

GONADOTROPHIN PROFILES AND OVULATION RATE OF MANTIQUEIRA COWS PASSIVELY IMMUNIZED AGAINST BOVINE FOLLICULAR FLUID

(NÍVEIS DE GONADOTROFINAS PLASMÁTICAS E TAXA DE OVULAÇÃO DE VACAS MANTIQUEIRA PASSIVAMENTE IMUNIZADAS CONTRA LÍQUIDO FOLICULAR BOVINO)

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SUMMARY

This study was conducted to investigate whether passive immunization against bovine follicular fluid (bFF) affects plasma gonadotrophin concentration and ovulation rate in cattle. Ten nonlactating Mantiqueira cows (a local *Bos taurus* breed) were randomly assigned to two groups. Eight to 12 d after estrus, 5 cows were given an iv injection of 100 ml immune serum (from ovariectomized ewes immunized with charcoal-extracted bFF) while 5 control cows were given serum of nonimmunized ovariectomized ewes. Cloprostenol (1 mg) was given 48 h later to induce luteal regression. Plasmatic gonadotrophin concentrations were assayed by radioimmunoassay. There were no significant differences within or between the two groups for plasma concentrations of FSH or LH. Ultrasound scanning used for counting corpora lutea 10 d after cloprostenol treatment showed two cows from the immunized group having multiple ovulations (one cow with two ovulations and another with three) while all control cows had single ovulations. Therefore, results suggest that passive immunization against bFF failed to significantly alter plasma gonadotrophin concentrations but can increase the ovulation rate in some cows.

KEY-WORDS: follicular fluid, bovine, FSH, LH, passive immunization, ovulation rate

RESUMO

O presente estudo teve como objetivo analisar o efeito da imunização passiva contra líquido folicular bovino na concentração plasmática de gonadotrofinas e na taxa de ovulação em bovinos. Dez vacas secas do ecótipo Mantiqueira foram distribuídas aleatoriamente em dois grupos de 5 vacas cada. Oito a 12 dias após o cio, os animais do grupo 1 receberam, por via intravenosa, 100 ml de anti-líquido folicular bovino (produzido em ovelhas ovariectomizadas) enquanto que os animais do grupo 2 receberam soro de ovelhas ovariectomizadas não imunizadas. Quarenta e oito horas após, foi aplicada uma injeção intramuscular de 1 mg de cloprostenol para induzir a luteólise. As concentrações plasmáticas de gonadotrofinas foram analisadas por radioimunoensaio. Não foi observada diferença significativa dentro o entre os grupos para as concentrações plasmáticas de FSH ou LH. A avaliação do número de corpos lúteos do grupo imunizado, realizada

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por palpação retal e ultra-sonografia 10 dias após o tratamento de cloprostenol, mostrou três vacas com ovulação simples, uma com ovulação dupla e outra com três ovulações, enquanto que todas as vacas do grupo controle apresentaram ovulação simples. Em conclusão, os resultados observados sugerem que a imunização passiva contra líquido folicular bovino não altera as concentrações plasmáticas de gonadotrofinas mas provoca um aumento na taxa de ovulação de alguns animais.

PALAVRAS-CHAVE: líquido folicular bovino, FSH, LH, imunização passiva, taxa de ovulação

INTRODUCTION

Ovulation rate in cattle is influenced by peripheral FSH concentrations (TAKEDOMI *et al.*, 1995). Release of FSH from the pituitary is controlled by hypothalamic GnRH and modulated by endocrine and paracrine factors, including steroids and peptides (FINDLAY, 1993). Estrogen and progesterone are potent inhibitors of FSH release and suppress serum FSH concentrations in ovariectomized heifers (PRICE & WEBB, 1988). Likewise, the synthesis and release of FSH in both intact (PRICE & WEBB, 1988) and ovariectomized heifers (IRELAND *et al.*, 1983, JOHNSON & SMITH, 1985; PRICE & WEBB, 1988; BEARD *et al.*, 1989; PRICE, 1991) are also suppressed by treatment with charcoal-extracted bovine follicular fluid (bFF). Because bFF has very low concentrations of steroids, their biological action on suppressing FSH has been attributed to substances others than steroid hormones. Now, there is evidence that inhibin and related peptides are involved (FINDLAY *et al.*, 1991). Active (GLENCROSS *et al.*, 1992 and 1994; MORRIS *et al.*, 1993; O'SHEA *et al.*, 1994; HILLARD *et al.*, 1994) or passive (TAKEDOMI *et al.*, 1995) immunization against inhibin increases ovulation rate in heifers although peripheral FSH concentrations were increased in some (GLENCROSS *et al.*, 1994; TAKEDOMI *et al.*, 1995) but not all (GLENCROSS *et al.*, 1992, MORRIS *et al.*, 1993) of these studies. However, inhibin as immunogen is somewhat limited by cost and availability. Also, there is the inconvenient of variability of ovarian response to immunization against inhibin (HILLARD *et al.*, 1994; MORRIS *et al.*, 1997). On the other hand, bFF which can be readily aspirated from abattoir-derived ovaries, is effective to delay estrus when given at luteolysis (LAW *et al.*, 1992) and suppresses emergence of the first follicular wave when given at estrus (QUIRK & FORTUNE, 1986; TURZILLO & FORTUNE, 1990) or ovulation (KASTELIC *et al.*, 1990). In sheep, immunization against bFF increases the ovulation rate (CUMMINS *et al.*, 1986). In cattle, however, the lack of antigenicity of bFF makes it ineffective to induce an immune response. Alternatively, there is the possibility of injecting directly into the circulatory stream some bFF antibodies raised in another species (passive immunization). When effective, passive immunization results in immediate neutralization of specific hormones at a very precise moment and has the

advantage of a rapid reversion to normal hormone concentration after ceasing antibody injection. However, passive immunization against bFF in cattle has not yet been reported.

The objective of the present experiment was to determine whether passive immunization against bFF affects plasma gonadotrophin concentrations and ovulation rate in cows.

MATERIAL AND METHODS

The procedure to obtain ovine bFF antibodies was described previously (ALVAREZ *et al.*, 1997). In brief, bovine ovaries were collected at a local abattoir. Follicles < 22 mm in diameter were aspirated and the follicular fluid frozen. After thawing, follicular fluid was pooled, centrifuged (700 g) for 15 min at 4°C to remove cellular debris, and incubated with 10 mg ml⁻¹ activated charcoal (Norit A, Sigma Chemical Company, St. Louis, MO, USA) for 2 h at 4°C, followed by sequential centrifugation (17000 g, 1h at 4°C) and filtration through sterile 45 µm nylon tissue. The resultant bFF had a protein content of 80 mg ml⁻¹ and a 99.8% reduction in steroid content (ALVAREZ *et al.*, 1997). Five ml of bFF were emulsified in 2 ml of Freund's complete adjuvant (Sigma Chemical Company, St. Louis, MO, USA) and injected subcutaneously on four points of the dorsal region of six ewes which had been ovariectomized six months previously. Five boosters were similarly given at 15 d intervals using bFF prepared in Freund's incomplete adjuvant (Sigma Chemical Company, St. Louis, MO, USA). Serum from four ewes which showed non-specific precipitation lines against bFF (determined by Ouchterlony plate technique) was pooled, filtered through 45 µm nylon tissue and lyophilized.

Ten nonlactating Mantiqueira cows (a local *Bos taurus* breed) were randomly assigned to two groups. The cows weighted an average of 370 kg (s.e.m. ± 45) and were of body score between 3 and 3.5 (in a 5 point scale). Eight to twelve days after synchronized estrus, cows were given either immune or nonimmune ewe serum (100 ml iv; five cows per group) and all were given 1 mg im cloprostenol (Ciosin, Lab. Cooper, Brazil) 48 h later.

The cows were catheterized in the jugular vein and blood samples were collected into evacuated tubes

containing heparin. Blood samples were collected 2 h prior to the immune or nonimmune serum administration and at 2-h intervals for the next 24 h and at 2 h intervals from 36 to 60 h after cloprostenol injection. After that, additional seven blood samples were collected by jugular venipuncture at 8 h intervals. Blood samples were centrifuged for 30 minutes at 700 g and plasma was recovered and frozen (-20 °C). The number of corpora lutea (as a measure of ovulation rate) was determined by rectal palpation and by ultrasonography (Pie Medical Model 480 Vet scanner with 5/7.5 Mhz transducer) ten days after cloprostenol administration.

Plasma concentrations of gonadotrophins were subsequently measured using validated radioimmunoassays as described by BOLT & ROWLING (1983) for FSH and NISWENDER *et al.* (1969) for LH. The sensitivity of the assays was determined according to RODBARD (1978). Highly purified bovine FSH (USDA-bFSH-1-2) was used as standard and for iodination and the FSH antiserum was anti-oFSH NIDDK-oFSH-I-1 (AFP-C5288113). Intra and interassay coefficient of variation (CV) averaged 4.2 and 12.2%, respectively. Sensitivity for bFSH was 1.4 ng ml⁻¹. Plasma LH concentrations were measured using USDA -bLH-I-1 as standard and for iodination and the LH antiserum was NIADDK-oLH-1 (AFP-192279). Intra and interassay CV averaged 4.7 and 8.6%, respectively. Sensitivity for bLH was 0.1 ng ml⁻¹.

Plasma gonadotrophin concentrations were examined for two intervals: the post-immunization period (from 2 h before to 24 h after treatment with immune or nonimmune serum) and the preovulatory period (from 18 h before the preovulatory LH surge to 18 h after the surge). The criteria used to define the preovulatory surge of LH was two SD exceeding the mean of 5 to 10 values preceding the onset of the surge, determined visually. Data for plasma gonadotrophin concentrations were log-transformed and realigned around the time of the preovulatory LH surge (0 h), prior to the statistical analysis.

Data were analyzed using the Statistical Analysis System (SAS, 1993). Student's t test was used to determine differences between the two treatments for the number of ovulations and for the interval from administration of cloprostenol to the preovulatory surge. Split-plot analysis of variance was used to determine the effect of treatment, time, and the treatment by time interaction for plasma gonadotrophins concentrations for both the post-immunization and preovulatory periods. A preovulatory rise in plasma gonadotrophins concentrations was not detected in one cow and she was therefore excluded from the statistical analysis of the hormone study.

RESULTS

The mean (\pm s.e.m.) cloprostenol-LH surge interval was $56 \pm 8,1$ and $52 \pm 9,6$ for control and bFF immunized cows, respectively ($P > 0.05$). There was no significant effect of treatment or treatment by time interaction for plasma concentrations of FSH or LH for either the post-immunization (Figure 1) or preovulatory (Figure 2) intervals. Though not significant, the average number of ovulations was greater for treated than control cows (1.6 ± 0.6 versus 1.0 ± 0.0). Similarly, the proportion of cows with multiple ovulations was greater for treated cows (2 of 5; one with two ovulations and another with three) than for control cows (0 of 5).

DISCUSSION

In the present study, passive immunization against bFF did not significantly affect serum gonadotrophin concentrations nor ovulation rate. However, a moderate increase of ovulation rate was observed in 2 immunized cows (one animal with 2 and another with 3 ovulations). It is probable that the corpora lutea found in the 2 immunized cows were originated from the treatment because rectal palpation of 125 cows of the same herd had single ovulation and there were no reports of cows delivering twins in the last 5 years before the present study. One of the immunized cows did not present LH surge but had a corpus luteum. The absence or delayed LH surge (and ovulation) in this cow could be due to high levels of progesterone resulting from incomplete luteolysis as reported in other studies with cows treated with cloprostenol (BAISHYA *et al.*, 1980). Therefore, the lack of significant difference between control and immunized cows could be due to the small number of animals used in this study.

Previous studies in cattle (GLENCROSS *et al.*, 1992 and 1994; MORRIS *et al.*, 1993 and 1997; SCANLON *et al.*, 1993; TAKEDOMI *et al.*, 1995) and other large domesticated species, such as sheep (WRATHALL *et al.*, 1992; WHEATON *et al.*, 1992), horses (McCUE *et al.*, 1992) and swine (BROWN *et al.*, 1990) have demonstrated that active immunization against inhibin or inhibin peptide fragments increases ovulation rates. Peripheral FSH concentrations in cattle were increased following immunization in some (GLENCROSS *et al.*, 1994; TAKEDOMI *et al.*, 1995) but not all (GLENCROSS *et al.*, 1992; MORRIS *et al.*, 1993) of these studies. Because basal concentrations of FSH were not altered in the immunized animals which had increased ovulation rate, there is the possibility of a direct effect on the ovary of inespecific antibodies to bFF. In fact, besides inhibin, bFF contains a variety of substances which affect follicular development (TONETTA & diZEREGA, 1989). It

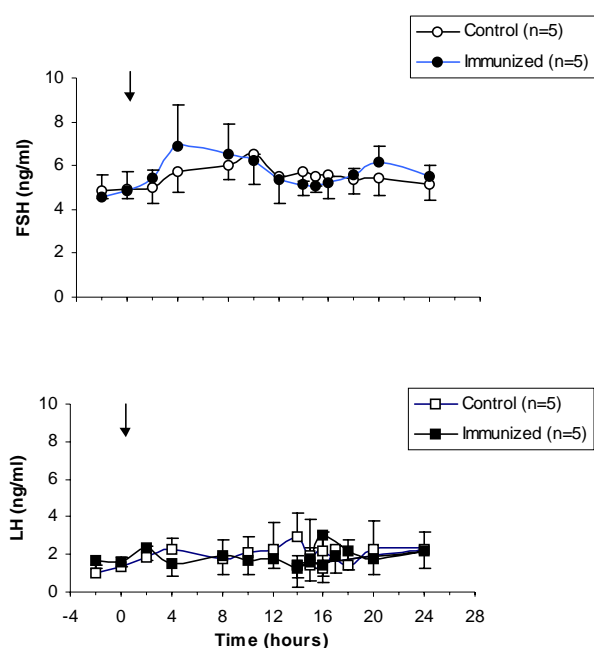


Figure 1 - Mean (\pm SEM) plasma concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in cows treated with nonimmune (control group) or immune anti bovine follicular fluid (immunized group) serum. The arrow indicates the timing of the serum injection.

has been reported that administration of inhibin-reduced (>90%) bovine (LAW *et al.*, 1992) or ovine (CAMPBELL *et al.*, 1991) follicular fluid to both heifers and ewes, respectively, suppressed follicular development and delayed the return to estrus following luteolysis; these effects were not attributable to reduced peripheral concentrations of FSH.

The failure in detecting increases in plasma FSH concentration after bFF immunization may be related to the frequency of blood sampling or the assay used. However, the present assay detected a decline in plasma FSH concentrations in ovariectomized prepuberal heifers treated with bFF (ALVAREZ *et al.*, 1998). Nevertheless, in that study, the FSH assay was unable to detect plasma FSH alterations in non-ovariectomized heifers treated with bFF.

There are indications that both bFF and inhibin may stimulate the release of LH in sheep (MUTUKRISHNA & KNIGHT, 1990; MEYER *et al.*, 1991). In cattle, however, the results of the present study showed no effect of passive immunization on plasma LH concentrations or on the interval from cloprostenol treatment to the LH surge according with previous reports (RHIND *et al.*, 1991; SCANLON *et al.*, 1993; GLENCROSS *et al.*, 1994).

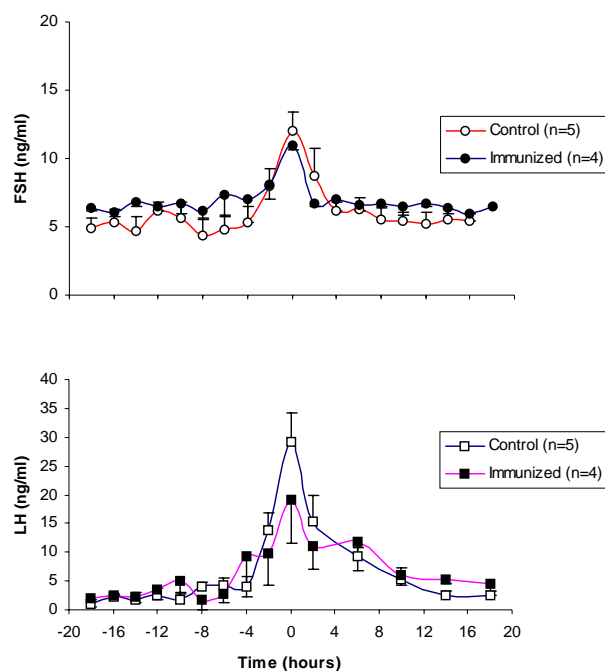


Figure 2 - Mean (\pm SEM) plasma concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in bFF immunized and control cows during the LH surge (0 h).

CONCLUSION

In conclusion, passive immunization against bFF did not significantly affect plasma gonadotrophin concentrations but increase the ovulation rate in some cows. Therefore, further studies using a higher number of cows are indicated to validate the present findings.

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REFERENCES

ALVAREZ, R.H., ARCARO, J.R.P., RIBELA, M.T.C.P., PERONE, C.N. Effect of charcoal-treated bovine

- follicular fluid on secretion of FSH in ovariectomized and intact prepubertal heifers. **Brazilian Journal of Veterinary Research and Animal Science**, v.35, 1998 (in press).
- ALVAREZ, R.H., IAPICHINI, J.E.C.B., PIATTI, R.M. Produção de anticorpos contra líquido folicular de origem zebuina nas espécies *Bos taurus taurus* e *Ovis aries*. **Boletim de Industria Animal**, v.54, p. 33-8, 1997.
- BAISHYA, B.N., BALL, P.J.H., LEAVER, J.D., POPE, G.S. Fertility of lactating dairy cows inseminated after treatment with cloprostenol. **British Veterinary Journal**, v.136, p.227-39, 1980.
- BEARD, A.J., SAVVA, D., GLENCROSS, R.G., MCLEOD, B.J., KNIGHT, P.G. Treatment of ovariectomized heifers with bovine follicular fluid specifically suppresses pituitary levels of FSH mRNA. **Journal of Molecular Endocrinology**, v.3, p.85-91, 1989.
- BOLT, D.J., ROLLINS, R. Development and application of a radiomunoassay for bovine follicle-stimulating hormone. **Journal of Animal Science**, v.56, p.146-54, 1983.
- BROWN, R.W., HUNGERFORD, J.W., GREENWOOD, P.E., BLOOR, R.J., EVANS, D.F., TSONIS, C.G., FORAGE, R.G. Immunization against recombinant bovine inhibin sub-unit causes increased ovulation rate in gilts. **Journal of Reproduction and Fertility**, v.90, p.199-205, 1990.
- CAMPBELL, B.K., PICTON, H.M., MANN, G.E., McNEILLY, A.S., BAIRD, D.T. Effect of steroid - and inhibin-free ovine follicular fluid on ovarian follicles and ovarian hormone secretion. **Journal of Reproduction and Fertility**, v.93, p.81-96, 1991.
- CUMMINS, L.J., O'SHEA, T., AL-OBAIDI, S.A.R., BINDON, B.M., FINDLAY, J.K. Increase in ovulation rate after immunization of merino ewes with a fraction of bovine follicular fluid containing inhibin activity. **Journal of Reproduction and Fertility**, v.77, p.365-72, 1986.
- FINDLAY, J.K. An update on the roles of inhibin, activin, and follistatin as local regulators of folliculogenesis. **Biology of Reproduction**, v.48, p.15-23, 1993.
- FINDLAY, J.K., CLARKE, I.J., LUCK, R.M., RODGERS, R.J., SUKOVSKI, L., ROBERTSON, D.M., KLEIN, R., MURRAY, J.F., SCARAMUZZI, R.J., BINDON, B.M., O'SHEA, T., TSONIS, C.G., FORAGE, R.G. Peripheral and intragonadal actions of inhibin-related peptides. **Journal of Reproduction and Fertility**, v.43, p.139-50, 1991. (Supplement).
- GLENCROSS, R.G., BLEACH, E.C., MCLEOD, B.J., BEARD, A.J., KNIGHT, P.G. Effect of active immunization of heifers against inhibin on plasma FSH concentrations, ovarian follicular development and ovulation rate. **Journal of Endocrinology**, v.134, p.11-8, 1992.
- GLENCROSS, R.G., BLEACH, E.C., WOOD, S.C., KNIGHT, P.G. Active immunization of heifers against inhibin: effects on plasma concentrations of gonadotrophins, steroids and ovarian follicular dynamics during prostaglandin-synchronized cycles. **Journal of Reproduction and Fertility**, v.100, p.599-605, 1994.
- HILLARD, M.A., BINDON, B.M., WILKINS, J.F., CUMMINS, L.J., TSONIS, C.G., FINDLAY, J.K. Inhibin vaccines for increased ovulation rate and fecundity in cattle. In: MEETING AUSTRALIAN SOCIETY ANIMAL PRODUCTION, 1994, Western Australia. **Proceedings...**v.20, p. 27-8.
- IRELAND, J.J., CURATO, A.D., WILSON, J. Effect of charcoal-treated follicular fluid on secretion of LH and FSH in ovariectomized heifers. **Journal of Animal Science**, v.57, p.1512-6, 1983.
- JOHNSON, S.K., SMITH, M.F. Effect of charcoal-extracted, bovine follicular fluid on gonadotropin concentrations, the onset of estrus and luteal function in heifers. **Journal of Animal Science**, v.61, p.203-9, 1985.
- KASTELIC, J.P., KO, J.C.H., GINTHER, O.J. Suppression of dominant and subordinate ovarian follicles by a proteinaceous fraction of follicular fluid in heifers. **Theriogenology**, v.34, p.499-509, 1990.
- LAW, A.S., BAXTER, G., LOGUE, D.N., O'SHEA, T., WEBB, R. Evidence for the action of bovine follicular fluid factor(s) other than inhibin in suppressing follicular development and delaying oestrus in heifers. **Journal of Reproduction and Fertility**, v.96, p.603-16, 1992.
- McCUE, P.M., CARNEY, N.J., HUGHES, J.P., RIVIER, J., VALE, W., LASLEY, B.L. Ovulation and embryo recovery rates following immunization of mares against an inhibin alpha-subunit fragment. **Theriogenology**, v.38, p.823-31, 1992.
- MEYER, R.L., CARLSON, K.M., RIVIER, J., WHEATON, J.E. Antiserum to an inhibin alpha-chain peptide neutralizes inhibin bioactivity and increases ovulation rate in sheep. **Journal of Animal Science**, v.69, p.747-54, 1991.
- MORRIS, D.G., BROWNE, D., DISKIN, M.G., SREENAN, J.M. Effects of peptide to carrier ratio on the immune and ovarian response to inhibin immunization in cattle. **Animal Reproduction Science**, v.48, p.1-8, 1997.
- MORRIS, D.G., McDERMOTT, M.G., DISKIN, M.G., MORRISON, C.A., SWIFT, P.J., SREENAN, J.M. Effect of immunization against synthetic peptide sequences of bovine inhibin a-subunit on ovulation rate and twin-calving rate in heifers. **Journal of Reproduction and Fertility**, v.97, p.255-61, 1993.
- MUTUKRISHNA, S., KNIGHT, P.G. Effects of crude and highly purified bovine inhibin (Mr 32000 form) on gonadotrophin production by ovine pituitary cells *in*

- vitro*: inhibin enhances gonadotrophin-releasing hormone-induced release of LH. **Journal of Endocrinology**, v.127, p.149-59, 1990.
- NISWENDER, G.D., REICHERT Jr, L.E., MIDGLEY Jr, A.R., NALBANDOV, A.V. Radiomunoassay for bovine and ovine luteinizing hormone. **Endocrinology**, v.84, p.1166-73, 1969.
- O'SHEA, T., HILLARD, M.A., ANDERSON, S.T., BINDON, B.M., FINDLAY, J.K., TSONIS, C.G., WILKINS, J.F. Inhibin immunization for increasing ovulation rate and superovulation. **Theriogenology**, v.41, p.3-17, 1994.
- PRICE, C.A. The control of FSH secretion in the large domestic species. **Journal of Endocrinology**, v.131, p.177-84, 1991.
- PRICE, C.A., WEBB, R. Steroid control of gonadotropin secretion and ovarian function in heifers. **Endocrinology**, v.122, p.222-31, 1988.
- QUIRK, S.M., FORTUNE, J.E. Plasma concentrations of gonadotrophins, preovulatory follicular development and luteal function associated with bovine follicular fluid-induced delay of estrus in heifers. **Journal of Reproduction and Fertility**, v.76, p.609-21, 1986.
- RHIND, S.M., SCHEMM, S.R., SCHANBACHER, B.D. Effects of active immunization against inhibin, androstenedione, separately or together, on hormone profiles, ovarian follicle populations and ovulation rate in heifers. **Animal Production**, v.52, p.563-4 1991.
- RODBARD, D. Statistical estimation of the minimal detectable concentration ("sensitivity") for radioligand assays. **Annals of Biochemistry**, v.90, p.1-12, 1978.
- SAS User's Guide, Statistics. Cary: SAS Institute, 1993.
- SCANLON, A.R., SUNDERLAND, S.J., MARTIN, T.L., GOULDING, D., O'CALLAGHAN, D., WILLIAMS, D.H., HEADON, D.R., BOLAND, M.P., IRELAND, J.J., ROCHE, F. Active immunization of heifers against a synthetic fragment of bovine inhibin. **Journal of Reproduction and Fertility**, v.97, p.213-22, 1993.
- TAKEDOMI, T., KANEKO, H., AOYAGI, Y., NAKANISHI, Y., TAYA, K. Effects of passive immunization against bovine inhibin on ovulation rate and circulating FSH levels in Holstein heifers. **Theriogenology**, v.43, p.333, 1995. (Abstract).
- TONETTA, S.A., diZEREGA, G.S. Intraovarian regulation of follicular maturation. **Endocrine Review**, v.10, p.205-29, 1989.
- TURZILLO, A.M., FORTUNE, J.E. Suppression of the secondary FSH surge with bovine follicular fluid is associated with delayed ovarian follicular development in heifers. **Journal of Reproduction and Fertility**, v.89, p.643-53, 1990.
- WHEATON, J.E., CARLSON, K.M., KUSINA, N.T. Active and passive immunoneutralization of inhibin increases follicle stimulating hormone levels and ovulation rate in ewes. **Biology of Reproduction**, v.47, p.361-7, 1992.
- WRATHALL, J.H.M., McLEOD, B.J., GLENCROSS, R.G., KNIGHT, P.G. Effects of active immunization against a synthetic peptide sequence of the inhibin a-subunit on plasma gonadotrophin concentrations, ovulation rate and lambing rate in ewes. **Journal of Reproduction and Fertility**, v.95, p.175-82, 1992.