Use of Computer-Assisted Image Analysis to Determine the Interval Before and After Ovulation

Elaine M. Carnevale, DVM, PhD; Celina M. Checura, DVM; Marco A. Coutinho da Silva, DVM, MS; Gregg P. Adams, DVM, PhD; and Roger A. Pierson, PhD

Computer-assisted image analysis was used to evaluate sequential ultrasound images from preovulatory follicles and ovulation sites. Image attributes were associated with the interval before or after ovulation. Authors’ addresses: Equine Reproduction Laboratory, Department of Biomedical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523 (Carnevale, Checura, Coutinho da Silva); Institute for Reproductive Science and Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada (Adams, Pierson). © 2002 AAEP.

1. Introduction

The development of ultrasound for use in equine reproduction has resulted in an invaluable tool for the clinician. Interpretation of ultrasound images has been limited to subjective visual assessment; however, use of advanced computer imaging systems could greatly enhance the amount and type of information available to the practitioner.

Ultrasound images are composed of an array of pixels (picture elements) that are displayed as a function of differences in tissue densities resulting from the refraction or transmission of high-frequency sound waves. Thousands of pixels collectively form the image. Each pixel represents the ability of a small, discrete unit of tissue to refract sound. The resulting image is displayed in shades of gray between 0 (black) to 255 (white). In contrast to the subjective evaluation of limited gray levels by the naked eye, use of a computer to analyze images results in the objective quantification of all pixel values (255 shades), providing sensitive and quantifiable end points.

Proper reproductive management of the mare is essential to maximize success and minimize costs in the equine industry. In recent years, mare management has become increasingly important, because more mares are being inseminated with cooled semen, frozen semen, or bred by high-demand stallions. A method to identify the number of hours before or after ovulation would help to determine when and if insemination or breeding would be beneficial and would reduce labor associated with mare management.

The study was conducted to test the hypothesis that computer-assisted image analysis could be used to determine the interval, within hours, before or after ovulation in the mare. Objectives of the study were: 1) to evaluate changes in image attributes occurring within 14 h before and 24 h after ovulation, and 2) to determine if image attributes could be
used to accurately predict the time before and after ovulation.

2. Materials and Methods

Light-horse mares were used to study sequential ultrasound images of preovulatory follicles (n = 12) or ovulation sites (n = 11). Reproductive tracts of mares were examined by using an ultrasound scanner4 that was maintained on standardized settings for focus and gain. Reproductive tracts were examined daily during estrus for the following criteria: 1) a follicle ≥ 35 mm in diameter, 2) uterine edema, and 3) relaxed tone to the uterus and cervix. When all criteria were observed, ovulation was induced through the administration of human chorionic gonadotropinb (hCG, 2500 IU, IV). Ultrasound images from preovulatory follicles were captured at 2-h intervals for at least 14 h before ovulation. For images of ovulatory sites, ovaries were scanned at 2-h intervals, beginning 32 h after administration of hCG and until 14 h after detection of ovulation. Ovulation was documented as the acute disappearance of the preovulatory follicle and visualization of the developing corpus luteum. A final image was collected at 24 h after ovulation. During scans, images of preovulatory follicles and ovulatory sites were captured on a computer interfaced with the ultrasound scanner and equipped with an image grabbing board with a resolution of 640 × 480 pixels and 255 shades of gray and stored on the local hard drive in a non-destructive image format. Images were subsequently transferred using a standardized internet file transfer protocol (ftp) to image analysis workstations in the Women’s Health Imaging Research Laboratory of the Royal University Hospital in Saskatoon, Canada. Images were analyzed by using a SparcStation 10SX and a customized program that was specifically designed for analysis of ultrasound images.

Images of preovulatory follicles or ovulation sites were divided into four roughly equal segments. Quantitative echotextural analyses were performed on the pixels comprising the images. Numerical pixel values (means of gray-scale values from 1 to 255) and pixel heterogeneity (standard deviation of gray-scale values) of sites were measured by a computer-generated spot analysis that used a 20° circular area to interrogate tissues in each of the quadrants. Changes over time were analyzed by using General Linear Models for repeated measures. When the overall model was significant, differences between time points were analyzed by protected least significant differences.

3. Results

Images of preovulatory follicles were analyzed at 2-h intervals for the 14 h before ovulation. The main effects of peak echoicity (p = 0.05), area (p = 0.01), slope of fluid and follicle interface (p = 0.05), and area and peak of area (p = 0.001) were different over time.

For images of ovulatory sites, mean pixel values changed during the experimental period (p < 0.02). Values declined between intervals 0–2 and 2–4 h (p = 0.09) and 2–4 and 4–6 h (p = 0.03) after ovulation, before increasing from 4–6 to 6–8 h (p = 0.01). Mean values between 6 and 24 h were not different. Heterogeneity of pixel values was not different (p = 0.3) over time. The numerically lowest mean pixel heterogeneity was observed at 2–4 h after ovulation, with the mean values at 2–4 h different than at 0–2 h (p = 0.01) and 6–8 h (p = 0.06).

4. Discussion

Computer-assisted image analysis of the preovulatory follicle and developing luteal gland revealed consistent changes in image attributes associated with the number of hours before or after ovulation. During the 14 h before ovulation, image attributes of the preovulatory follicle increased in an approximately linear fashion; therefore, characteristics of images could be used to closely predict ovulation time. Mean pixel values of ovulatory sites were lowest before 6 to 8 h after ovulation; therefore values could be used to aid in determining if ovulation had occurred before this time.

The ability to assess the time before or after ovulation would be beneficial for the equine practitioner. Insemination of mares close to the time of ovulation is important to maximize fertility with frozen semen or with semen from stallions with limited sperm longevity. Ability to predict the time of ovulation would assist the veterinarian in scheduling timing of insemination or of natural cover for stallions breeding large numbers of mares. Timed inseminations are usually based on the interval after administration of hCG or a GnRH agonist, which must be administered at the proper time during estrus. Failure to determine the time of ovulation occurs if the follicle does not respond, the follicle ovulates too early, or the follicle becomes abnormal (e.g., persistent anovulatory follicle). Image analysis of the preovulatory follicle could assist the practitioner in determining if the predicted ovulation time, based on hCG administration, is accurate and to reduce the use of ovulation-inducing agents. In the present study, hCG was administered to synchronize ovulations in small groups of mares. In previous research,1 follicles were monitored during estrus at 12-h intervals by palpation and ultrasound to determine changes in follicular characteristics associated with time to ovulation. Preovulatory changes in follicular characteristics were consistent with the present study. Although administration of hCG does not appear to affect follicular characteristics before ovulation, a direct comparison between mares that have or have not received hCG has not been conducted.

The success of postovulatory insemination in mares is dependant on the timing of insemination in relation to ovulation. Insemination of mares with fresh semen at 0–6 h after ovulation resulted in a
high pregnancy rate (11/14, 79%), but pregnancy rates declined in a linear fashion with progressing time after ovulation (6–12 h, 13/20, 69%; 12–18 h, 7/14, 50%; 18–24, 7/21, 33%). The recommended interval for the postovulatory insemination of mares with frozen semen is within 6 h after ovulation. Using computer-assisted image analysis, it is possible to determine if insemination after ovulation might be cost and labor effective.

In other species, computer-assisted image analysis has been used as a diagnostic and research tool. In heifers, attributes of ultrasound images were correlated with the functional and endocrine status of follicles. Differences in follicular attributes were observed in growing versus atretic follicles of heifers and women. Images of the bovine corpus luteum were reflective of histomorphological characteristics and progesterone production. Use of computer-assisted image analysis is now being investigated as a research and clinical tool in equine reproduction. Currently, image analysis is being evaluated as a method to diagnose and evaluate abnormal follicles, such as persistent anovulatory follicles.

In the present study, three clinicians performed ultrasound exams. Images were captured at approximately the largest cross-sectional area of the preovulatory follicle or ovulation site. No apparent effect of clinician was observed; however, all clinicians were experienced and were careful to minimize artifacts during image collection. For the experiment, an ultrasound machine with good image quality and a computer were required. Additional costs were minimal. However, images had to be transferred through the internet to a laboratory equipped for image analysis. Further development is needed to establish the system for use on farms or in veterinary clinics.

Results of this study illustrate the potential of computer-assisted image analysis as a diagnostic technique in equine reproduction.

The study was funded through the Colorado Equine Racing Commission through the Research Council of the College of Veterinary Medicine and Biomedical Sciences. Original research in RAP laboratory was funded by the Canadian Institutes for Health Research.

References and Footnotes

aSSD-900V, Aloka Co. Ltd., Wallingford, CT, 06492.
bIntervet Inc., Millsboro, DE, 19966.
cSun Microsystems, Mt. View, CA, 94040.
dSynergyne 1©, Saskatoon, Canada.