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Influence of the development phase of the dominant follicle on the superovulatory response in Nelore heifers*

Influência da fase de desenvolvimento do folículo dominante sobre a resposta superovulatória em novilhas da raça Nelore

CORRESPONDENCE TO:
José Antonio Visintin
Departamento de Reprodução Animal
Faculdade de Medicina Veterinária e Zootecnia da USP
Cidade Universitária Armando de Salles Oliveira
Av. Orlando Marques de Paiva, 87.
05508-900 - São Paulo - SP
e-mail: visintin@usp.br

1-Departamento de Reprodução Animal da Faculdade de MEdicina Veterinária e Zootecnia da USP - SP

Mayra Elena Ortiz D'Avila ASSUMPÇÃO[1]; Ed Hoffmann MADUREIRA[1]; Rubens Paes de ARRUDA[1]; Eneiva Carla Carvalho CELEGHINI[1]; Pedro Paulo Gimenez GUSMÕES[1]; Pedro Henrique CANDINI[1]; José Antônio VISINTIN[1]

SUMMARY

The aim of this study was to evaluate the influence of the dominant follicle during it's growing, static and regression phases on the superovulatory response in 18 estral cycles of nelore heifers. The follicular development was monitored by ultrasound from Day 6 to Day 10 (Day 0=estrus) of the oestrus cycle, when the diameter of the largest follicle was measured and the number of subordinate follicles counted. The animals where superovulated with 400 or 500IU of FSH/LH twice daily for 4 days begining on day 10 of the oestrus cycle I was injected PGF_2a concomitantly with the fifth dose of FSH/LH. Artificial insemination was done 12 and 24 hours after the begining of the estrus. Embryos were recovered on day 6.5 after the first insemination. If a dominant follicle was present before superovulation, it was observed subordinate follicles atretic and low response to superovulatory treatment. The best result of transferable and total embryos was observed when the dominant follicle was in regression phase (3.67 and 10.17) at the beginning of the superovulatory treatment.

UNITERMS: Dominant follicle; Superovulatory response; Nelore.

INTRODUCTION

Recently ultrasonic monitoring of the follicular dynamic has been of great value in the attempt to elucidate the variability of the superovulatory response. It can indicate the best moment to begin the superovulatory treatment, depending on the presence or absence of the dominant and subordinate follicles.

It is known that the follicular dynamics occurs in wave patterns according to Rajakoski, quoted by Taylor; Rajamahendran[13], based on the counting of follicles from animals slaughtered in different phases of the oestrus cycle.

Ginther *et al.*[6] have shown the existence of follicular wave patterns and have classified the dominant follicle in different categories (growing, static and regression phases). According to these authors, these wave patterns are characterized by the recruitment of great number of follicles, selection and growth of a dominant follicle, ovulatory or not, which suppresses the subordinate follicles.

The dominant follicle produces great amount of oestradiol and inhibin that stops endogenous FSH secretion (negative feedback) causing regression of the subordinate follicles. The dominant follicle has FSH and LH receptors, which can continue its development even though with low FSH concentration[3].

The inhibition exerted by the dominant follicle over the subordinated follicles is due to the production of steroidal substances (estradiol valerate) and non steroidal substances (inhibin) that block the production of gonadotrophins. This turned off in na insufficient FSH plasmatic level for the development of antral follicles. This does not happen to the dominant follicle who, through autocrinal factors, amplifies

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the FSH action, managing to develop even in the presence of low FSH levels. This supports the theory that the dominant follicle, during its growing phase, stops the emergence of a new follicular wave[1].

Ko *et al.*[9] observed that the subordinate follicles stop growing a few days after the emergence of the follicular wave. They also noted that a new wave does not emerge while the dominant follicle is in the growing or in the beginning of the static phase.

Fortune[5] mentions the existence of a morphological and functional dominance. This study shows that the morphological dominance is longer than the functional dominance, but both are necessary for ovulation.

Huhtinen *et al.*[8] and Bungartz; Niemann[3] have shown that superovulatory treatment in the presence of the dominant follicle diminishes the superovulatory response. According to Guilbault *et al.*[7] and Pierson; Ginther[11] superovulatory treatment in the absence of the dominant follicle results in a larger number of ovulations and less varied responses.

Bungartz; Niemann[3] concluded that the presence of the dominant follicle alters the superovulatory response by allowing, when absent, a greater number of ova, eggs and transferable embryos. They have suggested that one ultrasonic examination can detect the dominant follicle on the day that superovulatory treatment begins. This can be done by counting the number of subordinate follicles (>10 small follicles: no dominance; <10 small follicles: dominance).

Rhodes *et al.*[12] compared the size of the ovulatory follicle in *Bos taurus indicus* and *Bos taurus taurus* herds and concluded that Bos taurus indicus follicles are smaller. Figueiredo *et al.*[4] observed that Nelore follicular dynamics are similar to European breeds. Barros *et al.*[2] in a short review compared the information about follicular dynamics in Nelore breed with the European breed, showing that they are similar.

The aim of this study was to verify the behavior of the dominant follicle in Nelore heifers between Day 6 and Day 10 (Day 0= estrus) of the oestrus cycle to establish the optimum moment to begin superovulatory treatment based in the presence or absence of dominant and subordinate follicles.

MATERIAL AND METHOD

For this study nine Nelore heifers, two years old, were used as embryo donors, which were on grazing and supplemented with comercial concentrate and mineral salt.

Eighteen oestrus cycles were monitored by ultrasound from Day 6 to Day 10, a Scanner 480, Pie Medical (The Netherlands) 5 and 7.5 MHz. The ovaries were evaluated by the presence or absence of the corpus luteum (CL), by the measurement of the first and second largest follicle and by the

Table 1

Average number of follicles at artificial insemination and average numbers of corpus luteum and embryos at the embryos recovery in the different development phases of the dominant follicle between Day 6 and Day 10 of the oestrus cycle in Nelore heifers. São Paulo, 1997.

Dominant Follicle	Follicles			Corpus luteum			Embryos	
(Phase)	RO	LO	Total	RO	LO	Total	Viable	Total
Growth (n=6)	7.17	7.17	14.33	6.17	6.00	12.17	1.17	3.00[b*]
Static (n=6)	8.40	8.60	17.00	6.83	6.00	12.83	2.00	3.83[b]
Regression (n=6)	7.33	8.50	15.83	7.03	6.97	14.00	3.67	10.17[a]
Variation coefficient			21.13			24.33	112.49	00.01

RO = Right Ovary - LO = Left Ovary. *Different letters in the same column means statistical difference for $p<0.05$.

Table 2

Average size of dominant follicle and average number of subordinate follicles in the different development phases of the dominant follicle between Day 6 and Day 10 of the oestrus cycle in Nelore heifers. São Paulo, 1997.

Dominant Follicle (Phase)	Average size of Dominant Follicle (cm)					Average number of Subordinate Follicles					
	Day 6	Day 8	Day 9	Day 10	Mean	Day 6	Day 7	Day 8	Day 9	Day 10	Mean
Growth (n=6)	0.59	0.77	1.10	1.13	0.86	5.83	7.70	6.67	7.83	8.00	7.21
Static (n=6)	1.13	1.09	1.01	0.97	1.05	7.33	8.50	8.50	7.50	6.17	7.60
Regression (n=6)	1.12	1.04	1.01	0.77	1.00	8.83	6.00	8.00	9.67	8.00	8.10

[a]Pueset - Serono Pharmaceutical Products Ltda

[b]Ciosin® - Coopers Brasil Ltda

counting of the subordinated follicles.

These exams show whether the follicle is dominant or not and in which phase (growing, static and regression) it was. The follicle was considered dominant when concomitant increase in its diameter and diminishing numbers of subordinate follicles were observed.

At the time of insemination and embryo recovery, an ultrasound examination was done in order to evaluate the superovulatory response by the number of follicles and the ovulation rate by the number of corpus luteum, respectively.

The animals were superovulated with 400 or 500IU of FSH/LH[a] beginning on Day 10 of the oestrus cycle. The superovulatory treatment was in decreasing doses for 4 days at 12 hour intervals. On the fifth dose of FSH/LH (3rd day) prostaglandin[b] was injected. Animals were inseminated twice after heat detection (12 and 24 hours after the beginning of the estrus signs).

The embryos were recovered 6.5 days after the first insemination with PBS (Phosfate Buffered Saline) plus 1% FCS (Fetal Calf Serum) and classified for morphology and quality according to Lindner; Wright[10].

Statistical analysis was done by analysis of variance by GLM program from SAS. The variables that showed statistic significance (5%) where separated by Tukey's test. Since the variable "number of total embryos" did not follow the variance normality nor the residual homogeneity, it was analyzed with non-parametric order statistics by Kruskal Wallis' test (procedure NPAR 1 WAY from SAS).

RESULTS

Of 18 oestrus cycles evaluated, six (6) showed a dominant follicle in the growing phase, 6 in the static phase and 6 in the regression phase (Tab. 1 and 2).

According to the Kruskal-Wallis test there is significant statistical difference between the total number of embryos of the animals superovulated in the presence of the dominant follicle at regression phase and those superovulated in the growing and static phases of the dominant follicle ($p<0.05$). There was no statistical difference between the total number of embryos from the animals superovulated in the presence or absence of the dominant follicle in growing and static phases ($p>0.07$).

DISCUSSION

The verification of the follicular development wave pattern caused the revolution of the techniques of synchronization of the estrus cycle and superovulation[5]. The importance attributed to the follicular dynamics is related to its influence on the superovulatory response in the presence or absence of the dominant follicle and its suppressive activity over the subordinate follicles[3,9].

According to Ko *et al.*[9] the complete regression of the subordinate follicles, only occurs when the suppressive effect of the dominant follicle is constant. Nevertheless, when the dominant follicle is removed, the second largest becomes dominant. Guilbault *et al.*[6] observed that only morphological evaluation of the dominant follicle is not satisfactory. So, our procedure included the number of subordinate follicles to determine a physiological dominance of the dominant follicle. Even though the protocol described by Huhtinen *et al.*[8] was not possible in the routine work at the field it was chosen to conduct ultrasonic monitoring between Day 6 and Day 10 of the oestrus cycle. This period being ideal to establish the optimum moment to begin the superovulation process, since the superovulatory response is optimized in the presence of a larger number of follicles measuring 2 to 4mm.

The best superovulatory responses in this study were observed when the dominant follicle was in regression phase. This agrees with Bungartz; Niemann[3], who characterized as dominant follicle those with diameters greater than 9mm in growing phase or for <3 days in plateau phase and in the presence of less than 10 subordinate follicles. These principles were adopted for this study, adapted to different follicle size ratios, as mentioned by Rhodes *et al.*[12], who states that the follicular dynamics of the Brahman heifers is similar to the *Bos taurus taurus* with the exception of the size of the dominant follicle and the corpus luteum which are smaller. Figueiredo *et al.*[4] also mentioned this similarity, in spite of the smaller dominant follicles and corpus luteam in Nelore breeds and a two wave pattern prevalence. In the review done by Barros *et al.*[2], they confirmed in Nelore breed that the ovulatory follicles are smaller and the estrus behavior are shorter.

According to statistical analysis no difference was observed in the total number of embryos between animals superovulated with dominant follicles in the growing and static phase. This fact can be explained by the difficulties in classifying the dominant follicle on the transition from the growing to the static phase. It was also noted a certain suppression of the subordinate follicles in the static phase. As the classifying criteria was morphological, we cannot determine if the dominant follicle was in the beginning, middle or ending the static phase. This would need a physiological classification.

The analysis of Tab. 2 confirms the studies of Guilbault *et al.*[7] and Bungartz; Niemann[3] indicating that the number of subordinate follicles is superior in the absence of a dominant follicle. It was observed that during the regression phase there was a tendency for larger numbers of subordinate follicles (8.10), in comparison with the growing (7.21) and static phase (7.60).

CONCLUSION

Superovulation in the absence of a dominant follicle resulted in better embryos recovery ratios. However, new studies must be conducted to broaden the knowledge about the morphological and physiological behavior of the dominant follicle in the Nelore heifers.

RESUMO

O objetivo deste trabalho foi avaliar a influência do folículo dominante nas fases de crescimento, estática e de regressão sobre a resposta superovulatória em 18 ciclos estrais de 9 novilhas da raça Nelore. Avaliaram-se os ovários entre o dia 6 e o dia 10 do ciclo estral pela técnica de ultra-sonografia, medindo-se o diâmetro do maior folículo e contando-se o número de folículos subordinados. Os animais foram superovulados com 400 ou 500 UI de FSH/LH, iniciando-se no dia 10 do ciclo estral, em subdoses decrescentes, por 4 dias consecutivos. Aplicou-se $PGF_2\alpha$ concomitante com a quinta subdose de FSH/LH e realizaram-se inseminações artificiais às 12 horas e às 24 horas após o início dos sintomas de estro. Os embriões foram colhidos no dia 6,5 após a primeira inseminação artificial, obtendo-se 3,00; 3,83 e 10,17 embriões, respectivamente, nas fases de crescimento, estática e regressão. Observaram-se melhores resultados quanto ao número de embriões quando o folículo dominante se encontrava em fase de regressão.

UNITERMOS: Folículos dominantes; Resposta superovulatória; Nelore.

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