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Factors that Affect the Conception Rate in Fixed-Time Insemination of Crossbred Cattle

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ABSTRACT

Aim: To evaluate the effects of body condition, female category, reproductive state, and insemination technician, on the conception rate, using a fixed-time insemination protocol in crossbred beef cows.

Methods: A number of 711 crossbred beef females were treated with an intravaginal progesterone device + estradiol benzoate (day 0), withdrawal of the device + estradiol cypionate + eCG + PgF2α (day 8), TAI, 46-50 hours later, and diagnostic of gestation (ultrasonography), 30 days post insemination. The effects (female category, body condition, ovarian cyclicity, and insemination technician) on the conception rate at first service were evaluated with the chi-square test, and the pairwise comparison method with Bonferroni correction ($P<0.05$), validated through forward stepwise binary logistic regression (Wald) with simple contrasting.

Results: The conception rate in the first service was only significantly influenced by the insemination technician, with an odds ratio of 3.35, 4.62, and 1.94, insemination technicians 2, 3, and 4, respectively, compared to the reference.

Conclusions: The efficiency of the insemination technician (measured by the gestation rate at first service, and the odds ratio), was pivotal for the results, with significant differences among them. The methodology used for systematic evaluation of insemination technicians is recommended to measure the results of implemented programs and TAI.

Key words: bovine, pregnancy rate, progesterone, reproduction, estrus synchronization (*Source*: *DeCS*)

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INTRODUCTION

One alternative to estrus detection, fixed-time artificial insemination (TAI), is widely diffused (Salgado-Otero, Vergara-Avilés, and Vergara-Garay, 2015), since a potential increase of service rates can be achieved 100%, and therefore, pregnancy tests and cost-effectiveness are higher (Baruselli, SáFilho, Ambrósio, and Ferreira, 2016). This is a useful practice to make a more rational use of cattle, since a larger number of cows can be inseminated in a work session, without detecting estrus (de Graaff and Grimard, 2017), which has obvious economic advantages.

The success of TAI protocols in cattle is influenced by several factors, such as the stud, the insemination technician, female category, suckling (Sá Filho *et al.*, 2009; Aba *et al.*, 2013), follicle size, body condition, parity, breed, herd (Sá Filho *et al.*, 2010), and estrus expression (Nogueira *et al.*, 2009).

Under Cuban conditions, TAI protocols in commercial cattle raising have not been embraced, so considering the above rationale, the aim of this research paper was to evaluate the influence of body condition, female category, reproductive state, and insemination technician on the conception rate achieved in fixed-time artificial insemination, in crossbred beef cows.

MATERIALS AND METHODS

Location, duration, and animal selection

This study took place between May and August 2018. Out of 875 crossbred females from 16 herds, 711 were chosen, according to the requisites below: Minimum 60 days after calving, body condition (BC) over 2 in a 1-5 scale, where 1= emaciated cow, and 5= obese (Houghton *et al.*, 1990) , with no reproductive issues during the ultrasonographic test (Kinzal Vet., equipped with multi-frequency transducer set at 5.0 MHz).

The chosen animals were distributed at random on three farms at Maraguan Company, municipality of Najasa (21°5′1″ N, 77°44′50″ O), province of Camagüey, Cuba. The farms were readied as gestation centers, where the animals stayed for forty days under similar feeding conditions, based on natural grass, with no supplementation, and free access to water. The females were classified according to their category (lactating cows, n=129; non-lactating cows, n=468; and heifers, n=114), body condition (2-3, n=413; 3-3.5, n=226; and greater than 3.5 BC, n=72), and ovarian cyclicity (deep anestrus, ovarian follicle smaller than 0.9 mm; n=133; shallow anestrus, ovarian follicle greater than 0.9 mm; n=478), and cyclical, pro-estrus or estrus corpus luteum, n=100).

Treatment

At the beginning of the treatment (day 0), an intra-vaginal device containing 0.5 g progesterone (DIB®, Syntex SA, Argentina) was placed in all the females, along with an injection (IM) of 2 mg estradiol benzoate (Gonadiol®, Syntex SA, Argentina), according to Cutaia, Tríbulo, Moreno, and Bo (2003), modified by Cutaia (2018). On day eight, the device was withdrawn, and

1 mg estradiol cypionate (Cipiosyn®, Syntex SA, Argentina), 400 UI of eCG (Novormon® 5000, Syntex SA, Argentina) were administered (IM) with 250 µg PgF2α (Ciclase® DL Syntex SA, Argentina).

AI was performed 46-50 h after the removal of the intra-vaginal device (Figure 1), with frozen/thawed semen in 0.5 mL pills, collected from studs with demonstrated fertility, based on laboratory tests. At 30 days post-insemination, a diagnostic to detect gestation was performed (presence of embryonic vesicle with a viable embryo), through ultrasonography (Kayzal Vet., equipped with a multifrequency transducer set to 5.0 MHz).

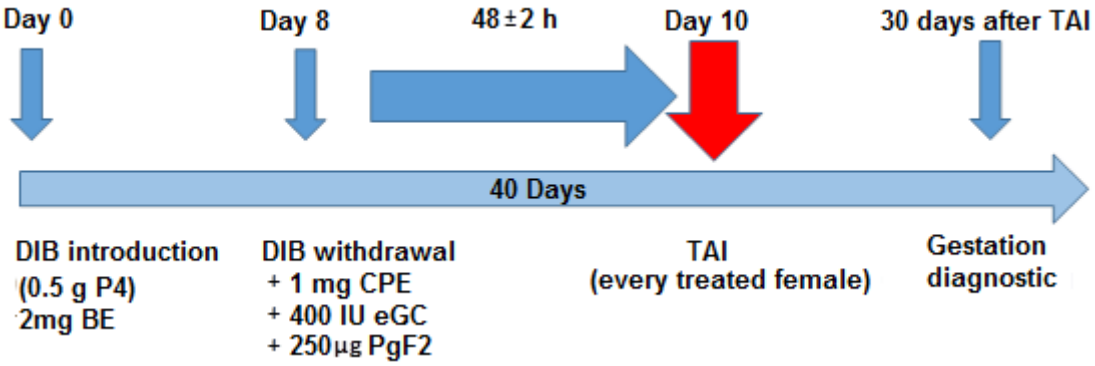

Figure 1. Protocol used for TAI.DIB™, P4 progesterone-releasing intra-vaginal bovine device, Progesterone. SB, estradiol benzoate. SCP, estradiol cypionate, eGC, equine chorionic gonadotropin.

Statistical analysis

The conception rate achieved after the first service was compared (number of gestating mothers 30 days after insemination to the total inseminated animals, expressed in percent), with factors (body condition, female category, reproductive state, and insemination technician), using the Chi-square test. Pairwise comparisons, with Bonferroni correction were performed ($P < 0.05$).

The forward stepwise multivariate binomial logistic regression model (Wald) was used to validate the previous results, through simple contrast, in which every category of the predictor was compared with the reference category and a definitive univariate binomial logistic regression model, to set the odds ratio (OR) corresponding to each insemination technician.

IBM™ SPSS™ version 24 (2016) was used to conduct the statistical analyses.

RESULTS AND DISCUSSION

A number of 321 (45.1%) females tested positive in the gestation diagnostic (Table 1), which is similar to the reports of selected Cuban companies (Hernández Marrero *et al.*, 2016), though on a reduced number of animals (45.26%), and in Argentina (Aba *et al.*, 2016), with 47.1%, using the eGC protocol, in lactating *Bos taurus* animals. This result falls within the group of international

reports for this type of protocol, varying between 40 and 60% in beef cattle (Campos *et al.*, 2016). The only significant effect was associated to inseminating technician. Technician No. 1 (25.9%) achieved a significantly lower gestation rate at first service ($P < 0.05$), and technician No. 3 (61.8%) achieved the greatest rate.

Table 1. Gestation rate achieved by factors using TAI

Factors	Levels	Diagnostic results		Total	First service gestation rate (%)	Prob.
		Non-gestating animals	Gestating animals			
Body condition	2.0-2.75	229	184	413	44.6	0.652
	3.0-3.5	119	107	226	47.3	
	> 3.5	42	30	72	41.7	
Category	Lactating cow	74	55	129	42.6	0.751
	Non-lactating cow	256	212	468	45.3	
	Heifer	60	54	114	47.4	
Reproductive state	Deep anestrus	64	69	133	51.9	0.468
	Shallow anestrus	279	199	478	41.6	
	Cycling	47	53	100	53.0	
Insemination technician	Technician No. 1	146	51	197	25.9[a]	0.000
	Technician No. 2	70	82	152	54.0[b]	
	Technician No. 3	74	120	194	61.8[b]	
	Technician No. 4	100	68	168	40.5[c]	
Total		**390**	**321**	**711**	**45.1**	

Unequal superscript letters mean a subset of insemination technician whose proportions in the column differ significantly between them ($P < 0.05$) (Pearson Chi square).

The multivariate binomial logistic regression analysis validated the outcome through the contingency tables, since in a preliminary step run, the same insignificant factors, according to Chi-square, were excluded. Technician No. 1 was the reference category to determine the odds ratio, since it was the lowest gestation rate at first service. Insemination technicians No. 4, 2, and 3 were higher, compared to the reference (Table 2). This ranking coincides with the gestation rates achieved by every insemination technician, due to the non-significance of the remaining factors in the model.

Table 2. Insemination technician effect on gestation rate at first service

Insemination technician	OR*	Confidence interval (95%)		Sig.
		Inferior limit	Superior limit	
Technician (1)	Reference	Reference		0.000
Technician (2)	3.35	2.136	5.264	0.000
Technician (3)	4.64	3.018	7.142	0.000
Technician (4)	1.95	1.250	3.033	0.003

***OR= Odds ratio**

This result coincides with other authors who reported rates between 15.1% and 81.8% (Sá Filho *et al.*, 2009), for the worst and best technicians, respectively, and 25.0% and 53.8% (Aba *et al.*, 2016). The above confirms the decisive role of the insemination technician, both in TAI (Russi, Costa-e-Silva, Zúccari, and Recalde, 2010) and traditional AI (Horrach *et al.*, 2017). The variations observed in the pregnancy rates are an important practical limitation to successful AI, and the fertility of the herd, since some technicians are less efficient than others (López-Gatius, 2013), which corroborates the need to keep a constant process of qualification and re-qualification of inseminating technicians, in order to achieve high conception rates in TAI.

An evaluation of BC effects on TAI pregnancy rate showed diverging results. It has been reported that a positive change in BC favors the gestation rate in the first service to *Bos indicus* females (Peralta-Torres, Aké-López, Centurión-Castro, and Magaña-Monforte, 2010), in synchronization protocols with CIDR, and BC at the beginning of the protocol (Sá Filho *et al.*, 2010). Other studies did not show significant effects. For instance, in Brahman cows, in Colombia (Correa-Orozco, Uribe-Velásquez, and Pulgarín-Velásquez, 2013).

In lactating beef cows with low BC, which received P4 on the fourth day after AI, Nishimura *et al*. (2018) did not produce increases in gestation rates, which is a warning against the effects of body condition in TAI programs (Riveros-Pinilla *et al.*,2018). In this study, BC and ovarian activity (OA), showed no significant effects on the gestation rate at first service, though the conception rate was greater in cycling cows with BC < 2.75 (pro-estrus or estrus corpus luteum).

Among the gestating cows, there was a predominance of animals with low body condition (57.3%), including animals in shallow or deep anestrus (69.61%), which may be attributed to the combination of eGC with estradiol cypionate, and the beginning of the rainy season. They contributed to increases in body condition, due to the beneficial effects of higher forage availability (Guevara *et al.*, 2012; Soto *et al*., 2014).

The most important effect of eGC is the stimulation of the dominating follicle, and an ensued increase in ovulation rate, since high concentrations of P4 during synchronization of ovulation have a negative influence on follicle diameter, and vascularization (Núñez-Olivera *et al.*, 2014). Its administration has been reported not to affect the rate of pregnancy in pasture-based systems (Randi *et al.*, 2018), and Ferraz *et al.* (2019), recommend its use as part of a strategy to favor better follicle and luteal phase response in crossbred cows, with high P4 concentrations, under TAI protocols.

Although the heifers showed 47.4% gestation, no differences were observed among the female groups by category, which, according to De Rensis and López-Gatius (2014), treatment with eGC controls the reproductive activity of cows during the initial post-calving stages, increases ovulation and gestation rates in non-cyclical cows, and improves the conception rate in cows with delayed ovulation.

The binary logistic regression analysis contributed to the idea that the insemination technician is the most important factor in relation to TAI. Additionally, it permitted quantification of the odds

ratio to achieve gestation that could be compared to the one made by the technician with lower results. This definition corroborates the importance of continuous training and control of insemination personnel (López-Gatius, 2013), and the need to assess other factors associated to technical work.

CONCLUSIONS

The behavior of conception rate in crossbred beef bovine females following a TAI protocol based on DIB^{TM} and estradiol cypionate was similar to findings of other international reports.

The female category, body condition at the beginning of the treatment, and ovarian cyclicity, had no negative effects on the conception rate at first service.

The efficiency of the insemination technician (measured by the gestation rate in the first service, and the odds ratio), was pivotal in the results, with significant differences among them.

RECOMMENDATIONS

To utilize OR for systematic evaluation of inseminating technicians, as a fundamental element to achieve stability of TFAI program results.

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AUTHOR CONTRIBUTION

Research design and idea: MNHJ, JABV, RVMO, MGD, data analysis and interpretation: RVMO, JABV, MNHJ redaction of the manuscript: MNHJ, MGD, JABV, RVMO

CONFLICT OF INTERESTS

The authors declare no conflict of interests.