ULTRASONOGRAPHIC FEATURES OF THE OVARIAN FOLLICLES IN APPARENTLY HEALTHY LOCALLY-RAISED BREEDING OSTRICHES *(Struthio camelus)*

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ABSTRACT

Eight apparently healthy sexually mature 4-6 years old locally-raised female ostriches were used to determine the ultrasonographic features of the ovarian follicles. Transcutaneous scanning of the featherless ventrolateral part of the left abdomen caudal to the thigh was conducted using an ultrasound machine equipped with a 3.5 MHz convex array scanner. The stage, shape, size and echogenicity of the ovarian follicles were determined. Four types of follicles were observed: a) large follicles (6.4-9.8 cm in diameter); b) small follicles (2.2-5.8 cm in diameter); c) atretic follicles (3.5-5.4 cm in diameter); and d) developing ova (2.1-2.7 cm in diameter). Large follicles appeared as anechoic round to oval structures with anechoic to hypoechoic walls. Small follicles appeared as irregular anechoic structures with hypoechoic walls, which were much smaller than large follicles. Atretic follicles appeared as irregular structures with hypoechoic center and hyperechoic walls while developing ova appeared as oval structures with hyperechoic center and hypoechoic walls. Developing ova had the highest while large follicles had the lowest echo mean values. The data obtained in the study provide baseline parameters for the ultrasound features of the ovarian follicles in apparently healthy locally-raised sexually mature female breeding ostriches which can be used for diagnosis of diseases.

Keywords: follicles, ostrich, ovary, ultrasound

INTRODUCTION

In the Philippines, ostrich farming has become a rapidly expanding industry (Salting, 1999). At present, ostrich farms are considered to be among the most profitable agricultural projects where there has been a large variety of possible products from meat, eggs, hide and feathers which leads to high profit potential for the industry. In comparison with traditional livestock, ostrich farming rates highly (Shanawany, 1999). However, it also faces significant problems, specifically in marketing, slaughter and production. One of the major challenges for ostrich producers is to achieve an optimal sized operation of efficient breeders (Michael, 2000). Conversely, knowledge about the basic morphological and functional

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changes of the reproductive organs during the production cycle, which could help maximize reproductive potential, is lacking.

Ultrasonography in animal reproduction has been proven useful as a diagnostic tool for veterinary practitioners and researchers in reproductive biology and animal science (Taverne and Willemse, 1989). Applications of ultrasound for reproductive purposes have been developed, not only in domestic animals but also in zoo and wildlife species, including some avian species. Recent advances in ultrasound technology have made it possible to non-invasively determine the ovarian status in poultry *in vivo*, without the need of sacrificing the hen (Anthony *et al.,* 2002). Researchers have been able to use ultrasound to assess the reproductive potential, primarily in hens, of broiler breeder and layer type chickens, suggesting that ultrasound can be used as a tool for selecting individuals with well formed, normal, follicular hierarchies in females (Bronneberg and Taverne, 2003).

However, the use of this diagnostic tool to visualize the female reproductive organs in the ostriches has not yet been described and explored exhaustively (Christen *et al.*, 2006). The potential for application of ultrasonography in these species is tremendous and merits further evaluation. Over the years, there have been limited investigations performed on the ovarian development of the ostrich species (Bronneberg and Taverne, 2003). The lack of standardized normal baseline data makes interpretation of images obtained by examiners difficult (Sued, 2006). More studies are needed to create a set of reference avian ultrasonographic values and to relate them to clinical and laboratory findings (Naldo and Samour, 2007).

This study aimed to explore an approach using diagnostic ultrasonography to provide information on the ovarian status of the female ostriches used in breeding. This study could also provide preliminary data which could demonstrate the potential value of the technique in evaluating the reproductive efficiency on the basis of ovarian morphology.

MATERIALS AND METHODS

Eight 4-6 years old, mature, female breeding ostriches randomly selected from a total population of 25 ostriches were utilized for ultrasound examination. Only apparently healthy animals, based on medical history and physical examination, were used. The subjects for this study were obtained from the Zoobic Safari, Subic, Zambales where standard management procedures are applied.

In order to facilitate an easy scanning procedure, the ostriches were kept on one corner of a small enclosed area one at a time. Around four to five persons held the animal in place, opposite to the examiner. A cotton hood was placed over the head to calm the animals while the examination procedure was being conducted. The procedure was done in a confined and shaded examination area to allow more accurate on-screen observation of the images.

The ostriches were subjected to transcutaneous ultrasonography using an ultrasound machine (Aloka US Diagnostic Equipment, Aloka® SSD-500, Aloka Co. Ltd., Tokyo, Japan) equipped with a 3.5 MHz convex array scanner. Copious amount of ultrasound gel (Trans-gel, Rothmeier Laboratories, Inc. Philippines) was placed over the transducer to maximize contact with the skin. Transcutaneous ultrasonography was performed by placing the transducer over the ventrolateral featherless part of the abdominal skin caudal to the left thigh (Figure 1), scanning the whole examination area until the acoustic window was identified and a detailed orientation of the reproductive organs was attained. The ultrasonograms of the different ovarian follicles were recorded using a video graphic printer (Sony® UP – 895 MD, Sony Corp., Tokyo, Japan).

Figure 1. Transcutaneous scanning over the ventrolateral featherless portion of the abdominal skin beneath the thigh on the left side of the body.

The ultrasonograms of the different ovarian follicles observed were described based on their number, size, shape and echogenicity. The ovarian follicles imaged in the ultrasound were tallied depending on the stage of the follicle per animal and the percentage of which was determined. The diameter (largest obtained in cm) of each ovarian follicle was measured using the ultrasound machine's electronic caliper and manually using Adobe Photoshop CS7 (Adobe System Incorporated, 1998 – 2003). Digital analysis of the ultrasonograms was performed using Adobe Photoshop CS7. Echo mean (Emean) values were calculated, using histogram analysis, and compared.

The protocol used in this study was approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Veterinary Medicine, University of the Philippines Los Baños.

RESULTS

In all of the ostriches, the ultrasonograms were obtained by transcutaneous scanning of the left flank. The acoustic window was determined to be the ventrolateral part of the abdomen caudal to the thigh on the left flank of the ostrich. It is limited cranially by the last vertebral rib, dorsally by the cranial pole of the left kidney (located at the ventral surface of synsacrum) and ventrally and caudally by the abdominal air sac (located to left of the mesentery and cranioventral to the pubis) and gastric diverticulum of the clavicular air sac.

The ovarian follicles visualized varied in stage, number and size (based on the largest obtained diameter in cm). Among the ovarian follicles imaged were large follicles, small follicles, atretic follicles and developing ova. Table 1 shows the number and percentage of occurrence of the different types of follicles. Among the follicles, large follicles had the highest number of observations (13 or 39.39%), while small and atretic follicles had the least (4 or 12.12%). For the birds, large follicles were observed in 7 of 8 birds, small follicles in 4 out of 8 birds, while the rest of the follicles were observed only in 3 out of 8 birds.

Table 1. Number and percentage of various ovarian follicles visible on the ultrasonograms of sexually mature female ostriches.

Parameter	Follicles		Birds with follicles	
	No.	%	No.	%
Large follicle	13	39.4	7	87.5
Small follicle	4	12.1	4	50.0
Atretic follicle	4	12.1	3	37.5
Developing ovum	12	36.4	3	37.5

The diameter of visible follicles ranged from 2.1 cm in developing ova to 9.8 cm in large follicles (Table 2). Large follicles had the biggest diameter while developing ova had the smallest diameter. The diameter of small follicles and atretic follicles were similar.

Table 2. Diameter and echo mean values (mean and standard deviation {S.D.}) of the different stages of ovarian follicles in sexually mature female ostriches obtained through ultrasonography.

Parameter	Diameter		Echo mean values	
	Mean	SD	Mean	SD
Large follicle	7.7	1.22	42.3	4.4
Small follicle	4.4	1.63	44.2	2.4
Atretic follicle	4.3	0.94	84.4	21.3
Developing ovum	2.3	0.16	168.6	14.9

Large follicles or mature follicles were seen in the ultrasonogram as anechoic round to oval structures with anechoic to hypoechoic walls (Figure 2). In some large follicles imaged, inner reverberations and corpuscular echogenicities artifacts were also observed. In other follicles, the walls were not smooth, probably due to interference by adjacent structures, like the intestines. An ovum with a developing shell was also imaged in one bird. The calciferous material causes a more intense echogenicity of the periphery.

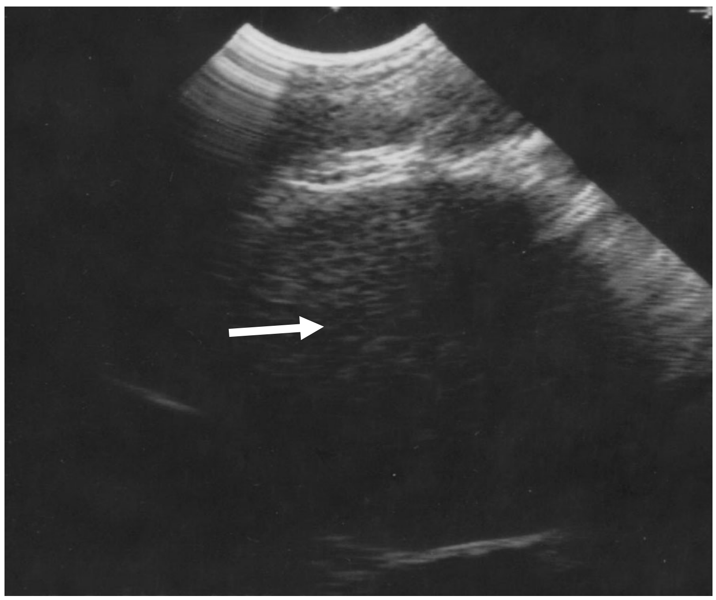

Figure 2. Ultrasonogram of a large mature follicle in a sexually mature female ostrich showing an anechoic oval structure with hypoechoic wall. Corpuscular echogenicities (arrow) were also observed.

The small follicles (Figure 3) appeared as irregular anechoic round to oval structures with hypoechoic walls. These follicles were relatively smaller than the mature follicles.

Atretic follicles were recognized by their greater echogenicity and lack of well -defined shape (Figure 4). The center and the outline of this follicle, as observed in the ultrasonogram, were hypoechoic and hyperechoic, respectively.

An irregularly-shaped developing ovum has a central echogenic spot clearly distinguished from a hypoechoic wall (Figure 5).

After quantitative measurement of the ovaries in mature breeding females, echo means were obtained through histogram analysis (Table 2). Developing ovum had the highest echo mean value (168.6) due to the hyperechoic nature of the follicle, followed by atretic follicle. Large and small follicles had almost similar echo mean values (42.3 and 44.2, respectively) due to the anechoic nature of the follicles.

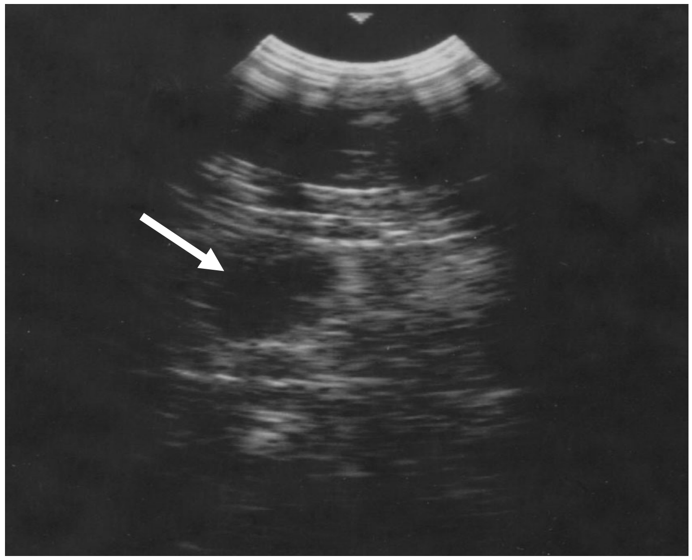

Figure 3. Ultrasonogram of a small follicle (arrow) in a sexually mature female ostrich showing an anechoic structure with irregular walls.

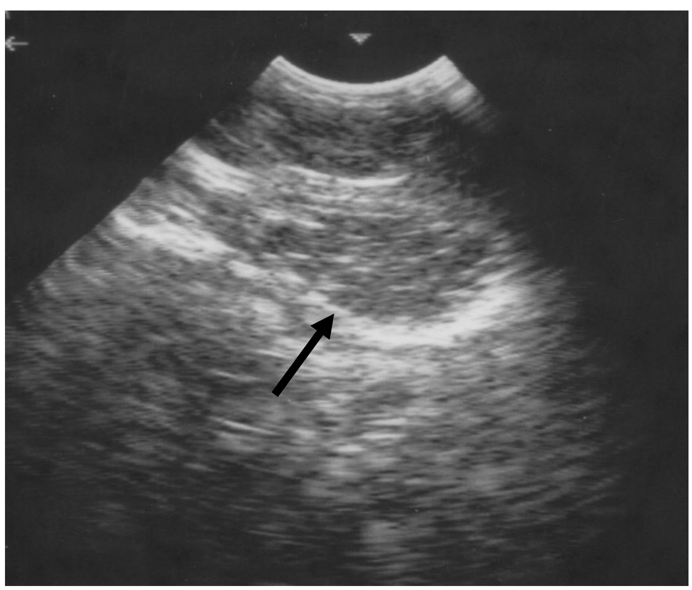

Figure 4. Ultrasonogram of an atretic follicle (arrow) in a sexually mature female ostrich showing an irregular structure with hypoechoic center and hyperechoic walls.

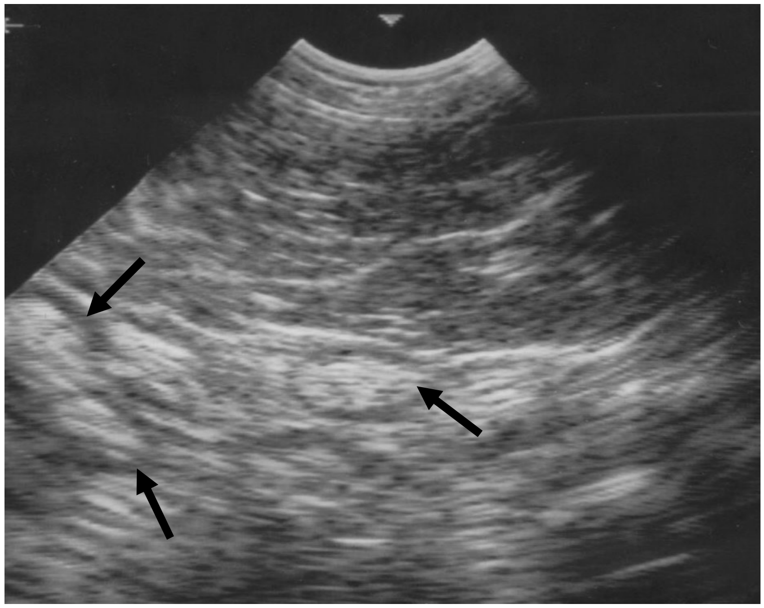

Figure 5. Ultrasonogram of developing ova (arrows) in a sexually mature female ostrich showing ovoid structures with hyperechoic center and hypoechoic walls.

DISCUSSION

Proper restraint is essential in obtaining desirable ultrasound images since an adult ostrich can be fractious, capable of injuring both the handler and itself. In this study, retraint was accomplished by placement of a "hood" made up of a cotton sack with holes over the head then holding the body of the ostriches by three to four personnel. Placement of a hood serves to impair the vision of the ostriches and is a practical way of keeping the animals calm. Holding the ostrich neck and head down low and horizontal to the ground while another assistant applies upward and forward pressure to the bird's pelvis from the rear has been also recommended (McKeown, 2007), but this was not done in the study to avoid injury to the birds. In addition, the birds were not held by their wings since restraining adult ostriches by the wings alone is a discouraged practice due to the potential for injury (McKeown, 2007).

The acoustic window for visualizing the ovarian follicles was determined to be the featherless area of the caudoventral side of the left lateral abdomen of the animal limited by the last vertebral rib cranially, cranial pole of the left kidney dorsally, and abdominal air sac and gastric diverticulum of clavicular air sac ventrally and caudally. In cases where there was impedance of the ultrasound waves by the intestines, the scanner was manipulated until an improved condition of the scanning and improved image was produced. The ovarian follicles were well-visualized in the study. However, Wagner and Kirberger (2001) reported that they were not able to observe the ovary in ostrich through transcutaneous ultrasonography. This was due to certain limitations including size of the ostriches, massive leg and dorsal muscles, large sternum, the extensive air sac system, compact convoluted intestines and varying amounts of gastrointestinal gas.

The female reproductive tract of ostrich consists of a single ovary and oviduct, of which usually the left develops (Babić *et al.*, 2004). The left ovary is suspended from the dorsal body wall, located ventral to the cranial pole of the left kidney and dorsal to the abdominal air sacs. As the breeding cycle of the hen progresses, the size, shape and the position of the ovary within the coelomic cavity undergo changes (Fowler and Miller, 2003). In mature birds, the ovary resembles a bunch of grapes, consisting of a stroma in which numerous follicles of varying sizes are embedded. During the breeding season, from 12 to 16 ova attain maturity. Each mature ovum is contained in a capsule and is attached to the surface of the ovary by its own stalk (Soley and Groenewald, 1999). It is released during ovulation as the largest follicle in the hierarchy and ruptures through the stigma, an avascular area of the follicular wall. The other structures of the ovary are the ruptured or post-ovulatory follicle (POF) and the atretic follicles, which regress before ovulation (Bronneberg and Taverne, 2003). Inactive ovaries have not been visualized as well since the reproductive tract of the ostriches can only be seen clearly in the mature breeding hen (Naldo and Samour, 2007).

Ultrasonographic features of the ovarian follicles observed in this study appear to be consistent with the anatomical descriptions in the literature. According to Bronneberg and Taverne (2003), the ovary of a mature breeding female consisted of numerous follicles ranging from 1-9 cm in diameter, varying in shape and embedded in a stroma. The present study was able to determine the size of the ovarian follicles in locally-raised sexually mature female ostriches. Each individual follicle was assigned as small and large follicle based on the follicle-size categories from the study of Bronneberg and Taverne (2003): a) 3.1-6.0 cm and b) 6.1-9.0 cm for small follicles and large follicles, respectively. Although ≥3 cm ovarian follicle is typical of a developing ovum, minor adjustment in the classification of a 2.2 cm ovarian follicle was made due to the features observed in the ultrasonograms, which is a consistent characteristic of a small ovarian follicle. Developing ova and atretic follicles were readily identified according to the ultrasonographic features.

The total number of ovarian follicles was difficult to determine because of obstruction in the scanning of some of the follicles caused by various coelomic structures such as air sacs and intestines which interfere with the visibility of the ovarian follicles. Over and underestimation of the count of the follicles – due to possible repetition of recognition or insufficient area scanned, respectively – is a problem in this kind of study. Based on the results of the study, large follicles were recognized the most among the different types of follicles, while small follicles were recognized the least. A comparison with the gross count of the follicles by sacrificing the hen would be highly recommended. Counting the total ovarian follicles in sexually mature ostriches would be a helpful reference in the future for a thorough representation of the ovaries of sexually mature female ostriches.

According to Bronneberg and Taverne (2003), the center of the follicle consists of white yolk which forms the core of the yolk formation. The white yolk in the center is deposited first as maturation of the follicle starts. After entry into the yolk-filled hierarchy class of follicles, deposition of yellow yolk occurs rapidly in concentric layers. In the present study, the details of the ultrasonographic images of the small and large follicles show that these follicles are rounded to oval with hypoechoic center. The hypoechoic center is attributed to the yolk. The presence of disc-like structures in large follicles (represented by internal reverberations) were observed in this study, in agreement with Bronneberg and Taverne (2003) who observed alternating hypo- and hyperechogenic rings representing the hyperechoic core of white yolk and alternating hypo-hyperechoic layers of yellow yolk with differing composition.

The walls of the large follicle varied from anechoic, hypoechoic and hyperechoic depending on the deposition of calciferous material (shell). In one bird, the shell and the uterine wall could be recognized. This is highly suggestive that follicular hierarchy and egg formation can be monitored with ultrasonography. Serial ultrasonography is recommended in order to quantify the interval of follicular stages and egg formation.

Ovarian follicles normally collapse and exhibit atresia, especially during non-breeding seasons. In atretic follicles, the wall ruptures and yolk is distributed. This explains the ultrasonogram of an atretic follicle showing increase in the echogenicity of the periphery and a hypoehoic center.

Developing ova are the smallest follicles in the ovarian hierarchy. The ultrasonogram of a developing ovum imaged in this study shows irregularly shaped structure with hyperechoic center clearly distinguished from a hypoechoic periphery. The ovum has an irregular shape at this stage of development since shell membranes have not yet developed.

Histogram analysis of the ultrasonograms was conducted after quantitative measurements were made. A histogram is a bar graph of frequency distribution in which the widths of the bars are proportional to the classes into which the variable has been divided and the heights of the bars are proportional to the class frequencies. This tool also measures the contrast of an ultrasonogram, enabling the examiner to arrive to a conclusion relative to the value of the echo means obtained. The higher the echo mean value, the more contrast there is and the more hyperechoic the ultrasonogram is, and vice-versa. In this study, the echo mean values were highest in developing ova, followed by atretic follicles and lowest in small follicles and large follicles. This result shows that a developing ovum is more echogenic, or hyperechoic, than the other types of follicles. Large follicles, on the other hand showed anechoic areas in the ultrasonogram.

The study demonstrated that transcutaneous ultrasonography of the ovarian follicles of sexually mature female ostrich is feasible. The study was able to describe the percentage of follicles, diameter of follicles, stages of follicles, ultrasound features and echo mean values of the different stages of follicles in sexually-mature female breeding ostriches. Ultrasonography provides a non-invasive imaging technique for determining the ovarian status in ostriches, allowing visualization of the ovarian follicles and determining the possible position of the ova within the female reproductive tract. These data would be helpful in the development of breeding program for the ostriches and for diagnosis of disorders of the ovaries. The use of ultrasonography, therefore, might allow prediction of the ovarian activity during the breeding season and assist the ostrich farmer in the selection of productive breeders.

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