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Expression of vascular endothelial growth factor and transforming growth factor alpha in rat testis during chronic renal failure

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Abstract

Introduction. Vascular endothelial growth factor (VEGF) is known to influence testis function. Transforming growth factor alpha (TGF-α) is expressed in the postnatal testis, and has been demonstrated to stimulate testis development. Systemic diseases such as chronic renal failure (CRF) interfere with hypothalamic-pituitary-gonadal axis, which may cause defective steroidogenesis and gonadal functions. The aim of this study was to investigate the expression and localization of VEGF and TGF-α in testicular tissues of experimental CRF model.
Material and methods. Experimental CRF was induced in rats by the resection of more than 85% of renal mass. The expression of VEGF and TGF-α in testicular tissues were assessed by immunohistochemistry on paraffin sections of control, CRF-nondialysed and CRF-dialysed rats.
Results. The microscopic evaluation of the testicular structure showed that CRF did not affect testicular histology. Immunohistochemical evaluation showed that VEGF was expressed in the cytoplasm of primary and secondary spermatocyte series as well as the early spermatids. Staining intensity was lower in spermatocytes going through the first meiotic division. TGF-α was expressed in the nuclei of spermatogonia and primary spermatocytes with stronger staining intensity in spermatogonia. The intensity of VEGF staining was similar in control and experimental animals, however, TGF-α expression was lower in the CRF group.
Conclusions. The continuous expression of VEGF in spermatocytes and spermatids suggests that the applied model of CRF does not directly disrupt morphology of seminiferous epithelium, thus also spermiogenesis. However, difference between control rats and CRF group in TGF-α immunopositivity, which was localised in spermatogonial mitosis step, may suggest the interference of CRF with early stages of spermatogenesis. *(Folia Histochemica et Cytobiologica 2014, Vol. 52, No. 4, 308–316)*

Key words: chronic renal failure; peritoneal dialysis; rat; testis; spermatogenesis; TGF-α; VEGF; IHC

Introduction

Hypothalamic-pituitary-gonadal axis is important in the regulation of testis function; however, receptors for the pituitary-derived gonadotropins are expressed exclusively on somatic cells [1]. The hormonal regulation of testis growth is mediated by paracrine and autocrine effects of different growth factors [2]. Therefore, locally secreted regulatory factors are necessary to modulate cell functions in spermatogenesis [3] and steroidogenesis [4]. Male gonad is regulated by growth factors both inducing specific differentiation steps, and acting primarily as environmental or survival factors in genetically predetermined steps [5, 6]. Leydig cells and possibly also other testicular cells secrete vascular endothelial cell growth factor (VEGF), whereas the receptors, VEGF-R1 and VEGF-R2, are expressed on testicular blood vessels [7–9]. VEGF is one of the most potent angiogenic and permeabili-

ty-increasing factors [10]. VEGF may be involved in mediating testicular growth and regression in seasonally breeding animals [11].

Transforming growth factor-alpha (TGF-α) and epidermal growth factor (EGF) bind to a common membrane receptor (EGFR) and exert identical biological effects on proliferation, differentiation and differentiated functions in a wide variety of target cells [12–15]. In the postnatal testis, TGF-α and EGFR were found to stimulate proliferation, differentiation, and migration of Sertoli, Leydig, and peritubular cells [16, 17].

At the onset of puberty (around days 10–15 in the rat), Sertoli cells cease to divide and become postmitotic [18]. Sertoli cells play a key role in the initiation of spermatogenesis. Sertoli-Sertoli and Sertoli-germ cell interface form the blood-testis barrier (BTB) and organizes the seminiferous epithelium into a basal epithelial and an adluminal (apical) compartment to accommodate the rapid changes in germ cell morphology during spermatogenesis, in particular spermiogenesis, as well as germ cell transport across the BTB [19]. Spermatocytes and spermatids are not motile cells *per se*, instead cytoskeletal F-actin organization in the mammalian testis which is maintained *via* the combined action of epidermal growth factor receptor pathway substrate 8 (Eps8, an actin filament barbed end capping and bundling protein) [20, 21], palladin (an actin cross-linking and bundling protein) [22] and espin [23] which help in the transporting of developing spermatids across the seminiferous epithelium.

Foetal Leydig cells originate by differentiation from mesenchyme-like precursors and once differentiated, no longer divide under normal conditions [18]. Leydig cells differentiate and secrete testosterone during early foetal life which is required for embryonic development and sexual maturation. Observations suggest that all the testis cell populations provide potential targets for locally produced growth factors (*e.g.* TGF-α). [24] or neurotropin receptors such as low affinity neurotropin receptor (p75/LNGFR) [25]. However, besides the general knowledge that growth factors participate in different cellular compartments of the testis, little is known about the codistribution of TGF-α and VEGF in seminiferous tubuli.

In chronic stresses, testicular dysfunction is due to primary testicular failure with reduced production of testosterone and semen and elevated gonadotropin levels [26]. Chronic renal failure (CRF) is associated with impaired spermatogenesis and testicular damage, often leading to infertility. A decreased volume of ejaculate combined with low or complete azoospermia and a low percentage of motility has been shown in semen analysis [27]. Leydig and Sertoli cells are prone to defects in their hormonal regulation. This latter effect might occur with either gonadotropin deficiency or resistance, rather than being a cytotoxic effect of uraemia when spermatogonia would be most affected [28]. The disorders of the pituitary-gonadal axis rarely normalize with initiation of haemodialysis or peritoneal dialysis and, in fact, often progress [29].

Although stimulatory or inhibitory effects of VEGF and TGF-α on different compartments of testis have been demonstrated, to our best knowledge, no data describe the simultaneous expression of these growth factors in CRF. We hypothesize that VEGF and TGF-α would have spatially and temporally specific roles in rat testis under CRF conditions. Therefore, we decided to investigate the expression and localization of VEGF and TGF-α in rat testicular tissue in an experimental model of CRF.

Material and methods

Animals, surgical technique and peritoneal dialysis. The study was carried out on 24 adult male Wistar rats, aged between 10 and 12 weeks, weighing 200–330 g. Akdeniz University Animal Ethics Committee approved the research protocol. CRF was induced by partial nephrectomy under general anaesthesia with pentobarbital 50 mg/kg (*i.p.*). The left kidney was exposed through a flank incision, and both the upper and lower poles and approximately one third of the remaining cortical tissue of that kidney was excised with the help of bipolar cauterization. During the same session, after mobilizing the adrenal gland, the right kidney was removed after ligation of the renal artery, vein, and ureter. This procedure resulted in a resection of more than 85% of the renal tissue as previously described [30].

Four weeks later, a silicon catheter (Kawasumi venous fistula set, 16-gauge, 35-mm, Lot No. 9718A7) was introduced into the abdominal cavity modifying the protocol described by Miller et al. [31]. The catheter length was determined for each animal, and wedge-shaped smooth holes were made using a sterile surgical blade 3–4 cm from the distal end of the catheter. The catheter was pushed over a trocar, creating a subcutaneous track ending at the back of the animal's neck. The catheter was closed by a Luer-Lock adaptor. Each morning, under sterile conditions, a catheter was passed through the permanent indwelling catheter, and the peritoneal cavity was rinsed with 20 mL pre-warmed saline. Afterwards, ceftazidime (125 mg/L) and gentamicin (8 mg/L) were given intraperitoneally. This procedure was continued until the beginning of chronic peritoneal dialysis (PD) [30]. Each morning, the Luer-Lock adaptor was removed and a sterile catheter was passed through the permanent indwelling catheter and rinsed with 20 mL pre-warmed saline. Five minutes later, this fluid was drained and 20 mL dialysis fluid, containing either 1.36 or 3.86%

glucose, was given infused intraperitoneally for 4 h in the anaesthetized animal.

After the first dwell time of 4 h, the dialysis fluid was drained as much as possible, the peritoneum was rinsed with 20 mL pre-warmed saline, and the Luer-Lock adaptor was closed. Each evening, after removing the adaptor and rinsing with 20 mL pre-warmed saline, 20 mL icodextrin was given for 10 h, and then it was drained as completely as possible, and the peritoneum was rinsed with pre-warmed saline. Ceftazidime (125 mg/L) and gentamicin (8 mg/L) were added to every dialysate exchange. The dialysis procedure was performed for 8 weeks.

Before chronic PD begun, the rats were divided into three groups. The first group consisted of 6 normal rats and served as the control group (group 1). In 8 rats, CRF was induced and the catheter was implanted, but the dialysis procedure was not performed until the end of the study (group 2). In 10 rats with CRF, chronic PD was performed by dialysis fluids containing 1.36% glucose and icodextrin (group 3). Each animal was housed in a metabolic cage and the daily fluid and food intake, 24-hour urine volume, and body weight were followed for the entire duration of the experiment. On Tuesdays of each week, blood samples were taken by puncture of the tail vein to measure creatinine and urea concentrations. Creatinine clearance was monitored every 2 weeks throughout the study period. Creatinine clearance (CCr) is calculated from the creatinine concentration in the collected urine sample (a 24-hour urine collection) (UCr), urine volume (24-hour volume), and the plasma concentration (PCr) (CCr = UCr × 24 hour volume/PCr × 24 × 60 min). Animals with normal serum creatinine levels before the start of PD were considered as failures.

We chose to evaluate the animals at 16 weeks postoperatively, because this time point permitted the evaluation of a cohort of animals in which uraemia had been produced in a stable homeostatic state.

Tissue collection. At the end of the PD procedure, both testes were delivered into the abdomen and bilateral orchiectomy was performed. The animals were then sacrificed using a lethal overdose of anaesthesia. The testicular samples were fixed by immersion in Bouin's fixative (75 mL of saturated aqueous solution of picric acid; Sigma-Aldrich, Steinheim, Germany), 25 mL of formalin (Merck, Whitehouse Station, NJ, USA) and 5 mL of glacial acetic acid (Sigma-Aldrich) and routinely processed for paraffin embedding.

Immunohistochemistry. For immunohistochemical analysis, 5- to 6-μm-thick tissue sections were mounted on poly-L-lysine-coated slides. After deparaffinization, slides were boiled in citrate buffer (pH 6.0) for 10 min for antigen retrieval and cooled for 20 min at room temperature. The following single and sequential immunoenzymatic double staining methods were performed according to Petraki et al. [32] and the manufacturer's instructions.

VEGF. After washes in phosphate-buffered saline (PBS), an endogenous alkaline phosphatase (AP) inhibiting reagent, containing 0.0002 mol/L levamisole (Dako X3021, Dako, Glostrup, Denmark) was applied for 10 min at room temperature. The primary antibody, mouse anti-human VEGF (sc-7269, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) was incubated overnight at 4°C. After several washes in PBS, the antibody was detected with alkaline phosphatase (LabVision, Fremont, CA, USA) detection system and visualized with Fast Red as substrate (Dako). The sections were counterstained with Mayer's haematoxylin (Dako), mounted with Kaiser's gelatine (Merck) without any further dehydration.

TGF-α. After PBS washes, the endogenous peroxidase activity was inhibited by incubation in methanol containing 3% H_2O_2 for 10 min. After several washes in PBS, the sections were blocked with a universal blocking serum (LabVision) for 7 min at room temperature in a humidified chamber. Excess serum was drained and mouse monoclonal TGF-α primary antibody (GF10; Calbiochem, Cambridge MA, USA) was applied as 2 μg/mL for 1 h at room temperature. After several washes in PBS, the antibody was detected with an avidin-biotin, horse-radish peroxidase complex (LabVision). The sections were rinsed with PBS and antibody complexes were visualized by incubation with 5,5'-diaminobenzidine tetrahydrochloride (DAB) chromogen (BioGenex, Fremont, CA, USA). Sections were not counterstained to prevent masking of the nuclear immunolabelling, dehydrated, mounted with Entellan (Merck), and visualized by light microscopy.

Double labelling. A sequential immunoenzymatic double staining method was also performed, in order to compare the localization of the two studied antigens. The tissue sections were incubated using the first primary antibody (TGF-α), and detected with avidin-biotin, horse-radish peroxidase complex (LabVision) as described above; then rinsed in distilled water and sequentially incubated with the second primary antibody (VEGF) and detected with alkaline phosphatase (LabVision) system. The sections were not counterstained, mounted with Kaiser's gelatine (Merck) without any further dehydration.

Tissues from control and experimental groups were processed at the same time. For controls, sections were treated with appropriate mouse IgG and diluted to the same final protein concentration as the primary antibody. Immunohistochemical staining was evaluated in seminiferous tubules in a blinded fashion to the groups of the tissues by 2 independent observers (GA, YSC) and expressed as the intensity of positively stained cells. Briefly, for each slide, 10 different areas were evaluated using the light microscope (Zeiss Axioplan, Oberkochen, Germany) at 200 × magnification, and the intensity of VEGF and TGF-α immunolabellings
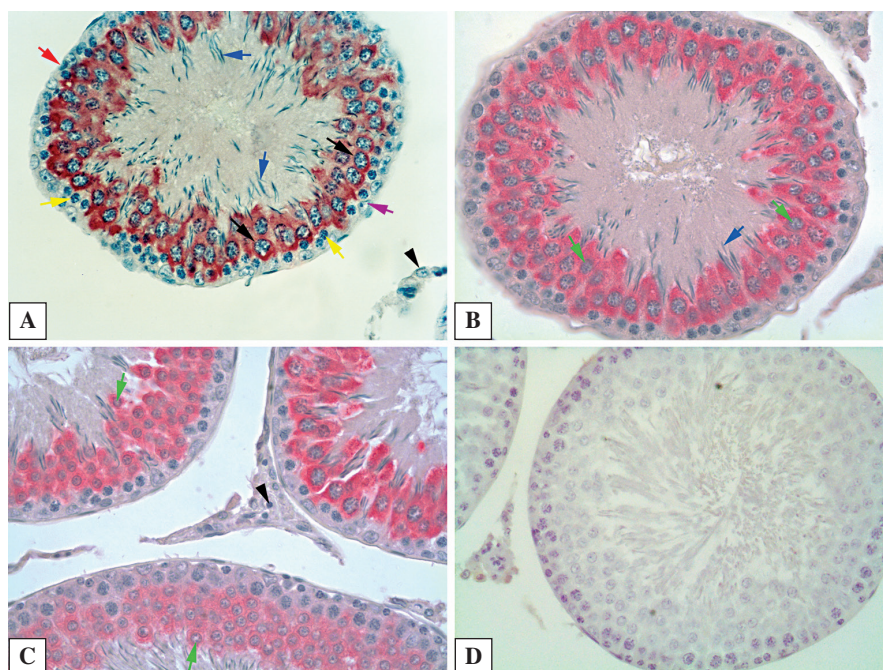
Figure 1. Immunohistochemical localization of VEGF in rat testis. Control (**A**), CRF (**B**) and CRF+PD (**C**) groups. Representative negative control section (**D**). Abbreviations as for Table 1. Pachytene spermatocytes and round spermatid cytoplasms stained positively for VEGF in the three rat groups. Blue arrow: elongated spermatid; green arrow: round spermatid; black arrow: pachytene spermatocyte; yellow arrow: preleptotene spermatocyte; red arrow: spermatogonium; pink arrow: Sertoli cell; black arrowhead: Leydig cell. Chromogen: Fast Red. Counter stain: Mayer's haematoxylin. Original magnification × 40

was expressed semiquantitatively as negative (–), weak (+), moderate (++) or strong (+++).

Periodic acid-Schiff (PAS) staining. In order to identify glycoproteins within the acrosome of spermatids, we performed PAS staining. Routinely deparaffinized and rehydrated sections were oxidized in 0.5% periodic acid solution for 5 min and rinsed in distilled water. Then, the sections were placed in Schiff's reagent for 15 min and washed in tap water for 5 min. Finally, they were counterstained in Mayer's haematoxylin for 1 min, washed in tap water, dehydrated, cleared and mounted.

Statistical analysis. Statistical evaluations were performed using SPSS (Statistical Package for Social Sciences) for Windows 13.0 software (SPSS Inc., Chicago, IL, USA). Normality was tested by Shapiro-Wilk test. The Student's t test was used to compare the body weights, serum creatinine and urea concentrations, and creatinine clearances between the CRF groups and the control group. A p value of < 0.05 was considered statistically significant.

Results

Induction of chronic renal failure

Of the 24 rats in this study, six died (two and four in groups 2 and 3, respectively) because of peritoneal trau-

ma, peritonitis attacks, and other problems. A significant decrease in the creatinine clearance value was measured in the CRF groups (groups 2 and 3; n = 12, mean 0.43 ± 0.11 mL/min) compared with control group (group 1; n = 6; 2.88 mL/min, p< 0.05). The mean creatinine clearance value in the CRF group represented 14.8% of normal, thus we could easily conclude that end-stage renal failure was successfully induced in these rats. All CRF animals had low creatinine clearance values. Although the body weight initially increased (by 10%) in the CRF group during the first week after surgery, before PD began, there was eventually a body weight loss (by 15%) in the same group. However, before start of PD, no statistically significant difference was found in the body weight of the CRF (groups 2, 3) and non-CRF (group 1) animals.

Microscopic evaluation of VEGF presence in rat testis of control and CRF groups

Immunohistochemical analysis showed that VEGF was expressed in the cytoplasm of spermatocyte series that were most likely pachytene spermatocytes as well as early spermatids forming up the adluminal compartment of the testis (Figure 1). The cells which morphologically appeared to be preleptotene

Table 1. Localization of VEGF and TGF-α in control, chronic renal failure (CRF) and CRF + peritoneal dialysis (CRF+PD) groups

Cell type	Control		CRF		CRF+PD	
	VEGF	**TGF-α**	**VEGF**	**TGF-α**	**VEGF**	**TGF-α**
Elongated spermatid	–	–	–	–	–	–
Round spermatid	+	–	+	–	+	–
Pachytene spermatocyte	+++	–	++	–	++	–
Preleptotene spermatocyte	–	+++	–	+	–	++
Spermatogonium	–	++	–	+	–	+
Sertoli	–	–	–	–	–	–
Leydig	–	+	–	+	–	+

Immunohistochemical detection of VEGF and TGF-α was performed as described in Methods. The intensity of immunoreactivity was assessed as negative (–), weak (+), moderate (++), and strong (+++)

spermatocytes residing in the basal compartment of the seminiferous tubules were negative for VEGF immunostaining. Staining intensity exhibited variations among spermatocytes which were probably committed to form round spermatocytes (Figure 1). VEGF immunoreactivity was not observed in mature spermatozoa. Also VEGF staining intensities in the seminiferous tubules and testicular cells were similar in control (Figure 1a), CRF (Figure 1b) and CRF+PD (Figure 1c) groups, which is outlined in Table 1.

Leydig cells and Sertoli cells were negative for VEGF in all studied groups (Table 1).

Microscopic evaluation of TGF-α presence in rat testis of control and CRF-animals

TGF-α was expressed in the nuclei of basal cells which were presumably spermatogonia and preleptotene spermatocytes (Figure 2a). TGF-α immunoreactivity was present in perinuclear proacrosomal granules in the round spermatids (Figure 2a). We also observed periodic acid-Schiff (PAS) staining, in parallel with immunohistochemistry, to mark the acrosome by a conventional method. Serial cross sections of testis were used for TGF-α labelling and PAS staining. PAS staining was observed in proacrosomal granules that have coalesced and formed one big granule as well as in basement membrane of tubuli and spermatozoa heads (Figure 2a, insert). Mature spermatozoa were immunonegative for TGF-α. When compared with the control group, TGF-α staining intensity was lower in the CRF (Figure 2b) and CRF+PD groups (Figure 2c, Table 1).

Leydig cells stained positively for TGF-α in all animal groups, however, Sertoli cells were immunonegative (Table 1).

Double immunohistochemical staining

Double immunohistochemical labelling of the control, CRF and CRF+PD groups evidently showed that VEGF immunopositive spermatogenic cells comprised the adluminal compartment and TGF-α immunopositive cells comprised the basal compartment of the seminiferous tubule (Figure 3). The adluminal compartment specific VEGF immunolabelling and basal compartment specific TGF-α immunostaining did not overlap in control, CRF and CRF + PD testes sections (Figure 3a–c).

Discussion

In the present study, we examined the immunolocalization of VEGF and TGF-α in a rat CRF model. Our results demonstrate that the testis histology was not directly affected by CRF. The expression of VEGF in pachytene spermatocyte and spermatids suggests that CRF may not affect late spermatocyte stages. However, decreased TGF-α expression in spermatogonia and preleptotene spermatocytes suggests that CRF probably exerts an inhibitive effect on spermatogonia. Since we observed no co-localization of the two growth factors when a single immunochemistry was performed, we ascertained this observation by applying a double labelling approach which clearly identified the VEGF and TGF-α immunopositivities in the adluminal compartment and the basal compartment, respectively.

Interestingly, there was an exception for TGF-α immunolocalization in the acrosomal vesicles of developing pachytene and round spermatids. In rats, classification of the seminiferous epithelium cycle by Leblond and Clermont [33] defines 14 stages (desig-
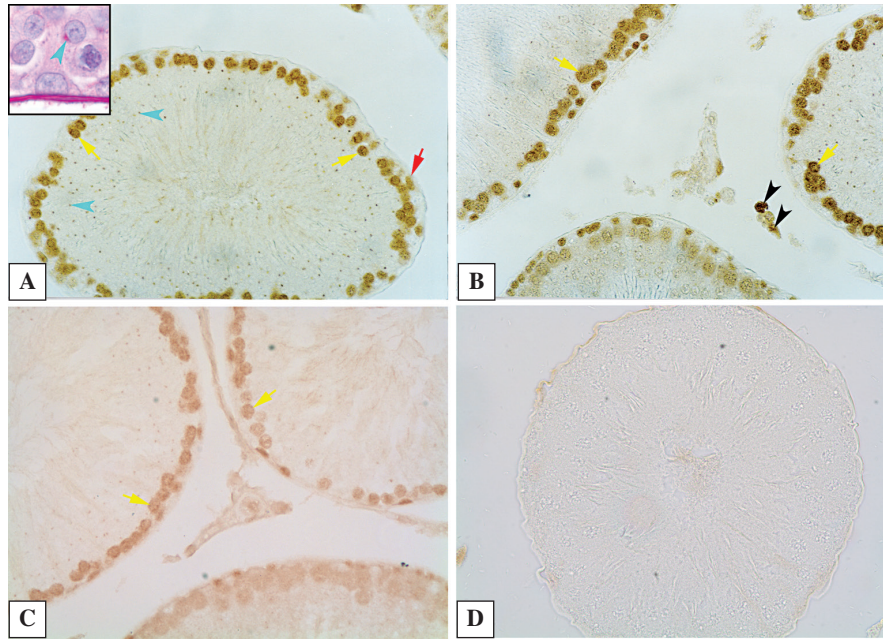
Figure 2. Immunohistochemical localization of TGF-α in rat testis. Control (**A**), CRF (**B**) and CRF+PD (**C**) groups. Representative negative control section (**D**). Periodic acid Schiff (PAS) staining (insert in Fig. 2a). Abbreviations as for Table 1. Preleptotene spermatocytes and spermatogonium nuclei as well as the Leydig cells showed TGF-α immunoreactivity. TGF-α staining was weak in the CRF group. Turquoise arrowhead: acrosomal vesicles; yellow arrow: preleptotene spermatocyte; red arrow: spermatogonium; black arrowhead: Leydig cell. Chromogen: DAB. Counter stain: not applied. Original magnification × 40 (A–D), × 100 (insert)

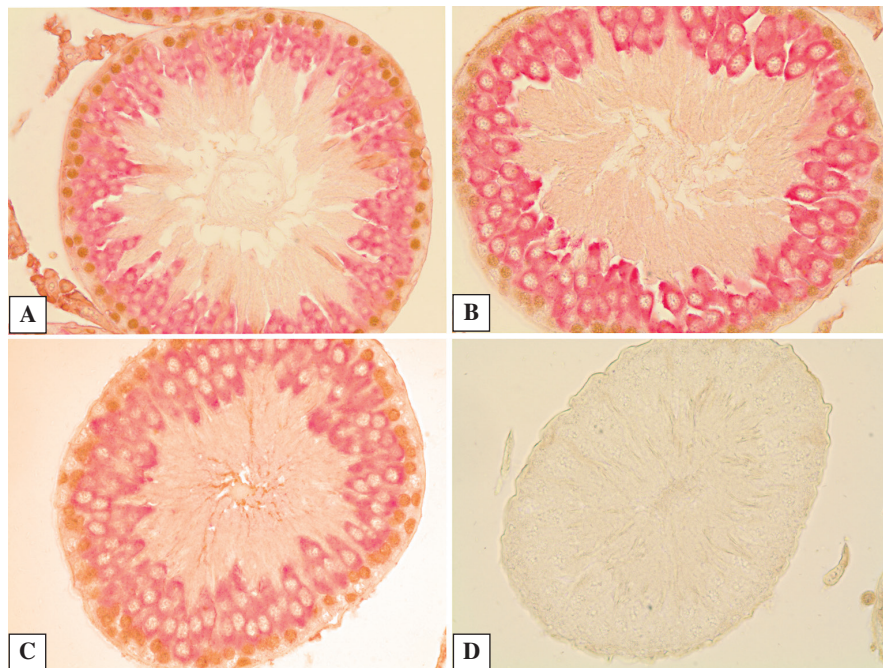**Figure 3.** Representative double immunohistochemical labelling of rat testis for VEGF and TGF-α. Control (**A**), CRF (**B**) and CRF+PD (**C**) groups. Representative negative control section (**D**). Abbreviations as for Table 1. VEGF and TGF-α immunohistochemical labellings were visualized using the chromogens, Fast Red (red) and DAB (brown) respectively. Counter stain: not applied. Original magnification × 40

nated I–XIV) in which spermiogenesis, which refers to the morphological transformation of spermatids into spermatozoa, involves 19 differentiation steps (from 1 to 19). Periodic Acid Schiff (PAS) reaction is an accepted conventional method of labelling the acrosome [34] and studying the seminiferous tubules histology [35]. The SP-10 protein, whose intra-acrosomal localization was found in mammalian male germ cells [36], has been recently applied for staging the mouse seminiferous cycle [37]. According to our findings, TGF-α is similarly present in proacrosomal granules of rat spermatids suggesting the role of TGF-α in normal spermatid development. The further differentiated forms of spermatids and spermatozoa were not labelled which may suggest posttranslational modifications of TGF-α during acrosome formation.

Spermatogenesis can be broadly divided into several discrete events: (1) renewal of spermatogonial stem cells and spermatogonia *via* mitosis, (2) proliferation (*via* mitosis) and differentiation of spermatogonia through type A and type B, and eventually preleptotene spermatocytes, (3) meiosis, (4) spermiogenesis (transformation of round spermatids to elongated spermatids and spermatozoa) and (5) spermiation (the release of sperm from the epithelium into the tubule lumen) [38]. Spermatogenesis and steroidogenesis can be affected by chronic renal failure causing deterioration of testicular function [39]. Renal failure is associated with the retention of a variety of toxic compounds responsible for the uremic syndrome. One of the most encountered complications is CRF-associated testicular dysfunction, which often causes infertility [40]. The exact pathophysiological mechanism involved in CRF-induced testicular damage is not fully understood. Haemodialysis patients have reduced testicular volume, indicating impaired spermatogenesis [41], however, recovery of hormonal and spermatogenic abnormalities occur after renal transplantation [27, 39]. Spermatozoa obtained from uremic patients show improved ultrastructural features after renal transplantation, and a low dose of immunosuppressant is suggested to prevent degenerating effects of immunosuppressive therapy on seminiferous epithelia [42]. However, Adachi et al. applied a stepwise-nephrectomy procedure and demonstrated that testicular histology has not been affected by CRF in rats [43]. This observation is compatible with our previous results that the testicular histology between the CRF and control groups was not statistically different based on the Johnsen's scores [30].

Several studies have found that VEGF is involved in both neonatal [44] and postnatal [45] rodent testis development. It was demonstrated that altering VEGFA isoform activity in the testis *in vivo* resulted in significant changes in the ability of spermatogonial stem cells to self-renew and colonize seminiferous tubules [44]. Recently, in a mouse VEGFA knockout model it has been observed that although testis weight did not change, the seminiferous epithelium was disorganized which suggested that VEGFA isoforms secreted by Sertoli cells and germ cells are necessary for the maintenance of undifferentiated spermatogonia, sperm numbers and normal male fertility [46].

Locally produced testicular factors are of potential physiological significance [47]. Epidermal growth factor (EGF)/TGF-α is a family of growth factors that has attracted attention as putative regulators of embryonic [48] and testicular [24] development. Earlier results [49] supported by more recent findings [50] indicate that EGF/TGF-α family members are also directly involved in the regulation of spermatogenesis.

It was reported that CRF models based on 75% surgical nephron reduction resulted in FVB/N mice strain with severe tubulo-interstitial kidney lesions, whereas C57BL6xB6D2F1 strain is resistant to early renal deterioration [51]. Additionally, it was shown that TGF-α expression markedly increased after nephron reduction exclusively in FVB/N mice, and this increase preceded the development of renal lesions [52]. Furthermore, pharmacologic inhibition of the EGFR prevented the development of renal lesions in the sensitive FVB/N strain [52]. These data suggest that variable TGF-α expression may explain, in part, the genetic susceptibility to the progression of chronic kidney disease (CKD). Thus, EGFR inhibition may be a therapeutic strategy to counteract the genetic predisposition to CKD. Similarly, administration of VEGF was found to be effective in reducing long-term histologic damage resulting from testicular ischemia-reperfusion, and preservation of histologic parameters, such as mean seminiferous tubular diameter and germinal epithelial cell thickness [53].

Growth factors are considered to participate in creating a suitable niche for spermatogenesis [50]. Mammalian testis is a self-renewing cell system in which proliferation and differentiation of stem cells, *i.e.* spermatogonia, takes place in microenvironments that are described as niche. Rat spermatogonia are preferentially located near the interstitial regions [54]. These regions were considered as spermatogonial niches where Leydig cells, macrophages, and lymphatic and blood vessels are commonly found in topographically critical locations. Similarly in human testes, peritubular wall cells contribute to the spermatogonial stem cell (SSC) niche which is supported by the production of glial cell line-derived neurotrophic factor (GDNF), and its receptor GDNF family receptor alpha-1 (GFRα1), responsible for maintaining

SSCs [55]. Specific factors emanated by those niches such as epidermal growth factor (EGF)-like growth factors, heparin binding (HB)–EGF, amphiregulin, and TGF-α are considered to support human spermatogenesis [50]. Our results supported the hypothesis that VEGF and TGF-α have cell-specific expressions through the seminiferous epithelia and chronic renal failure neither affects these localizations nor deteriorates testicular histology. In our study the continuous expression of VEGF in pachytene spermatocytes and spermatids not only in normal but also in CRF rats suggests that chronic kidney disease may not affect spermiogenesis. However, TGF-α might be important in spermatogonial mitosis step and CRF might have temporary influence on spermatogonia causing the impairment in the spermatogonial phase, during which stem cells divide to replace themselves and provide a population of committed spermatogonia. These spatial and temporal expressions of VEGF and TGF-α remain to be elucidated in further research and clinical conditions.

Acknowledgments

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