ULTRASONOGRAPHIC STUDIES ON CYCLIC AND CYSTIC OVARIES IN COWS

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SUMMARY

The objective of this study is to characterize and to evaluate the ovarian structures in the cow by ultrasonography and ultrasound image analysis (echotexture) and to correlate the results with the physiologic status of the ovarian structures (i.e. follicles, CL, and ovarian cysts) under the study. Computer-assisted image analysis was used to evaluate ultrasound images of ovarian structures. The ovaries of 15 postpartum dairy cows were examined by transrectal ultrasonography. Seven cows were examined (Group I) for two estrous cycles for monitoring of follicular activity and CL development, while the other 8 cows (Group II) were examined for characterization of ovarian cysts (follicular versus luteal). Ultrasonographic images were saved to a disk, and were subsequently digitized for computer analysis of echotexture [mean pixel value (MPV) and pixels heterogeneity (PH)] of the antrum and wall of ovulatory and cystic follicles and the CL. MPV and PH of the antrum for the dominant anovulatory follicles were low during the early static phase (Day 6), then increased at onset of the regressing phase (Day 13). MPV and PH of the follicle wall for the dominant ovulatory follicles were relatively high compared with the dominant anovulatory follicles. Serum estradiol reached highest levels when the ovulatory follicle was at its maximum diameter.

A correlation was observed between MPV of the follicle wall of ovulatory follicles and serum estradiol levels. MPV decreased ($P<0.01$) in the regressing CL compared to the growing and static CL. PH increased ($P<0.01$) in static CL compared to growing and regressing CL. A significant correlation ($r=0.69$, $P<0.01$) was observed between MPV of the CL and progesterone concentrations. A significant correlation was also observed between MPV of the wall of luteal cysts and plasma progesterone level ($r=0.62$, $P<0.01$). There was a significant increase ($P<0.01$) in MPV of the antrum and PH

of the wall of luteal cysts compared to follicular cysts. It could be concluded that ultrasonography and computer-assisted analysis of ovarian ultrasound images revealed significant changes in the structure and echotexture of: ovarian follicles, CL, and ovarian cysts in cows. These changes were concurrent with changes in functional and endocrine characteristics of such ovarian structures.

INTRODUCTION

Ultrasonography would not be considered

practice have aided in the advancement of reproductive technologies. Continuous and rapid evolution of computer technology and software has created exciting new experimental and diagnostic capabilities related to analysis of visual data. Quantitative data analysis of ultrasonographic images has been used to evaluate the physiologic status of ovarian follicles, the corpus luteum, the follicular and luteal cysts, and the ovarian response to pharmacologic superstimulation (Adams and Pierson, 1995; Pierson and Adams, 1995; Singh et al., 1997; Singh et al., 1998; Tom et al., 1998; Schrick et al., 2001, Duggavathi et al., 2003).

Ultrasonographic images are composed of picture elements (i.e. pixels) resulting from the refraction or transmission of high-frequency sound waves. Each pixel represents the ability of a small, discrete unit of tissue to refract or transmit ultrasound waves, resulting in an image displayed in various shades of gray. Collective numerical pixel values (i.e., brightness) and heterogeneity are functions of differences in tissue densities and macro-molecular composition (Pierson and Adams, 1995; Singh et al., 1997; Tom et al., 1998; Kot and Ginther, 1999). It has been demonstrated that pixel analysis (i.e., echotextural characteristics) of images representing follicles and CL in cattle reflects discrete changes in their morphology and secretory function (Pierson and Adams, 1995). The follicular development in cattle occurs in a wave-like pattern (Adams and Pierson, 1995; Noseir, 2003). A wave of follicular growth has been characterized as the simultaneous growth of a group of follicles one of which becomes dominant and continues to grow, while the subordinates cease growing and regress. A dominant follicle either become atretic and regresses (anovulatory follicle) or ovulates and releases an oocyte (ovulatory follicle). Development of dominant follicles may be divided into growing, static, and regressing phases (Ginther et al., 1989).

Ultrasonography is used for determining various physiological abnormalities such as diagnosis of ovarian cysts. Cysts are common in postpartum cows and determination of type (follicular versus luteal) can be difficult with rectal palpation. Failure to correctly classify the type of cystic structure may result in improper treatment for removal of the cyst. Utilizing ultrasonography and echotexture for determination of cyst type, these structures can be separated into follicular and luteal cysts (Schrick et al., 2001). Ultrasound imaging has been also used to detect and measure the CL throughout the bovine estrous cycle. Changes in CL diameter, as detected by ultrasonography, are highly correlated with progesterone production; however, functional demise of the CL precedes physical regression by 1-2 days (Kastelic et al., 1990; Assey et al., 1993). Hence, determination of luteal function based on diameter alone may be accomplished only retrospectively (after evaluating data from serial examinations). Also, identification of the physiological status of ovarian follicle type (dominant Vs subordinate) and phase (growing, static, and regressing) may only be accomplished retrospectively. There is no reliable way to determine the physiological status of ovarian structures based on a single ultrasound examination.

The present study was designed to evaluate the structural and functional correlation of cyclic and cystic ovaries in cows, as determined by serial ultrasound examinations, ultrasound image analysis (echotexture), and measurement of hormonal concentrations; including: 1) ovarian follicular activity during estrous cycle, 2) Monitoring of CL development during estrous cycle, 3) Characterization of follicular and luteal cysts.

MATERIALS AND METHODS

Animals:

The present work was carried out on 15 postpartum dairy cows (Native breed), aged 3-7 years. Animals from the farm and clinic of Faculty of Veterinary Medicine (Alexandria University) were studied during the period from September to December. Animals were divided into 2 groups as follows;

Group I:

Seven cycling cows were examined once every other day by transrectal ultrasonography throughout 2 consecutive estrous cycles, thus 14 interovulatory intervals (7 cows x 2 estrous cycles), to monitor ovarian follicular activity and CL development.

Follicular activity:

Follicles were retrospectively identified as dominant or subordinate of successive waves. The day of ovulation at the beginning of an interovulatory interval was designed as day 0. A follicular wave was identified by detecting a dominant follicle attaining a diameter > 6 mm. The day of first detecting a follicle that was retrospectively identified as a dominant follicle, was taken as the first day of a wave. The *growing phase* of the dominant follicle was defined as the period extending from the day of wave emergence to the day that the follicle was appeared to cease its progressive increase in diameter. The *static phase* was defined as the period extending from the last day of the growing phase to the first day that the follicle began a progressive decrease in diameter. While, the *regressing phase* was defined as the period from the last day of the static phase to the day the follicle was no longer detectable (Ginther et al., 1989). For the ovulatory follicle, the period from wave emergence to ovulation was considered the growing phase.

CL development:

Similar to the criteria established for ovarian follicles, the *growing phase* of the CL (metestrus) was defined as the period from the day of ovulation (day 0) to the day that the CL appeared to cease its progressive increase in diameter. The *static phase* (diestrus) was defined as the period from the last day of the growing phase to the first day that the CL appeared to start its progressive decrease in diameter. While, the *regressing phase* (proestrus) was defined as the period from the last day of the static phase till the CL was no longer detectable (Ginther et al., 1989; Tom et al., 1998).

During each ultrasound examination, a single blood sample was drawn by jugular venepuncture from all cows. Samples were analyzed by RIA for estradiol and progesterone using Coat-A-Count Kits (DPC, Diagnostic Products Cooperation, USA).

Group II:

Eight cows with cystic ovaries were used in this group. Ovaries were diagnosed by palpation per rectum and ultrasonography and were considered cystic when it contained fluid-filled structures more than 25mm in diameter and persisted for 10 days (Schrick et al., 2001). Ultrasonography examination was performed twice at 7 days interval in the absence of a corpus luteum (Lopez_Gatius and Lopez_Bejar, 2002). Retrospective confirmatory diagnosis was done by progesterone determination. Blood samples were collected twice; at the time of initial diagnosis and 7-14 days later. The level of progesterone was <1.0ng/ml for follicular cyst and >1.0ng/ml for luteal cysts. Progesterone was analyzed by RIA using Coat-A-Count Kit (DPC, Diagnostic Products Cooperation, USA).

Ultrasonography and echotexture analysis of ultrasound images:

Ultrasonography examinations were performed using a real-time ultrasound scanner (Pie Medical, Scanner100LC, The Netherlands) equipped with a 6/8 MHz linear-array transrectal transducer. The scanner produced high-resolution images. During each ultrasound examination, the distance between the transducer face and the ovary, and settings on the ultrasound scanner were standardized throughout the examination period. Ultrasound images were saved to a floppy disk for later computer analysis. Special software was used for analysis of ultrasound images (Scion Image for Windows V 4.0, based on NIH Image for Macintosh, National Institute of Health, USA). The program was designed to quantify characteristics of user-selected regions. Pierson and Adams (1995) defined echotexture in terms of mean pixel value (MPV) and pixels heterogeneity (PH). Mean pixel value (average of gray-scale values) was quantified using values ranging from 0 (black) to 255 (White), and the degree of deviation from the mean (standard deviation) of pixel values within the user-defined region was used as indicator of heterogeneity. Greater standard deviation was taken as indication of greater heterogeneity among pixel values within selected regions. Evaluation of the follicle antrum involved sampling a circular region covering 80% of each quadrant for each image of the antrum (Figure 1, left). Evaluation of images for the follicle wall involved sampling from two circle regions (Figure 1, middle) within 10 and 2 o'clock positions (Kremkau, 1989; Ginther, 1995). While, evaluation of images of the CL involved dividing the image into quadrants, and a circular region was used to sample MPV and PH from each quadrant (Figure 1, right). Sample regions included only luteal tissue, avoiding ovarian stroma and fluid-filled cavities when present (Tom et al., 1998).

Statistical analysis for MPV, PH, and hormonal concentrations were carried out using ANOVA, while multiple comparisons were made by LSD. Pearson's correlation coefficients were used to examine the relationship between ultrasound image attributes (Norusis, 1986).

RESULTS

Group I:

Follicular activity:

The number of observations studied was 14 interovulatory intervals. Wave 1 was first detected on day 0 (day of ovulation). The growing phase of the dominant follicle of wave 1 was days 0 to 6, the static phase was days 6 to 12 followed thereafter by the regressing phase. The mean day of emergence of wave 2 was Day 8.7±0.3 while the mean day of emergence of the ovulatory wave was Day 10.8±0.3.

Echotexture of the follicle wall:

MPV and PH of the follicle wall were relatively high ($P<0.01$) in the dominant ovulatory follicles compared with the dominant anovulatory follicles (Table 1 and Figure 3). There was a correlation between MPV and PH of the follicle wall for the dominant anovulatory follicles ($r=0.56$, $P<0.01$).

Echotexture of the follicles and estradiol correlates:

Serum estradiol values showed a gradual increase ($P<0.01$) during the growth phase (3.94±1.54 pg/ml) till the end of the static phase (5.68±0.97 pg/ml) of dominant anovulatory follicles, followed by a decrease ($P<0.01$) during the regressing phase (4.14±1.11 pg/ml). Estradiol levels were highest (6.82±0.18 pg/ml) at day of ovulation when the dominant ovulatory follicle was at its maximum diameter (10.99±0.18 mm). There was a correlation between the MPV of the follicle wall of the dominant ovulatory follicles and serum estradiol levels ($r=0.76$, $P<0.01$), but no significant correlation was detected between PH and estradiol values.

CL development:

The number of observations studied was 14 interovulatory intervals. The length of growing phase of the CL was from days 0 to 6, the static phase was days 7 to 15, and the regressing phase was days 16 to 20.

CL compared to growing and regressing CL (Table 2 and Figure 4, right). A significant correlation (r= -0.56, P<0.01) was observed between MPV and the stages of CL development (growing, static, and then regressing).

Echotexture of the CL and progesterone correlates:
Plasma progesterone concentration showed gradual increase during the growing phase of the CL, and reached the maximum values during the static phase then started to decrease at the end of the static phase (Table 2). A significant correlation (r=0.69, P<0.01) was observed between MPV of the CL and progesterone concentrations. A significant correlation was also observed between the CL size and progesterone concentrations (r=0.65, P<0.01).

Group II:

Results of echotexture analysis of ultrasound images of cystic ovaries revealed that there was a significant (P<0.01) increase in MPV and PH of the antrum of luteal cysts compared with follicular cysts (Table 1 and Figure 5, left). A significant increase (P<0.01) was also observed in MVP and PH of the wall of luteal cysts compared with follicular cyst (Table 1 and Figure 5, right). There was a significant correlation between MPV and PH of the follicle wall (r=0.84, r=0.81, respectively, P<0.01) and antrum (r=0.80, r=0.79, respectively, P<0.01), with the advancement of cyst development [follicular, and then luteal]. A significant correlation was also observed between MPV of the wall of luteal cysts and plasma progesterone level (r=0.62, P<0.01).

DISCUSSION

Computer-assisted echotexture offers the potential of such a highly sensitive and quantitative method of assessing follicular and luteal status (Pierson and Adams, 1995; Tom et al., 1998; Duggavathi et al., 2003). MPV obtained by spot analysis of the antrum, is a measure of the overall (average) gray-scale value of the pixels falling under the measuring circle, while PH of the antrum is a measure of the variation in gray-scale value of pixels falling under the measuring circle. The earliest signs of follicular atresia were decreased diameter, degeneration, and detachment of granulosa cells from the basal lamina (Yang and Rajamahendran, 2000). During the static and regressing phases of the dominant anovulatory follicles, granulosa cells are sloughed into the antrum as sheets or individually (Singh, 1997). Cellular detachment may result in accumulation of debris within the follicle antrum during the regressing phase, a process that has been documented by histomorphological assessment (Singh and Adams, 1998). It is likely that these cells or their degeneration products were responsible for the observed increase, in the present study, in MPV and PH of the follicle antrum of the dominant anovulatory follicles. These observations are in complete agreement with earlier studies (Tom, 1996; Singh et al., 1998) in which significant increase in MPV and PH of the antrum was detected during the late static and regressing phases of the dominant anovulatory follicles. This significant increase in pixel heterogeneity reflected the functional demise of the dominant anovulatory follicle. Tom et al. (1998) observed that atretic bovine ovarian follicles had an increased follicle antrum heterogeneity compared to ovulatory follicles. Changes in follicle image attributes may have been due to changes in histomorphology of the follicle during the interovulatory interval. The ovulatory dominant follicle had the thickest wall among all follicles and the number of theca interna cell layers was great, while the granulosa layer was not as compact as that of the ovulatory follicle (Singh and Adams, 2000). This may account for the high MPV and PH, in the present study, of the follicle wall of ovulatory follicles, and for the correlation that was observed between estradiol concentration and MPV of the follicle wall of ovulatory follicles, since estrogen is secreted form the cells of the theca interna. Similar results were reported in mares (Gastal et al., 1999) in which changes in echotexture of the follicle wall (increased thickening and echogenicity of granulosa) as well as increase in estrogen production, were observed with the approach of ovulation.

Pixel values of ultrasound images of the bovine corpus luteum were correlated with plasma progesterone and morphometric characteristics (Singh et al., 1997). In the present study a significant correlation was observed between both MPV and size of the CL and progesterone concentration. Similar results were obtained by Tom et al. (1998) who stated that plasma progesterone concentration increased during the growing and early static phase, remained constant during the late static phase, then decreased during the regressing phase. They added that luteal pixel value decreased during the regressing phase of the CL. Duggavathi et al. (2003), found that mean pixel value and pixels heterogeneity of the CL and serum progesterone concentration increased ($P<0.05$) from 6072 h after ovulation. They added that echotexture of the CL was closely associated with its morphological and functional characteristics. Changes in the distribution of tissue reflectors within the CL, due to changes of cells comprising the CL, could be responsible for the changes of MPV and PH of the CL during its developmental stages. An increase in PH of static phase and decrease in MPV during regressing phase of the CL was observed in this study. This could be contributed to the changes in the intraluteal blood flow at the start of and during the process of luteolysis (Acosta et al., 2002).

In the present study, a significant increase was observed in MPV of the wall and antrum of luteal cysts compared to follicular cysts. Similar results were reported by Schrick et al. (2001), who stated that luteal cyst has an obvious wall with a rougher inner lining and has the echotexture of a mature corpus luteum. They added that the antrum of luteal cysts have an echoic band-like network. In this study, there was an increase in MPV of the antrum of luteal cysts. A significant positive correlation between MPV of the wall of luteal cysts and serum progesterone level was also observed. Similar finding was reported by Singh et al. (1997) who stated that pixel values of ultrasound images were highly correlated to plasma and luteal tissue progesterone concentrations.

In conclusion, ultrasonography and computer-assisted analysis of ovarian ultrasound images revealed significant changes in the structure and echotexture of: the antrum and wall of ovarian follicles, CL, and ovarian cysts in cows. These changes are concurrent with changes in functional and endocrine characteristics of these ovarian structures. Prediction of future ovulation of the dominant follicle, growth and luteolysis of the CL, and differentiation between follicular and luteal ovarian cysts, could be concluded from echotextural characteristics of these structures. Computer-assisted ultrasound image analysis of ovarian structure in cows may be developed into not only a diagnostic but also a prognostic tool to assess the structural and functional status of the bovine ovaries.

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Table 1: Ultrasound image attributes of cyclic and cystic ovaries

Criteria	Follicle Antrum		Follicle Wall	
	Mean pixels value (MPV)	Pixels heterogeneity (PH)	Mean pixels value (MPV)	Pixels heterogeneity (PH)
Cyclic ovaries				
Ovulatory follicles	1.04 [a]	0.21 [a]	7.52 [a]	3.78 [a]
Anovulatory follicles	1.26 [b]	0.65 [b]	4.18 [b]	2.56 [b]
Subordinate follicles	1.24 [b]	0.50 [a]	4.36 [b]	2.48 [b]
Cystic ovaries				
Follicular cysts	42.73 [a]	10.34 [a]	7.65 [a]	12.79 [a]
Luteal cysts	63.66 [b]	40.92 [b]	94.30 [b]	38.93 [b]

Values are means. Means with different letters (a,b) in the same *column* and same *criteria* are significantly different (P<0.01)(LSD).

Table 2: Ultrasound image attributes of the CL and plasma progesterone concentration

	Mean pixels value (MPV)	Pixels heterogeneity (PH)	Plasma progesterone (range ng/ml)
Growing CL	12.21 [a]	3.83 [a]	0.76-1.03
Static CL	13.69 [a]	4.30 [b]	1.21-1.78
Regressing CL	07.83 [b]	3.34 [a]	0.14-0.84

Values are means. Means with different letters (a,b) in the same *column* are significantly different (P<0.01)(LSD).

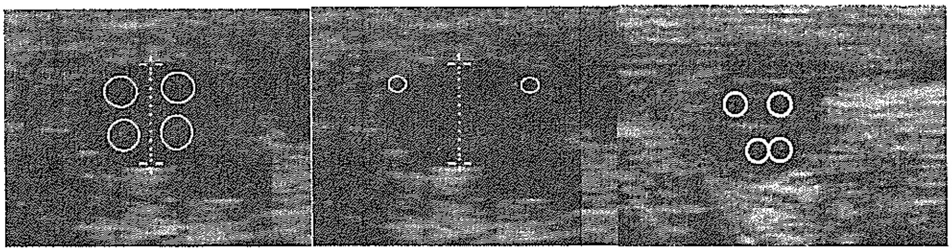

Figure 1: Sample regions for echotexture analysis of ultrasound images of: the follicle antrum (left), wall (middle), and the CL (right).

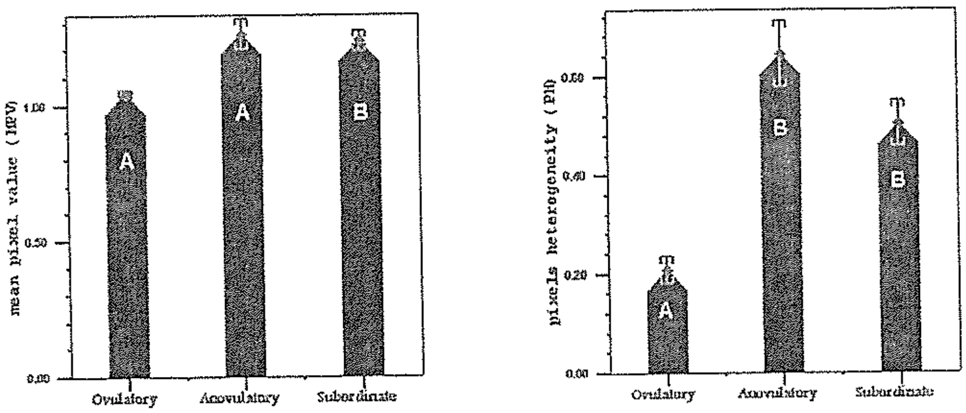

Figure 2: Mean pixel value (left) and pixels heterogeneity (right) for the antrum of dominant (ovulatory versus anovulatory) and subordinate follicles.
significantly different (LSD).

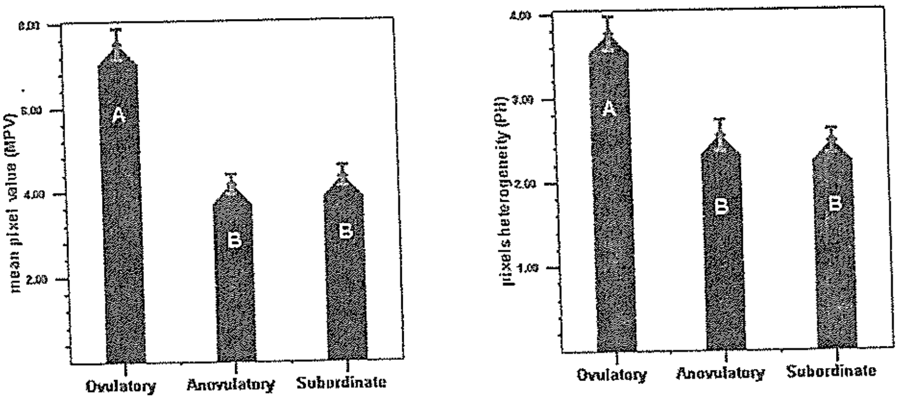

Figure 3: Mean pixel value (left) and pixels heterogeneity (right) for the wall of dominant (ovulatory versus anovulatory) and subordinate significantly different (LSD).

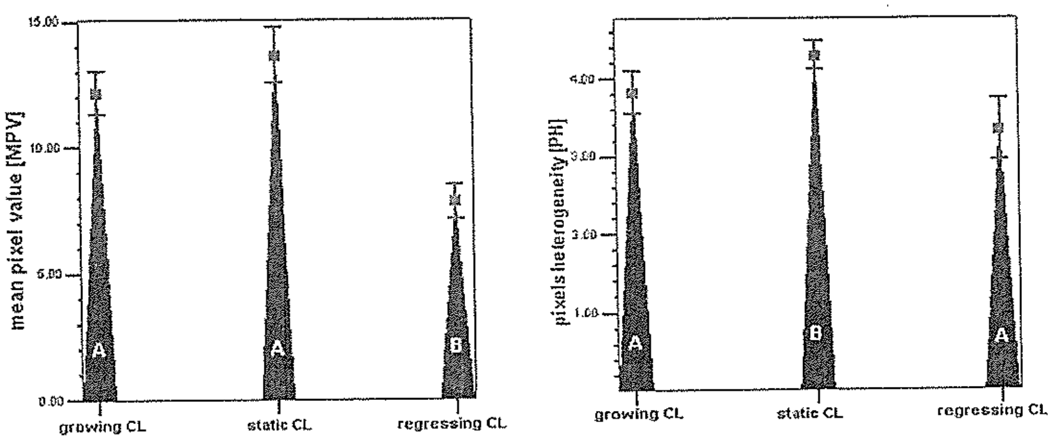

Figure 4: Mean pixel value (left) and pixels heterogeneity (right) for the significantly different (LSD).

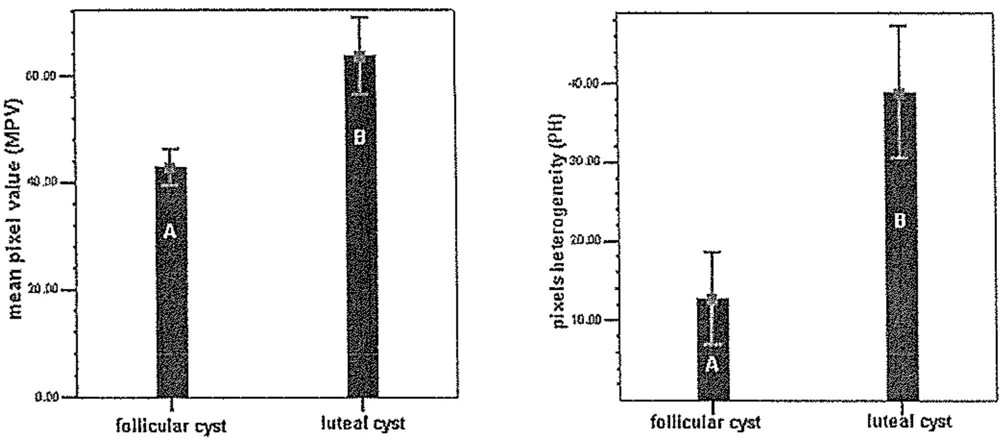

Figure 5: Mean pixel value of the antrum (left), and pixels heterogeneity of the wall (right)
no common letters are significantly different (LSD).

الملخص العربى

دراسات بالموجات فوق الصوتية على المبايض ذات النشاط الدورى والمتكيسة فى الابقار

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قسم الولادة، كلية الطب البيطرى-جامعة الاسكندرية

تهدف هذه الدراسة الى تقييم وتوصيف المركبات المبيضيه فى الابقار بواسطة التصوير بالموجات فوق الصوتية وتحليل هذه الصور (بتحليل التركيب المحاكى) ، و ربط النتائج بالحالة الوظيفية لهذه المركبات المبيضيه (الجريبات المبيضيه،الجسم الاصفر،التكيسات

المبيضـــيه). تم تقييم صور الموجات فوق الصوتيه للمركبات المبيضيه بواسطة تحليل الصور بالحاسب الالى. تم فحص المبايض فى 15 من الابقار الحلابة فى فترة ما بعد الولادة بواسطة التصـــوير بالموجات فوق الصوتية عن طريق المستقيم. فحصت سبعة من الابقار (المجموعة الاولـــى) خلال دورتى شبق وذلك لمراقبة نشاط الجريبات المبيضية وتطور الجسم الاصفر ، بيـــنما فحصـــت ثمانـــية من الابقار (المجموعة الثانية) لتوصيف التكيسات المبيضيه. تم حفظ صور الموجات فوق الصوتيه على قرص كومبيوتر وذلك لتحليل البنية المحاكى [متوسط قيمة البيكســـل (وحـــدة قـــياس الصـــور)، تجانس البيكسلات] لجدار و تجويف كل من الجريبات و التكيســـات المبيضـــيه و الجســـم الاصفر. متوسط قيمة البيكسل و تجانس البيكسلات لتجويف الجريبات اللاتبويضيه السائدة كانت منخفضة خلال بداية طور الثبات (اليوم 6) ثم زادت القيم مـــع بدايـــة طـــور التراجع (اليوم 13). بينما كان متوسط قيمة البيكسل و تجانس البيكسلات لجـــدار الجريـــبات التبويضيه السائدة مرتفعة بالمقارنة بالجريبات اللاتبويضيه السائدة. وصل تركـــيز هرمون الاستراديول فى السيرم الى اعلى مستوى عندما كانت الجريبه التبويضيه فى اكـــبر قطر لها. كان هناك ارتباطا بين متوسط قيمة البيكسل لجدار الجريبات التبويضيه السائدة و مســـتوى هـــرمون الاستراديول. كان هناك نقص معنوى فى متوسط قيمة البيكسل فى طور الـــتراجع للجســـم الاصفر مقارنة بطورى النمو والثبات. بينما زاد تجانس البيكسلات فى طور الثـــبات للجســـم الاصـــفر مقارنة بطورى النمو والتراجع. وقد وجد ترابط ايجابى معنوى بين متوســـط قيمة البيكسل للجسم الاصفر وتركيز هرمون البروجيستيرون. كذلك كان هناك ترابط ايجـــابى معنوى بين متوسط قيمة البيكسل لجدار التكيسات المبيضية وهرمون البرجيستيرون. مـــن هـــذه النتائج يمكن الاستنتاج ان التصوير بالموجات فوق الصوتية وتحليل صور المبيض بالحاســـب الالى ادى الى ظهور تغيرات معنوية فى التركيب و التركيب المحاكى للجريبات و التكيسات المبيضيه و الجسم الاصفر فى الابقار. هذة التغيرات كانت متزامنه مع التغيرات فى الخصائص الوظيفية والهرمونيه لتلك المركبات المبيضية.

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جامعة المنوفية

كلية الطب البيطرى

بمدينة السادات

مجلة البحوث الطبية البيطرية

العدد الثالث - ابريل ٢٠٠٤

عدد خاص بالمؤتمر العلمى الثالث

فى الفترة من ٢٠ - ٢١ ابريل ٢٠٠٤

تحت عنوان

الطب البيطرى والتوجهات المستقبلية لتنمية الثروة الحيوانية والداجنة والسمكية

تصدر عن كلية الطب البيطرى

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