Jurnal Veteriner
pISSN: 1411-8327; eISSN: 2477-5665
Terakreditasi Nasional, Dirjen Penguatan Riset dan Pengembangan, Kemenristek Dikti RI S.K. No. 36a/E/KPT/2016

Maret 2020 Vol. 21 No. 1 : 124-135
DOI: 10.19087/jveteriner.2020.21.1.124
online pada http://ojs.unud.ac.id/index.php/jvet

The Differences of Ultrasonography Imaging Between Green Turtles (*Chelonia mydas*) and Olive Ridley Turtles (*Lepidochelys olivacea*) in Bali

(*PERBEDAAN CITRA ULTRASONOGRAFI ANTARA PENYU HIJAU (CHELONIA MYDAS) DAN PENYU LEKANG (LEPIDOCHELYS OLIVACEA) DI BALI*)

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ABSTRACT

Ultrasonography is a rapid and non-invasive method for assessing internal organs. The purpose of this study is to determine the differences in ultrasonographic imaging of internal organs between green turtle (*Chelonia mydas*) and olive ridley turtles (*Lepidochelys olivacea*). This study used two female green turtles (*C. mydas*) and two olive ridley turtle (one male and one female) (*L. olivacea*) with body condition index ranging from Average-Very Good. Micro-convex transducer ultrasonography with frequencies 4.5-8 MHz was used in this study. Acoustic windows were used in this study from cervical dorsal, cervical ventral, cervicobrachial, sinister-dexter, axillary sinister-dexter, prephemoral sinister-dexter and postphemoral sinister-dexter. The necropsies were performed to provide reference data. The results of ultrasonography imaging showed that the jugular vein of the olive ridley turtles (*L. olivacea*) was wider than the jugular vein of the green turtles (*C. mydas*). The ultrasonography imaging also showed that the stomach of green turtles (*C. mydas*) was smooth folds but the stomach of olive ridley turtles (*L.olivacea*) was sharp folds according to necropsy. There were no differences in the heart, stomach, liver, small intestine, large intestine, and kidneys. Vitellogenic follicles ultrasonography imaging was found from the olive ridley turtles (*L. olivacea*) which had shown in the period of premating and mating.

Keywords: *Chelonia mydas*; *Lepidochelys olivacea*; internal organs; ultrasonography.

ABSTRAK

Ultrasonografi adalah metode yang cepat dan tidak invasif untuk menilai organ internal. Penelitian ini bertujuan untuk mengetahui perbedaan pencitraan ultrasonografi organ internal penyu hijau (*Chelonia mydas)* dan penyu lekang (*Lepidochelys* olivacea). Penelitian ini menggunakan dua ekor penyu hijau (*C. mydas*) dan dua ekor penyu lekang (*L. olivacea*) dengan indeks kondisi tubuh dari Sedang-Sangat Baik. Jendela akustik yang digunakan yaitu *cervical dorsal*, *cervical ventral*, *cervicobrachial sinister-dexter*, aksilaris *sinister dexter prefemoral sinister-dexter*, dan *postfemoral sinister-dexter*. Hasil nekropsi ditampilkan untuk melengkapi data referensi. Hasilnya menunjukkan bahwa citra ultrasonografi vena jugularis pada penyu lekang (*L. olivacea*) memiliki ukuran lebih lebar daripada vena jugularis pada penyu hijau (*C. mydas*). Citra ultrasonografi pada lambung juga menunjukkan bahwa penyu hijau (*C. mydas*) memiliki lipatan lebih halus daripada penyu lekang (*L. olivacea*) yang memiliki lipatan yang lebih tajam sesuai dengan nekropsi. Tidak terdapat perbedaan pada organ jantung, lambung, hati, usus halus, usus besar, dan ginjal. Ditemukan juga citra ultrasonografi folikel vitellogenik pada penyu lekang (*L. olivacea*) yang menunjukkan dalam periode sebelum kawin dan kawin.

Kata-kata kunci: *Chelonia mydas*; *Lepidochelys olivacea*; organ intenal; ultrasonografi.

INTRODUCTION

Indonesia's ocean is the habitat of six out of seven species of sea turtles in the world. Those species are green turtles (*Chelonia mydas*), olive ridley turtles (*Lepidochelys olivacea*), loggerhead turtles (*Caretta caretta*), hawksbill turtles (*Eretmochelys imbricata*), leatherback turtles (*Dermochelys coriacea*) and flatback turtles (*Natator depressus*) (Karnan, 2008).

Green turtles (*C. mydas*) are the most commonly found sea turtles in Indonesia, however olive ridley turtles (*L. olivacea*) are widely distributed in the Arafuru Sea. According to IUCN (2004), green turtles are listed as endangered animals (EN A2bd), due to the high level of human consumption, especially in Bali. However, according to IUCN (2008), olive ridley turtles are listed as vulnerable animals (VU A2bd) after being listed as endangered animals until 1996. The main purpose of sea turtle conservation is to increase the population of sea turtle from being exposed to eat fish hooks, get trapped in fishing nets, get hit by boat propellers and eat trash which results in various wounds and diseases (Bugoni *et al*., 2001; Magalhães *et al*., 2012). To diagnosis of a disease in a sea turtle is difficult, because it is recently done only based on clinical examination. When the sea turtles get disease infection, they generally do not show clear clinical signs. This condition might be due to the presence of carapace and plastron which contribute to the difficulties to make the diagnosis. Physical examination is an example of health status requirement which requires complementary diagnostic procedures to provide useful information for clinical diagnosis (Penninck *et al*., 1991; Lieberman dan Rosskopf, 1984).

Ultrasonography (USG) is a rapid, non-invasive, and inexpensive method for assessing internal organs. Usage of ultrasonography can show some imaging of pathological features, for the example is calculi in the gallbladder (Majo *et al*., 2016). It is also valid for detecting the abnormalities of internal organs, therefore ultrasonography is a very useful tool for clinical diagnosis at sea turtle conservation. The imaging of ultrasonography in green turtles and olive turtles is important to be done in order to support in making the diagnosis of them in the Turtle Conservation and Education Center (TCEC). Turtle Conservation and Education Center is one of the conservation places of turtle stranded and sea turtle health examination. The study of USG is important to be done, because each species of sea turtle has differences anatomical related on ultrasonography imaging.

A study on ultrasonography imaging on green turtles and olive ridley turtles is needed for a basic reference for related knowledge and as a milestone for related further studies.

RESEARCH METHODS

This study used two female green turtles (*C. mydas*) and two olive ridley turtles (one male and one female) (*L. olivacea*), with a minimum straight carapace length (SCLmin) in green turtles 59 cm to 70 cm and olive ridley turtles 62 cm to 63.5 cm. This study has been approved ethically by Ethic commission of using animals for research and education, Faculty of Veterinary Medicine, Udayana University with the number of Ethical Clearence of 1593/UN 14.2.9/PD/2019). The green turtles from the confiscation of the Gianyar Police and olvie ridley turtles from the rescue of Kuta Beach because got caught by fishing nets. All sea turtles have been measured of their Body Condition Index (BCI) on green turtles with results in 1.02-1.23, while for the olive ridley turtles with results in 1.02-1.44. Based on the standard of BCI measurement, all of the sample sea turtles are categorized as Average-Very Good.

Ultrasonography examinations were performed with a real-time, B-Mode (Sogata 10) using micro-convex transducer with frequencies 4.5 MHz – 8 MHz. During the ultrasonography examinations at Turtle Conservation and Education Center (TCEC) Serangan Island, Denpasar, Bali, the turtles were restrained in ventral recumbence position and placed on a bucket and tire. No sedation was given. The head, neck or limbs were extended as needed and the body surface was kept wet using a sopping towel. Acoustic windows (Fig 1) were used in this study from cervical dorsal, cervical ventral, cervicobrachial, sinister-dexter, axillary sinister-dexter, prefemoral sinister-dexter and postfemoral sinister-dexter (Majo *et al.,* 2016*)*. The results of ultrasonography imaging were saved on USB flash disk to be analyzed.

Anatomical sections used each green turtles and olive ridley turtles to be compared with ultrasonography imaging. Necropsies were done from deceased sea turtle. The necropsies were used for comparison of morphological data: *Anatomical terminology* by Wyneken (2001).

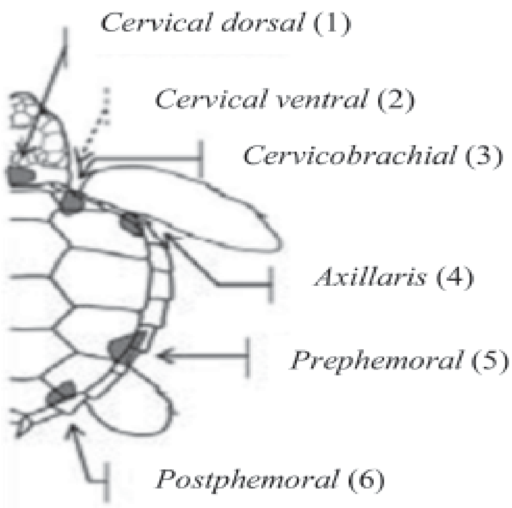
<table>
<tr><th>Acoustic windows</th><th>Echo access</th></tr>
<tr><td rowspan="6">
</td><td>1. The echoes pass obliquely cranial to the edge of the carapace (nuchal scute).</td></tr>
<tr><td>2. The echoes pass in parallel dorsally to the plastron, between the cranial edge (epiplastron bone), medial ends of coracoid bones and ventral face of the cervical vertebra.</td></tr>
<tr><td>3. The echoes pass between the coracoid bone and the acromion process of the scapula.</td></tr>
<tr><td>4. The echoes pass between the shoulder joint and the cranial border of the bridge joining the carapace of the plastron.</td></tr>
<tr><td>5. The echoes pass between the caudal border of the bridge joining the carapace and the plastron and cranial face of the femur.</td></tr>
<tr><td>6. The echoes are limited dorsally by the carapace and the proximal and femur.</td></tr>
</table>

Fig 1. Acoustic windows and echo accesses were used for ultrasound. (Valente *et al*., 2007)

RESULTS AND DISCUSSION

The results of ultrasonography imaging on green turtles (*C. mydas)* and olive ridley turtles (*L. olivacea*) were clearly seen in transverse and oblique on the cervical dorsal acoustic window showed cervical vertebrae was hyperechoic followed by acoustic shadowing due to strong reflection from the osseous, spinal cord was hyperechoic, vertebral vein, and jugular vein were hypoechoic (Fig 2). Vertebral vein and spinal cord can be used as a landmark to identify the left and right cervical dorsal sinuses. The left and right jugular vein was seen through the anastomosis with vertebral vein. The width of the jugular vein on olive ridley turtles was 5.36 cm and green turtles was 4.56 cm. It showed that the jugular vein on olive ridley turtles wider than green turtles. The jugular vein of *C. mydas* is relatively small in diameter and has fewer branches than the anatomy of other cheloniids (Wyneken, 2001).

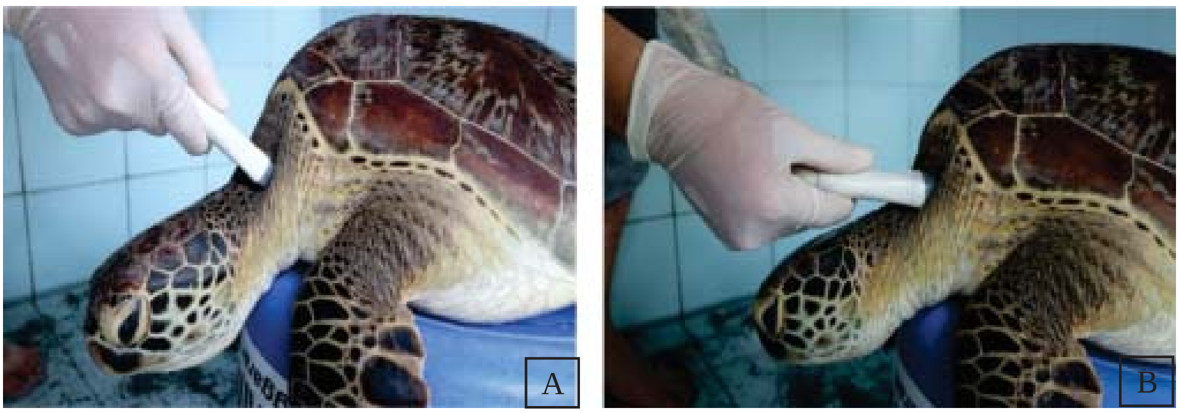


Fig 2A and 2B. Ultrasound evaluation of the cervical dorsal acoustic window (Fig 2A) Ultrasonography imaging was taken by Transverse 90°. (Fig 2B) Ultrasonogaphy iImaging was Taken by Trasnverse 45°.

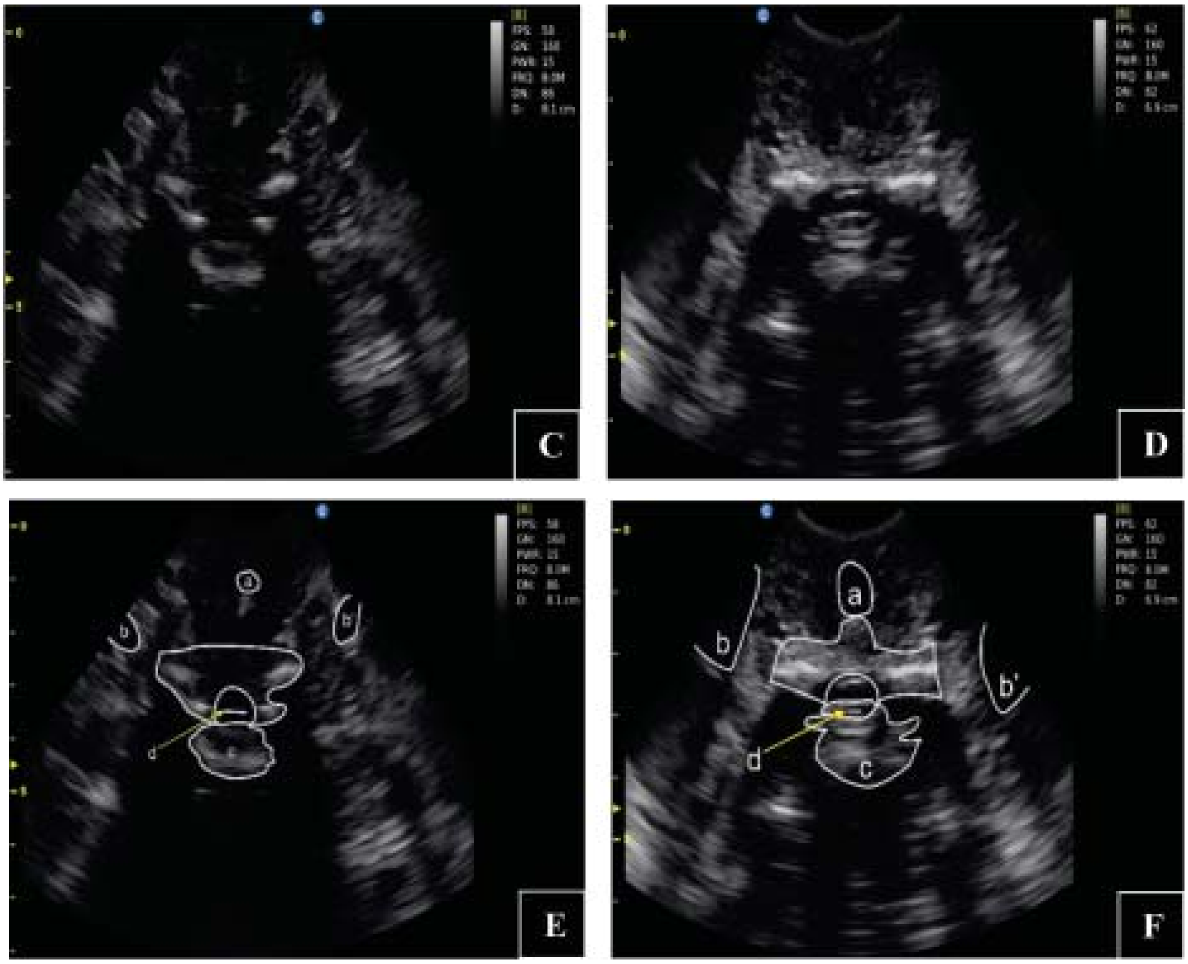


Fig 2C, 2D, 2E, and 2F. Ultrasound evaluation of the cervical dorsal acoustic window: a. Vertebral Vein, b-b'. Jugular Vein, c. Vertebra Cervical, and d. Spinal Cord. (Fig 2C and Fig 2E) Transverse ultrasonography imaging on green turtles. (Fig 2D and Fig 2F), Transverse ultrasonography imaging on olive ridley turtles.

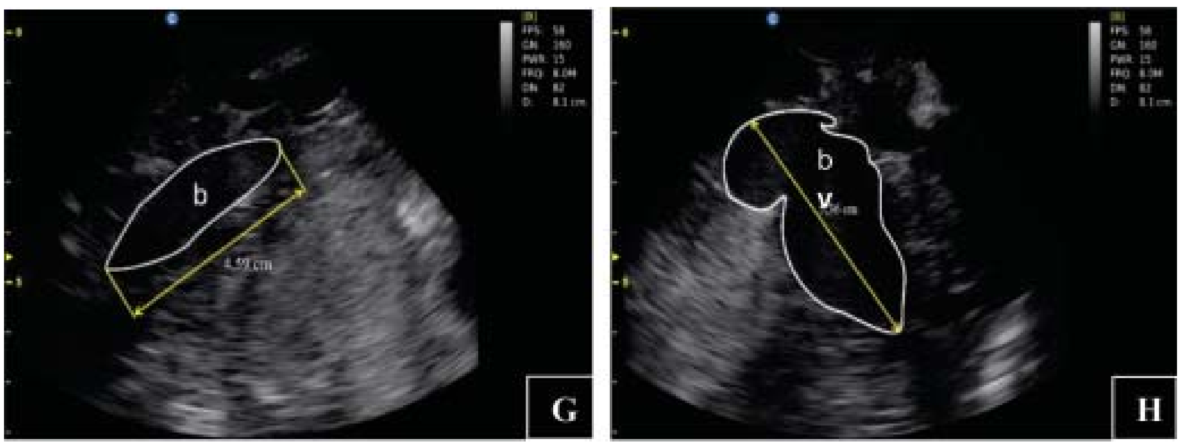


Fig 2G and 2H. Ultrasound evaluation of the cervical dorsal acoustic window: b. Jugular Vein. (Fig 2G) Oblique Ultrasonography Imaging on Green Turtles. (Fig 2H) Oblique Ultrasonography Imaging on Olive Ridley Turtles.

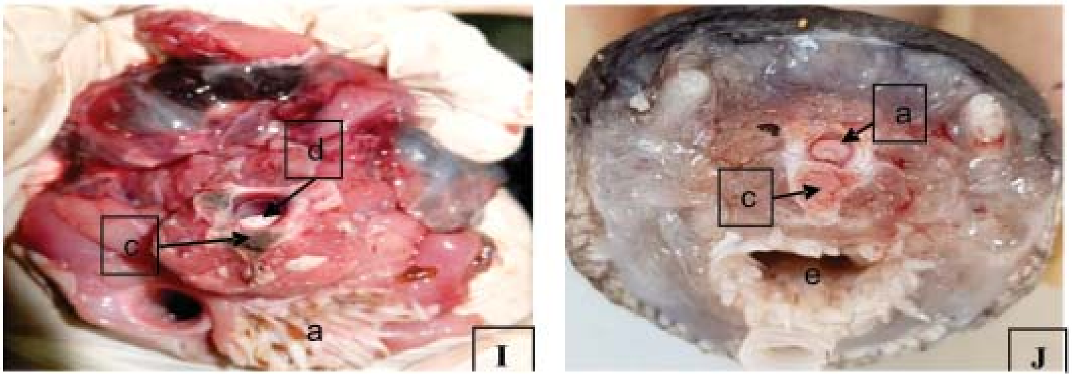


Fig 2I and J. Anatomical transverse cross section: c. Vertebra Cervical, d. Spinal Cord, and e. Oesophagus. (Fig 2I) Anatomical Transverse Cross Section on Green Turtles. (Fig 2J) Anatomical Transverse Cross Section on Olive Ridley Turtles.

Cardiovascular system on olive ridley turtles and green turtles can be seen in cervical ventral acoustic window. The heart of green turtles has a similar ultra-sonography imaging with olive ridley turtles. The atrial systole (Fig. 3C and E) was identified with hyperechoic wall, but when the ventricle systole (Fig 3B and D) showed hyperechoic wall. The heart of green turtles and olive ridley turtles corresponded to the anatomy of turtles by Wyneken (2001).

Fig 3A. Ultrasound evaluation of the cervical ventral acoustic window. (Fig 3A) Ultrasonography Imaging was Taken by Transverse.

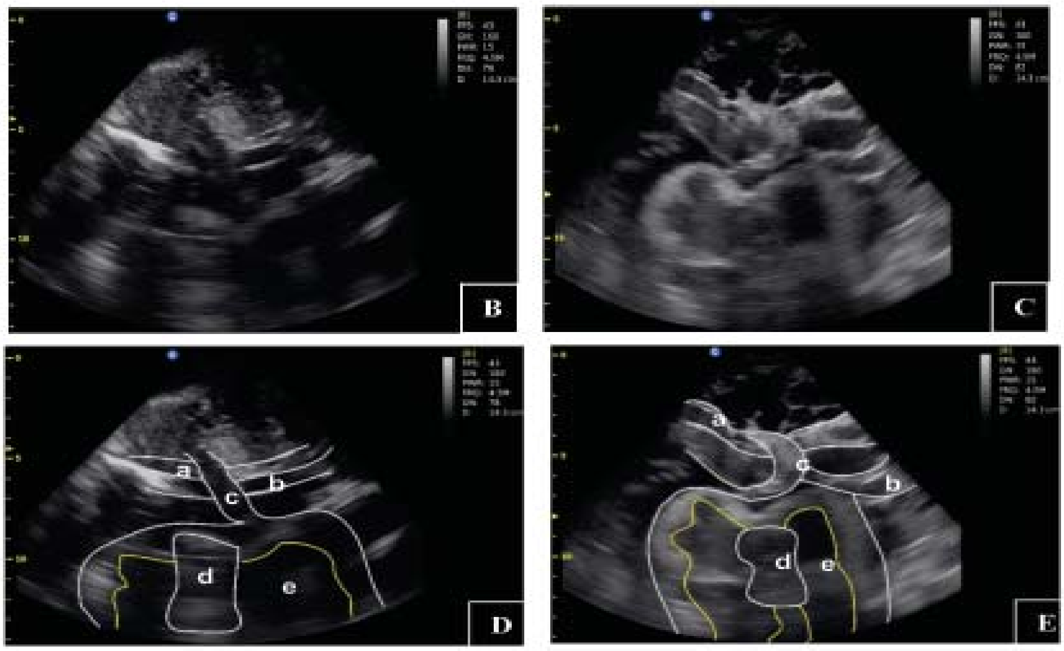


Fig 3B, 3C, 3D and 3E. Ultrasound evaluation of the cervical ventral acoustic window: a. Brachiocephalic Trunk, b. Pulmonary Trunk, c. Left Aorta, d. Ventricle, and e. Atrial. (Fig 3B and Fig 3D) Transverse Ultrasonography Imaging on Green Turtles. (Fig 3C and Fig 3E) Transverse Ultrasonography Imaging on Olive Ridley Turtles.

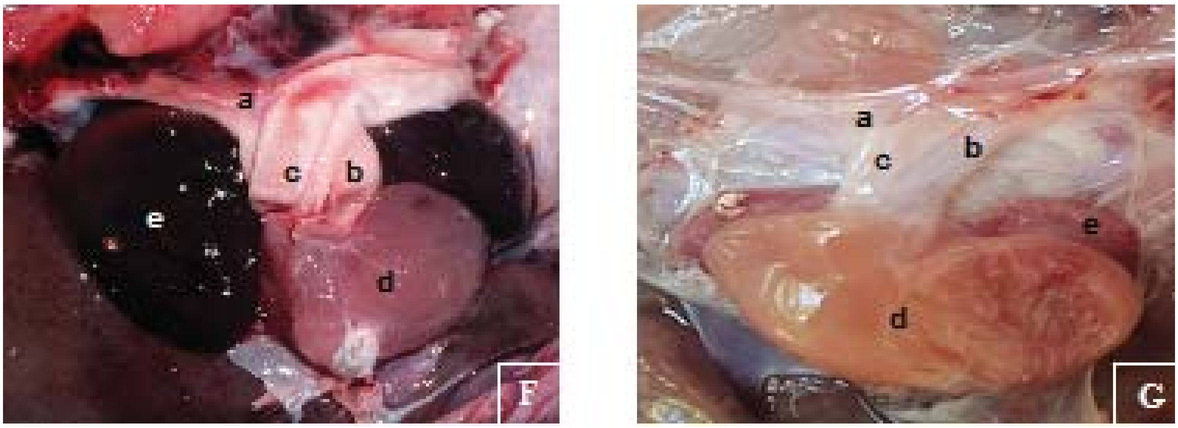


Fig 3F and 3G. Anatomical transverse cross section: a. Brachiocephalic Trunk Vertebral, b. Pulmonary Trunk, c. Left Aorta, d. Ventricle, and e. Atrial. (Fig 3F) Anatomical Trasnverse Cross Section on Green Turtles. (Fig 3G) Anatomical Transverse Cross Section on Olive Ridley Turtles.

The stomach of green turtles and olive ridley turtles was located on the left side. Although it could be seen in the left cervicobrachial, axillary and prefemoral acoustic window, it was more frequently found in the left axillary. The determination of the stomach could be seen on subscapular muscle which located dorsally from the stomach. The stomach showed a thin wall with hyperechoic wall, the lumen showed poor images due to the presence of intraluminal gas. The stomach of green turtles has smooth folds on fundus area. The parallel folds of green turtles begin weaving, branching and converging, and finally changing to the transverse folds. The stomach of olive ridley turtles (Fig. 4G) showed longitudinal folds, the pattern and distribution of the gastric folds are various depends on the species which may associate with the gastric dynamic and dietary of animals.

Fig 4A. Ultrasound Evaluation of the Left Axillary Acoustic Window. (Fig 4A) Ultrasonography Imaging was Taken by Transverse.

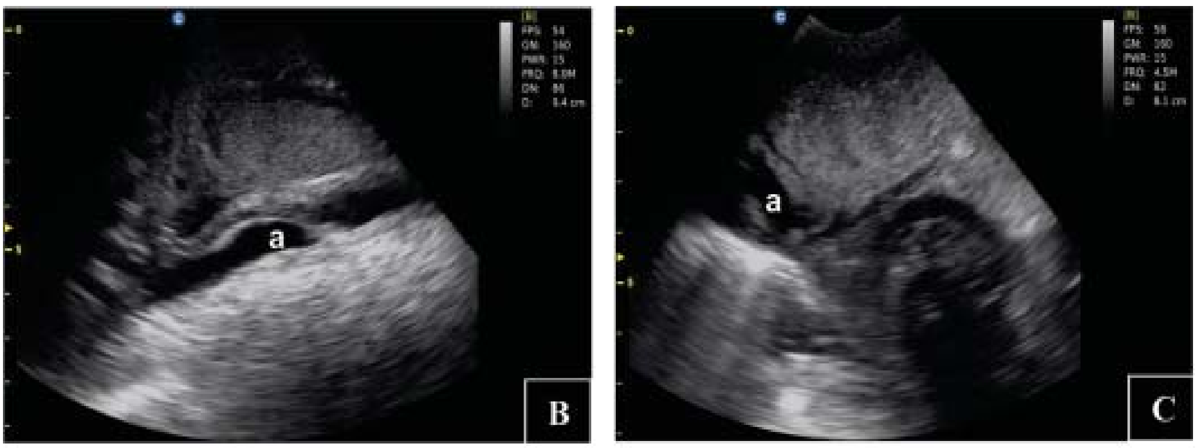


Fig 4B,4C, 4D, AND 4E. Ultrasound evaluation of the left axillary acoustic window: a. Lumen and b. Subscapular Muscle. (Fig 4B and 4D) Transverse Ultrasonography Imaging on Green Turtles. (Fig 4C and Fig 4E) Transverse Ultrasonography Imaging on Olive Ridley Turtles.

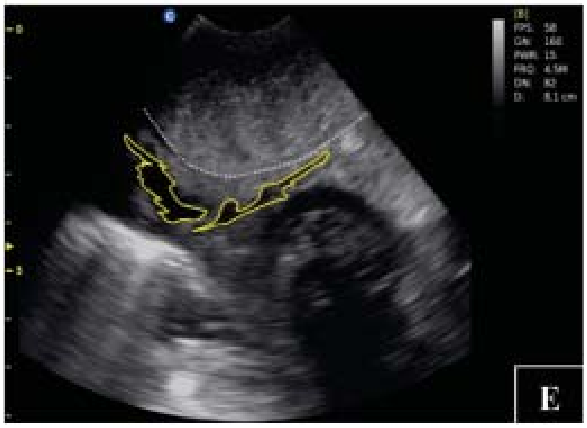


Fig 4B,4C, 4D, AND 4E. Ultrasound evaluation of the left axillary acoustic window: a. Lumen and b. Subscapular Muscle. (Fig 4B and 4D) Transverse Ultrasonography Imaging on Green Turtles. (Fig 4C and Fig 4E) Transverse Ultrasonography Imaging on Olive Ridley Turtles.

Fig 4F and 4G. Anatomical Transverse Cross Section Stomach on Green Turtles. (Fig 4G) Anatomical Transverse Cross Section Stomach on Olive Ridley Turtles

The liver of green turtles and olive ridley turtles was located in the right side of sea turtles internal body, it can be evaluated in the right cervicobrachial and axillary. The best position to see this organ is from the right axillary. The liver of green turtles and olive ridley turtles had no difference. The gallbladder showed an anechoic oval appearance, was surrounded by granular parenchyma with hypoechoic. The hepatic vein was seen anechoic on the lumen without the echogenic borders The portal vein was seen anechoic on the lumen with the echogenic borders. The liver of green turtles and olive ridley turtles corresponded to Wyneken (2001).

Fig 5A. Ultrasound evaluation of the right axillary acoustic window. (Fig 5A) Ultrasonography Imaging was Taken by Transverse

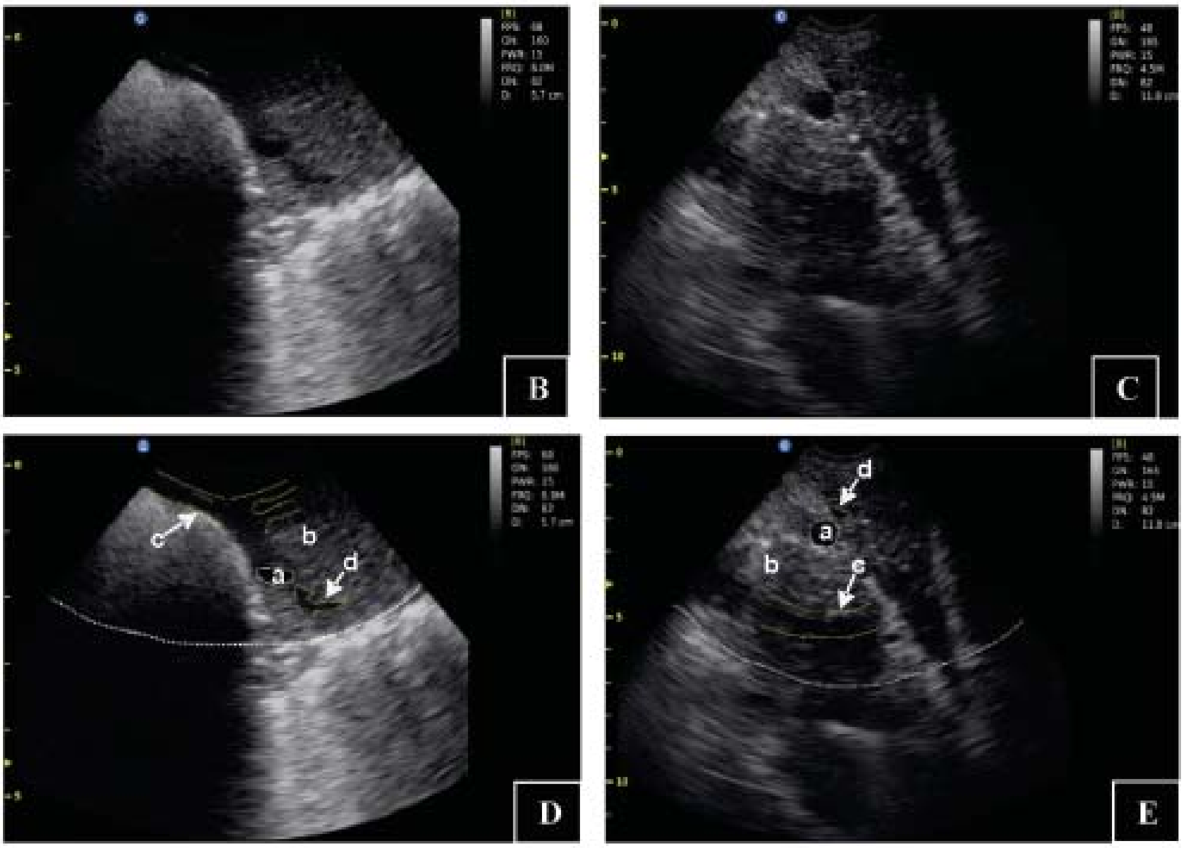


Fig 5B, 5C, 5D, and 5E. Ultrasound Evaluation of the Right Axillary Acoustic Window: a. Gallbladder, b. Granular Parenchyma, c. Portal Vein, and. d. Hepatic Vein. (Fig 5B and Fig 5D) Transverse Ultrasonography Imaging on Green Turtles (Fig 5C and Fig 5E) Transverse Ultrasonography Imaging on Olive Ridley Turtles.

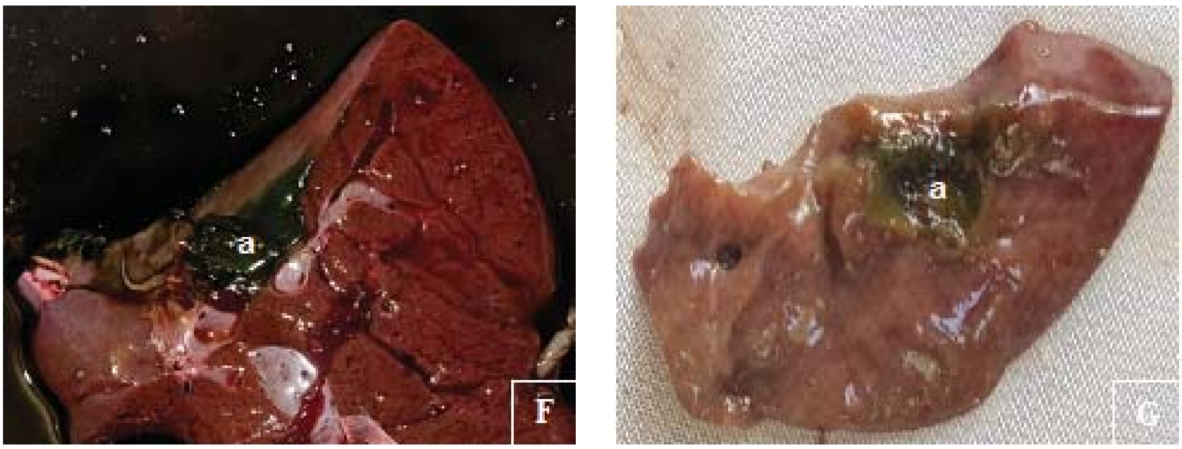


Fig 5F and 5G. Anatomical Transverse Cross Section Liver on Green Turtles. (Fig 5G) Anatomical Transverse Cross Section Liver on Olive Ridley Turtles.

The small and large intestine can be seen in the prefemoral acoustic window from both sides. The small intestine was more frequently seen on the right side. The ultrasonography imaging of the intestine was similar to mammals, and stratification in five layers could be recognized (Noviana *et al.,* 2018). In all the intestine of sea turtles species consisted of a long and convoluted tube with a constant diameter throughout its length. In *C. mydas*, *L. olivacea*, *C. caretta* and *E. imbricata* mucosa of the duodenum was characterized by the presence of reticular folds. The jejunum/ ileum region had rectilinear longitudinal folds (Magalhaes *et.al.,* 2012). The presence of folds considerably enlarges the surface of this organ, thus increasing the area available for absorbing nutrients. (Porter, 1972; Romes dan Parsons, 1985; Legler 1993; Wyneken 2001). In the large intestine of the species *C. mydas*, *L. olivacea*, *E. imbricata* and *C. caretta*, regions with flat mucosa were observed, probably due to the accumulation of fecal matter (Magalhaes *et.al.,* 2012).

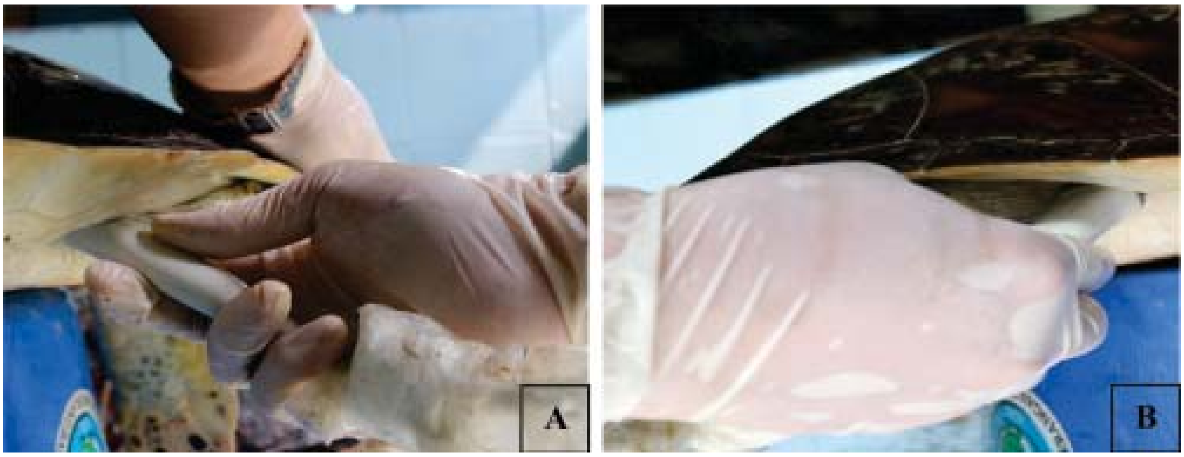


Fig 6A and 6B. Ultrasound Evaluation of the Prefemoral Acoustic Window. (Fig 6A) Ultrasonography Imaging of Left Prefemoral was Taken By Transverse (Fig 6B) Ultrasonography Imaging of Right Prefemoral was Taken By Transverse.

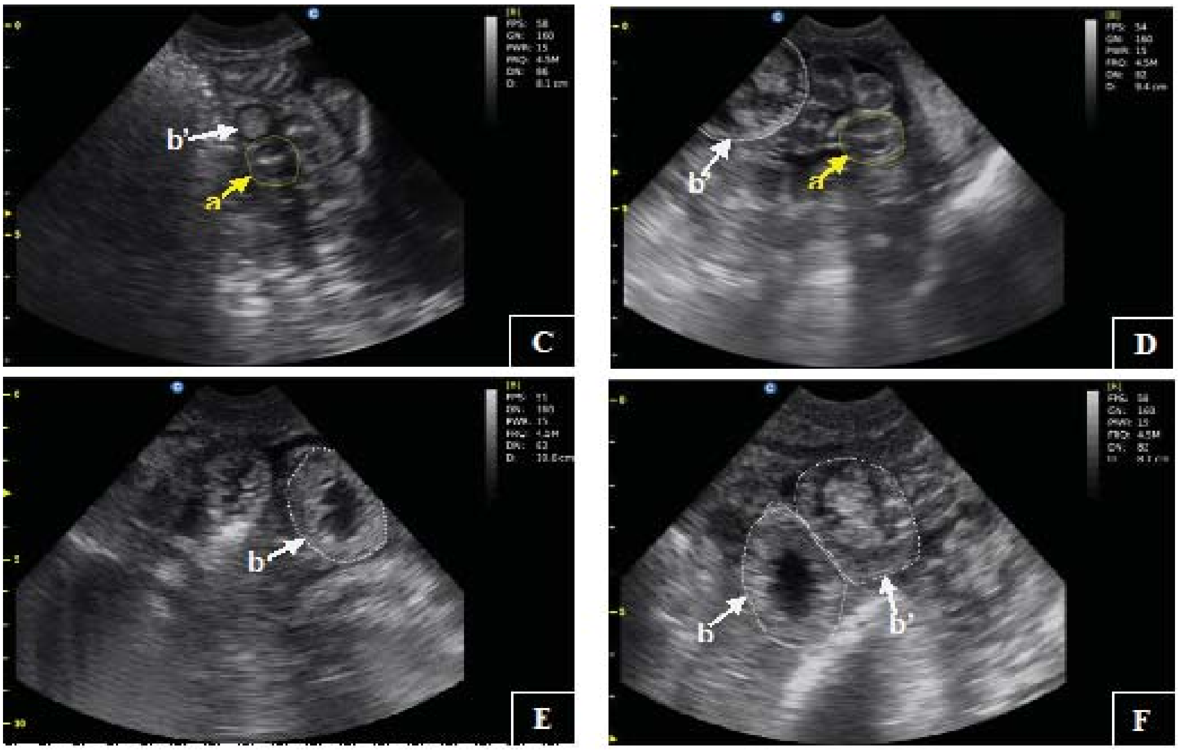


Fig 6C, 6D, 6E and 6F. Ultrasound evaluation of the prefemoral acoustic window: a. small intestine, b. Large Intestine, and b'. Large Intestine Containing Mass (Fig 6C and Fig 6E) Transverse Ultrasonography Imaging on Green Turtles. (Fig 6D and Fig 6F) Transverse Ultrasonography Imaging on Olive Ridley Turtles.

Vitelogenic follicle on female olive ridley turtles were seen in the left and right prefemoral acoustic window. The diameter of vitelogenic follicle are 2.44 cm; 3.14 cm; 3.30 cm; 3.80 cm; and 3.93 cm, and the echogenicity was hyperechoic. Vitelogenic follicle was made in the process of vitellogenesis, which consists of the enlargement of ovaries and follicles before reproduction, during which yolk proteins incorporate with the oocyte within the follicle (Rostal 2007). In the beginning stage (premating period), The ovary was filled with vitellogenic follicles (Blanco *et al.*, 2012). This follicle continues to increase in size during the period of premating and mating. As female sea turtles get closer to the nesting season, they become receptive to the mating advances of males. At this stage, the ovaries are completely developed (Rostal *et al.,* 1998) and the final stages of follicular maturation begin (Owens 1980).

Fig 7. Ultrasound Evaluation of Vitelogenic Follicle of the Prefemoral Acoustic Window.

The kidney was identified in the prefemoral acoustic window. The results of ultrasonography imaging showed that no difference between green turtles and olive ridley turtles. The kidney could be seen in the prefemoral and postfemoral acoustic window. The best access to see the kidney from the prefemoral acoustic window, due to strong reflection from coxae ossea made acoustic shadow in the postfemoral acoustic shadow. The kidney of green turtles and olive ridley turtles have no difference and showed hypoechoic. Based on FIG. 8E and 8F shows that the kidneys are paired, lobular, elliptical, pink or red structures according to Wyneken (2013).

Vesica urinaria (VU) could have seen anechoic on USG if it was full of urine. In this research, VU was not seen.

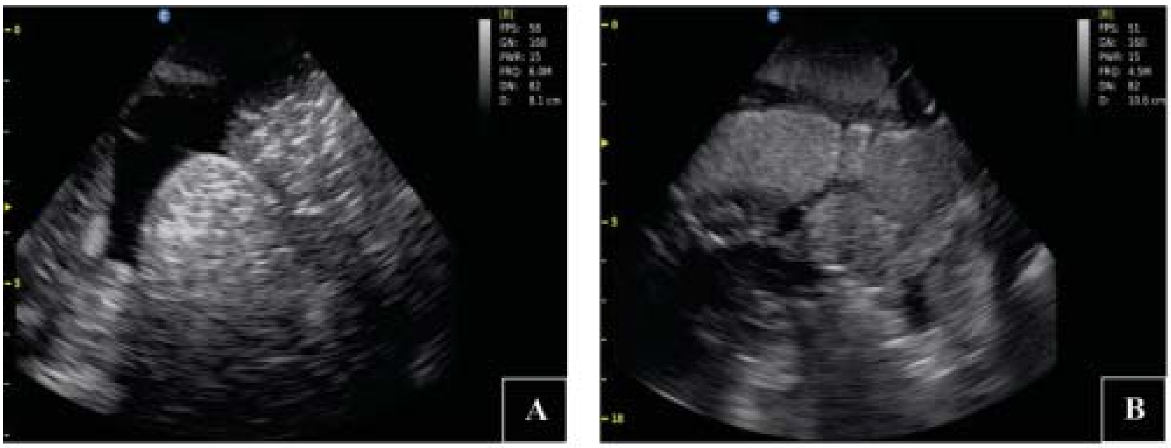


Fig 8A, 8B, 8C, and 8D. Ultrasound Evaluation of Kidney of the Prefemoral Acoustic Window. (Fig 8A and Fig 8C) Transverse Ultrasonography Imaging on Green Turtles. (Fig 8B and Fig 8D) Transverse Ultrasonography Imaging on Olive Ridley Turtles.

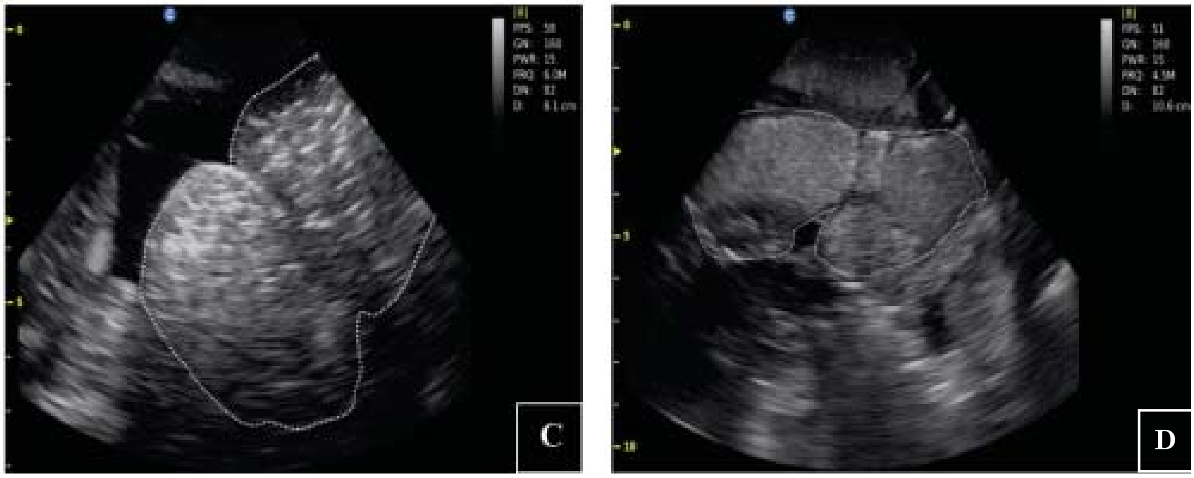


Fig 8A, 8B, 8C, and 8D. Ultrasound Evaluation of Kidney of the Prefemoral Acoustic Window. (Fig 8A and Fig 8C) Transverse Ultrasonography Imaging on Green Turtles. (Fig 8B and Fig 8D) Transverse Ultrasonography Imaging on Olive Ridley Turtles.

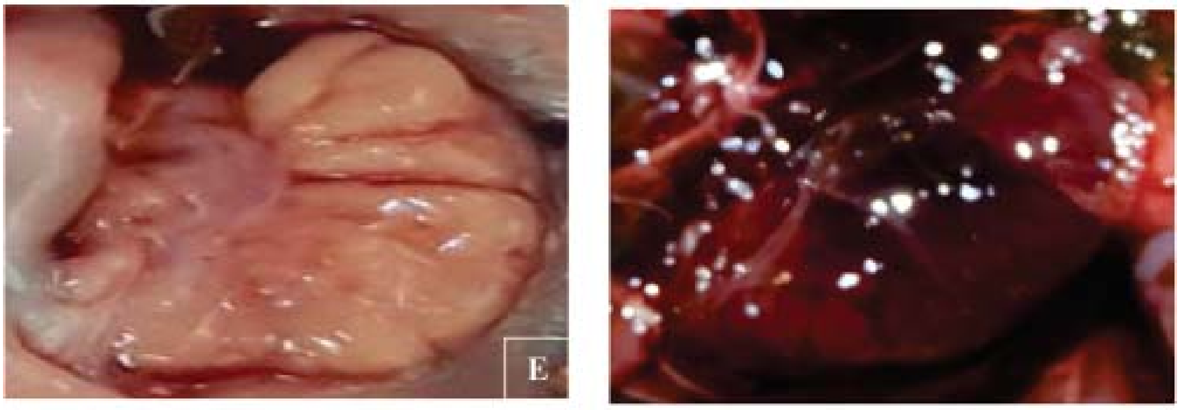


Fig 8E and 8F. Anatomical transverse cross section kidney on Green Turtles. (Fig 8F) Anatomical Transverse Cross Section Kidney on Olive Ridley Turtles.

CONCLUSION

Based on the results of this study, it is concluded that the cervical dorsal acoustic window showed that the jugular vein of olive ridley turtles wider than green turtles, ultrasonography imaging of the stomach showed that green turtles had smooth folds than olive ridley turtles which had sharper folds according to necropsy, there were no difference in the stomach; heart; small intestine; large intestine; and kidney on green turtles and olive ridley turtles, and ultrasonography imaging of vitelogenic follicle showed that the turtle was being in a period premating and mating.

SUGGESTION

The suggestions for further are using USG with interval frequencies 2,5-3.5 MHz for big sea turtle, doing very good necropsy for the best results, and doing further research to measure growing of ovarium follicle.

ACKNOWLEDGMENTS

The researchers would like to thank the staff and management of TCEC for place of doing research and for Gianyar police and all TCEC Staff for the support of finishing this research.

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