TITLE: Nephrogenesis and the renal renin-angiotensin system in fetal sheep: effects of intrauterine growth restriction during late gestation


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RUNNING TITLE: Nephrogenesis and restricted fetal growth

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ABSTRACT

Previous studies have shown that intrauterine growth restriction (IUGR) can impair nephrogenesis, but uncertainties remain about importance of the gestational timing of the insult and the effects on the renal renin-angiotensin system (RAS). We therefore hypothesised that induction of IUGR during late gestation alters the RAS and this is associated with a decrease in nephron endowment. Our aims were to determine the effects of IUGR induced during the later stages of nephrogenesis on (a) nephron number, (b) mRNA expression of angiotensin AT\textsubscript{1} and AT\textsubscript{2} receptors, angiotensinogen and renin genes in the kidney, and (c) the size of maculae densae. IUGR was induced in fetal sheep (n=7) by umbilical-placental embolization (UPE) from 110-130d of the ~147d gestation; saline-infused fetuses served as controls (n=7). Samples of cortex from the left kidney were frozen and the right kidney perfusion fixed. Total kidney volume, nephron number, renal corpuscle volume, total maculae densae volume and the volume of macula densa per glomerulus were stereologically estimated. mRNA expression of AT\textsubscript{1}, AT\textsubscript{2} receptors, angiotensinogen and renin in the renal cortex were determined. In IUGR fetuses at 130d, body and kidney weights were significantly reduced and nephron number was reduced by 24%. There was no difference in renin, angiotensinogen or AT\textsubscript{1} and AT\textsubscript{2} receptor mRNA expression levels in the IUGR kidneys compared to controls. We conclude that fetal growth restriction late in nephrogenesis can lead to a marked reduction in nephron endowment, but does not affect renal corpuscle or macula densa size, or renal RAS gene expression.

Key words: IUGR, kidney, nephron endowment, macula densa, stereology

[Word count: 250]
INTRODUCTION

It is now apparent that intrauterine growth restriction (IUGR), which affects up to 10% of pregnancies, can alter fetal organ development, leading to changes that increase the risk of cardiovascular and renal disease in adulthood (2, 18, 35, 37). A major factor that may contribute to the increased risk of adult renal disease after exposure to IUGR is a reduction in nephron endowment (19, 24). Indeed, recent experimental studies suggest that a congenital nephron deficit renders the kidney more vulnerable to the effects of secondary postnatal insults such as high circulating levels of advanced glycation endproducts (as observed in diabetes) and induction of acute glomerulonephritis (33, 44). In humans, the number of nephrons has been related to birthweight, suggesting that IUGR inhibits nephrogenesis (15, 20, 21). A reduced nephron endowment has also been reported in the kidneys in many experimental models of IUGR (3, 22, 25, 26, 42, 44). However, there are still uncertainties as to critical periods during which nephrogenesis can be impaired by IUGR.

In humans, ~60% of nephrons are formed in the last trimester of pregnancy with nephrogenesis being completed by 36 weeks (~0.9) of gestation; no new nephrons are formed after this time (15). So, although there may be catch up in growth of the IUGR infant after birth the nephrons cannot increase in number, but they may enlarge. The sheep is an excellent model for studying the effects of IUGR on the fetal kidney; like humans, the sheep has a long gestation (term, 147-150 days) with much of renal ontogeny occurring prior to birth (10). In sheep, IUGR can be induced by umbilical-placental embolization (UPE) which replicates key aspects of the placental insufficiency associated with IUGR in humans during the later stages of gestation (23). In a recent study using sheep we found that UPE from 120 days to
140 days gestation led to a marked reduction in birth weight and kidney size but had no effect on nephron endowment (26). This is not surprising since the fetal growth restriction occurred after the completion of nephrogenesis; in sheep, nephrogenesis is complete by about 120 days (~0.8) of gestation (10). However in twins, in which IUGR occurs during at least the latter half of gestation, there was a reduction in nephron number (26). This earlier study has highlighted the importance of the timing of the gestational insult when considering the effects of perturbations in utero on renal ontogeny. Therefore, in the present study we have induced UPE from 110 days (~0.75) of gestation in order to determine if IUGR coinciding with the later stages of nephrogenesis can affect nephron number. This is important as IUGR in humans occurs predominantly during the third trimester.

The renal renin-angiotensin system (RAS) is considered to play a key role in nephrogenesis and in the development of renal vascular and tubular structures (8, 11, 14). Angiotensin II (Ang II) is the main effector hormone in the RAS cascade. In humans and sheep, Ang II acts via two main receptors: the Ang II type 1 (AT\(_1\)) and the type 2 (AT\(_2\)) receptor. However, little is presently known about the effects of IUGR on the renal RAS, including the number of AT\(_1\) and AT\(_2\) receptors and the level of angiotensinogen, the precursor for renal angiotensin. In the ovine fetal kidney the expression of the renal AT\(_1\) receptor is normally low during early gestation and increases during the last third of gestation (36). In contrast, the AT\(_2\) receptors are abundantly expressed in the metanephric mesenchyme and down-regulated late in gestation, when nephrogenesis is complete; the AT\(_2\) receptors are then predominantly expressed in the macula densa (10, 40). The maculae densae consist of specialized epithelial cells that lie within the cortical thick ascending limb of the juxtaglomerular apparatus, between the afferent and efferent arterioles (4).
main role is to monitor NaCl concentration in the distal tubule and to regulate the release of renin (32). Since the maculae densae cells are intimately associated with renin release, they play a key role in the renal RAS. We recently observed that the maculae densae appeared enlarged in sections from the kidneys of fetuses that were experimentally or spontaneously growth-restricted (26).

We hypothesize that induction of IUGR during late gestation leads to alteration of the RAS and this is associated with a decrease in nephron endowment. Hence, our aims were to determine the effects of IUGR induced during the later stages of nephrogenesis on (a) nephron number, (b) mRNA expression of the AT₁ and AT₂ receptors, angiotensinogen and renin genes in the kidney, and (c) the size of maculae densae.

**MATERIALS AND METHODS**

**Animals and surgery**

All surgical and experimental procedures were approved by the Monash University Animal Welfare Committee. Details are provided in Bubb et al. (7), and only a brief account will be given here. Fourteen time-mated Border Leicester x Merino ewes were anesthetized at 105-106 days of gestation and prepared for surgery; term is ~147 days. The uterus was incised and the hind quarters of the fetus exposed to allow insertion of catheters into a femoral artery and vein and the amniotic sac. The arterial catheter was positioned such that its tip lay in the fetal descending aorta below the renal arteries and above the umbilical arteries (9). After 4-5 days of postsurgical recovery, IUGR was induced in 7 fetuses (3 female, 4 male); another 7 fetuses (4 female, 3 male) were allowed to grow normally and served as controls. IUGR was induced by UPE from 110 to 130 days of gestation by daily injections of
microspheres into the fetal aortic catheter. The microspheres become embedded into the fetal tissue of the placenta where they impair the exchange of oxygen and nutrients to the fetus (9). The control group received daily saline (2-5ml) via the aortic catheter.

At 115 and 125 days of gestation (5 and 15 days after commencing embolisation, respectively) a 2ml sample of fetal blood was collected via the femoral artery catheter for measurement of fetal plasma renin activity and Ang II levels using radioimmunoassay (ProSearch Australia).

At necropsy (130 days of gestation) the fetal kidneys were excised and weighed. From the left kidney, pieces of cortex were snap frozen in liquid nitrogen and stored at -75 °C. The right kidney was perfusion fixed at 80mmHg with 4% formaldehyde (pH=7.4) and then stored in 10% buffered formalin.

**Kidney sampling and tissue processing**

The right kidney was cut through the hilus in the longitudinal plane followed by a transverse section through the hilus, resulting in four quarters. Two diagonally opposite quarters were randomly selected and cut into slices 2mm thick; every second slice was embedded in glycolmethacrylate resin (Technovit 7100 resin, Heraeus Kulzer, Hanau, Germany). These blocks were sectioned at 20µm using a Leica RM 2165 microtome (Leica Microsystems, Nussloch, Germany) fitted with glass blades. We collected the 10\textsuperscript{th} and 11\textsuperscript{th} sections, starting from a random number, and stained them with Periodic Acid Schiff’s reagent.

In the remaining slices, small cubes of cortex (1 per kidney slice; ~8 cubes per kidney) were cut from a randomly chosen site of cortex, and then embedded in epon-araldite. These blocks were sectioned at 1µm using a Leica OmU3 Ultramicrotome
(C.Reichert, Vienna, Austria) and stained with toluidine blue in order to examine the size of the maculae densae (see below).

Measurement of kidney volume, nephron number and renal corpuscular volume

In the glycolmethacrylate sections, total kidney volume \( V_{\text{kid}} \), nephron number and renal corpuscle volume were stereologically estimated. The sampled kidney sections (every 10\(^{th}\) section) from each block were used for the estimation of kidney volume using the Cavalieri principle (13).

Nephron number \( N_{\text{glomerulus}, \text{kid}} \) was estimated in the pairs (10\(^{th}\) and 11\(^{th}\) sections) of intact kidney sections using an unbiased physical disector/fractionator technique (5, 26). The total number of glomeruli (and thereby nephrons) in the kidney was determined by the formula:

\[
N_{\text{glomerulus}, \text{kid}} = 4 \times 10 \times (P_s/P_t) \times (1/2f_a) \times Q^- \]

where 4 represents the inverse of the slice sampling fraction, 10 is the inverse of the section sampling fraction, \( P_s/P_t \) and \( 1/2f_a \) represent the fraction of the total section area used to count glomeruli, \( Q^- \) is the total number of glomeruli counted from all selected pairs.

Mean renal corpuscle volume \( V_{\text{corp}} \) was determined stereologically by dividing the volume density of renal corpuscles in the kidney \( V_{V_{\text{glomerulus}}, \text{kid}} \) by the numerical density of glomeruli in the kidney \( N_{V_{\text{glomerulus}}, \text{kid}} \) (5, 26).

Estimation of the total volume of maculae densae and volume of macula densa per glomerulus
The epon-araldite sections (1 per block; 8 per kidney) were used for the stereological determination of the total volume of maculae densae. The sections were sampled using a uniform step length of 250 µm in the horizontal and vertical direction. This allowed for uniform systematic sampling throughout the kidney sections commencing at a random start point. An unbiased counting frame was superimposed over the sections at a magnification of x1250. In each section, grid points that fell on maculae densae (P$_{MD}$) and on the renal corpuscle (P$_{corp}$) were counted. Maculae densae were identified as closely packed columnar epithelial cells in the wall of the distal tubule which is in direct contact with the glomerulus at the vascular pole. The total volume of maculae densae in the kidney (Total V$_{MD}$) was calculated using the formula:

$$\text{Total V}_{MD} = \frac{V_{MD}}{V_{corp}} \times \text{Total V}_{corp}$$

where Total V$_{MD}$ was determined from multiplying $\frac{V_{MD}}{V_{corp}}$ by total volume of renal corpuscle (Total V$_{corp}$) (17).

The volume of macula densa per glomerulus (V$_{MD}$) was calculated by division of the total volume of maculae densae in the kidney (Total V$_{MD}$) by the total number of glomeruli in the kidney (N$_{glom, kid}$) (17):

$$V_{MD} = \frac{\text{Total V}_{MD}}{N_{glom, kid}}$$

**Gene expression studies: real-time PCR**

Total RNA was extracted from the cortex of the left kidneys (control n=7, IUGR n=6) using an RNeasy Midi Kit (QIAGEN® Australia) and 1 µg of RNA was then reverse transcribed into cDNA. Negative reactions were prepared to check for genomic DNA contamination. Reverse transcription reactions were run in a PCR
machine (Biorad i-cycler) at 25˚C for 10 min, 48˚C for 30min, 95˚C for 5min and then held at 4˚C. Relative mRNA expression levels of Ang II receptors type 1 (AT₁) and type 2 (AT₂), angiotensinogen and renin were determined using a 25 µl TaqMan multiplex real time PCR assay in the ABI PRISM 7700 Sequence Detection System (Perkin-Elmer Applied Biosystems, Foster City, CA, USA). Table 1 shows the primer and probe sequences used in this study. The sequences used for the primer and probes for the AT₁, and AT₂ receptors, angiotensinogen and renin were optimized previously (28).

A comparative cycle of threshold (Ct) method was used for mRNA quantitation of genes of interest as described previously (28), where 18S was used as a housekeeping gene (endogenous / internal control). Each sample was run and analyzed in triplicate. To calculate relative gene expression, the Ct value for 18S was subtracted from the Ct value of the gene of interest to give a delta Ct (ΔCt) for each sample. The ΔCt value of a “calibrator” (in this case the mean ΔCt value of the control group), was then subtracted from the ΔCt of each sample to give a ΔΔCt. This was put into the equation, $2^{-\Delta\Delta C_t}$ to give relative gene expression. The values obtained for each gene (AT₁ and AT₂ receptors, angiotensinogen and renin) were then compared between the treatment groups. The intra-assay coefficients of variation (34) were calculated for all genes of interest: they were 5%, 12%, 8% and 11% for AT₁, AT₂, angiotensinogen and renin respectively.

**Statistical analysis**

Data are presented as mean ± SEM. Statistical analyses were performed using Graphpad Prism for Windows (ver.3.00, Graphpad Software, San Diego). For all data, gender was included in an initial ANOVA to identify gender differences. As no
significant gender differences were detected, data from male and females were pooled and effects of treatment (IUGR vs control) compared using an unpaired two tailed student t-test. If data were not normally distributed a Mann-Whitney correction was applied. Within each group, linear regression analyses of fetal body weight versus kidney weight, kidney volume and nephron number were determined as well as kidney weight versus nephron number. Statistical significance was accepted where probability (p) values were equal to or less than 0.05.

RESULTS

Fetal body weight, kidney weight and kidney volume

There was a 25% reduction in body weight (p<0.05) and right kidney weight (p<0.05) in IUGR fetuses compared to controls (Table 2). Kidney volume was also significantly reduced (by 31%) in the IUGR group (Table 2) compared to controls. In both the control and IUGR groups, linear regression analyses showed a strong linear correlation between fetal body weight and kidney weight (R²=0.765, p=0.011 in controls and R²=0.676, p=0.023 in the IUGR group; Figures 1A and 1B, respectively.

Plasma renin activity and Ang II levels

The mean plasma renin activity and Ang II levels at 115 days gestation and 125 days gestation are shown in Table 3. There was a wide range in plasma renin activity and Ang II concentrations within both the control and IUGR fetuses at the gestational ages studied. There was no significant change within either the control or the IUGR groups in the levels of renin activity or Ang II from 115 days gestation to 125 days gestation; in some fetuses there was a rise in renin activity and Ang II from 115 days gestation to 125 days gestation whereas in others the levels decreased.
There was wide variability among individual measurements within groups which is reflected in the absence of a significant difference ($p = 0.07$) between groups in renin activity and Ang II levels at 115 days of gestation. At 125 days of gestation there was no difference in either renin activity or Ang II levels between groups.

**Nephron number**

The number of glomeruli (and thereby nephrons) was significantly reduced in IUGR fetuses (Figure 2); the IUGR kidneys had 24% fewer nephrons compared to controls. In the control group, linear regression analysis demonstrated a strong linear relationship between nephron number and birth weight ($R^2=0.791$, $p=0.007$); interestingly, there was no such association in the IUGR group ($R^2=0.169$, $p=0.359$). In the control group, nephron number and kidney weight were strongly associated ($R^2=0.689$, $p=0.021$; Figure 3A), whereas no such association was found in the IUGR group ($R^2=0.002$, $p=0.929$; Figure 3B). The number of glomeruli per unit of kidney volume (numerical density) was not different between groups (Table 2).

**Renal corpuscle size**

The mean volume of renal corpuscles was not significantly different between groups (Table 2). There was no correlation between nephron number and renal corpuscle volume in either the control group ($R^2=0.453$, $p=0.098$) or the IUGR group ($R^2=0.344$, $p=0.220$).

**Maculae densae volume**

In general, the maculae densae in the IUGR kidneys when examined microscopically appeared to be longer than those in controls (Figures 4B and 4A,
respectively). However, stereological analysis demonstrated that the total volume of maculae densae was not different between groups (Table 2). Likewise, there was no difference in the volume of the macula densa per glomerulus between groups (Table 2).

Relative expression of AT$_1$ and AT$_2$ receptors, angiotensinogen and renin genes

In the renal cortex the mRNA levels for the AT$_1$ and AT$_2$ receptor genes were not different between groups (Figures 5A and 5B, respectively). There was also no difference in angiotensinogen or renin mRNA expression in the renal cortex between groups (Figure 5C and 5D, respectively).

DISCUSSION

In this study, IUGR induced during late gestation led to significant reductions in both fetal body weight and kidney weight and this was accompanied by a substantial decrease in nephron endowment. Our findings show that a brief period of placental insufficiency, coinciding with the later stages of nephrogenesis, can markedly reduce renal growth and nephron endowment. However we found no evidence that the renal RAS was altered by late gestational IUGR and found no difference in the total volume of the maculae densae or in the volume of the macula densa per nephron.

Nephrogenesis in the sheep closely resembles that in humans in that it commences early in gestation when the ureteric bud first invades the metanephric mesenchyme and is complete prior to birth (10). Nephrogenesis occurs as a result of branching of the ureteric bud and induction of nephron formation within the
metanephric mesenchyme at the branch tips (39). The first branching of the ureteric bud has been observed at day 27 in the ovine fetal kidney (27), and nephrogenesis is completed at ~120 days, after which no further nephron formation has been observed (10). The nephrogenic zone often remains visible up to about 130 days gestation in sheep as many of the recently formed nephrons undergo maturation. Thus, in the present study in which IUGR began at day 110 of gestation, there was a developmental window of only about ten days in which nephrogenesis could be affected by the IUGR. Importantly, our results demonstrate that nephron endowment can be reduced substantially by fetal growth restriction in a relatively short developmental window during late gestation. This is likely linked to the extensive level of branching of the ureteric bud in the kidney during the latter stages of nephrogenesis (39).

There is mounting evidence that a primary congenital nephron deficit can render the kidney susceptible to secondary postnatal insults (30, 33, 44). Furthermore, the majority of IUGR infants show catch up growth within the first 2 years of life (1) resulting in a disproportionately low number of nephrons in relation to adult body weight. Hence, our findings are clinically important, as our IUGR model mimics the growth restriction often observed in human pregnancies which occurs as a result of late gestational placental insufficiency. Our findings suggest that IUGR babies may be at risk of reduced nephron endowment at birth and to subsequent renal disease, especially if there is catch up in body growth. Interestingly, in a previous study in our laboratory, in which IUGR was induced in the same manner from 120 days to 140 days of gestation, there was no difference in nephron endowment between the treatment groups; we concluded that this was because nephrogenesis was already complete in these fetuses (26). The results from both of
these studies demonstrate that the timing of the insult leading to IUGR during fetal development is crucial to the effects on nephron endowment.

We have recently shown in the primate kidney that kidney size is directly correlated with nephron number during normal gestation (12). In accordance with this concept, in the present study there was a strong linear relationship between nephron number and kidney weights and nephron number and kidney volumes in the control fetuses. Importantly, however, this regulation of nephron number was not evident in the kidneys of the IUGR fetuses. In these IUGR fetuses, a high kidney weight did not necessarily infer a greater nephron complement and similarly a low kidney weight did not infer a reduced nephron endowment. Apparently the number of nephrons is not directly linked to kidney size in the IUGR fetuses, suggesting that other factors which have been altered in the intrauterine environment are affecting nephrogenesis. Potential factors that lead to the dysregulation of nephrogenesis in the IUGR fetus are the fetal hypoxia and hypoglycemia that are seen in association with IUGR (7). However, the mechanisms leading to the de-regulation of nephron endowment within the kidneys of the IUGR fetuses cannot be determined from the present study, but it is an important area for future research.

Interestingly, there was no difference between groups in the mean corpuscular volume, although there were fewer nephrons in the IUGR group. Under normal circumstances, the postnatal kidney compensates for the loss of nephrons through an increase in the size of glomeruli (6, 29, 31) but this relationship is not always observed in the fetal or early postnatal kidney, implying that filtration is sufficient for body size at this age. It is expected that if the IUGR fetuses were born and allowed to grow to adulthood, especially if catch up growth occurred, there would be induction of glomerular hypertrophy in order to maintain renal filtration.
surface area, which may in turn lead to adverse effects in the kidney as a result of chronic glomerular hyperfiltration (16).

Prior to the commencement of this study we had made the observation in the kidneys of two other cohorts of growth restricted ovine fetuses that the maculae densae appeared enlarged (26); preliminary analyses demonstrated an increase in the length of the macula densa per nephron. Since the maculae densae, and in particular the juxtaglomerular cells, play a key role in renin production in the kidney, in the present study we compared the total volume of the maculae densae and the volume of the macula densa per nephron in the IUGR and control kidneys. Using this stereological approach we were able to avoid the difficulties of anisotropy associated with sampling of the anisotropic maculae densae. Interestingly in the present study, as before, we observed that microscopically the maculae densae appeared longer in the IUGR kidney sections compared to controls, yet our stereological measurements demonstrated no difference in total volume of the maculae densae or in the volume of the macula densa per nephron.

Prior to this study, there have been a number of studies in other species on the effects of IUGR on the fetal renal RAS (38, 41, 43). In the case of the IUGR rat there are reports of down-regulation of the renal RAS at birth (41, 43), whereas in newborn spontaneously growth-restricted piglets it has been recently reported that there is up-regulation of the renal RAS (38). The differences in findings between species may relate to the relative developmental maturity of the kidneys at birth; in the rat the kidneys are still undergoing nephrogenesis at birth whereas in the piglet it is complete. In this regard, our ovine model resembles the human and piglet, with nephrogenesis being complete late in gestation (about day 120 in the fetal sheep). However, unlike the findings in piglets (38) we found no difference in the mRNA
expression of either the AT₁ or the AT₂ receptor or angiotensinogen in the IUGR kidneys at 130 days gestation within the IUGR group compared to controls. Of particular relevance to the present study, the effects of IUGR induced by placental restriction in sheep on the intrarenal RAS have also been previously described (45). In that study there was down-regulation of renal angiotensinogen and renin expression in the IUGR fetus at ~140d gestation; the authors therefore suggested that reduced activity of the intrarenal RAS may lead to impaired renal growth and development in the IUGR fetal sheep. In the present study although there was obvious impairment of nephrogenesis as a result of late gestational IUGR, there was no clearly defined suppression of the intra-renal RAS. However, it must be noted, that the mRNA expression of the components of the intrarenal RAS and plasma concentration of Ang II and renin activity varied considerably between animals within groups which makes detection of subtle changes difficult.

We conclude that fetal growth restriction that coincides with the later stages of nephrogenesis can markedly reduce renal growth and nephron endowment. This may render the kidney increasingly vulnerable to renal insults later in postnatal life, especially if catch up in body growth occurs, potentially contributing to the excess of renal disease in individuals born with low birthweight.

ACKNOWLEDGEMENTS
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19. *Hoy WE, Hughson MD, Singh GR, Douglas-Denton R, Bertram JF.*


28. **Moritz KM, Koukoulos I, Alibston A, Wintour EM.** Angiotensin II infusion to


FIGURE LEGENDS

Figure 1. Linear regression analyses of fetal body weight versus kidney weight at 130d gestation in (A) the control group and (B) the IUGR group. There was a significant correlation between fetal body weight and kidney weight in both groups, demonstrating that kidney weight was proportional to fetal weight in both groups.

Figure 2. Stereological estimates of total nephron number in kidneys from ovine fetuses at 130d gestation in the control (n=7) and IUGR (n=7) groups. There was a significant 24% reduction in nephron number in the IUGR group compared to the controls; asterisk signifies p<0.05.

Figure 3. Linear regression analyses of nephron number versus kidney weight at 130d gestation in (A) control fetuses and (B) IUGR fetuses. There was a direct association between nephron number and kidney weight in the control kidneys but this was not observed in the IUGR kidneys.

Figure 4. Representative light micrographs of cortical glomeruli and associated maculae densae in (A) control and (B) IUGR kidneys at 130d gestation. The arrows point to the macula densa. The size of the image corresponds to 1250x magnification and the sections are stained with toluidine blue.

Figure 5. mRNA expression of the genes for (A) the AT₁ receptor, (B) the AT₂ receptor and (C) angiotensinogen and (D) renin in the cortex of control (n = 7) and IUGR (n = 6) fetal kidneys at 130d gestation. There were no significant differences between groups.
**Table 1.** Primer and probe sequences used in real time PCR. Nucleotide position is shown in brackets.

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Table 2. Body weights, right kidney weights, and stereological estimates of right kidney volumes, numerical density of glomeruli, renal corpuscle volumes, total volume of maculae densae and volume of the macula densa per glomerulus in control and IUGR fetal sheep at 130d gestation (term ~147 d).

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<tr>
<td>Body weight (kg)</td>
<td>3.64 ± 0.30</td>
<td>2.74 ± 0.09</td>
<td>0.014</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>11.57 ± 1.06</td>
<td>8.71 ± 0.68</td>
<td>0.043</td>
</tr>
<tr>
<td>Kidney volume</td>
<td>7.19 ± 0.40</td>
<td>4.94 ± 0.44</td>
<td>0.003</td>
</tr>
<tr>
<td>(mm$^3 \times 10^3$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Numerical density</td>
<td>51.63 ± 4.62</td>
<td>60.31 ± 6.80</td>
<td>0.310</td>
</tr>
<tr>
<td>(glom/ mm$^3$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean corpuscle volume</td>
<td>9.35 ± 1.51</td>
<td>8.17 ± 0.67</td>
<td>0.458</td>
</tr>
<tr>
<td>(mm$^3 \times 10^{-4}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total volume of maculae densae</td>
<td>48.91 ± 6.74</td>
<td>44.39 ± 8.18</td>
<td>0.668</td>
</tr>
<tr>
<td>(mm$^3$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macula densa volume per glomerulus</td>
<td>1.39 ± 0.23</td>
<td>1.57 ± 0.25</td>
<td>0.604</td>
</tr>
<tr>
<td>(mm$^3 \times 10^{-4}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM.
**Table 3.** Plasma Renin activity and ANG II levels in control and IUGR fetuses at 115 and 125 days of gestational age (term ~147 d).

<table>
<thead>
<tr>
<th></th>
<th>Renin (ngAngI / ml / h)</th>
<th>ANG II (pg / ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>115 d</td>
<td>125 d</td>
</tr>
<tr>
<td>Control</td>
<td>n = 7</td>
<td>n = 7</td>
</tr>
<tr>
<td></td>
<td>9.07 ± 2.52</td>
<td>8.34 ± 2.03</td>
</tr>
<tr>
<td>IUGR</td>
<td>3.64 ± 0.98</td>
<td>6.56 ± 1.11</td>
</tr>
</tbody>
</table>

*p value*  
0.068 0.453 0.085 0.799

Values are means ± SEM.
(A) Control \[ R^2 = 0.765, \ p = 0.011 \]

(B) IUGR \[ R^2 = 0.676, \ p = 0.023 \]
(A) Control  \[ R^2 = 0.689, \ p = 0.021 \]

(B) IUGR  \[ R^2 = 0.002, \ p = 0.929 \]
(A) **AT\textsubscript{1}**

AT\textsubscript{1} mRNA expression (arbitrary units)

Control | IUGR
--- | ---
1.0 | 1.5

(B) **AT\textsubscript{2}**

AT\textsubscript{2} mRNA expression (arbitrary units)

Control | IUGR
--- | ---
1.0 | 1.5

(C) **Angiotensinogen**

Angiotensinogen mRNA expression (arbitrary units)

Control | IUGR
--- | ---
1.0 | 1.5

(D) **Renin**

Renin mRNA expression (arbitrary units)

Control | IUGR
--- | ---
2.0 | 1.0