Research Note

Use of Ultrasonography to Characterize Ovarian Status in Chicken

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ABSTRACT Much research has been conducted to investigate the effects of environmental and nutritional treatments on ovarian development in poultry. However, to investigate the ovary, the hen must be killed, and thus, lifelong egg production can only be inferred. To date, the ability to noninvasively determine ovarian status has not been available. Improvements in ultrasound technology now make it possible to observe ovarian condition in vivo, thereby allowing for repeated sampling of the same bird over an entire egg production cycle. In the current study, large yellow follicles (LYF; diameter greater than 10 mm) were characterized in broiler breeder hens using Aloka® ultrasound diagnostic equipment. Ultrasound images were used to determine the number and diameter of the LYF as well as the presence of an egg in the oviduct. Immediately following ultrasonography, hens were killed and dissected to determine the number and diameter of LYF. From the ultrasound images, the number of LYF ± 1 was predicted with 96.3% accuracy in Experiment 1 and 93.3% accuracy in Experiment 2. Diameter measurements were used to classify follicles hierarchically. Of the birds determined via dissection to have multiple hierarchies, 77.8% were identified with ultrasound. All regressing or regressed ovaries were correctly identified with ultrasound.

(Key words: ultrasound, ovary, follicle, hen)

INTRODUCTION

With increasing genetic selection for growth and yield of broilers, reproductive fitness of parents is compromised. With broiler breeder hens, there is a negative correlation between BW and egg production (Siegel and Dunnington, 1985; Robinson et al., 1993). Ovarian morphology has been shown to affect egg production and laying sequence (Hocking et al., 1987; Hocking, 1993). An excess of large yellow follicles (LYF) in the ovary increases the potential for cull eggs and erratic laying (Jaap and Muir, 1968; van Middelkoop, 1971; Yu, 1992). Unfortunately, determination of ovarian morphology is limited in that it is terminal, and thus, lifelong egg production can only be inferred.

Management strategies, such as feeding (Robinson et al., 1991, 1993, 1995; Hocking, 1993) and lighting (Robinson et al., 1996) programs during pullet rearing, significantly affect egg production. The pullet-rearing period is critical, as significant determinants of reproductive fitness occur prior to peak egg production (Robinson et al., 1995). Obesity in broiler breeders results in increased follicular recruitment (Hocking et al., 1987, Hocking and Whitehead, 1990; Yu et al., 1992). During lay, seven to eight LYF (greater than 10 mm diameter) are present in the ovary of a feed-restricted broiler breeder, compared to 12 LYF in the ovary of a typical full-fed breeder hen (Yu et al., 1992). Excess follicles are arranged in double or triple hierarchies in which more than one follicle occupies the same position in the hierarchy (Hocking et al., 1987, 1989). In 1971, van Middelkoop was the first to coin the term EODES (erratic oviposition and defective egg syndrome) to describe reproductive problems of broiler breeders that result from multiple hierarchies. This term includes conditions such as follicular atresia, internal ovulation, internal laying, production of soft-shelled or membranous eggs, multiple-yolk eggs, multiple-egg days, and ovipositions occurring out of sequence.

Understanding the variation that exists in ovarian development would be advantageous for developing new management strategies to maximize production. Noninvasive methods of measuring ovarian status through puberty and the egg production cycle have not been available. Ultrasound techniques have been used with other livestock such as swine, horses, and cattle to evaluate reproductive status. Through the use of ultrasound techniques, hens with multiple follicular hierarchies, and thus a propensity toward poor egg production, can be identified. This paper will demonstrate that ultrasound techniques can be used to count and measure in vivo the number and diameter of LYF present in the ovary.

Abbreviation Key: LYF = large yellow follicle.
MATERIALS AND METHODS

Equipment

Aloka® ultrasound diagnostic equipment (Model SSD-100V)\(^2\) was used to noninvasively determine the number and diameter of LYF in vivo. Images were obtained using an endovaginal panoramic radial transducer (5 Mhz, 7.5 Mhz) with a diameter of 20 mm. Images and measurements were printed for each follicle by a black-and-white thermal printer connected to the ultrasound console.

Imaging Procedure

Each hen was immobilized by placing the bird breast down with legs restrained by an assistant. Ultrasound transmission gel\(^3\) applied to the surface of the transducer ensured a noninterrupted conductance of the sound waves. The transducer was inserted at, approximately, a 30-degree angle to the dorsal wall, 3 to 5 cm into the cloaca. To standardize positioning of the transducer, the directional marker on the transducer was always inserted in line with the dorsal midline of the chicken.

Ultrasound examinations were conducted in B-mode. The region surrounding the ovary was scanned. To completely scan the ovary, the transducer must sweep the entire area once a follicle has been located. The LYF appeared as dark circles or ovals with light concentric rings and narrow white borders (Figure 1). Concentric rings on the image of the follicle were produced when the sound waves emitted from the transducer were perpendicular to the fullest part of the follicle (Figure 1). An ovum in the oviduct appeared at the top of the image and did not display marked concentric rings (Figure 2). Ova in the oviduct were typically located separately from clusters of follicles and were surrounded by a wide, dark band (Figure 2). Identification of all of the LYF required the designation of a marker follicle. Marker follicles were determined arbitrarily but were usually those that were readily imaged upon insertion of the transducer. Once designated, the operator was able to scan in all directions relative to the marker follicle to identify the remaining follicles. Unfortunately, not all of the follicles will be apparent in a single image. follicles often appear as clusters, depending upon the position of the transducer. Once concentric circles in the center of the follicle were viewed, the follicle was measured, and a printed image was made.

The presence of a hard-shelled egg in the vagina impeded ultrasound examination, as it was not possible to move the transducer into position. To minimize the likelihood of a hard-shell egg being in the vagina, ultrasound examinations were performed after 1300 h. If a hard-shelled egg was detected in the vagina, examinations were postponed until the egg was laid. Oviposition usually occurred within an hour of detection. Eggs or ova detected in other regions of the oviduct did not impede ultrasound identification of the LYF.

Measurement

Two experiments were conducted by the same operator to evaluate the feasibility of using ultrasound to assess ovarian status. A second experiment was conducted to determine the effects of operator experience on precision. In each of the two experiments, broiler breeder hens were used. Experiment 1 consisted of 31, 36-wk-old broiler breeder hens, and Experiment 2 consisted of 30, 40-wk-old broiler breeder hens. By using calibrated measurement functions of the ultrasound console, diameter measurements were made for each follicle in Experiment 1. Only one follicle could be measured at a time in each image. The ultrasound image provides a slice image through the

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\(^2\) Aloka Co. Ltd. 10 Fairfield, Wallingford, CT 06492.
\(^3\) Parker Laboratories, Inc., Orange, NJ 07050.
follicle. Thus, in order to accurately measure the follicles in rapid development, the slice showing maximal diameter for each follicle was printed. Individual pictures of each follicle were printed and later used to identify follicular hierarchy position.

Measurements of the longest axis, shortest axis, circumference, and area were recorded on the printed image. To determine the diameter of the follicle, the circumference measurement was divided by $\pi$. This method resulted in a circumference that was equivalent to averaging the long axis and short axis measurements.

After ultrasound examinations, the hens were euthanized, and the ovaries were removed. The number of LYF and, in Experiment 1, the diameter of each of the LYF were recorded. LYF were considered those with a diameter of 10 mm or greater (Robinson and Etches, 1986).

In Experiment 1, the number of LYF and the diameters of the follicles for both methods were used to determine ovarian status. Follicles were assigned to the same position in the hierarchy if they differed in diameter by 1.0 mm or less for ultrasound and manual measurements. The potential for multiple ovulations was assessed by determining the total number of positions and the proportion of positions containing two or more follicles of similar size.

Statistical Analysis

The number of LYF was tested for agreement of the ultrasound and manual methods using the Kappa Statistic (Fleiss, 1982). A statistically significant positive value for Kappa indicates greater agreement than that expected by chance. Results were considered to be statistically significant if $P < 0.05$. All statistical analyses were carried out using SAS® Version 7.4.

RESULTS AND DISCUSSION

New developments in ultrasound technology now make it possible to monitor changes in ovarian status throughout a hen’s life. Prediction of the number of LYF and the follicular hierarchy status can provide valuable information to assess egg production potential as affected by strain or management strategies. Ultrasound provides the ability to make repeated measures on the same bird and to relate ovarian status with egg production.

The value of ultrasound technology to determine ovarian status depends on the purpose for which the information is intended. As a research tool, ultrasound analysis is an accurate method of evaluating the mean effects of a treatment on the number of ovarian follicles for a sample of birds. In both experiments, the mean number of LYF predicted by ultrasound techniques was not significantly different from the number of LYF determined via dissection. In Experiment 1, the mean number of LYF predicted by ultrasound was $6.7 \pm 0.27$ LYF and by dissection was $6.9 \pm 0.26$ LYF. Similarly, the mean number of LYF predicted by ultrasound in Experiment B was $6.1 \pm 0.21$ LYF compared with $6.4 \pm 0.21$ LYF determined by dissection. Four birds in Experiment 1 and one bird in Experiment 2 were found to have regressing or regressed ovaries upon dissection and, thus, were removed from the analysis.

Many studies that have investigated the effects of feeding and lighting programs on ovarian development in broiler breeder females might have benefited from the ability to monitor an individual’s ovarian development as related to egg production. Previously, the only method of determining changes in ovarian status was to kill a sample of birds periodically and then make inferences regarding the ovarian status of the flock. Flock egg records were then related to the ovarian development of each of these sample groups. Ultrasonography enables relationships to be made directly between the effects of ovarian development and egg production, rather than being inferred through sample populations.

Through ultrasound analysis, it is possible to identify those birds that have an overabundance of LYF and multiple hierarchies. Many studies have shown that an abundance of LYF commonly results in a multiple hierarchy arrangement (Hocking et al., 1987; Yu et al., 1992; Hocking, 1993; Robinson et al., 1995). Ovaries containing more than seven or eight LYF usually have a more than one follicle in a single position of the hierarchy (Hocking and Whitehead, 1990; Robinson et al., 1991, 1993, 1995, 1996; Hocking, 1993). Thus, the ability to count follicles in vivo is valuable in predicting the potential for multiple hierarchies. By using ultrasound, prediction of the number of LYF within one LYF was 96.3% accurate in Experiment 1 and 93.3% accurate in Experiment 2. In Experiment 1, a Kappa statistic of 0.215 with a 95% confidence interval of 0.022 to 0.408 showed that agreement between the two methods was significantly greater than that expected by chance. With operator experience, Kappa value increased in Experiment 2 to 0.478 with a 95% confidence interval of 0.249 to 0.706. In both experiments, when the number of follicles estimated by ultrasound did not exactly match the number determined by dissection, ultrasound measurements underestimated the number of LYF more often than overestimated them. In Experiment 1, the number of LYF was underpredicted, by 1 LYF, 40% of the time, whereas in Experiment 2 underestimation occurred 27% of the time. Ultrasound overestimation by 1 LYF occurred 29 and 17% of the time in Experiments 1 and 2, respectively.

In Experiment 1, diameter measurements were used to classify follicles into hierarchy position and thereby identify multiple hierarchies. Ultrasound and manual measurements provided similar conclusions regarding ovarian hierarchy status. With ultrasound, the reproductive status (single hierarchy vs. double hierarchy) was correctly assessed (as verified by dissection) for 77.7% of the hens. Ultrasound measurements incorrectly classified four hens as having multiple hierarchies and two birds as having normal hierarchies, which were not supported.

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by the findings obtained by dissection. Thus both measurements result in the same conclusions regarding the magnitude of the condition in which multiple follicles occupy the same position.

All birds with regressing or regressed ovaries were correctly identified by ultrasound (Experiment 1, four birds; Experiment 2, one bird). Regressing follicles appeared less dense, with less defined borders, and were lacking concentric rings as compared with normal follicles. Completely regressed ovaries were identified indirectly by a lack of LYF. The ability to identify multiple hierarchies is an asset, as the fate of LYF can be determined when monitored regularly and then related to egg production.

Statistical analysis regarding the accuracy of ultrasound diameter measurements was not possible. Because LYF are slightly elliptical, it is difficult to say with complete certainty that, although diameter measurements may be similar, the same follicle was being measured with both techniques. The inability to view and measure all of the follicles in one image makes it difficult to know with certainty the orientation of each follicle. This limitation contributes to the overestimation and underestimation by one follicle. Follicles appear differently, depending on the angle of the transducer. Operator experience aids in the reduction of these errors.

The use of ultrasonography is noninvasive. In another study, birds measured repeatedly by ultrasound did not show any differences in egg production or livability as compared to those that were not evaluated by ultrasound (unpublished data). Duration of the procedure varies with ultrasound operator. Care should be taken to minimize the time taken to use ultrasound in order to minimize stress and possible damage to a hen. As expected, more experienced operators enable shorter durations of restraint for hens.

The ability to noninvasively determine ovarian status in vivo provides a valuable tool to monitor ovarian status over time. Many studies can be contemplated to evaluate the effects of strain or management strategies on reproductive development. This tool now offers the ability to make repeated measures on the same bird and to relate an individual’s egg production to ovarian status. With this ability, the number of birds used to conduct such studies can be reduced compared to previous experimental designs. The value of an individual as breeding stock can be determined by identifying those hens with regressing or regressed ovaries or those with a propensity toward poor egg production due to abundance of LYF and multiple hierarchies. Ultrasound techniques may be valuable in evaluating the effectiveness of various feeding programs and management strategies for pullets entering sexual maturity and throughout the production cycle.

REFERENCES