THE UNIVERSITY OF ZAMBIA

OESTRUS RESPONSE OF COWS TREATED WITH VARIOUS DOSES OF PROSTAGLANDIN $F_{2\alpha}$

BY

SIBESO I. KATUNDU

UNZA, LUSAKA AUGUST, 2014

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A RESEARCH PROJECT REPORT SUBMITTED TO THE SCHOOL OF AGRICULTURAL SCIENCES IN PARTIAL FUFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF AGRICULTURAL SCIENCES DEGREE

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SUPERVISED BY DR. P.C. SIANANGAMA

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ABSTRACT

The oestrus response to various doses of Prostaglandin $F_{2\alpha}$ in dairy cows was investigated to determine the effectiveness of doses lower than the recommended dose. Prostaglandin analogues are used in reproductive management to induce oestrus in cows. They also have pharmacological application in the correction of a number of reproductive disorders in cows. While prescription of a therapeutic dose is expected for the correction of reproductive disorders, the dose for inducing oestrus and associated ovulation is expected to be comparatively lower. However, the current dose recommendation prescribes a blanket application rate of the hormone for all these purposes. The implication of this is the use of pharmacological doses of Prostaglandin $F_{2\alpha}$ where physiological dose should surfice. Consequently, costs that would otherwise be eliminated through the use of a minimum required dose are incurred. In an experiment with the objective of lowering the Prostaglandin $F_{2\alpha}$ dose used in oestrus synchronization, the effectiveness of lower doses in inducing oestrus in dairy cows was investigated. Four (4) Friesian cows were randomly allocated to receive either 2.0 mL (recommended dose and positive control), 1.5 mL, 1.0 mL or 0.5 mL of Alfaglandin, an analogue of Prostaglandin $F_{2\alpha}$, given intramuscularly on day seven of the oestrous cycle. There were no differences in response to various doses of Alfaglandin administered ($\chi^2 = 3.1$, df = 3, $P = 0.4$). There was 100% oestrus response among cows treated with 2.0 or 0.5 mL, 50% at 1.5 mL, and 66.7% at 1.0 mL. All the cows that responded positively to treatment did so within a period of 2.5–3.5 days ($P>0.05$). In conclusion, lower doses of Prostaglandin $F_{2\alpha}$ were just as effective as those recommended for oestrus synchronization in cows. Additionally, the time taken to oestrus was unaffected by the dose of Prostaglandin $F_{2\alpha}$ administered.

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DEDICATION

This research project report is dedicated to my mother Sandra Etambuyu Mushiba Katundu. Words are not enough to express my gratitude to you. You have made sacrifices as far back as I can remember, enabling me to pursue my education and get to where I am today.I'll forever be grateful to you.

CHAPTER 1

1.0. INTRODUCTION

Oestrus is a period that a female mammal exhibits sexual desire and acceptance of the male by standing to be mounted. Oestrus constitutes a phase of the oestrous cycle which takes an average of 21 days in cattle.

It starts with the generation of follicles under the stimulus of follicle stimulating hormone (FSH). These follicles undergo selection, from which the selected go on to grow. The follicles produce oestradiol 17β (E_2) in increasing amounts as they grow. Among these follicles, a few from each ovary continue developing and increasing in size and ultimately one mature, dominant follicle, referred to as a graafian follicle ovulates. Oestrus precedes ovulation which is triggered by a surge in Luteinising Hormone (LH) that is in turn stimulated by the high levels of E_2 produced by the follicles. Oestrus lasts for up to 18 hours before ovulation occurs.

The corpus luteum (CL) is a transient gland that is produced following the rupture of the ovulated graafian follicle. This serves the purpose of Progesterone (P_4) production which is necessary for maintenance of pregnancy in the event that the animal is successfully bred. In the absence of pregnancy, the CL regresses around the 16^{th} day of the oestrous cycle, in order to restart the cycle.

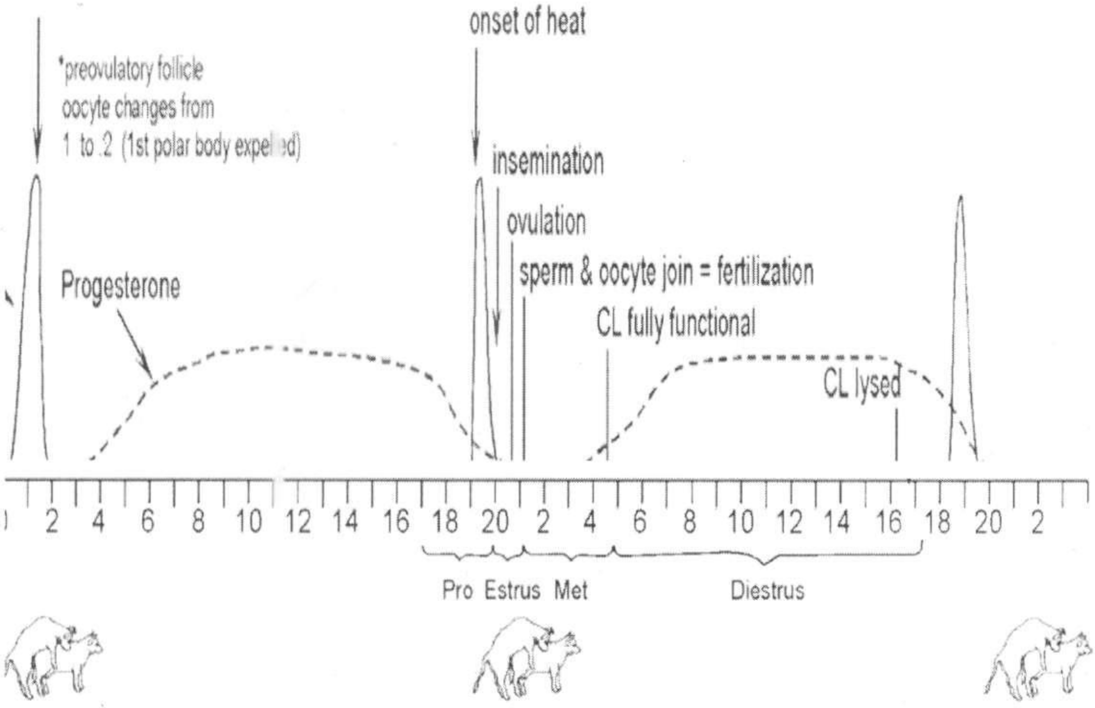

Figure 1. Schematic s ages of the oestrous cycle, serum progesterone concentrations and serum luteinizing horn one concerntrations. *Image taken from Larson and Randle, 2008*

Knowledge of the oestrous cycle is essential to successful reproductive management of cattle herds. This has practical application in animal reproduction and more importantly in artificial insemination (AI). Artificial insemination is a technique that employs artificial means of depositing spermatozoa from a male animal, in this case a bull, into the female reproductive tract, in this case a cow, for the purpose of fertilisation and consequently pregnancy. A vast array of benefits comes from the use of AI such as introduction of genetic superiority into herds, reduction in venereal disease transmission, economic utilisation of sperm and the elimination of requirement to keep bulls all year round, applicable in particular to the dairy enterprise.

Artificial insemination is implemented timely to coincide with ovulation in order to maximise conception rates. Oestrus, therefore, serves as an important factor for this exercise. In fact, oestrus detection has been cited as one of the most important factors affecting the reproductive success of AI programmes (Raja *et al.*, 2001). The signs of oestrus range from sniffing the rump of other animals, mucous discharge from the vulva, bellowing, restlessness, trying to mount other animals to the most significant, standing to be mounted.

Most commercial farms or ranches, however, will not utilise AI technology unless this investment can be confined to a period of less than five to seven days because of the substantial investment of time and labour involved (Holm *et al.*, 2008).

Oestrus synchronization has been proposed as a means of reducing the labour cost associated with AI by concentrating the labour utilisation into brief periods (Holm *et al.*, 2008).

Oestrus synchronization is the manipulation of the oestrous cycle so that female animals can be bred around the same time. It also gives many cattle producers the opportunity to capture the economic benefits of AI.

There are basically two principles of controlling oestrus and ovulation in cattle. The first principle is to prolong the lifespan of the CL, thus delaying oestrus, and the second principle is to shorten the lifespan of the CL in order to hasten the onset of oestrus. These principles are implemented as protocols individually or incorporated into protocols combining both principles. Some examples are the Ovsynch (GnRH given 7 days before $PGF_{2\alpha}$ and then GnRH again 48 hours later), Cosynch (GnRH given 7 days before $PGF_{2\alpha}$

and then GnRH again 48–64 hours later), single shot (given between days 6 to 15 of the oestrous cycle) or double shot (10 to 14 days apart during the luteal phase) Prostaglandin $F_{2\alpha}$ protocols.

The following are some of the brand names and mechanisms of commercially available hormones used to synchronize oestrous (Adapted from Larson and Randle, 2008):

Cystorelin®, Factrel®, and Fertagyl®, are the equivalent of Gonadotrophin Releasing Hormone (GnRH). These act to increase the release of FSH and LH from the anterior pituitary gland. This in turn triggers follicular development and ovulation of most dominant or largest of the growing follicles and the subsequent luteinisation of the resultant structure.

Pregnant Mare Serum Gonadotrophin (PMSG), also known as Equine Chorionic Gonadotrophin (eCG is produced by the deciduate cells of the equine placenta. It exhibits predominantly FSH activity but also some LH activity, and is used in the superovulation of cattle.

Human Chorionic Gonadotrophin (hCG) is produced by chorionic epithelial cells of the human placenta. This hormone exhibits predominantly LH activity but also some FSH activity, and is used in the superovulation of cattle.

Progesterone (P_4) is administered in a Controlled- Intravaginal Drug Release (CIDR) device, releasing P_4 over period of time after which it is removed. The P_4 from the device acts like natural P_4 from the CL preventing increase in blood LH levels until removal of the device. This prevents the increase in LH and E_2 both of which are necessary for the final maturation of the ovulatory follicle, ovulation, and oestrus.

Melengestrol Acetate (MGA®) is a synthetic progestogen. When administered in the feed at 0.5 mg / head / day, this synthetic progestogen mimics progesterone produced naturally by the CL. For as long as the progestogen is present in the blood at a minimum level, it prevents the spike in LH necessary to initiate ovulation and also prevents E_2 concentrations increasing to the level necessary to initiate oestrus behaviour.

Lutalyse®, Estrumate®, ProstaMate® and InSynch® are Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or analogues of naturally occurring $PGF_{2\alpha}$. They function by regressing the CL of actively cycling females and thus decrease the blood P_4 levels and subsequently return the cow to oestrus. They are among the most commonly used methods of synchronization (Lamb *et al.*, 2010) and have been indicated for both reproductive biotechniques and the treatment of reproductive disorders (Mokhtari *et al.*, 2011).

Alfaglandin (cloprostenol sodium) is a synthetic $PGF_{2\alpha}$ analogue. Each ml of the aqueous solution contains 0.2 mg of cloprostenol sodium. It also contains sodium chloride, sodium citrate, citric acid, chlorocresol, and sodium hydroxide in fewer amounts. Alfaglandin causes functional and morphological regression of the CL in cattle. In normal, non-pregnant cycling cows, oestrus results in two to three days following injection.

Alfaglandin is highly effective in certain applications animals such as:

i. Non detectable oestrus (NDO) is a condition where an animal does not show signs of oestrus but has normal ovarian cyclicity. This makes heat detection and consequently AI difficult to conduct. Alfaglandin corrects this.
ii. Parturition can be induced with Alfaglandin in the period around normal term.
iii. Normal pregnancy can be terminated in cattle one week after conception to about 150 days of gestation, provided the pregnancy is still dependant on the CL for its maintenance.
iv. Alfaglandin can be used to expel a mummified foetus which can be removed via the vagina and allow the animal to resume normal cycling.
v. Chorionic endometritis (pyometria) can successfully be treated with Alfaglandin.
vi. Alfaglandin has proved effective in correcting cystic ovaries associated with persistent luteal tissue and absence of oestrus.

The use of a hormone with pharmaceutical properties as has been illustrated above brings up the question of whether or not the same dose is appropriate for use in management of a physiological process. From a physiological point of view, the dose that is required to synchronize oestrus must be less than that required for the treatment of the aforementioned conditions. The implication of this is that costs are reduced by spreading the recommended dose over a relatively larger number of cows. This would be translated into higher economic profits for the farmer and an added benefit of employing synchronization of oestrus and AI technology as a whole.

1.1 AIM:

To reduce the cost of prostaglandin $F_{2\alpha}$–based oestrus synchronization in dairy cows.

1.2 OBJECTIVE:

To establish the oestrus response of dairy cows treated with various doses of Prostaglandin $F_{2\alpha}$

1.3 HYPOTHESES:

Research hypothesis: Reducing the dose of $PGF_{2\alpha}$ administered to dairy cows does not alter oestrus response.

Statistical hypothesis:

Ho : Doses lower than 2 mL of $PGF_{2\alpha}$ are not effective in inducing oestrus in dairy cows.

HA : Doses lower than 2 mL of $PGF_{2\alpha}$ are effective in inducing oestrus in dairy cows.

CHAPTER 2

2.1 LITERATURE REVIEW

2.1.1 Economic benefit of synchronization

Economic returns from a properly managed average synchronization programme can more than pay for its cost (De Jarnette, 2004). Beef cows that conceive early in the breeding season produce calves that weigh more at weaning simply because they are older. At 0.9 Kg/day of calf growth×K30/Kg, each day a calf is older at weaning means an additional K27 in the farmer's pocket. A calf that is conceived one week earlier is worth K189/head more in calf weaning weight. A calf conceived on the first of a 60-day breeding season will be worth K1,620 more than one conceived on the last day. Through the use of lower doses of the hormones for oestrus synchronization, economic returns like these would be even higher than is currently being realised.

The cost of acquiring GnRH that is used in the Ovsynch protocol was successfully cut down through the use of a dose lower than recommended (Fricke *et al.*, 1998). A lower dose of GnRH for synchronization was justified since the same dose of GnRH is also used in the treatment of cystic follicles. This was concluded in a study with primiparous and multiparous lactating Holstein cows (n= 237) assigned to one of two treatment groups. Ovulation was synchronized for cows in the first group using IM injections of GnRH and $PGF_{2\alpha}$ as follows: Day 0, 100 μg GnRH; Day 7, 25 mg $PGF_{2\alpha}$; Day 9, 100 μg GnRH. Ovulation was synchronised in the second group of cows using the same injection schedule and dosage of $PGF_{2\alpha}$ but only 50 μg GnRH per injection. Synchronization rates yielded for both groups were 84%. Decreasing the dose of GnRH to 50% of the recommended dose therefore, reduces synchronization costs per cow and per pregnancy without compromising the efficacy of the protocol.

2.1.2 Alternative routes of $PGF_{2\alpha}$ administration

Various routes of administration of $PGF_{2\alpha}$ have been used in an effort to reduce the dose of $PGF_{2\alpha}$ given to cows for synchronization of oestrus and ovulation (Rovani *et al.*, 2012). Research carried out in Brazil evaluated the effectiveness of lower than recommended doses of $PGF_{2\alpha}$ administered via the intravulvosubmucosal (IVSM) route in oestrus behaviour (Rovani *et al.*, 2012). Cycling, non-lactating beef cows (*Bos taurus taurus*) were used to validate the efficiency of 5 mg (one fifth of the standard dose) of dinoprost tromethamine under the brand name Lutalyse®. Beef cows (n=1937) were synchronized with the 10-day, 1-injection management system (Donaldson *et al.*, 1982). Briefly, oestrus detection was performed twice a day, at 12 hour intervals for five days, which was considered as the control period. In the morning of day five, only the cows that were not considered to be in oestrus (n= 1440) received a dose of 5 mg of dinoprost (IVSM).

The rate of detection of oestrus in cows over the first five days (control) was 25.6% (497 out of 1937). By five days after the injection of 5 mg dinoprost via IVSM route, 68.2% of cows were considered to be in oestrus (983 out of 1440).

In another aspect of the same experiment to compare the effectiveness of the intramuscular (IM) route to the IVSM route in oestrus behaviour, non-pregnant cyclic beef heifers (n=251; 2[illegible]–30 months old) were observed for oestrus behaviour twice a day for five days. The animals considered not to be in oestrus during the first five days received 5 mg of dinoprost and were randomly placed in either the IM group (n=95) or the IVSM group (n=97). Oestrus behaviour was observed and recorded for five days.

At the end of five days, 23.5% of cows were detected to be in oestrus. Treatment with 5 mg of dinoprost via IM or IVSM route resulted in 54.7% and 47.4% of cows being in oestrus, respectively, during the five days after PGF_{2a} administration.

Therefore, the implication of the findings of this research is that the IVSM route of administration of $PGF_{2\alpha}$ is effective in inducing oestrus behaviour since there was no significant difference in effectiveness of the route of administration of $PGF_{2\alpha}$ between IM and IVSM routes.

2.1.3 Work with other hormones

The use of doses of hCG in excess of 3 ml was dispelled through earlier research.Subsequent work with reduced doses has demonstrated the efficacy of the reduced doses. (Rajamahendran and Sianangama, 1992) reported ovulation of the large follicles in cows consequence to injection with 1.0 mL of hCG. A random selection of 18 lactating and regularly cycling cows (2½ to 5 years old) 60 to 90 days postpartum were assigned at standing oestrus, to receive treatments of no hCG for the control and 1.0 mL hCG to the remainder of the cows. This was given intramuscularly on day seven of the oestrous cycle. The choice of the day was in accordance with previous studies that ascertained that the dominant follicle attains maximum diameter between day seven and day eight of the oestrous cycle (Taylor and Rajamahendran, 1990) and that it possesses more hormone receptors than any other follicle in the luteal phase (Ireland and Roche, 1983). Ovulations induced by hCG were verified, as defined to be the complete disappearance of large follicles that were present at the time of oestrus administration and the subsequent emergence of a luteal structure on a site previously occupied by the disappeared follicles. Administration of lower than recommended doses of hCG on day seven of the oestrus cycle, therefore, resulted in the ovulation of large follicles.

Similarly, (Buttrey *et al*. 2006) observed that 1.0 mL of hCG was effective in inducing ovulation in dairy cows. This was based on the findings of a study performed to compare the effectiveness of hCG to GnRH in inducing ovulation. Cows were assigned randomly to treatments of saline, 100 μg of GnRH or 0.5, 1.0, 2.0, or 3.0 mL of hCG. Based on diameter of ovarian structures, it was observed that a dose of at least 1 mL of hCG resulted in a greater ovulatory response than saline, GnRH or 0.5 mL of hCG.

CHAPTER 3

3.1 MATERIALS AND METHODS

The research was conducted at the University of Zambia (UNZA) Department of Animal Science Research Field Station.

3.1.1 Selection of animals:

The animals were palpated per rectum (Pregnancy Diagnosis Gloves; Kruuse Limited, Denmark) and then followed up using an ultrasound scanner (Chison 600VET, Veterinary Ultrasound Scanner; Chison Veterinary Imaging, China) to determine the reproductive state of the cows. Only the animals found to be open and actively cycling were included in the research. This amounted to a total of four Friesian cows each one of which was reused.

3.1.2 Weighing of animals:

All the cows selected for the research were weighed at the start of the experiment (Weight Tape Rondo, Kruuse Limited, Denmark). The range of weights of the cows was 482 Kg–553 Kg

3.1.3 Oestrus synchronization:

The cows selected were synchronized for the experiment with 2 mL of commercially acquired Estrumate® (Schering- Plough Animal Health, Germany) to gain control of the time frame of the treatments. All injections were administered via the IM route into the gluteal tissue (18-gage 3.2 cm needle, 5 ml syringe; Kruuse Limited, Denmark). The day of observed oestrus following synchronization was counted as day 0. Commercially acquired Alfaglandin (0.25 mg cloprostenol sodium; Alfasan Nederland BV, The Netherlands) was given at the respective treatments on day seven after observed oestrus. The choice of day seven was based on earlier research ascertaining that the dominant follicle in the first wave of follicular growth is capable of ovulating when luteolysis is initiated by day seven of the oestrous cycle (Kastelic *et al.*, 1990). This implies that the follicle has the morphological and functional maturity (Taylor and Rajamahendran, 1990), to respond to an injection of $PGF_{2\alpha}$ and regress to return the cow to oestrus.

Each cow was randomly allocated to receive treatments of 2.0 mL, 1.5 mL, 1.0 mL or 0.5 mL of Alfaglandin.

Checking for oestrus following treatment was done for five days by an experienced herdsman. The signs of oestrus considered were trying to mount others or standing to be mounted.

For the duration of the research, the cows were fed on a diet of natural veldt and supplements provided at milking time.

The variable of interest was the response of the cows at each dose administered.

Oestrus response was analysed using Chi-square in Statistical Analysis Software (SAS) package (2004, SAS Institute Incorporated, NC, USA, version 9.1.3)

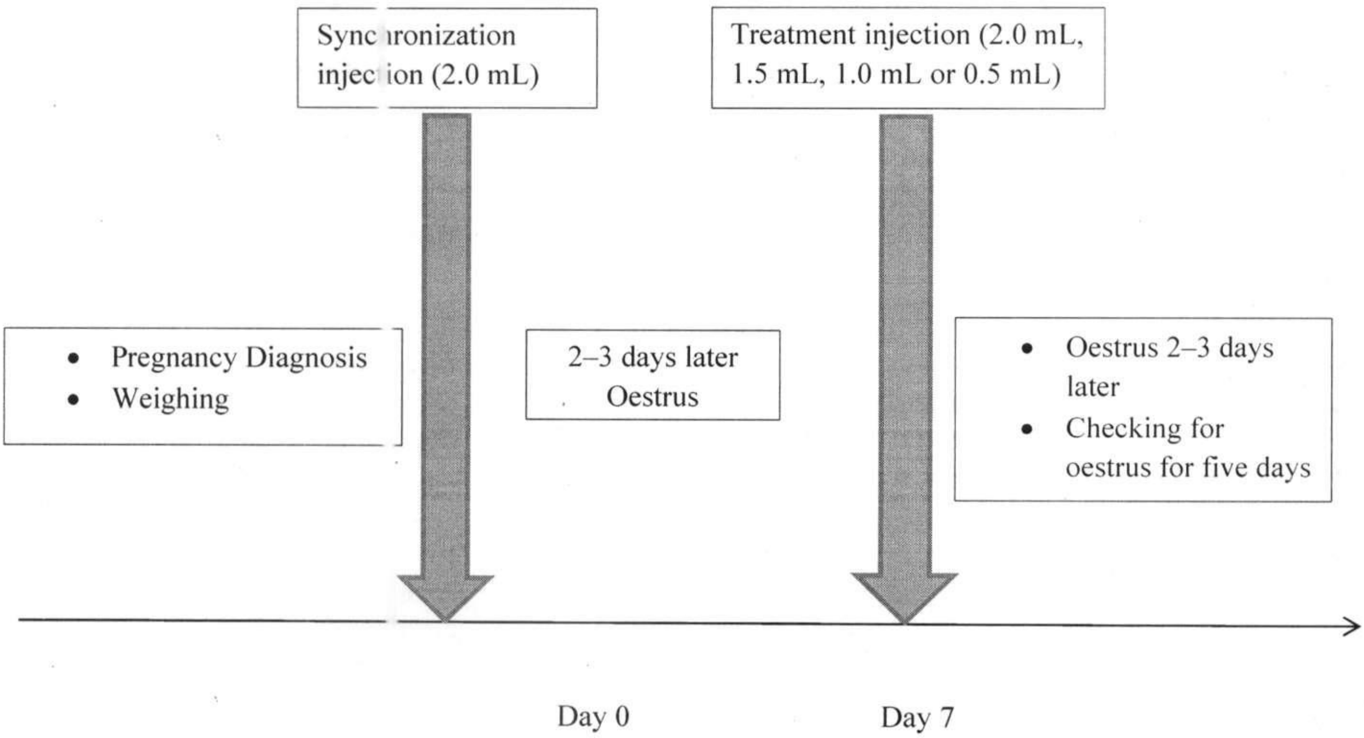

Figure 2. Summary of procedure of oestrus synchronization of dairy cows treated with doses of $PGF_{2\alpha}$ lower than the recommended (2.0 mL) given on day seven of the oestrus cycle.

CHAPTER 4

4.1 RESULTS

Table 1. Treatment sc edule and dates of response of dairy cows treated with 0.5 mL, 1.0 mL, 1.5 n L or 2.0 mL of $PGF_{2\alpha}$ given on day seven of the oestrous cycle.

Cow Number	Date treated	Dose (mL)	Date of oestrus	Days taken to oestrus
1	20/06/2014	1.0	23/06/2014	3.0
2	29/06/2014	0.5	04/07/2014	5.0
3	09/07/2014	0.5	11/07/2014	2.0
4	09/07/2014	2.0	11/07/2014	2.0
5	11/07/2014	1.5	–	–
6	11/07/2014	2.0	14/07/2014	3.0
7	19/07/2014	1.5	23/07/2014	4.0
8	19/07/2014	1.5	23/07/2014	4.0
9	21/07/2014	1.5	–	–
10	21/07/2014	1.0	24/07/2014	3.0
11	31/07/2014	0.5	02/08/2014	2.0
12	31/07/2014	1.0	–	–

Table 2. The oestrus response of cows treated with 0.5 mL, 1.0 mL, 1.5 mL or 2.0 mL of $PGF_{2\alpha}$ given on day seven of the oestrus cycle.

Dose (mL)	Active ingredient delivered (mg)	Number of cows treated (n)	Oestrus response (%)	Interval taken to oestrus (Days)
0.50	0.125	3	100.00	3.00
1.00	0.250	3	66.67	3.00
1.50	0.375	4	50.00	3.50
2.00	0.500	2	100.00	2.50

There were no significant differences between oestrus response of cows treated with the recommended dose of 2.0 mL and the lower doses of 1.5 mL, 1.0 mL or 0.5 mL ($\chi^2 = 3.1$, df = 3, $P = 0.3748$). However, it was observed that one cow failed to respond to any of the doses of $PGF_{2\alpha}$ lower (1.0 and 1.5 mL) than the recommended 2.0 mL to which it responded positively. The time taken to oestrus was found to be statistically the not different ($P>0.05$) at all the doses of $PGF_{2\alpha}$ administered, including the recommended dose of 2.0 mL.

On the basis of these results, the null hypothesis "Doses lower than 2.0 mL of $PGF_{2\alpha}$ are not effective in inducing oestrus in dairy cows" is therefore rejected and the alternate hypothesis "Doses lower than 2.0 mL of $PGF_{2\alpha}$ are effective in inducing oestrus in dairy cows" is accepted.

Similarly, research with Ongole cows yielded about 81% oestrus rates consequence to double injection of 5 mL Lutalyse (25 mg dinoprost tromethamine), given intramuscularly. The current study utilised 2 mL and less (0.5 mg cloprostenol sodium and below) but yielded similar oestrus rates.

Furthermore, oestrus rates of 54.7% were reported in non-lactating beef cows given 5 mg dinoprost tromethamine (one fifth of the recommended dose) via IM route (Rovani *et al.*, 2012). The current study however, utilised less doses (0.5 mg cloprostenol sodium and below) but obtained higher oestrus response of up to 100%. The difference in response, may again be attributed to the choice of day seven of the oestrous cycle for administration of $PGF_{2\alpha}$.

The results of the current study are much better compared to contemporaries partly due to the day of the oestrous cycle when $PGF_{2\alpha}$ was administered. In order to assess the effectiveness of lower doses in inducing oestrus in dairy cows, a specific period during the oestrous cycle was chosen. The seventh day of the oestrus cycle is hypothesised as the day providing the most optimum conditions for the dominant follicle of the first follicular wave to ovulate. This is in accordance with earlier studies ascertaining that the dominant follicle in the first wave of follicular growth is capable of ovulating when luteolysis is initiated by day seven of the oestrous cycle (Kastelic *et al.*, 1990). Furthermore, at this time, the follicle has morphological and functional maturity (Taylor and Rajamahendran, 1990).

CHAPTER 6

6.1 CONCLUSION

On the basis of these results, there are indications that the administration of lower doses of $PGF_{2\alpha}$ is as effective as the use of the recommended dose in inducing oestrus in dairy cows. Additionally, the time taken to oestrus is unaffected by the dose of $PGF_{2\alpha}$ administered. These results also point to potential application of the findings in an embryo transfer programme for synchronizing donor cows after embryo collection has been undertaken.

6.2 RECOMMENDATIONS

This study used a very limited number of animals, therefore, an extension of the study is recommended in order to add to the sample size for the generation of more conclusive results.

Different days of the oestrous cycle may be used in future research to demonstrate the efficiency of giving $PGF_{2\alpha}$ on day seven of the oestrous cycle.

Future research can incorporate radioimmunoassay (RIA) and ultrasonography into data collection for an in-depth analysis of the response at the hormonal profiles level and of the internal reproductive tract, respectively.

An economic analysis of the additional income realised from the use of lower doses in oestrus synchronization is recommended. This would ultimately reveal the economic benefit of the use of lower doses than the recommended dose of $PGF_{2\alpha}$ in the synchronization of oestrus

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APPENDIX 1

Table 3. Frequency distribution of response of dairy cows treated with 0.5 mL, 1 mL, 1.5mL or 2 mL of $PGF_{2\alpha}$ given on day seven of the oestrus cycle.

Dose Frequency Percent Row percent Column percent	Response		
	No	Yes	Total
0.50	0.00 0.00 0.00 0.00	3.00 25.00 0.00 33.33	3.00 25.00
1.00	1.00 8.33 33.33 33.33	2.00 16.67 66.67 22.22	3.00 25.00
1.50	2.00 16.67 50.00 66.67	2.00 16.67 50.00 22.22	4.00 33.33
2.00	0.00 0.00 0.00 0.00	2.00 16.67 100.00 22.22	2.00 16.67
Total	3.00 25.00	9.00 75.00	12.00 100.00

APPENDIX 2

Table 4. Chi-Square analysis of the oestrus response of dairy cows treated with 0.5 mL, 1.0 mL, 1.5 mL or 2.0 mL of $PGF_{2\alpha}$ given on day seven of the oestrus cycle.

Statistic	DF	Value	Probability
Chi-square	3	3.1111	0.3748
Likelihood Ratio Chi-Square	3	4.1318	0.2476
Mantel-Haenszel Chi-Square	1	0.2129	0.6445
Phi Coefficient		0.5092	
Contingency Coefficient		0.4537	
Cramer's V		0.5092	