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AN UROLOGICAL MODEL: VOLUME ESTIMATION OF THE BOVINE RENAL PARENCHYMA

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Abstract

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The bovine kidney has recently been used as a model for urological techniques in human health. The volume of the renal parenchyma (cortex-medulla) criteria is especially important in the morphological, developmental and toxicological experiments for the determination of the health status of the kidney. However, there are no recent, adequate morphological data about the bovine renal parenchyma. Because of this reason, the present study aimed to estimate the volume of the renal parenchyma by Cavalieri method in two bovine breeds. All lobes of the left kidneys of the Brown Swiss and Holstein bulls were evaluated individually. The mean volume of the cortex and medulla of the Brown Swiss and the Holstein bulls per lobe were estimated as 13.8 cm^3, 5.8 cm^3 and 15.4 cm^3, 7.2 cm^3, respectively. The weight, length, width, total volume and the volume of the medulla fraction between breeds was statistically significant ($p<0.05$). However, no statistical differences were observed at the volume of the cortex fraction and the cortex/medulla ratio ($p>0.05$). The volume fraction of the Brown Swiss bull left kidney was 70.41% cortex, 29.59% medulla, while the Holstein bull left kidney was 68.14% cortex, 31.86% medulla. The researchers believe that the findings may facilitate the future researches and will contribute to the existing literature.

Key words: Bovine, Cavalieri Method, Morphology, Renal Parenchyma, Stereology

Introduction

The bovine kidney is a multilobar kidney with lobated surface (Dyce et al., 1999; König and Liebich, 2004). In structure, the cortex and the medulla are of the multipyramidal type and have no *pelvis renalis*. A continuous cortex caps the separate medullar pyramids. The *apex* of the medulla named as *papilla renalis* and fits into *calices renales* of the *ureter* (Dyce et al., 1999; Fradson et al., 2003; Nav, 2012). In the kidney, the cortical and medullar regions could be distinguished in gross sections by the much lighter colour of the former (Dyce et al., 1999). In ruminants, the left kidney is mobile and rumen pushes it through the right half of the abdomen thus protecting it from pressure (König and Liebich, 2004).
Recently, it was proved that the bovine kidney can be used as a urologic model, especially on the laparoscopic partial nephrectomy (Carvalho et al., 2008; Harty et al., 2011; Moinzadeh et al., 2005a,b). But the hilar region in the bovine kidney limits its utility for other urologic procedures (Carvalho et al., 2008). Because the hilar region of the kidney contains very important structures like artery, vein, ureter and lymphatic vessel (Shalgum et al., 2012; Carvalho et al., 2008). However the human kidney is much smaller and its hilar region is much more narrow than the bovine kidney (Weld et al., 2005; Simmons et al., 2007; Carvalho et al., 2008). Therefore, the application to the bovine kidney in the urologic model does not satisfactory model except for the laparoscopic partial nephrectomy (Carvalho et al., 2008).

This could clearly say that bovine kidney would be especially useful as a laparoscopic partial nephrectomy model in the human kidney researches (Moinzadeh et al., 2005a, b). There are approximately 65.000 new cases of renal tumours diagnosed in 2012 (Siegel et al., 2012). The small and amenable kidney tumours extirpated by partial nephrectomy (Moinzadeh et al., 2005a, b). The first challenge of the partial nephrectomy is haemostasis (Moinzadeh et al., 2005a, b). Af-

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ter clamping the renal vasculatures the warm ischemia time becomes essentially important for the organ and the surgeon, if this time is exceeded it may be harmful for the renal parenchyma (Moinzadeh et al., 2005a, b). Therefore, the scientists' trial new techniques instead of hilar clamping to avoid haemostasis (Moinzadeh et al., 2005a, b). These techniques were potassium-titanyl-phosphate laser laparoscopic partial nephrectomy and water jet assisted laparoscopic partial nephrectomy (Moinzadeh et al., 2005a, b). Both techniques also performed without hilar clamping (Moinzadeh et al., 2005a, b) and the preferred animal model of these studies was bovine (Moinzadeh et al., 2005a, b). After these applications, the kidney tissue was evaluated by retrograde pyelography, renal arteriography and histological analyses (Moinzadeh et al., 2005a, b). In addition to this analysis, the gross anatomical inspection of the volume of the renal parenchyma will be beneficial and contribute to obtaining accurate results.

The renal volume is clinically important, because the renal mass is directly related with the renal functional reserve (Coulam et al., 2002). The mass weight change and the inspection of morphological changes of the kidney is a sensitive indicator of chemically induced changes to organs and an especially important requirement in toxicological experiments (Michael et al., 2007; Bailey et al., 2004). Moreover, because renal diseases affect the size of the kidney, the volume comparison of the kidney components and thickness evaluation of the fractions may produce useful data of hypertrophy, atrophy and tumour formation in the kidneys (Pazvant et al., 2009; Adibi et al., 2008; Coulam et al., 2002). Thus, total kidney volume and kidney fraction estimations have been suggested as an index for studying the health status of the kidney (Adibi et al., 2008).

The volume of the renal parenchyma could accurately estimate *in vitro* by the Cavalieri method (Bolat et al., 2011; Gundersen et al., 1988; Nyengaard, 1999; Pazvant et al., 2009; Pereira-Sampaio et al., 2008); unbiased systematic sampling using the Cavalieri method provides accurate, unbiased, efficient, cheap, and simple quantitative estimates of the objects (Agashiwala et al., 2008; Akosman et al., 2011; Gundersen et al., 1988). According to Cavalieri method, the volume of any object (V) may be estimated from parallel sections separated by a known distance (t), by summing up the areas of all sections of the object and multiplying the distance (t) (Gundersen et al., 1988). The transparent test probe superposed could estimate the areas of the sections. This probe consists of the regularly spaced points in a known distance. For estimates of the areal fraction of profile, count all points that hit the profile (Gundersen et al., 1988). This method was once applied successfully on the sheep, rabbit, pig and horse kidneys because it reached the volume fractions of the kidneys (Bahar et al., 2011; Bolat et al., 2011; Pazvant et al., 2009; Pereira-Sampaio et al., 2008).

There has been great progress in the science of medicine recently and because of this progress, the area needs more morphological studies on the bovine kidney (Carvalho et al., 2008). According to our investigations, no detailed morphological research on the bovine renal parenchyma (cortex and medulla) was found. In this frame, we decided to estimate the volume of the renal parenchyma of the bovine kidney. This study evaluated the Holstein and Brown Swiss breeds' volume of the kidney parenchyma. In Turkey, the Holstein cattle are raised especially for their milk yield and the Brown Swiss breed are raised for their combined milk and beef features. The Brown Swiss breed's adaptation to the hard conditions is much better than the Holstein cattle. The males of each breed are raised for the brood stock and beef (Alpan and Arpacik, 1998). Researchers believe that the findings will be useful for the future scientific researches.

Materials and Methods

Animals

This study was performed on five Holstein and five Brown Swiss bulls' (2 – 2.5 years old, 550-700 kg) left kidneys. All kidneys were removed immediately from their carcass after slaughter in the abattoir.

Tissue Preparation and Sampling Protocol

Firstly, the adipose tissue was removed and all left kidneys were weighted (Kern, Balingen-Germany). The left kidney length was measured between the cranial and caudal polar region and the width was measured between the hilar region and the opposite side by vernier caliper (Labomar, 304B-01- Turkey). After these measurements, through the renal artery neutral buffered 10% formalin administered and the kidney perforated by an insulin needle for the immersion fixation of the kidney and left in neutral buffered 10% formalin for one week.

Volume Estimation

Surface fissures into many lobes divide the bovine kidneys. In this study, in the guidance of these fissures, the kidneys were dissected and all lobes were obtained per kidney. All lobes per kidney individually sliced into 0.3 cm intervals (Figure 1) and the area on the slices was measured by 0.25 cm transparent point grid (Figure 2).

The volume of the renal parenchyma of the kidney was calculated by the formula below:

$V = (t \cdot a/p \cdot \sum P)$ cm^3

t : section thickness (0.3 cm),

a/p : the area represented by a point in the grid (0.25 cm x 0.25 cm),

$\sum P$: total number of points hitting the surface area of sections.

The coefficient of error (CE) of the study was calculated according to Nyengaard (1999).

Statistics

All estimations between the breeds were compared using Mann-Whitney U test.

Results

The mean kidney weight of the Brown Swiss bulls and the Holstein bulls were found as 442±34 g and 618±55 g, respectively. The weight of the kidney between the breeds was statistically significance ($P<0.05$). The mean length of the kidney of the Brown Swiss bulls was 16.8±1.5 cm and the mean width was 7.2±0.4 cm. The mean length of the kidney of the Holstein bulls was 18.4±0.8 cm and the mean width was 8.6±0.5 cm (Table 1). Significant statistical differences in the length and width were observed ($P<0.05$).

Stereological Estimations

The volume of the renal parenchyma of the kidney was estimated by Cavalieri method. All lobes were individually evaluated per kidney (Table 2). The mean cortex-medulla volume fraction per kidney was recorded in Table 2. The mean volume of the cortex, medulla and the cortex/medulla ratio per lobe for Brown Swiss and Holstein bulls were 13.8 cm^3, 5.8 cm^3, 2.4 and 15.4 cm^3, 7.2 cm^3, 2.2, respectively. The mean volume of the medulla fraction between the breeds was found statistically significant ($P<0.05$). However, the mean volume of the cortex fraction and the cortex/medulla ratio between the breeds were not found statistically significant ($P>0.05$). The mean volume fraction percentage share of the cortex and medulla in a left kidney of the Brown Swiss and Holstein Bulls were 70.41%, 29.59% and 68.14%, 31.86%, respectively.

Coefficient error of the study

The CE of the cortex, medulla was calculated as 0.02, 0.03 for the Brown Swiss and 0.03, 0.03 for the Holstein bulls, respectively.

Discussion

The importance of the renal parenchymal volume has been recognized over the years. The renal parenchymal researches have concentrated on morphometrical, developmental ana-

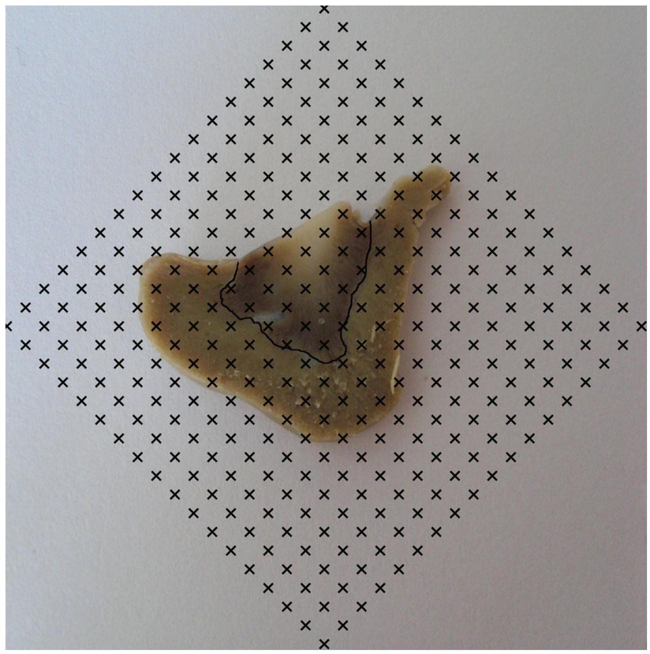

Fig. 2. The point grid was used to estimate the area of the fractions of the kidney

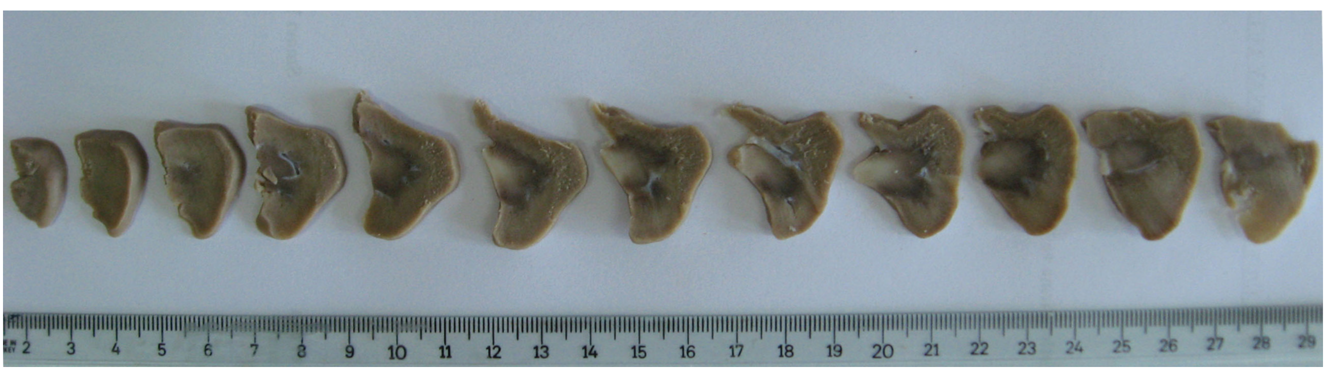

Fig. 1. The sliced one lobe of the kidney

Table 1
The weight, length and width of the kidneys

Brown Swiss	Weigth	Length	Width	Holstein	Weigth	Length	Width
1	414 g	16.2 cm	7.0 cm	1	546 g	17.4 cm	8.0 cm
2	486 g	18.7 cm	7.8 cm	2	678 g	19.2 cm	8.6 cm
3	410 g	14.7 cm	7.0 cm	3	635 g	18.6 cm	9.2 cm
4	432 g	17.3 cm	7.0 cm	4	577 g	17.8 cm	8.5 cm
5	468 g	17.0 cm	7.3 cm	5	652 g	19.0 cm	8.9 cm
Mean	442 g	16.8 cm	7.2 cm	Mean	618 g	18.4 cm	8.6 cm

Table 2
The mean cortex and medulla volume fractions per kidney lobe of the Bovines and cortex-medulla ratios

Brown Swiss	Cortex	Medulla	C/M Ratio	Holstein	Cortex	Medulla	C/M Ratio
1	16.1 cm^3	6.4 cm^3	2.5	1	18.0 cm^3	6.6 cm^3	2.7
2	13.1 cm^3	5.7 cm^3	2.3	2	13.7 cm^3	8.6 cm^3	1.6
3	12.0 cm^3	5.0 cm^3	2.4	3	13.5 cm^3	6.9 cm^3	2.0
4	14.2 cm^3	5.9 cm^3	2.4	4	18.1 cm^3	6.9 cm^3	2.6
5	13.7 cm^3	5.9 cm^3	2.3	5	13.8 cm^3	7.1 cm^3	1.9
Mean	13.8 cm^3	5.8 cm^3	2.4	Mean	15.4 cm^3	7.2 cm^3	2.2

tomical and also experimental researches. If we investigate the results of the morphometrical researches also included the present research, it was found in this study that the mean volume fraction of the cortex and medulla per lobe in the left kidney of the Brown Swiss and Holstein bulls were 70.41%, 29.59% and 68.14%, 31.86%, respectively. It has been reported by the researchers that the kidney of the rams consisted of 70.2% cortex, 26.4% medulla, and 3.4% pelvis (Pazvant et al., 2009), the horses' left kidney consisted of 57% cortex, 41% medulla and 2% pelvis (Bahar et al., 2011), rabbits' left kidney consisted of 59.7% cortex, 36.4% medulla, 3.8% pelvis (Bolat et al., 2011), and the renal cortex and medulla fraction in pigs was estimated at 83.79% and 16.21-12% (Coulam et al., 2002; Pereira-Sampaio et al., 2008). Beside those, when human renal parenchyma was compared with the bovine renal parenchyma, a similarity is observed in the results, because the cortico-medullar fraction of the human is around 70%-30% and in the bovine it is same (Coulam et al., 2002). From the developmental anatomical aspect, the renal cortex medulla volume ratios was estimated on the prenatal and postnatal rats and the volume fraction of the cortex in fetal kidneys (86%) was higher than in newborn (54%) or adult rats (77%) (Simsek et al., 2009). Beside, the similar research on the volume of the renal cortex medulla has been performed by Vlajković and colleagues (Vlajković et al., 2005). Moreover, the experimental researches on the volume of the renal cortex and medulla has focused e.g., the diabetic kidney induced by different doses of streptozotocin (Heidari et al., 2003), the comparison of the MRI and morphometric kidney measurements in diabetic and non-diabetic rats to determine the applicability of the MRI (Christiansen et al., 1997), and the effects of high a fat diet on the rat renal structure (Altunkaynak et al., 2008).

In this study, the length and the width were also measured on both breeds' left kidney. The mean kidney length and width of the Holstein bulls and the male mixed-breed cattle was around 18.4 cm-8.6 cm and 18.5 cm-8.6 cm respectively, and these values were in accordance with Holstein bull's values but not with Brown Swiss bulls (Carvalho et al., 2008). In another study, the reported length of the left kidney of the male buffalos was 18.6 cm. This finding when compared with Holstein bulls showed that the lengths are close but that the buffalo kidneys' width was around 12.5 cm; this value was so wide that it did not correspond with the Holstein and Brown-Swiss bulls. Moreover, the mean weight of the buffalo kidneys were 937 g and heavier than reported weights in this research (Hussain et al., 2006).

The scientists believed that the bovine kidney modelling is more appropriate instead of the other animals such as cats, dogs and pigs (Moinzadeh et al., 2005a, b). Actually, the dimensions of the bovine kidney is greater than that of the human kidney where the length is 11.25 cm and the width is 6 cm wide (Graaff, 2001). However, from the laparoscopic aspect the bovine model is more representative of the human kidney: in pulsatile fashion, the bovine kidney bleeds more profusely, like human kidneys. Its vascularity, laparoscopic technical difficulty and the potential for hemorrhagic complications are similar to the human kidney (Moinzadeh et al., 2005a, b).

The partial nephrectomy may affect the renal parenchyma (Moinzadeh et al., 2005a, b). The size of the renal parenchyma and the total and the cortical volumes of the kidney are important factors in creatinine clearance and indicate the potential functional capacity of the kidney (Pereira-Sampaio et al., 2008). The volume fraction parameters of the kidney are generally used in biomedical researches (Bolat et al., 2011; Heidari et al., 2002; Ozden et al., 2007; Altunkaynak et al., 2008; Simsek et al., 2009). It is used to express the proportion of a phase or component within the whole structure and does not require standardizing the body weight or height of the subject in the groups. Besides this, approaching to the volume fraction is a big advantage than the total volume (Pazvant et al., 2009).

The volume of the renal parenchyma could be estimated by the Cavalieri method *in vitro.* The method works simply and produces efficient and current results (Pazvant et al., 2009; Gundersen et al., 1988). *In vitro* total volume estimation of the kidney could also perform by Archimedean principle (Pazvant et al., 2009). However, this method may produce erroneous results especially on the small and hollow organs and does not allow to estimating the volume of the renal parenchyma (Akosman and Ozdemir, 2010; Gouletsou et al., 2008). MRI (Coulam et al., 2002) could achieve the volume estimation of the kidneys *in vivo*. The human medullary fraction of the kidney is obtained from the MRI measurements (Coulam et al., 2002). It has been reported that the MRI measurements of the total renal volume is accurate, however the fractional measurements are limited but promised (Coulam et al., 2002). Today an MRI allows estimating the glomerular number and size in the whole kidney. Variations in the number and size of the glomeruli are also the indicator of many renal and systemic diseases (Beeman et al., 2011).

The present studies CE values were found so appropriate. Generally, less than 5% CE is an acceptable value for a study and shows the quality of the sampling procedure (Bolat et al., 2011; Pazvant et al., 2009). The obtained lower CE level achieved via the kidney lobes were sliced into very thin slices to increase the number of sections and decrease the distances between two points in the grid for counting a large amount of points per kidney lobe. After all, the obtained results in this study are believed to facilitate the future anatomopathological, surgical and physiological researches. The data will also contribute to the researchers who are interested in experimental urologic models.

Conclusions

The importance of the volume of the renal parenchyma; cortex and medulla, is recognized. In addition, the renal modelling is especially important on the trial treatments of the renal tumours. The surgical treatments affect the renal parenchyma and the volume of the renal parenchyma is an important criteria determination of the health status of the kidney. The volume of the renal parenchyma should be considered in the evaluation of the results of the laparoscopic partial nephrectomy.

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