

Estrous synchronization and fertility of cross-bred dairy cows using prostaglandin F2 α and gonadotropin releasing hormone

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ABSTRACT: A comparative hormonal study of prostaglandin, gonadotropin releasing hormone and their combination was conducted on 30 randomly selected cross-bred dairy cows of SEMEX project for artificial insemination in Hillat Kuku, Khartoum North, Sudan. The study attempts to determine the response of the animals to hormonal induction and synchronization of estrous as well as fertility following fixed time artificial insemination. Three treatment groups of 10 cows each were undertaken. Groups 1, 2 and 3 involved administration of prostaglandin F2 α (PGF2 α), gonadotropin releasing hormone (GnRH) and a combination of PGF2 α + GnRH, respectively. Descriptive statistical analysis was performed. The results revealed that all the three protocols induced and synchronized estrous were almost synchronous in all the treated groups. However, protocol 3 revealed a significantly ($p < 0.05$) better result of 70% pregnancy rate compared to other two protocols that equally provided 50% pregnancy rates. The study resolves that protocol 3 be highly recommended to change the mindset of farmers about the spread of reproductive technology in Sudan. Protocols 1 and 2 provided acceptable pregnancy rates that can be enhanced with the improvement of management.

Keywords: Cross-bred dairy cows, estrous regime, gonadotropin, prostaglandin, pregnancy rate.

INTRODUCTION

The use of endocrine/hormonal treatments in sustainable development of livestock is very imperative for improvement of pregnancy rates in cattle (Bisinotto and Santos, 2011). However, the recent adoption of farmers to reproductive technologies in the Sudan and their perception to fertility of the cross-bred animals cannot be enhanced without a serious scrutiny. The fact that farmers have embraced negative perceptions about estrous synchronization and Artificial Insemination (AI) in the Sudan need mitigation measures including a change of mind set. The original objectives behind the development

of controlled breeding techniques are to promote a system that removes completely any need for heat detection. Precise control of the timing of ovulation leading to AI at a fixed and predominant time has been considered as an essential requisite for any commercially accepted system.

Animal reproduction remains the top of animal production pyramid. Nevertheless, good reproduction is a milestone for a successful dairy operation with highest fertility (Roelofs and Kooij, 2015; Ahlawat et al., 2015). It has been reported that estrous synchronization and AI programmes are sides of a coin (Lijalem et al., 2015) and

that estrous synchronization is one of the realistic solutions for reproductive management and control of the estrous cycle (Islam, 2011; Ahlawat et al., 2015). However, the synchronization requires adequate nutrition, good body condition, high quality semen, health, efficient estrous detection technique, appropriate postpartum period and efficient technician. As such, any deviation from one of the pre-requisites can result to failure of the programme (Troxel, 2012; Smith et al., 2012; Lamb, 2013; Gizaw et al., 2016).

The use of prostaglandin F_{2α} (PGF_{2α}) hormone in cattle reproduction has been firstly reported by Rowson et al. (1982). Moreover, the luteolytic properties of prostaglandin such as dinoprost tromethamine salts and its analogues such as cloprostenol estrumate have been described to effectively control Corpus Luteum (CL) function and estrous in di-estrous cattle (Rowson et al., 1982). Furthermore, PGF_{2α} hormone and its potent analogues which are described as the effective luteolytic agents for estrous synchronization have been reviewed (Bihon and Assefa, 2021). Nowadays, a variety of protocols for estrous synchronization in cattle is designed and implemented (Gizaw et al., 2016).

A number of estrous synchronization protocols are available in cattle using PGF_{2α} hormone alone or in combination with other hormones such as GnRH, progesterone and estrogen (Islam, 2011; Troxel, 2012). However, the selection of technically right and practically feasible protocol is the base for any success (Gizaw et al., 2016). Protocols based on the administration of a combination of hormones such as GnRH and PGF_{2α} have been used over the past two decades. It has been observed that three main protocols, namely; Ov-synch, Co-synch and Select-synch are used either alone or in combination with progesterone supplementation. Melendez et al. (2006) have observed a higher pregnancy rate when progesterone is supplemented with Ov-synch. This Ov-synch protocol uses sequential injections of GnRH, PGF_{2α}, and GnRH prior to timed AI. Evidence has shown that GnRH causes the pituitary gland to release Luteinizing Hormone (LH) that acts to transform a large follicle containing an unfertilized egg into a CL; thus, it has been recently incorporated into synchronization programmes. GnRH is always used 6 to 7 days prior to PGF_{2α} because it increases the percentage of cows that have been synchronized in response to PGF_{2α}. Usually the GnRH analogues; Cystorelin, Factrel, or Fertagyl are used. The first injection of GnRH is given to all cows without any knowledge of the stage of the estrous cycle. Depending on the stage, GnRH can either luteinize or ovulate the largest follicle in about 85% of all cows, forming a CL. After a 7 day waiting period, PGF_{2α} is given. Administration of PGF_{2α} regresses the CL induced by GnRH. A new dominant follicle grows and is available for ovulation by the second GnRH injection. This injection is given 36 to 48 hours after the PGF_{2α} injection. Recently,

Mekonnin et al. (2016) have modified GnRH-PGF_{2α} protocol and successfully used in cross-bred dairy cows and heifers in Mekelle, Ethiopia.

Evidence has shown that failure of estrous detection results to infertility in cattle (Cirit et al., 2008; Gebrehiwot et al., 2015; Paul et al., 2015). Generally speaking, strict follow up and the use of heat detection devices and technologies help increase a detection ability to have successful breeding (Jemal et al., 2016). Detecting estrous many times a day as much as possible is better than detecting once a day (Gebrehiwot et al., 2015). For the past seven decades, the standard for the time of insemination has been recognized as 12 hours after onset of estrous (Dorsey et al., 2011; Legesse, 2015).

In the Sudan, the use of prostaglandin as a tool to induce luteolysis has been practised only at academic level and research institutions. Therefore, the need for developing system of controlled breeding becomes very crucial especially as more specialized dairy farming under intensive management systems are gaining momentum. Hence, this study is an attempt to determine the effects of prostaglandin F_{2α}, GnRH hormones and their combination at different levels on the induction and synchronization of estrous and enhancement of fertility rate in the intensively managed cross-bred dairy cows.

MATERIALS AND METHODS

Study area

This study was conducted at SEMEX project for artificial insemination Hillat Kuku, Khartoum North, Sudan. It lies at geographical coordinates of 15 to 36°N latitude, 32 to 33°E longitude and a 380 m altitude. Maximum temperature ranges between 41 to 48°C and minimum is 10 to 15°C during winter. The temperature ranges as maximum during summer season. While a minimum air temperature is reported during winter season, with highest relative humidity during autumn as 78% with average rainfall in the same season as 770 mm. A series of experimental tests were carried out that is, the animals were subjected to rectal examination to eliminate pregnant cows.

Study animals

A total of thirty (30) cows were selected irrespective of age, milk yield, parity, and no infection with post-partum sub estrous diseases. Non-pregnant cross-bred with exotic blood ranging between 50 to 75%, apparently clinically healthy, were randomly selected from a herd ranging between 200 to 500 cattle. Pretreatment rectal palpation of reproductive system to determine cyclicity and non-cyclicity by the presence of mature CL, reproductive tract abnormalities and pregnancy were done.

Study design

Cows were divided into three treatment groups; 10 each as follows:

Group 1: In which cows were given 2 ml (25 mg) intramuscular injections of prostaglandin F_{2α} (PGF_{2α}) 11 days apart, then inseminated in the presence or absence of estrous and again after 12 hours.

Group 2: The second group was injected with 2.5 ml (75 µg gonadotropin (GnRH) alone then inseminated in the presence or absence of estrous and again after 12 hours.

Group 3: The third group was injected with 2 ml (25 mg) PGF_{2α} then GnRH 2.5 ml of 75 µg after 9 days followed by the second injection of PGF_{2α} on day 11 and then inseminated in the presence or absence of estrous and again after 12 hours. These treatments aimed at inducing and synchronizing estrous as the basis for insemination.

Heat detection

A painstaking observation three times a day was followed to detect estrous (early morning, late evening and mid night).

Insemination

Animals were blanket inseminated whether they exhibited signs of estrous or not. Insemination was repeated again after 6 to 12 hours from the first one.

Pregnancy diagnosis

Pregnancy diagnosis was determined by non-return rate to estrous after 24 days, followed by rectal palpation 60 days after insemination.

Animals feeding

Animals were fed on cut and carry system of green fodders comprising mainly sorghum bicolor, Abu 70, plus concentrated mixture of crushed sorghum grains, cotton seed, concentrate and wheat bran (Table 1). Drinking water was supplied *ad libitum*.

Data analysis

Descriptive statistics in form of frequencies means and proportions, and Student's Test (t-test) were used for data analysis. Level of significant differences was made at $p < 0.05$.

Table 1. Ration used for feeding animals during the experiment.

Feed type	Percentage (%)
Sorghum	40
Abu 70	30
Cotton seed	13
Wheat bran	12
Concentrate	5
Total	100

RESULTS AND DISCUSSION

Table 2 shows that the three hormonal protocols induced and synchronized estrous in all animals under the study (100%). Time of response to estrous was almost synchronous in all the treated groups with animals under protocol 3 (GnRH + PGF_{2α}) responded earlier than the other two protocols (Mean $\pm$ SE 46.2 $\pm$ 3.46 hrs). The same protocol also resulted to a significant ($p < 0.05$) pregnancy rate (70%) following pregnancy diagnosis 60 days post-insemination. The recent adoption of farmers to reproductive technologies in the Sudan and their perception to fertility of the cross bred animals, cannot be enhanced without a serious scrutiny. The fact is that farmers embrace negative perceptions about estrous synchronization and AI in the Sudan. This is in line with similar report of Legesse (2015). As such, dissemination of results obtained in this study among the stakeholders may help mitigate such concept and push toward adoption of reproductive technologies among farmers as a strong base for genetic improvement and increase production and productivity of cross-bred animals.

Not surprisingly, fertility is the consequence of many factors or combination of one or two factors under tropical conditions, and there were considerable difficulties encountered by investigators in determining the importance of dietary requirements for reproduction, effect of breed on reproductive traits, and estrous cycle as a sequence of physiological events under hormonal control which is a part of reproductive chain and they do significantly affect the pregnancy rate of all types of breed. In the present study, despite the fact that the dairy cows were well-nourished and kept under an intensive system of management, they exhibited a marked variation in the pregnancy occurrence. However, hormones can improve estrous synchronization in all types of dairy cows' breeds and enhance conception rate (Macmillan, 1978).

Over the past several decades strong efforts have been made across the world to eliminate the need of heat detection in cattle (Bihon and Assefa, 2021), using different hormonal combinations and to breed the females either according to estrous or by fixed time AI. In pursuit of this concept, the present study has revealed encouraging and promising results in estrous response and synchrony in addition to conception rates.

Table 2. Response to synchronizing protocol and pregnancy rate after insemination (N = 30).

Synchronizing Protocol	Cows	Response (100%)	Time of response (hours)	Pregnancy occurrence (-ve %)	Pregnancy occurrence (+ve %)	T-test
PG F2 α (N=10)	10	100%	48-75 (55.0 $\pm$ 06)	50	50	NS
GnRH (N=10)	10	100%	52-96 (68.8 $\pm$ 1.24)	50	50	NS
GnRH +PGF2 α (N=10)	10	100%	36-72 (46.2 $\pm$ 3.46)	30	70	*

NS = Not significant; * = significant at $P < 0.05$; SE = Standard Error.

Troxel et al. (2012) reported that combination of two hormones may induce better cyclicity in cows experiencing postpartum anestrus. This is in line with the findings of this study, where combination of two hormones revealed better synchronous estrous and conception rate (70%) than that of the single hormone injections (50%). These three protocols almost rested in compacted time of response to estrous ranging from 46 to 68 hours which was shorter than that reported by Chanyalew et al. (2018) in Ethiopia.

The optimal time for insemination considered by Dorsey et al. (2011) is 4 to 12 hours after estrous detection which concurred with this study in which insemination was done at detection of estrous zero hour and the second insemination 12 hours after estrous detection (0 to 12 hours). Moreover, Legesse (2015) and Jemal et al. (2016) reported the achievement of high pregnancy rate after inseminating animals from 7 to 12 hours and 10 to 20 hours, respectively after estrous.

Prostaglandin is one of the reproductive hormones used for treatment of some reproductive disorders and synchronization of estrous cycles as well as Follicular Stimulating Hormone (FSH), Pregnant Mare Serum Gonadotropin (PMSG) and progesterone or combination of them (Horton and Poyser, 1976). This finding agrees with Zhil et al. (1987) who found that 84.45% of cows treated with prostaglandin had increases in conception rate. Several reports have revealed a higher rate of estrous synchronization and 80% when PGF2 α is combined with GnRH than when prostaglandins are used alone (50%) (Johnson and Stevenson, 2003). In contrast, the reports did not agree with this study where all the hormonal regimes provided 100% response to estrous. Seemingly, it could be due to improved management especially flush feeding before breeding during the time of trial. However, the result is in line with that revealed by Small et al. (2001) and Zeuh et al. (2014) who reported estrous response rate of 94.7%.

It is observed that prostaglandins alone either as a single or double dose schedule have been used for a long time for estrous synchronization in cattle (Kristula et al., 1992) with varying results of estrous and conception. Its use in this study revealed 50% pregnancy rate lower than similar treatment in Ethiopia by Chanyalew et al. (2018), who reported high pregnancy rate of 63.1%. Ostensibly, the discrepancy may be due to some factors responsible for early embryonic death as pregnancy diagnosis was done

on day 60 post-insemination. It seems that hot summer temperature during the study could be one of the key factors. The use of GnRH in this study gave a synchronous estrous with 100% response and somewhat prolonged time for response in estrous (68.8 hours.). In this study the lower conception rate (50%) may be attributed to the low single dose administered, which did not concur with the interpretations of Fricke et al. (1998), Hall et al. (2002) and Yamada et al. (2003) who argued that the reproductive performance of dairy cows is not affected when the dose of GnRH is reduced.

Conclusion

The three protocols revealed a synchronous estrous and acceptable pregnancy rate. However, combination of hormones provided better pregnancy rate compared to a single used hormone. Hence, the use of combination of two hormones for induction and synchronization of estrous is recommended to provide promising pregnancy rate.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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