

Anencephalic fetuses can be an alternative for kidney transplantation: a stereological and histological investigation

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Summary. In the study, stereological, histological, and anatomical techniques were used to investigate structural and morphometrical features of anencephalic and normal fetal kidneys.

Twenty human fetal kidneys (5 male and 5 female anencephalic fetuses, and 5 male and 5 female normal fetuses) at gestational ages 30 to 35 weeks were examined. Our study used two basic research methods. One was conventional anatomical measurement at the macroscopic level, such as volume, length, weight, etc. The other consisted of conventional and modern microscopic techniques. The microscopic techniques were based on two research methods: histopathological examination at light microscopic level and stereological estimations, including mean kidney volumes, obtained by the Cavalieri method, and the total number and mean height of the glomeruli via the physical dissector method.

There was no statistical difference between the two groups in terms of width, height, weight, and fluid replacement volumes. Microscopic quantitative assessment found no statistical differences either, in terms of the kidney volumes and the number and height of the glomeruli.

Our findings suggest that kidneys from anencephalic infants may be a suitable alternative for renal transplantation.

Key words: Anencephalic donors, Kidney, Stereology, Anatomy, Light Microscopy

Introduction

Neural tube defects (NTD) are a group of unique and complex congenital anomalies of the central nervous system (CNS), such as anencephaly, spina bifida, and encephaloceles (Padmanabhan, 2006). Anencephaly is a lethal malformation that occurs when the head end of the neural tube fails to close (Moore and Persaud, 2007). Few children with anencephaly live to the end of the pregnancy. They usually die after delivery; 50% have a life expectancy of between a few minutes and 1 day, and 25% live up to 10 days (Lemire and Siebert, 1990).

In recent years, researchers have focused on finding suitable organ sources due to increasing transplantation needs. The most important reason for this increase may be explained by advances in surgery techniques for transplantation procedures, as well as developing laboratory processes. These include easy determination of HLA-matched family members, new treatment alternatives preventing rejection, and investigation of structural features of interesting organs supporting surgical applications.

These structural properties, as is the case of the kidney in the present study, may be divided into two groups. The first group contains morphometrical parameters. They can be examined at the macroscopic level, such as water replacement volume, length, weight, etc., and the microscopic level, such as the number, size,

volume, and surface area of the nephrons, which are structural and functional units of the kidney, or as the volume and surface area of the whole kidney. The second group is composed of histological features consisting of Bowman's capsule investing a capillary tuft, the glomerulus. Furthermore, the mesangium consists of mesangial cells embedded in the mesangial matrix and podocytes that bulge into the urinary spaces. These structural elements are important factors in detecting the most appropriate embryological stage of a kidney for renal transplantation. However, there is still a need for specific research methods to correctly evaluate the complex composition of the kidney.

In the last few decades, stereology, which is an unbiased sampling technique whereby the object of interest is sectioned into a series of two-dimensional slices, has become the most popular method used in quantitative studies. The current study, thus, aimed to explore the anatomical, morphometrical, and histological structures of kidneys from anencephalic fetuses, and to detect whether these kidneys were different in nature from those of normal fetuses or not. The possibility of anencephalic donors for kidney transplantation was studied. Subsequently, macroscopic measurements, including width, height, weight, and fluid replacement volumes of the kidneys, were first examined. They were then analyzed stereologically in terms of the number and height of the glomeruli, and the volume of the kidney at the light microscopic level. Histopathological examinations were performed at light microscopic levels.

Materials and methods

Experimental Design

Our study was approved by the Ethics Committee of the Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey. Twenty fetuses of gestational ages ranging from 30 to 35 weeks were used in this study. Gestational ages were determined according to the criteria as follows: i.) Crown-rump length (CRL-mm) was 380-470 mm, ii.) foot-length (FL-mm) was 50-78 mm, and iii.) (Deshpande et al., 1980; Scher and Barmada, 1987). Fetal weight (FW) was 1300-2400 g for 30-35-week fetuses. Necropsies were performed, and gross developmental features (normal or anencephalic) and anatomic characteristics (width, height, weight, and fluid replacement volumes) were determined at the Department of Anatomy, the Faculty of Medicine, Karadeniz Technical University. Likewise, stereological and histopathological properties (glomerule number-height, kidney volume, and light microscopical assessment) were examined in the Departments of Histology and Embryology and Pathology, the Faculty of Medicine, Ataturk University, Erzurum, Turkey, and in the Department of Histology and Embryology, the Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey. Clinical aspects were also evaluated in

the Department of Obstetrics and Gynecology, Faculty of Medicine, Kafkas University, Kars, Turkey. The specimens were divided into four groups based on normal males (n: 5) and females (n: 5), and anencephalic males (n: 5) and females (n: 5). No developmental abnormalities were observed during the macroscopic inspections of the normal fetuses. In addition, no developmental abnormalities were observed in the anencephalic fetuses other than the apparent anencephaly seen on macroscopic inspections. Based on the absence of the brain and calvarium superior to the orbits, it was decided whether fetuses were anencephalic or not.

Tissue preparation procedures

Twenty fetal kidneys weighing between 1300 and 2400 g and at gestational ages 30 to 35 weeks were used in this experiment, which was carried out in an ethically proper way.

Light microscopy

Kidneys were fixed by 10% formaldehyde, dehydrated in graded alcohol series, embedded in paraffin wax, and serially sectioned using a Leica RM2125RT microtome (Leica Microsystems, Wetzlar, Germany). Serial sections of 5- μ m thickness in the transverse plane were mounted onto glass slides. Sections were stained with Hematoxylin-Eosin (H-E), and were used for stereological and light microscopic examination.

Tissue Sampling Procedures for Stereology

Based on a pilot study, approximately 1800 sections were obtained. It was decided to select every 31st kidney section pairs from consecutive sections using the systematic random sampling approach (Fig. 1). Thus, approximately 12-14 section pairs were used for each sample. Both section pairs and one of a section pair only were used for estimating the object number (total glomerulus number) and total kidney volume, respectively (Figs. 1, 2). In the number estimation, the first chosen section and its adjacent section, called the dissector pair, were separated by 25 μ m (5 section thickness: 25 μ m distance) as a rule of the physical dissector. According to this rule, the distance between section pairs must be about 30-40% of the average projected height of the related object to be estimated. The number of sections for both the number estimation procedure with the physical dissector method and the volume estimation procedure with the Cavalieri method were predicted to receive a coefficient of error (CE) within an acceptable range (Gundersen and Jensen, 1987).

The Cavalieri and physical dissector methods were applied to the light microscopic images for the stereological estimation of the volume and glomeruli

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number of the kidneys, respectively, by the use of stereology software 6.0 (version 6.0, Microbrightfield, Colchester, VT).

Number estimation with physical dissector method

Area sampling procedure of section and physical dissector application

In the study, the area of interest was located in one of the section pairs called the reference section. The external counter of this area was traced by Stereo Investigator[®], and the determined section area was estimated. It was divided into equal fields in a square manner at the x- and y-axes of the microscope. Finally, photographs of the previously described field were taken via motorized stages, and positions encoded through moving the x-y axes. The same procedure was applied to the other section pairs, called the look-up sections. Consequently, in each dissector pair, considerable fields were carefully found (Fig. 2). At this point, a major difficulty is to provide identical orientation of the same areas in dissector pairs. After the identical field had been obtained, the images of the dissector pairs were transferred to another PC.

The adjacent vision fields were first located on the PC screen. Then, a suitable unbiased counting frame was manually replaced on identical structures on these sections with a fixed rule. Finally, the dissector-counting method rules were easily applied in these section pairs. These rules consisted of two steps associated with each other:

i.) One of these is associated with unbiased counting frame. The bottom and left-hand edges of the frame are considered the forbidden (exclusion) lines together with the extension lines. Other boundaries of the frame and the top-right corner were considered inclusion points, and any particle that hit these lines or was located inside the frame was counted as a dissector particle.

ii.) The other rule is associated with section pairs. The relevant objects (glomeruli in our study) were counted when they were visible in the reference section but not in the look-up section (Fig. 3). The reference and look-up sections were reversed in order to double the number of dissector pairs without taking new sections (Fig. 4).

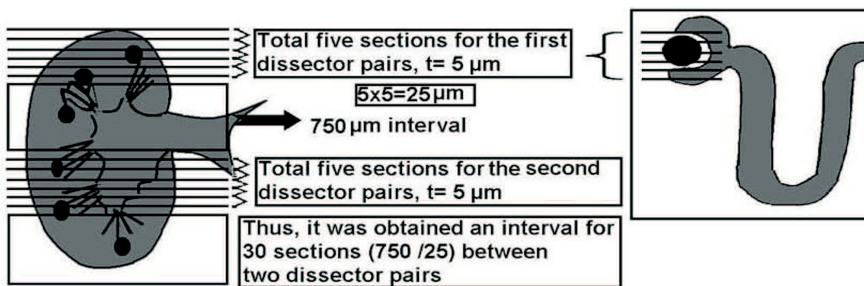
The mean numerical density of glomeruli ($NV_{\text{glomeruli}}$) per cm^3 was estimated using the following formula (Sterio, 1984):

$$N_{V\text{Glomerule}} = \frac{\sum Q_{\text{glo}}^-}{t \cdot A}$$

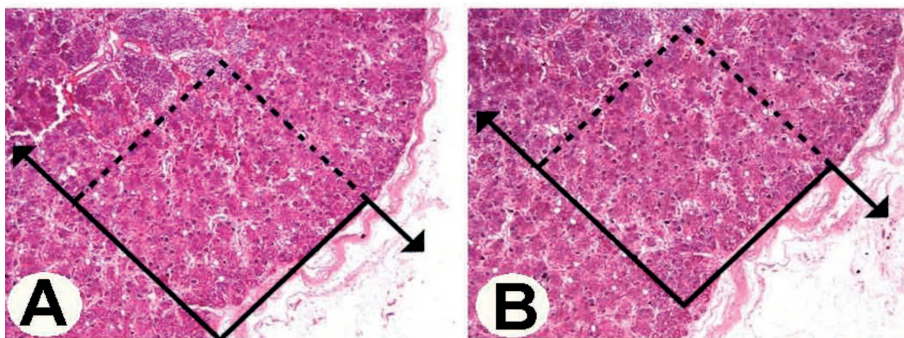
where $\sum Q_{\text{glo}}^-$ the total number of counted glomeruli profiles that appeared only in the reference sections, t is the section thickness, and A is the area of the counting frame.

The mean glomerulus height (H_{glo}), which is a measure of the size of the section plane, was estimated from the following equation (Sterio, 1984):

$$H_{\text{glo}} = \left(\frac{\sum Q_{\text{glo}}}{\sum Q_{\text{glo}}^-} \right) \cdot t$$



-I-



-II-

Fig. 1. I; Sampling strategy used in this study, II **A, B**. Dissector pair (reference and look-up section, respectively), used in the number and height estimations of glomeruli.

where ΣQ_{glo} is the total number of glomeruli in the reference section. Since the glomeruli that were not considered dissector particles were also recorded, the mean glomerulus height was estimated from the same data obtained by counting without any additional work. The estimations were performed for each type of glomerulus at all developmental stages.

Volume estimation with the Cavalieri Method

Volume estimation of any structure that has an arbitrary shape and size may be efficiently obtained by the principle of Cavalieri (Gundersen and Jensen, 1987). According to the first important rule of this principle for unbiased estimation of the volume of any object of

interest, serial and parallel planes must be cut, separated by a fixed distance (t) (Gundersen, 1986; Cruz-Orive and Weibel, 1990). In the study, a modified point-counting grid was used for the area estimation of the section profiles ($a/p = 1-\mu m^2$ interval for kidney). The point density of the point grid was designed to obtain an appropriate coefficient of error (CE) for the serial paraffin section of our study. The coefficient of error and coefficient of variation (CV) were estimated in accordance with the indicated formula (Gundersen and Jensen, 1987).

A test grid with a systematic array of points was randomly placed on a PC screen, and the appropriate point per area of interest (total kidney areas) was carefully calculated for each individual. The volumes of

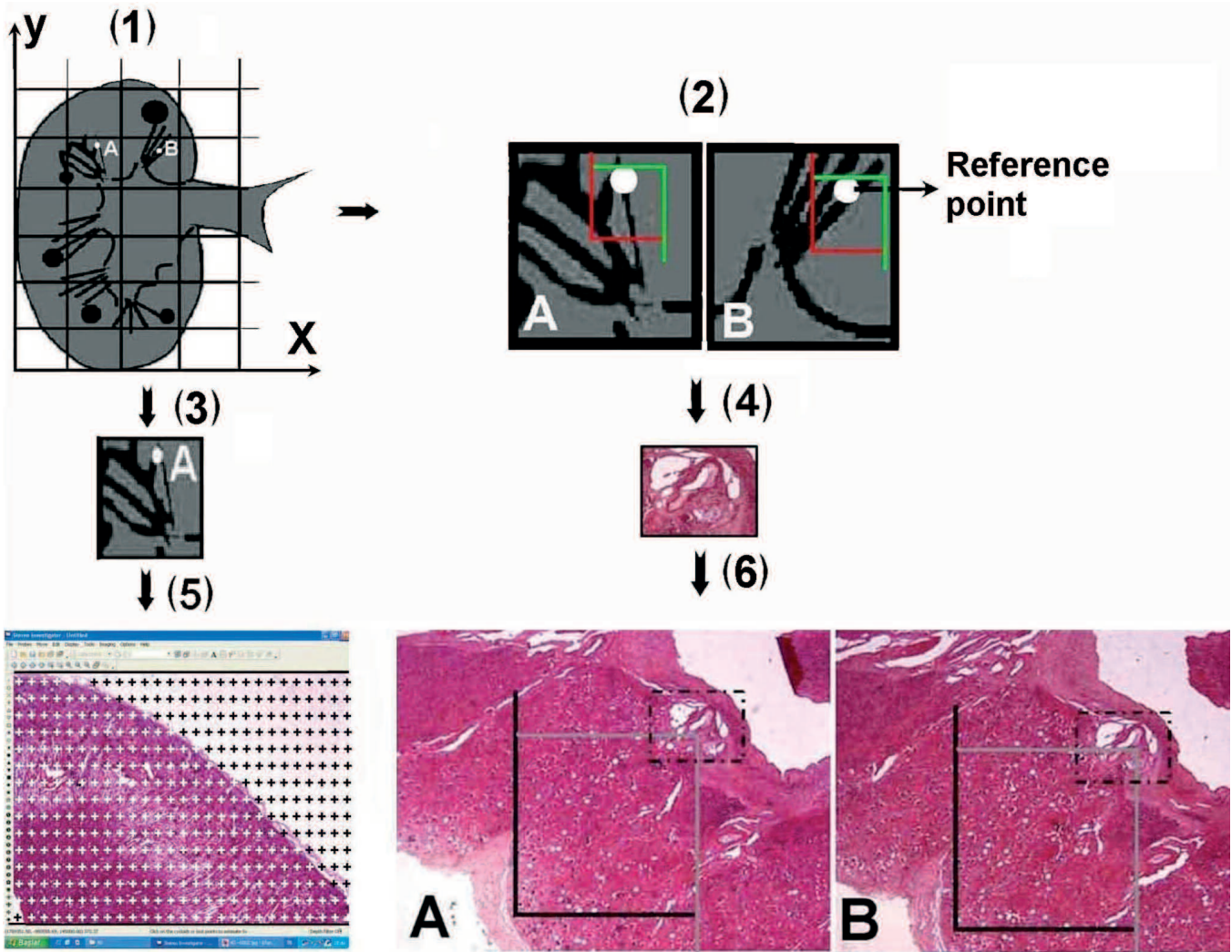


Fig. 2. (1). Distribution of sampling areas in kidney. (2). A disector pair, consisting of consecutive areas with detected reference points and unbiased counting frames at the illustrated reference (A) and look-up sections (B). (3). A selected area for volume estimation. (4). Detected reference point at high magnification. (5). Volume estimation at PC with stereo-investigator software. (6). A, B. An original disector pair with an unbiased counting frame, used in number estimations.

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the kidney for each section were estimated by the following formula:

$$\hat{V} = t \times \frac{a}{p} \times \sum_{i=1}^m P_i$$

where V is the volume of the object of interest (kidney) in one section plane, t is the section thickness, a/p is the inter-point area, and ΣP is the number of points hitting the kidney in that section. After the same formula had been applied for the entire section, the total volume was obtained from

$$\hat{V}_{\leftarrow total} = V_1 + V_2 + \dots + V_n$$

Error predictions for the Cavalieri estimation

The number of sections and the point density of the point-counting grid were designed to obtain an appropriate coefficient of error (CE) for the images of the serial sections. The CE and coefficient of variation (CV) were estimated according to Gundersen and Jensen's formula (Gundersen and Jensen, 1987):

$$Noise = 0.0724 \times (b/\sqrt{a}) \times \sqrt{nx} \times \sqrt{\Sigma P}$$

Noise is the value of information on the complexity of the examined cut surface area of the specimen, b/\sqrt{a} is equivalent to the mean boundary length of the profiles divided by the square root of their mean area, n is the number of sections examined, and ΣP is the number of points hitting the kidney in whole sections.

$$Var_{SRS} \left(\sum_{i=1}^n a \right) = (3 \cdot (A - Noise) - 4 \cdot B + C) / 12$$

where $Var_{SRS} \left(\sum_{i=1}^n a \right)$ indicates the variance of the total area in the systematic random sampling (SRS). These data give information on the sufficient section number required to obtain an appropriate variation for section samples.

$$A : \left(\sum_{i=1}^n (Q_i^-)^2 \right), B : \left(\sum_{i=1}^{n-1} Q_i^- \cdot Q_{i+1}^- + 1 \right), C : \left(\sum_{i=1}^{n-2} Q_i^- \cdot Q_{i+2}^- + 2 \right)$$

are the total numerical values for the data.

$$TotalVar = Noise + Var_{SRS}$$

$$CE(\Sigma P) = \frac{\sqrt{TotalVar}}{\Sigma P}$$

CE is the last calculated value. The generally accepted highest limit of CE is 5% (Gundersen and Jensen, 1987).

Error predictions for the physical dissector estimation

$$Nugget = \sum_{i=1}^n Q_i^-$$

where Nugget is the estimated total dissector particles for each sample.

$$VAR_{SRS} = \frac{3(A - Nugget) - 4B + C}{12}$$

where $Var_{SRS} \left(\sum_{i=1}^n a \right)$ indicates the variance of the total number in the systematic random sampling (SRS). These data give information on the sufficient number of dissector particles required to obtain an appropriate variation for section samples.

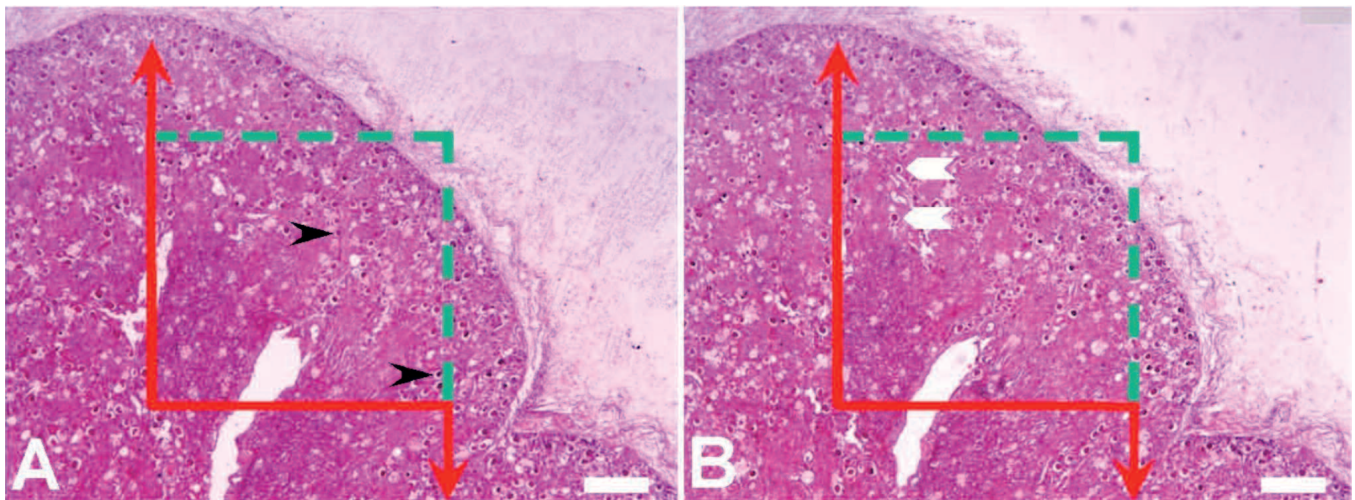


Fig. 3. A, B. The two figures belong to an application of the physical dissector counting method. The same areas of the two adjacent sections, separated by 5 μm. **A.** Reference section. **B.** look-up section. Nuclei hitting the inclusion lines or located inside the frame in **A** (black arrowhead) and **B** (white arrowhead) were counted as dissector particles when their profiles were not seen in the look-up section (**B and A**). Scale bar: 800 μm.

$$A : \left(\sum_{i=1}^n (Q_i^-)^2 \right), B : \left(\sum_{i=1}^{n-1} Q_i^- Q_i^- + 1 \right), C : \left(\sum_{i=1}^{n-2} Q_i^- Q_i^- + 2 \right)$$

are the total numerical values for the data.

$$\text{TotalVar} = \text{Nugget} + \text{VARSRs}$$

$$CE = \frac{\sqrt{\text{TotalVar}}}{\text{Nugget}}$$

CE is the last calculated value. The generally accepted highest limit of CE is 5% (Gundersen and Jensen, 1987).

Statistical analysis: Differences in gross anatomical and stereological data (number/height of glomeruli and volume of kidneys) between the two groups were tested using the independent samples t-test (two tailed, with a significance limit of p=0.05 in this test). The statistical calculations were performed using SPSS 13.0 software for Windows.

Results

Macroscopic results

The macroscopic results were summarized in Table 1. Gross anatomical data were evaluated by comparing two groups according to sex and localization (left or right). No significant differences were found in the anatomical characteristics of the kidneys (length, weight, etc.) between the groups (p>0.05).

Stereological results

Our stereological study contained the numerical density of glomeruli - total number-mean height and total kidney volume. No significant differences in glomerulus density, total glomerulus number, mean glomerulus height (µm), and mean kidney volume (cm³) were observed between the normal and anencephalic

fetuses, in depending only on gender but also by not depending on gender (p>0.05) (Tables 2-5, and Figs. 5-8).

Numerical Density of Glomeruli

The results of the numerical density of glomeruli are summarized in Table 2 and Figure 5.

Total Number of Glomeruli

The results of the total number of glomeruli are summarized in Table 3 and Figure 6.

Mean Glomerulus Height

The mean glomerulus height of kidneys in normal and anencephalic fetuses is summarized in Table 4 and Figure 7.

Kidney Volume

The kidney volumes of normal and anencephalic fetuses are summarized in Table 5 and Figure 8.

Histological analysis

Although histological assessment of normal (Fig. 9A-H for males and Fig. 10A-H for females) and anencephalic (Fig. 9A₁, B₁, C₁, D₁, E₁, F₁, G₁, and H₁ for males and Fig. 10A₁, B₁, C₁, D₁, E₁, F₁, G₁, and H₁ for females) fetal kidneys at the 30-35th week of gestation determined both newly developed and developing glomerular structures, mature glomeruli were clearly predominant in the inner and outer regions of the renal cortex (Fig. 9-10). In the developing glomerular structures, the glomeruli were smaller, and the glomerular capillaries were not developed completely (Fig. 9D, C₁, D₁ and Fig. 10D, C₁, D₁). In most of the

Table 1. The dimensions, weight, and volumes of both kidneys and ± SEM.

Measurements	Fetus (Female) Right Kidney		Fetus (Female) Left Kidney		Fetus (Male) Right Kidney		Fetus (Male) Left Kidney	
	N; n=5	A; n=5	N; n=5	A; n=5	N; n=5	A; n=5	N; n=5	A; n=5
Length-1	39,6±0,22	36,42±0,86	36,87±0,77	35,91±1,09	36,80±0,28	34,21±0,08	34,68±0,07	34,60±0,32
Length-2	21,8±0,11	19,16±0,11	22,93±0,05	22,45±0,13	20,46±0,13	19,59±1,29	19,94±0,56	20,34±0,63
Length-3	18,6±1,28	17,09±1,93	19,08±1,34	18,94±0,16	17,40±1,77	15,29±0,58	18,41±1,18	16,84±0,17
Depth-1	19,1±0,45	20,99±2,04	17,44±1,09	18,77±1,11	14,87±0,65	13,62±1,08	14,71±0,66	13,28±1,34
Depth-2	20,5±0,94	23,45±1,25	16,47±0,61	17,76±1,12	13,86±0,38	14,90±0,15	15,89±0,61	13,24±0,43
Depth-3	13,8±0,68	13,63±0,97	14,04±1,23	13,56±0,22	12,14±0,98	11,72±1,65	12,31±0,56	11,12±0,48
Weight (gr)	8,52±0,54	6,55±1,32	8,29±0,83	7,8±1,18	6,45±0,85	5,60±1,31	6,27±0,30	5,37±1,14
Water Replacement Volume (ml)	8,75±0,42	8±1,15	8,75±0,46	8,5±0,28	6,5±0,29	5,5±0,29	6,33±1,19	5,75±0,52

N: Normal; A: Anencephalic fetuses. Length-1 is the longest margin of each kidney between the extremitas superior and inferior. Length-2 is the margin between the extremitas superior and the middle line. Length-3 is the margin between the extremitas inferior and the middle line. Depth-1 is the longest antero-posterior margins of the uppermost kidney pieces divided from three equal parts. Depth-2 is the longest antero-posterior margin of the middle kidney pieces divided from three equal parts between the hilus renalis and the margo lateralis. Depth-3 is the longest antero-posterior margin of the last kidney pieces divided from three equal parts.

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renal corpuscles, the Bowman's capsule was lined with a simple squamous epithelium (Fig. 9 B, D and Fig. 10C, D1). However, there was still a simple cuboidal epithelium that was smaller (Fig. 9C1, D1 and Fig. 10C, D). Bowman's space was detected clearly, and it was

Table 2. Numerical density of glomeruli (total glomerulus number/ total kidney volume- cm3) of kidneys in normal and anencephalic fetuses.

Groups	Numerical Density of Glomeruli (Number/Volume)	Standard Error Mean
Normal Female	128.644	3.724
Anencephalic Female	137.029	3.553
Normal Male	101.299	2.041
Anencephalic Male	96.654	2.082

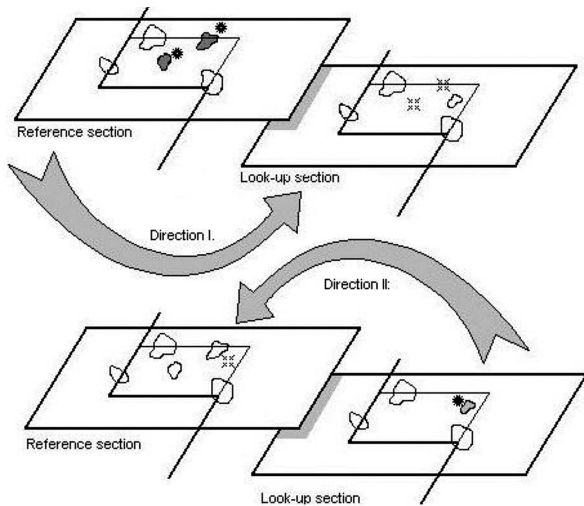


Fig. 4. Reference and look-up sections were reversed in order to double the number of dissector pairs without taking new sections.

Table 3. Total number of glomeruli of kidneys in normal and anencephalic fetuses.

Groups	Total Number of Glomeruli	Standard Error Mean
Normal Female	845.929	4516.363
Anencephalic Female	793.490	13122.206
Normal Male	598.388	7811.785
Anencephalic Male	601.095	7123.927

Table 4. Mean glomerulus height of kidneys in normal and anencephalic fetuses and ± SEM.

Groups	Mean Glomerulus Height (µm)	Standard Error Mean
Normal Female	121.917	0.557
Anencephalic Female	118.333	1.001
Normal Male	113.9	1.22
Anencephalic Male	115.225	0.49

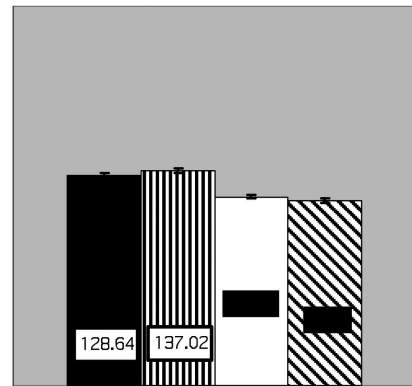


Fig. 5. Numerical density of glomeruli of kidneys in normal and anencephalic fetuses and ± SEM.

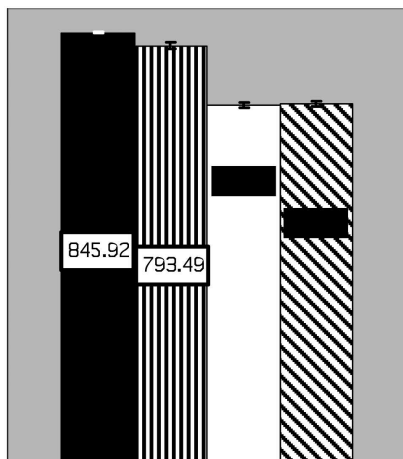


Fig. 6. Total number of glomeruli of kidneys in normal and anencephalic fetuses and ± SEM.

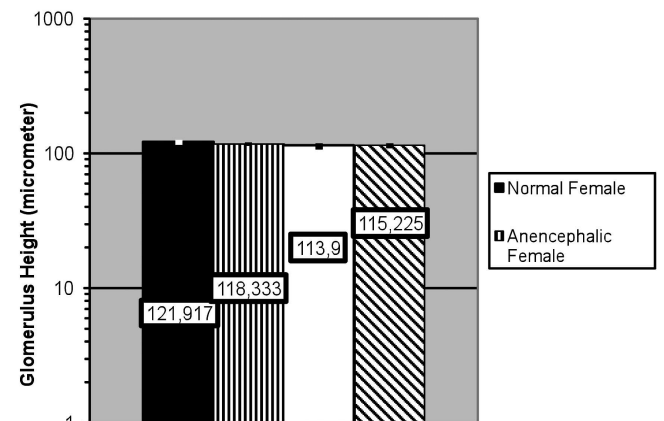


Fig. 7. Mean glomerulus height of kidneys in normal and anencephalic fetuses and ± SEM.

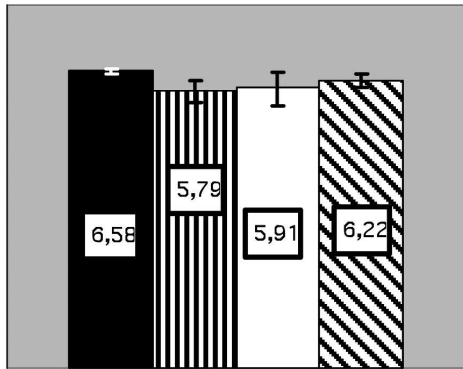


Fig. 8. Kidney volume of normal and anencephalic fetuses and \pm SEM.

separated from other structures of the glomerulus (Fig. 9B, C, D, B1, C1, and D1 and Fig. 10B, C, D, B1, C1, and D1).

Table 5. Kidney volume of normal and anencephalic fetuses.

Groups	Mean Kidney Volume (cm ³)	Standart Error Mean
Normal Female	6.576	0.09
Anencephalic Female	5.791	0.42
Normal Male	5.907	0.63
Anencephalic Male	6.219	0.26

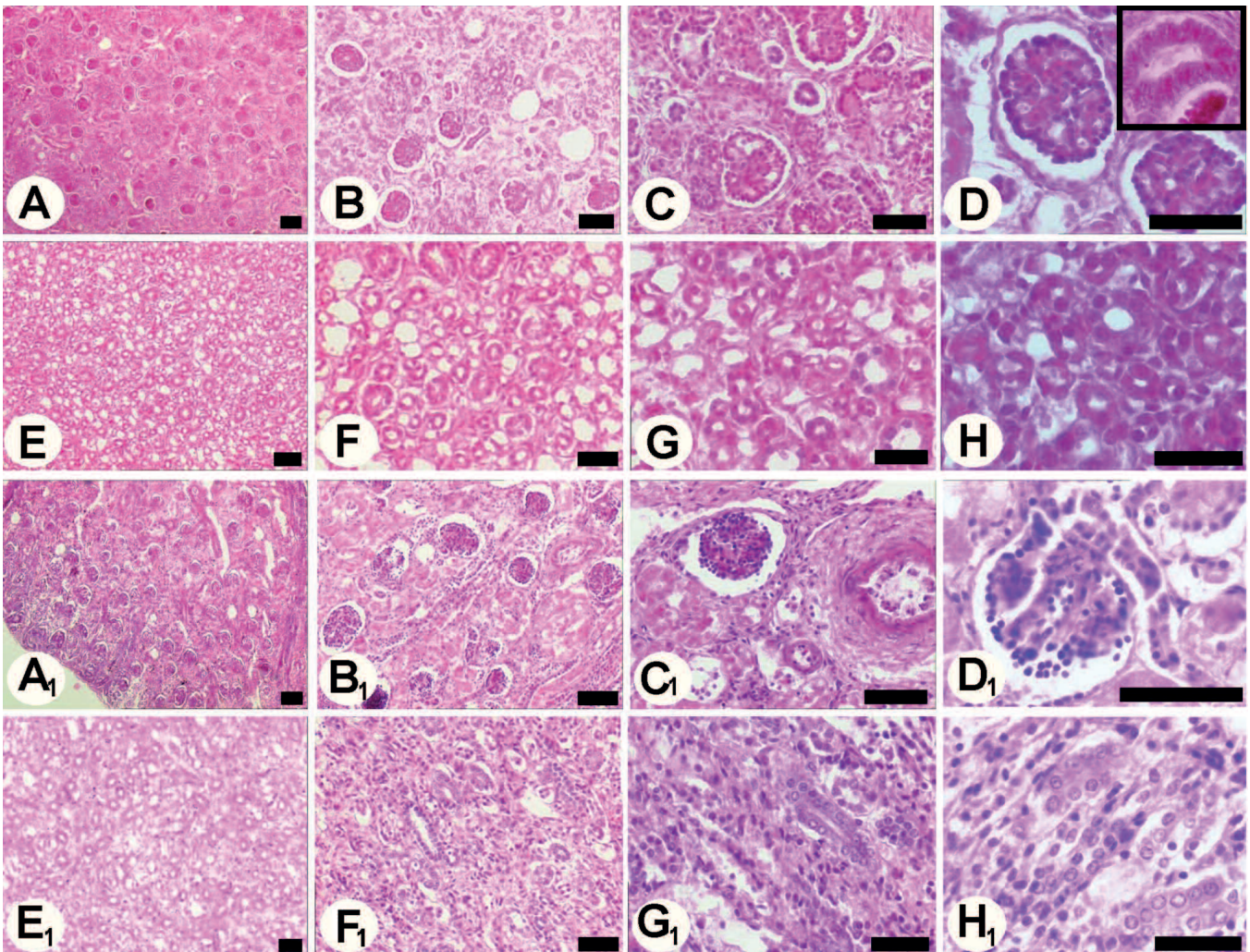


Fig. 9. Light micrographs of kidneys from normal male and anencephalic male fetuses. **A, B, C, D** and **E, F, G, H** were obtained from the cortex and medulla of the kidneys of normal fetuses, respectively. **A1, B1, C1, D1** and **E1, F1, G1, H1** were obtained from the cortex and medulla of the kidneys of anencephalic fetuses. Dye: H.E.; Bars: 120 μ m.

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Discussion

Due to the short survival period, anencephalic infants have been considered possible organ donors for other infants. If anencephalic infants receive intensive care, their solid organs usually would not undergo irreversible hypoxic injury during the process of dying, and become suitable for donation. Literature reports have documented successful kidney transplant cases where the donors are anencephalic neonates (Gutierrez-Carreno et al., 1989; Gomez-Campdera et al., 1990). Clinical experiences have also suggested that most organs from anencephalic donors have good function (Peabody et al., 1989). Furthermore, it has been reported that anencephalic donors are acceptable options for kidney transplantation (Gomez-Campdera et al., 1990). A Japanese case study has supported this idea, in which a kidney transplantation was performed from an

anencephalic newborn to a young girl. This report also evaluated the advantages and circumstances of anencephalic neonates, brain death criteria, and organ donation (Kimura, 1989). The results of our study hereby also indicate the validity of kidney transplantation from anencephalic neonates.

In humans, the urinary system consists of the kidneys, ureters, bladder, and urethra. The human body has two kidneys (one on either side of the middle back) that filter wastes from the blood and produce urine. The urinary system goes through three phases (pronephros, mesonephros, and metanephros) on its way to becoming fully functional kidneys. The last of these phases, the metanephros (adult kidney), begins as an aggregate of mesenchymal cells that are detectable by the fifth gestational week as two small areas in the intermediate mesoderm close to the pelvic aorta (Davies, 2002). It is approximately at this time that the nephric duct produces

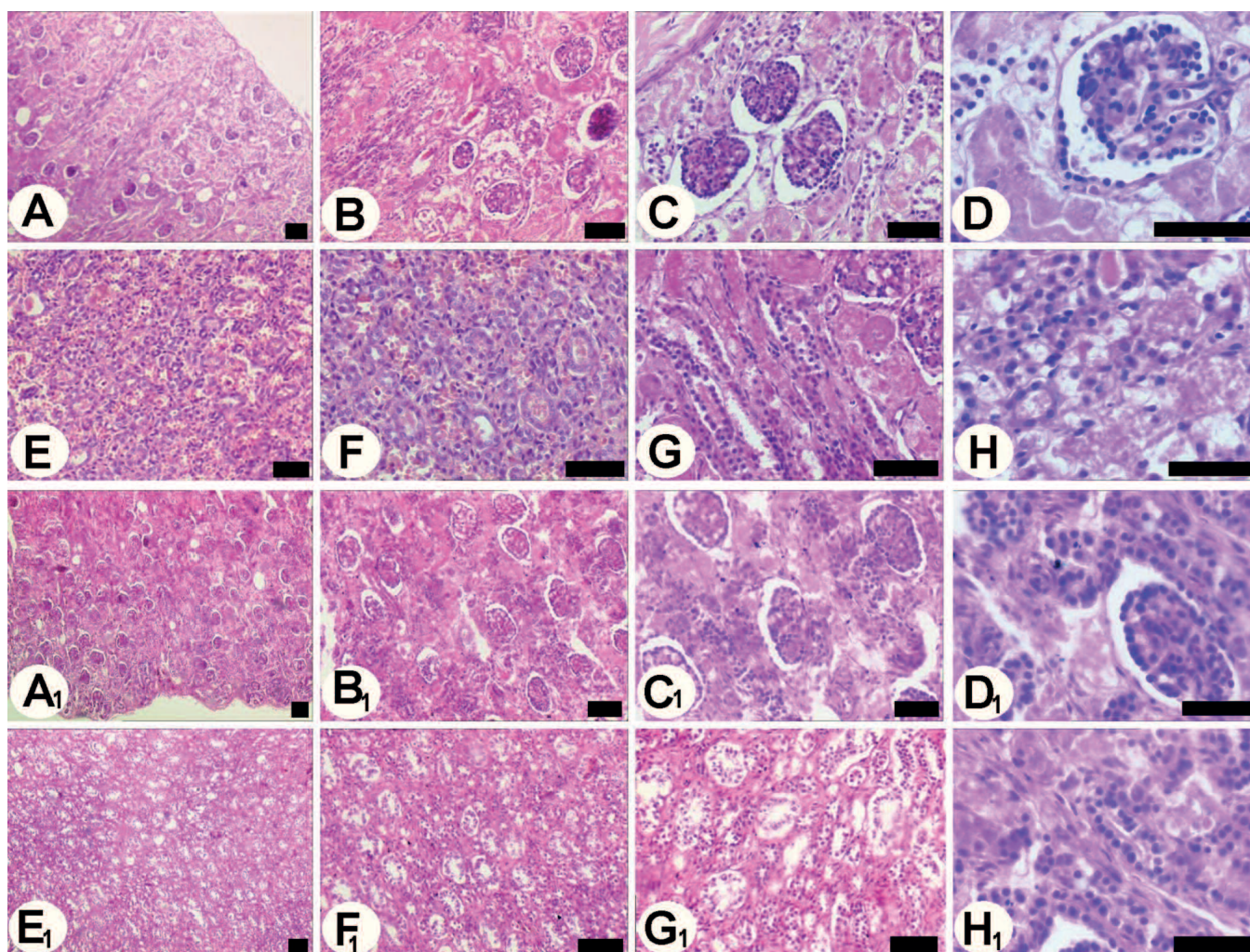


Fig. 10. Light micrographs of kidneys from normal female and anencephalic female fetuses. A, B, C, D and E, F, G, H were obtained from the cortex and medulla of the kidneys of normal fetuses, respectively. A₁, B₁, C₁, D₁ and E₁, F₁, G₁, H₁ were obtained from the cortex and medulla of the kidneys of anencephalic fetuses. Dye: H.E.; Bars: 120 μ m.

a finger-like projection called the ureteric bud that has, or will soon, invade the aggregate of cells known as the metanephric mesenchyme (Caruana et al., 2006). The ureteric bud of the nephric duct is stimulated by genetic signals emanating from the metanephric mesenchyme, and the ureteric bud brings with it new genetic signals that will help kidney formation (Nagata, 2006). When the ureteric bud has invaded the metanephric mesenchyme, it will stimulate certain cells within the metanephric mesenchyme to condense around the tips of the branches of the ureteric bud, and these cellular condensations will eventually form nephronic units, vascularized glomeruli (Potter and Osathanondh, 1966). All of the branches of the ureteric bud and the nephronic units have been formed by 32 to 36 weeks of gestation (Luzi et al., 1996). However, these structures are still immature, and will continue to grow after birth. Humans have been estimated to possess approximately one million mature nephronic units (approximately 500,000 per kidney) (Dakovic-Bjelakovic et al., 2005).

In this study, three basic research methods were performed. One is conventional anatomical measurements at the macroscopic level, the second one is the modern stereological method, and the last one is the histological examination at the light microscopic level.

Stereology is a group of advanced and unbiased techniques for collecting information on quantitative data. These techniques section the object of interest into a series of two-dimensional slices with known height and interval. They are sampled to produce estimates of required geometric or mathematical features, such as length, number, numerical density, surface area, and volume. Morphometrical properties are handled by probes that depend on the interested dimension. These probes are points for volume estimations; cycloids for the surface area and dissectors for numerical density and number estimations. For our stereological analysis, a point-counting grid and dissector were used.

Although there are many useful properties of stereology, its practice may sometimes be tedious, complicated, and confusing. Consequently, we used Stereo Investigator to prevent human error to get the most reliable results, quickly and easily.

According to our results, there was no statistical difference between the macroscopic measurements of both groups, regarding length, depth, weight, and fluid replacement volumes. When microscopic quantitative results were considered, no statistical difference was found among the kidney volumes obtained with the Cavalieri method. There were also no statistically significant differences in the number and height of the glomeruli, acquired by the physical dissector method.

In the histological evaluation at the light microscopic level, we did not find any abnormalities in the kidneys of the anencephalic fetuses. The kidneys were very similar to those of the cephalic fetuses.

We showed in this study that there was no significant difference between anencephalic and normal fetal kidneys in terms of anatomical, morphometrical, or

histological features. Thus, in our opinion, anencephalic infants can be used as organ donors for kidney transplantation.

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