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Pregnancy Rates in Timed Artificial Insemination in Nelore Cows (Bos Taurus Indicus) Using Estradiol Benzoate or Estradiol Cipionate as Ovulation Inducers Associated WitheCG or FSHp.

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Abstract: *The aim of this study was to evaluate the effects of two different esters of estradiol, associated with equine chorionic gonadotropin (eCG) or Follicle Stimulating Hormone (FSHp) on conception rates of Nelorecows in the Amazon,northern Brazil.Nelore cows (n= 1098) at 40-60 days postpartum, with body condition score (BCS) of 3.17 were used.In experiment 1, cows were assigned in groups EB-eCG (n= 279) or EB-FSHp (n= 280). In experiment 2, cows were assigned in group EC – eCG (n= 270) and in group EC –FSHp (n= 269). Cows received 300 IU i.m of eCG or 10mg i.m of FSHp. Insemination was performed 56h after progesterone intravaginal releasing device (PRID) removal. In a subset of cows (n=102) ultrasound (US) examination were performedto evaluate the presence of largest follicle (LF), ovulatory follicle (OF) and ovulation (OV) from D8 to D10 every 24h and from D10 to D12 every 12h until detection of ovulation. (LF), (OF),(OV) and interval of PRID removal to ovulation did not differ between groups.The eCG treatment in group EB increased CR compared with FSHp.In EC groupsthere were no differences in CR. Thus, the estradiol esters EB and EC seem to be efficient as ovulation inducers in TAI programs, and the use of eCG was greater than FSHp in TAI programs in Nelore Cows.*

Keywords: *Amazon, anestrous, artificial insemination, genetic improvement.*

I. Introduction

In different tropical areas of the world, as in the case of the Brazilian Amazon, beef production is made using zebu cattle (*Bostaurusindicus*) due to its greater resistance to the humid tropical climate and its adaption to grazing in extensive management systems [1]. Under these circumstances, Brazil exported in 2015, 1,399,259 tons of beef, corresponding to the value of USD $ 5.939 million to more than 100 countries [2]. Furthermore the Brazilian cattle herd reached a record 215.2 million head in 2015, an increase of 1.3% compared to the previous year. Moreover, in the last decade beef production in the Amazon has contributed strongly to the expansionary scenario of animal production in Brazil. Actually the cattle herd in the Brazilian Amazon represents 36.95% of the national herd with current 80,046,890 head, with the growth ofregional livestock representing 85% of the national herd increase [3]. Therefore the participation of the Amazon region in the production of beef in Brazil, has been increasing year after year. Although these numbers are significant it is known that the cattle herd has a low reproductive efficiency which compromises this economic activity [4].In this respect reproductive biotechnologies (RB) have been used and have significantly increased the CR through the use of protocols for Timed Artificial Insemination (TAI) which has proven efficient to ensure improvement of the sustainabilityofcattle ranching [5, 3]. In addition to above, the use of RB has been an important tool for increasing the efficiency of cattle production systems in the Amazon region, avoiding deforestation and increasing the sustainability of agricultural systems [5]. The results of protocol combinations using progesterone and estrogens are very well consolidated in the literature [6, 1, 7, 8], showing CR near ~50%. However, in tropical conditions based on seasonal grasses, anestrous postpartum, which is quite common, when used in primiparous cows, the use of hormones that improve ovulation rate and final follicular growth is required [9]. Furthermore, the same results have been obtained at a local level using protocols combining progestinsand estrogens and have demonstrated tostimulate an increase in pregnancy rates to ~ 50 percent,and such increase is attributed to the positive effect of these hormones on anestrous postpartum primiparous cows, due the enhancement of growth and follicular maturation and consequently higherovulation rates [9, 5].Considering that in the literature there are still gapswith respect to the use of equine chorionic gonadotropin (eCG) and follicle stimulating hormone (FSHp), the aim of the present study was to test protocols of TAI using hormone variations of Estradiol benzoate (EB) or Estradiol cipionate (EC) as ovulation inducers, combined with eCG or FSHp in multiparous Nelore cows in an early postpartum period. The hypothesis was that protocols using eCG,

independent of ovulation inducers, would enhance final follicular growth and better pregnancy rates than the use of FSHp.

II. Materials and methods

Study Location

The experimentwas conducted in a commercial farm located in northern Brazil(S = 04° 13 1.3s W = 56° 9 19.3s) in the municipality of Itaituba, Pará state, which has a long tradition in cattle production. The experiment was done from July 2014 through August 2016.

Animals

Multiparous suckled Nelore cows (n= 1098) at 40-60 days postpartum and average body condition score (BCS) of 3.17 (1-5 points scale,were used). Cows were kept on pasture (*Brachiariabrizanhta var. Marandu*) with *ad libitum* access to water and mineral supplement during the experimental period.The entire herdwas subjected to routine health control composed of vaccination against brucellosis, Foot and Mouth Diseases, rabies and deworming twice a year.

Experimental design

Experiment 1

On D0 all cows received a PRID (Sincrogest® OuroFino® 1g P4) and 2.0 mg i.m. estradiol benzoate-EB (Gonadiol®; Intervet/Schering-Plough, Brazil). On D8 PRID were removed and administered 4.0mg of dinoprosttromethamine (Lutalyse® Pfizer® 4mg PGF2α)i.m. Cows were assigned according BCS in groups EB-eCG(n= 279) and received 300 IU i.m. (Novormon®, Syntex, Buenos Ayres, Argentina) or EB-FSHp(n= 280) and received 10mg i.m. (Folltropin®, Bioniche, Canada). On D9 1mg of EB im(Gonadiol®; Intervet/Schering-Plough, Brazil)were administered. Insemination was performed 56h after PRID removal. Figure 1.

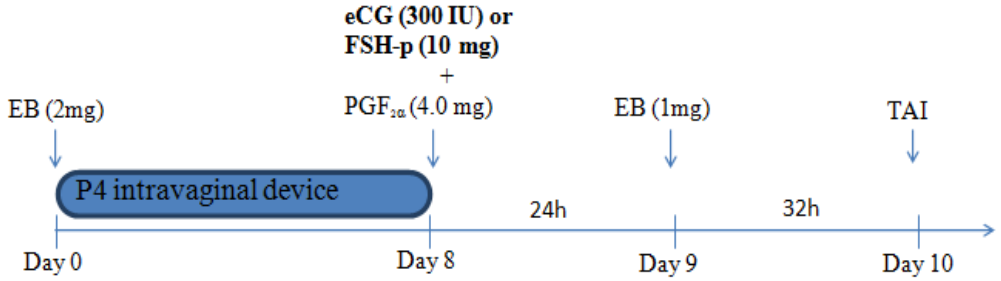

Fig.1.Experimental design group EB-eCG or EB-FSHp.

Experiment 2.

On D0 all cows received a PRID (Sincrogest® OuroFino® 1g P_4) and 2.0 mg i.m. of estradiol benzoate-EB (Gonadiol®; Intervet/Schering-Plough, Brazil). On D8 PRID were removal and 1mg of estradiol cipionate –EC (Ecp® Pfizer® 1.0 mg) and 4.0 mg of dinoprosttromethamine (Lutalyse® Pfizer® 4mg PGF2α) were administered i.m.. Cows were assigned according BCS in groups EC-eCG or EC-FSHp. Cows in group EC - eCG (n= 270) received 300 IU i.m. (Novormon®, Syntex, Buenos Ayres, Argentina) and in group EC -FSHp (n=269) received 10mg i.m. (Folltropin®, Bioniche, Canada). In these groups insemination was performed 56h after PRID removal. Figure 2.

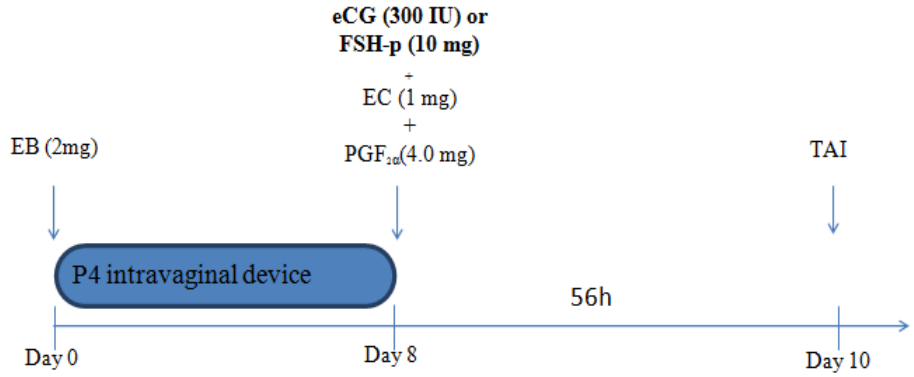

Fig.2. Experimental design group EC-eCG or EC-FSHp.

All inseminations were performed by the same AI technician and frozen straws of four sires with proven fertility were homogenously distributed among groups. To detected pregnancy, ultrasound examinations (US) (Mindray DP-2200VET, 5-MHz linear transducer, China) were performed 35 days after timed artificial inseminations.Furthermore, in a subset of multiparous cows (n=102)US (Mindray DP-2200VET, 5-MHz linear transducer, China) was done to evaluate the presence of largest follicle (LF), ovulatory follicle (OF) and ovulation (OV). The cows were assigned according BCS in groups EC-eCG, EC- FSHp, EB-eCG, EB-FSHp. The US examinations were performed from D8 to D10 every 24h and from D10 to D12 every 12h until detection of ovulation. The moment of ovulation was defined as the time of disappearance of the LF between successive US examinations.

Statistical Analysis

Statistical analyses were performed using the Statistical Analysis Systems for Windows version 8.2 (SAS 2001). The Chi-square procedure was used to determine significant differences between groups in experiments 1 and 2. Treatment differences are considered significant at P <0.001.To evaluate the diameter of largest follicle (LF), ovulatory follicle (OF) and ovulation (OV) rates the GLM procedure with SNK adjustment was used to determine significant differences among groups.

III. Results and discussion

The largest follicle, maximum diameter of the LF, maximum diameter of the ovulatory follicle, ovulation and interval P4 device removal to ovulation did not differ between groups EB and EC. Data are show in Table 1. These results were similarlyto those from previous studies[7, 9]. Despite the high ovulation rate in this study, both estradiol esters had similar effects on ovulation rate,and this allows cows to be inseminated at a predetermined point in time thereby avoiding the need for estrus detection [1].

Table 1 Effects of ovulation induction associated with eCG or FSH on ovarian responses of suckled multiparous (*Bostaurusindicus)*Nelorecows submitted to estrus cycle synchronization through TAI protocols.

Items	Group EB		P	Group EC		P
	eCG	FSH		eCG	FSH	
N. of animals	24	26		23	25	
Diameter of LF on Day 8 (mm)	12.15	11.09	0.094	12.30	13.24	0.07
Maximum diameter of LF (mm)	13.5	12.63	0.0183	13.85	14.66	0.114
Maximum diameter of OF (mm)	16.0	14.50	0.030	16.23	16.47	0.079
Interval PRID removal to ovulation (h)	68.5	64.88	0.045	65	63.84	0.052
Ovulation rate (%)	100	100	0.088	96	96	0.076

Abbreviations: LF, largest follicle; OF, ovulatory follicle; TAI, timed articial insemination; P4, progesterone.

Experiment 1 - Conception rate throughTAI in suckled Nelore cows using EB as an ovulation inducer associated with eCG or FSHp.In the present experiment the eCG treatment increased CR compared with FSHp (65.23% versus 48.57%), (Table 2). Thus, the initial hypothesisis clearly supported, and data show the capacity of the eCG to enhance the final follicular growth, ovulation as well as demonstrates the positive effects of TAI. [5]foundsimilar results in pregnancy rates in TAI in the Amazon region (58.8%) when TAIwas performed in multiparous cows. Indeed the optimum results of eCG on CRfound in the study are in agreementwith other authors and strengthens the positive function of gonadotropins in cows in anestrous or in early postpartum period [1, 9]. Therefore, it seems obvious that eCG acts as a potent final gonadotropic support leading to a greater ovarian response [10]. Despite the similar effects of FSHp in ovarian response, there was a smaller increase in the CR when compared with eCG,and [11]found similarresults. It is know that the half-life of FSHpis about 5 hours, and this probably led to a limitation in TAI protocols. BCS did not have any effect in pregnancy rates in this study, probably due to the greater BCS of the cows at the beginning of the study (~3.17), and these results were higher than those reported by [12] and [5] whoreported 56.9% and 61.0% of P/AI,respectively.

Table 2 P/AI following EB or EC as ovulation inducers associated with eCG or FSHp on suckled multiparous *Bosindicus*cows submitted to progesterone-based TAI protocols.

Group		No. Ofanimals	P/AI	(%)	P
EB	eCG	279	182	65.23[a]	0.001
	FSH	280	136	48.57[b]	
EC	eCG	270	163	60.37 [a]	3.4669
	FSH	269	141	52.42 [a]	

Abbreviations: EB, estradiol benzoate; EC, estradiol cipionate; TAI, timed articial insemination.

Experiment 2: Conception rate through TAI in suckled Nelorecows using EC as an ovulation inducer associated with eCG or FSHp.When EC was used as an ovulation inducerthere were no differences between groups eCG and FSHp in P/AI. (Table 2). Therefore, our main hypothesis in experiment 2 was refuted. Group EC-eCG had 60.37% pregnancy rates, greater than the 52.42% of the EC-FSHp group, but not statistically significant. [13]explainedthat eCG effects are more pronounced in primiparous cows then in multiparous cows, and in their study cited P/AI in primiparous cows of 20.0% in the control group versus 45.7% in eCG group, and for multiparous cows 39.9% in the control group versus 52.0% in the eCG group.The high BCS in our study possibly contributedto these results. It is well-known in the literature that cows with greater ovulatory follicles have positive correlations with ovulation rate and fertility with TAI [8], and in the current studythe EC-eCG group had 16.23 mm and the EC-FSHpgroup had greater OFthan all groups (16.47mm).Thus, the use of FSHp in protocols of TAI in multiparous cows with greater BCS and good OF may be a plausible alternative to eCG. Moreover, the use of eCG at the time of PRID removal in a TAI program improves the conception rates and these treatments may facilitate the application of genetic improvement programs more efficiently in *Bostaurusindicus* herds under Amazonian tropical conditions [5].

IV. Conclusions

- The estradiol esters, estradiol benzoate and estradiol cipionate, are efficient as ovulation inducers in TAI programs;
- The use of eCG with EB as an ovulation inducer was greater than EB-FSHp in P/AI in Nelore multiparous cows;
- The use of eCG with EC as an ovulation inducer was similar to the EC-FSHp in P/AI in Nelore multiparous cows with good BCS and OF.

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