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Superovulatory response, production and quality of embryos of cows fed on linseed or canola seed supplemented diets

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ABSTRACT. Superovulatory response, production and embryo quality and the effects of seasonal changes on embryo production of Nellore cows fed on either linseed (n-3) or canola seeds (n-6) were evaluated. Sixteen Nellore cows (550 ± 48.0 kg) were fed on three diets: control diet (CON), diet with linseed (LIN) and diet with canola seed (CAN) during four seasons. There was no difference in superovulatory response and in total corpora lutea produced when diets are taken into consideration. The number of cows that responded to superovulatory treatment was higher in the winter (93.8%) than in the summer (62.5%). No difference with regard to the three diets existed on the average number of total structures (6.20, 4.96 and 6.50), unfertilized structures (2.53, 1.17 and 1.60) or congealable embryos (3.40, 1.30 and 3.80). Average degenerated embryos were higher for cows fed on LIN diet (2.48) than for those fed on CON diet (0.32); the CAN diet presented an intermediate response (1.10). Mean total structures produced were lower during the winter (3.57) and summer (3.50) than during the autumn (8.75) and spring (7.25).

Keywords: season of the year, diets, embryos, fatty acids, fertility, omega 3, omega 6.

Resposta super ovulatória, produção e qualidade de embriões de vacas suplementadas com semente de linhaça ou grãos de canola

RESUMO. Este estudo avaliou a resposta superovulatória, produção e qualidade de embriões e o efeito da estação do ano sobre as alterações hormonais e na produção embrionária de vacas Nelores alimentadas com semente de linhaça (*n*-3) ou semente de canola (*n*-6). Dezesseis vacas Nelores foram usadas neste estudo (550 ± 48,0 kg). Nas quatro estações do ano as vacas receberam diferentes dietas: controle (CON), com semente de linhaça (LIN) e com semente de canola (CAN). Não houve diferença na resposta superovulatória e número total de corpos lúteos produzidos em função das dietas. O número de vacas que respondeu ao tratamento superovulatório foi maior na primavera (93,8%) do que no verão (62,5%). Não houve diferença para as três dietas sobre o número médio de estruturas colhidas (6,20; 4,96 e 6,50), de estruturas não fertilizadas (2,53; 1,17 e 1,60) ou de embriões congeláveis (3,40; 1,30 e 3,80). O número médio de embriões degenerados foi maior para as vacas com a dieta LIN (2,48) do que para as vacas da dieta CON (0,32). A dieta CAN mostrou uma resposta intermediária (1,10). O número de estruturas produzidas foi menor no inverno (3,57) e verão (3,50) do que no outono (8,75) e primavera (7,25).

Palavras-chave: estação do ano, dietas, embriões, ácidos graxos, fertilidade, ômega 3, ômega 6.

Introduction

Animals of pure origin (PO) are important for the cattle-raising industry since the value of cows and their products (embryos or calves) is high. The group, which includes animals with high genetic potential for the production of milk and meat, justifies their expensiveness. Since biotechnologies such as embryo transfers (ET) and *in vitro* fertilization (IVF) are necessary for the rapid and profitable diffusion of genetic material, these techniques increase the female reproduction index, selection intensity and the availability of replacement animals and decrease the time between generations.

Protein and energy have complex effects on ET and an excess of energy may have negative effects on the development of embryos (BOLAND et al., 2001). On the other hand, a proper increase in the diet's energetic level may increase the number of follicles that respond to the superovulatory diet (RIGOLON et al., 2003; ALBUQUERQUE et al., 2009). Moreover, the dietary source used to feed the donors may also influence their superovulatory response and the quality of their embryos (CAPOVILLA et al., 2006; CAVALIERI et al., 2005; PETIT et al., 2002; YAAKUB et al., 1999).

Recent research highlights the benefits with sources rich in essential fatty acids in the diet. Müller et al. (2009) observed that the number of viable embryos increased when grains of canola (linoleic acid *n*-6) were added to the diets of Nellore heifers. Likewise, Capovilla et al. (2006) found that supplementing the diets of *Santa Ines* sheep with Lac 100® (linoleic fatty acid *n*-6) resulted in a higher number of viable embryos. In addition to nutritional factors, weather may also affect the superovulatory response and embryo production. According to Pinto Neto et al. (1999), most investigations on technologies associated with embryo production have studied on European breeds, with few studies on Zebu animals. Although there are several differences between Zebu and European cows, little information is available on the performance of Zebu breeds, particularly Nellore, in programs involving embryo transfer and conservation (PINTO NETO et al., 1999).

Current trial was carried out during the four seasons of the year to evaluate the superovulatory response and the total production of congealable and viable structures of Nellore cows fed with linseed grains or canola seeds.

Material and methods

Animal management

The experiment was carried out at the cattle sector of the Iguatemi Experimental Farm of the State University of Maringá, Maringá, Paraná State, Brazil. Sixteen 5-year-old Nellore cows, average weight 550 kg ± 48.0 kg and average body score of 3 (on a scale from 1 to 5) were used. The cows were kept in individual 10 m^2 pens enclosed with square iron bars and concrete floors, and partially covered with zinc tiles. Each pen had a feed box (2 meters long) where food was placed twice a day (at 8 am and 4 pm). Feeding lasted for 46 days (30 days for the initial feeding and 16 days until embryo collection).

Isoprotein and Isoenenergy diets, following NRC (1996) (Table 1), and control values were used to estimate Total Digestible Nutrients (TDN). Whereas Table 2 shows the feed percentages used in the experimental diets, Table 3 demonstrates the composition of fatty acids. The diets' bromatologic and fatty acids composition analyses were undertaken at the Animal Nutrition Laboratory of the State University of Maringá.

Table 1. Chemical composition of ingredients and experimental diets.

Ingredients	DM[a]	CP[b]	NDF[c]	ASH	EE[d]	TDN[e]
Cottonseed hulls	92.5	3.64	83.0	1.62	0.90	48.8
Cottonseed meal	93.3	34.4	51.3	4.34	1.62	63.0
Ground corn	90.7	9.51	14.7	1.22	2.56	80.0
Corn germ	84.6	14.0	26.2	3.08	6.30	77.0
Linseed	94.4	24.5	42.3	3.75	28.43	127
Canola seed	95.4	30.0	45.0	3.57	29.30	127
Mineral salt	97.8	-	-	-	-	-
Limestone	98.0	-	-	-	-	-
CON[f]	90.6	9.44	51.4	2.00	2.43	67.2
LIN[g]	92.9	9.10	69.1	2.08	5.96	72.1
CAN[h]	92.9	9.09	67.9	1.92	5.75	71.9

[a]Dry matter; [b]Crude protein; [c]Neutral detergent fiber; [d]Ether extract; [e]Total digestible nutrients; [f]Control diet; [g]Linseed diet; [h]Canola diet.

Table 2. Percentage composition of experimental diets (%DM).

Ingredients (%DM)	CON[a]	LIN[b]	CAN[c]
Cottonseed hulls	47.5	69.9	69.6
Cottonseed meal	8.03	4.83	1.99
Ground corn	23.3	4.93	9.50
Corn germ	19.1	0.00	0.00
Linseed	0.00	18.4	0.00
Canola seed	0.00	0.00	16.9
Mineral salt	1.07	1.05	1.04
Limestone	1.07	1.05	1.04

[a]Control diet; [b]Linseed diet; [c]Canola diet.

Table 3. Fatty acids percentage of the experimental diets.

Fatty Acids	CON[a]	LIN[b]	CAN[c]
16:0 (hexadecanoic acid)	12.9	5.38	5.51
18:0 (octadecanoic acid)	7.01	8.17	2.82
18:1 *n*-9 (9-octadecenoic acid)	40.9	29.1	64.8
18:1 *n*-7 (11-octadecenoic acid)	0.28	0.23	0.00
18:2 *n*-6 (9,12-octadecadienoic acid)	38.1	19.3	21.3
18:3 *n*-3 (9,12,15-octadecatrienoic acid)	0.85	37.8	5.53
PUFA (Polyunsaturated fatty acids)	38.9	57.1	26.8
MUFA (Monounsaturated fatty acids)	41.2	29.4	64.8
SFA (Saturated fatty acids)	19.9	13.6	8.33
n-6 (Omega-6)	38.1	19.3	21.3
n-3 (Omega-3)	0.85	37.8	5.53
PUFA/SFA	1.95	5.03	3.23
n-6/*n*-3	44.9	0.52	3.86

[a]Control diet; [b]Linseed diet; [c]Canola diet.

Treatments and sampling

Treatments consisted of three experimental diets: control diet (CON), diet with linseed (LIN) and diet with canola seed (CAN). Cotton hull was included as roughage in all three diets.

The experiment was conducted during autumn, winter, spring and summer. During the superovulation treatment, the cows' rectal temperature was measured in the morning.

Each experimental period lasted 46 days; on average, there were 25 days between the periods, so that each season had one experimental period. The cows were randomly distributed into three groups. In each season, two groups contained five animals and one contained six animals, the cows were supplied with the respective diets for each treatment. At the end of each period, the cows were kept on a pasture of star grass (*Cynodon nlemfluensis*) until the next experimental period began. In the following season, the cows were again distributed randomly for another treatment which was different from the previous one. Thus, all of the cows received all three diets, albeit in different seasons.

The superovulation treatment started 30 days after the cows' adaptation to the nutritional program. Donor's estrus was synchronized with an auricular implant containing 5.0 mg of Norgestomet (Crestar® - Lab. Intervet Ltda) (day zero – D0) and 2.0 mg of estradiol benzoate (Estrogin® - Lab. Farmavet Ltda) (D1). On D5, a total of 250 UI of FSH (Pluset® - Lab. Serono Ltda.) were administered to each cow twice a day in decreasing doses (100/100 UI, 75/75 UI, 50/50 UI, 25/25 UI). Whereas in the afternoon of D7, 2.0 mL of prostaglandin F2α (Ciosin® - Lab. Coopers Ltda) were given intramuscularly, in the morning of D8, the auricular implant was removed and 24 hours later (D9) they received 2.500 UI of hCG, IM (Chorulon® - Lab. Intervet Ltda). The fixed-time artificial insemination (FTAI) was performed 12 and 24h after hCG administration with semen (only one sire) with motility equal to or higher than 35% and with vigor 3.

The superovulatory response was determined by the presence of corpora lutea in the right and left ovaries.

The embryos were collected seven days after the first artificial insemination by the transcervical method. The uterine wash was performed with 500 mL of modified medium Dulbecco (DMPBS) (Nutricell Nutrientes Celulares Ltda) at 37ºC. The medium was introduced into the cervix by a Folley Catheter 20 and the effluent was collected with a 75 μm filter and transferred to a checkered petri dish. The embryos were located with a stereoscopic microscope at an 8-fold magnification level and were transferred to a mini petri dish containing a holding medium (Holding Plus® - Lab. AB Technology Ltda). Through a 40 magnification level, the developmental stage and quality of the embryonic structures were classified according to the system proposed by the International Embryo Transfer Society (IETS, 1999). The system includes four levels: degree 1 (excellent or good), degree 2 (regular), degree 3 (poor) and degree 4 (dead or degenerate). However, in current trial, all congealable embryos classified at levels 1, 2 or 3 were taken into account, regardless of their development stage.

Experimental design and statistical analysis

The distribution function of binomial probability and the logarithmic connection function were used to examine the variable superovulatory response. Whereas the Poisson probability distribution function analyzed the number of corpora lutea in the ovaries (right and left), total structures, unfertilized structures, degenerate and congealable embryos, the logarithmic function of connection was estimated by the generalized linear model methodology (NELDER; WEDDERBURN, 1972) using GLIM 4.0 software. Mean rates were compared to those by Tukey's test at 10% probability. The statistic model was the same in all analyses and treatment type and period were included.

The statistical model was the following:

$Y_{ijk} = \mu + T_i + E_j + TE_{ij} + e_{ijk,}$ $Y_{ijk} =$ observation with regard to animal "k," on treatment i (i = 1, 2 and 3), during period "j" (1, 2, 3 and 4);

T_i = Effect of the treatment i (i = 1, 2 and 3);

E_j – Effect of the season j (j = 1, 2, 3 and 4);

TE_{ij} – Effect of the interaction between the treatment and the season;

e_{ijk} = Random error associated with each observation.

To determine the superovulatory response, the number of corpora lutea in the left and right ovaries and the number of total structures, unfertilized structures, degenerate embryos, and congealable embryos as a function of the rectal temperature were deployed in an orthogonal polynomial using SAS (2006) software.

Results and discussion

Superovulatory response and total corpora lutea with the addition of n-3, n-6 and n-9

The addition of linseed grains or canola seed in the diets of Nellore cows did not have any effect ($p > 0.10$) on superovulatory response and on the corpora lutea (Table 4). The superovulatory response was not constant among the cows stimulated for embryo collection. Moreover, high variation in the superovulatory response is one of the biggest problems related to embryo transfer (BOLAND et al., 2001) According to Gong et al. (1997), this variation might be related to the population of follicles responsive to gonadotrophins in the superovulatory period. Likewise, an increase in the energy density of the diet may increase the number of responsive follicles (GUTIÉRREZ et al., 1997; RIGOLON et al., 2003).

The high proportion of cereal grains in the diets of ruminants may alter the population of microorganisms (NUSSIO et al., 2006). A high amount of amylolitic bacteria may increase the production of pronionic acid (NUSSIO et al., 2006) which, after being absorbed by the ruminal wall, goes to the liver and metabolized until it is transformed into glucose through glucogenesis (O'CALLAGHAN; BOLAND, 1999). This raises the level of glucose in the blood stream and subsequently increases the level of sanguine insulin (GUTIÉRREZ et al., 1997). Spicer and Echterkamp (1995) suggest that insulin and IGF-I, acting on follicular growth, stimulate the proliferation and differentiation of granulose cells and the production of progesterone. Müller et al. (2009) observed that heifers fed on canola seed presented a higher number of corpora lutea in the left ovary, a lower number of cysts in the right ovary and fewer total cysts. These results were similar to those observed by Cavalieri et al. (2005) in their research on Holstein cows that received linseed grains or Megalac®. The amount of fat in the diets of cows increases the number of follicles, although this increase occurs regardless of fatty acid profile (CAVALIERI et al., 2005).

Table 4. Superovulatory response and number of corpora lutea in the ovaries (left and right) of Nellore cows fed on linseed or canola diets.

Cows	CON[a]	LIN[b]	CAN[c]
Number of cows	16	14	16
Number of responsive cows	13	12	13
Variables			
Superovulatory response %	81.3	85.7	81.3
Average corpora lutea	10.0	10.0	11.0

[a]Control diet; [b]Linseed diet; [c]Canola diet.

Number of total structures, unfertilized structures, degenerate embryos and congealable embryos produced with the addition of n-3, n-6 and n-9

There was no difference ($p > 0.10$) among the treatments (CON, LIN and CAN) in the total number of structures produced or in the number of unfertilized structures, degenerate embryos and frozen structures (Table 5).

Table 5. Means and percentage of total structures and means of unfertilized structures, degenerated embryos and congealable embryos of Nellore cows fed on linseed or canola seeds.

	CON[a]	LIN[b]	CAN[c]
Number of cows	16	14	16
Variables			
Structures, numbers	6.20	4.96	6.50
Unfertilized structures, numbers	2.53	1.17	1.60
Degenerate embryos, numbers	0.32a	2.48b	1.10ab
Degenerate embryos (%)	40.8a	23.6b	24.6ab
Congealable embryos (%)	5.16a	49.8b	16.9ab
Frozen structures, numbers	3.40	1.30	3.80
Frozen structures (%)	54.8	26.2	58.5

[a]Control diet; [b]Linseed diet; [c]Canola seed diet. Different letters on the same line show different rates ($p < 0.10$).

Mean total structures (5.89) were less than those observed by Donaldson (1984) who found an average of 9.20 total structures in Zebu cows. Moreover, in their studies on Zebu cows, Gradela et al. (1996) found an average of 11.5 structures. This amount was higher than that reported in current experiment. Müller et al. (2009) found that when linseed or canola seed were included in the diets of Nellore donors, a greater number of total structures resulted from treatments without fat additions (10.4) and from treatments with canola seeds (8.70) than from those with linseed (5.30). Similarly, Cavalieri et al. (2005) found higher values for this variable (42.0% for linseed and 32.0% for Megalac®).

The mean degenerate embryos were higher ($p < 0.10$) with LIN treatment (2.48 – 23.6%) than with CON treatment (0.32 – 40.8%), whereas CAN treatment produced an intermediate rate (1.10 – 24.6%) (Table 5) The mean number of degenerate embryos was lower than that observed by Müller et al. (2009) who found a higher number of degenerate embryos in diets without fat addition (73.0%) and with linseed (69.8%) when compared to diets with canola seed (23.0%). Featuring similar values of 21.6% and 18.9%, respectively, Cavalieri et al. (2005) did not observe any difference in the number of degenerate embryos from treatments with linseed or Megalac®. While current rates corroborate results by Cavalieri et al. (2005), Capovilla et al. (2006) observed higher percentages of unfertilized structures and degenerate embryos from linseed treatment (33.6%) than from treatment with Megalac-100® (0%). This fact suggests that sheep supplemented with linseed produce fewer and lower quality embryos. Müller et al. (2009) have also reported a higher percentage of viable structures from canola seed treatment (76.9%) when compared to no fat (26.9%) and linseed (22.0%) treatments.

Superovulatory response and number of total corpora lutea as a function of the seasons of the year

Table 6 shows that there was a higher ($p < 0.10$) response to superovulatory treatment in the winter (93.8%) than in the summer (62.5%); response was intermediate in the autumn (87.5%) and spring (78.6%). However, the ovulation rate was highest ($p < 0.10$) in autumn (13.0) and lowest in summer (8.00).

During the experiment, the average pen temperature (RT) ranged between 28.8 and 32.4°C; relative humidity (RH) ranged between 56.4 and 44.0%; temperature and humidity rates (THI) ranged between 75.1 and 81.6 units; the Index of Globe Temperature (WBHT) ranged between 21.8 and 21.0 units in the shade and between 22.70 and 27.10 units in the sun. Overall average values were: 30.6°C (RT), 50.2% (RH), 78.4 units (THI) and 24.9 units (WBHT). THI rate (78.4) demonstrates that the animals are in a state of alert. According to classification by Rosenberg et al. (1983), THI between 68.0 and 74.0 may cause production loss in the animals; when values are between 75.0 and 78.0, the farmer must be alert to animal death; values between 79.0 and 84.0 are a danger to confined cattle in particular; if level exceeds 85.0, death is likely, unless urgent action is taken. Despite the development of this index for bovine living in North-American conditions, Hahn (1999) reports that THI values below 75.0 may not cause damage to animal production since the night temperature does not exceed 23°C.

Table 6. Superovulatory responses and ovulation rates of Nellore cows in Autumn, Winter, Spring and Summer.

Season	Autumn	Winter	Spring	Summer
Number of cows	16	16	14	16
Number of responsive cows	14	15	11	10
Variables				
Superovulatory response, %	87.5ab	93.8a	78.6ab	62.5b
Total corpora lutea	13.0a	11.0ab	11.0ab	8.00b

Different letters on the same line show different rates ($p < 0.10$).

Although THI was above 75.0, the mean night temperature lay between 16.0 and 18.1°C for September and October, respectively. Nevertheless, a 78.35 THI may have affected the animals' general performance regardless of diet treatment. This corresponds to the information presented by Silva et al. (2002) who stated that this index is an important parameter since THI over 72.0 results in a decline in milk production in Holstein cows. Conflicting information exists about the possible influence of the seasons on the superovulatory response of cows (KAFI; McGOWAN, 1997). According to these authors, features of seasons may vary from one year to another and differ in various regions, coupled to the fact that it is difficult to control such factors.

One research project was carried out with Zebu breeds (Gyr and Nellore) and aimed to assess the effects of the season (winter and summer) on follicular dynamics and on the moment of ovulation (BORGES et al., 2004). The researchers noticed the predominance of estrus cycles with three follicular waves that were not influenced by the season of the year. On average, ovulation occurs 23.1 hours after the detection of estrus; this average does not differ much among breeds and seasons. When the interaction of diet and season was examined, no difference ($p > 0.10$) was found in superovulatory response averages or in the number of total corpora lutea.

Unfertilized structures and degenerate embryos as a function of seasons of the year

Mean number of total structures collected in autumn (8.75) and spring (7.25) were higher ($p < 0.10$) than the mean number collected in the winter (3.57) and summer (3.50) (Table 7).

Table 7. Means and percentage of total structures, unfertilized structures, degenerate embryos and congealable embryos in Autumn, Winter, Spring and Summer.

	Autumn	Winter	Spring	Summer
Number of cows	16	16	14	16
Variables				
Total structures	8.75a	3.57b	7.25a	3.50b
Unfertilized structure	2.00	1.22	3.30	0.38
Unfertilized structures, %	22.9	34.2	45.5	10.9
Degenerates embryos	2.25	0.50	0.63	2.00
Degenerate embryos, %	25.7	14.0	8.69	57.1
Congealable embryos	4.50a	1.90b	3.40ab	1.10b
Congealable embryos, %	51.4	53.2	46.9	31.4

Different letters on the same line have different rates ($p \leq 0.10$).

Mean unfertilized structures and degenerate embryos were similar ($p > 0.10$) throughout the seasons of the year (Table 7). However, the number of congealable embryos was lower ($p > 0.10$) in the summer (1.10) and winter (1.90) than in the autumn (4.50).

As rectal temperature (RT) increased, a linear increase ($p < 0.10$) in the number of animals that responded to the superovulatory treatment was reported. Rectal temperatures of adult Zebu cows ranged between 38.5 and 39.7°C, with a mean 39.1°C. The thermal comfort zone for this subspecies is between 7 and 35°C (or room temperature). These values are considered to be the lowest and highest critical temperatures, respectively. When room temperature is lower than ICT, the animal experiences a stress condition caused by the cold, whereas heat causes stress when the temperature exceeds SCT (SILVA et al., 2002).

However, in current experiment, RT did not have a significant influence ($p > 0.10$) on the number of corpora lutea or on mean total structures, unfertilized structures, degenerate embryos and congealable embryos. These results were obtained because the mean temperatures and relative humidity measured during the four seasons were within the thermal comfort zone for Zebu cows.

Mean total structures influenced the mean number of congealable embryos. Therefore, a lower mean number of total structures would result in a lower mean number of congealable embryos. Research by Massey and Oden (1984) and Shea et al. (1984) examined the influence of the seasons of the year on embryo production in Zebu and European cows. These studies, however, have not reported seasonal differences for any of the subspecies under analysis. Badingaa et al. (1993) worked with Holstein cows during the summer in Florida and observed that thermal stress condition was associated with a lower diameter of the dominant follicle and a lower volume of follicular liquid (14.5 mm and 1.10 mL) than that observed in thermo-neutral cows (16.4 mm and 1.90 mL). The authors suggested that calorie stress may alter the efficiency of follicular selection and dominance and therefore jeopardize follicle quality.

The THI in current research was 78.4, which suggests that the cows were in an alert condition. In fact, this value is at the upper limit of the classification system developed by Rosenberg et al. (1983). On the other hand, Hahn (1999) used this classification system and argued that THI values below 75.0 cannot cause damage to the production of animals in feedlots since night temperatures do not exceed 23.0°C. Even with the mean night temperature at 23.0°C in this experiment, it is possible that 78.4 THI level impaired the total performance of the animals regardless of dietary treatments.

Putney et al. (1998) examined the ET process in Holstein cows and observed differences in the quality of the embryos. During the cold seasons, the quality of the embryos was superior to those collected during the summer. The authors therefore recommend that embryo collection be done during the winter. According to Hasler (1992), a mean of 5 transferable embryos per collection from a donor hides the variability of the response; extremes may range from 0 to over 50 structures. This variability may be related to several factors and is still not fully understood (KAFI; McGOWAN, 1997). Further, the mechanisms involved in the variation among individuals cannot be easily determined (HASLER, 1992). Thermal stress may result in an increase or a decrease in body temperature (hyperthermia or hypothermia, respectively). However, estrus also results in an increase in body temperature. An increase in body temperature between 1.0 and 1.5°C is considered normal when close to the ovulation period. Putney et al. (1998) observed that only 20.7% of the 82 embryos recovered from Holstein cows under heat stress were normal when compared to 51.5% of the 68 embryos recovered from thermo-neutral cows.

Conclusion

The addition of linseed or canola seeds to the diets of Nellore cows did not alter the superovulatory response or the production of total structures, unfertilized structures and congealable embryos. However, mean degenerate embryos were higher from cows fed on linseed-supplemented diet. Thus, the use of omega-3 fatty acids as a source of energy may cause poor performance in Nellore cows. The seasons of the year did not influence the superovulatory response or the production and quality of embryos from Nellore cows under subtropical conditions.

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