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Comparison of Two Insemination Programs in Synchronized Early Postpartum Zebu Cows under Tropical Conditions: An Economic Appraisal

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Abstract

To evaluate an artificial insemination program in postpartum *Bos indicus* cows and by using deterministic models, to estimate the possible economic advantages of insemination by appointment over detected estrus, sixty-three multiparous animals were synchronized using a progestagen device inserted at day 31 and withdrawn after 9 days (average time for withdrawal was 40.9 ± 6.8 days). Cows were observed continuously from 0700 to 1800 during 72 h. The percentage of animals detected positive to estrus by riding behavior was 43.4%, transrectal palpation, 22% and heat detector patches, 4.6%. The percentage of cows ovulating as evidenced by progesterone samples taken at 9 and 11 days after the average estrus response was 73%. The economical assessment by using the values obtained in the present study afforded that the cost of semen, ovulation rate and fertility point to a definitive advantage of using AI at fixed time over a program based on estrus detection.

Keywords

Insemination, Estrus Detection, Behavior, *Bos indicus*

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1. Introduction

There have been several efforts to evaluate the economical impact of the use of AI, especially in Holstein cows raised under intensive systems where insemination following estrus detection (AIED) or at fixed time (AIFT) are common procedures [1] [2]. However, the difficulties in estrus detection in beef cattle has forced technologists to use the latter method to eliminate the need for detecting overt signs of estrus facilitating management during the postpartum period in beef cattle raised under semi or extensive pastoral systems in the tropics [3]-[5]. On the other hand, scarce efforts have been vested to assess the economical feasibility of using either method (AIED vs. AIFT) in beef cows. For example, Rodgers *et al.* [6] compared the efficacy inseminating at fixed time versus natural mating with the weight of the calves at weaning finding an economical advantage in the former method. This is particularly important in postpartum beef cows in the tropics where the response can be misleading as the development of a corpus luteum (CL) following induced estrus, and has afforded doubts to whether this technique is viable at least under tropical conditions. Effectively, Díaz *et al.* [7] and later Pérez-Torres *et al.* [8] found that almost 30% of the animals could display behavioral estrus while not ovulating, as shown by serum progesterone values indicative of the formation of a mature CL. Moreover, fertility results following AI by appointment are inconsistent. For example, Alonso-Alanuza *et al.* [9] in a field trial evaluating fixed AI at 48 and 72 h but observing cows continuously for overt signs of estrus found that eighty-six percent of the pregnancies occurred in cows displaying estrous behavior regardless of the time of fixed AI versus applying this technique by appointment.

With this in mind, the objective of the present study was to undertake a field trial to estimate the physiological values on estrus detection efficacy, the formation of a CL indicative of ovulation and the use of external aids to increase the number of animals detected in estrus, in order to assess the convenience of inseminating post partum *Bos indicus* cows at observed estrus and the data compared with the hypothetical use of AI at fixed time using a modeling procedure to assess the economical value of both procedures.

2. Material and Methods

2.1. Ethical Statement

The methods used during the present work were approved by the Animal Care Internal Committee (CICUA) of the Faculty of Veterinary Medicine and Zootechnics of the National Autonomous University of Mexico in accordance to The Code of Ethics of the World Medical Association (Declaration of Helsinki).

2.2. Location

The study was carried out at the Centre for Teaching, Research and Extension in Tropical Animal Husbandry of the Faculty of Veterinary Medicine of the National Autonomous University of Mexico, located in the state of Veracruz, Mexico at 20˚4'N and 97˚3'W. The climate is hot and humid, without a defined dry season. Average yearly rainfall is 1840 mm and average temperature range between 14˚C and 35˚C. The animals were at pasture at all times, with two or three days of grazing, 50 to 70 days of recovery, stocking rate of 1.25 animal units [AU = 450 kg of live weight]/ha). The pasture was composed of an average of 39% of introduced grasses, mainly Stargrass (*Cynodon nlemfuensis*); 48% for native grasses, mainly *Paspalum* spp. and *Axonopus* spp.; 3% for broad-leafed weeds; 6% for narrow-leafed weeds and 5% for native legumes, mainly *Desmodium* spp. Mineralized salt was offered *ad libitum*.

2.3. Animals and Sampling Procedures

In order to have all animals as close as possible to 35 days postpartum in a calving season of about 60 days, a total of 63 cows with a body condition score of 3 to 4 on a scale of 1 - 5 [10]. Cows were allotted to five groups which were formed as they reached the target date. The study was undertaken in the months of May and June 2014 (average temp 24.9 and humidity 95.7%). A progesterone releasing device (CIDR® Zoetis Mexico) was inserted at day 31 and withdrawn after 9 days (average time for withdrawal of the device was 40.9 ± 6.8 days for all groups) according to the procedure previously described [8] (**Table 1**). The five groups of cows remained together throughout the study. The resumption of ovarian activity was evaluated by measuring the concentrations of progesterone on days 21 and 26 postpartum to assess the presence of ovarian activity prior to treatment.

Table 1. Frequencies of cows in the five groups displaying visible estrous behavior, as opposed detection by transrectal palpation at 56 h after CIDR withdrawal or animals non visible in estrus.

Group	n =	Interval of days from calving to CIDR removal	Estrus			Patch		
			Visible	Palpation	Non visible	Visible	Doubtful	Negative
1	11	44.5 ± 8.3	18	27	55	0	0	100
2	11	43.9 ± 3.0	45	36	18	9	9	82
3	13	34.2 ± 4.5	46	31	23	8	0	92
4	13	41.4 ± 6.2	38	15	46	0	23	77
5	15	40.7 ± 12.1	69	0	31	6	6	88
Total	63	40.9 ± 6.8	43.4%	22.0%	34.6%	4.6%	7.7%	87.7%

Three days after CIDR removal, blood samples were taken at day 9 and 11 to verify the presence of a corpus luteum at the time when overt signs of estrus would be expected to appear. The samples were handled according to the recommendations of Pulido *et al.* [11] and centrifuged at 1500 rpm for 10 minutes and kept frozen according to the procedure previously described [8]. Serum was analyzed using a solid phase radioimmunoassay in 100 µl of serum using commercial kits (Pharmaceuticals, Diagnostic Division). The intra and inter-assay coefficients of variation were 7.41% and 6.18%, respectively. When the concentration of progesterone was above 0.5 ng/ml in two or more successive samples, the animal was considered to have a functional corpus luteum [11].

2.4. Observations for Signs of Estrus

Cows were observed continuously immediately after implant withdrawal. The period of observation extended from 0700 to 1800 for 72 h. Signs of estrus were classified according to the following criteria: a) showing overt signs: sexual behaviors (e.g. mounting and intent to mount) and courtship [12] [13] including head bumping flehmen, sniffing, following, licking and assisted head resting [14]; b) transrectal palpation: cows which did not display overt signs were detected in estrus by transrectal palpation at 56 hours by gently massaging the uterus per rectum and observing the presence of mucous discharge or a wet and congested vulva [15]; c) finally, ESTROTECT (Rockway Inc., Spring Valley, WI, USA) heat detector patches were used as an aid for riding behavior, according to the company' specifications [8]. Cows were detected by showing overt signs of estrus and palpation, or triggering off the paint from the patches. The gold standard test was the observation of overt signs of estrus.

2.5. Calf Management

At the moment of implant withdrawal all cows were started on a regime of restricted suckling allowing calves to suckle for a period of two hours in the morning. Visual contact was possible at all times.

2.6. Statistical Analysis

Relative frequencies were calculated using the five groups of cows displaying overt signs of estrus or detected by transrectal palpation after 56 h from implant withdrawal, together with those not showing signs of estrus. A further calculation was done only with the animals detected in estrus and ovulating either as displaying overt signs or those detected through the patch.

2.7. Modeling Procedure

Deterministic models were used to estimate the possible economic advantages of insemination by appointment over detected estrus using the values obtained in the present study.

Associated cost for pregnancy rates after insemination and the results obtained in observational estrus and ovulation were compared. Two strategies of AI were defined: at observed estrus (AIOE), and by appointment (AIA). In the case of AIOE, an estrus detection percentage of 45% (obtained in the present study) was taken as

an average scenario. Additionally, two other situations were included, one with a lower estrus detection efficacy (30%) and the other with a higher rate (67%), which are the minimum and maximum values obtained in the field study. These values are in agreement with those previously reported [14]. Also, we evaluated a scenario where 96% of the cows in estrus ovulated, as observed in the present study, and compared this parameter to lower (85%) and higher (100%) ovulation rates after observed estrus.

To model AIA, three scenarios for ovulation rates were considered, average (71%), low (60%) and high (80%) [7]. Finally, for AIA and AIOE, three hypothetical fertility rates were used, average (70%), low (65%) and high (75%), and three costs for commercial semen, $20.00, $30.00 and $40.00 US dollars, fixing the labor cost of IA at $5.00/cow, and open day at $3.00/cow/day. The cost of labor was estimated based on data published in Mexico and Colombia [16]; on the other hand, the cost for days open was an approximation taken from the study published by Vargas *et al.* [17] for dairy cattle under tropical conditions. It was assumed that cows not pregnant after the first service will have on average a second opportunity after at least 21 additional days. All modeling procedures were established using a baseline of 100 animals.

3. Results

Only four out of 63 cows were cycling in the samples taken prior to the synchronization scheme. As shown in **Table 1** the global mean of days between calving and CIDR removal was 40.9 ± 6.8. On average, 43.4% of the animals displayed estrous behavior and 22% were detected as positive by palpation. The use of patches was not consistent, of the 29 cows displaying riding behavior only two of the animals were positive to the heat detector device and three were considered doubtful. Of the 13 animals detected by palpation only one was positive to the heat detector device, and two animals were negative to estrous behavior, but doubtful to the patch.

Figure 1 displays the percentage of cows either showing overt signs of estrus or confirmed positive to estrus by transrectal palpation. There was a significant ($p < 0.05$) difference only between cows in group 1 versus group 5 in relation to the detection of overt signs of estrus.

Figure 2 shows the percentage of animals per group in relation to estrus response and ovulation; differences are not significant ($p > 0.05$). Two features are apparent, the first is the significant number of cows detected in estrus by visual observation that subsequently ovulated, as indicated by progesterone levels above 0.5 ng/ml), along with the limited number of errors in detecting animals on the basis of imitation behavior (cows riding other
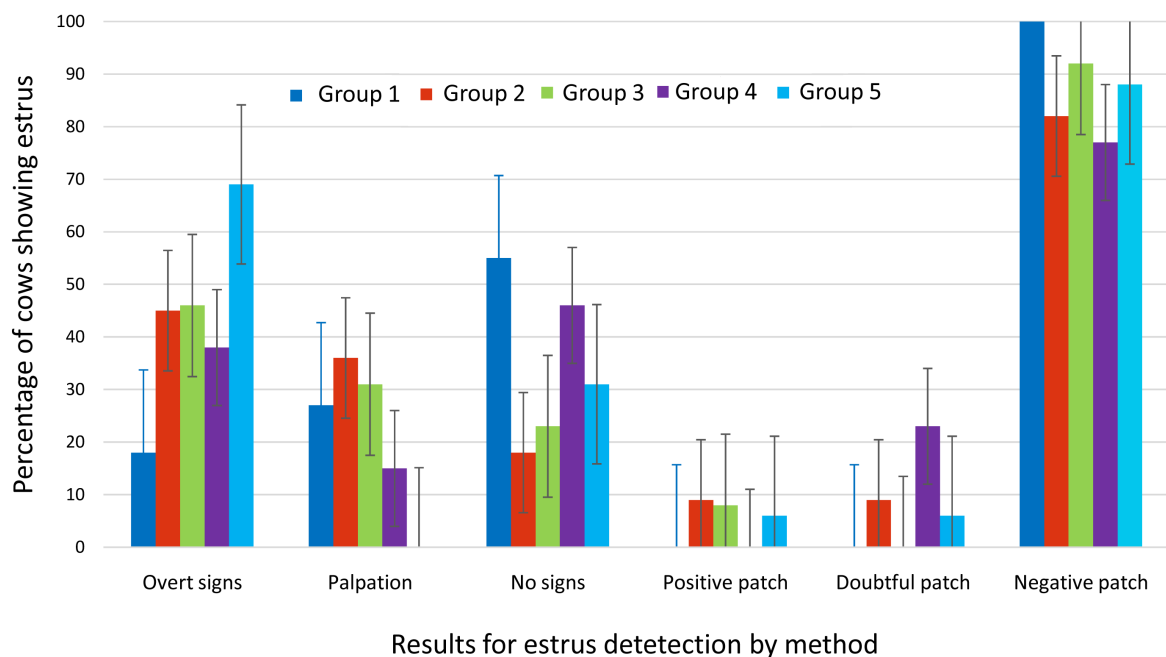

Figure 1. Percentage of cows displaying overt signs of estrus, or detected by transrectal palpation at 56 h after implant withdrawal or by the use of a patch to evidence mounting behavior. The typical error bar is shown.
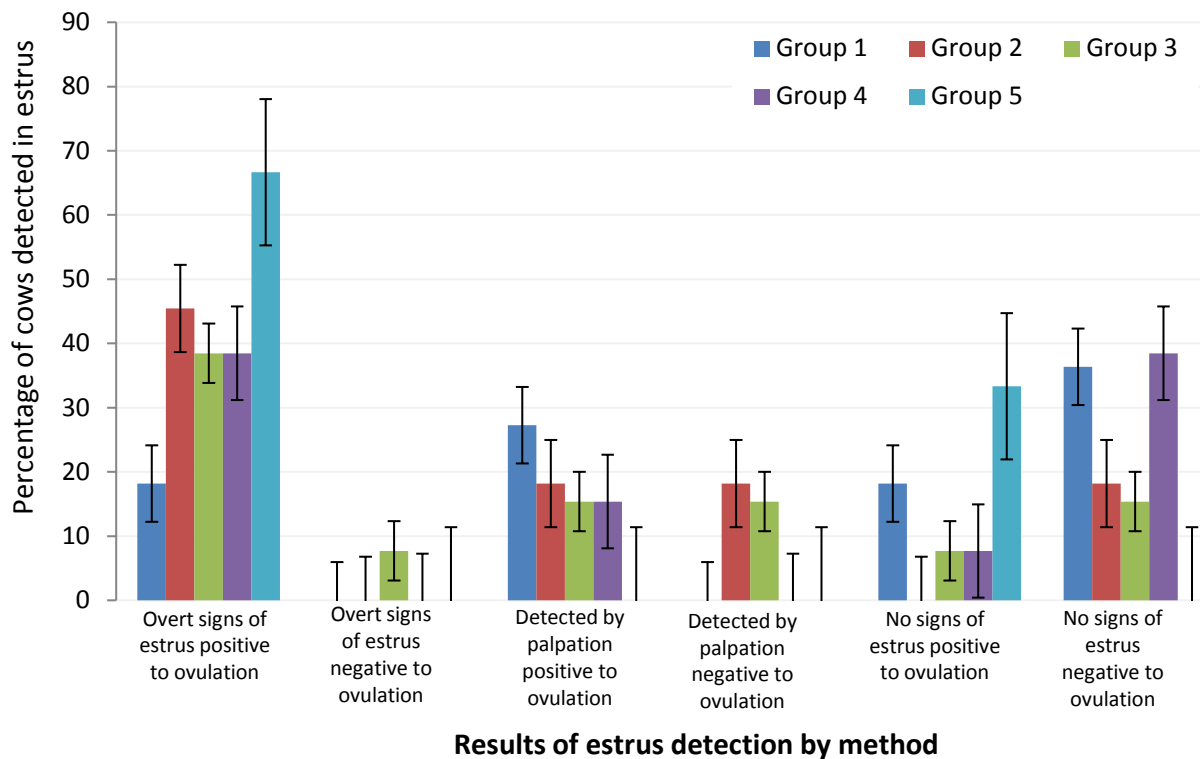
Figure 2. Percentage of cows detected in estrus by overt signs, transrectal palpation or positive patches, events followed by an ovulation measured by progesterone concentration above 0.5 ng/ml in the five treatments.

cows or accepting being ridden). Secondly, the number of cows detected in estrus by palpation that were subsequently tested positive for ovulation (true positives) compared to the number of animals diagnosed in estrus but not ovulating. When calculating the ratio between the animals positive to ovulation through palpation, there was a probability of 30% error for every cow diagnosed as a true positive.

In reference to the simulation studies, considering different rates of estrus observation, ovulation, fertility and the price of semen, IA by appointment was the better option than IA at observed estrus, using a balance between the expenses for open days less IA at fixed cost. An exception was found in the scenarios with higher reproductive efficiency where the difference between expenses for AIOE and AIA were about $60.00 USD (when price for semen was $20.00 USD) and $600.00 USD (when price for semen rises to $40.00 USD). In addition, the difference could reach $2800.00 in the scenario with lower reproductive efficiency, when the semen price was set at about $40.00 USD (**Table 2**).

Figure 3 shows the variations between the costs for open days less expenses in the two AI programs. Independent of the price of semen, when reproductive efficiency is high, AIA has higher costs than AIOE. In the case of semen at $20 the difference was almost $300, at $30 it was around $100 and for $40 it was up to $600.

Likewise, using the cost of per pregnant cow after first service as an indicator for comparison in the different scenarios, AIA always resulted more profitable, regardless of the cost of semen. The differences can reach up to $230.00 in the worst case scenario of reproductive efficiency, whereas in the cost of semen the difference can increase to $40.00 USD. In the average situation, the cost of a pregnancy using AIA is about half that of AIOE independent of the cost of semen (**Table 3**).

The variation in the cost per pregnant cow, according to the different reproductive efficiencies and the various costs for semen can be observed in **Figure 4**. Regardless of the price of semen and reproductive efficiency, the cost for a pregnant cow is less in AIA than in AIOE.

4. Discussion

The number of cows cycling prior to CIDR removal (average 40.9 ± 6.8) was only 6%, this result agrees with
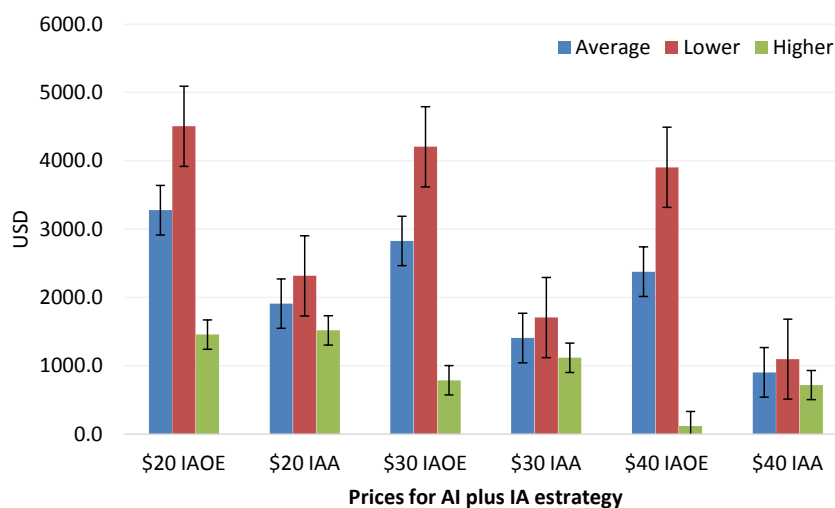
Figure 3. Cost balance per open days—This analysis includes the cost of artificial insemination (AI) at different prices for semen, assuming three scenarios of reproductive efficiency (AIOE = AI at observed estrus; AIA = IA by appointment).
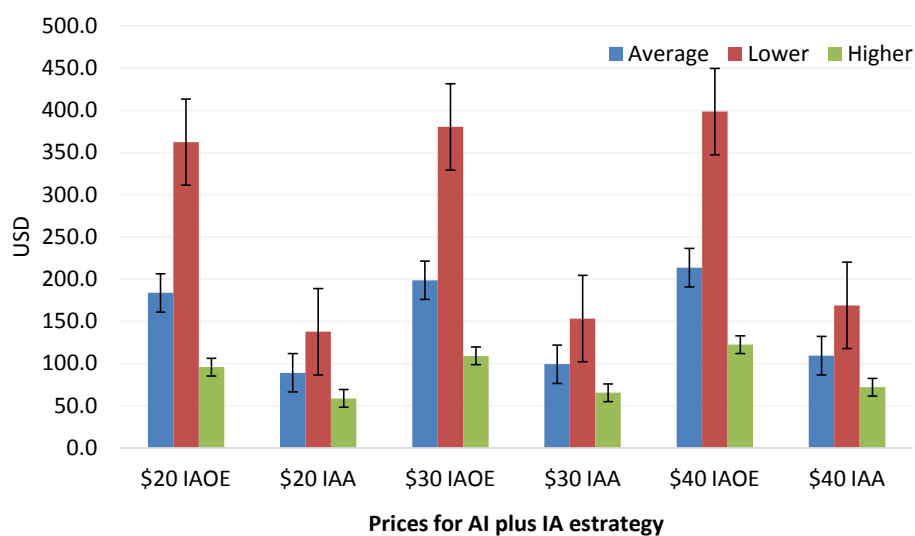

Figure 4. Comparison of cost per pregnant cow in different scenarios and price for semen.

Table 2. Evaluation of costs in relation to open days less IA expenses. This analysis was calculated at different costs for semen for the strategies of AIOE versus AIA, in three scenarios of reproductive efficiency (amounts in USD).

Cost of semen	IA	Scenario Reproductive Efficiency		
		Average 70%	Lower 65%	Higher 75%
20.0	AIOE	3278.7	4505.8	1459.3
	AIA	1911.4	2318.0	1520.0
	Difference	1367.3	2187.8	−60.8
30.0	AIOE	2828.7	4205.8	789.3
	AIA	1408.4	1708.0	1120.0
	Difference	1420.3	2497.8	−330.8
40.0	AIOE	2378.7	3905.8	119.3
	AIA	905.4	1098.0	720.0
	Difference	1473.3	2807.8	−600.8

Table 3. Differences in the cost for a pregnant cow after first IA using different costs for semen. Calculations based on the strategies of AIOE versus AIA, in three scenarios of reproductive efficiency (amounts in USD).

Cost of semen	IA	Scenario Reproductive Efficiency		
		Average 70%	Lower 65%	Higher 75%
20.0	AIOE	183.7	362.3	95.7
	AIA	89.1	137.6	58.7
	Difference	94.6	224.7	37.0
30.0	AIOE	198.6	380.4	109.0
	AIA	99.2	153.3	65.3
	Difference	99.4	227.2	43.7
40.0	AIOE	213.6	398.5	122.4
	AIA	109.3	168.9	72.0
	Difference	104.3	229.6	50.4

the abundant information in the literature indicating late onset of ovarian activity when the cows are kept with their calves at all times [18]. On average, 43.4% of the animals displayed estrous behavior and 22% were detected as positive by palpation. The combination of these two procedures has previously proven to be quite efficient to improve detection rates and has been recommended by others [15]. On the contrary, the use of patches was not consistent as only 5% of the animals were confirmed in estrus with the use of this commercial aid. It is difficult to explain this finding as in previous experiments this method has been quite efficient [8]. We have ruled out the possibility that the patches were too old as they had been kept in a warehouse at all times since their purchase three months prior to use.

As can be observed in **Figure 2**, the majority of cows detected in estrus by visual observation did in fact ovulate indicating that these animals were likely to become pregnant. Moreover, the limited number of errors in detecting animals by imitation behavior (cows riding other cows or accepting being ridden) suggests above average precision and accuracy in estrus detection in this experiment and compares quite favorably with other studies [14] [19]. This finding could be the consequence of the limited number of animals detected in estrus (about 50%) with less than 14% of animals not detected in estrus and ovulating. This is consistent with data published for Holstein cattle, where detecting cows in estrus solely on the basis of standing to be mounted is seriously questioned [20] [21]. López-Gatius and Camón-Urgwl [15] recommended the use of transrectal palpation as an aid in estrus detection, however, when calculating the ratio between the animals positive to ovulation by palpation, for every cow was diagnosed as a true positive, we found a probability error of 30% making this technique less than accurate. Fewer than 15% of animals entering the study and detected positive to estrus by palpation, subsequently ovulated while more than 6% did not.

Simulation studies in Holstein cows [2] calculating an index of estrus detection efficacy between 85% and 95% showed a better economical return if AI was applied at detected estrus as opposed to AI at fixed time. In the case of our study, the values for detected estrus were not above 60% which was in accord with several studies of cattle raised under tropical conditions [14]. Thus, with the present efficacy in detecting overt signs of estrus, it does not seem economically feasible to recommend this method over insemination by appointment.

All the deterministic economic modeling procedures utilized in the present study point to a definitive advantage of using AI at a predetermined time versus a program based on estrus detection in postpartum cows. This is particularly important in cattle raised under extensive or semi intensive conditions [3] [5]. Basically, even with a reasonable level of accuracy in detecting cows in estrus, the number of animals not displaying overt signs adds to the cost of using AI. This inconvenience, plus the time spent in observing animals, is contrary to the recommendation of using AI at detected estrus [22]. Unless fertility improves [23], AI at a predetermined time is a better recommendation.

A certain bias was present in our fertility calculations following AI as 70% of animals conceiving is quite a high figure when compared to values reported in the literature [24] [25]. A more realistic figure would be around

30% to 40%. However, even when fertility was calculated at 65% IA by appointment was less expensive than IA at detected estrus.

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