

# Cadherin and vimentin immunoexpression in the testis of normal and induced infertility models of albino rats

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[Received 18 January 2014; Accepted 3 March 2014]

**Background:** Sertoli cells are important in determining the fate of spermatogenic cells by providing nutrition and structural support via cell junctions. Adhesion between Sertoli and germ cells is important for spermatogenesis. Cadherin are transmembrane proteins that mediate cell-cell adhesion, while, vimentin, the cytoskeletal intermediate filament plays an important role in spermatogenesis. The aim of the present study was to investigate cadherin and vimentin immunoexpression in the normal testis and in two types of altered spermatogenic states: the cyclophosphamide (CP) treatment and the cryptorchidism (Cx) models.

**Materials and methods:** Twenty four male albino rats were divided into control group: 6 rats receiving saline orally and the other 6 were sham-operated. CP group (n = 6): given 6 mg/kg/day of CP orally for 4 weeks. Cx group (n = 6): the left testis was surgically freed from the scrotum and fixed in the abdomen. Animals were sacrificed and the left testis dissected and prepared to be stained with haematoxylin and eosin stain and immunohistochemical stain against cadherin and vimentin. Morphometric measurements and statistical analysis were done.

**Results:** In CP-treated group there was degeneration of spermatocytes, vacuolations of Sertoli cells and absence of spermatozoa. These changes were more prominent in Cx group, in addition to interstitial hypercellularity. There was also a significant decrease in cadherin and vimentin immunostaining in CP-treated group that was more marked in the cryptorchidism group.

**Conclusions:** A downregulation of both cadherin and vimentin was associated with both models of impaired spermatogenesis. This impairment could be attributed to disruption of the junctions between Sertoli and germ cells. (Folia Morphol 2014; 73, 3: 339–346)

**Key words:** testis, cadherin, vimentin, immunohistochemistry

## INTRODUCTION

Adherens junctions (AJs) form connections between neighbouring cells and are responsible for the maintenance of tissue integrity. AJs are actin-based or intermediate filaments-based junctions [6]. Adhesion between Sertoli cells and germ cells is thought to be

crucial for spermatogenesis. The dynamic nature of AJs in the testis permits the passage of developing germ cells from the basal to the adluminal compartment during spermatogenesis, as well as contribute to the orientation, positioning and correct shape of sperms [20].

The cadherin/catenin complex is an essential regulator of intercellular adhesion and is critical for the establishment of epithelial cell polarity [12]. The cadherin population in the testis varies. At the mRNA level at least 24 cadherins were identified in rat testis including 7 classical cadherins, such as P-, N- and E-cadherin [13]. However, only few studies were concerned with the expression and localisation of P-cadherin protein in adult rat testis, especially during impaired spermatogenesis [15, 20]. P-cadherin was detected in rat Sertoli cell culture [27] and reported to participate in the architecture of AJs in the testis and to play an important role in maintaining normal spermatogenesis [20].

Unlike in most other epithelia where the intermediate filaments are of the keratin type, intermediate filaments in mature Sertoli cells are of the vimentin type. The patterns of filament distribution are consistent with a role in maintaining tissue integrity when the epithelium is mechanically stressed [26].

Vimentin occurs in the basal and perinuclear region of the Sertoli cells and radiates toward the apical cytoplasm where they become associated with some specialised membrane structures "desmosome-like junctions" between Sertoli cells and adjacent germ cells. It is thought that they play a role in anchoring germ cells to the seminiferous epithelium [3]. The role of vimentin in elongate spermatid movement within the seminiferous epithelium has been postulated [28]. Vimentin filaments play an important role in the maintenance of spermatogenesis through their distribution in the Sertoli cells; their damage is associated with the seminiferous epithelium disintegration and their restoration with a recovery of spermatogenesis after the unfavourable conditions subside [16].

Cyclophosphamide (CP) as an alkylating agent is the most commonly used anticancer and immunosuppressant drug [22]. A variety of adverse effects have been implicated in patients and experimental animal models receiving CP treatment, such as reproductive toxicity [11]. However, the precise mechanism by which CP causes testicular toxicity is undefined [18].

Cryptorchidism (Cx) is the failure of the testis to descend in the scrotum exposing it to body heat that severely affects spermatogenesis [8]. Moreover, this elevated temperature was also shown to cause degenerative changes both in Sertoli and germ cells and has also been associated with decreased sperm motility [21]. In addition, it was shown that Cx lead to AJs disintegration [20]. Cx in man usually leads

to clinical infertility due to severe decrease in sperm production. Although the pathways for germ cell apoptosis have been reported, the molecular mechanism is still not well characterised [1].

The aim of the present work was to investigate 2 AJ components cadherin and vimentin immun-expression in the normal testis and in 2 types of altered spermatogenic states: the first is the chemical CP model and the second is the surgical Cx models. The use of 2 distinct models leading to hypospermia was chosen to find whether they have the same or different effects on AJ proteins. Our hypothesis postulates that whatever injurious agent, the mechanism of oligospermia starts from dehiscence of the spermatogenic cells from the nurturing Sertoli cells due to lack of these proteins. This might offer better understanding of the mechanism of hypospermia and infertility.

## MATERIALS AND METHODS

### Experimental design

The present study was carried out on 24 adult male albino rats, weighing 170–220 g. They were kept on a 12 h light-dark cycle and given food and water *ad libitum*. The animals were randomly divided into three groups (all rats survived the experimental period until sacrifice):

- Control group (n = 12) — it was subdivided into: 6 animals that received daily oral 0.5 mL of saline and the other 6 were sham operated.
- Cyclophosphamide group (CP group) (n = 6): cyclophosphamide (Endoxan, Baxter Oncology, USA) was purchased as 50 mg tablets. The tablets were crushed to powder and dissolved in 20 mL saline. It was given to the animals at daily oral dose of 6 mg/kg/day for 4 weeks [7].
- Cryptorchidism group (Cx group) (n = 6): the rats were anaesthetised by intraperitoneal injection of pentobarbital. A small inguino-scrotal incision was made and the left testis was freed from the scrotum to be displaced into the abdomen. Testis descent was prevented by suturing the inguinal canal. The right testis was kept in the scrotum [21].

The applied procedures were approved by the research ethical committee, Faculty of Medicine, Cairo University.

One month from the beginning of the experiment, all animals were sacrificed using deep chloroform anaesthesia. The left testis was removed and rapidly

fixed in bouin's solution, processed to obtain paraffin blocks and cut at 5–6 micron thickness sections. Sections were stained with haematoxylin and eosin and immunohistochemical staining using either anti-cadherin or anti-vimentin antibodies [4].

Immunostaining required antigen retrieval by boiling tissues sections in citrate buffer pH 6.0 in microwave for 2 min followed by cooling at room temperature. Then they were incubated with the ready to use primary antibody for 1 h. The primary antibodies used were: anti-Cadherin rabbit polyclonal antibody, cat. #RB-9036-R7, positive control: tonsil or squamous epithelium and anti-vimentin monoclonal mouse antibody (J144), cat. #MA3-745, positive control: human rhabdosarcoma cell line JR1. Then, ultravision universal detection system was used to detect the immunoreaction. This was formed of biotinylated anti-polyvalent secondary antibody, streptavidin peroxidase and DAB. The sections were then counterstained using Mayer's haematoxylin. All these materials were purchased from Labvision Corporation, ThermoScientific (USA).

#### Morphometric study and statistical analysis

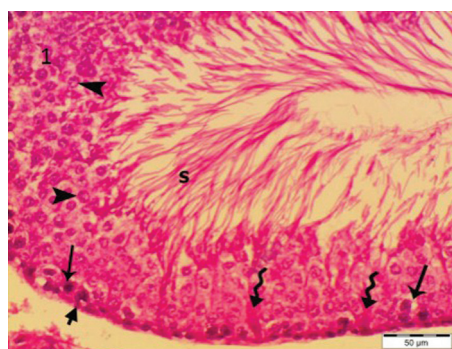
The area percent of cadherin and vimentin immunostaining were measured using the image analyser computer system "Lecia Qwin 500 C" (Cambridge, UK). This was done in 10 non-overlapping fields for each animal at  $\times 400$ . The data obtained were statistically analysed by comparing the mean values of different groups by analysis of variance ANOVA test using "SPSS 9" software. P value  $< 0.05$  were considered statistically significant [19].

## RESULTS

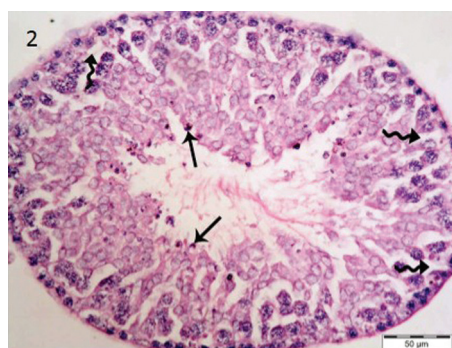
### Light microscopic results

#### Haematoxylin and eosin results

Sections in control testis showed seminiferous tubules lined with spermatogenic cells in the form of spermatogonia in the basal region, primary spermatocytes in the intermediate region with the characteristic dispersed chromatin, rounded spermatids in the apical region and spermatozoa attached to the apical part of Sertoli cells and extending their tails inside the lumen. Sertoli cells were seen in between the spermatogenic cells having elongated acidophilic cytoplasm and vesicular oval nucleus (Fig. 1). The CP-treated group showed seminiferous tubules with markedly detached spermatocytes and spermatids

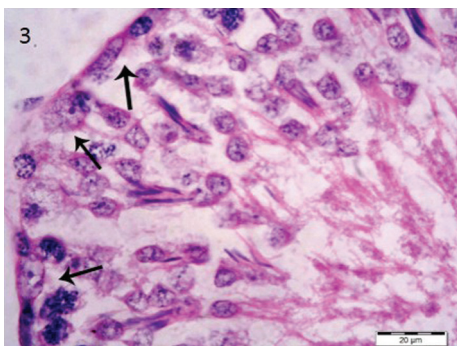


**Figure 1.** A section in the testis of a control group rat (stage VIII spermatogenesis) showing seminiferous tubule with normal distribution of spermatogonia (short arrow) in the basal region, primary spermatocyte (long arrow) in the intermediate region, spermatids (arrow heads) in the apical region and spermatozoa inside the lumen (S). Note the Sertoli cells (wavy arrow) in between the spermatogenic cells with their elongated acidophilic cytoplasm and oval vesicular nuclei (haematoxylin and eosin).

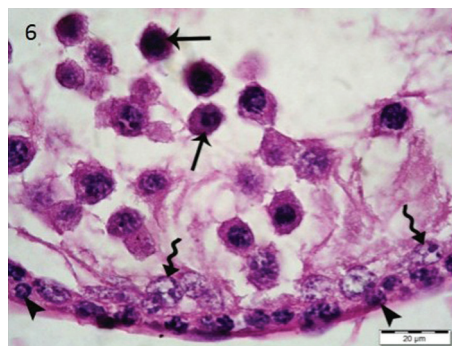


**Figure 2.** A section in the testis of a cyclophosphamide group rat (stage VIII) showing seminiferous tubule with markedly detached spermatocytes and spermatids from the Sertoli cells (wavy arrows). Few residual cytoplasmic bodies are seen after spermiation attached to the apices of Sertoli cells (thin arrow) (haematoxylin and eosin).

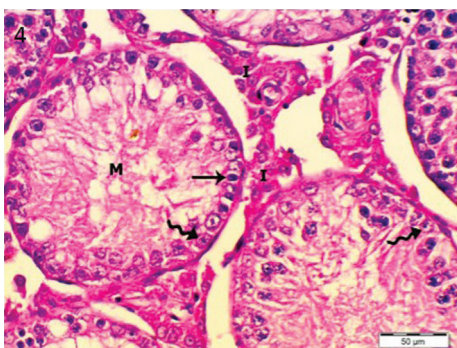
from the Sertoli cells. Few spermatozoa were hardly seen in the lumen (Fig. 2). The lateral walls of Sertoli cells were seen detached from the spermatids and spermatozoa, so that the Sertoli cells appeared sometimes bordering empty spaces (Fig. 3). In the Cx group there was degeneration of the germinal epithelium as shown by the marked decrease of spermatogenic cells some of them had pyknotic nuclei and only few spermatogonia were seen in the basal part of the tubules. There was also absence of spermatozoa in the lumen. The Sertoli cells were widely separated and sometimes appearing like holding large vacuoles. A degenerative acidophilic material obliterating the



**Figure 3.** A section in the testis of a cyclophosphamide group rat (stage VII) showing seminiferous tubule with markedly detached spermatocytes and spermatids from the Sertoli cells (arrows) (haematoxylin and eosin).



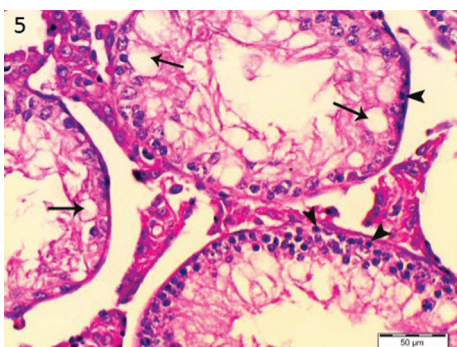
**Figure 6.** A section in the testis of a cryptorchism group rat (stage II) showing marked degeneration of the germinal epithelium with only few spermatogonia at the base (arrowheads). The remaining germ cells are widely detached from the sertoli cells (wavy arrow) and some of them have pyknotic nuclei and darkly acidophilic cytoplasm (thin arrow). No spermatozoa can be observed in the lumen of seminiferous tubules (haematoxylin and eosin).



**Figure 4.** A section in the testis of a cryptorchism group rat (stage II) showing only few spermatogonia (arrow) and Sertoli cells (wavy arrow). There is obliteration of the lumen with degenerative acidophilic materials (M). The interstitium showed marked expansion with proliferation of cells (I) (haematoxylin and eosin).



**Figure 7.** A section in the testis of a control group rat (stage VIII) showing strong positive cadherin immunoreactivity as dense bands circumferencing the basal part of the seminiferous tubule (thin arrow) and as thickened streaks in the Sertoli cells (wavy arrow) attaching their lateral walls to the spermatocytes. Cadherin immunopositivity is also seen at the apex of Sertoli cells (short arrow) adhering to spermatozoa (S) inside the lumen of the tubule (cadherin immunostaining).

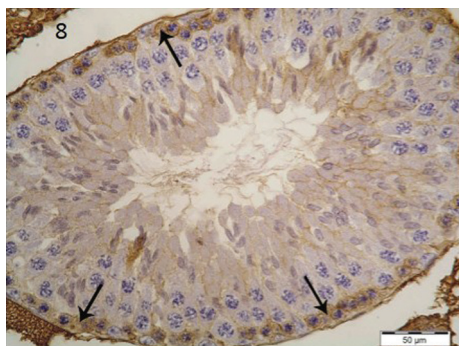


**Figure 5.** A section in the testis of a cryptorchism group rat (stage II) showing marked degeneration of the germinal epithelium with only few spermatogonia at the base and some of them show darkly stained pyknotic nuclei (arrowheads). There is absence of spermatozoa and spermatids in the lumen of seminiferous tubules. The Sertoli cells are widely separated appearing like holding large empty vacuoles (arrows) (haematoxylin and eosin).

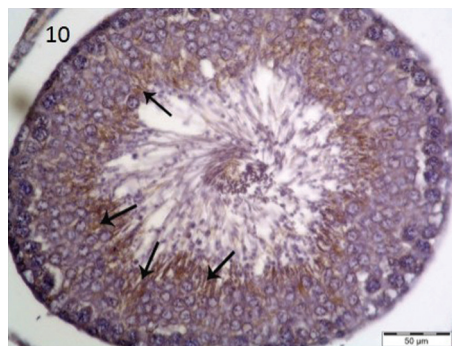
lumen of the tubules, in addition to hypercellularity of interstitium could also be observed (Figs. 4–6).

### Immunohistochemical results

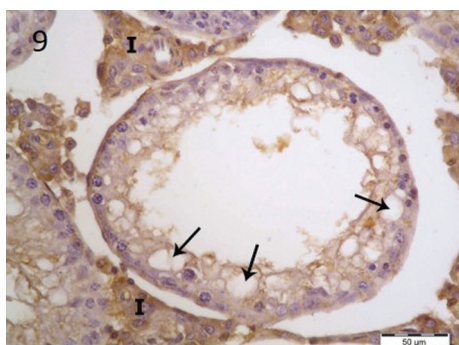
**Cadherin immunostaining.** In the control group, cadherin immunoreaction was seen as dense bands circumferencing the tubules and as thickened streaks within the Sertoli cells attaching them to the spermatocytes. Cadherin immunostaining was also seen at the apex of Sertoli cells adhering to spermatids and in the wall of spermatozoa inside the lumen of the tubules (Fig. 7). Cadherin immunostained section in CP-treated group showed markedly reduced reaction in the midportion area of the seminiferous tubules and only a mild reaction in the basal region (Fig. 8). In Cx group there was very faint cadherin immunoreactivity and wide spaces in the place of the degenerated



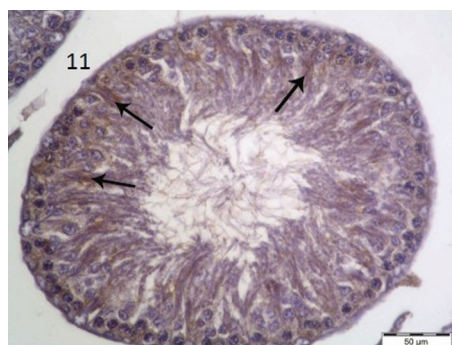
**Figure 8.** A section in the testis of a cyclophosphamide group rat (stage VIII) showing reduced cadherin immunoreactivity being confined to the basal region of the seminiferous tubule (arrow). Markedly diminished cadherin immunostaining in the midportion region of the seminiferous tubule is observed (cadherin immunostaining).



**Figure 10.** A section in the testis of a control group rat (stage VIII) showing the distribution of vimentin immunostaining (arrow) in the midportion and apices of Sertoli cells at their attachments to the germ cells and spermatozoa (vimentin immunostaining).



**Figure 9.** A section in the testis of a cryptorchidism group rat (stage II) showing very faint immunostaining and empty wide spaces (arrow) occupying the places of the degenerated spermatocytes and spermatids. Note the positive immunoreactivity in the interstitial cells (I) (cadherin immunostaining).



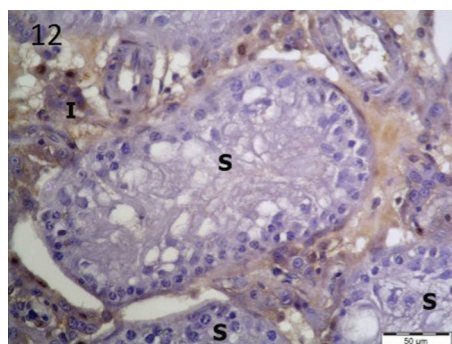
**Figure 11.** A section in the testis of a cyclophosphamide group rat (stage VIII) showing mild vimentin immunostaining (arrow) as streaks in the midportion of Sertoli cells (vimentin immunostaining).

germinal epithelium. Positive immunoreactivity was detected in the interstitial cells (Fig. 9).

**Vimentin immunostaining.** The control group showed positive vimentin immunostaining in the midportion and apices of Sertoli cell walls and their adjoining germ cells and spermatozoa (Fig. 10). Meanwhile, in the CP-treated group faint vimentin immunoreaction was seen as streaks in the midportion of Sertoli cells in the contact areas between them and spermatocytes (Fig. 11). Cx-group revealed absence of vimentin immunostaining in some seminiferous tubules, with large vacuoles occupying the place of degenerated spermatocytes, while mild immunoreactivity was seen in the interstitial cells (Fig. 12).

**Morphometric results**

The area percent of both cadherin and vimentin immunostaining declined significantly in both CP- and



**Figure 12.** A section in the testis of a cryptorchidism group rat showing (stage II) negative vimentin immunostaining in seminiferous tubule (S). Mild positive immunoreactivity can be detected in some interstitial cells (I) (vimentin immunostaining).

Cx-groups as compared to the control. The values for the Cx-group were much lower (Table 1, Fig. 13).

Also, the optical density of both cadherin and vimentin immunoreactions was significantly reduced

**Table 1.** Mean area percent  $\pm$  SD of cadherin and vimentin immunopositivity of the different groups and their statistical significance.

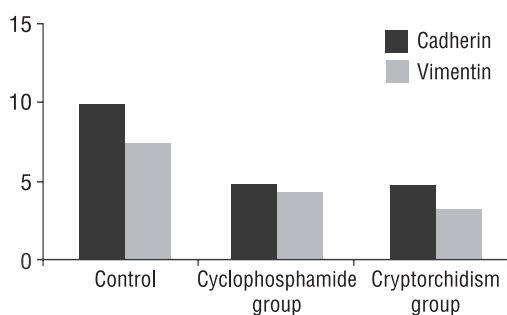
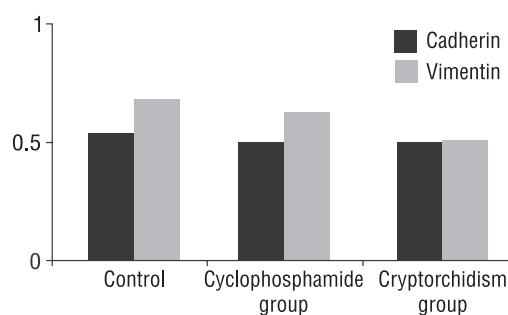
	Cadherin		Vimentin	
	Mean $\pm$ SD	P	Mean $\pm$ SD	P
Control	12.114 $\pm$ 0.628		9.602 $\pm$ 0.866	
Cyclophosphamide (CP) group	5.764 $\pm$ 0.488	0.001*	4.910 $\pm$ 0.965	0.001*
Cryptorchidism (Cx) group	5.562 $\pm$ 0.342	0.001*	3.864 $\pm$ 0.357	0.001*

\*Statistically significant as compared to control ( $p < 0.05$ ); SD — standard deviation

**Table 2.** Mean optical density  $\pm$  SD of cadherin and vimentin immunoreactivity of the different groups and their statistical significance.

	Cadherin		Vimentin	
	Mean $\pm$ SD	P	Mean $\pm$ SD	P
Control	0.6262 $\pm$ 0.0063		0.8285 $\pm$ 0.0081	
Cyclophosphamide (CP) group	0.5944 $\pm$ 0.0166	0.001*	0.7455 $\pm$ 0.0153	0.001*
Cryptorchidism (Cx) group	0.5604 $\pm$ 0.0187	0.001*	0.5832 $\pm$ 0.0135	0.001*

\*Statistically significant as compared to control ( $p < 0.05$ ); SD — standard deviation

**Figure 13.** Mean area percent of cadherin and vimentin immunostaining in the different groups.**Figure 14.** Mean optical density of cadherin and vimentin immunostaining of the different groups

in both CP- and Cx-groups as compared to control (Table 2, Fig. 14).

## DISCUSSION

Intercellular interactions are vital in male reproductive biology. During gametogenesis, Sertoli cells provide developing germ cells with essential structural support via adhesion junctions and nutritive support via secreted factors [14]. Both surgically induced Cx and administration of toxic compounds enable the study of adhesion marker expression during impaired spermatogenesis [20].

The aim of this work was to detect two of the cytoskeletal integral proteins, cadherin and vimentin immunopositivity in normal and altered testicular conditions both quantitatively and qualitatively. Two

models were used to induce testicular alteration and impaired spermatogenesis: CP model, a famous cytotoxic alkylating drug and surgically induced oligospermia by conducting Cx procedure.

CP is used in the treatment of various malignant and nonmalignant tumours [24]. It was chosen in the current study as it was previously shown to provide a suitable model for observing processes occurring in the seminiferous epithelium during impairment and restoration of spermatogenesis. It is also commonly used to examine degenerative changes in the seminiferous epithelium and spermatogonial stem cell function [9].

On the other hand, Cx is a common congenital anomaly usually leading to clinical infertility in man due to severe decrease in sperm production [1], owing to alterations in the maturation of germ cells possibly

caused by inguinal heat stress [25]. Thus it offered another suitable model for examination of testicular impairment.

In the current study, the histological findings in haematoxylin and eosin stained sections of the CP-treated group, included sloughing of the germ cells from the apices of Sertoli cells and mild to marked gaping between these cells and the spermatocytes lodged to the banks in their lateral wall. Sertoli cells also showed cytoplasmic vacuolations and the attached germinal epithelium showed pyknotic nuclei. In agreement with these results, a previous study found hyalinised seminiferous tubules with thickened basement membrane, impaired spermatogenesis and damaged Sertoli cells with some central debris in the tubules of the CP-induced group [23].

CP is cytotoxic to rapidly dividing cells which makes the highly proliferative testis a good target for the damaging effects of this drug. The testicular dysfunction associated with CP has been known for many decades, yet the cellular/biochemical mechanisms by which CP causes reproductive toxicity is poorly understood. However, it was proposed that an oxidant mechanism may be involved in CP-induced toxicity in which CP and its metabolite acrolein causes inactivation of microsomal enzymes and result in increased intracellular reactive oxygen species generation and lipid peroxidation in the rat [23].

On the other hand, the present histological changes detected in the Cx-group were more marked than those of the CP-treated group. They were in the form of degeneration of the germinal epithelium and only few spermatogonia were seen in the basal part of the tubules with absence of spermatozoa in the lumen. The Sertoli cells were vacuolated in addition to hyperplasia of interstitial cells. In accordance with these results, recent study reported spermatogenic cellular desquamation, epithelial vacuolisation and pyknosis in the testes of cryptorchid rats [25]. Interestingly, these findings were also mirrored by the electron microscopic changes in the form of large vacuoles between Sertoli and adjacent germ cells, leading to disruption of corresponding cell junctions, 24 h after heat treatment [5]. Accordingly, the deleterious effect of Cx could be attributed to increased testicular temperature and inguinal heat stress. It has long been known that, in most mammalian species, the testis, being placed in the scrotum, is kept at 4–5°C below body temperature. This lower temperature has been shown to be essential for normal germ cells deve-

lopment and testicular functions [21]. Furthermore, the severe decrease in sperm production associated with Cx could be due to heat-induced germ cell apoptosis evidenced by the involvement of anti- and pro-apoptotic genes in response to heat stress [1].

In the current study, cadherin immunoreaction was detected at the base of the tubules and within the Sertoli cells attaching them to the spermatocytes, spermatids and spermatozoa. The CP-treated group showed mild basal reaction and markedly reduced in the midportion area of the seminiferous tubules. The decreased immunostaining was more pronounced in the Cx-group with gaps between the Sertoli cells and the germinal epithelium. This is concomitant with the previous results who also detected decreased cadherin immunoexpression in testis of cryptorchid rats up to complete absence 22 days after cryptorchidism. P-cadherin is involved in the Sertoli cell-spermatocyte and Sertoli cell-spermatid adhesion in adult rat testis and the sloughing of germ cells in the cryptorchid testes is probably associated with injury of AJs structure and function [20]. Comparable to our findings cadherin immunoreaction was weakly visible in dihydrotestosterone deficient rats after finasteride treatment. The decline of this junctional protein immunoexpression in sloughed germ cells could ultimately lead to impaired fertility [15].

Moreover, vimentin immunopositivity, in the present work, showed significant decline in both CP- and Cx-groups as compared to the control one. This was also supported by the morphometric measurements, with a decrease in both the area percent and the optical density of vimentin immunopositivity being more marked in the Cx-group. These findings are in agreement with the work of Kopecky et al. [16] as they reported that both Cx and the treatment with the anticancer drug Busulphan led to collapse of vimentin filaments and their disorganisation in the basal region of the Sertoli cells. They concluded that the Sertoli cell vimentin filaments play an important role in the maintenance of spermatogenesis, where the damage and restoration of spermatogenesis are related to the disintegration or recovery of these filaments. The adverse effect of this decline in vimentin expression associated with both models of testicular injury was due to the collapse of Sertoli cell vimentin filaments away from the cell membrane. This might lead to detachment of spermatogenic cells, then the detached cells might undergo apoptosis because of loss of the support and nurture provided by Sertoli cells [2].

CP-treatment induced microfilament aggregation, marginalisation and regionalisation. This impairment of the cytoskeleton is likely caused by induction of the oxidative stress response [17]. The altered immunoexpression of the junctional proteins cadherin and vimentin observed in the current study provides evidence and supports these data. Altered expression of junction proteins could be related to insufficient testosterone production and/or excessive oestradiol synthesis, which might result from impaired Leydig cell function in response to testicular alteration [10].

## CONCLUSIONS

It could be concluded, from the findings of this study, that the junction proteins cadherin and vimentin are important for normal spermatogenesis and spermiation of the germ cells. Decreased density or delocalisation of these proteins away from the cell membrane is crucial for affection of spermatogenesis by cutting the germ-Sertoli mutual relationship depriving the different sets of developing germinal epithelium from maturation and nourishment. The alterations of these proteins could also be expected to lead to leak of the blood-testis barrier and this could be the focus of future studies.

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