

USE OF ANTIOXIDANTS TO IMPROVE THE PREGNANCY RATE IN BEEF CATTLE SUBMITTED TO A SYNCHRONIZATION PROTOCOL WITH PROGESTERONE (P4)

RICARDO ZANELLA,¹ CARLOS BONDAN,² JULIO CESAR MENDES SOARES,² ERALDO LOURENÇO ZANELLA² E MARINA RAGAGNIN DE LIMA³

1. Veterinary Doctor, doctorate student at Washington State University, Department of Animal Science Pullman WA, 99163.

E-mail: zanellar@wsu.edu

2. Veterinary Doctors, professors of Veterinary Medicine at Faculty of Agronomy and Veterinary Medicine, Universidade de Passo Fundo

3. Veterinary Doctor, Caçapava do Sul, RS.

ABSTRACT

Estrus synchronization with progesterone is commonly used in farms with better reproductive control. Free radicals production, i.e. oxidative stress, is associated with progesterone levels. The oxidative stress is responsible for aggression to the cellular membrane, leading to a lise and lipoperoxide formation. In this work, the antioxidant compensatory effect (Vitamins C and E) associated with the exogenous progesterone implant (P4), used in estrus synchronization protocols in cattle, was evaluated. Twenty-five cows were randomly selected in 5 different groups:

1) control without P4, 2) control with P4, 3) P4 + vitamin C and E, 4) P4 + vitamin E, 5) P4 + vitamin C. The lipid lipoperoxidation was measured through thiobarbituric acid reactive substances (TBA-RS) and glutathione peroxidase enzyme (GSHpx) at days 0 and 7 of the estrus synchronization protocol. The use of vitamin E, in this experiment, showed a better pregnancy rate, however, the results must be validated before orienting the use of the vitamin in cows synchronized with P4.

KEYWORDS: Antioxidants, cows, oxidative stress, pregnancy rate, progesterone.

RESUMO

USO DE ANTIOXIDANTES PARA MELHORAR A EFICIÊNCIA REPRODUTIVA DE REBANHO BOVINO SUBMETIDO A PROTOCOLO DE SINCRONIZAÇÃO COM PROGESTERONA (P4)

Progestágenos para a sincronização de estro têm sido utilizados como rotina em propriedades com melhor controle reprodutivo. A produção de radicais livres, estresse oxidativo, está relacionada com os níveis de progesterona circulante. O estresse oxidativo é responsável pela agressão à membrana celular, causando lise e a formação de lipoperoxídeos. Neste trabalho, buscou-se avaliar o efeito compensatório de antioxidantes (vitaminas C e E) associados ao implante de progesterona exógena (P4), utilizada em protocolos de sincronização de estro em fêmeas bovinas. Vinte e cinco fêmeas bovinas foram divididas aleatoriamente em cinco

grupos: 1) controle sem implante de P4; 2) implante de P4; 3) implante de P4 + vitamina C e E; 4) implante de P4 + vitamina E; 5) implante de P4 + vitamina C. Avaliaram-se a lipoperoxidação lipídica, através da mensuração de substâncias reativas ao ácido tiobarbitúrico (TBA-RS), e a enzima glutathione peroxidase (GSHpx), nos dias zero e sete do protocolo de sincronização de estro. A utilização de vitamina E, neste experimento, apresentou uma maior taxa de prenhez; entretanto, existe a necessidade de validar esses resultados, antes que seu uso seja preconizado em fêmeas bovinas sincronizadas com P4.

PALAVRAS-CHAVES: Antioxidantes, estresse oxidativo, progesterona, taxa de prenhez, vacas.

INTRODUCTION

The development and use of reproductive biotechniques, synchronization and estrus induction in beef cattle are fundamental for the Brazilian production system. The concentration of mating activities with good conception rates allows females to show a good parturition rate at the most appropriate moment of the year. (BAYARD et al., 2002). Researches have demonstrated that the levels of success of estrus synchronization varies according to the protocol used, but they are usually effective. However, the level of animal fertility is reduced with the use of exogenous progesterone implants, as described by AINSWORTH & SHRESTHA (1983). Recent studies of estrus synchronization presented pregnancy rates between 37.68 and 57.6% (CÂMARA et al., 2006; BORGES et al., 2009).

In bovine culture, management flaws, such as estrus misidentification, are one of the great problems which lead to low pregnancy rate in the herd. Because of that, the use of hormone therapy, with the intent of synchronizing estrus, and of fixed-time artificial insemination (FTAI) became important (BARROS et al., 1995). Exogenous progesterone (P4), estrogen (E2), equine chorionic gonadotropin (eCG) and GnRH have been broadly used in estrus synchronization and ovulation protocols, including in animals in anestrus (ROCHE et al., 1992; JOLLY et al., 1995; MORAES & JAUME, 1997; CÂMARA et al., 2006; BORGES et al., 2009). The purpose of utilizing these hormones is to mimic estrous cycle, as well as stimulate the hypothalamus-hypophyseal-gonadal axle of the females and the cells of such structure (GONZALEZ-PADILHA et al., 1975; TROXEL et al., 1993; KESLER & FAVERO, 1996; WILTBANK et al., 1996; BAYARD et al., 2002). LUCY et al. (2001) mention that the pregnancy rate of cows synchronized with vaginal device presented higher indices than the control group, without the device. SIMONETTI et al. (1999), in an experiment with ovises, obtained 92.86% of estrus, but only 50.43% of pregnancy. The low reproductive efficiency indices were related to the use of progestans as estrus modelling; however, the reasons are not

entirely known yet.

The increase of the P4 levels circulating is directly related to the increase/elevation of the levels thio-barbituric acid (TBA-RS) (MÁRQUEZ et al., 1997). On the other hand, E2 has a contrary effect to the ones of P4, inhibiting the oxidative stress process (EJIMA et al., 1999; CHENG et al., 2006). The levels of TBA-RS indicate damages to the cellular membrane due to the production of reactive oxygen species (ROS), which may have a deleterious effect on eggs and embryos. MILLER & BRZEZINSKA-SLEBODZINSKA (1993) documented the negative effect of the reactive oxygen species (ROS) on metabolism and health of dairy cows. Nevertheless, such effect may be neutralized or reduced by enzymatic and non-enzymatic antioxidative mechanisms.

In this study, an ovulation synchronization protocol, with intravaginal devices with medroxi-progesteron (MPA), associated with intramuscular administration of estradiol benzoate, prostaglandin and equine chorionic gonadotropin (eCG), was used for the synchronization of ovulation and estrus. At the same time, antioxidative vitamins were administrated aiming at evaluating their effect on the oxidation level and pregnancy rate in beef cattle. The hypothesis of this study is that the utilization of exogenous P4 as a synchronization method increases the lipid peroxidation in preovulatory follicles, leading to problems in the oocyte development, causing, hence, low pregnancy rate.

MATERIAL AND METHODS

Crossbred Nellore X Angus cows (n=25), from Agropecuária Zanella (Zanella Livestock), municipality of Paim Filho, North of Rio Grande do Sul State, were used in this experiment. The animals were classified according to their body condition score (BCS) from one to five, being one considered thin and five obese (GRAHAM, 1982). The animals were randomly divided into five different groups, with five animals each: (1) control group without P4 implant; (2) P4 implant; (3) P4 implant with vitamins C and E administration; (4) P4 implant with vitamin E administration;

(5) P4 implant with vitamin C administration. Estrus synchronization protocol was carried out according to description by BAYARD et al. (2002), with the application of medroxyprogesteron (MAP 250mg) by an intravaginal device for seven days. An injection of 2.5 mg estradiol benzoate, 3.0 ml of vitamin C (150 mg/mL; BRAVET Laboratories, RJ) and 5.0 ml of vitamin E (100 mg/mL; BRA-VET laboratories, RJ) was applied intramuscularly (IM) on day zero (d0). On day six, eCG (100mg) was applied intramuscularly (IM) and PGF2a (25 mg) via vulvar mucosa. All the procedures were carried out according to the ethic principles for animals experimentation recommended by the Colégio Brasileiro de Experimentação Animal (COBEA, Brazilian School of Animal Experimentation).

Blood samples were collected by venipuncture of the jugular vein after antisepsis, using disposable needles and sterilized sample tubes. The collected heparinized blood was conserved under refrigeration at 4°C and used for analysis right after the collection for measuring the levels of lipoperoxide on days zero and seven of the synchronization protocol.

The quantification of the cellular peroxidation was carried out by the thiobarbituric acid reactive substances (TBA-RS) measurement technique. The heparinized samples were centrifuged at 2000 g for five minutes and the supernatant was discarded and replaced by a 0.9% NaCl physiological solution with same volume. This procedure was carried out twice, and, after that, the blood cells concentrate was resuspended with an hematocrit of 50%. After erythrocytes washing with NaCl 150 mM, the samples were precipitated with acetic acid 40% in a 1:2 proportion and centrifuged at 2000 g for the separation of the supernatant. Lipid peroxidation was determined according to the method of thiobarbituric acid reactive substances (TBA-RS) formation, used by OHKAWA et al. (1979), and non-proteic sulfhydryl groupings (NPSH), by ELLMANS (1959). Sixty days after the insemination of the animals, pregnancy was detected by transrectal palpation. The values of TBA-RS and GSHpx production were submitted to ANOVA test, followed by Tukey test, considering the differences were significant when $p < 0.05$.

RESULTS AND DISCUSSION

Hormone therapy is an indispensable tool for manipulation and improvement of the use of biotechnology in beef cattle. The greatest challenge for the use of such biotechnology is the obtainment of better reproductive rates in livestock production. Different alternatives have been utilized, such as the P4, to try to concentrate the estrus and ovulation synchronization activities and, hence, parturition period in a small interval. However, when this method is used, pregnancy rate is under the expectation, considering the increase of the cost with animal production.

In this experiment, on the general, pregnancy rate was 52%. This index is compatible with the results observed by BORGES et al. (2009), who obtained 57.6% of pregnancy rate. It should be considered that in this research non-suckling cows were used, that is, cows without the calves, whereas in the research mentioned suckling cows were used, with the calves removal for four days. Besides, the number of cows in this experiment was small when compared with the other work.

The pregnancy rate in the control groups (with and without P4) was 40%. The production levels of TBA-RS in the control group with synchronization protocol with and without P4 were higher on day seven than on day zero ($p < 0.0002$) in the same group, confirming, thus, the activity of exogenous and endogenous progesterone with the production of ROS, already described by Márquez et al. (1997). Consequently, there was a proportional increase of the production of GSHpx enzyme, suggesting a compensatory mechanism to inhibit ROS activity ($p < 0.0002$).

The pregnancy rate in the group treated with vitamin C was 40%. In this group, the production levels of TBA-RS were smaller on day seven than on day zero ($p < 0.05$), what is likely to suggest whether an antioxidative effect of vitamin C or that the animals were in estrous period between days seven and eighteen of the first collect, when there is a greater production of endogenous progesterone. This result corresponds to the description by Márquez et al. (1997), in which high levels of progesterone are found in the presence of a

functional and mature corpus luteum, what is directly related to the increase of the levels of thiobarbituric acid reactive substances (TBA-RS). There is a significant increase of oxidative stress with the mature corpus luteum than with luteal regression, showing low levels of TBA-RS and serum progesterone (MÁRQUEZ et al., 1997). Even the reduced levels of TBA-RS on day seven, GSHpx levels were not higher than on day zero. Although this increase was not significant, it may suggest a compensatory mechanism against ROS production.

The association of vitamins C and E caused an increase of TBA-RS production on day seven compared to day zero, suggesting a pro-oxidative effect of vitamins C and E. In this case, the GSHpx enzyme had also a compensatory effect to keep ROS production balanced. Nevertheless, even with the pro-oxidative effect the pregnancy rate was not different (40%), comparing to control-groups and treated with vitamin C and vitamins C and E. High doses of antioxidative vitamins may have a deleterious effect on the animal's organism. For instance, ascorbic acid (vitamin C) might act as a pro-oxidative, leading to the production of radical hidroxila, which is highly reactive, causing oxidative damages to the cellular membrane.

In the group treated with vitamin E, the levels of TBA-RS and GSHpx were higher on day seven than on day zero, demonstrating, thus, the compensatory effect this enzyme has on the production of ROS. Pregnancy rate of the group with vitamin E was 100%, demonstrating the efficiency of associating this enzyme to the protocol ($p < 0.05$). The use of vitamin E as an antioxidative resulted in higher pregnancy rates. These data are in accordance with the ones previously published by LAFLAMME & HIDIROGLOU (1991) and RICHARDSON et al. (2008), when a significant increase of pregnancy rates in cows and heifers, supplemented with vitamin E, was observed.

The interaction mechanism between antioxidative vitamins and the reduction of embryony losses is still unknown. Nevertheless, associations between vitamin E and uterine health have been observed. Such results confirm the ones reported by EVANS & BISHOP (1922), who administered vitamin E in fema-

le rats and observed reduction of embryony losses and fetal absorption. The findings of this experiment support the hypotheses of the inhibiting association of antioxidative vitamins and the levels of lipidic peroxidation. It can be concluded, thus, that the association of vitamin E to the estrus synchronization protocol with the use of P4 is beneficial for the obtainment of good reproductive results. The carry-out of further experiments with a higher number of animals is suggested for a better evaluation of such results.

ACKNOWLEDGEMENTS

To the owners of Agropecuária Zanella (Zanella Livestock), Mr. Ipenor Zanella and Mrs. Nair Zuleika Zanella, for supplying the animals for this study..

REFERENCES

- AINSWORTH, L.; SHRESTHA, J. N. B. Effect of type of intravaginal progestagen treatment on estrous response and reproductive performance of ewes. **Theriogenology**, v. 19, p. 869-875, 1983.
- BARROS, C. M.; FIGUEIREDO, R. A.; PINHEIRO, O. L. Estro, ovulação e dinâmica folicular em zebuínos. **Revista Brasileira de Reprodução Animal**, v. 19, p. 9-22, 1995.
- MORAES, J. C. F.; SOUZA, C. J. H.; GONÇALVES, P. B. D.; FREITAS, V. J. F.; LOPES JÚNIOR, E. S. Controle do estro e da ovulação em bovinos e ovinos. In: BAYARD, P. D. G.; FIGUEIREDO, J. R.; FREITAS, V. J. F. **Biotécnicas aplicada à reprodução animal**. 2. ed. ROCA: São Paulo, 2002. p. 33-56.
- BORGES, L. F. K.; FERREIRA, R.; SIQUEIRA, L. C.; BOHRER, R. C.; BORSTMANN, J. W.; OLIVEIRA, J. F. C.; GONÇALVES, P. B. D. Artificial insemination system without estrous observation in suckled beef cows. **Ciência Rural**, v. 39, n. 2, p. 496-501, 2009.
- CÂMARA, D. R.; FIGUEIRA, R. F.; PORTELA, O. C.; MENDONÇA, L. B. R.; PINTO, L. C.; MELLO, M. M. C. Efeito de diferentes protocolos hormonais sobre a taxa de prenhez de vacas Nelore inseminadas em tempo fixo. **Ciência Veterinária nos Trópicos**, v. 9 n. 2/3, p. 74-78, 2006.
- EJIMA, K.; NANRI, H.; ARAKI, M.; UCHIDA, K.; KASHIMURA, M.; IKEDA, M. 17 β -Estradiol induces protein thiol/disulfide oxidoreductases and protects cultured bovine aortic endothelial cells from oxidative stress. **European Journal of Endocrinology**, v. 140, p. 608-613, 1999.

- ELLMAN G. L. Tissue sulphhydryl groups. **Archives of Biochemistry and Biophysics**, v. 82, p. 70-77, 1959.
- EVANS, H. M.; BISHOP, K. S. On the existence of a hitherto unrecognized dietary factor essential for reproduction. **Science**, v. 56, p. 650-651, 1922.
- GONZALEZ-PADILHA, E.; NISWENDER, G. D.; WILTBANK, J. N. Puberty in heifers. II. Effect of injections of progesterone and estradiol 17-beta on serum LH, FSH and ovarian activity. **Journal of Animal Science**, v. 40, p. 1105-1109, 1975.
- GRAHAM, J. F. Condition scoring for beef cattle. **Victorian Department of Agriculture and Technology**, 1982.
- JOLLY, P. D.; MACDOUGALL, S.; FITZPATRICK, L. A.; MACMILLAN, K. L.; ENTWISTLE, K. W. Physiological effects of undernutrition on postpartum anoestrus in cows. **Journal of Reproduction and Fertility**, v. 49, p. 477-492, 1995.
- KESLER, D. J.; FAVERO, R. J. Estrus synchronization in beef females with norgestomet and estradiol valerate. **Agricultural Practice**, v. 17, p. 12-17, 1996.
- LAFLAMME, L. F.; HIDIROGLOU, M. Effects of selenium and vitamin E administration on breeding of replacement heifers. **Annals of Veterinary Research**, v. 22, p. 65-69, 1991.
- LUCY, M. C. H. J.; BILLINGS, W. R.; BUTLER, L. R.; EHNIS, M. J.; FIELDS, D. J.; KESLER, J. E.; KINDER, R. C.; MATTOS, R. E.; SHORT, W. W.; THATCHER, R. P.; WETTEMANN, J. V.; YELICH AND H. D. HAFS. Efficacy of an intravaginal progesterone insert and an injection of PGF2a for synchronizing estrus and shortening the interval to pregnancy in postpartum beef cows, peripubertal heifers and dairy heifers. **Journal of Animal Science**, v. 79, p. 982-995, 2001.
- MÁRQUEZ, Y. C.; FERRARO, S. LÓPEZ-ORTEGA, A. Malondialdehyde concentration in bovine corpus luteum. **Revista Unellez de Ciencia y Tecnología**, volumen especial, p. 79-85, 2001.
- MILLER, J. K.; BRZEZINSKA-SLEBODZINSKA, E. Oxidative stress, antioxidants, and animal function. **Journal of Dairy Science**, v. 76, p. 2812-2823, 1993.
- MORAES, J. C. F.; JAUME, C. M. Sincrobovi: um pessário para sincronização deaios em bovinos. **Revista Brasileira de Reprodução Animal**, v. 21, p. 99-101, 1997.
- OHKAWA, H.; OHISHI, N.; YAGI, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. **Analytical Biochemistry**, v. 95, p. 351-3b58, 1979.
- RICHARDSON, M. J.; LEMENAGER, R. P.; PYATT, N.; LAKE, S. L. Natural source vitamine E supplementation and reproductive efficiency in beef cows. **Proceedings, Western Section, American Society of Animal Sciences**, v. 59, p. 339-342, 2008.
- ROCHE, J. F.; CROWE, M. A.; BOLAND, M. P. Postpartum anestrus in dairy and beef cows. **Animal Reproduction Science**, v. 28, p. 371-8, 1992.
- SIMONETTI, L.; GARDON, J. C. Y.; RAMOS, G. Residual levels on medroxyprogesterone acetate (MAP) impregnated sponges after estrus synchronization treatment in cyclic ewes. **Brazilian Journal of Veterinary Research and Animal Science**, v. 36, 1999. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1413-95961999000500003&lng=en&nrm=iso>. ISSN 1413-9596. doi: 10.1590/S1413-95961999000500003.
- TROXEL, T. R.; CRUZ, L. C.; OTT, R. S.; KESLER, D. J. Norgestomet and gonadotropin-releasing-hormone enhance corpus luteum function and fertility of postpartum suckled beef cows. **Journal of Animal Science**, v. 71, p. 2579-2585, 1993.
- WILTBANK, M. C.; PURSLEY, J. R.; FRICKE, P. M.; VASCONCELOS, J.; GUENTHER, J. N.; GIBBONS, J. R.; GINTHER, O. J. Development of AI and ET programs that do not require detection of estrus using recent information on follicular growth. **Proceedings of the American Embryo Transfer Association**, v. 15, p. 23-44, 1996.

Submitted on April 30, 2008. Accepted on November 23, 2009