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## Ultrasonography as Aid for Early Pregnancy Diagnosis in Zebu Cattle in a Natural Mating Programme

L.L. Galicia<sup>1</sup>, K.S. Estrada<sup>2</sup>, C.S. Galina<sup>1\*</sup>, G.E. Pérez<sup>2</sup>,  
J.J. Romero, S.R. Molina<sup>3</sup>

<sup>1</sup>Departamento de Reproducción  
Facultad de Medicina Veterinaria y Zootecnia  
Universidad Nacional Autónoma de México, Mexico

<sup>2</sup>Programa de Investigación en Medicina Poblacional  
Escuela de Medicina Veterinaria  
Universidad Nacional de Costa Rica, Costa Rica

<sup>3</sup>Departamento de Agronomía  
Sede Regional Santa Clara  
Instituto Tecnológico de Costa Rica, Costa Rica

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### Abstract

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*To utilise ultrasonography in Zebu cattle to test early pregnancy, multiparous **Bos indicus** cows (n=74) were allocated with 2 mature Brahman bulls, which were replaced with new ones every four weeks. Cows were bled for radioimmunoassay progesterone analysis at the time of ultrasound and four days prior or after this event. The efficiency of the ultrasound findings and progesterone levels were compared with Kappa index using the computer programme (WIN) EPISCOPE. Ultrasound precision for early pregnancy diagnosis was 96%. Moreover, animals not clearly defined as pregnant, following examination revealed that only 54% were actually pregnant. The efficiency to detect the lack of ovarian activity was only 25% as revealed by progesterone concentrations lower than 1ng/ml. In contrast, the association between a detectable CL by ultrasound and progesterone values higher than 1ng/ml was 90%. The incidence of embryonic death was 15.4%. Based on these results the efficacy for detecting early pregnancy by ultrasound and the presence of an active CL is satisfactory, whereas the precision to detect non-cycling animal is questionable and the incidence of embryonic death is high.*

Key words: Pregnancy diagnosis, *Bos indicus*, cattle, ultrasonography.

### Introduction

Pregnancy diagnosis by rectal palpation of the uterus in cattle is inexpensive and,

therefore, commonly used. However, when carried out before the 40th day of conception a slightly increased incidence of embryonic mortality may result (Alexander *et al.*, 1995). The advantage of transrectal ultrasonography over rectal palpation is that the presence of

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\*E-mail: cgalina@servidor.unam.mx; Phone: (525) 56225860.

the embryo can be detected earlier and direct physical manipulation of the gravid reproductive tract is not necessary (Beal *et al.*, 1992) thus potentially reducing the risk of embryonic death. Most of the work on ultrasonography has been carried out in dairy herds, in which the exact date of insemination or mounting is recorded. In contrast, in grazing beef cattle the date of mating is usually unknown.

In *Bos indicus* cattle embryonic mass and heartbeat can be accurately determined from day 23 (Rosiles *et al.*, 2005). The precision of ultrasound for pregnancy diagnosis increases as long as pregnancy continues (Pieterse *et al.*, 1990b, Rosiles *et al.*, 2005).

There is a need for prompt evaluation of conception during a breeding programme, so that reproductive interventions in non-pregnant animals can be implemented as soon as possible. The objective of the present study is to evaluate how early animals can be detected via ultrasound following a natural mating programme.

### ***Materials and Methods***

Seventy-four Brahman and cross-bred cows (*Bos indicus*), with  $161 \pm 94$  days postpartum and  $2.4 \pm 1.7$  calvings on average were used. At the beginning of the trial, 58% had a body condition score from 1-1.5 on a scale from 1 to 5 (Edmonson *et al.*, 1989). Animals were kept in a single group along with two adult Brahman bulls (3-5 years, previously evaluated for reproductive soundness) for 4 week periods at the end of which the bulls were replaced by two new bulls as previously described (Molina *et al.*, 2002). The experiments lasted for four months so in all 8 bulls were used.

Ultrasonic pregnancy diagnosis was carried out using an Aloka SD-500 with a 5.0 MHz transrectal probe and recorded on a VHS cassette for further analysis. Ultrasound examination was performed 31

days after the first bulls were introduced and again every 16 days making a total of 8 examinations. The first identified cows that became pregnant in the first seven days after the bulls were added to the herd and the rest to sequentially detect pregnancies occurring during the mating period according to the methodology proposed by Rosiles *et al.* (2005).

On ultrasound examination animals were classified as: 1) not cycling, cows without ovarian structures; 2) cycling, cows with a corpus luteum defined as a highly echogenic structure (Pierson and Ginther, 1984); 3) pregnant, cows with an amniotic vesicle, embryonic mass and heartbeat (Curran *et al.*, 1986); 4) possibly pregnant, cows with fluid filled uterus and corpus luteum but not clearly identifiable embryo.

Embryonic mortality occurred when the cows diagnosed as pregnant were detected later as cycling or non-pregnant.

In addition, serum samples were collected for progesterone determination from 74 cows for 4 days prior and 4 days after ultrasound examinations. Serum was separated in aliquots and kept at -20C until processed for progesterone concentration by RIA (Diagnostic Products Corporation). Levels above 1 ng/ml were considered as indicative of the presence of a corpus luteum.

Embryos were aged according to the scale developed for *Bos indicus* cattle by Rosiles *et al.* (2005).

Descriptive statistics were used to determine the accuracy of ultrasound in pregnancy diagnosis in the cows. The agreement between the ultrasound results and progesterone concentrations was determined by the *Kappa* index, using a computer program WinEpiscope 2.0 (Thrusfield *et al.*, 2001). Sensitivity, specificity and predictive values for pregnancy diagnosis by ultrasound were calculated by 2 x K tables using the same program.

## Results and Discussion

The first embryos were observed on day  $31 \pm 5$ . Embryo size for *Bos indicus* cattle is similar to that reported for *Bos taurus* up to day 50 of pregnancy. The females became pregnant on average  $40 \pm 29$  days after the programme started. Fifty per cent of the pregnant animals were cows calving for the first and second time. Average days postpartum when pregnancy occurred were  $206 \pm 97$  and body condition  $2.0 \pm 0.5$ . The time post-service when a positive diagnosis can be achieved with certain degree of confidence was  $31 \pm 5$ . This is similar to the findings reported by Rajamahendran *et al.* (1994). Other authors mention different times for positive diagnosis, for example around day 20 (Curran *et al.*, 1986; Pierson and Adams, 1994) or from day 26 to 29 is when embryos can be observed for the first time with 98.3% accuracy (Pierson and Ginther, 1984). The discrepancies between these studies and the present findings might be that in earlier communications the breeding date was known. In a recent study carried out in *Bos indicus* with known breeding dates, the embryonic vesicle was first observed between days 18 and 20 and heartbeat detected by day 23 (Rosiles *et al.*, 2005).

Early embryonic mortality rate in this study was 15.4% (8/52) and cows averaged  $183.7 \pm 96.7$  days postpartum. Gregory *et al.* (1996) found that embryonic mortality diagnosed by rectal palpation to confirm earlier ultrasound findings, was 13.3%. Body condition plays an important role and is linked to nutritional status. Animals with a negative energy balance may start cycling but later undergo anestrus after embryonic mortality takes place (Butler, 1995). In our study, embryonic mortality occurred in cows calving for the second or third time (75%), which agrees with Hafez (1996), who found that embryonic mortality is more common in young cattle than in those 6 years or older.

In 58% of the cases where cows were diagnosed as not having ovarian structures, the same diagnosis was obtained at the next check. Similarly, when the cows were diagnosed as cycling, 50% were similarly diagnosed at the next examination, and only 18% were diagnosed as pregnant. Animals were diagnosed as pregnant by ultrasound, were confirmed as pregnant on the next examination in 96% of the cases. Furthermore, when animals were diagnosed as possibly pregnant, only 54% of them were in fact pregnant.

The potential of the ultrasonographic examination as a pregnancy test has several advantages. For instance, embryos can be detected earlier than by rectal palpation and the manual manipulation of the reproductive tract is less harmful than ultrasound (Beal *et al.*, 1992). Another advantage is the early diagnosis of a live embryo.

Ultrasound examination showed that 4.6% of the females diagnosed as not cycling, were pregnant at the next examination. This might be due to the corpus luteum being too young or immature for detection (Pieterse *et al.*, 1990a). Another possibility is that some females were not cycling but began to cycle between testing periods.

Sensitivity was 54% (Table 1) and specificity 87.5%. The positive predictive value of females detected as having a corpus luteum and those actually had one was 96% and the negative predictive value indicating the ratio of females diagnosed as not having corpus luteum and those actually did not have one was 25%.

The accuracy of ultrasound for the diagnosis of a corpus luteum was 10% lower than that reported earlier (Pierson and Ginther, 1987) for *Bos taurus* cows between days 12 and 14 of the cycle. This discrepancy is probably related to the young corpus luteum appearing as a bright-grey echogenic structure, two to four days after ovulation

Table 1

Sensitivity and specificity values for predictive values for the positive and the negative diagnosis by ultrasound for the presence of corpus luteum compared to progesterone concentrations

| Corpus luteum      | P4 levels at RIA |                 |
|--------------------|------------------|-----------------|
|                    | P4>1ng/ml        | P4<1ng/ml       |
| Present            | 96 <sup>a</sup>  | 4 <sup>b</sup>  |
| Not present        | 84 <sup>c</sup>  | 28 <sup>d</sup> |
| Total              | 180              | 32              |
| Sensitivity        | 53.3%            |                 |
| Specificity        | 87.5%            |                 |
| + Predictive value | 96.0%            |                 |
| - Predictive value | 25.0%            |                 |

and difficult to distinguish from the ovarian stroma. Thus, in some cases the old corpus luteum of a previous cycle might be seen besides a young developing corpus luteum. Usually, the corpus luteum of an earlier cycle is not detected probably as it has the same echogenicity as the ovarian stroma (Pieters *et al.*, 1990a). Pieterse *et al.* (1990a) reported a sensitivity of 64.3% for corpus luteum detection with a lower positive predictive value of 67.9%. Besides, Gutiérrez *et al.* (1996) mentioned a higher sensitivity value than that found in the present study with a similar positive predictive value and a higher negative predictive value. One factor affecting the accuracy of corpus luteum detection in *Bos indicus* is its small size. Diagnosis is made even more difficult by the fact that it does not exceed the limits of the ovarian stroma (Aguilar *et al.*, 1983). Another factor is the time postpartum when the diagnosis is established. Studies indicate that detection is easier as the postpartum period progresses from 37.5% up to day 70 after calving to 73.7% from day 71 to day 110 postpartum (Silva *et al.*, 1992).

The sensitivity of ultrasound to detect gestation is 82.8% and its specificity for the detection of not pregnant females was 89.7% (Table 2). The positive predictive value

Table 2

Sensitivity and specificity values for positive and negative predictive values of ultrasound for diagnosis of early pregnancy when compared to progesterone concentrations

| Early pregnancy at ultrasound | P4 levels at RIA |                 |
|-------------------------------|------------------|-----------------|
|                               | P4>1ng/ml        | P4<1ng/ml       |
| Yes                           | 135 <sup>a</sup> | 11 <sup>b</sup> |
| Not                           | 28 <sup>c</sup>  | 96 <sup>d</sup> |
| Total                         | 163              | 107             |
| Sensitivity                   | 82.8%            |                 |
| Specificity                   | 89.7%            |                 |
| + Predictive value            | 92.5%            |                 |
| - Predictive value            | 77.4%            |                 |

indicates that the proportion of females diagnosed as pregnant which were actually pregnant, was 92.4%. The negative predictive value corresponding to the proportion of females diagnosed as non-pregnant, and found not be pregnant was 77.7%.

Ultrasound precision for pregnancy diagnosis was 96% which agrees with the figure of 98% reported by Pierson and Ginther (1984) in dairy herds between days 26 to 29 of gestation. Ultrasound precision may depend on the pregnancy age at which ultrasound is performed (Pieterse *et al.*, 1990b). Most studies suggest increasing precision as pregnancy advances from an estimated 25 days, in which the precision was of 96%.

Sensitivity of 83% obtained in the present study is the same as that reported by Badtram *et al.* (1991) for dairy herds but below the 97.7% found by Pieterse *et al.* (1990b) for days 26 to 33 of pregnancy and above 44.8% published by the same authors for days 21 to 25 of pregnancy. This difference might be due to the fact that in dairy unlike beef herds, pregnancy diagnosis is performed earlier as the exact dates of breeding or service are known. Therefore, the diagnosis of the amniotic vesicle as a positive sign of

early pregnancy might be confused with the fluid that is naturally accumulated during proestrus and estrus.

## References

- Aguilar, A., Galina, C.S. and Hummel, J. 1983. Estudio morfológico comparativo de los ovarios de la vaca cebú y la vaca Holstein. *Veterinaria México*, 14: 133-136.
- Alexander, B., Johnson, M., Guardia, W., Van de Graff, W., Senger, P. and Sasser, R. 1995. Embryonic loss from 30 to 60 days post breeding and the effect of palpation per rectum on pregnancy. *Theriogenology*, 43: 551-556.
- Badtram, G., Gaines, J., Thomas, C. and Bosu, W. 1991. Factors influencing the accuracy of early pregnancy detection in cattle by real-time ultrasound scanning of the uterus. *Theriogenology*, 35: 1153-1167.
- Beal, W., Perry, R. and Corah, L. 1992. The use of ultrasound in monitoring reproductive physiology of beef cattle. *J. Anim. Sci.*, 70: 924-929.
- Butler, W. 1995. Postpartum negative energy balance, ovarian activity and fertility in dairy cows. *Memorias del Sexto Curso Internacional de reproducción de los bovinos*, México, Pág. 30-35.
- Curran, S., Pierson, R. and Ginther, O. 1986. Ultrasonic appearance of the bovine conceptus from 20 through 60 days. *J. Amer. Vet. Med. Assoc.*, 189: 1295-1302.
- Edmonson, A.J., Lean, I.J., Weaver, L.D., Farver, T. and Webster, G.A. 1989. Body condition scoring chart for Holstein cows. *J. Dairy Sci.*, 75: 68-78.
- Gregory, R.M., Mattos, R.C. and Lamprecht, M. 1996. Embryonic mortality in bovine ultrasonographic evaluation. *Arq. Fac. Vet. UFRGS, Porto Alegre.*, 24: 25-29.
- Gutiérrez, C., Zarco, L., Galina, C.S., Rubio, I. and Basurto, H. 1996. Predictive values of palpation per rectum for detection of the cl in Zebu cattle as evaluated by progesterone concentrations and ultrasonography. *Theriogenology*, 46: 471-479.
- Hafez, E.S. 1996. *Reproducción e Inseminación Artificial en Animales*. México, Editorial Interamericana. Pág. 439-444.
- Molina, R., Galina, C.S., Camacho, J., Maquivar, M., Diaz, G.S., Estrada, S. and Martínez, L. 2002. Effect of alternating bulls as a management tool to improve the reproductive performance of suckled Zebu cows in the humid tropics of Costa Rica. *Anim. Rep. Sci.*, 69: 159-173.
- Pierson, R. and Adams, G. 1994. Uso de la ultrasonografía para el estudio de los eventos reproductivos en el bovino. *Memorias del I Curso internacional sobre Transplante de Embriones en Bovinos*, Costa Rica.
- Pierson, R. and Ginther, O. 1984. Ultrasonography for detection of pregnancy and study of embryonic development in heifers. *Theriogenology*, 22: 225-233.
- Pierson, R. and Ginther, O. 1987. Follicular populations during the estrous cycles in heifers. I. Influence of day. *Anim. Reprod. Sci.*, 14: 165-176.
- Pieterse, M., Taverse, M., Kruij, A. and Willemsse, A. 1990a. Detection of corpora lutea and follicles in the cows: A comparison between transvaginal ultrasonography and rectal palpation. *Veterinary Record*, 126: 552-554.
- Pieterse, M., Szenci, A., Willemsse, A., Bajosy, C., Dieleman, S. and Taverne, M. 1990b. Early pregnancy diagnosis in cattle by means of linear-array real time ultrasound scanning of the uterus and a qualitative and quantitative milk progesterone test. *Theriogenology*, 33: 697-708.
- Rajamahendran, R., Divakar, J. and Burton, B. 1994. Clinical and research applications of real-time ultrasonography in bovine reproduction: A review. *Can. Vet. J.*, 35: 563-572.
- Rosiles, V.A., Galina, C.S., Maquivar, M., Molina, R. and Estrada, S. 2005. Ultrasonographic screening of the embryo development in cattle (*Bos indicus*) between days 20 and 40 of pregnancy. *Anim. Reprod. Sci.* (in press).
- Silva, E., Galina, C.S., Porras, A.A. and Galina, M.A. 1992. Evaluación de la actividad ovárica por medio de la palpación rectal, observación de calores y los niveles de progesterona en vacas lecheras explotadas en el trópico seco. *Ciencias Veterinarias (Costa Rica)*, 14: 5-11.
- Thrusfield, M., Ortega, C., de Blas, I., Noordhuizen, J.P. and Frankena, K. 2001. WIN EPISCOPE 2.0: Improved Epidemiological software for Veterinary Medicine. *Vet. Rec.*, 148: 567-572.

एल०एल० गैलिसिया, के०एस० एस्ट्रेडा, सी०एस० गैलिना, जी०ई० पेरेज़, जे०जे० रोमेरो, एस०आर० मोलिना।

भारतवंशी गो पशुओं में प्राकृतिक मैथुन कार्यक्रम में आरंभिक गर्भ निदान हेतु अल्ट्रासोनोग्राफी की सहायता।

भारतवंशी गो पशुओं में आरंभिक गर्भ जांचने हेतु अल्ट्रासोनोग्राफी के उपयोग के लिए बहुब्यांती भारतवंशी गायों (72 बॉस इंडिकस) को दो प्रौढ़ ब्राह्मनी साड़ों के साथ रखा गया, जिन्हें प्रत्येक चार सप्ताह के अंतराल पर बदल दिया गया। अल्ट्रा साउन्ड के समय और उसके चार दिन पूर्व या चार दिन पश्चात् रेडियोइम्युनोएस्से प्रोजेस्ट्रान विश्लेषण के लिए गायों से रूधिर निकाला गया। कम्प्यूटर कार्यक्रम (डब्ल्यू आई एन) ईपीआईएससीओपीई के काप्पा सूचकांक के उपयोग से अल्ट्रासोनोग्राफी परिणामों और प्रोजेस्ट्रान स्तरों की

तुलना की गयी। आरंभिक गर्भ निदान के लिए अल्ट्रासाउण्ड की परिशुद्धता 96% थी। वैसे इस जांच से अगर्भित परिणाम वाली गायों में भी 54% वास्तव में गर्भित थे। अंडधानी सक्रियता की अनुपस्थिति ज्ञात करने में प्रोजेस्ट्रान सांद्रता की दक्षता मात्र 25% थी, जो कि प्रोजेस्ट्रान के प्रति मिली 1 मैनो ग्राम से भी कम थी। इसके व्यतिरेक, अल्ट्रासाउण्ड से अभिज्ञेय कार्पस ल्यूटियम और 1 नैनोग्राम प्रति मिलि से अधिक प्रोजेस्ट्रान सांद्रता में

90% से अधिक संगुणन था। भ्रूण मृत्यु की घटना 15.4% थी। परिणामों के आधार पर अल्ट्रासाउण्ड से आरंभिक गर्भ ज्ञात करने की और सक्रिय कार्पस ल्यूटियम की उपस्थिति जानने की दक्षता संतोषजनक थी, जबकि अचक्री पशुओं में ज्ञात करने की परिशुद्धता संदेहास्पद है और भ्रूणीय मृत्यु की घटना अधिक है।