

Light and Electron Microscopic Examination of the Single Kidney of Unilateral Nephrectomized Rats during Pregnancy

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Abstract: The light microscopic and ultrastructural changes observed in the single kidney of pregnant rats were detected during mid-term pregnancy and after parturition and the results were compared with those of the pure nephrectomized group. The experimental studies were performed on 24 female virgin rats and all of them were subjected to unilateral nephrectomy. No pathology was observed in the light microscopic examination of the kidneys of the groups. In the transmission electron microscopic examination, in the pure nephrectomized group, cell swellings were present in the endothelial cells of the interlobular arteries and arterioles. Additionally, a mild cell swelling was seen in the cytoplasm of a few of the mesangial cells. In the mid-term pregnancy group, a mild cell swelling was observed in the cytoplasm of the podocytes and more prominent cell swellings were seen in the endothelial cells of the interlobular arteries and arterioles. These more prominent cell swellings were also present in some of the mesangial cells. In the post-parturition group, in addition to these findings, focal thickenings were observed in the glomerular basement membranes.

Key Words: Nephrectomy, pregnancy, light microscopy, transmission electron microscopy

Unilateral Nefrektomize Sıçanların Geriye Kalan Tek Böbreklerindeki Işık ve Elektron Mikroskopik Değişikliklerin Gebelik Sırasında Değerlendirilmesi

Özet: Bu çalışmada, unilateral nefrektomi ile tek böbrekleri çıkarılıp gebe bırakılmış sıçanların geriye kalan tek böbreklerindeki ışık ve elektron mikroskopik değişiklikler gebelik ortası dönemde ve gebelik sonrasında incelendi. Elde edilen sonuçlar sadece unilateral nefrektomi yapıp gebe bırakılmamış olan sıçanlar ile karşılaştırıldı. Çalışma sırasında 24 adet dişi sıçan kullanıldı. Doku örneklerinin ışık mikroskopik incelenmesinde herhangi bir patolojik bulgu gözlenmedi. Transmission elektron mikroskopik incelemede; sadece unilateral nefrektomi yapılan grupta a. interlobularis ve arteriollerdeki endotel hücrelerinde şişme ve nadiren bazı mezangial hücrelerin sitoplazmalarında hafif bir şişme izlendi. Gebelik ortası dönemde incelenen grupta; podositlerin sitoplazmasında hafif bir şişme vardı. Bununla birlikte; a. interlobularis ile arteriollerdeki endotel hücrelerinde ve bazı mezangial hücrelerde daha belirgin bir şişme mevcut idi. Gebelik sonrası dönemde değerlendirilen grupta ise tüm bu bulgulara ilave olarak glomeruler bazal membranda fokal kalınlaşmalar mevcut idi.

Anahtar Sözcükler: Nefrektomi, gebelik, ışık mikroskopi, transmission elektron mikroskopi

Introduction

Pregnancy has several effects on the normal kidney. These include alterations in glomerulotubular balance; dilation of the renal calyces, pelvis and ureter; increases in the glomerular filtration rate and renal plasma flow due to chronic renal vasodilation; and increases in aldosterone and renin production and proteinuria (1-3). However, in unilaterally nephrectomized animals, in addition to these findings, a compensatory growth was observed in the remaining kidney during pregnancy (4-6).

Although there are many studies in the literature related to uninephrectomy and pregnancy, none of them have examined the single kidney during pregnancy at the electron microscopic level (7-12). Additionally, there are no studies in the literature related to the single kidney of unilateral nephrectomized rats during mid-term pregnancy. Therefore, in this study, the light microscopic and ultrastructural changes observed in the single kidney of pregnant rats were detected during mid-term pregnancy and after parturition and the results were compared with those of pure nephrectomized rats.

Materials and Methods

The experimental studies were performed on 24 female virgin Sprague-Dawley rats. Their ages varied between 4 and 6 months. All these rats were subjected to unilateral nephrectomy. During the operations, the animals were anesthetized by ketamine hydrochloride (Ketalar) 30 mg/kg intramuscularly. For muscle relaxation, 6 mg/kg xylazine (Rompun) was used. The left kidney of each animal was excised as atraumatically as possible and the incisions were closed with sutures. Then, following nephrectomy, the rats were divided into three groups (eight animals in each group). The animals in group I remained virgins throughout the study. Seven days after nephrectomy, the rest of the animals were put for 72 h with adult male rats. Sets of three females and one male were put into separate cages, but in one of the cages there were four females and one male. After this procedure, the male rats were removed from the cages. These 16 animals were divided into two groups (groups II and III). The animals in group II were caged from day one of their pregnancy (which was established by the detection of spermatozoa in the morning vaginal smear) up to day 11 of their pregnancy. Then their solitary kidneys were excised surgically and the tissue samples were put into 2.5% gluteraldehyde (group II: mid-term pregnancy group). The animals in group III were caged until the end of their pregnancy and, following the delivery of the litters, their solitary kidneys were excised surgically. The single kidneys of rats in group I were excised surgically together with this group and all the tissue samples were put into 2.5% gluteraldehyde for fixation.

The specimens, which were taken from the cortex and medulla of each kidney, were fixed in 2.5% gluteraldehyde for 24 h, washed in phosphate buffer (pH 7.4), post-fixed in 1% osmium tetroxide in phosphate buffer (pH 7.4) and dehydrated in increasing concentrations of alcohol. Then the tissues were washed with propylene oxide and embedded in epoxy-resin embedding media. Semi-thin sections about 2 µm in thickness and ultrathin sections about 60 nm in thickness were cut with a glass knife on a LKB-Nova (Sweden) ultramicrotome. In this study, five semi-thin and five ultrathin sections were taken from the cortex, and five semi-thin and five ultrathin sections were taken from the medulla of each kidney. All of these sections were examined under a light microscope and transmission electron microscope. Semi-thin sections were stained with methylene blue and examined under a Nikon Optiphot (Japan) light microscope. Ultrathin sections were collected on copper grids, stained with uranyl acetate and lead citrate and examined under a Jeol JEM 1200 EX (Japan) transmission electron microscope.

Results

Pure nephrectomized group: No pathology was observed in the light microscopic examination of the tissue specimens in this group (Fig. 1A). In the transmission electron microscopic examination of the kidneys, glomerular basement membranes and podocytes were normal (Fig. 1B). However, cell swellings were observed in the endothelial cells of the interlobular

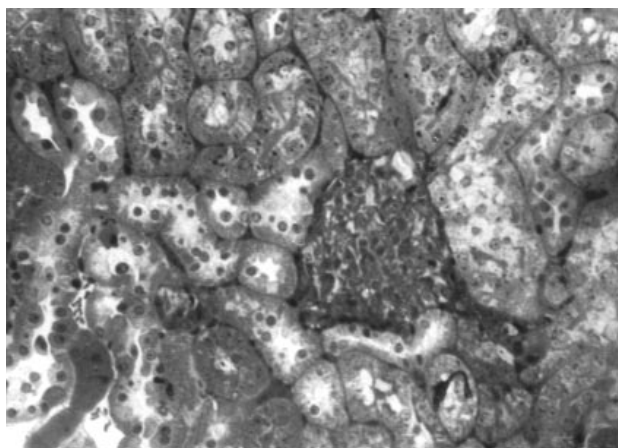


Fig. 1A. Photomicrograph showing no pathological changes in the pure nephrectomized group (X 100).

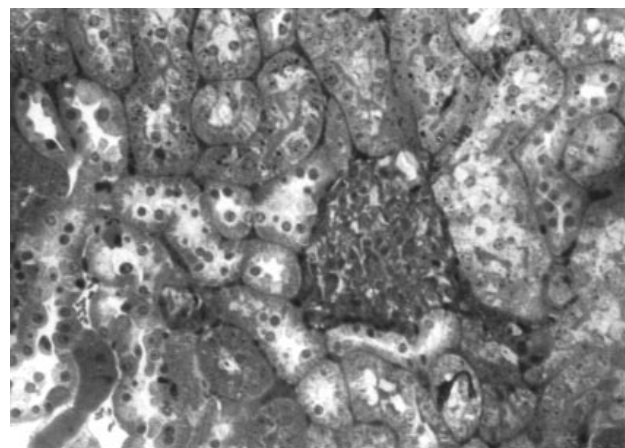


Fig. 1B. Electron micrograph showing the glomerular basement membrane (*), podocytes (p) and an endothelial cell with cell swelling (o) in the pure nephrectomized group (Original magnification X 12,000).

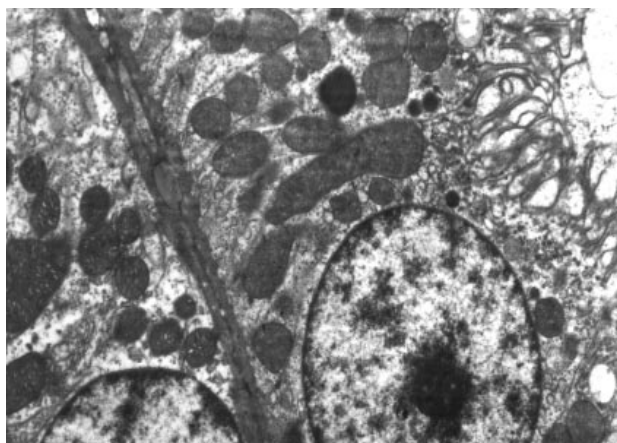


Fig. 1C. Electron micrograph showing the normal tubular epithelial cells in the pure nephrectomized group (Original magnification X 5000).

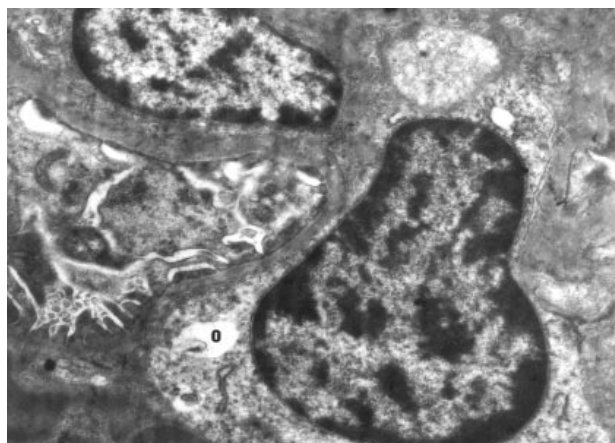


Fig. 1D. Electron micrograph showing the mild cell swelling (o) in the cytoplasm of a mesangial cell (Original magnification X 7500).

arteries and arterioles (Fig. 1B). The tubules were found to be normal, ultrastructurally (Fig. 1C). In addition, a mild cell swelling was observed in the cytoplasm of a few of the mesangial cells (Fig. 1D). The present cell swellings were seen in every section in this group.

Mid-term pregnancy group: No pathology was observed in the light microscopic examination of the tissue specimens in this group (Fig. 2A). In the transmission electron microscopic examination of the kidneys, the glomerular basement membranes were normal (Figs. 2B and 2C). However, a mild cell swelling was observed in the cytoplasm of the podocytes (Fig. 2B). A more prominent cell swelling was seen in the

endothelial cells of the interlobular arteries, arterioles and in some of the mesangial cells (Fig. 2C). The tubules were found to be normal, ultrastructurally (Fig. 2D). The present cell swellings were seen in every section in this group.

Post-parturition group: No pathology was observed in the light microscopic examination of the tissue specimens in this group (Fig. 3A). In the transmission electron microscopic examination of the kidneys, focal thickenings were observed in the glomerular basement membranes (Fig. 3B). Cell swellings were present in the cytoplasm of the podocytes (Fig. 3C), endothelial cells of the interlobular arteries and arterioles (Fig. 3D), and in

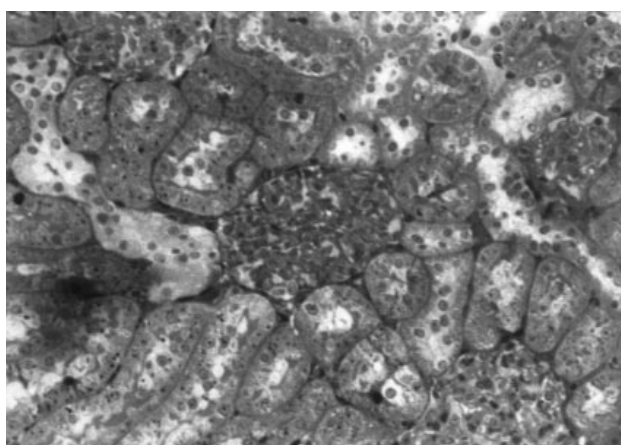


Fig. 2A. Photomicrograph showing no pathological changes in the mid-term pregnancy group (X 100).



Fig. 2B. Electron micrograph showing the normal glomerular basement membrane (*) and the mild cell swelling in the cytoplasm of a podocyte (o) in the mid-term pregnancy group (Original magnification X 10,000).

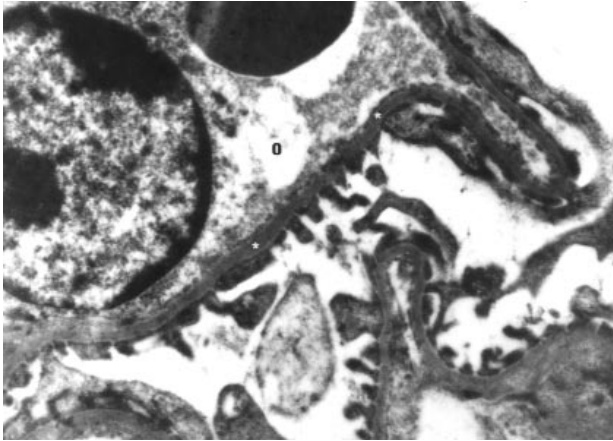


Fig. 2C. Electron micrograph showing the normal glomerular basement membrane (*) and the cell swelling in the endothelial cell (o) in the mid-term pregnancy group (Original magnification X 12000).

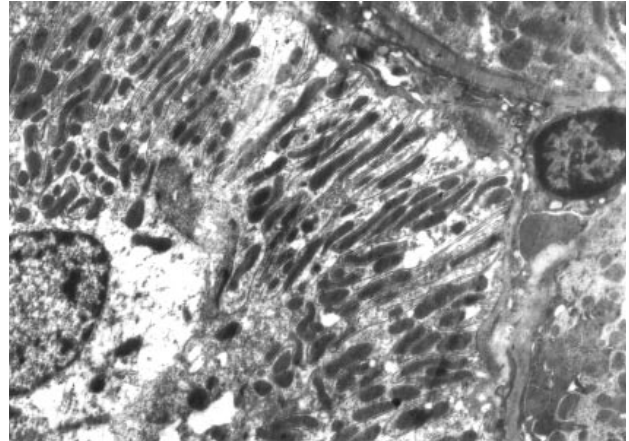


Fig. 2D. Electron micrograph showing the normal tubular epithelial cells in the mid-term pregnancy group (Original magnification X 5000).

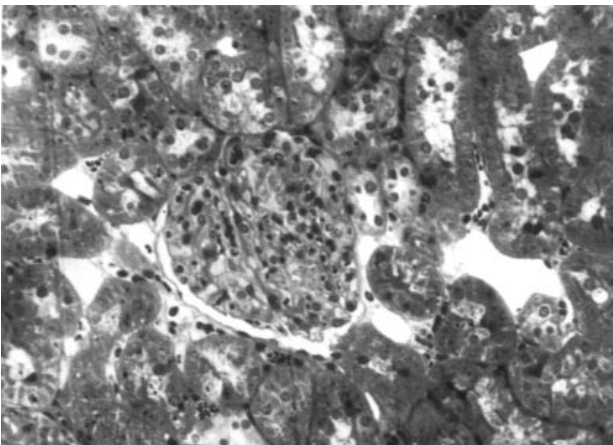


Fig. 3A. Photomicrograph showing no pathological changes in the post-parturition group (X 100).

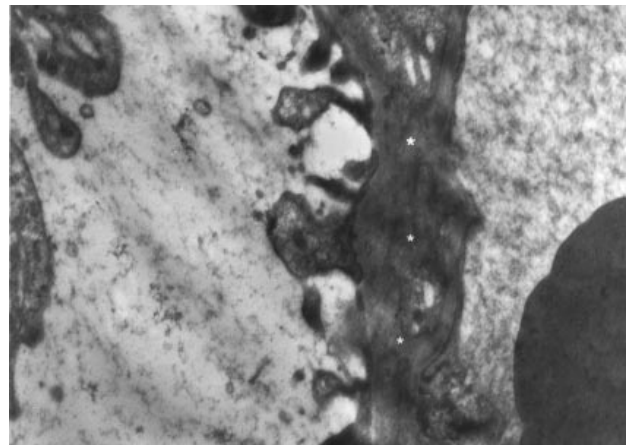


Fig. 3B. Electron micrograph showing the thickening of the glomerular basement membrane (*) in the post-parturition group (Original magnification X 12,000).

some of the mesangial cells (Fig. 3E). The tubules were found to be normal, ultrastructurally (Fig. 3F). The present cell swellings were seen in every section in this group.

Discussion

The clinical features of patients with solitary kidneys were studied by Rugiu et al. (13). In their study, they observed proteinuria, hypertension, hyperuricemia and deterioration of renal function. In one patient, focal glomerular sclerosis was seen. Following ablation of renal tissue, Gutierrez-Miller et al. (10) reported focal

glomerular sclerosis and proteinuria in patients with solitary kidneys. In rats with surgically induced reduction of renal mass, a syndrome of proteinuria and progressive renal failure occurred, usually associated with focal glomerular sclerosis (14,15). However, Baylis and Rennke (16) found no histological abnormalities in the glomeruli of rats at the end of five consecutive cycles of pregnancy and lactation. Similarly, in the study of Averbukh et al. (7), the glomeruli of the mice were normal on light microscopy at the end of a continuous period (19 weeks) of a dual challenge for glomerular hyperfiltration induced by uninephrectomy and repetitive pregnancies.

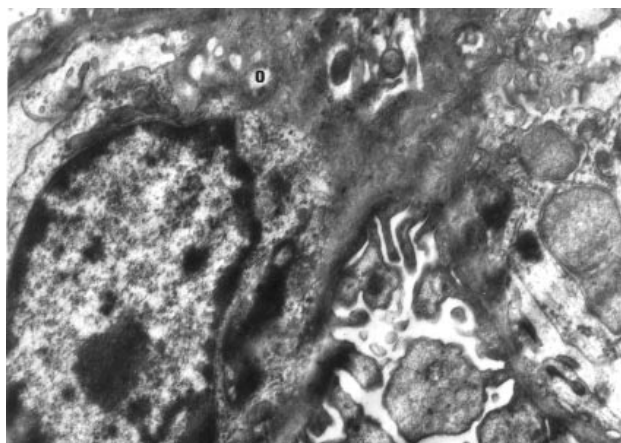


Fig. 3C. Electron micrograph showing the cell swelling in the cytoplasm of a podocyte (o) in the post-parturition group (Original magnification X 10,000).

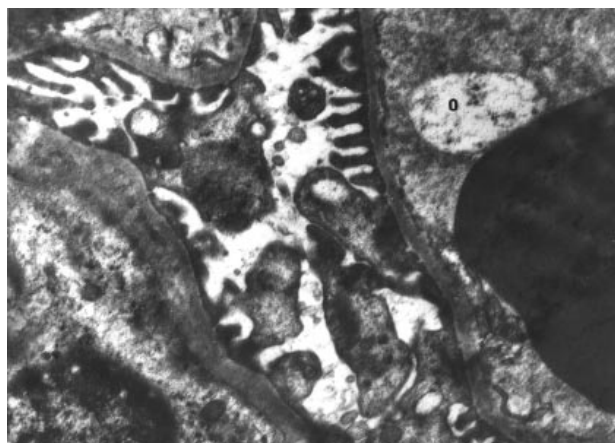


Fig. 3D. Electron micrograph showing the cell swelling in the cytoplasm of an endothelial cell (o) in the post-parturition group (Original magnification X 12,000).

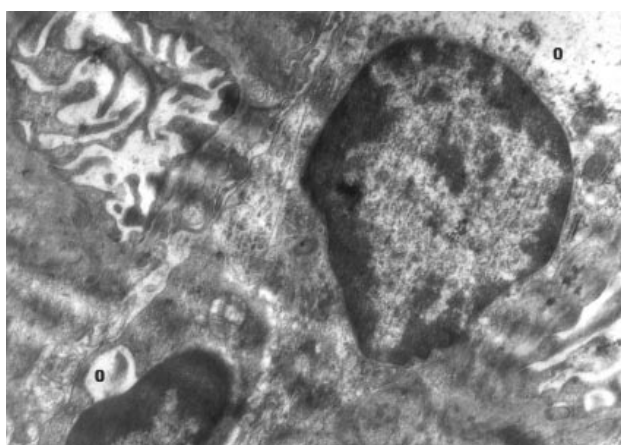


Fig. 3E. Electron micrograph showing the cell swelling in the cytoplasm of two mesangial cells (o) in the post-parturition group (Original magnification X 10,000).

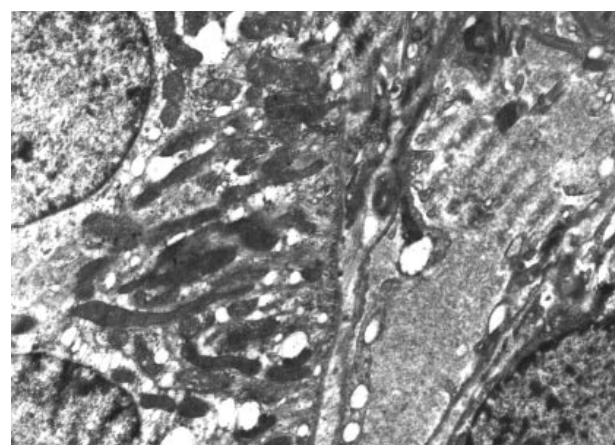


Fig. 3F. Electron micrograph showing the normal tubular epithelial cells in the post-parturition group (Original magnification X 5000).

The light microscopic results of the present study are in accordance with the studies by Baylis and Rennke (16) and Averbukh et al. (7). However, the transmission electron microscopic results of the present study are of great importance, as there are no ultrastructural studies in the literature related to uninephrectomy and pregnancy. Additionally, this is the first study reporting the light microscopic and ultrastructural changes in the kidneys of uninephrectomized animals during mid-term

pregnancy. In conclusion, cell swellings were observed in the endothelial cells and mesangial cells in the pure nephrectomized group. Additionally, cell swellings were observed in the podocytes in the mid-term pregnancy group. In the post-parturition group, in addition to all these findings, focal thickenings were observed in the glomerular basement membranes and these focal thickenings occurred during the second half of the pregnancy.

References

1. Berman, L.B.: The pregnant kidney. *J. Am. Med. Assoc.* 1974; 230: 111.
2. Lindheimer, M.D., Katz, A.I.: Renal physiology in pregnancy. In: Seldin, D.W., Geibisch, G. (eds) *The Kidney: Physiology and Pathophysiology*, vol 2. Raven, New York. 2017-2042, 1985.

3. Baylis, C.: Glomerular filtration and volume regulation in gravid animal models. In: Lindheimer, M.D., Davison, J. (eds) *Renal Disease in Pregnancy: Baillieres Clinical Obstetrics and Gynecology*. Saunders, London. 789-813, 1987.
4. Malt, R.A.: Compensatory growth of the kidney. *N. Engl. J. Med.* 1969; 280: 1446-1459.
5. Galla, E.H., Klein-Robbenharr, T., Hayslett, J.P.: Influence of age on the compensatory response in growth and function to unilateral nephrectomy. *Yale J. Biol. Med.* 1974; 47: 218-226.
6. Hayslett, J.B.: Functional adaptation to reduction in renal mass. *Physiol. Rev.* 1979; 59: 137-164.
7. Averbukh, Z., Weissgarten, J., Cohn, M., Rosenmann, E., Modai, D.: Dual challenge for glomerular damage in mice: Uninephrectomy and repetitive pregnancies. *Israel J. Med. Sci.* 1990; 26: 545-547.
8. Robitaille, P., Mongeau, J., Lortie, L., Sinnassamy, P.: Long-term follow-up of patients who underwent unilateral nephrectomy in childhood. *Lancet* 1985; 1 (8441): 1297-1299.
9. Brenner, B.M., Meyer, T.W., Hostetter, T.H.: The role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation and intrinsic renal disease. *N. Engl. J. Med.* 1982; 107: 652-659.
10. Gutierrez-Miller, V., Nieto, J., Praga, M., Usera, G., Martinez, M.A., Morales, J.M.: Focal glomerular sclerosis and proteinuria in patients with solitary kidneys. *Arch. Intern. Med.* 1986; 146: 705-709.
11. Baylis, C., Wilson, C.B.: Sex and the single kidney. *Am. J. Kid. Dis.* 1989; 13: 290-298.
12. Winkler, J., Boner, G.: Is pregnancy deleterious in females with one kidney? *Isr. J. Med. Sci.* 1990; 26: 575-576.
13. Rugiu, C., Oldrizzi, L., Lupo, A., Valvo, E., Loschiavo, C., Tessitore, N., Gammara, L., Ortalda, V., Fabris, A., Panzetta, G., Maschio, G.: Clinical features of patients with solitary kidneys. *Nephron* 1986; 43: 10-15.
14. Shimamura, T., Morrison, A.B.: A progressive glomerulosclerosis occurring in partial five-sixths nephrectomized rats. *Am. J. Pathol.* 1975; 79: 95-101.
15. Grond, J., Schiltuis, M.S., Koudstaal, J., Elema, J.D.: Mesangial function and glomerular sclerosis in rats after unilateral nephrectomy. *Kidney Int.* 1983; 22: 338-343.
16. Baylis, C., Rennke, H.G.: Renal hemodynamics and glomerular morphology in repetitively pregnant aging rats. *Kidney Int.* 1985; 28: 140-145.