells and cell classes of germinal cells) (Tung and Fritz, 1990).

Using α.-SMA as specific marker of smooth muscle differentiation (Skalli et al., 1986), seminiferous peritubular α- smooth muscle actin (α.-SMA) has been detected early in the first few days in rat testis (Tung and Fritz, 1990 and Palombi et al., 1992) and early postnatal ovine testis (Steger and Wrobel, 1994).

In rat myoid cells, actin filaments run both longitudinally and circularly around the long axis of the seminiferous tubules exhibiting a lattice work pattern (Tung and Fritz, 1990 and Palombi et al., 1992). Changes in arrangement of actin filaments during postnatal development was detected by Maekawa et al., 1995.They suggested a relationship between SMA and testicular development and function.

In rat testis, proliferation of sertoli cells starts from day 16 of fetal life and reaches its maximum 2 days before birth. Approximately one million sertoli cells are present in the rat testis at birth with continued proliferative activity of these cells but in a declining rate (Orth, 1982). The number increases reaching about 40 million at day 15 of postnal life then proliferation ceases and differentiation commences, however the number remains stable throughout adulthood (Wang et al., 1989).

Sertoli cells possess a highly organized cytoskeleton. Vimentin, is the most common intermediate filaments present in sertoli cells, located around the nucleus and provides it with structural support (Amuller et al., 1992; Steger and Wrobel 1994; Steger et al., 1994 and Zhu et al., 1997). Many authors reported that morphological transformation related to cytoskeletal changes takes place during postnatal development of mammalian sertoli cells (Sinowatz and Amsel-graber, 1986; Russel et al., 1989; Condos

and Berndston, 1993 and Zhu et al., 1997).

The aim of the present study is to detect the changes in distribution of α- SMA and vimentin(specific markers for myoid cells and sertoli cells respectively) in rat testis during postnatal development in order to throw light on their functional roles.

MATERIAL AND METHODS

The present study was carried out on 12 male albino rats at different age groups (one day, 7 days, 14 days, 28 days, 42 days and 56 days) 2 rats for each age group. The animals were sacrificed by ether inhalation. The testes were dissected and excised carefully, fixed in Buin's fixative for 1-2 days at room temperature then cut transversely into 3 pieces and post fixed for another 2-3 days in the same fixative. Fixed tissues were dehydrated in ascending grades of alcohol and cleared in xylol, followed by paraffin impregnation and embedding. Paraffin sections were cut 5μm thickness, coated on to gelatin coated slides and dried at 50°C for 3 hours. Some sections were stained with hematoxylin and eosin (H&E) for observation of the general histology. Immuno-histochemical staining for αSMA and vimentin was carried out using avidin-biotin peroxidase complex method (Bancroft and Gamble, 2002).

Kits used :

1. Primary antibody monoclonal mouse antihuman alpha smooth muscle actin (N 1584 -Dacocytomation).
2. Mouse anti primary vimentin (N 1521 -Dacocytomation).
3. Universal LSAB detection kit (K 0673, Dacocytomation).

Methods of the immune-staining :

Sections were dewaxed in xylol and hydrated in descending grades of alcohol down to distilled water. Sections were immersed in 0.3% hydrogen peroxide for 15 minutes to block the endogenous peroxidase activity. Following each step, 2 times washes in phosphate buffer saline (PBS) was allowed. Antigen was retrieved by boiling the sections in citrate buffer (PH6) for 10 minutes followed by coaling and washing in PBS.

Primary antibody (dilution 1:100),

was applied to the sections in humidity chamber at room temperature of 60 minutes, then secondary antibody (biotinylated goat anti polyvalent) was applied to the slides for 10 minutes.

Bounded antibody was visualized by applying the streptavidin biotin peroxidase conjugate to the sections for 15 minutes. Freshly prepared 0.05% 3,3-diaminobenzidine, (DAB) was applied as chromogen. Harris hematoxylin was used as counter stain. Negative control sections were put under the same conditions after omitting the primary antibody.

In immuno-stained sections of the testes, the smooth muscles of the blood vessels were considered as positive control for α-SMA immune reactivity and the interstitial fibroblasts were considered as positive control for vimentin immune reactivity.

RESULTS

Alpha smooth muscle actin (α SMA) immune reaction :

Alpha smooth muscle actin (α-SMA) immune reaction was detectable in peritubular myoid cells (PMCs) of the rat testis at all developmental stages and in the smooth muscles of the blood vessels which represent the positive control.

α- SMA immune-reactivities appeared in PMCs of the rat testis from the 1st day of postnatal (p.n) life. PMCs of this age were few, relatively thick and sparse in distribution around the seminiferous tubules (Figs. 1, 2). On p.n day 7, α-SMA immune reaction appeared in increased number of PMCs (Fig. 3). On p.n day 14, PMCs exhibiting +ve reaction for αSMA were thinner, stretched and attained regular pattern around the circumference of the seminiferous tubules (Fig. 4).

On p.n day 28, α- SMA immune reaction was stronger in PMCs attaining adult appearance with more regular distribution around the seminiferous tubules (Fig. 5). On p.n day 42 and 56 α- SMA immune reaction attained adult appearance. It was strong in stretched myoid cells around the whole circumference of the seminiferous tubules (Figs. 6, 7).

Vimentin immune reaction :

On the first day of postnatal age,

sertoli cells were few with no detectable vimentin immune reaction (Fig. 8). On the 7th day of p.n age, there was relative increase in sertoli cells. Vimentin immune reaction was seen in a thin basal infranuclear area of sertoli cells (Fig. 9). On p.n day 14, there was more increase in sertoli cells, vimentin immune reaction was seen in the perinuclear region and attained minimal supranuclear extension (Fig. 10). On p.n day 28, sertoli cells were relatively differentiated, vimentin immune reaction was strong in the perinuclear region with more supranuclear extension (Fig. 11).

On p.n day 42, vimentin immune reaction attained adult appearance. It was intense around the nucleus with much more supranuclear extension (Fig. 12). On p.n day 56, sertoli cells appeared thin and compressed by the spermatogenic epithelium. Vimentin immune reaction was strong around the nucleus and exhibit flame like supranuclear extension of all cytoplasm (Fig. 13).

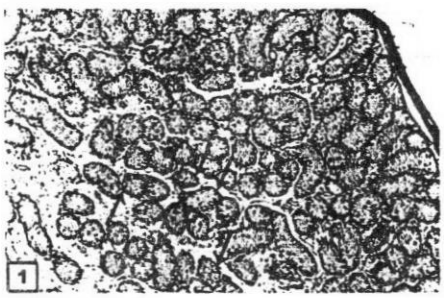


Fig. (1): A photomicrograph of one day old rat testis showing few peritubular myoid cells (PMCs) (arrows) with positive α-SMA immune reaction. Note positive α-SMA immune reaction in the wall of the blood vessels (V).
(α- SMA immune reaction x100).

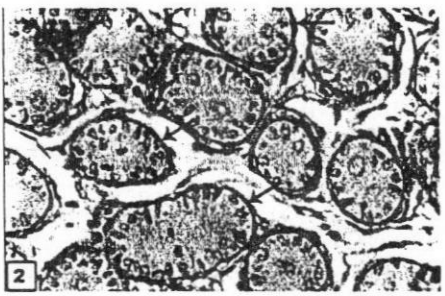


Fig. (2): A high power magnification of the previous figure showing few and relatively thick peritubular myoid cells (arrows) with positive α - SMA immune reaction.
(α- SMA immune reaction x 400).

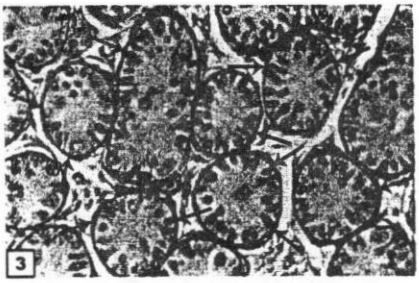


Fig. (3): A photomicrograph of 7 days old rat testis showing increase in peritubular myoid cells (arrows) with +ve α- SMA immune reaction. (α- SMA immune reaction x400).

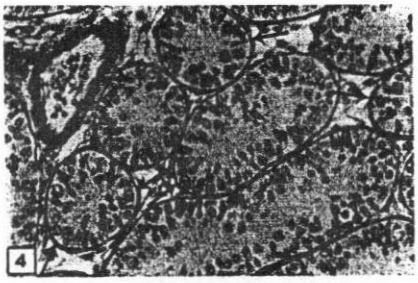


Fig. (4): A photomicrograph of 14 days old rat testis showing +ve α- SMA immune reaction in the peritubular myoid cells (arrows). PMCs appear numerous and stretched all around the circumference of the seminiferous tubules. Note +ve α- SMA immune reaction in the wall of the blood vessel (V). (α- SMA immune reaction x400).

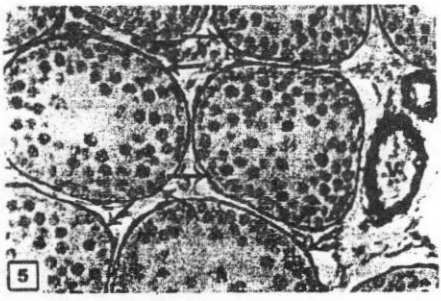


Fig. (5): A photomicrograph of 28 days old rat testis showing strong positive α- SMA immune reaction in the peritubular myoid cells (arrows). PMCs appear stretched all around the circumference of the seminiferous tubules. The blood vessel (V) shows strong +ve immune reaction. (α- SMA immune reaction x400).

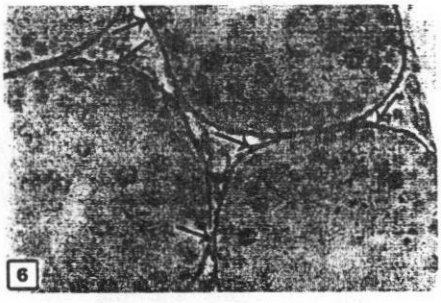


Fig. (6): A photomicrograph of 42 days old rat testis showing strong +ve α- SMA immune reaction in the peritubular myoid cells (arrows). PMCs appear more stretched around the seminiferous tubules comparing with the previous age. (α- SMA immune reaction x400).

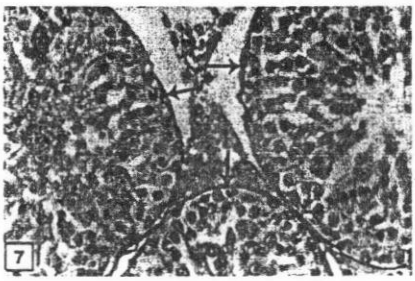


Fig. (7): A photomicrograph of 56 days old rat testis showing strong α-SMA immune reaction in the peritubular myoid cells (arrows). PMCs appear more stretched around the whole circumference of the seminiferous tubules.
(α- SMA immune reaction x400).

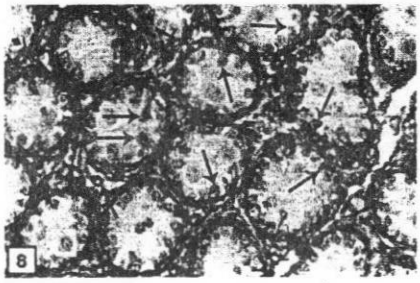


Fig. (8): A photomicrograph of one day old rat testis showing few sertoli cells (arrows) with no visible vimentin immune reaction. Note +ve vimentin immune reaction in the interstitial fibrablasts (crossed arrows).
(vimentin- immune reaction. x400).

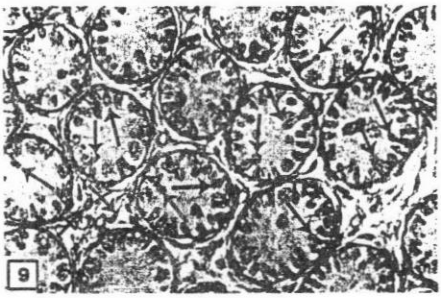


Fig. (9): A photomicrograph of 7 days old rat testis showing increased number of sertoli cells (arrows) exhibiting thin basal infranuclear vimentin immune reaction. Note +ve vimentin immune reaction in the interstitial fibrablasts (crossed arrows)
(vimentin- immune reaction x400).

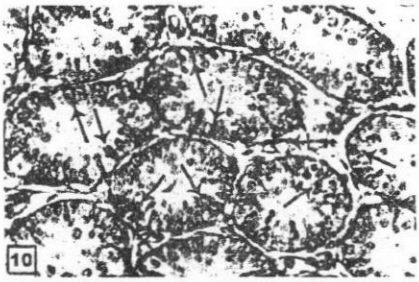


Fig. (10): A photomicrograph of 14 days old rat testis showing increased number of sertoli cells (arrows) exhibiting +ve vimentin immune reaction in the perinuclear region and minimal supranuclear extension. positive vimentin immune reaction is seen in interstitial fibroblasts(crossed arrows).
(vimentin- immune reaction, x400).

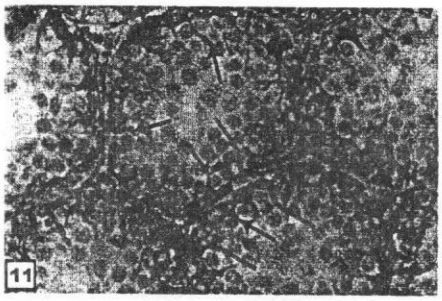


Fig. (11): A photomicrograph of 28 days old rat testis showing relatively differentiated sertoli cells (arrows) with +ve vimentin immune reaction in the perinuclear region and more supranuclear extension.
(vimentin- immune reaction, x400).

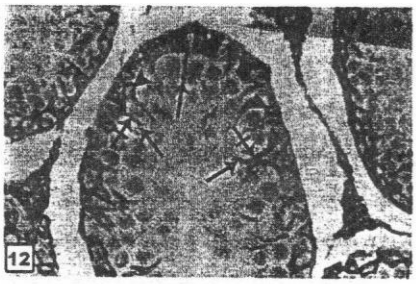


Fig. (12): A photomicrograph of 42 days old rat testis showing more differentiated sertoli cells (arrows) exhibiting strong +ve vimentin immune reaction in the perinuclear regions (arrow head) and supra-nuclear cytoplasm (crossed arrows).
(vimentin- immune reaction, x400).

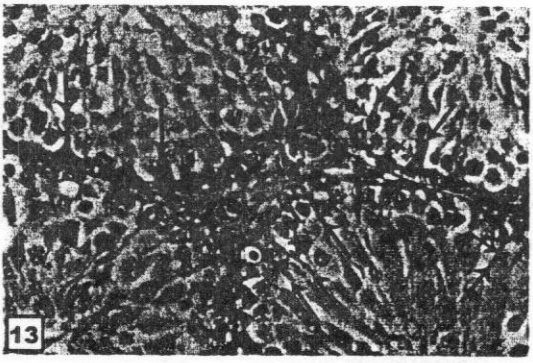


Fig. (13): A photomicrograph of 56 days old rat testis showing sertoli cells (arrows) with Strong +ve vimentin immune reaction in the perinuclear region (arrow head) with flame like extension in the supranuclear cytoplasm (crossed arrows).
(vimentin- immune reaction x400).

DISCUSSION

In the present study, α- SMA was demonstrated in the peritubular myoid cells of the rat testis early in postnatal days of age. It has been reported that Peritubular actin expression in the rat testis commences during gestation while spermatic cords are undergoing organization (Palombi et al., 1992). This is in contrast with postnatal detection of α- SMA in other species like bovine testis (Devkota et al, 2006) where it was observed around 4th month of age. It has been reported that, onset of α- SMA expression in peritubular myoid cells is correlated with major increase in seminiferous tubule diameter and testis weight (Holt et al., 2004). Species differences in onset of peritubular actin expression are therefore a biological interpretation must be taken in consideration.

In myoid cells of Sprague Dewaly rats actin filaments were described by Russel et al.(1989) as parallel filaments at 10 days of age and by 22 days actin cables formed a meshwork pattern, a characteristic of adult myoid cell. In the present study, peritubular α- SMA expression in the developing rat testis progressively increased with age and adult distribution pattern was fully established on p.n day 28.

The presence of F-actin in peritubular myoid cells has previously been reported as a correlate of testicular maturation in mammals for example in bovine and ovine testis. In these species actin expression represents one aspect of the formation and completion of the blood testis barriers, an essential feature of seminiferous tubule organization (Steger and Wrobel, 1994 and Wrobel, et al., 1995).

Earlier studies (Amuller et al., 1992) using immunohistochemical techniques and electron microscope for Ifs of sertoli cells of rat during postnatal development revealed appearance of the Ifs central around the nucleus, where they apparently terminate in the nuclear envelope providing a perinuclear stable core area, from this area they radiate to plasma membrane. The increase in vimentin content and appearance of axially oriented vimentin filaments was explained as a co-incidence with acqui-

sition of the columnar shape of the sertoli cells (Paranko et al., 1986).

Vimentin type IFs in rat sertoli cells has been described by Zhu et al. (1997) as a delicate endocellular network, which is centered in the perinuclear region and extends to the apical region of the cell. They mentioned that during postnatal development, Ifs gradually increase in number and the main distribution area is transferred from basal nuclear to the perinuclear and supranuclear region. In the present study, vimentin Ifs first appeared in the basal nuclear regions of sertoli cells on p.n day 7 and the first appearance in supranuclear or apical region was noted on p.n day 14 followed by gradual increase where adult appearance was observed on p.n day 42.

In the present study, increase in numbers of setoli cells in rat testis during 1st 14 days of p.n age was noticed, however, fully differentiated sertoli cells and adult appearance of vimentin immune reaction was noticed on p.n day 42. This is in consistent with what mentioned by Wang et al. (1989), that the number of sertoli cells inceases to a maximum of around 40 million at day 15 of postnatal life. After postnatal day 15 prolifo-ration ceases and differentiation commences, however the number of sertoli cells remains stable throughout adulthood

α- SMA expression in peritubular myoid cells was noted early in p.n age of rat (Palombi et al., 1992)and human (Arenas et al., 1997) followed by rapid attainment of mature pattern which might help to increase contractility of seminiferous tubules and might help in the secretion of paracrine factors required for regulation of sertoli cell function (Skinner, 1991 and skinner, et al., 1998). This agreed with our findings in the present study, where α- SMA expression in peritubular myoid cells attained mature pattern early on p.n day 28 while sertoli cells attained mature pattern for vimentin immune reaction late on p.n day 42. This indicates the importance of myoid cells for early contraction of seminiferous tubules and early regulation of sertoli cell function that are vital for spermatogenic process.

An appropriate testicular organogenesis involves a cascade of gene activation and differentiation of the component cell types. Sertoli cells organize themselves into testicular cords surrounded by peritubular myoid cells and enclosing fetal germ cells (Skakkeback et al., 2001). Myoid peritubular cells engage in a dialougue with sertoli cells (Hadley et al., 1985), which is crucially important for structural formation of the blood testis barriers (Hadley and Dyme, 1987). Peritubular myoid cells are known to stimulate total protein production by sertoli cells and to increase sertoli cell production of androgen-binding protein and transferrin. They produce a protein named P-mod-S (peritubular factors that modulate sertoli cell function) that has been shown to be an important regulator of sertoli cell function in vitro and also considered to be highly influential in modulating the effects of androgens on the seminiferous tubules(Anthony et al., 1991).

CONCLUSION

From this study, we can postulate that, during postnatal development of rat testis, there was sequestral functional relationship between myoid cells and sertoli cells indicated by the sequestral qualitative changes in expression of α- SMA of the peritubular myoid cells and in vimentin of sertoli cells. In order to confirm this postulation further electron microscopic study may be needed.

REFERENCES

1. **Anthony, C.T.; Rosselli, M. and Skinner, M.K. (1991) :** Actions of testicular paracrine factors (P-mod-S) on sertoli cell transferrin secretion throughout pubertal development. J. Endocrinol., 129: 353-366.

2. **Arneas, M.I.; Bethencourt, F.R.; Miguel, M.P.; Fracile, B.; Romo, E. and Paniagua, R. (1997) :** Immunocytochemical and quantitative study of actin, desmin and vimentin in the peritubular cells of the testes from elderly men. J. Repord. Fertil.; 110: 183-193.

3. **Amuller, G.; Schulze, C. and Viebahn, C. (1992) :** Intermediate filaments in sertoli cells.

Microsc. Res. Tech.; 20: 50-72.

4. **Bancroft, J.D. and Gambie, M. (2002)** : Theory and practice of histological techniques. 5th ed., churchil Livingstone, New York, Philadelphia, st Luis and Toronto, pp: 171-173.

5. **Benzonana, G.; Skalli, O. and Gabbiani, G. (1988)** : Correlation between distribution of smooth muscle or non muscle myosins and α-smooth muscle actin in normal and pathological soft tissue. Cell. Motil. Cytoskeleton, 11: 260-74.

6. **Clement, S.; Chaponnier, C. and Gabbiani, G. (1999)** : A subpopulation of cardiomyocytes expressing alpha-skeletal actin is identified by a specific polyclonal antibody. Circ. Res.; 85: e51-e58.

7. **Clement, S.; Orlandi, A.; Bocchi, L.; Pizzolato, G.; Foschini, M.P.; Eusebi, V. and Gabbiani, G. (2003)** : Actin isoform pattern expression: a tool for the diagnosis and biological characterization of human rhabdomyosarcoma. Virchows Arch.; 442: 31-38.

8. **Clement, S; Stouffs, M.; Bettiol, E.; Kampf, S.; Heinz Krause, K.; Chaponnier,C. and Jaconi, M. (2006)** : Expression and function of α-smooth muscle actin during embryonic-stem-cell-derived cardiomyocyte differentiation. Journal of cell science,120:229-238.

9. **Devkota, B.; Sasaki, M.; Takahashi, M.; Matsuzaki, S.; Matsui, M.; Haneda, S.; Takahashi, M.; Osawa, T. and Miyake J.I. (2006)** : Postnatal developmental changes in immunhistochemical localization of α-smooth muscle actin (α-SMA) and vimentin in bo-

vine testes. J. Reprod. Dev.; 52: 43-49.

10. **Franke, W.W.; Schmid, E.; Vandekerckhove, J. and Weber, K. (1980) :** A permanently proliferating rat vascular smooth muscle cell with maintained expression of smooth muscle characteristics including actin of the vascular smooth type. J. Cell. Biol.; 87: 594-600.

11. **Franke, W.W.; Stehr, S.; Stumpp, S.; Kuhn, C.; Heid, H.; Rackwitz, H.R.; Schnolzer, M.; Baumann, R.; Holzhousen, H.J. and Mall, R. (1996)** : Specific immunohistochemical detection of cardiac / fetal alpha actin in human cardiomycytes and regenerating skeletal muscle cells. Differentiation, 60: 245-250.

12. **Gimona, M.; Vandekerckhove, J.; Goethals, M.; Herzog, M.; Lando, Z. and Small, J.V. (1994) :** Beta-actin specific monoclonal antibody. Cell motil. Cytoskeleton, 27: 108-116.

13. **Gondos, B. and Berndston, W.E. (1993) :** Postnatal and pubertal development. In: Russel LD, Friswold MD (eds.), The sertoli cell. Clearwater, FL: Chache River Press; 115-153.

14. **Hadley, M.; Byers, S.; Suarez-Quian, C.; Kleinman, H. and Dym, M. (1985)** : Extracellular matrix regulate sertoli cell differentiation, testicular cord formation and germ cell development in vitro. J. Cell Biol.; 101: 1511-1522.

15. **Hadley, M.A. and Dyme, M. (1987)** : Immuno-cytochemistry of extracellular matrix in the lamina propria of the rat testis electron microscopic localization. Biol Reprod.; 37: 1283-89.

16. **Holt, W.V.; Waller, J.; Moore, A.; Jepson, P.D.; Deaville,**

R. and Bennett, P.M. (2004) : Smooth muscle actin and vimentin as markers of testis development in harbour porpoise (phocoena phocoena). J Anat.; 250 (3): 201-11.

17. **Maekawa, M.; Kazama, H.; Kamimura, K. and Nagano, T. (1995) :** Changes in the arrangement of actin filaments in myoid cells and sertoli cells of rat testes during postnatal development and after experimentally cryptorchidism. Anat. Rec.; 241: 59-69.

18. **Maekawa, M.; Kamimura, K. and Nagano, T. (1996) :** Peritbular myoid cells in the testis: their structure and function. Arch histocytol.; 59(1): 1-13.

19. **Orth, J.M. (1982) :** Proliferation of sertoli cells in fetal and postnatal rats. A quantitative autoradiographic study. Anat. Record.; 203: 485-492.

20. **Owens, G.K.; Loeb, A.; Gordon, D. and Thompson, M.M. (1986) :** Expression of smooth muscle-specific α-isoactin in cultured vascular smooth muscle cells: relationship between growth and cytodifferentiation. J. Cell. Biol.; 102: 343-52.

21. **Palombi, F.; Farini, D.; Salanova, M.; de Grossi, S. and Stefanini M. (1992) :** Development and cytodifferentiation of peritubular myoid cells in the rat testis. Anat. Rec.; 233: 32-40.

22. **Paranko, J.; Kallajoki, M.; Pelliniemi, L.J.; Lehto, V.P. and Virtaness, I. (1986) :** Transient co-expression of cytokeratin and vimentin in differentiating rat sertoli cells. Dev Biol.; 117(1): 35-44.

23. **Russell, L.D.; Bartke, A. and Groh J.C. (1989) :** Postnatal development of the sertoli cell barrier, tubular lumen and cytoskeleton of

sertoli and myoid cells in the rat and their relationship to tubular fluid secretion and flow. Am J Anat.; 184: 179-189.

24. **Sinowatz, F. and Amselgruber, W. (1986) :** Postnatal development of bovine sertoli cells. Anat. Embryol.; 174: 413-423.

25. **Skakkebaek, N.E.; Rajpert-De Meyts, E. and Main, K. (2001) :** Testicular dysgenesis syndrome: an increasingly developmental disorder with environmental aspects. Hum. Reprod.; 16: 972-978.

26. **Skalli, O.; Ropraz, P.; Trzeciak, A.; Benzonana, G.; Gillessen, D. and Gabbiani, G. (1986) :** A monoclonal antibody against α-smooth muscle actin: a new probe for smooth muscle differentiation. J Cell Biol.; 103: 2787-2796.

27. **Skinner, M.K. (1991) :** Cell-cell interaction in the testis. Endocr. Rev.; 12: 45-77.

28. **Skinner, M.K.; Fetterolf, P.M. and Anthomy, C.T. (1998) :** Purification of a paracrine factor P-Mod-S, produced by testicular peritubular cells that modulates sertoli cell function. J. Biol. Chem.; 263: 2884-2890.

29. **Steger, K. and Wrobel K.H. (1994) :** Immunohistochemical demonstration of cytoskeletal proteins in the ovine testis during postnatal development. Anat. Embryol. (Berl.); 189: 521-530.

30. **Steger, K.; Schimmel, M. and Wrobel, K.H. (1994) :** Immunocytochemical demonstration of cytoskeletal proteins in seminiferous tubules of adult rams and bulls. Arch Histol. Cytol.; 57: 17-28.

31. **Tung, P.S. and Fritz, J.B., (1990) :** Characterization of rat testicular peritubular

myoid cells in culture: alpha-smooth muscle isoactin is a specific differentiation marker. Biol. Reprod.; 42: 351-365.

32. **Wang, Z.X.; Wreford, N.G. and De kretzer, D.M. (1989) :** Determination of sertoli cell numbers in developing rat testis by stereological methods. Int. J. Androl.; 12: 58-64.

33. **Wrobel, K.H.; Bickel, D. and Kujat, R. (1995) :** Distribution pattern of f-actin, vimentin and alpha-tubulin in the bovine testis during postnatal development. Acta. Anat. (Basel); 135(4): 263-72.

34. **Zhu, L.J.; Zong, S.D.; Philips, D.M.; Moo-Young, A.J. and Bardin, C.W. (1997) :** Changes in the distribution of intermediate filaments in rat sertoli cells during the seminiferous ephtihelial cycle and postnatal development. Anat. Rec.; 248: 391-405.

الملخص العربي

دراسة هستوكيميائية مناعية للخلايا شبه العضلية وخلايا سرتولى فى خصية الفأر أثناء التطور بعد الولادة

نـــوال عـــوض حسنـــين

قسم الأنسجة والخلايا - كلية طب المنصورة

تعتبر الخيوط الدقيقة والخيوط المتوسطة من العناصر الهامة فى الجهاز الهيكلى للخلية وفى الشدييات ومنها الفئران تحتوى الخلايا شبه العضلية المحيطة بقنوات نقل المنى على خيوط دقيقة من نوع أكتين ألفا للعضلة الملساء وتحتوى خلايا سرتولى على خيوط متوسطة من نوع الفيمنتين. وفى دراسات سابقة تم ذكر بعض الجوانب عن توزيع هذه الخيوط فى هذه الخلايا ولكن ذكر القليل عن التغيرات فى توزيعها أثناء التطور بعد الولادة .

وفى هذه الدراسة تم استخدام الطرق الهستوكيميائية المناعية لتحديد التغيرات التى تطرأ على توزيع أكتين ألفا للعضلة الملساء فى الخلايا شبه العضلية المحيطة بقنوات نقل المنى وكذلك على الفيمنتين فى خلايا سرتولى وذلك فى خصية الفأر أثناء التطور بعد الولادة(يوم ، ٧ أيام ، ١٤ يوم ، ٢٨ يوم ، ٤٢ يوم ، ٥٦ يوم) .

لوحظ ظهور أكتين ألفا للعضلة الملساء فى القليل من الخلايا شبه العضلية المحيطة بقنوات نقل المنى منذ اليوم الأول بعد الولادة . أما فى اليوم السابع فكانت هناك زيادة فى الخلايا المحيطة بقنوات نقل المنى إيجابية التفاعل لأكتين ألفا للعضلة الملساء والذى أصبح أكثر وضوحا وامتدادا بهذه الخلايا حول محيط قنوات نقل المنى عند اليوم الرابع عشر وأصبح مشابها لمرحلة البلوغ عند اليوم الثامن والعشرين .

لوحظ ظهور الفيمنتين فى الجزء القاعدى من خلايا سرتولى ابتداء فقط من اليوم السابع بعد الولادة ، أما الظهور الأول حول النواة وفوقها فكان فى اليوم الرابع عشر بعد الولادة وأصبح

اكثر وضوحا وانتشارا بخلايا سرتولى عند اليوم الثامن والعشرين ومشابها لمرحلة البلوغ عند اليوم الثانى والأربعين.

نستخلص من هذه الدراسة أنه أثناء تطور خصى الفأر فى مرحلة ما بعد الولادة تطرأ تغيرات جوهرية متتابعة على توزيع أكتين ألفا للعضلة الملساء الموجود بالخلايا شبه العضلية المحيطة بقنوات نقل المنى وكذلك على خيوط الفيمنتين الموجودة بخلايا سرتولى موضحة بذلك وجود علاقة وظيفية متتابعة بين الخلايا شبة العضلية و خلايا سرتولى.