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Efficacy of Artificial Insemination and Natural Service in Gudali Cows (*Bos indicus*) Synchronized with Progesterone Releasing Intravaginal Device

[1]Justin Kouamo, [1]Sehbo Diddiwa, [1]Victor Ngu Ngwa, [2]David Rakansou, [3]Souley Hayatou, [4]Freddy Ngah Osoe and [4]Augustave Kenfack

[1]School of Veterinary Medicine and Sciences,
The University of Ngaoundere, P.O. BOX: 454, Ngaoundere, Cameroon
[2]SIMEL, Wakwa, Ngaoundere, Cameroon
[3]CAMVET-CO SARL, Ngaoundere, Cameroon
[4]Animal Physiology and Health Research Unit,
Department of Agronomy and Agricultural Sciences, University of Dschang, Cameroon

Abstract: An experimental study was carried out at the Wakwa zootechnical station, Ngaoundere, (Cameroon), with the aim of comparing the effectiveness of artificial insemination (AI) and natural service in Gudali cows (*Bos indicus*) synchronized with intravaginal progesterone (PRIDND DELTA), prostaglandin F2α and GnRH. Gudali cows (*N*=40) divided into two equal groups the first one bred using artificial insemination and other group received natural service with two Gudali bulls identified suitable for reproduction. The estrus induction and synchronization rates were 100% for both groups. The natural service group achieved 80% conception rate and the AI rate was 100%. Depending on the progesterone assay at day 22, pregnant cows from the AI group was lower (45%) than those bred with natural service (56.25%). Ultrasound examination performed 53 days after AI and 47 days after the last wave of natural service, revealed a pregnancy rate of 45% with 10% twin gestations for the inseminated cows, compared to 56.25% with 6.25% embryonic mortalities for cows placed in natural service. The two techniques had specificity, sensitivity, a positive predictive value and a negative predictive value of 100%, respectively. The induction and synchronization of estrus based on intravaginal progesterone, prostaglandin F2α and GnRH are therefore applicable in local cows for the techniques of artificial insemination and natural service.

Key words: Gudali Cow · Estrus · Assisted Reproductive Technology · Gestation · Adamawa · Cameroon

INTRODUCTION

The low productivity of cattle herds generally observed in tropical regions is due to several factors including diet, pathologies, environment, breeding method and genetic potential. The control of reproduction is a necessary practice in breeding, because it implements strategies, methods or techniques allowing optimization of the reproduction (natural service or artificial insemination) in particular during an anestrous period. However, the reproductive control strategies put in place aim at the choice of the birthing period, the grouping of births, the reduction of unproductive periods and finally maximizing production with health, zootechnical and genetic advantages. To control this variability, many estrus induction and synchronization protocols based on molecules such as progesterone, estrogens or prostaglandins have been in common use for several years. Improving production performance at one calf per cow per year is not achieved by most breeders, as they have constraints to adhere to artificial insemination programs which require skilled labor; some farms are often located in difficult-to-access areas and the probable role of the male in the determinism of sterility of the livestock is less regarded. To achieve this, it is necessary to use estrus induction and synchronization techniques and the selection of bulls to optimize the reproductive potential in cattle by natural service and artificial insemination to be

Corresponding Author: Prof. Justin Kouamo, School of Veterinary Medicine and Sciences, The University of Ngaoundere, P.O. BOX: 454, Ngaoundere, Cameroon. Tel: +237 675376954.

able to reach production targets for one calf per cow per year. These techniques proved to be of capital importance for the purpose of genetic improvement, to maximize the productivity of the livestock and the conservation of local breeds in extensive, semi-intensive and semi-extensive breeding like most farms in the northern regions of Cameroon [1]. The skillful application of these technologies has an immediate effect on contemporary animal production efficiency and a permanent effect on future generations through alteration of selection differentials and generation length. The main causes that limit the use and success of artificial insemination following synchronization are the lack of knowledge of estrus cycle and poor estrus detection, which is particularly important in breeds raised under tropical conditions [2]. It is in this context that this study was carried out with the general objective of comparing the effectiveness of artificial insemination (AI) and natural service in Gudali cows (*Bos indicus*) induced with intravaginal progesterone (PRIDND DELTA), prostaglandin F2α and GnRH. More specifically, we will have to evaluate the production performance of the selected sires, to assess the rate of induction and synchronization of estrus induced with intravaginal progesterone in the two groupes; to determine and compare the gestation rates for the two reproductive methods using immunoenzymatic assay of progesterone and ultrasound examinations and finally to determine and compare the factors of variation of the two reproductive methods.

MATERIALS AND METHODS

Period and Study Area: The study was carried out from November 2019 to April 2020 in the region of Adamawa, department of Vina (Ngaoundere). All the activities related to insemination, natural service as well as the various samples took place at the Wakwa Zootechnical Station and the treatments, conservation and blood analysis were carried out at the Wakwa Regional Center for Agricultural Research.

Selection of Breeding Bulls: The selection was carried out according to the methods of evaluation of the state of fertility of bulls described by Mopoundza *et al*. [3]: age, weight (Y = 0.00016 (TP) 2.847; TP = thoracic perimeter), libido, scrotal circumference, testicular heights, torsion, injury to the hoof, limbs and sheath; general examination of the reproductive system including inspection of the penis, inspection, measurement and palpation of the scrotum and a rectal palpation were the criteria evaluated. To test the libido, a cow in estrus was restrained in a pen and a bull was turned in for 10-15 minutes. If one or more services were completed during this time interval, libido was not considered to be a problem. These selection criteria allow selecting two Gudali bulls suitable for reproduction.

Selection of Cows and Animal Management: The selection of non-pregnant females in good health depended on the evaluation of the general animal health status and the rectal palpation. In the end, 40 Gudali cows were selected for the study. After an acclimatization period of about three weeks, the selected cows were divided into two equal groups or treatments, one for cows to be inseminated (n = 20) and the other for natural service (n = 20). The fattening status was assessed by assigning a body condition score (BCS) on the scale of 1 to 5 and only females with BCS between 2 and 4 were selected and body weigh was determined before installing the PRIDND DELTA.

The animals were driven in a semi intensive mode. After selection, they were housed in a well-delimited plot allowing them to be isolated from other herds and fed on the basis of natural pasture and cultivated fodder (*Bracharia sp*.) for around 8 hours of grazing per day. Supplementation was made with a ration based on cottonseed meal at a rate of 1 to 2 kg respectively per animal and per day. Salt was distributed once a week and the animals were watered ad libitum. They were vaccinated beforehand against pasteurellosis, contagious bovine pleuropneumonia (CBPP), lumpy disease and symptomatic anthrax during the national vaccination campaign organized by MINEPIA (ministry of livestock, fisheries and animal industry). All the selected females have been dewormed beforehand. External deworming was carried out using cypermethrin (Vectoclor Plus ®) with insecticidal, acaricidal action and repellent effect against flies and all other harmful insects. In addition, all selected cows received treatment with Ivermectin subcutaneously. This treatment was administered 10 days before the installation of intravaginal progesterone (PRIDND DELTA).

Estrus Synchronization, Artificial Insemination and Natural Service Protocols: The protocol for induction and synchronization of estrus in the two treatments was carried out as presented in Figure 1. It consisted of the intra-vaginal application of PRIDND DELTA for 8 days and an injection of 2ml (100 mcg) of GnRH (CYSTORELINEND) intramuscularly at the time of application of PRIDND

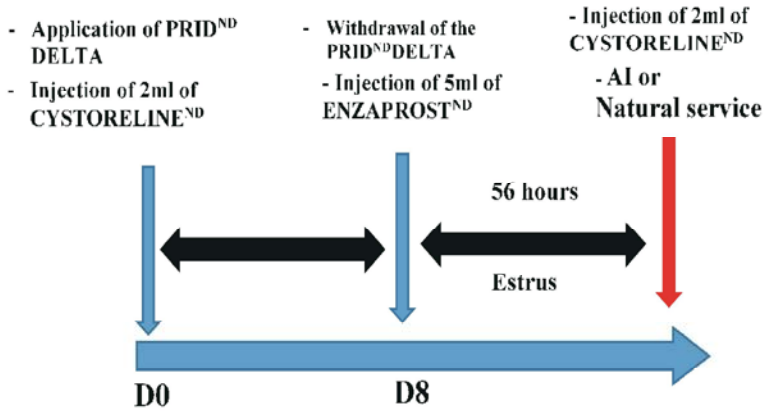

Fig. 1: Protocol for induction and synchronization of estrus, AI or natural service. D=day.

DELTA. An injection of 5ml of PGF2α (ENZAPROSTND) was given intramuscularly at the time of removal of PRIDND DELTA. The appreciation of the response to the synchronization treatment, in particular the monitoring of the signs and behavior of estrus (vulvar congestion, cervical mucus, tail deflection, acceptance of overlap or standing heat) was put in place the day after the spiral was removed by observing grazing animals. Females (n = 20) were inseminated using the recto-vaginal method 56 hours after withdrawal of PRIDNDDELTA using the CASSOU insemination gun with the Montbeliard semen (FABLO), thawed at a temperature varying between 36- 37 °C for 30 seconds. After the insemination, each cows received a 2 ml (100 mcg) dose of CYSTORELINEND (GnRH) intramuscularly (IM). The same synchronization protocol was used during natural service (Fig. 1) and CYSTORELINEND (100 mcg) was administered after mating. The natural services were organized in waves of 5 cows and 2 bulls selected for an interval of 12 days.

Pregnancy Diagnosis: Two pregnancy diagnostic methods have been implemented: the enzyme-linked immunosorbent assay of progesterone using a commercial DRG brand ELISA kit on blood samples taken on D0 (Day of AI or natural service), D11 and D22, post IA or natural service; and the ultrasound examination carried out using an IMAGO brand ultrasound system, fitted with a linear probe with adjustable frequency from 5 to 7.5 MHz. It was carried on the 54th day after AI for the inseminated cows and the 47th day after the last wave of the natural service. The intrinsic (age, body condition score (BCS), postpartum duration, number of parturitions, thoracic perimeter, weight, cyclicity) and extrinsic (dates and time of placement of PRIDND DELTA, withdrawal of PRIDND DELTA, injections of GnRH, injection of PGF2α, AI or natural service) parameters to the selected cows were collected.

Statistical Analysis: All of the data collected related to AI and natural service operations were entered into the Microsoft Excel spreadsheet and analyzed with SPSS 16.0 software for Windows. The measurement was based on the Chi-square test between -1 and +1, the results were expressed as an average ± standard deviation and the differences were considered to be significant at the 5% probability level. Cramer's V test was used to measure the intensity of association or bonding between nominal variables.

RESULTS

Characteristics of Selected Bulls: All the selected bulls were healthy, without abnormalities in their reproductive tract. Mean ± standard deviation (Minimum-maximum) of BCS was 4.25±0.35 (4-4.5), mean age was 6±1.4 (5-7) years and mean body weight was 586±46.6 (553-619) kg of the two selected bulls for the natural service. The means of the scrotal circumference, the height of the left testicle, the height of the right testicle and the testicular volume were 35.5±2.1 (34-37) cm, 19±1.4 (18-20) cm, 20±1.4 (19-21) cm and 979.12±187.0 (846.8-1111.3) cm3, respectively.

Characteristics of the Selected Cows: The majority of cow selected for AI or natural service were included in the range 3-7 years. The inseminated and naturally serviced cows consisted of 45% and 55% of cycling and non-cycling cows, respectively (Table 1).

Table 1: Characteristic of the cows according to the group

		Group of inseminated cows (n = 20)		Group of cows in natural service (n = 20)	
Parameters	Variables	Number	%	Number	%
Age (years)	]3-7[	9	45	11	55
	[7-9[	4	20	4	20
	[9-12]	7	35	5	25
	Total	20	100	20	100
BCS	[2-3[	4	20	13	65
	[3-4[	16	80	7	35
	Total	20	100	20	100
Number of calvings	[0-1[	0	0	4	20
	[1-3]	17	85	12	60
	[4-7]	3	15	4	20
	Total	20	100	20	100
Postpartum duration (months)	0	0	0	4	20
	[2-3]	8	40	3	15
	]3-7]	7	35	10	50
	]7-20]	5	25	3	15
	Total	20	100	20	100
Live weight (kg)	[285-350]	8	40	16	80
	]350-400]	5	25	1	5
	]400-480]	7	35	3	15
	Total	20	100	20	100
Cyclicity	Cyclic	9	45	9	45
	Acyclic	11	55	11	55
	Total	20	100	20	100

Table 2: Means ±standard deviation, minimum (min) and maximum (max) values of age, BCS, calving number, postpartum duration and weight of the selected cows.

	Inseminated cows Test value = 0.05 Confidence interval = 95%			Cows in natural service Test value = 0.05 Confidence interval = 95%			
Parameters	M±SD	Min.	Max.	M±SD	Min.	Max.	P-value
Age/year	7.60±2.13	3	9	7.05±2.92	5	9	0.373
BCS	3.02±0.34	2.5	3.5	2.62±0.39	2.5	3	0.21
Calving Number	2.65±0.98	2	3	2.3±1.92	1	3	0.262
Postpartum duration month	5.55±3.591	4	7	5.45±5.56	3	8	0.145
Body weight/kg	371.32±54.76	345	397	269.37±48.99	312	358	0.89

The overall averages of age, BCS, number of lactations, postpartum duration and live weight of cows in the inseminated and natural service groups are presented in Table 2. No significant difference ($P > 0.05$) was observed between the two groups for parameters studied.

Estrus Induction and Synchronization Rates: All of the females in both groups exhibited at least one sign of estrus, representing a 100% estrus induction and synchronization rates, respectively (Table 3).

Pregnancy Rate

Progesterone Assay: No difference was observed in the concentration of progesterone in the two groups ($P > 0.05$) (Table 4).

The values at D22 determined that 45% and 56.25% of the inseminated and natural service females, respectively, were presumed to be pregnant (Table 5).

Ultrasound Examination: Ultrasound examinations (53 and 47 after AI and natural service, respectively) revealed that 45% (n=9) of inseminated females were pregnant with 10% (n=2) twin gestations against 56.25% (n=8) of pregnant females after natural service with 6.25% (n=1) embryonic mortalities.

Compliance Between Progesterone Test and Ultrasound Examination: The compliance of the 2 methods of pregnancy diagnosis was 100% (Table 6).

Table 3: Signs of estrus observed.

		Inseminated cows		Cows in natural service	
Sign of estrus	Variable	Number	%	Number	%
Mounting other females (overlap)	Yes	13	65	2	10
	No	7	35	18	90
	Total	20	100	20	100
Standing when mounted	Yes	7	35	3	15
	No	13	65	17	85
	Total	20	100	20	100
Cervical mucus	Yes	20	100	18	90
	No	0	0	2	10
	Total	20	100	20	100
Moo	Yes	1	5	1	5
	No	19	95	19	95
	Total	20	100	20	20
Nervousness	Yes	1	5	5	25
	No	19	95	15	75
	Total	20	100	20	100
Agitation	Yes	1	5	13	65
	No	19	95	7	37
	Total	20	100	20	100
Vulvar temperature	Yes	20	100	/	/
	No	0	0		
	Total	20	100		
Uterine tonicity	Yes	20	100		
	No	0	0		
	Total	20	100	/	/

Table 4: Variations in progesterone levels in selected cows

		Inseminated cows				Cows selected for natural service				
Days following AI	N	M±SD	Min.	Max.	N	M±SD	Min.	Max.	P-value	Cramer's Coef
D0	9	0.69±9.33	0.10	0.99	8	1.91±2845	0.05	8.80		
D11	20	1.528±0.60	0.71	2.41	16	4.392±8.102	0.09	32.00		
D22	20	7.351±9,331	0.10	31.00	16	6.46±9,910	0.38	33.60	0.108	1.00

D = day. N=number. Coef.= coefficient. M=mean. SD=standard deviation. Min=minimum. Max=maximum

Table 5: Results of presumed gestation from the concentration of progesterone

	Inseminated cows		Natural served cows	
Progesterone assay	Number	%	Number	%
Doubtful (progesterone between 1 and 2 ng / ml)	7	35	4	25
Negative (progesterone <1 ng / ml)	4	20	3	18.75
Presumed positive (progesterone >2 ng / ml)	9	45	9	56.25
Total	20	100	16	100

Table 6: Contingency table for progesterone assay and ultrasound examination in cows from the two lots.

	Inseminated cows		Cows in natural service	
Pregnancy diagnosis	Gestation confirmed by ultrasound examination	Non gestation confirmed by ultrasound examination	Gestation confirmed by ultrasound examination	Non gestation confirmed by ultrasound examination
Presumptive gestation by progesterone assay	9 (TP) 45%	0 (FP) 0%	9 (TP) 56.25 %	0 (FP) 0 %
Non gestation presumed by the assay of progesterone	0 (FN) 0%	4 (TN) 20%	0 (FN) 0%	3 (TN) 18.75 %

TP: true positive. FP: false positive. FN: false negative. TN: true negative.

Table 7: Variation in gestation rate regarding intrinsic cow parameters.

Intrinsic parameters related to the selected cows	Variables	N	Gestation rate of inseminated cows (%)	N	Gestation rate of cows in natural service (%)	P- Value
Age (years)	]3-7[	9	33.33 (3)	11	27.27 (3)	0.202
	[7-9[	4	75 (3)	4	75 (3)	
	[9-12]	7	42.85 (3)	5	60 (3)	
	Total	20		20		
BCS	[2-3[	4	25 (1)	13	30.76 (4)	
	[3-4[	16	50 (8)	7	71.42 (5)	
	Total	20		20		0.559
Number of calvings	[0-1[	0	0	4	0	
	[1-3]	17	47.05 (8)	12	50 (6)	
	[4-7]	3	33.33 (1)	4	75 (3)	
	Total	20		20		0.000
Postpartum duration (months)	0	0	0	4	25 (1)	
	[2-3]	8	50 (4)	3	33.33 (1)	
	]3-7]	7	57.14 (4)	10	60 (6)	
	]7-20]	5	20 (1)	3	33. 33 (1)	
	Total	20		20		0.488
Weight (kg)	[285-350]	8	25 (2)	16	37.5 (6)	
	]350-400]	5	80 (4)	1	100 (1)	
	]400-80]	7	42.85 (3)	3	66.66 (2)	
	Total	20		20		0.56
Cyclicity	Cyclic	9	100 (9)	9	77.77 (7)	
	Acyclic	11	0	11	18.18 (2)	
	Total	20		20		0.000

N = number.

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) in the two groups were 100%, respectively.

Factors Affecting Gestation Rate Using Ultrasonography: Among the intrinsic factors related to the selected cows, the number of lactations and the cyclicity significantly influence the gestation rate (Table 7).

Among the extrinsic factors (Interval between application and withdrawal of PRIDND DELTA in minutes (P= 0.084), time interval between 56 hrs after removal and AI or natural service in hours (P= 0.475) and placement of semen into the genital tract (P= 0.409) of the selected cow, none significantly influence the gestation rate.

DISCUSSION

The average scrotal circumference of the selected bulls (35 cm) is greater than 31.35 ± 0.40 cm, obtained by Bah [4] in Gudali bulls of 5-6 years, 27.16 ± 0.84 cm and 27.2 ± 0.8 cm, respectively, reported by Chicoteau [5] in Baoulé bulls aged 5.3 years. On the other hand, it is close to that of the Norman of 15 months (34.63 cm) and the Holstein of 15 months (36.73 cm) found by Thibier and Colchen-Bourlaurd [6]. The means of the heights of the left and right testicles (19 and 20 cm respectively) are much greater than the means of the left and right testicles reported by Mopoundza *et al*. [3] corresponding respectively to 10.20 ± 2.1 and 9.96 ± 2, 4 cm in Ndama bulls in Congo Brazzaville. These differences observed at the level of each parameter would be due, to the breed on one hand and on the other hand to the difference in age. Taking into account the average of the testicular volume which was calculated from the heights of the testes and the scrotal circumference, the means of the parameters as well as the excellent libidos reveal that the selected bulls are suitable for reproduction. According to Blockey [7] and Kamga-Waladjo *et al*. [8], the factors permitting to determine the quality of the bull's fertility go through the control of parameters such as the morphology, the potential of its reproductive organs, the quality sperm, libido, diet and management mode.

Where AI or hand-mating is being used, estrus detection is the most important limiting factor for optimum reproduction performance. Insufficient and/or inaccurate estrus detection leads to delayed insemination (within estrus and also post-partum), reduced conception rates and thus extended calving intervals. All the cows in the two groups showed at least one sign of estrus. The flow of cervical mucus, the vulvar temperature and the uterine tone have a rate of 100% for the inseminated cows and the rates of 65% and 90% were obtained

respectively for agitation and flow of cervical mucus in cows placed in natural service. The differences may be due to the fact that all the cows in the inseminated group were observed and handled during AI while the cows in the natural service group were not handled. Overall induction and synchronization rates of 100% in each of the two groups are higher than the rates of 80.54% obtained by Okouyi [9] with the classic PRIDND; 75.77% of Issoufou [10] with PRIDND DELTA in local cows in Senegal; 96.9% of Pitala *et al*. [11] with CRESTARND combined with prostaglandin and PMSG in Burkina Faso in Gudali zebus and 98.05% by Blagna *et al*. [12] out of a total of 617 cows of the Zebu Peul, N'Dama and Gudali breeds synchronized with CRESTARND. These results obtained on local breeds (Gudali) are in agreement with Kouamo [2] who reported that the GnRH-based protocol seems to be a useful method of synchronization. In cattle, synchrony of estrus and fertility with a combination of GnRH and prostaglandin F2a are good for cyclic females and this combination may induce cyclicity in cows experiencing postpartum anoestrus [2].

Gestation rates of 45% and 56.25% using progesterone assay and ultrasound examination were obtained to the group of inseminated cows and those placed in natural service, respectively. These rates are higher than the results obtained with the Zebu Gobra (40%) after artificial insemination and in Ndama (43.75%) placed in natural service by Delahaut *et al*. [13]; 44.3% obtained by Kouamo *et al*. [14] in female Zebu Gobra and F1 crossbred in Senegal; 42.7% reported by Zongo *et al*. [15] among Gudali zebus in Burkina Faso. In addition, Ikhar [16] obtained a conception rate of 40% in a group of inseminated cattle after synchronization by double injections of PGF2α 11 days apart. The results are comparable to the 52% obtained by Bayemi *et al*. [17] on Red Fulani zebus in Cameroon. Furthermore, these results are below the standards defined at 60% (conception rate at first insemination), representing the objective for dairy herds and 72% obtained by Parmar *et al*. [18] in Indonesia.

The twinning rate (10%) in the group of inseminated cows is higher than the results obtained by Kouamo *et al*. [14] in zebus females Gobra and F1 crossbred after artificial insemination in Senegal (6.89%) and Ahmadou *et al*. [19] in a dairy herd in Zambia (1.4%). Kouamo *et al*. [14] have reported that twinning is a genetic factor that varies prolificacy considerably. The use of Pregnant Mare Serum Gonadotrophin (PMSG) in estrus induction protocols is often associated with twinning in the herd because its action is dose dependent. Indeed, it induces ovulation (400-500 IU) and/or super ovulation (2000 IU). These variations could also be explained by the use of GnRH twice at the beginning and end of the estrus induction protocol which may cause a superovulation and consequently cases of twin. In dairy cow, twin birth is associated with a higher calf mortality, retained placenta, longer calving to conception intervals and reduced milk yield. If done by careful management these problems can be controlled, the induction of twin pregnancy may have economic advantages. In beef cattle, where milk yield is not the primary source of income, twinning has been shown to be economically interesting.

In the group of cows placed in natural service, an embryonic mortality rate of 6.25% was obtained using ultrasound examination. This rate is lower than that reported by Mouiche *et al*. [20] in Senegal. Santos *et al.* [21] estimate that, among dairy cows, this rate would be between 5 and 12% according to studies. Silke *et al*. [22] observed a 7.2% loss of conceptus for cows, but comparable to that of 6.1% in heifers between days 28 and 84 post gestation. Genetic, endocrine, immunological, nutritional, environmental and pathological predisposing factors may be responsible for this high rate of embryonic mortalities [23, 24].

Cows whose ages were included in the interval [7 – 9 years] have the highest gestation rate (75%) for the two groups combined (P= 0.2). Issoufou [10] in Senegal reports a higher gestation rate in cows aged 11 to 14 years (60%) compared to those between the ages of 7 and 10 years (35.71%). On the other hand, the results of this study are comparable to those obtained by Humblot [25], which found a decrease in the gestation rate with age and attributes it to the increase in late embryonic mortalities in older cows but also to failures observed during early gestation.

Cows that have given birth at least once respond best to artificial insemination, while the gestation rate is higher (75%) from the fourth lactation in cows placed in natural service. This result differs from that reported in previous tropical studies [10, 26] where the number of lactations had no significant influence on the gestation rate. In addition, the results of this study are superior to those obtained in a study of dairy cows in Rwanda by Nishimwe *et al*. [27]. In fact, the latter reported a gestation rate of 69.2% in cows of 4 to 6 lactations otherwise significantly higher compared to cows of 1 to 3, 7 to 9 and more than 10 lactations. This may indicate that prior uterine activity increases the chances of successful gestation. In breviary, for better induction and synchronization of the estrus, selection should exclude heifers.

Cycling cows have 100% and 77.77% gestation rates in inseminated cows and those placed in natural service after estrus induction, respectively (P= 0.00). This result is similar to many studies [28-32]. In anestrus case, the cow is not observed in estrus either because she has not come on heat (non-cyclic cows) or because the estrus was not detected (cyclic cows). Subestrus or failure to observe estrus is the major cause of post-partum anestrus. It includes animals with normal estrus behaviour, animals with weak estrus behaviour and animals without estrus behaviour. In the group of cows placed in natural service, only females that evoke a standing reflex are pregnant. Off all signs the standing reflex (standing when mounted) is a truly reliable indication of estrus.

CONCLUSION

In order to improve reproductive performance and maximize the productivity of cattle, induction and synchronization of estrus using the PRIDND DELTA, prostaglandin F2α and GnRH protocol to achieve AI and natural service (using established criteria for bulls) may be a way to improve herd fertility.

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