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Ultrasonographic Imaging of Reproductive Organ in Indonesian Native Chicken Hen (*Gallus Domesticus*)

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Abstract

Hen's native chicken reproductive organ health status evaluation is important for raising Indonesian native chicken in order to support protein supply for people demand. This study aimed to discover the images of Indonesian hen's native chicken reproductive organ using ultrasonography. There were 18 hens that used in this study and divided into two groups, 15 hens for brightness-mode ultrasonography imaging and three hens for color Doppler ultrasonography imaging. The 15 hens that were used for brightness-mode ultrasonography divided into three groups based on body weight i.e. 1.2 kg, 1.3 kg, and 1.5 kg, and it's also divided in two groups based on their age less and more than one year old. The transcutaneous ultrasonography performed in this study. The transducer lubricated by ultrasound transmission gel and positioned on caudal femur with an approximately angle 30 degree against abdomen wall. The results showed that follicles were ease to found and appeared as anechoic oval to round form confined by a hypoechoic layer at left position of ultrasound scanning. Furthermore, follicles were difficult to scanning in hens with age under than a year. The number and size of follicles and eggs were affected by body weight. In-vivo ultrasound image of eggs showed enhancement artifact and specular reflection, while in-vitro ultrasound image of eggs showed acoustic shadowing. The uterine organ showed as an anechoic structure with several parts having multiechoic that representing plica uterina. Further, the color Doppler ultrasound images can be used for identification of ovarium vascularisation profile. In general, it can be stated that ultrasonography is a useful non-invasive supporting diagnostic tool to evaluate the health status of hen's reproductive organ.

Introduction

Native chicken (*Gallus domesticus*) is one of chicken breeds in Indonesia. The origins of Indonesian native chickens are still yet to be confirmed, even though there are two theories that explains it. The first theory named monophyletic origin; it explains that Indonesian native chicken originated from one ancestor which is the red jungle fowl (*Gallus gallus*) (Sulandari *et al.,* 2008). The second theory explains that Indonesian native chicken come from several origin and is historically the result of domestication of four wild chicken species i.e. green jungle fowl (*Gallus varius*), red jungle fowl (*Gallus gallus*), Indian grey jungle fowl (*Gallus soneratti*), and ceylon orange jungle fowl (*Gallus lafayetti*) (Hidayat and Asmarasari, 2015). As an animal protein source, native chicken has a significant role in providing more than 200 million Indonesian people requirements for animal protein. Recently, some Indonesian local breeders have been starting to apply for breeding and selection program on local chickens to support high consumer demand on native chicken products (Ulfah *et al.*, 2015) and it makes natives chicken reproductive organ evaluation is important.

Before ultrasonographic scanning is used to evaluate ovaries and reproductive tract health of live birds, such as broiler chickens (Melnychuk *et al.*, 2002), turkeys and cassowaries (Thielebein and Kozlowski, 2010), and ostriches (Bronneberg and Taverne, 2003), the evaluation was done invasively

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through post-mortem method. The chicken is killed first and dissected, and then the target reproductive organ is isolated (Salang *et al.*, 2015). Ultrasonography as advanced non-invasive technology can give an image whose clarity is near to the one produced by post-mortem method, but without killing the chicken. Ultrasonography in broiler's reproductive organs has the accuracy of 93.3%-96.3% (Melnychuk *et al.*, 2002), thus it can be an effective device to monitor reproductive health.

Ultrasonography of birds' reproductive organs can be done by three techniques, which are transcutaneous, transvaginal, and transintestinal ultrasonographies. Transcutaneous ultrasonography can be done while the bird is still alive by restraining and handling the bird mechanically (Bronneberg and Taverne, 2003). However, it has difficulties in observing inactive gonads and follicles with certain sizes, because the air sacs will blur the image produced by transcutaneous ultrasonography (Thielebein and Kozlowski, 2010). Transvaginal ultrasonography is good to determine avian reproductive tract because it will allow a very close view of the pelvic organs, but it works only in big avian such as ostrich and it also needs euthanasia (Aryanti, 2017). Transintestinal ultrasonography can reduce the spreading of wave by air sacs, but this technique also needs anaesthesia (Bronneberg and Taverne, 2003). This study was then used transcutaneous ultrasonography method to observe reproductive organs images of 18 Indonesian native hens.

Materials and Methods

Animals used and procedures were approved by the Animal Ethic Commission of Bogor Agricultural University (number: 127 - 2018 IPB). The study was done in Teaching Animal Hospital, Bogor Agricultural University, West Java, Indonesia. All of B-mode ultrasonographic images from 15 native hens was scanned using B-mode ultrasonography console (SOGATA SG10®, PT Tunas Daya Veteriner, Indonesia), while the color Doppler ultrasonographic images of the 3 other chickens was scanned using color Doppler ultrasonography console (Chison Q8®, PT Mega Utama Medica, Indonesia). All Indonesian native hens used in this study were adults aged over one year old, except for two of the total of 15 native hens used for B-mode ultrasonography scanning. Transducer that was used is small convex transducer with frequency setting of 8.0 MHz. Two methods were used in this study as following:

Method 1: In-vivo transcutaneous B-mode ultrasound imaging in live chickens

Chickens were not habituated beforehand. Restrain and handling of the chicken hens were done physically without anaesthesia, using two hands; one hand holding the legs and the other hand holding the hen's head (Figure 1A). The chicken was positioned in right lateral recumbency to ease the ultrasound scanning. The base of the chicken's left thigh was kept away from the body to ease the ultrasound scanning at the left part of the abdomen, which is normally covered by the thigh (Figure 1B). The transducer was smeared by ultrasound transmission gel to minimize wave interruption. The transducer position for assessment of follicle, uterine, and egg in-vivo ultrasound is at the caudal of femur with the angle of around 30 degree against the abdomen wall, 3-5 cm from the cloaca (Melnychuk *et al.*, 2002). The in-vivo transcutaneous ultrasound method was done to collect B-mode and color Doppler data in right and left position scanning.

Method 2: In-vitro B-mode ultrasound imaging of eggs

In-vitro ultrasonography was only done in eggs in order to image their image in ultrasonogram. The egg was placed in a container that filled with tap water. The transducer was then placed at the water's surface above the egg (Figure 1D).

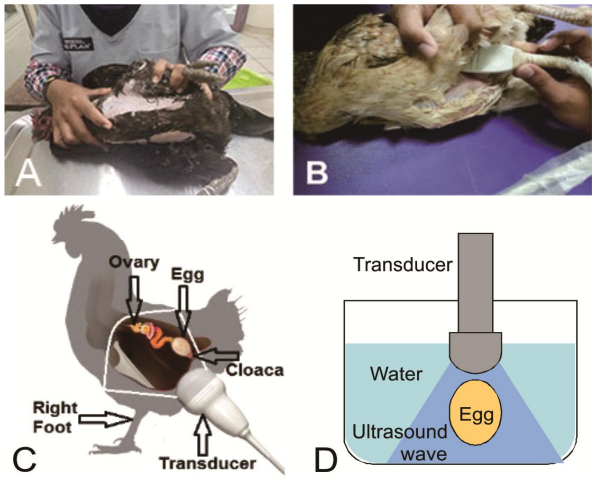

Figure 1. Ultrasonography image scanning methods in Indonesian native hens reproductive organ system. (A) restraining method, (B) transducer positioning, (C) illustration of chicken reproductive organs in in-vivo ultrasound scanning, (D) illustration of ultrasound image scanning of egg in in-vitro imaging.

The obtained ultrasound images of different scanning position and both of in-vitro and in-vivo were firstly compared using ImageJ software (NIH, USA), and then analyzed using Microsoft Excel 2016 and Statistical Product and Service Solutions (IBM, USA) applications was used for post hoc Duncan test statistical analysis with the level of significant was $P < 0.05$.

Results and Discussion

Ultrasonogram of Hens' Ovaries Based on Transducer Position

Figure 2 shows the ultrasonogram of hen's ovary that scanned at left and right side of hen's body. Ultrasonogram of hen's ovarium with transducer positioned in left-side abdomen showed as anechoic follicle image with hypoechoic center and margins (Figure 2A). Prehierarchical follicles (PhF) still could be good observed in this position as a cluster. Meanwhile, ovary ultrasonogram with transducer position in right-side abdomen showed hypoechoic follicle image with hypoechoic center and unclear margins (Figure 2B). Prehierarchical follicles was not well imaged with transducer positioned at the right-side abdomen. Functional ovaries were blurred when scanning was done at right-side abdomen because ultrasound wave is blocked by digestive tract and air sacs (Thielebein and Kozlowski, 2010). Transcutaneous ultrasound scanning in native hens is better done in base of thigh, at the left-side of abdomen (Figure 2).

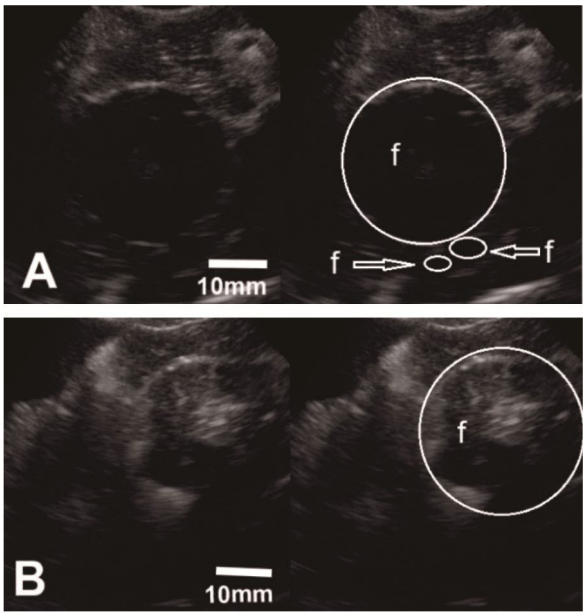

Figure 2. Ultrasonogram of hen's ovary that scanned at left-side (A) and right-side (B) of abdomen. f = follicles.

Ultrasonogram of Hens' Ovaries

Ultrasonograms of ovaries of three samples, hen's number 10, 11, and 15, did not show any follicle, despite the ages, which were already over one year old and the ovaries only showed as an almost well-distributed hypoechoic tissue (Figure 3A). Ultrasonogram of native hen number 1's ovary, aged under 1 year old, also did not show any sighting of the follicle (Figure 3B). Ultrasonograms of other native hens' ovaries showed that the ovaries have oval to rounded shape (Figure 3C). There are two echogenicities at follicles ultrasonograms, most of them are anechoic with hypoechoic walls (Figure 3D). Moreover, several follicles show higher echogenicity by having a hypoechoic center. Ultrasonogram of hens' follicles were similarity with ultrasonogram of ostrich's follicles (Gonzales and Acorda, 2014). However, this finding is different from the ultrasonogram of ostrich's follicles which showed a more hyperechoic center when a probe with frequency of 3.5-5.0 MHz was used (Bronneberg and Taverne, 2003).

The total range of PhF scanned was between 0-5 follicles (Table 1), with the largest amount of follicles found in hens weighed 1.5 kg (Table 2).

There was not found any PhF image in hens weighed 1.2 kg (Table 2). The absolute diameter of PhF was 4.53±1.27 mm, with the largest diameter of 4.93±1.38 mm that found in hens weighed 1.3 kg (Table 3). The total range of pre-ovulatory follicle (PoF) scanned was between 0-4 follicles (Table 1). The largest amount of PoF was found in hens weighed 1.5 kg, and the least amount was found in hens weighed 1.3 kg (Table 2). The largest PoF diameter was also found in hens weighed 1.5 kg (Table 3). Likewise, the smallest PoF diameter was also found in hens weighed 1.2 kg (Table 3). The PoF sized 30-40 mm was also found in native hens

weighed 1.2 kg (Table 1). Hens weighed 1.5 kg were significantly different ($P < 0.05$) in absolute and relative diameters of PoF (Table 3). The absolute and relative diameters in PhF and PoF were also significantly different between groups ($P < 0.05$). Atretic follicle was not found in all ultrasonography images of samples that used in this study.

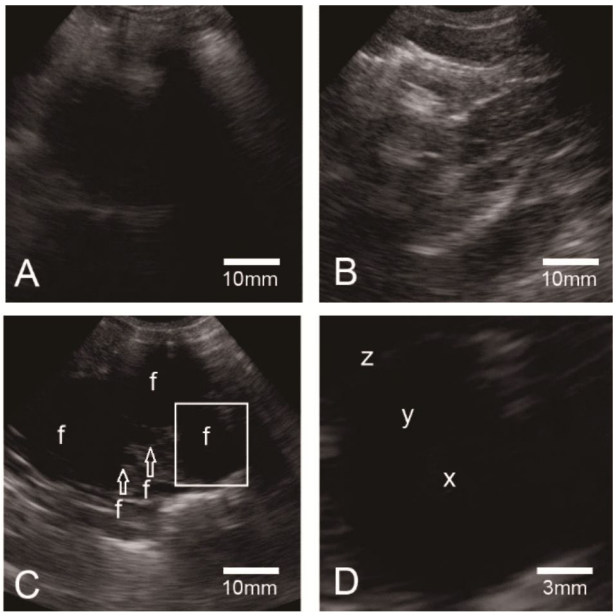

Figure 3. Ultrasonogram (A) ovary of adult hen number 10, (B) ovary of young hen number 1, (C) ovary of adult hen number 6, and (D) magnification of one of the (f) follicles. The parts of the follicle consist of (x) hypoechoic center, (y) anechoic part, and (z) hypoechoic margin.

Table 1. Pre-hierarchical follicle (PhF) and pre-ovulatory follicle (PoF) profile of hen's ovary that found by ultrasonographic imaging.

Hen's number	Age (year)	Body weight (kg)	PhF (follicles)	PoF (follicles)				PhF+PoF (follicles)
			1-8 mm	8-20 mm	20-30 mm	30-40 mm	Sum	
1	<1	<1	n.a	n.a	n.a	n.a	n.a	n.a
2	<1	<1	n.a	n.a	n.a	n.a	n.a	n.a
		Total	0	0	0	0	0	0
		Range	0	0	0	0	0	0
3	>1	1.2	n.a.	1	2	n.a.	3	3
4	>1	1.2	n.a.	2	n.a.	1	3	3
5	>1	1.2	n.a.	1	1	n.a.	2	2
6	>1	1.3	1	1	3	n.a.	4	5
7	>1	1.3	3	n.a.	1	n.a.	1	4
8	>1	1.3	3	2	1	n.a.	3	6
9	>1	1.3	n.a.	1	2	n.a.	3	3
10	>1	1.3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
11	>1	1.3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
12	>1	1.3	n.a.	n.a.	1	n.a.	1	1
13	>1	1.5	n.a.	2	2	n.a.	4	4
14	>1	1.5	5	2	2	n.a.	4	9
15	>1	1.5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		Total	12	12	15	1	28	40
		Range	0-5	0-2	0-3	0-1	0-4	0-9

Note: PhF=*Pre-hierarchical Follicle*, PoF=*Pre-ovulatory Follicle,* n.a.= not available.

Every female chick that just hatches from the egg already has ovary full of ova, which amount around 10000 ova, but they are very small in size and will start developing when the chick enters adulthood (Jacob and Pescatore, 2013), thus they are hardly visible in ultrasound images (Bronneberg and Taverne, 2003). This is proven by the ultrasound images of chicken hen number 7 and 8, which were under one year old in this study (Table 1).

Table 2. Number of pre-hierarchical follicle (PhF) and pre-ovulatory follicle (PoF) of hens with different body weight that found in ultrasonographic imaging.

Age (year)	Body weight(kg)	n	PhF (follicles)	PoF (follicles)			
			1 - 8 mm	8-20 mm	20 - 30 mm	30 - 40 mm	Subtotal
<1	<1	2	n.a.	n.a.	n.a.	n.a.	n.a.
>1	1.2	3	n.a.	1.33±0.58	1.50±0.71	1.00±0.00	2.67±0.58
	1.3	7	2.33±1.15	1.33±0.58	1.60±0.89	n.a	2.40±1.34
	1.5	3	5.00±0.00	2.00±0.00	2.00±0.00	n.a	4.00±0.00

Note: Data served in mean with standard deviation, PhF=*Pre-hierarchical Follicle*, PoF=*Pre-ovulatory Follicle,* n.a=not available.

The varying size of follicles in current experiment is in line with findings of Apperson *et al.* (2017) who reported that in one time an reproductively active bird's ovary has varying sizes from all stage follicles. Further, Lovell *et al.* (2003) classified layer hen's follicles' growth and development into three phases, which are prehierarchial follicle (PhF), preovulatory follicle (PoF), and postovulatory follicle (PooF). The size of a PhF is around 1-8 mm (Lovell *et al.*, 2003). The PhF is a follicle which already has two layers of theca cells, i.e. theca interna and externa, and already gets a small portion of yolk (Dong *et al.,* 2014). Adult and productive domestic native birds have PhFs with the average size of 2-3 mm, and around ten PhFs can be found each day in each ovary (Waddington and Walker, 1988).

Table 3. Absolute and relative diameter of pre-hierarchical follicle (PhF) and pre-ovulatory follicle (PoF) of hens with different body weight that found in ultrasonographic imaging.

Age (year)	Body weight(kg)	n	Ø PhF (mm)			Ø PoF (mm)		
			Absolute	Relative	P-value	Absolute	Relative	P-value
< 1	<1	2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
> 1	1.2	3	n.a.	n.a.		20.45 ± 8.40^{b}	$17.04\pm7.00^{a,b}$	
	1.3	7	4.93 ± 1.38^{b}	$3.80\pm1.06^{a,b}$	0.018	21.31 ± 4.26^{b}	$16.39\pm3.28^{a,b}$	0.019
	1.5	3	4.20 ± 0.77^{b}	2.80 ± 0.51^{a}		21.35 ± 4.48^{b}	14.23 ± 2.98^{a}	
	Total		4.53±1.27	3.38±0.98		21,08±5.57	15.93±64.55	

Note : Data served in mean with standard deviation, Ø=Diameter, n.a.=not available, n=number of hen, PhF=*Pre-hierarchical Follicle*, PoF=*Pre-ovulatory Follicle*, P-value=Significance, number followed with different letter in the same row is stated to have a significant difference ($P < 0.05$).

There are 5 phases of PoF in chickens. The phase starts from f5 with the smallest diameter, which is 9 mm, followed by f4, f3, f2, and f1 with the largest diameter (Navara, 2018). The granulose cells in PoF expand so that more yolk precursor can enter and enlarge the follicle, surpassing the size of PhF (Navara, 2018). The f1 will ejected from the ovary to infundibulum to be processed as an egg is the largest follicle with diameter coming near to 40 mm and has undergone 20 day-development inside the ovary (Lovell *et al.*, 2003).

Active hen's ovary has millions of follicles, but not all of them will be ovulated. Some follicles within the prehierarchical stage will undergo atresia via the process of apoptosis (Johnson, 2014). Atretic follicle has an irregular shape with hypoechoic center and hyperechoic margins. The follicle can be observed in ostrich's ultrasound image (Gonzales and Acorda, 2014) but it was difficult to find it in the native hens that performed in this study (Figure 2 and 3). The small and overlapping follicles are difficult to image in real time using ultrasound technique using probe with frequency of 3.5-5.0 MHz (Bronneberg and Taverne, 2003).

The average layer hens who actively lays eggs have 5 PoFs in their ovary (Salang *et al.*, 2015). The total PhFs and PoFs from ultrasound image in this study are fewer than the literature. The most differ in productivity is shown by chicken hen number 10, 11, and 15, which does not show any follicle in the ultrasound images, even though they were not incubating (Table 1). The follicles will stop growing and many follicles will undergo atresia when the incubation period begins because ofthe increasing prolactin which have an antisteroidogenic effect (Hrabia *et al.,* 2004). The different productivity can be caused by difference in ovary cycle or disturbances in the reproductive system. Reproduction disturbance in hens is directly proportional to the incidence of diseases, environmental source, nutritional problems and genetic problems such as immunity (Sudarisman, 2009). Abnormalities in reproductive tract and egg-forming materials also reduce the frequency of egg forming (Rahman, 2013).

The follicle size observed in the ultrasound image is contingent on the hen's body weight (Table 3). In this study, the diameter of absolute and relative follicles in ultrasound image are significantly different between groups ($P < 0.05$). It means there is

a positive correlation between follicle or egg size and body weight. Najib and Al-Yousif (2014) also stated that many factors may affect egg size, and body weight considered as one of the greater influence. The heavier the chicken's body weight, the bigger the size of their egg.

In-vivo and In-vitro Ultrasonogram of Hen's Eggs

Figure 4 depicts in-vivo and in-vitro ultrasonogram of chicken egg. In-vivo ultrasonogram of eggs inside the hen's body is observed as a white or hypoechoic shadow in the median and margins of the egg (Figure 4A). However, in-vitro ultrasonogram of the eggs looks similar with in-vivo ultrasonogram but has black or anechoic shadow at the median (Figure 4B). Table 4 shows the amount of eggs scanned in ultrasonogram of native hen.

Table 4. Hen's egg profile that found at in-vivo ultrasonographic imaging

Age (year)	Body weight (kg)	n		Ø Egg (mm)		
		Chicken hen	Egg	Absolute	Relative	P-value
<1	<1	2	n.a.	n.a.	n.a.	n.a.
>1	1.2	3	n.a.	n.a.	n.a.	
	1.3	7	1.00±0.00	32.87±9.18[a]	25.28±7.06[a]	0.26
	1.5	3	1.00±0.00	35.94±0.13[a]	23.96±0.08[a]	
	Total		1.00±0.00	34.09±6.71	24.75±5.04	

Data served in mean with standard deviation form. n=number, Ø=diameter, n.a.=not available, P-value=significance, number followed with different letter in the same row is stated to have a significant difference (P<0.05).

The eggs scanned are in native hens weighed 1.3 kg and 1.5 kg. The largest diameter, which almost reaches 36 mm is found in native hen's body weighed 1.5 kg. The egg diameter in this study is not affected by the hens' weight (P > 0.05) (Table 4).

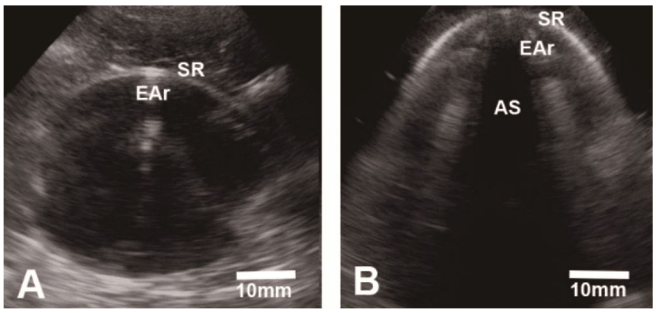

Figure 4. (A) In-vivo and (B) in-vitro ultrasonogram of chicken egg. Note: specular reflection (SR), enhancement artifact (EAr), and acoustic shadowing (AS).

Mature follicles in productive native hens which already entered infundibulum will continue their process into eggs, fertilized or not fertilized (Rahman, 2013). The follicles will undergo an increase in albumin when they reach magnum, then a formation of inner and outer shell in isthmus. The shell that formed in uterus contains 95% hard crystal from mineral of calcium carbonate (Rahman, 2013). The shell-forming hard crystal reflects sound waves in ultrasound, so specular reflection and enhancement artifact are observed in egg ultrasonogram (Bronneberg and Taverne, 2003). The curved hyperechoic shadow at the margins of the egg is the specular enhancement, while the hyperechoic shadow at the median is the enhancement artifact. The in-vivo ultrasonogram of the egg inside the hen's body is different from the in-vitro ultrasonogram of the egg inside the water (Figure 4). The in-vitro egg ultrasonogram shows acoustic shadowing, which is an anechoic shadow formed under a structure because the egg structure absorbs or reflects the whole sound wave it receives (Bolliger *et al.*, 2008).

There was varying size of the eggs inside the hen's body in this study. There was no significant difference between the absolute and relative diameters of the egg (P > 0.05) (Table 4). Aside from the limited samples, this difference can also be caused by the difference in hens' productivities, weights, egg positions inside the oviduct, or ages. The eggs produced during the early laying stage are smaller than the following eggs. Further, the younger hen which starts laying egg also having the smaller egg (Hocking *et al.,* 2003).

Ultrasonogram of Hen's Uterine

Figure 5 illustrated the ultrasonogram of hens' uterine with longitudinal and transversal views scanning. Ultrasonogram of longitudinal view of uterine lumen

is observed as an anechoic to hypoechoic image, with hypoechoic to hyperechoic walls (Figure 5A). Ultrasonogram of transversal view of uterine shows as rounded structure with anechoic to hypoechoic echogenicities, bordered by walls with hypoechoic to hyperechoic echogenicity (Figure 5B). The transversal view of uterine diameter was 18.17±3.00 mm, and the longitudinal view diameter was 11.19±5.42 mm. The uterine of hens under one year old was hard to found with ultrasound in this study.

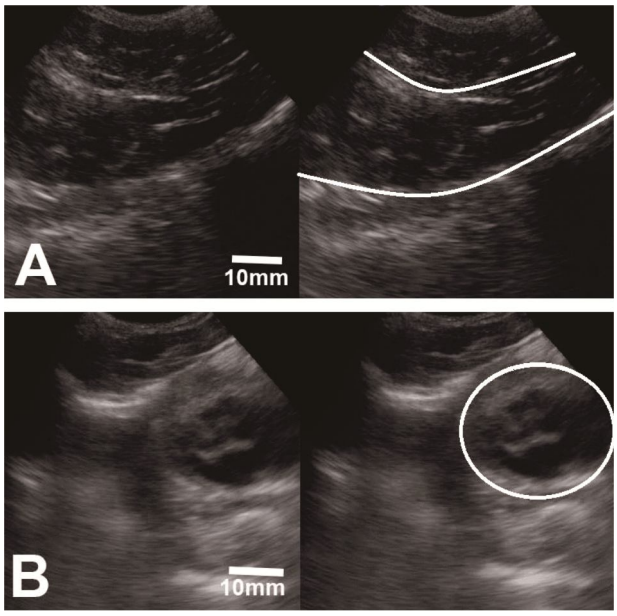

Figure 5. Ultrasonogram of hens' uterine with longitudinal (A) and transversal (B) view scanning

The varying echogenicities in the ultrasonogram of uterine lumen are caused by the plica, or longitudinal folds in uterine mucus membrane (Figure 5). This causes the sound wave is not come simultaneously because the secretive glandular area (shell gland) of uterine mucosal folds that have different heights (Berg *et al.,* 2001). Echogenicity of uterine also changed following by their contraction and time movement. Anechoic image represents uterine lumen, while the hypoechoic image at the margins of the uterine represents the epithelial cells.

According to the post-mortem evaluation of Uttarakhand native hen's reproductive organs (Khan *et al.*, 2017), the shell gland has the diameter of 5.28±0.55 mm to 22.91±0.77 mm, the magnum has the diameter of 3.31±0.25 mm to 10.84±0.62 mm, and the isthmus has the diameter of 2.16±0.28 mm to 6.35±0.33 mm. The longitudinal and transversal view of the uterine in the ultrasonogram (Figure 5) can be considered as the shell gland, while magnum and isthmus were not scanned in this study.

Color Doppler Ultrasonogram of Hens' Ovary

Figure 6 shows the ultrasonogram of hen's ovarium using color Doppler scanning. Color Doppler ultrasound scanning in three native hens shows the blood flow at the margins of the follicle. The blood flow is shown by bright to dark red coloring (Figure 6B and 6E). The blood flow of ovary in hen number 17 is shown more colorings (Figure 6H). Table 5 shows the number and diameter of follicles in hen using color Doppler ultrasound scanning.

Table 5. Number and diameter follicle of hen in color Doppler ultrasonographic imaging

Hen's number	Age (year)	Body weight (kg)	PhF		PoF				
			1-8 mm	Ø (mm)	8-20 mm	20-30 mm	30-40 mm	Total	Ø (mm)
16	>1	1.92	6	4.63±1.87	4	2	n.a.	6	18.68±4.86
17	>1	1.19	n.a.	n.a.	1	1	n.a.	2	20.51±4.96
18	>1	1.55	4	3.17±1.16	1	1	n.a.	2	20.67±9.49

Note: Data served in mean with standard deviation. PhF = *Pre-hierarchical Follicle,* PoF = *Pre-ovulatory Follicle,* Ø=Diameter, n.a.=not available

Peak Velocity (PV), Mean Pressure Gradient (MPG), and Pulsatility Index (PI) values as pulsed wave Doppler profiles in hen are presented in Table 6. The PV, MPG, and PI values can be determined in hen number 16 and 18. Pulsed wave Doppler profile is hard to observe in ultrasonogram of hen number 17.

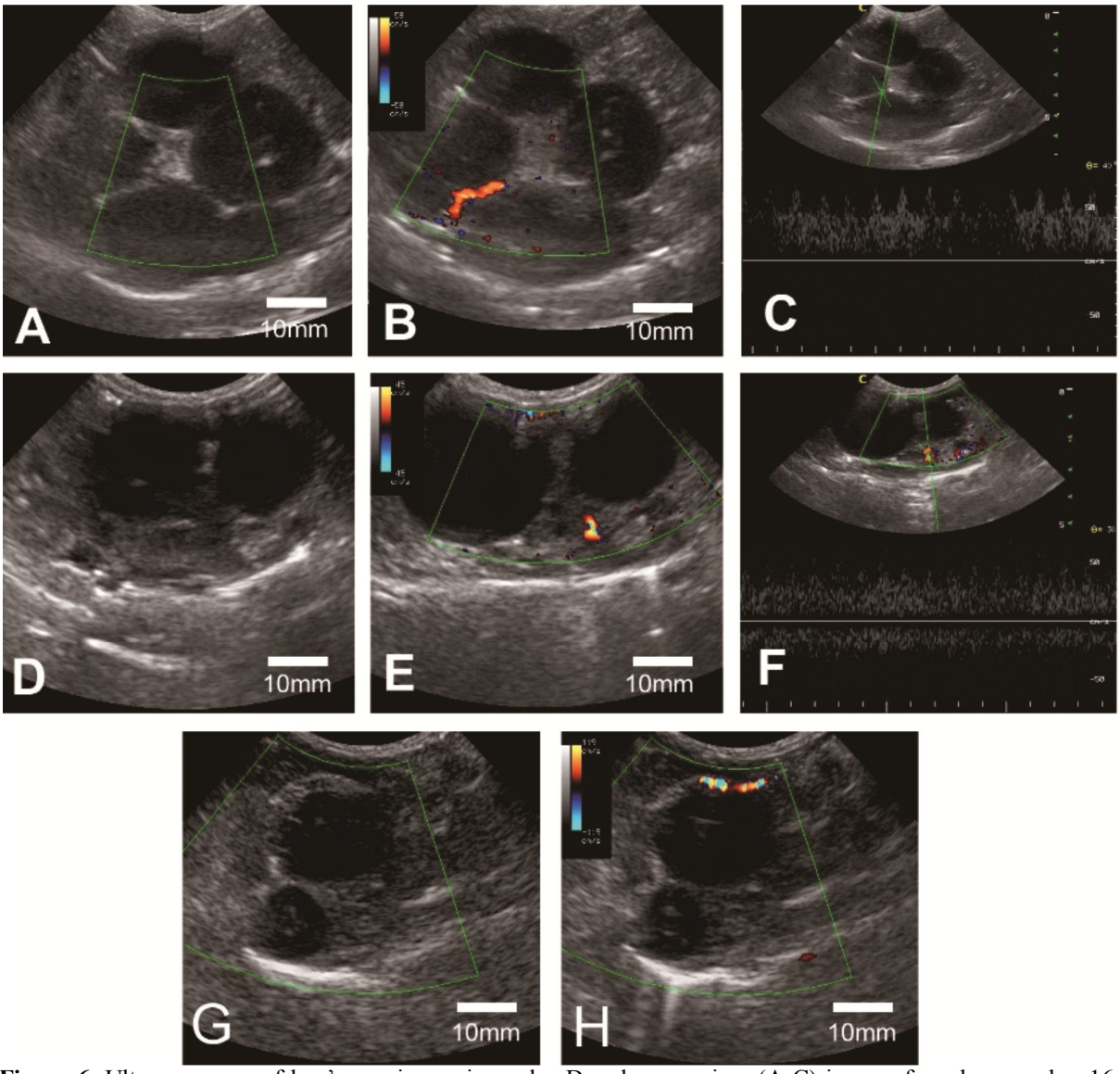

Figure 6. Ultrasonogram of hen's ovarium using color Doppler scanning. (A-C) images from hen number 16, (D-F) images from hen number 18, and (G, H) images from hen number 17.

In normal breed hens, the blood flow is in follicles growth area (Barua *et al.*, 2007). Most of blood is supplied by the left cranial renal artery, which enters the ovary at the ovarian hilus (Apperson *et al.*, 2017). The difference in blood flow velocity in ovarium is caused by the follicular cycle. The increase of follicles' size and number will increase the vascularization, except at the stigma area (Apperson *et al.*, 2017). This caused the chicken hen number16 having PV higher than chicken hen number 18 (Table 6). The red coloring shows that the blood flows approaching to transducer (Hwang, 2017). High MPG value is an indicator of the vascular or ventricular stiffness with high pressure at the end of diastole, and is usually used to indices of stenosis severity (Baumgartner *et al.*, 2009). Meanwhile PI value indicates that there is an increased resistance of blood vessel (Desai and Desai, 2016).

Table 6. Pulsed wave Doppler in the vessel around the follicles

Hen's number	Peak Velocity (cm/s)	Mean Pressure Gradient (mmHg)	Pulsatility Index Value (cm/s)
16	68.8±61.30	0.044±0.001	4.49±0.64
17	n.a.	n.a.	n.a.
18	54.10±1.33	0.055±0.001	4.46±0.80

Note: Data served in mean with standard deviation. n.a.=not available.

Color Doppler ultrasound image of chicken hen number 17 in this study shows that the blood vessel has varying flows, characterized by varying colors (Figure 6H). Doppler pulsed wave image is hard to obtain because of the difficulty in determining Doppler angle and precise Doppler sample volume position, or if a stenosis is occurred at the blood vessel (Lui *et al.*, 2005). The usage of color Doppler ultrasound in hens is very beneficial for early detection of ovary tumor (Barua *et al.*, 2007). Aside from giving difference values in PV, MPG, and lower PI in pulsed wave Doppler, ovaries with tumor will show varying blood flow from ovary's center to the periphery. The blood flow will appear inconstant and the main tumor blood flow can be observed (Barua *et al.*, 2007).

Conclusion

Transcutaneous ultrasound scanning using B-mode of Indonesian native hen's reproductive organs can show follicles, uterine, and eggs. Most of the follicles appear to be anechoic, have hypoechoic margins and center. Ultrasonogram of eggs inside the uterine is different than the one taken outside the uterine. Ultrasonogram of uterine lumen has anechoic to hypoechoic echogenicity and hypoechoic to hyperechoic walls. The size of follicles and eggs is affected by the hen's body weight. Color Doppler scanning can be used to identify vascularization profile in ovaries.

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