

RESPONSE OF GRAZING COWS TO SYNCHRONIZATION PROTOCOLS AND FIXED TIME ARTIFICIAL INSEMINATION

A.O. Adeyina¹, A.S. Akanbi², A.O., Salako³, S.B. Salau⁴ & F. Falana⁵
^{1,2,3,4,5}Department of Animal production, University of Ilorin, Ilorin, Nigeria.

*E-mail of Correspondence Author: aadeyina@yahoo.com

ABSTRACT

This study investigated the response of grazing cows to PGF2 α + PGF2 α , and GnRH +PGF2 α synchronizing protocols with fixed time artificial insemination (FTAI). A total of 52 cows with 26 assigned to each treatment as groups A and B were used. Group A was administered with 500 μ g (2ml) prostagladins (PGF2 α , 1M cloprostenol sodium) on day 1 and day 12 respectively followed by FTAI 60-72 hours later, while group B was synchronized with 10 μ g (1ml) gonadotropin releasing hormone (GnRH, IM, Buserelin acetate) on day 1 followed by 500 μ g(2ml) prostaglandin PGF2 α (PGF2 α , IM, cloprostenol sodium) and on day 8 and the second dose of GnRH (10 μ g) on day 10 followed by FTAI 8-24hr later. Pre-synchronization revealed that the range of FSH (8.38-13.18), LH (8.19-11.28) and Estrogen (2.61-3.85) for the two groups. While the hematological values showed that, the range of WBC (8.87-9.82), RBC (5.45-5.66) and PCV (32.71-33.38) for the two groups. With synchronization protocols, PGF2 α +PGF2 α (group A) resulted in significantly ($p>0.05$) high value of LH, WBC and RBC and significantly low PCV and pregnancy rate of 57.69% compared with GnRH +PGF2 α (group B) which also had a pregnancy rate of 46%. It can be concluded that grazing cows are better adapted to double dose of PGF2 α and could be recommended,

Keywords: Grazing Cows, Synchronization, Artificial insemination, Pregnancy

1.0 INTRODUCTION

Fulani are good animal husbandry people and their pastoralist expertise could be a good foundation for the development of cattle production system in Africa. The complexity of the system and the problems of developmental efforts have risen within the constraint of pastoral land – tenure system and agricultural system which has resulted in many conflicts across the sub tropics. Many animals with low reproductive potentials are kept for natural mating thereby increasing the grazing density over feed availability. The use of ART has made a major improvement in the cattle industry among the developed countries. ARTs are techniques designed to improve reproductive efficiency, genetic gain and reduce generation intervals. Applications of ARTs such as estrus synchronization and artificial insemination (AI) have not been fully utilized in the pastoralist system. Estrus synchronization optimizes labour and time and improves ease of using AI (Lamb *et al.*, 2009). Artificial insemination allows access to superior genetics, accelerate genetic changes within a herd and it is less expensive than natural services (Johnson and Jones, 2004)

The use of artificial insemination in cattle is expanding worldwide however, variability in reproductive performance could occur due to difference in interval between onset of estrus and its detection (Foote, 1979). Recent advances in protocols for fixed-time Artificial insemination (FTAI) in cow has limited its use worldwide due the difficulties in the estrus detection and in finding an adequate moment for this procedure (Baruselli *et al.*, 2007). Therefore, an alternative to increase the number of cow inseminated is the use of synchronization protocols that allow AI without the need of estrus detection, usually called fixed-time AI (FTAI). Follicular wave development can be controlled by treatments with GnRH or estradiol and progestogen/progesterone in combination. Treatment of buffalo with GnRH in combination with prostaglandin F2 α (PGF2 α) at 7 day and a second GnRH 48 h after PGF2 α (known as Ovsynch) has resulted in acceptable pregnancy rates after FTAI in cycling buffalo during the breeding season (Baruselli *et al.*, 2007).

2.0 LITERATURE REVIEW

Konyves *et al.*(2009) reported a delayed renewal of the ovarian activity and an increased interval between the parturition and the first ovulation for synchronized cow. Synchronization of ovulation with GnRH and PGF2 α brought a major impact on managing lactating cows by allowing timed AI and ascertained ovulation which eliminated estrus detection (Pursley *et al.*, 1997). Synchronized females exhibit estrus at a controlled time, have increased calf uniformity within the season (Perry, 2004). Resent research has shown fixed time AI (FTAI) to have similar pregnancy rates to heat detection and AI (Tibbitts *et al* 2017). Many approaches may be adopted for natural services, herd detection and timed AI (TAI) however, prostaglandins and GnRH have

been used at different degrees to achieve success in AI with high percentage of pregnancy. Synchronization of estrus and fertility with a combination of GnRH and PGF2 α are good for cyclic females (Prusley *et al* 1994). This treatments control cycle length with the manipulation of follicular development in order to achieve precise estrus and ovulation for timed insemination. The nature of grazing cattle in pastoralism requires that responses of the cows to PGF2 α +PGF2 α and PGF2 α +GnRH protocols be carried out for a successful AI, irrespective of their physiological state.

3.0 METHODOLOGY

3.1 Materials and methods

The experiment was conducted at YAO farms located in Afon, Nigeria, characterized with mean annual rainfall of 900mm and mean annual temperature of 32.5⁰C from the month of November to April, the following year. A total of 52 dry and non-pregnant cows of white Fulani breed weighing 250kg $\pm$ 5.6kg, age 3-6 years) were selected. The body scores of the cows were determined in accordance with the methods described by Natumyana *et al* (2008). They were confirmed non pregnant through palpation by an experienced veterinary personnel. The animals were tagged and arranged into groups A and B respectively. The animals were herded together in an improved nomad system in which the animal was taken out in the morning to graze on mixed pasture field consisting of *Panicum maximum*, *Pennisetum purpureum*, *Brachiaria spp.* and *legume spp.* The animals were also offered supplemental feed consisting cassava peels and cowpea chaff when they return to base in the evening. Prior to the commencement of the experiment, the animal was administered with Ivomec super (ivomectin 10mg/ml) manufactured by Menal, France for reducing the parasite load and oxytetracycline LA (Oxytetracycline 200mg/ml manufactured by Invesa, Spain. The drugs were administered by a veterinarian

3.2 Blood collection

Blood samples were collected from all the animals via jugular venipuncture using a 10 ml syringe and 5ml from each sample was added into plastic tube containing EDTA and the remaining 5 ml was added the plain plastic tube without anticoagulant for hormonal assessment. The blood was collected 2 weeks before synchronization and 2 weeks after artificial insemination. Blood samples were also obtained from the pregnant and non-pregnant animals resulting from the experimental procedure. The blood samples were analyzed using haematology analyzer (HA) model 6000. Hormonal profile (follicle stimulating, luteinizing and estrogen hormones were determined using the procedure of Chauhan and Agarwal, (2008).

3.3 Synchronization protocols

Animals in group A (n=26) were administered with progladins (2M administration of 500ug PGF2 α analogue produced by Estrumate, Schering-Plough, Germany) on day 1 while the second dose PGF2 α (500ug) was administered on day 12. Animal showing signs of estrus were observed but all the animals were inseminated between 60-72 hours after the second dose of PGF2 α .

Animals in group B (n=26) were administered GnRH on day 1. On day 8, they were administered with PGF2 α while on day 11 administration of GnRH was repeated. The animals were inseminated 24 hours after the last administration of GnRH. Artificial insemination was carried out by an experienced veterinary doctor using the procedure described by Sorensen, (2014). The semen used for insemination were obtained from international fertilizer development centre (IFDC) partner of Freilandcapinal (WAMPCO) milk collection centre, Iseyin, Nigeria while the synchronizing protocols were obtained from Agric project concept international limited, Kaduna, Nigeria. Pregnancy Diagnosis was carried out using return to heat and rectal palpation at 60 days post insemination methods as described by Sorensen, (2014).

3.4 Statistical Analysis

Data were analyzed using a 2-way Analysis of Variance in a 2 $\times$ 2 factorial arrangement. Significant ($p < 0.05$) differences among treatment means were determined using Duncan Multiple Range Test as contained in Statistical Analysis Software (SAS, 2010) package. While, the number of dams inseminated, on estrus and pregnant was determined using descriptive statistic (percentile).

4.0 FINDINGS AND DISCUSSION

Table 1. Hormonal pattern of cows before and after synchronization and AI

Source of Variations		FSH(miu/ml)	LH(miu/ml)	E(pg/ml)
TREATMENTS				
EFFECT				
A	BEFORE	13.18 ^b	11.28 ^b	2.61 ^b
	AFTER	15.49 ^a	17.29 ^a	3.86 ^a
B	BEFORE	8.38 ^c	8.19 ^d	3.85 ^a
	AFTER	13.30 ^a	10.60 ^c	3.84 ^a
P-value		<.0001	<.0001	<.0001
TREATMENTS (T)				
A		14.33 ^a	14.28 ^a	3.23 ^b
B		10.84 ^b	9.39 ^b	3.84 ^a
P-value		<.0001	<.0001	<.0001
PROTOCOL (P)				
BEFORE		10.78 ^b	9.73 ^b	3.23 ^b

	AFTER	14.40 ^a	13.95 ^a	3.85 ^a
P-value		<.0001	<.0001	<.0001
T*P		NS	NS	S
SEM±		0.301	0.992	0.768

a-b Means on the same row having different superscript were significantly (p<0.05) different. SEM= Standard Error of Mean, FSH= Follicle Stimulating Hormone, LH= Lutealizing Hormone, E= Estrogen

Table 2: Haematological profile of cows before and after synchronization and AI

Source of Variations	WBC (x10 ⁹ /l)	RBC (x10 ¹² /l)	PCV (%)	HB (g/dl)	PLT (x10 ⁹ /l)	MCV (fl)	MCHC (g/dl)	MCH (pg)
TREATMENTS EFFECT								
A BEFORE	9.82	5.66 ^a	32.71 ^b	11.36 ^a	15.61 ^a	57.89 ^b	34.72 ^b	20.09 ^{bc}
AFTER	8.86	5.21 ^c	22.39 ^d	7.51 ^c	12.80 ^c	43.03 ^c	33.54 ^b	14.42 ^d
B BEFORE	8.87	5.45 ^{bc}	33.38 ^a	10.49 ^b	14.84 ^b	61.48 ^a	31.41 ^c	19.29 ^c
AFTER	7.95	4.72 ^d	27.64 ^c	9.96 ^b	12.97 ^c	58.58 ^b	36.03 ^a	21.11 ^a
P-value	0.6710	0.0024	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
TREATMENTS (T)								
A	9.34 ^a	5.43 ^a	27.55 ^b	9.43 ^b	14.21 ^b	50.46 ^b	34.13 ^a	17.26 ^b
B	8.41 ^b	5.08 ^b	30.51 ^a	10.22 ^a	14.90 ^a	60.30 ^a	33.72 ^b	20.20 ^a
P-value	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002	<.0001	<.0001
PROTOCOL (P)								
BEFORE	9.34 ^a	5.55 ^a	33.04 ^a	10.92 ^a	15.22 ^a	59.68 ^a	33.07 ^b	19.69 ^a
AFTER	8.40 ^b	4.97 ^b	25.01 ^b	8.74 ^b	13.89 ^b	50.80 ^b	34.79 ^a	17.76 ^b
P-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
T*P	NS	NS	NS	NS	NS	NS	S	S
SEM±	0.896	0.706	0.230	0.976	0.581	1.897	0.765	2.346

a-c Means on the same row having different superscript were significantly (p<0.05) different WBC= White Blood Cell, RBC=Red Blood Cell, PCV= Packed cell Volume, PLT= Platelet, MCH= Mean Corpuscular Volume, MCH= Mean Corpuscular Haemoglobinand MCHC= Mean Corpuscular Haemoglobin Concentration.

Table 3. Hormonal pattern of pregnant and non-pregnant cows synchronized and AI

Source of Variations	FSH(miu/ml)	LH(miu/ml)	E(pg/ml)
TREATMENTS EFFECT			
A PREGNANT	10.50 ^c	12.50 ^b	2.46 ^b
NON-PREG	10.70 ^c	4.50 ^d	2.02 ^b
B PREGNANT	12.16 ^b	14.67 ^a	4.72 ^a
NON-PREG	14.42 ^a	5.33 ^c	2.50 ^b
P-value	0.001	<.0001	0.002
TREATMENTS (T)			

A	10.60 ^b	8.50 ^b	2.24 ^b
B	13.29 ^a	10.00 ^a	3.61 ^a
P-value	<.0001	<.0001	<.0001
PREGNANCY (P)			
PREGNANT	11.33 ^b	13.56 ^a	3.60 ^b
NON-PREG	12.46 ^a	4.92 ^b	2.62 ^a
P-value	<.0001	<.0001	<.0001
T*P	S	NS	S
SEM±	1.421	0.646	0.218

a-b Means on the same row having different superscript were significantly (p<0.05) different. FSH= Follicle Stimulating Hormone, LH= Luteinizing Hormone, E= Estrogen

Table 4: Haematological profile of pregnant and non-pregnant cows synchronized and AI

Source of Variations	WBC (10 ⁹ /l)	RBC (10 ¹² /l)	PCV (%)	HB (g/dl)	PLT (10 ⁹ /l)	MCV (fl)	MCHC (g/dl)	MCH (pg)
TREATMENTS EFFECT								
A PREGNANT	8.48	4.73 ^b	32.90 ^a	9.78 ^a	3.32 ^a	69.56 ^a	29.72 ^d	21.58 ^a
NON-PREG	8.49	4.40 ^b	24.10 ^d	8.88 ^b	3.12 ^a	54.77 ^b	36.85 ^a	21.54 ^a
B PREGNANT	8.29	4.32 ^b	29.67 ^b	8.94 ^b	3.14 ^a	68.68 ^a	30.13 ^c	16.94 ^c
NON-PREG	8.38	6.39 ^a	27.33 ^c	9.01 ^b	2.43 ^b	42.77 ^c	32.93 ^b	18.63 ^b
P-value	0.812	0.0010	<.0001	<.0001	0.002	<.0001	<.0001	0.001
TREATMENTS (T)								
A	8.45	4.57 ^b	28.5	9.33	3.22	62.17 ^a	33.29 ^a	21.57 ^a
B	8.34	5.36 ^a	28.5	8.98	2.29	58.23 ^b	30.53 ^b	17.79 ^b
P-value	0.701	<.0001	0.765	0.091	0.089	0.000	<.0001	<.0001
PRREGNANCY (P)								
PREGNANT	8.39	4.53 ^b	31.29 ^a	9.36 ^a	3.18 ^a	69.07 ^a	29.93 ^b	19.26 ^b
NON-PREG	8.44	5.40 ^a	25.72 ^b	8.95 ^b	2.78 ^b	48.77 ^b	34.91 ^a	20.09 ^a
P-value	0.061	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.005
T*P	NS	S	NS	S	NS	NS	NS	S
SEM±	0.816	0.576	0.245	1.075	0.489	2.112	1.966	1.995

a-b Means on the same row having different superscript were significantly (p<0.05) different. WBC= White Blood Cell, RBC=Red Blood Cell, PCV= Packed cell Volume, PLT= Platelet, MCH= Mean Corpuscular Volume, MCH= Mean Corpuscular Haemoglobinand MCHC= Mean Corpuscular Haemoglobin Concentration,

Table 5: Effect of Synchronization protocols and AI on percentage of pregnant and non-pregnant cows

Parameters	A (%)	B (%)
No Pregnant	57.69	46
No of Estrus	42.30	31
No Inseminated	100	100

Table 1. Hormonal pattern of cows before and after synchronization and AI.

The follicle stimulating hormone (FSH) and leutinizing hormone (LH) of the cows significantly ($p>0.05$) increased with the synchronization protocols. The value of FSH and LH before synchronization is a reflection of hormonal environmental threshold for reproduction. According to Crowe and Muller (2013), FSH and LH facilitate the transition of female animals between period of reproductive non receptivity to receptivity, enabling mating and subsequent pregnancy. Animals are in a certain state of reproductive hormone during physiological conditions, these hormones surge in the event of cyclicity by natural negative or positive feedback control. The significant increase in LH and FSH after synchronization could have been induced by surge from hormone of the protocols. Sunderland et al, (1994) reported that GnRH surge induces coincidental LH and FSH surge. The value of LH is higher than FSH because, FSH is stored for a short time while LH is stored for a longer period during estrous cycle (Farnworth, 1995) However, synchronization exogenously manipulates the hormonal profile of estrous cycle to the threshold level of estrus. It could be said that, protocol A of PGF2 α reduces CL much faster than protocol B resulting in reduced FSH and increasing LH. The level of estrogen was significantly ($p>0.05$) higher in animals administered with B protocol than A. however; the values were relatively low with the two protocols indicating that estrogen level is low prior to estrus. The protocols were to bring the animals to a state of estrus therefore; it appeared that estrogen was not affected by the protocols.

Table 2. haematological parameters

The values of WBC, RBC, PCV, HB, PLT, MCH were observed to be significantly ($p>0.05$) higher before synchronization than after synchronization in the two groups. However, these values were within the range for normal healthy cows as reported by Chauhan and Agarwal, (2006). This implies that synchronization from the two protocols did not alter the health status of the cows. According to Oyawoye and Ogunkunle, (2004) haematological components, which consist of RBC, WBC, MCV, MCH are valuable indices in monitoring the blood as well as the health status of farm animals. WBC, HB and PCV are health indicators for animal wellbeing and Protocol A, (PGF2 α), resulted in higher value of WBC than protocol B. PGF2 α administered twice could have presented an antigenic like nature capable of stimulating antibodies in the cows thereby increasing the level of WBC in protocol A. than in the cows administered with GnRH in protocol B.

Table 3. Hormonal profile of pregnant.

The value of FSH was significantly higher for animals administered with protocol B than A. The significantly ($p>0.05$) high value of FSH in non- pregnant animals could be an indication

of state of preparedness for estrus. While the high value of LH for pregnant animals may be a reflection of natural release of LH for the maintenance of pregnancy through progesterone. The level of Estrogen which was significantly ($p > 0.05$) lower in non-pregnant animals than the pregnant ones corroborates Hendricks (1976) that estrogen is lower in non-pregnant cow and were only detectable during late pregnancy. In the pregnant cow's estrogen recorded was lower than 5 ug/ml reported by Hendricks et al, (1972).

Table 4. Haematological indices of pregnant and non-pregnant

The protocols did not alter the WBC of both pregnant and non-pregnant. The value of RBC was significantly ($p > 0.05$) lower while the HB was significantly ($p > 0.05$) higher in pregnant than non-pregnant. The low RBC in pregnant could have resulted from nutritional status, stress, physiological state and fetal demand. The Fulani cattle are in constant stressful state of grazing to meet the nutritional need which could affect the blood profile.

Table 5 shows the conception rate of 57% for synchronized cow with protocol A was higher than 22% reported by Fogwell et al, (1986) at timed insemination following single administration of PGF 2α . Moody and Lauderhals, (1977) also reported 59% of conception rate for cow synchronized with prostaglandins. Cows synchronized with B (GnRH) had 46% of conception rate. This value is supported by Pursley et al, 1995b who reported 45% pregnancy rate using GnRH-PGF 2α -GnRH protocol

5.0 CONCLUSION

The future direction of estrous synchronization is to focus on combination of traditional methods of reproduction and management with manipulation of follicular development. The immediate goal is to utilize treatment that synchronizes and control the time of ovulation to allow single insemination with or without heat detection or behavioral signs coupled with nutritional inadequacies experienced by grazing cattle. Protocol A proved to support the idiosyncrasies of grazing cattle with high percentage of pregnancy than protocol B and it is therefore recommended.

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