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Pharmacological Control of Estrus in Tropical Cattle, an Economical Assessment of Different Synchronization Protocols

——Economics of Estrus Control in Tropical Cows

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ABSTRACT

To compare the fertility results and to assess the cost-effectiveness of several synchronization protocols applied under the conditions of beef cattle enterprises in Colombia, 1658 multiparous zebu crossbred cows mostly Brahman and Nelore ranging between 2 - 6 parities were used. Five protocols of pharmacological treatments varying in hormones used, dosage and the time of application were tested. All cows were inseminated at 52 h by appointment. Pregnancy diagnosis was undertaken over 45 d after insemination by rectal palpation. The total cost per cow and the total cost per gestation, for each protocol, were calculated. Taking a herd of one-hundred cows as baseline for calculations, it was estimated the cost of the total amount of pregnancies possibly obtained in each protocol; then, the excess between the costs of a pregnant cow and the cost of a treated cow was estimated. Additionally, the costs due to cows empty after four services were calculated. A total of 874 pregnancies were registered (52.7%), with pregnancies per protocol varying between 46.9% and 66.2% ($p < 005$). The cost per treated cow, varied between $64.08 and $97.47 and the cost per gestation from $126.01 to $177.26, without association between the cost of the treatment and the pregnancy rate. Protocol A was the best cost-effective with the lowest additional costs, the lowest amount of open days (2107.7 to 2231.7 d) and IA straws (average = 134), with an additional costs of $6940.00. Synchronization of estrus using pharmacological products seems to have a place in the management of cattle; however, caution should be called upon a careful assessment both from the part of the farm and the professional in charge of the enterprise to avoid using the technique indiscriminatively thus propitiating the use of a method that might not be cost efficient.

Keywords: Tropics; Bos Indicus; Cost-Benefit; Estru; Synchronization

1. Introduction

The use of Artificial Insemination in the tropics has been hampered by the difficulties in accurate detection of signs of estrus in Zebu type cattle, the most popular crossbred animal in the beef industry of the area. One possible solution to implement the technique is to manipulate pharmacologically the estrous cycle and coupling this procedure with fixed-time artificial insemination (F-TAI) regardless the signs of estrus, so called insemination by appointment. In relation to animals kept at pasture under tropical conditions, the rate for detecting animals in estrus does not go beyond 40% [1,2]. The reasons for this low detection rate is multifactorial mainly related to social behavior in the herd [3], age, breed of the animals and the presence of the male [4,5]. Because of these limitations, the number of protocols to synchronize estrus is abundant [5,6], the same can be noted as to the hour(s) where F-TAI is applied following removal of the treatment [7,8]. However, few studies are published whilst comparing protocols under the same conditions and with the same clinicians applying the procedure, particularly under beef cattle enterprises in the tropics.

Interventions in the beef cattle industry have to be cost

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effective and render economic dividends to the producer otherwise they will become an academic exercise. For example Tenhagen *et al*. [9] showed that synchronization of estrus in dairy cows in the USA was more cost-effective than inseminating animals displaying overt signs of estrus and this was mainly due to more cows that were culled and more open days among cows not culled. However, Hoff-Sousa and Ferrugem-Moraes [10] in beef cattle raised in southern Brazil, showed that whilst the total cost for a pregnancy using natural mating was 5 dollars, this cost increased to 20 dollars if AI was to be applied and 24 if AI was accompanied with an estrous synchronization protocol. Oltenacu *et al*. [11] in the USA working with dairy cows undertook an economic evaluation of several factors that affect pregnancy. Accurate estrus detection was among the most profitable managerial decisions. Thus, economic calculations will certainly differ according to the type of enterprise and production item to be evaluated.

The objective of the present study is to compare the fertility results and to assess the cost-effectiveness of several protocols applied under the conditions of beef cattle enterprises in Colombia.

2. Material and Methods

2.1. Animals

A total of 1658 multiparous zebu crossbred cows, mostly Brahman and Nelore, were chosen from 4 farms located in the Department of Antioquia-Medellin, Puerto Berrío, Colombia. The site is located at 125 m above sea level, at 06˚29'40" latitude north and 74˚24'24" longitude west. Average temperature is around 29˚C and pluvial precipitation around 2300 mm. Animals ranged from 4 to 12 years old, with 2 to 6 parities. The selection was done by the same two practitioners based on: 1) having at least 40 days since calving when the synchronization procedure was implemented; 2) the body condition score of the cows, including only those with a minimum of 2.5 in a scale 1 to 5; and 3) presence of a corpus luteum, or traces of follicular activity, detected by rectal palpation.

2.2. Treatments

Five protocols of pharmacological treatments were observed on field conditions. All used a synthetic progestagen (Crestar®, Intervet Schering Plough Animal Health) as base at day 0, with several variations between them like the hormones used, dosage and the time of application (**Table 1**). Not all protocols were used in each farm because that depended of the willingness of the farmers; however, all of them were applied under the supervision of the same two veterinarians. Moreover, in order to distribute similarly the experimental error, all cows were inseminated by the same veterinarians following the FTAI at 52 h. In farms where two or more protocols were applied, cows were randomly allocated in each treatment.

No experimentation was performed during this study, this was an observational study of the estrus synchronization on field conditions. The approval of the Animal Welfare and Bioethics Committee of the Veterinary School of the National University in Costa Rica was obtained.

2.2.1. Pregnancy Diagnosis

In all protocols the pregnancy diagnosis was done over 45 d after insemination by rectal palpation by the same veterinarians that selected and treated the cows. They have at least ten years of experience in this kind of reproductive procedures.

2.2.2. Cost Analyses

To assess the monetary cost of each protocol, it was calculated the cost of the pharmaceutical treatments, adding the costs for the professional labor of the veterinarians. The rate of each hormone was estimated using the current price in Mexico quoted in US dollars as currency. So, the price for each dose of drug was the following: Norgestomet (Crestar® implant) + estradiol valerate (Crestar® injection), $15.87; equine chorionic gonadotropin (Folligon®), $16.27; estradiol benzoate (Mesalin®), $4.65; gonadorelin (Fertagyl®), $18.30; cloprostenol (Estrumate®), $14.48; estradiol ciprionate, $9.58. All these products are manufactured by Intervet Schering Plough Animal Health. Additionally, the cost for the professional labor of the veterinarian was estimated in a total of $20.00 per cow for the whole treatment. At last, the mean price for the semen was fit on $10.00. The total cost for veterinary services during the complete protocol, including all the visits, was estimated on $20.00 per cow.

With these data, the total cost per cow (TCPC) for each protocol (TCPP), was calculated. Once the pregnancy rate (PR) of each protocol was obtained, a calculation of the cost of each gestation (TCG) was done as follows: $TCG = TCPC/PR$.

Taking a herd of one-hundred cows as baseline for calculations, it was estimated the cost of the total amount of pregnancies possibly obtained in each protocol (CTPP) using the formula: $CTPP = (100*PR)*TCG$. Finally, in order to have a relative estimation of the cost of a gestation per protocol, the excess between the cost of a pregnant cow in relation to the cost a treated cow (EPP) was calculated using the formulae $EPP = (TCG/TCPC)*100$.

The estimation of both costs for a treated cow and for a pregnant animal, at 95% of confidence was carried out using @RISK 5.7 software (Palisade®), using the mod-

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Table 1. Description of protocols used for heat synchronization in zebu cows on field conditions in the humid tropic of Colombia. The name of the product in bolds indicates the hormone that makes the treatment different.

Day	Protocol A Estradiol Benzoate		Protocol B Gonadorelin		Protocol C Equine Chorionic Gonadotropin	
	With	Without	With	Without	With	Without
0	Crestar® implant Crestar® injection	Crestar® implant Crestar® injection	Crestar® implant Crestar® injection	Crestar® implant Crestar® injection	Crestar® implant Crestar® injection	Crestar® implant Crestar® injection
9	Remove Implant Folligon® 400 UI	Remove Implant Folligon® 400 UI	Remove Implant Folligon® 400 UI	Remove Implant Folligon® 400 UI	Remove Implant Folligon® 400 UI	Remove Implant Folligon® 400 UI
11	FTAI 52 h.	FTAI 52 h.	FTAI 52 h. Fertagyl® 1ml	FTAI 52 h.	FTAI 52 h.	FTAI 52 h.
24	Crstar® Reimplant Mesalin® 1 mg	Crestar® Reimplant			Crestar® Reimplant	Crestar® Reimplant
30					Remove Implant Folligon® 150 UI	Remove Implant
31 - 33					FTAI 52 h.	FTAI 52 h.
32	Remove Implant	Remove Implant				
33 - 35	FTAI 52 h.	FTAI 52 h.				
40 - 45	Pregnancy diagnosis	Pregnancy diagnosis	Pregnancy diagnosis	Pregnancy diagnosis	Pregnancy diagnosis	Pregnancy diagnosis

Day	Protocolo D Crestar® new/used		Protocolo E Estradiol Ciprionate (ECP)	
	New	Used	With	Without
0	Crestar® implant	Crestar® implantEstradiol Benzoate 2.5 mg	Crestar® implant	Crestar® implant
8		Remove Implant Estrumate® 2 ml Folligon® 330 UI		
9	Remove Implant Folligon® 400 UI		Remove Implant Folligon® 400 UI ECP 0.5 ml (1 mg)	Remove Implant Folligon® 400 UI
11	FTAI 52 h.	FTAI 52 h.	IATF 52 h.	IATF 52 h.
22				
31 - 33			Heat detection	Heat detection
36				
36 - 47				
40 - 45	Pregnancy diagnosis	Pregnancy diagnosis	Pregnancy diagnosis	Pregnancy diagnosis

FTAI 52 h = Fixed time artificial insemination, Crestar®: Implant (Norgestomet 3 mg) + Injection (Norgestomet 3 mg, Estradiol Valerate 5 mg), Folligon®: eCG (equine Chorionic Gonadotropin), Fertagyl®: GnRH(Gonadotropin Release Hormone), Mesalin®: Estradiol beanzoate.

cost estimation assuming a variation of ±10% from the base price for each component of the treatment. The variation from demean was obtained after a run with 10,000 iterations. Additionally, an approach of the costs for the owner due to cows empty after four services, for each protocol was estimated by means of a deterministic model carried out in Microsoft Excel. For this purpose, it was assumed a constant pregnancy rate (the observed one in the protocol *i.e.* fertility at first insemination) for each time of AI and a heat interval of 21 d. This calculation included the total amount of days empty, AI straws projected to use and their associated costs assuming a cost of $3.00 US dollars per day empty and a cost of $10.00 US dollars per AI straw.

The costs for the remaining days empty and AI straws after four services were not estimated. Cost for management, feed, and others related to the maintenance of the herds was not taken into consideration due to the vari-

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ability in the sample.

3. Results

3.1. Reproductive Efficacy of the Protocols

A total of 874 pregnancies were registered from the 1658 cows studied (52.7%). The general percentage of pregnancies per protocol varied between 66.2% (protocol A) and 46.9% (protocol D). There were not differences between protocols B, C, D and E, but all of them were different to protocol A (**Table 2**).When segregating the results per treatment within each protocol, these percentages vary to 66.9% for protocol A with estradiol benzoate, and 43.7% for protocol D with the re-used implant of Norgestomet ($p < 0.05$) (**Table 2**).

3.2. Costs per Treated Cow (Gross Cost)

Regarding the cost per treated cow, in average, protocol D was the cheaper whilst protocol C was the most expensive. Even within each protocol, the total cost varied between 64.08 US dollars for protocols B and D; specifically without Gonadorelin and with the new Crestar®, respectively, while it was $97.47 for protocol C when using eCG (**Table 3**).

3.3. Costs per Pregnant Cow (Net Cost per Pregnancy)

When the pregnancy rate was taken into account, the cost for each gestation varied from $126.01 in protocol A without estradiol benzoate, to $177.26 in protocol C with eCG ($P < 0.05$). No statistical differences were found in the net cost per pregnancy within each protocol; however, the absolute difference in some protocols was quite important. For instance, in protocol B, there was an absolute difference of $29.97 while in protocol D it was $26.57 (**Table 3**). So, at the end, although protocol B without Gonadorelin was the cheaper regarding the gross cost of treatment ($64.08), it was, the second one with the higher excess cost between treated and pregnant cows ($121.27). Besides, the protocol D had excess costs of $99.10 and $128.86 when using new and re-used Crestar® (**Table 3**). These results indicate that there is not a direct association between the cost of the treatment and its efficacy measured as pregnancy rate.

Table 2. Percentage of gestations, by protocols used for heat synchronization, in zebu cows on field conditions in the humid tropic of Colombia.

Protocol	Treatment	Total	Gestations	%	95% CI		Diff.*
					IL	SL	
A	Total	394	261	66.2	61.6	70.9	a
Estradiol benzonate	With	305	204	66.9	61.6	72.2	
	Without	89	57	64.0	54.1	74.0	0.70
B	Total	280	131	46.8	40.9	52.6	b
Gonadorelin	With	145	70	48.3	40.1	56.4	
	Without	135	61	45.2	36.8	53.6	0.63
C	Total	82	44	53.7	43.9	64.5	a,b
Equine chorionic gonadotropin	With	40	22	55.0	39.6	70.4	
	Without	42	22	52.4	37.3	67.5	0.82
D	Total	601	282	46.9	42.9	50.9	b
Implant of norgestomet	New	301	151	50.2	44.5	55.8	
(new/used)	Used	300	131	43.7	38.1	49.3	0.12
E	Total	301	156	51.8	46.2	57.5	b
Estradiol ciprionate	With	198	104	52.5	45.6	59.5	
	Without	103	52	50.5	40.8	60.1	0.81

*Different letters indicate difference in the global percentage of gestations between protocols. The numbers indicate the exact P-value of the comparison within each protocol; both calculations at 95% of confidence.

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Table 3. Costs analyses for each protocol of synchronization used for heat synchronization in zebu cows on field conditions in the humid tropic of Colombia.

Parameter	Protocol A		Protocol B		Protocol C		Protocol D		Protocol E	
	Estradiol benzoate		Gonadorelin		eCG		Norgestomet new/used		ECP	
	With	Without	With	Without	With	Without	New	Used	With	Without
% of gestation	66.9	64.0	48.3	45.2	55	52.4	50.2	43.7	52.5	50.5
Total cost per treated cow[*♣]	85.33	80.59	82.70	64.08	97.47	80.62	64.12	67.39	73.94	64.21
Cost per gestation[β]	127.66	126.01	171.76	141.79	177.26	153.63	127.66	154.23	140.78	127.00
Excess cost pregnant/treated cow (%)[♣]	49.61	56.36	107.69	121.27	81.86	90.56	99.10	128.86	90.40	97.79
Cows pregnant after 4 services[**]	83	82	73	72	77	75	74	71	75	74
Total of AI straws until 4 services[**]	133	135	151	154	144	147	149	156	147	149
Total of days empty until 4 services[***]	2107.7	2231.7	3223.6	3409.6	2789.6	2975.6	3099.6	3533.5	2975.6	3099.6
Total cost due to days empty	6155.8	6695.1	9615	10191.5	8368.9	8852.5	9261.6	10470.4	8833.9	9205.8
Additional costs due to IA + days empty[****]	6648.3	7230.8	10384.3	11006.9	9038.5	9560.7	10002.6	11308.2	9540.7	9942.4

[*]In US Dollars. [♣]Based on a stochastic model using the cost estimation module in @Risk. [**]On a base of 100 cows treated per protocol. [***]This does not include the days before treatment. [****]This is the sum of the cost due to days open + the extra cost due to AI straws used, assuming a cost of $10.00 US Dollars per straw.

3.4. Estimated Economic Losses Associated to Reproductive Failure per Protocol

The failure of getting the cows pregnant at first service adds to the costs for days open and additional AI straws used until the pregnancy of the cows in a hypothetical fourth service. Protocol A was the best cost-effective because its additional costs were the lowest among all protocols and had the lowest amount of open days (2107.7 to 2231.7 d) and IA straws (average = 134), then, the additional costs reached almost $6940.00 on average. Protocols B and D were less efficient, with more than 3200 d of additional open days due to reproductive failure, reaching, on average, more than $10500.00 in additional costs after four simulated rounds of AI keeping constant the pregnancy rate observed in each protocol. On the other hand, the protocols C and E occupied an intermediate position; however, both had estimated additional costs over $9300.00 (**Table 3**). The sensitivity analysis indicated that the most important source of variation in the model assessing the economic losses was the pregnancy rate of the protocols.

4. Discussion

The use of pharmacological agents to promote estrous expression is a technique to ascertain a massive use of Artificial Insemination in cattle raised under tropical conditions. The value of this procedure has to be measured by the number of animals pregnant after an intervention. It is however, a rather difficult task to compare fertility results between different experiments for the variability that exists in each field trial. The protocols tested in the present study afforded an overall fertility of 52% which is rather similar to many of the studies reviewed; in effect, conception rates after treatment with hormonal combinations in different studies vary between 40% and 70% [12-17]. Reviews of several studies have been published by Bo *et al*. [5] and Barusselli *et al*. [18] and earlier by Galina and Arthur [19]. The ranking of all these studies established an average of around 40% ranging from 20% to 60% after first insemination. Nonetheless, the remaining non-pregnant animals had the inherent cost of being inseminated, especially if fixed time insemination is used, with negative results resulting in lengthier periods of days open. Most of the studies report the number of animals pregnant following an intervention but hardly any information if the non-pregnant animals were actually at the risk of becoming pregnant. Diaz *et al*. [20] in a survey where cows were sampled for progesterone following a protocol of synchronization observed that 30% of the animals did not form a CL. Moreover, in an old study Landivar *et al*. [21] compared fertility in different herds, either following spontaneous or synchronized estrus and using either natural mating or artificial insemination, reported no differences in fertility if AI or natural mating were used being the variation the

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animals utilized in the study rather than the technique to make them pregnant. So, the relationship of cost-benefit of synchronizing zebu cows with the goal of pregnancy should be an exercise that practitioners should undertake before embarking in costly protocols which are not cost-effective. Two of the main challenges of zebu cattle under tropical conditions are the long postpartum anestrus and the low estrus detection rate [5,19]; so the protocols for synchronization of ovulation can have large positive impacts on the reproductive efficiency in the herds raised under tropical conditions if adequately used [6].

Several protocols have been tested for estrus synchronization, since the early days of utilizing only prostaglandins, to those more elaborate using progestins, estrogens and GnRH [12,13,17,22-25]. Diverse studies have documented the success of several protocols applied in diverse climatic conditions, different breeds, time postpartum where the intervention was undertaken and feeding strategies. However, studies comparing the results of protocols used on the same field conditions applied routinely by practitioners in different management conditions are less frequent. Besides, several progesterone or progestins have been investigated for estrus synchronization in zebu cows in controlled conditions, varying considerably the success in synchronization and gestation rates [12,13,17,18,24].

Whilst rates of gestation of the protocols tested in the present report are similar to others, we are aware that the absence of the characteristics for each cow included in the study, did not allow us to carry out a mixed model including fixed and random effects, which could assess the effect of variables such as breed, number of parities, body condition score and time postpartum among others taking into consideration the random effect of the herd, on the synchronization and pregnancy rates. What we can assume is that there aren't differences between the body condition score, number of parities and time postpartum, as well as the breeds between protocols. In this scenario we can take for granted a global similarity of the groups treated. A favorable argument is that all cows of the study were selected by only two veterinarians who were the same that applied the treatments and undertook the pregnancy diagnosis hence the sources of error were equally distributed in all protocols.

Some studies report the costs of hormonal treatments for synchronization in beef cattle, most of them in *Bos taurus*; however, the calculations are based on the costs of hormonal treatment, without taking into consideration fixed cost such as labor and the possibility of applying various treatments in the case of cows having to be reinseminated. Also, fewer studies used mathematical models to simulate the effect of measures of management on the net income of a beef farm [26] the modeling of the cost-benefit of different protocols taking into account other aspects such as the cost of the IA straws, the added value of labor as well as the cost of the days open, are exercises that should be taken to understand the economic merit of hormonal treatments. Bolivar and Maldonado [27] and Alarcón *et al*. [28] have attempted to analyze the cost-benefit of using embryo transfer in cattle; their results indicate that the technique might be overvalued for the average farmer for it has its place in stud farming.

In conclusion, synchronization of estrus using pharmacological products seems to have a place in the management of cattle and its popularity has expanded in the last decade. However, caution should be called upon a careful assessment both from the part of the farm and the professional in charge of the enterprise to avoid using the technique indiscriminatively thus propitiating the use of a method that might not be cost-efficient.

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