

# FOLLICULAR DYNAMICS AND PLASMA FSH CONCENTRATIONS DURING FOLLICULAR DEVIATION IN THE FIRST POST-OVULATORY WAVE IN TABAPUA (*Bos indicus*) COWS TREATED WITH BOVINE SOMATOTROPIN (bST)\*

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**ABSTRACT.** Arnone B., Negrão C.B., Brito L.F.C., Giometti I.C. & Castilho C. **Follicular dynamics and plasma FSH concentrations during follicular deviation in the first post-ovulatory wave in tabapua (*Bos indicus*) cows treated with bovine somatotropin (bST).** [Dinâmica folicular e concentração plasmática de FSH durante a primeira onda pós-ovulatória em vacas Tabapuã (*Bos indicus*) tratadas com somatotrofina bovina (bST)]. *Revista Brasileira de Medicina Veterinária*, 35(4):318-322, 2013. Programa de Pós-Graduação em Ciência Animal, Universidade do Oeste Paulista, Rodovia Raposo Tavares, Km 572, Presidente Prudente, SP 19067-175, Brasil. E-mail: calie@unoeste.br.

The objective of the present study was to evaluate follicular dynamics and plasma FSH concentrations during the first post-ovulatory wave in *Bos indicus* cows treated with bovine somatotropin (bST). Diameters of ovarian follicles and plasma FSH concentrations were determined every 12 h until 5 d after ovulation in 10 Tabapua cows, half of which had been treated with 500mg bST. Treatment did not affect follicular diameters, follicular dynamics, ovulation-to-follicular deviation interval, or FSH concentrations. Follicular deviation was characterized primarily by a decrease in the growth rate of the largest subordinate follicle and occurred 54 h after ovulation when plasma FSH concentrations were decreasing. Mean diameters of the dominant and largest subordinate follicles at the time of deviation were 6.31 and 6.24 mm, respectively. In conclusion, bST did not affect follicular dynamics and plasma FSH concentrations in *B. indicus* cows. Follicular deviation occurred during a period of decreasing FSH concentration and no diameter differences between the dominant and largest subordinate follicle were observed prior to deviation.

**KEY WORDS.** GH, Dominant follicle, zebu cattle, ultrasound.

**RESUMO.** O objetivo do presente estudo foi avaliar a dinâmica folicular e a concentração plasmática de FSH durante a primeira onda pós-ovulatória em vacas *Bos indicus* tratadas com somatotrofina bovina (bST). Os diâmetros ovarianos e as concentrações plasmáticas de FSH foram determinados a cada 12 h até 5 dias após a ovulação em 10 vacas da

raça Tabapuã, metade das quais foram tratadas com 500 mg bST. O tratamento não afetou o diâmetro folicular, a dinâmica folicular, intervalo ovulação divergência folicular ou a concentração de FSH. A divergência folicular foi caracterizada primariamente pelo decréscimo na taxa de crescimento do maior folículo subordinado e ocorreu 54 h após a

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ovulação quando a concentração plasmática de FSH estava decrescendo. O diâmetro médio dos folículos dominante e maior subordinado, no momento da divergência, foram 6,31 e 6,24 mm, respectivamente. Concluí-se que a bST não afeta a dinâmica folicular e a concentração plasmática de FSH. A divergência folicular ocorreu durante um período de decréscimo na concentração de FSH e aumento no diâmetro folicular ou um diâmetro “crítico” alcançado pelo folículo dominante antes do folículo subordinado não foi observado.

PALAVRAS-CHAVE. GH, Folículo dominante, vaca zebuína, ultrassom.

## INTRODUCTION

Follicular deviation in cattle refers to the time at which differences in the growth rate between the dominant and subordinate follicles become apparent. Follicular deviation is characterized by decreased growth rate of the subordinate follicles and continuous growth of the dominant follicle (Ginther et al. 2003, Sartorelli et al. 2005 e Gimenes et al. 2008). Although decreasing circulating FSH concentration has been associated with follicular deviation in *Bos taurus* females (Gibbons et al. 1999, Ginther et al. 2003), the role of FSH in the timing of follicular deviation in *Bos indicus* females has not been established (Castilho et al. 2007).

Follicular IGF-I seems to be an important factor involved in follicular dominance. Studies comparing IGF-I concentrations in the two largest ovarian follicles during follicular deviation demonstrated that IGF-I concentration decreases in the second largest follicle and is unchanged in the largest follicle. These observations suggested that IGF-I might modulate follicular response to FSH, making the dominant follicle less sensitive to the decreased FSH concentrations (Beg et al. 2001 e 2002). Bovine somatotropin (bST) increases the synthesis and secretion of IGF-I and its carrier protein (Jones & Clemmons 1995). Treatment with bST in female cattle has been shown to increase the number of small follicles recruited during wave emergence and to stimulate follicular development; these effects are probably due to a combination of direct effects of bST and indirect effects of IGF-I (Pavlok et al. 1996, Kirby et al. 1997 e Lucy 2000).

The objective of the present study was to evaluate follicular dynamics and plasma FSH concentrations during follicular deviation in the first post-ovulatory wave in *B. indicus* cows treated with

bST. Our hypothesis was that bST treatment affects follicular dynamics (timing of deviation, follicular diameter at deviation) and circulating FSH concentrations.

## MATERIALS AND METHODS

### Animals and treatments

This study was reviewed and approved by the Animal Care and Use Committee of the Universidade do Oeste Paulista. Ten Tabapua (*B. indicus*) cows, 6- to 7-yr-old, with body condition score ranging from 2.5 to 3 (scale from 1 to 5) were used in this experiment. The cows were maintained in *Brachiaria decumbens* pasture and had *ad libitum* access to water and mineral salt. At a random stage of the estrous cycle (Day 0), the cows received a progestin ear implant (Crestar<sup>®</sup>; Intervet/Schering-Plough Animal Health, Boxmeer, The Netherlands) and were treated with 1 mg estradiol benzoate intramuscularly (Estrogin<sup>®</sup>; Farmavet Produtos Veterinários Ltda, São Paulo, Brazil). On Day 5, the cows were randomly divided into two groups; six cows received 500 mg recombinant bST (Boostin<sup>®</sup>; Intervet/Schering-Plough Animal Health) into the skin fold on the side of the tail head (subcutaneous) and five cows did not receive any treatment and served as controls. On Day 10, progestin implants were removed and all cows received 500 µg cloprostenol sodium intramuscularly (Sincrocio<sup>®</sup>; Ourofino Agronegócios, Cravinhos, Brazil). Also on Day 10, cows that presented follicles larger than 9 mm received 300 µg leirelin intramuscularly (GnRH analogue; Gestran Plus<sup>®</sup>; Tecnopec, São Paulo, Brazil).

### Follicular dynamics and FSH concentration

Ultrasound exams were performed every 12 h after GnRH treatment until 5 d after ovulation using an HS-2000VET ultrasound system (Honda Electronics Co. Ltd., Toyohashi, Japan) equipped with a 5-MHz transrectal transducer. During each examination, ovarian maps were drawn to record the diameter and relative position of follicles  $\geq 3$  mm. Ovulation was defined as the disappearance, from one examination to the next, of a previously identified follicle  $\geq 10$  mm. The diameters of the dominant follicle and largest subordinate follicle were evaluated retrospectively and 12-h growth rates were calculated. The differences in the growth rate between the dominant and subordinate follicles were investigated and deviation was defined as the time at which growth rate of the dominant follicle became greater than the growth rate of the subordinate follicle.

Blood samples were collected at the time of ultrasound exams from the jugular vein into 15 mL-heparinized tubes. Plasma was separated by centrifugation, and stored at -20°C until assayed. Quantification of FSH was performed by RIA validated for bovine FSH using USDA-bFSH for iodination and reference standards, and NIDDKanti-oFSH antiserum (Bolt & Rollins, 1983). Assay sensitivity was 0.01 ng/ml and intra-assay coefficient of variation was 8.15%.

### Statistical analyses

Repeated-measures ANOVA with Bonferroni test (GraphPad Prism; GraphPad Software, La Jolla, CA) was used to determine and locate treatment, time, and treatment-by-time interaction effects on dominant and subordinate follicles dia-

meter and growth rate, and on plasma FSH concentrations. Data were analyzed according to the time of ovulation and after normalization according to the time of follicular deviation. The effects of treatment on the intervals from ovulation until detection of the dominant and subordinate follicles, and until follicular deviation were determined using Student's t-test (Statistix; Analytical Software, Tallahassee, FL, USA).

**Ethics committee**

This project was approved by the ethics committee of the institution of origin (UNOESTE) under protocol 014/07.

**RESULTS**

There were time effects ( $p < 0.001$ ) on dominant and largest subordinate follicles diameter and growth rate, and on plasma FSH concentrations when data were analyzed either according to the time of ovulation or according to the time of follicular deviation; however, there were no treatment or treatment-by-time interaction effects on any of these

endpoints. Although the interval from ovulation until detection of the dominant and subordinate follicle was slightly shorter in the bST group ( $12 \pm 5.4$  and  $9.6 \pm 4.5$  h, respectively) when compared to the control group ( $16.8 \pm 2.9$  and  $14.4 \pm 2.4$  h, respectively), the differences were not significant. Follicular deviation occurred between 48 and 72 h after ovulation in the control group and between 36 and 60 h after ovulation in the bST, but the mean interval between ovulation and follicular deviation was not significantly different between the groups ( $57.6 \pm 5.5$  and  $50.4 \pm 5.5$  h, respectively; 54 h overall). When the combined, normalized data was plotted, it was evident that follicular deviation was characterized by a sudden and drastic reduction in the subordinate follicle growth rate, but the dominant follicle growth rate remained largely unchanged (Figure 1A). Follicular deviation occurred when plasma FSH concentrations were decreasing and the mean diameters of the dominant and subordinate follicles at the time of deviation were  $6.31 \pm 0.41$  and  $6.24 \pm 0.39$  mm, respectively (Figure 1B).

**DISCUSSION**

Although follicles seemed to be detected earlier and follicular deviation seemed to occur sooner after ovulation, bST treatment did not significantly affect follicular dynamics or circulating FSH concentrations during the first post-ovulatory wave. The lack of significant differences might have been due to the inability of the dose of bST used in the present study to sufficiently increase IGF-I to concentrations required in order to affect follicular growth in *B. indicus* cows. Kirby et al. (1997) also reported that treatment with different doses of bST did not affect follicular diameter in Holstein cows. Although these authors did not specifically study follicular deviation, bST treatment resulted in earlier emergence of the second post-ovulatory wave, an observation that suggests that the negative feedback effect of the dominant follicle of the first wave subsided earlier after ovulation in treated cows. If the *suppressive life-span* of the dominant follicle is more or less constant, earlier emergence of the dominant follicle in bST treated cows might be associated with the earlier cessation of negative feedback and emergence of the second follicular wave in these animals.

Consistent with the results obtained in the present study, follicular deviation in *B. indicus* females has been reported to occur between 2.5 to 2.7 d after ovulation when the diameters of the dominant

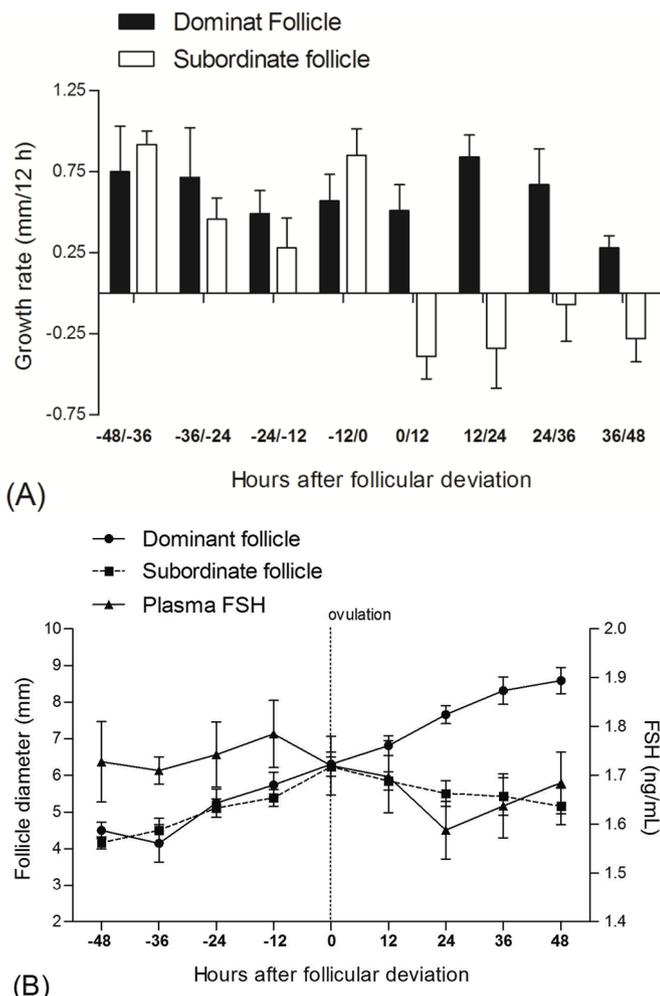


Figure 1. Mean ( $\pm$  SEM) dominant and largest subordinate follicles growth rates (A), and follicles diameters and plasma FSH concentrations (B) according to the time of follicular deviation in the first post-ovulatory wave in Tabapua (*Bos indicus*) cows.

and subordinate follicles are 5.4 to 6.2 and 5.3 to 5.9 mm, respectively (Sartorelli et al. 2005, Castilho et al. 2007 e Gimenes et al. 2008). Follicular deviation was characterized primarily by a decrease in the growth rate of the subordinate follicle. This observation is similar to those reported in *B. taurus* females (Ginther et al. 2001, Sartori et al. 2001), but is somewhat different from a previous report in *B. indicus* heifers (Nelore), in which the decrease in subordinate follicle growth rate was accompanied by an increase in the dominant follicle growth rate (Castilho et al. 2007). Although other studies in *B. taurus* and *B. indicus* females have reported that the future dominant follicle of the first post-ovulatory wave was identified slightly earlier than the subordinate follicle (Ginther et al. 2001, Castilho et al. 2007), the opposite was observed in the present study. The observation that the diameters of the dominant and subordinate follicles did not differ at the time of deviation provide further evidence that follicular size advantage and the attainment of a *critical* diameter by the dominant follicle earlier than the subordinate follicle does not explain deviation in *B. indicus* females (Sartorelli et al. 2005, Castilho et al. 2007).

There is great variation in plasma FSH concentrations during the first wave of follicular development in cattle among different studies. The concentrations observed in the present study (1.24 to 2.26 ng/mL) were similar to that observed in Holstein heifers (Ginther et al. 1999), but much greater than that observed in *B. indicus* heifers (0.2 to 0.8 ng/mL) (Buratine et al. 2000, Castilho et al. 2007). It has been demonstrated in *B. taurus* females that follicular deviation involves a shift from FSH to LH dependency, so that a follicle acquires the ability to respond to LH even with decreasing FSH concentrations to become dominant. The decline in FSH concentration is necessary for deviation because treatment with exogenous FSH prevents deviation (Gibbons et al. 1999, Ginther et al. 2003). The observation that follicular deviation occurred while FSH concentrations were decreasing indicates that a similar mechanism might be involved in follicular deviation in *B. indicus* cows, despite some inconclusive results from an earlier study in *B. indicus* heifers (Castilho et al. 2007).

In conclusion, bST did not affect follicular dynamics and plasma FSH concentrations in *B. indicus* cows. Follicular deviation occurred during a period of decreasing FSH concentration and no diameter

differences between the dominant and largest subordinate follicle were observed prior to deviation.

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