MAKERERE UNIVERSITY

EVALUATION OF THREE ESTRUS SYNCHRONIZATION PROTOCOLS AND CONCEPTION AMONG DAIRY CATTLE AT NJERU STOCK FARM, UGANDA

BY

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DECLARATION

I, **Jumah Magambo,** declare that this work is original and has not been submitted to any higher institution of learning for any award.

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DEDICATION

I dedicate this dissertation to my beloved family, whose unwavering love, encouragement, and patience have sustained me throughout this academic journey. Your belief in my potential has been my greatest source of strength.

I also dedicate this work to my colleagues and workmates, whose support, collaboration, and constant motivation contributed immensely to the successful completion of this study.

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LIST OF ABBREVIATIONS

Abbreviation	**:**	**Meaning**
AI	:	Artificial Insemination
BCS	:	Body Condition Score
CL	:	Corpus Luteum
COVAB	:	College of Veterinary Medicine, Animal Resources and Biosecurity
DB	:	Data Bank
df	:	Degrees of Freedom
et al	:	and others
FSH	:	Follicle Stimulating Hormone
FTAI	:	Fixed Time Artificial Insemination
GnRH	:	Gonadotropin-Releasing Hormone
HPO	:	Hypothalamus-pituitary-ovarian
LH	:	Luteinising Hormone
MAAIF	:	Ministry of Agriculture, Animal Industry and Fisheries
NAGRC & DB	:	National Animal Genetic Resources Center and Data Bank
NEB	:	Negative energy balance
$PGF_{2\alpha}$	:	Prostaglandin F2 Alpha
PRID	:	Progesterone Releasing Intra Vaginal Device
PRID DELTA	:	A commercial brand of progesterone-releasing intravaginal device.
TAI	:	Timed Artificial Insemination
X^2	:	Chi-Square Statistic

ABSTRACT

Reproductive performance is still one of the most important factors that influence the profitability of a dairy herd, but there is a lot of poor reproductive performance in elite dairy herds. In extensive pasture-grazed systems, low conception and delayed breeding are mainly due to unobserved heats and variable ovarian cyclicity; therefore, structured hormonal oestrous synchronization protocols are essential to evaluate the time when heats occur and to maximize conception. This study aimed to assess and compare the efficiency of the single-agent and multi-agent hormonal synchronization on the onset of oestrous and conception in dairy cattle at Njeru Stock Farm, Uganda. An experimental study design was implemented using 27 (Holstein-Friesian) and 27 (Jersey) dairy cows that were divided into three treatment groups of 18 each: Treatment Protocol 1 (T1: $PGF_{2\alpha}$ alone), Treatment Protocol 2 (T2: GnRH + $PGF_{2\alpha}$), and Treatment Protocol 3 (T3: PRID + GnRH + $PGF_{2\alpha}$). Treatment Protocol 3 gave a significantly higher oestrous response (83.3% (15/18 cows) than Treatment Protocol 2 (33.3% (6/18 cows)) and Treatment Protocol 1 (22.2% (4/18 cows)) (χ^2= 15.343, df = 2, p = 0.000). The difference in oestrous response between Treatment Protocol 3 and Treatment Protocol 2 was highly significant (p = 0.009) and between Treatment Protocol 3 and Treatment Protocol 1 was highly significant (p = 0.001), while the difference between Treatment Protocol 1 and Treatment Protocol 2 was not significant (p = 1.000). With regard to Day 30 and Day 45 post-AI, Treatment Protocol 3 resulted in a significantly higher conception of 77.8% (14/18 cows) than Treatment Protocol 2, 33.3% (6/18 cows) and Treatment Protocol 1, 22.2% (4/18 cows), with 2 cows in Treatment Protocol 3 conceiving during silent heat (χ^2= 12.600, df = 2, p = 0.002). The pairwise comparisons showed that conception was significantly higher for Treatment Protocol 3 than for Treatment Protocol 2 (at P = 0.024) and Treatment Protocol 1 (at P = 0.003) and that there was no significant difference between Treatment Protocol 1 and 2 (at P = 1.000). The study revealed that multi-agent progesterone-primed synchronization (Treatment Protocol 3) was superior in increasing both oestrous onset synchrony and conception compared to single-agent and two-agent protocols, as it controlled follicular wave dynamics and overcame the limitation of ‘silent heat' during pasture grazing. The study recommends implementing Treatment Protocol 3 (PRID + GnRH + $PGF_{2\alpha}$) along with normal health and nutrition care to achieve maximum reproductive efficiency in dairy cows.

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

The reproductive efficiency of dairy cattle is still the main determinant of profitability in dairy farms all around the world. However, in the last few decades, the breeding ability of top dairy cows has significantly declined (Royal *et al*., 2000; Lucy & Crooker, 2001; Butler, 2003; Softic *et al*., 2020). At the physiological level, the reproductive performance of a cow is the result of a multi-factorial network of interacting biological, environmental and management factors. The hypothalamus-pituitary-ovarian (HPO) axis plays a key role in orchestrating cyclic follicular development, luteolysis and ovulation through well-controlled endocrine signaling mediated by Gonadotropin-Releasing Hormone (GnRH), Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH), progesterone, and Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Crowe *et al*., 2018). But the functional integrity of the HPO axis is highly vulnerable to systemic physiological limitations, most important of which are nutrition, negative energy balance (NEB) in early lactation, metabolic demand of milk production, body condition score (BCS), parity and postpartum uterine health (Walsh *et al*., 2011; Fricke & Wiltbank, 2022). In addition, environmental factors (including increased heat loads and high humidity) reduce gonadotropin release and also influence the expression of behavioural oestrous and management issues (including inaccuracy during visual heat detection, handling facilities and inappropriate timing of insemination) further negatively impact herd conception (Bihon & Assefa, 2021). The key step for addressing the underlying subfertility is not relying on oestrous synchronisation, but adopting a specific reproductive management intervention that will increase the effectiveness of the use of artificial insemination (AI) and eliminate the visual heat detection bottlenecks (Merkelytė *et al*., 2025). This depends on the metabolic/physiological status of the cow and can only be implemented if the energy balance of the cow is not too negative or the feed is of poor quality.

In Sub-Saharan Africa, dairy farming has expanded rapidly, but repeat breeding and prolonged postpartum anestrus remain major impediments for farmers utilizing artificial insemination (AI) (Galukande *et al*., 2013; Mungube *et al*., 2019). These constraints have necessitated the adoption of oestrous synchronization as a reproductive management tool. Rather than replacing natural cyclicity, these hormonal treatments temporarily override spontaneous cycles to induce

luteolysis and coordinate follicular waves, thereby increasing circulating concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) to promote ovulation (Abebe & Alemayehu, 2021). In extensive grazing systems across the region, double-dose Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) protocols have been widely evaluated, showing that grazing cows with a functional corpus luteum respond favorably to luteolytic agents (Adeyina *et al*., 2021). However, the success of these single-agent protocols remains highly variable because they do not control follicular wave dynamics, making multi-agent combinations essential to evaluate reproductive outcomes (Haile *et al*., 2023).

The traditional reproductive management strategies of tropical grazing herds were predominantly based on single-agent luteolytic agents, principally prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Adeyina *et al*., 2021). Single-agent $PGF_{2\alpha}$ protocol physiologically functions only by regressing an active, functional corpus luteum (CL) (luteolysis). But $PGF_{2\alpha}$ has no effect on ovarian follicular wave activity and the stage of dominant follicle development when the injection is given (Noguchi *et al*., 2017). This is a wide, irregular period of time between oestrous and ovulation (48 to 120 hours after the $PGF_{2\alpha}$ treatment) which also makes it difficult to undertake intensive, labour-intensive, and subjective visual heat detection, and makes Fixed-Time Artificial Insemination (FTAI) impractical (Haile *et al*., 2023). In order to rectify these biological constraints, complex multi-agent coordination techniques have been developed to enable systematic control of the luteal regression and follicular wave development.

The multi-agent protocols make use of exogenous gonadotropin-releasing hormone (GnRH) analogues like the multi-agent protocols involve the use of exogenous gonadotropin-releasing hormone (GnRH) analogues that help in synchronizing follicular wave emergence as well as LH surges at ovulation (Morini *et al*., 2019; Tschopp *et al*., 2022) and exogenous progesterone devices for maintaining luteal phase progesterone and preventing premature LH surges and ovulation. Controlled ovulation is achieved through the use of $PGF_{2\alpha}$ (dinoprost tromethamine) that causes regression of the natural and/or GnRH-stimulated corpora lutea. For the $PGF_{2\alpha}$ approach, ovulation can be totally controlled through advanced multi-agent protocols (PRID, GnRH and $PGF_{2\alpha}$) that have not been explored and evaluated for grazing public stock farms in the tropics of Uganda.

Socio-economically, dairy farming constitutes an essential component of the agriculture industry and makes significant contributions to GDP (Gross Domestic Product) and the value of agricultural exports in Uganda (International Growth Centre [IGC], 2024). The National

Animal Genetic Resources Centre and Data Bank under the Animal Breeding Act (2001) is statutorily empowered to spearhead genetic characterisation, multiplication and commercialisation of high-performing animal breeding stocks across the country (Animal Breeding Act, 2001). Importantly, widespread implementation of the national Parish Development Model (PDM) has resulted in a growing demand from the public for high-yielding dairy heifers and good-quality breeding bulls well beyond NAGRC&DB's capacity to provide (NAGRC&DB, 2026). In Uganda, there are plans to scale up Assisted Reproductive Technologies (ARTs) that involve high-quality Artificial Insemination (AI) and mass oestrous synchronization as the most effective way to increase the production of superior local livestock genetics quickly, improve their productivity and reduce this deficit. The purpose of this study was to evaluate oestrous synchronisation and conception in dairy cattle at Njeru Stock Farm, Uganda.

1.2 Statement of the problem

The demand for high-quality heifers in Uganda is high because of the Parish Development Model therefore putting pressure on the National Animal Genetic Resources Centre and Data Bank to produce these high-quality heifers (Mbabazi, 2026). The technique of artificial insemination is utilized to increase genetic improvement (Oketcho, 2025); however, the conception of the reproduction processes of the government multipliers like Njeru Stock Farm is quite low. Traditional single-agent prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) synchronization programs are biologically problematic because single-agent $PGF_{2\alpha}$ will only regress a current corpus luteum and will not recruit or synchronize ovarian follicular waves (Fricke & Wiltbank, 2022). This causes ovulations that are not synchronous and cannot be located with the naked eye, which requires intensive, 24-hour visual heat detection, unfeasible for large grazing paddocks as in Njeru's case 1099 acres (NAGRC&DB, 2024). This translates to a lot of times where insemination is skipped, leading to poor single-service conception, higher services per conception, and longer days open while there are multiple advanced multi-agent synchronisations that can coordinate the follicular waves and luteolysis to maximize conception efficiency for Fixed-Time Artificial Insemination (FTAI); these have not been tested or optimised in high-yielding Friesian and heat-tolerant Jersey breeds under Ugandan pasture-grazed conditions to empirically demonstrate their physiological response, oestrous onset kinetics and conception efficiency. There are no well-designed and robust comparative trials in Uganda that have scientifically and reliably generated heifer synchronization between single-agent and multi-agent protocols for maximizing heifer production and genetic gains.

1.3 Objectives of the study

1.3.1 General Objective

To evaluate and compare the biological efficacy of single-agent ($PGF_{2\alpha}$) and multi-agent (GnRH + $PGF_{2\alpha}$; PRID + GnRH + $PGF_{2\alpha}$) hormonal synchronization protocols in evaluating the proportion of oestrous onset and 45-day post-insemination conception in pasture-grazed Friesian and Jersey dairy cattle at Njeru Stock Farm, Buikwe District, Uganda.

1.3.2 Specific objectives

i. To evaluate and compare the proportion and timing of oestrous onset (observed within a 9-day post-treatment monitoring window) in Friesian and Jersey dairy cows subjected to a single-agent protocol ($PGF_{2\alpha}$ alone) versus multi-agent protocols (GnRH + $PGF_{2\alpha}$ and PRID + GnRH + $PGF_{2\alpha}$) at Njeru Stock Farm.

ii. To determine and compare the 45-day post-insemination conception (confirmed via transrectal ultrasonography at 30 days and amniotic vesicle palpation at 45 days) in Friesian and Jersey dairy cows inseminated under a single-agent protocol ($PGF_{2\alpha}$ alone) versus multi-agent protocols (GnRH + $PGF_{2\alpha}$ and PRID + GnRH + $PGF_{2\alpha}$) at Njeru Stock Farm.

1.4 Research hypotheses

1. H_0: There is no effect on oestrous onset in cows synchronized with a single agent and those synchronized with multi-agent synchronization treatments.

2. H_0: There is no effect on conception in cows synchronized with a single agent and those synchronized with multi-agent synchronization treatments.

1.5 Justification of the study

Dairy farming is one of the key contributors to the overall gross domestic product in the country due to its substantial contribution to agricultural gross domestic product and significant involvement in dairy export earnings. Njeru Stock Farm, located at approximately 1,099 acres in the Buikwe district of Uganda, is directly under the supervision of the National Animal Genetic Resources Centre and Data Bank (NAGRC&DB, 2024).

Most importantly, the biological basis for the use of synchronization protocols needs to be closely related to physiological truths about conception. Hormonal oestrous synchronization

does not automatically lead to increased intrinsic biological fertility or increased conceptions per single service; indeed, oestrous synch cows (particularly those inseminated by fixed time insemination) are often found to have conception rates per insemination service that are similar or slightly lower than those of cows bred by natural spontaneous oestrous (Xu & Burton, 1999; Fricke & Wiltbank, 2022). The real value of synchronization is that it can dramatically increase the percentage of cows that are inseminated in a desired 10- to 21-day period (herd conception), which eliminates visual heat detection failures, batching inseminations, reducing the median interval to first service, and ultimately reduces herd days open.

Additionally, socio-economically, most of the milk producers in Uganda are smallholders who are very short of capital, do not have access to cold-chain facilities and the costs of veterinary inputs are high. The high costs of multi-agent synchronisation programmes that involve the use of intravaginal progesterone devices (PRIDs) and several injectable hormones are often too expensive and financially risky for the typical smallholder who may not be able to afford such high costs and the risks of using the drugs which would be required to ensure a successful synchronisation. The results of this study will serve as baseline comparative data for tiered extension approaches to reproduction, single agent versus multi-agent:

1. For Institutional Multiplier Farms (e.g., Njeru Stock Farm): Where the high costs of labour, large paddock grazing areas and tougher heifer multiplication goals make it worthwhile to invest more money, the superior oestrous synchrony and cumulative herd pregnancy throughput of the multi-agent PRID + GnRH + $PGF_{2\alpha}$ protocols are worth the investment.

2. For Smallholder Production Systems: This research evaluates the relative performance of the low-cost single-agent ($PGF_{2\alpha}$) protocols compared with multi-agent protocols, and identifies the specific physiological requirements (e.g., requirement for a functional corpus luteum) for the low-cost single-agent protocols to be successful. This allows vets to provide affordable, tailored synchronisation when they are unable to incur unnecessary multi-agent costs for smallholders with limited resources.

Thus, this study is directly related to its objectives by determining whether the increased input costs of multi-agent protocols (compared with single-agent protocols) are justified by increased input costs, operational efficiency and improved oestrous synchrony and conception in tropical grazing conditions in Uganda.

1.6 Significance of the study

The study findings would be of significance to many stakeholders in the dairy industry. First of all, policymakers would use the study findings to assess the effectiveness of three-hormone synchronization on reproductive performance in dairy breeds. They could base their policy proposals on the study findings. The findings would be of great use to veterinary staff. They would provide baseline information on the effectiveness of oestrous synchronization treatments. Then they could use the findings in the field, as they would help the farmers in breeding dairy animals. They, in most cases, would have problems with oestrous detection and breeding with artificial insemination. The findings show the farmers that it is possible to synchronize oestrous for breeding efficiency. The comparison of efficiency in different dairy breeds provides breed-tailored information on how best to synchronize oestrous and maximize conception in these breeds in Ugandan conditions.

1.7 Conceptual Framework

A conceptual framework used to guide the current study acknowledges that low oestrous onset and poor conception in dairy cows are caused by a combination of interacting biological, environmental, and management-related factors. On the biological side, poor nutrition, negative energy balance, postpartum metabolic stress, parities, and poor body condition also disrupt the operating hypothalamic-pituitary-ovarian axis, resulting in poor follicular growth, silent oestrous, and slow- or non-occurring ovulation. Additional environmental stress, like heat stress and disease burden, suppresses endocrine signaling and diminishes oestrous expression. Additionally, management-related variables (such as inefficient heat detection, delayed insemination, and insufficient access to reproductive technologies) are among the reasons why breeding opportunities are missed, and conception rates are decreased. (Bihon & Assefa, 2021).

Several solution pathways can be pursued to overcome these challenges. Improved herd health management and nutritional interventions can improve the metabolic status and ovarian responsiveness. Better ways of detecting the existence of heat and breeding management are capable of enhancing the probability of timely insemination. Genetic selection of fertility factors should be seen as a long-term solution, and hormonal regulation can be seen as both a short-term and medium-term response to reproductive processes. (Tschopp *et al*., 2022).

Among the wide range of possible interventions, multi-hormonal oestrous synchronization is the solution of this study, a targeted and scalable method for systems in which nutritional and environmental limitations cannot be easily addressed. In particular, the use of single-agent prostaglandin ($PGF_{2\alpha}$) regimens has demonstrated non-ideal results because of insufficient luteolysis and weak control of follicular wave dynamics (Lopez-Gatius, 2021). Conversely, multi-hormone oestrous synchronization interventions that include both progesterone (PRID) and gonadotropin-releasing hormone (GnRH), along with $PGF_{2\alpha}$, can provide luteal phase, follicular growth, and ovulation time controls that improve ovulation and successful conception (Mawadri *et al*., 2025).

Therefore, although it is evident that further research is possible across several directions, this study contributes by evaluating multi-hormonal oestrous synchronization as a viable route to enhance reproductive efficiency in dairy cattle.

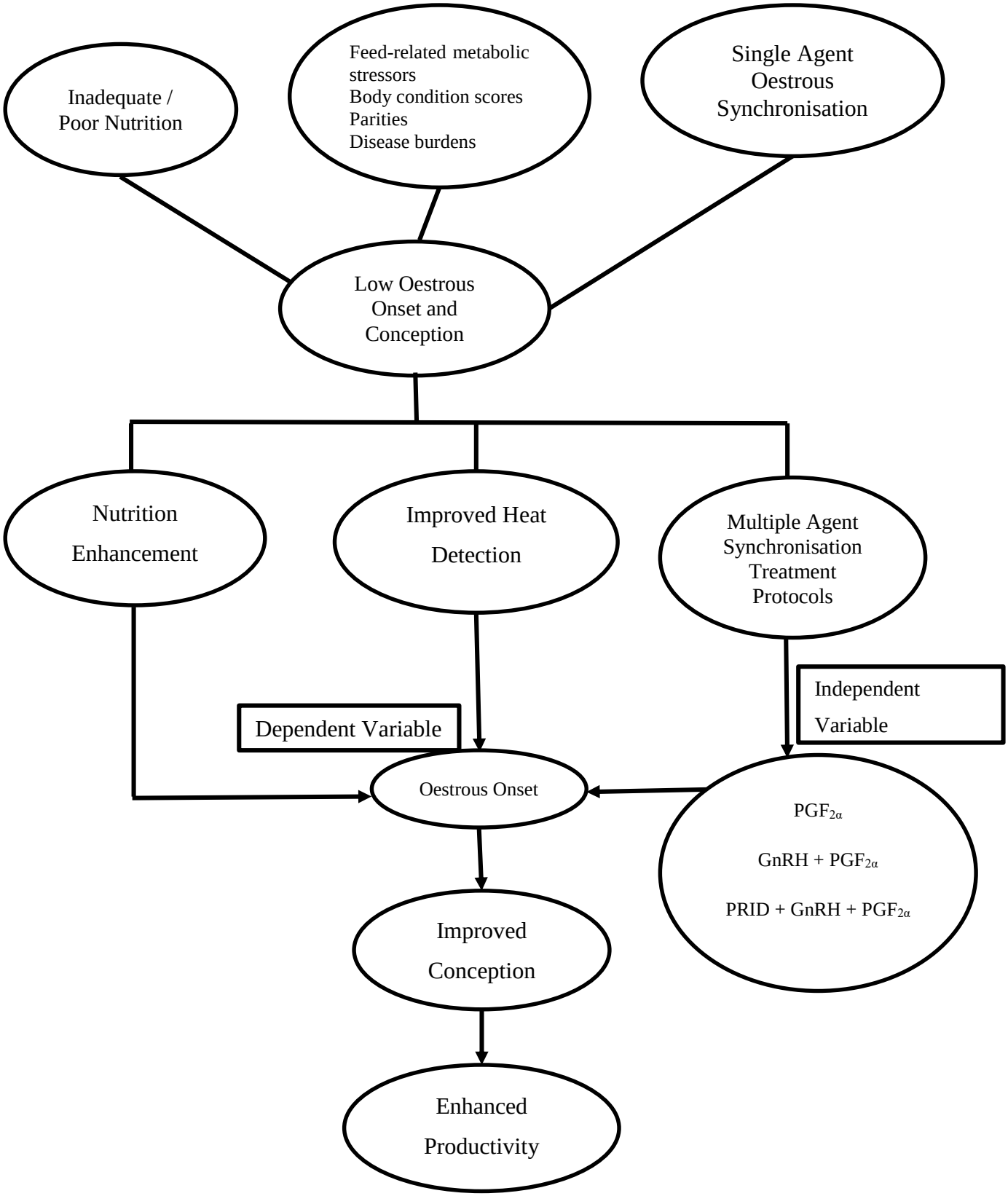


Figure 1: Conceptual Framework

CHAPTER TWO

LITERATURE REVIEW

2.1 Reproductive performance in dairy cattle

Reproductive efficiency is one of the essentials for the economic sustainability and productivity of dairy herd management worldwide (Lucy & Crooker, 2001; Butler, 2003). The high genetic breeding of dairy cows over the last 40 years has led to an increase in the amount of milk produced, and a significant decrease in the fertility of commercial dairy cows has been reported across the globe (Royal *et al*., 2000; Walsh *et al*., 2011; Fricke & Wiltbank, 2022). In a physiologic sense this subfertility is linked to a network of interacting biological factors. The most important of these is Negative Energy Balance (NEB), which occurs during early lactation when the metabolic requirement of the animals for energy to produce milk is higher than their energy intake from feed (Butler, 2003). NEB is correlated with a decrease in the frequency of Luteinizing Hormone (LH) pulses, a decrease in circulating levels of Insulin-Like Growth Factor 1 (IGF-1), delayed postpartum ovulation and a prolonged postpartum period of anestrus (Crowe *et al*., 2018). Furthermore, changes in the Hypothalamus-Pituitary-Ovarian (HPO) axis brought by BCS dynamics, parity and subclinical endometritis and ketosis result in changes in oestradiol levels and oestrous behavioural expressions (Bello *et al*., 2012; Softic *et al*., 2020).

These biological stressors are worsened greatly in Sub-Saharan Africa and tropical dairy production systems due to environmental and management limitations (Mungube *et al*., 2019). One of the significant challenges in smallholder and pastoral dairy systems is the lack of a proper diagnosis of spontaneous heat. Stockpersons only detect 50% – 65% of spontaneous heats due to the ambient temperature, high Temperature Humidity Index (THI), walking stress during the time spent in pasture and the short duration or the absence of observation of heats at night (Roelofs & Kooij, 2015; Abebe & Alemayehu, 2021). As a result, farms with no detection will have high days open, high numbers of services per conception and extended calving intervals, which will negatively impact farm profitability (Galukande *et al*., 2013).

In a few small commercial dairy farms in Bosnia and Herzegovina, Softic *et al*. (2020) tried to evaluate the reproductive performance indicators and found the average pregnancy rate (APR) to be 40.0%, average number of services per conception (ASC) 2.61 and average calving interval (ACI) 385 days. These parameters have been recorded by Softic *et al*. (2020) but other important factors of the operation (e.g. days open, days waiting for return-to-heat after AI, and

interactions with hormonal cycle manipulation) have not been included. Likewise, Eastern Kenya is a semi-arid region where Mungube *et al*. (2019) assessed the pregnancy rate and services per conception on small scale dairy farms and did not assess whether the pregnancy rate could be improved through the use of standardized hormonal oestrous synchronization protocols given the challenge of unobserved heat.

Mugisha *et al*. (2014) surveyed 450 smallholder dairy farms in six districts in the Central region of Uganda and found the mean age at first calving was 29.7 months, the mean service per conception was 1.7 services while the mean calving interval was 13.1 months. Although socio-economic factors affecting the utilization of breeding services were observed by Mugisha *et al*. (2014), they did not investigate the post-partum anestrus from a therapeutic and/or pharmacologic perspective in the public multipliers farm system in tropical pasture-grazed environment.

2.2 Oestrous synchronization and reproductive performance

Oestrous synchronization technologies were developed to systematically manipulate the bovine **o**estrous cycle, eliminate the reliance on visual heat detection, and enable Timed Artificial Insemination (TAI) or Fixed-Time Artificial Insemination (FTAI) (Islam, 2011; Kasimanickam, 2021). Over the past four decades, synchronization regimens have evolved from single-agent luteolytic treatments to multi- hormonal combination protocols designed to control both luteolysis and ovarian follicular wave dynamics (Fricke & Wiltbank, 2022; Bogdan *et al*., 2026).

2.2.1 Single-Agent Prostaglandin ($PGF_{2\alpha}$) Protocols

Traditionally, single drug synchronization is accomplished using either the exogenous administration of Prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$) or any of its synthetic equivalents (e.g., dinoprost tromethamine). On a physiological level, $PGF_{2\alpha}$ causes luteolysis, regression of the matured and functioning CL, thus reducing progesterone plasma concentration levels and consequently lifting negative feedback on the hypothalamus and causing an LH ovulatory spike (Noguchi *et al*., 2017; Umaña Sedó *et al*., 2022). Nevertheless, there exists an important biological limitation to the use of a single drug such as $PGF_{2\alpha}$ for synchronization, in that, $PGF_{2\alpha}$ acts solely on the luteal tissue within days 5-17 of the 21-day cycle and has absolutely no physiological control over follicular waves selection and development within the ovary (Lopez-Gatius, 2021).

For this reason, when a herd is treated with either single or double doses of $PGF_{2\alpha}$ (for example, 11 or 14 days apart), the interval between treatment and standing heat and ovulation may significantly vary (ranging from 48 to 120 hours), and will depend solely on the stage of development of the dominant follicle when luteolysis occurs (Chanyalew *et al*., 2018; Adeyina *et al*., 2021). In cyclic, prepubertal, or anestrus cattle without a healthy CL, $PGF_{2\alpha}$ is fully ineffective (Prajapati *et al*., 2019). As shown by the field studies of Chanyalew *et al*. (2018) carried out in Ethiopia under tropical conditions, double $PGF_{2\alpha}$ treatment produced higher pregnancy rates (63.1%) compared to single doses (55.8%), though both still involved intense manual heat detection and did not provide for the fixed-time AI technique without heat detection.

2.2.2 Two-Agent GnRH + $PGF_{2\alpha}$ (Ovsynch) Protocols

To counter the challenge of irregular follicular wave development, two-component systems using GnRH and $PGF_{2\alpha}$ (referred to as the Ovsynch protocol) have been developed (Cordoba & Fricke, 2001; Marques *et al*., 2024). With the use of a normal GnRH-$PGF_{2\alpha}$-GnRH scheme, the first dose of GnRH on Day 0 results in the secretion of LH and brings about either ovulation or luteinization of a dominant follicle followed by a synchronized appearance of a new wave of follicles 1.5 to 2 days later (Momcilovic *et al*., 1998; Alsuwaidawi & Alrawi, 2024). $PGF_{2\alpha}$ is then given on Day 7 to regress the existing or newly created CL. Subsequent administration of GnRH on day 9 causes a synchronous LH peak such that TAI can be carried out 12 to 24 hours after this without oestrous detection (Kwon *et al*., 2017; Morini *et al*., 2019).

While dual agent protocols offer considerable benefits over single $PGF_{2\alpha}$ treatments, studies show inconsistent outcomes regarding fertility obtained by using these protocols on high-producing or pasture-raised dairy cattle (Kothandaraman *et al*., 2023). The synchronization of wave emergence does not occur if the cow lacks a dominant follicle during the administration of the initial dose of GnRH. Most importantly, where the cow experiences premature luteolysis or low progesterone levels before the application of $PGF_{2\alpha}$, there will be premature LH surges that impact negatively on oocyte quality and conception rates (Colazo *et al*., 2013; Ayantoye *et al*., 2025).

2.2.3 Three-Agent Progesterone (PRID/CIDR) + GnRH + $PGF_{2\alpha}$ Protocols

To obtain complete physiological control of the luteal phase and of the follicular wave dynamics, three-agent multi-hormonal protocols have been developed combining the use of exogenous Progesterone (induced by the use of PRID or CIDR) with the use of GnRH and

$PGF_{2\alpha}$ (Herlihy *et al*., 2011; Bogdan *et al*., 2026). This protocol uses an intravaginal device with natural progesterone (such as 1550 mg progesterone) which is inserted on Day 0 along with an intramuscular injection of GnRH.

The exogenous progesterone device generates the presence of exogenous progesterone at high concentrations, which reduces LH pulsatile activity, prevents early onset of oestrous or ovulation, and helps in the development of a dominant, highly fertile follicular wave, physiologically (López-Gatius *et al*., 2015; Omar *et al*., 2025). Day 6 or Day 7 is the time for administration of the $PGF_{2\alpha}$ injection, which causes regression of the existing luteal tissue, and the PRID is removed 24 hours post the injection of $PGF_{2\alpha}$. With the withdrawal of the PRID, there is a rapid decrease in the progesterone concentration in the blood leading to a rapid pre-ovulatory development of the follicles, and finally GnRH is administered at AI time (Tschopp *et al*., 2022).

The investigations done across the world using either pasture or production system have shown that the multi-agent protocol works effectively much better than the double-agent and even the single-agent protocol in anestrus cows, repeat breeder and pasture grazed cows (Haile *et al*. 2023; Sundar 2024; Mawadri *et al*. 2025). A study conducted in Ethiopia, shows that the multi-agent protocols were effective much more than a single dose of $PGF_{2\alpha}$ in inducing both oestrous and pregnancies (Haile *et al.* 2023). In a similar manner, Herlihy *et al*. (2011) and Xu & Burton (1999) reported that the progesterone-based TAI protocols conducted in pasture dairy herds had a very high 24-day conception and increased pregnancy without causing any economic loss due to non-detection of the spontaneous heat cycle.

2.3 Contextual Profile and Agro-Ecological Reality of Njeru Stock Farm, Buikwe District

A major limitation in the current literature regarding Uganda and East Africa is the lack of adequate consideration of the setting under which the breeding experiments are carried out (Mugisha *et al*., 2014; Mawadri *et al*., 2025). The study was conducted at Njeru Stock Farm located in the Lake Victoria Crescent agro-ecological zone of the Central region of Uganda (NAGRC&DB, 2024).

From an agro-ecological perspective, Njeru Stock Farm has a bimodal rainfall ranging from an average of 1,200 mm – 1,500 mm per year, average relative humidity of 75% to 88% and temperatures ranging from 22°C to 30°C. This makes THI high and moderates all year round and presents high-yielding Bos taurus dairy cattle with chronic heat stress (Omar *et al*., 2025). Heat stress directly decreases the secretion of hypothalamic gonadotropin-releasing hormone

(GnRH); decreases the amplitude of the LH surge; decreases the amount of dominant follicle steroidogenesis; and decreases visual oestrous behavior (Fricke & Wiltbank, 2022).

From an operational perspective, Njeru Stock Farm consists of about 1099 acres of open grazing lands owned by the National Animal Genetic Resources Centre and Data Bank (NAGRC&DB), Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) (Animal Breeding Act, 2001; NAGRC&DB, 2024). It is the main national purebred Friesian and Jersey nucleus breeding farm that is responsible for producing and disseminating high-quality genetic material to the national poverty alleviation and agricultural transformation frameworks, namely the Parish Development Model (PDM) (Mbabazi, 2026; NAGRC&DB, 2026). The experimental cattle are kept under a free-range paddock grazing system based on a natural pasture (mainly Pennisetum purpureum, Brachiaria ruziziensis, Chloris gayana) with mineral licks.

Visual heat detection is severely affected under large-acreage, open-paddock conditions due to cattle dispersion, night-time heat, walking stress because of pasture, and tick-borne disease problems (Anaplasmosis, East Coast Fever etc.). In addition, with Njeru Stock Farm being a national semen distribution centre and liquid nitrogen hub for Eastern Uganda, a fixed-time AI (FT-AI) synchronization protocol, evaluated and highly efficient on the nucleus farm, has direct implications for the quality and quantity of high-pedigree dairy heifers multiplied for distribution to smallholder farmers across the country (IGC, 2024; Mbabazi, 2026).

2.4 Identification of the Knowledge Gaps

Many factors of the oestrous synchronization process, including biological, pharmacological, environmental, or breed-specific factors, should be correlated into one system. The following table demonstrates the interaction between all these aspects and their impact on the reproductive outcomes of the livestock in tropical pastures:

Table 1: Interaction between aspects in oestrous synchronization

Study Facet / Dimension	Single-Agent Protocol ($PGF_{2\alpha}$)	Two-Agent Protocol (GnRH + $PGF_{2\alpha}$)	Three-Agent Protocol (PRID + GnRH + $PGF_{2\alpha}$)

Biological & Pharmacological Mechanism	Regression of mature CL only (luteolysis). Zero control over follicular wave emergence.	Resets follicular wave emergence via 1st GnRH; luteolysis via $PGF_{2\alpha}$; ovulation surge via 2nd GnRH.	Exogenous progesterone priming suppresses LH pulses; GnRH resets wave; $PGF_{2\alpha}$ regresses CL; PRID removal triggers synchronized ovulation.
Interacting Environmental / Breed Stressors	Ineffective in anestrous/acyclic cows; high reliance on visual heat detection.	Premature luteolysis or low baseline progesterone compromises oocyte quality.	Overcomes negative energy balance, silent heats, heat stress (THI), and anestrus in pasture grazing.
Impact on Reproductive Outcome (Heat & Conception)	Highly variable heat onset (48–120 hrs); low conception; unsuitable for Fixed-Time AI.	Moderate synchronization; improved over $PGF_{2\alpha}$ alone; variable conception in pasture herds.	Tight heat onset window; high TAI conception; eliminates visual heat detection reliance.

Although considerable research has been conducted internationally in the field of bovine reproductive biotechnology, the existing literature has not been synthesized in an adequate manner to reveal three distinct areas of unaddressed knowledge gaps that are directly related to the goals of this dissertation:

Lack of Controlled Head-to-Head Comparative Protocol Evaluations in Standardized Tropical Pasture-Grazed Public Stock Farm Conditions in East Africa. There are no rigorous, controlled experimental data that have been undertaken to compare the effectiveness of three distinct protocols (single-agent ($PGF_{2\alpha}$), two-agent (GnRH+$PGF_{2\alpha}$), and three-agent (PRID+GnRH+$PGF_{2\alpha}$) simultaneously within a single, highly controlled tropical pasture-grazed public stock farm environment in East Africa. Previous regional studies conducted in Ethiopia (Chanyalew *et al*., 2018) and Kenya (Mungube *et al*., 2019) tested protocols within small farms that were broken up into small units and had significantly confounding variations in feeding, housing, and competence of the stockperson. In this study, the researcher fills this gap in a multi-protocol experimental trial with farm-to-farm standardization variables eliminated from the investigation conducted at Njeru Stock Farm.

Breed-Protocol Interactions under Tropical Pasture Management: Lack of Empirical Evidence of Holstein-Friesian x Jersey Heat-Tolerant Cattle with High Yielding Protocols in Uganda. The literature shows that there are differences in metabolic steroid clearance rates and heat

tolerance between large-framed Holstein-Friesian and moderate-framed Jersey cows (Sveberg *et al*., 2015; Prajapati *et al*., 2019). Empirical evidence on whether there is a significant difference in the physiological response to single-agent versus multi-agent synchronization between purebred Friesian and Jersey cattle in the same tropical paddock settings, however, does not exist in Uganda. The current national reports (Mugisha *et al*., 2014; Mawadri *et al*., 2025) grouped dairy breeds as a whole. This study fills exactly this gap by providing comparative response data on a per-breed basis to enable targeted reproductive management.

The relationship between Progesterone-Primed Synchronized Oestrous Onset Timing and 45-Day Post-AI Conception (PDM) amongst Public Nucleus Multiplier Herds (PNMHs) supporting National Policy Frameworks (NPFs) is not well understood.

National public stock farms (NAGRC&DB) are statutorily required under the Animal Breeding Act (2001) to increase the number of quality heifers for the Parish Development Model (PDM) but there is no published scientific evidence to give details of the exact timing of oestrous onset after PRID removal and the corresponding ultrasound-confirmed 45-day conception under open-paddock management. To fill this gap, this study was conducted to create empirical field data that relates the composition of hormonal protocols to strict windows of oestrous and ultrasound/rectal-defined conception.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This research was done at Njeru Stock Farm which is located in Buikwe District of Uganda. Njeru Stock Farm is a government livestock farm under the Ministry of Agriculture, Animal Industry and Fisheries, managed by the National Animal Genetic Resources Center and Data Bank. The choice of Njeru as an experimental site was based on several factors. First, it is the designated national Pure Friesian Breeding Centre in Uganda encompassing approx. 1099

Acres of fenced grazing land situated in the Lake Victoria Basin agro-ecological zone, characterized by a bimodal rainfall pattern averaging 1,200 to 1,500 mm annually, mean ambient temperatures ranging between 22°C and 30°C, and relative humidity between 75% and 88% (NAGRC&DB, 2024). Secondly, Njeru is Uganda's designated national nucleus breeding centre for purebred Holstein-Friesian and Jersey cattle, maintaining standardized herd management records (NAGRC&DB, 2024). Thirdly, the farm operates a uniform paddock-grazing management system across standardized open pastures (comprising Pennisetum purpureum, Brachiaria ruziziensis, and Chloris gayana), thereby controlling major nutritional and environmental confounding variables that typically compromise multi-farm field trials and the facility houses the Eastern Regional Liquid Nitrogen and Semen Distribution Hub, providing cold-chain infrastructure, standardized cryopreserved semen straws, and accredited Assisted Reproductive Technology (ART) technical personnel required for controlled reproductive research. Thus, enabling the provision of the necessary infrastructural facilities needed for carrying out mass reproductive technology trials.

3.3 Study Design

The study used an experimental design where 54 cows were selected by the researcher. Friesian and Jersey breeds were chosen due to being the two main foreign dairy breeds whose multiplication and distribution have been undertaken by the NAGRC&DB for improving the national herd in Uganda. These two breeds were selected because they represent the primary exotic dairy genetics multiplied and distributed nationwide by NAGRC&DB to support national livestock development initiatives, including the Parish Development Model (PDM). Mechanistically, comparing these breeds provided a valuable physiological contrast under identical tropical pasture-grazed conditions: Holstein-Friesians represent high-yielding, large-framed dairy cattle with elevated metabolic rates and rapid hepatic steroid clearance, whereas Jerseys represent moderate-framed, highly feed-efficient cattle with superior heat tolerance and distinct endocrine dynamics.

3.3 Experimental Animals, Pre-Trial Screening, and Pre-Treatment Conditioning

3.3.1 Voluntary Waiting Period, Bull Isolation, and Pre-Trial Rest Period

Rigorous animal selection and isolation protocols were performed before the animals were entered into experiments:

Postpartum Rest Period and Voluntary Waiting Period (VWP): All candidate cows selected for the trial were multiparous cows that had been given a minimum of 60 days of Voluntary Waiting Period (VWP) postpartum (mean = 74.5 ± 8.2 days). This allowed for complete uterine involution, histological recovery of the endometrium and stabilization of normal metabolic and endocrine functions before starting hormonal manipulation.

Njeru Stock Farm has an exclusive Artificial Insemination (AI) breeding policy known as Strict Isolation from Intact Bulls. All of the cows that were tested were kept in bull-free paddocks and received no contact or co-grazing with intact breeding bulls for at least 60 days before they were screened or for the pre-experimental and experimental periods. This complete separation removed all temptation for natural matings that had not been recorded and undetected pregnancies that might have occurred due to exposure to the bull before the start of the trial.

Review of Institutional Breeding and Clinical Records: The researcher, with the assistance of the resident NAGRC&DB Veterinary Officer, reviewed all individual animal health cards, calving records and breeding registers. Cows excluded were those which were known to have had dystocia, retained placenta, clinical endometritis, and repeat breeding (more than 3 previous unsuccessful inseminations).

3.3.2 Pre-Trial Reproductive Staging, Pregnancy Screening, and Prophylactic Conditioning

A strict two-stage pre-trial screening process was performed to ensure accurate oestrous staging, verify pregnancy cyclicity and rule out early pregnancy loss which might be caused by early treatment of the pregnancy with prostaglandin.

Two sequential transrectal ultrasonographic examinations were performed in candidate cows 10 days apart (Day -20 and Day -10 before Protocol Day 0) with a real-time ultrasound scanner fitted with a linear-array 5.0/7.5 MHz transducer (Ibex Pro, E.I. Medical Imaging, Loveland, CO, USA). The examinations were conducted by a senior theriogenologist who has 19+ years of experience working in bovine gynecology. Differentiated and excluded from the differential diagnosis of active ovarian cyclicity (presence of a functional Corpus Luteum ≥ 15 mm in diameter or dynamic antral follicles ≥ 8 mm) from acyclicity, persistent anestrus, ovarian follicular/luteal cysts and subclinical uterine pathologies (pyometra, endometritis).

Definitive Pregnancy Diagnostics: Definitive pregnancy determination was done using transrectal ultrasonography at Day -10, and was re-determined manually with transrectal palpation at Day -3 before trial onset. No animal with an early undetected gestation was allowed

to enter the study since early gestation (before 35–45 days) was not detected by manual rectal palpation. The risk of inducing an iatrogenic abortion by $PGF_{2\alpha}$ or other hormones was completely negated.

Prophylactic Health and Nutritional Conditioning: All eligible candidate cows were given Albendazole 10% w/v oral suspension (7.5 mg/kg body weight) and a trypanocidal injection of Diminazene aceturate (3.5 mg/kg body weight, intramuscularly) exactly 21 days (3wks) before day 0. Body Condition Scoring (BCS) was carried out independently and using the standard 5-point Wildman scale (1 = emaciated; 5 = obese). Only cows with moderate to good BCS (3.5 to 4.0) were selected for enrollment to ensure that those with severe negative energy balance (BCS < 3.0) did not suppress hypothalamic GnRH pulsatility and thus would not affect ovarian responsiveness.

This gave positive confirmation of the normal physiology and anatomy of the reproductive system of all candidates selected, hence no occurrence of false positives of oestrous or cysts affecting synchronization and conception results.

3.3.3 Sample size determination, factorial allocation and power justification

The selection of 54 multiparous dairy cows was established using a balanced 3X2 factorial stratified block design to compare three hormonal synchronization protocols across two primary exotic dairy breeds (Holstein-Friesian, n = 27; Jersey, n = 27). Allocating the animals equally across the three treatment protocols yielded 18 cows per treatment group: Treatment Protocol 1 ($PGF_{2\alpha}$ alone), Treatment Protocol 2 (GnRH + $PGF_{2\alpha}$), and Treatment Protocol 3 (PRID + GnRH + $PGF_{2\alpha}$).

Statistically, a sample size of n = 18 per treatment protocol (N = 54 overall) provides adequate statistical power (beta 0.80 at $\alpha = 0.05$) under non-parametric Chi-square analysis (df = 2) and Bonferroni-adjusted post-hoc pairwise testing to detect large proportional differences in categorical reproductive outcomes (oestrous onset and 45-day post-AI conception) between single-agent and multi-agent protocols. Furthermore, evaluating the experimental design using Mead's Resource Equation for animal research:

$(E) = N - T - B$

Where N = 54 (total experimental animals), T = 3 (treatment protocols), and B = 2 (breed stratification blocks):

$E = 54 - 3 - 2 = 49$

The resulting E value of 49 significantly exceeds the standard recommended threshold (10 <E < 20), confirming that 54 animals provide sufficient experimental degrees of freedom for valid statistical inference without excessive or unethical animal usage.

Biologically, N = 54 represented the complete eligible herd population at Njeru Stock Farm that satisfied all strict pre-trial inclusion criteria: multiparous cows (Parity 2–4), age 3.5–5.5 years, a minimum 60-day Voluntary Waiting Period (VWP), Body Condition Score (BCS 3.5–4.0), 60-day strict isolation from bulls, and double ultrasound-confirmed active ovarian cyclicity ($CL \geq 15\,mm$ or follicle $\geq 8\,mm$). This rigorous pre-trial screening eliminated confounding baseline variances in age (p = 0.85), parity (p = 0.88), and BCS (p = 0.09) across all three treatment protocol groups.

The cows were divided into three groups, with each group receiving Treatment Protocol 1, consisting of Prostaglandin ($PGF_{2\alpha}$); Treatment Protocol 2, which consisted of Gonabreed (GnRH) + Prostaglandin ($PGF_{2\alpha}$); and Treatment Protocol 3, which consisted of Progesterone (PRID) + Gonabreed (GnRH) + Prostaglandin ($PGF_{2\alpha}$). The animals were divided into groups as shown in Table 2.

Table 2: The study sample

Breed	**Treatment Protocol 1**	**Treatment Protocol 2**	**Treatment Protocol 3**
Jersey	9	9	9
Friesian	9	9	9
Total	**18**	**18**	**18**

3.3.4 Administration of Treatments

The prostaglandin hormone involved in the regression/lysis of the corpus luteum; its composition was Dinoprost tromethamine (natural $PGF_{2\alpha}$, 5 mg/ml; Enzaprost®, Ceva Santé Animale, Libourne, France). 5.0 ml (25 mg dinoprost) IM was administered to induce luteolysis (Sedo *et al*., 2022). GnRH hormone causes the release of both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and, in addition to this, it is responsible for the synchronization of the emergence of the follicle's waves and for inducing ovulation (Marques *et al*., 2024). Synthetic GnRH analogue (Gonadorelin diacetate, 0.05 mg/ml; Cystorelin® /

Gonabreed®, Ceva Santé Animale, Libourne, France). A single dose of 2.0 ml (0.1 mg gonadorelin diacetate) IM to trigger the development of a new follicular wave and the release of the LH/FSH surge (Tschopp *et al*., 2022). Exogenous hormone that is used to inhibit the early oestrous period and to coordinate the development of follicles. The administration was done using the Progesterone-releasing intravaginal device containing 1.55 g of natural progesterone (PRID Delta®, Ceva Santé Animale, Libourne, France) (Morini *et al*., 2019).

The first group (treatment protocol 1), which was treated with a single agent to synchronize the animals. This group was treated with Enzaprost ® ($PGF_{2\alpha}$). Intramuscular injection of 5,0 ml Enzaprost® (dino 25 mg) after the ultrasonographic detection of an active Corpus Luteum (CL ≥ 15 mm). Three checks were made every 24 hours over a 6-day period for visual heat detection. To lyse persistent or newly formed CLs, cows that failed to express standing heat in the first 6 days were given a second injection of 5.0 ml Enzaprost® (25 mg dinoprost) on Day 11. Standing heat cows after the first injection were inseminated 12 hours after the onset of standing heat (AM/PM rule). Cows that did not show visual heat signs were inseminated at a Fixed-Time Artificial Insemination (FTAI) window of exactly 72 hours after the second injection of $PGF_{2\alpha}$ on Day 14 (Pereira *et al*., 2016).

The second group received Treatment Protocol 2, which consisted of Gonabreed (GnRH) + Prostaglandin ($PGF_{2\alpha}$). This group was treated with Cystorelin ® (GnRH). On day 0, Cystorelin ® (GnRH) 2 mL was injected intramuscularly. On day 7, Enzaprost ® ($PGF_{2\alpha}$) 5 mL was injected, and on day 9, another injection of Cystorelin ® (GnRH) 2 mL was given, and the animals were served at a fixed time within 8-24 hours from the last GnRH injection.

The third group received Treatment Protocol 3, which consisted of Progesterone (PRID DELTA) + Gonabreed (GnRH) + Prostaglandin ($PGF_{2\alpha}$). This group was treated with PRID Delta ® (progesterone). On day 0, the cows were treated with intra-vaginal progesterone-releasing devices (PRID DELTA) and injected with Cystorelin ® (GnRH) 2 mL intramuscularly. On day 6, the cows were injected with Enzaprost ® ($PGF_{2\alpha}$) 5 mL intramuscularly (at the same time as when the first Cystorelin ® (GnRH) 2 mL was injected). On day 7, the PRID DELTAs were removed (at the same time as when the first Cystorelin ® (GnRH) 2 mL was injected). A fixed Artificial insemination was done after 48 to 56 hours of PRID DELTA removal, and Cystorelin ® (GnRH) 2 mL was administered at the service (Omar *et al*., 2025).

A cow in all three treatment protocols was determined to be in heat when she exhibited abundant vulval discharge, a lot of bellowing, a red and swollen vulva and stood for other cows to mount. Protocols 2 and 3 were conducted under the rule of Strict Fixed-Time Artificial Insemination (FTAI). All animals in Protocols 2 and 3 were inseminated at the exact time that was predetermined (16–20 h after the second injection of GnRH and 56 h after removal of the PRID, respectively), irrespective of whether they were observed to express visual standing heat. Protocol synchrony was quantified as a dependent variable (oestrous onset) with visual heat detection, and not as a decision trigger for AI timing, except in protocol 1 where visual heat detection was also used as an observation variable and was an operational decision trigger for AI timing during the initial 6 days post first $PGF_{2\alpha}$ injection. On observed heat (AM/PM rule) animals exhibiting standing heat were served. When the second injection of $PGF_{2\alpha}$ was given on Day 11, nonresponsive cows were treated as per a Fixed-Time AI decision rule at 72 hours after PGF injection. This standard operating procedure for decision-making eliminated decisional mix-ups and guaranteed strict and repeatable data collection.

3.4 Insemination Procedure, Artificial Insemination Techniques, and Semen Quality Control

Artificial insemination was standardized for all 54 experimental cows:

All 54 inseminations were carried out by a single accredited, senior NAGRC&DB Artificial Insemination Specialist with consistent placement of semen into the uterine body using the transcervical route.

High-Quality Frozen Semen: Inseminations were done with high-grade 0.25 ml French straws of high quality (high grade) inseminating semen from proven and disease-screened NAGRC&DB breeding bulls (Friesian and Jersey). Under strict hygienic conditions, the straws were thawed and dried in a temperature-controlled water bath at 37°C for 45 seconds and were loaded into the stainless-steel insemination guns (Cassou gun) with sterile sheaths and immediately deposited into the mid-uterine body.

Post-Thaw Semen Quality Verification: Immediate assessment of the post-thaw progressive motility of semen immediately before insemination batches indicated a progressive motility of >50% and a minimum dose of 20 × 10^6 viable spermatozoa, under phase-contrast microscopy.

3.5 Data collection

3.5.1 Monitoring and Staging of Oestrous Onset

Primary data collection was done using systematic observation of oestrous initiation and clinical diagnosis of pregnancy. Trained herd technicians observed all experimental cows visually for behavioural oestrous three times daily (06:00–08:00 h, 12:00–13:00 h and 17:00–19:00 h) for 30 minutes per observation time. A cow was considered to have reached 'oestrous Onset (YES)' if it showed standing heat (allowed a standing position to other cows in the herd). Clear, viscous vaginal discharge of cervical mucus, hyperactive bellowing, vulval hyperemia/edema and the flehmen reaction were recorded as secondary primary signs. In the case of Protocol 3, oestrous onset was recorded in 16-hour phase blocks after PRID removal. Within 9 days after the completion of the protocol (Tschopp *et al*., 2022), the number of cows in oestrous was calculated as a percentage of synchronized cows. All heats observed, dates of artificial insemination and diagnostic pregnancy results were entered in the structured study recording tool as shown in Appendix II.

3.5.2 Dual-Stage Diagnostic Verification of Conception

Conception status was determined using a rigorous dual-stage diagnostic verification procedure:

Stage 1: Primary Transrectal Ultrasonography (Day 30 Post-AI): Transrectal ultrasonography (Ibex Pro, 5.0/7.5 MHz linear transducer) was the first method used to diagnose the pregnancy 30 days after insemination. Diagnostic confirmation required good visualization of the fluid-filled gestational sac in the ipsilateral (same side) uterine horn to the ovulation site, an embryonic vesicle, and an active embryonic heartbeat.

Stage 2: Secondary confirmation of conception and gestational viability was attempted 45 days after insemination using manual transrectal palpation by the senior theriogenologist (Stage 2). The criteria for confirmation were the asymmetry of the uterine horns; the positivity of the membrane slip of the chorioallantois membrane and the palpation of the turgid amniotic vesicle. The percentage of cows that were inseminated and subsequently confirmed pregnant at Day 45 post-AI was used to determine conception.

3.6 Measurement of variables

The dependent variables in the current study were oestrous onset and conception. Oestrous onset was measured based on the number of animals initiating oestrous based on the protocol

used (López-Gatius *et al*., 2015). Fertility was assessed through a dual-test diagnostic procedure. Pregnancy status was first determined on day 30 following artificial insemination through the use of transrectal ultrasonography with the aid of a portable ultrasound machine fitted with 5.0/7.5MHz linear-array transducer (Ibex Pro, E.I. Medical Imaging, Loveland, CO, USA) to determine the presence of embryonic vesicles and viability of fetal heartbeat. A second confirmation of pregnancy was made on day 45 following artificial insemination through manual transrectal palpation of the amniotic vesicle and fetal membrane slip (Pereira *et al*., 2022). Confirmation of fertility status in the animal was done through palpation and ultrasound scans (Department of Energy, Environment and Climate Action, 2024).

The independent variable for this study was the different treatment protocols that were used for the study. The first treatment protocol received $PGF_{2\alpha}$ (Amin *et al*., 2023). The second treatment protocol received GnRH + $PGF_{2\alpha}$, while the third treatment protocol received PRID + GnRH + $PGF_{2\alpha}$.

3.7 Statistical Analysis

Descriptive analysis was used to analyze the conception and onset of oestrous in the two breeds of animals in the experiment. Results were shown in graphical format. Chi-square tests were done to test for significant differences in oestrous response and conception in cows based on the treatment protocols (Haile *et al*., 2023). Bonferroni pairwise tests were done to show significant differences within the treatment protocols and statistical significance was declared at $p < 0.05$.

3.8 Ethical considerations

In keeping with the general awareness about the need to observe animal welfare while executing studies that involve livestock, special attention has been paid to minimizing pain and stress during hormonal administration as well as ensuring proper handling practices for the subjects undergoing various interventions such as transrectal palpation, artificial insemination, etc.

All cattle used in this experiment were safely restricted within standard hydraulic-assisted cattle crushes to avoid them getting physically uncomfortable or harmed. Written administrative clearance and institutional consent have also been secured from the National Animal Genetic Resources Centre and Data Bank Management for conducting the experiment at their on-site facility (Njeru Stock Farm) as shown in Appendix I.

Therapeutic agents, frozen semen straws, and Intravaginal Devices (PRID Delta), where needed, used throughout this experiment have been certified veterinary products that are legally allowed and regulated by the National Drug Authority (NDA) as per the relevant stipulations of the Uganda Animal Breeding Act (2001).

Prior to starting the actual experiment, body condition scoring was carried out independently by the researcher and confirmed once again by the resident NAGRC&DB veterinary officer. The researcher restricted himself only to those body condition scoring categories which corresponded to 'Moderate-To-Good' scores (i.e., body condition scores ranging between 3.5 and 4.0). This step was important since cows having a very poor/lower than average score due to a state of severe negative energy balance (body condition scores<3.0) usually experience suppressed levels of pulsatility of luteinizing hormone secretion and may remain persistently in a state of post-partum anestrus irrespective of whatever intervention protocol one may subject them to, thus creating bias towards ovulation/conception outcome measures associated with such synchronization protocols. A baseline body condition scoring was done independently by the researcher using a standardized five-point Wildman scale [where: score = 1 is extremely thin/emaciated and score = 5 is obese].

CHAPTER FOUR

RESULTS

4.1 Oestrous Onset across the Three Treatment Protocols

The study evaluated oestrous onset among 54 cows assigned to three synchronization treatment protocols with 18 cows in each treatment group. Each treatment group contained nine Friesian and nine Jersey cows. Treatment Protocol 1 consisted of $PGF_{2\alpha}$, Treatment Protocol 2 consisted of GnRH + $PGF_{2\alpha}$ and Treatment Protocol 3 consisted of PRID + GnRH + $PGF_{2\alpha}$. The study population was selected and consisted of cows of comparable parity, age and body condition score that had been confirmed non-pregnant before commencement of the synchronization treatment protocols.

For cows given Treatment Protocol 1, 2 cows showed oestrous at 48 hours from the second $PGF_{2\alpha}$ injection. The other 2 cows showed oestrous at 60 hours from the $PGF_{2\alpha}$ injection; the remaining 14 cows under Treatment Protocol 1 did not show any signs of oestrous within the observation period. For cows given Treatment Protocol 2, 6 cows showed oestrous signs 12 hours from the GnRH second injection to artificial insemination; the remaining 12 cows in Treatment Protocol 2 did not express any visible oestrous signs during the monitoring period. For cows given Treatment Protocol 3, heat detection was grouped into 3-hour phases of 16 hours, whereby the hours were counted from the time of PRID DELTA removal. For the first phase of 16 hours, 2 cows showed oestrous. Then, for the second phase of 16 hours, 3 cows showed oestrous. For the third phase of 16 hours, 10 cows showed oestrous. Therefore, a total

of 15 cows under treatment protocol 3 exhibited oestrous signs, while the remaining 3 cows did not show oestrous signs.

The results presented in this chapter therefore describe the observed reproductive responses under the conditions and selection criteria of the study. The comparisons are between the three synchronization treatment protocols tested rather than between breeds as independent determinants of reproductive performance. This distinction is important because factors other than synchronization treatment protocols, including parity, individual reproductive history, ovarian status, nutrition, health status, semen quality, insemination technique and timing of insemination, may influence oestrous expression and conception. Where such factors were not specifically measured or statistically controlled in the present study, the results should be interpreted as treatment protocol-group associations rather than as definitive causal effects.

There were variations in oestrous onset in cows based on different treatment protocols, Treatment Protocols 1, 2, and 3, as shown in Figure 2. Treatment Protocol 3, comprising PRID + GnRH + $PGF_{2\alpha,}$ recorded the highest number of cows exhibiting oestrous, followed by Treatment Protocol 2 and Treatment Protocol 1.

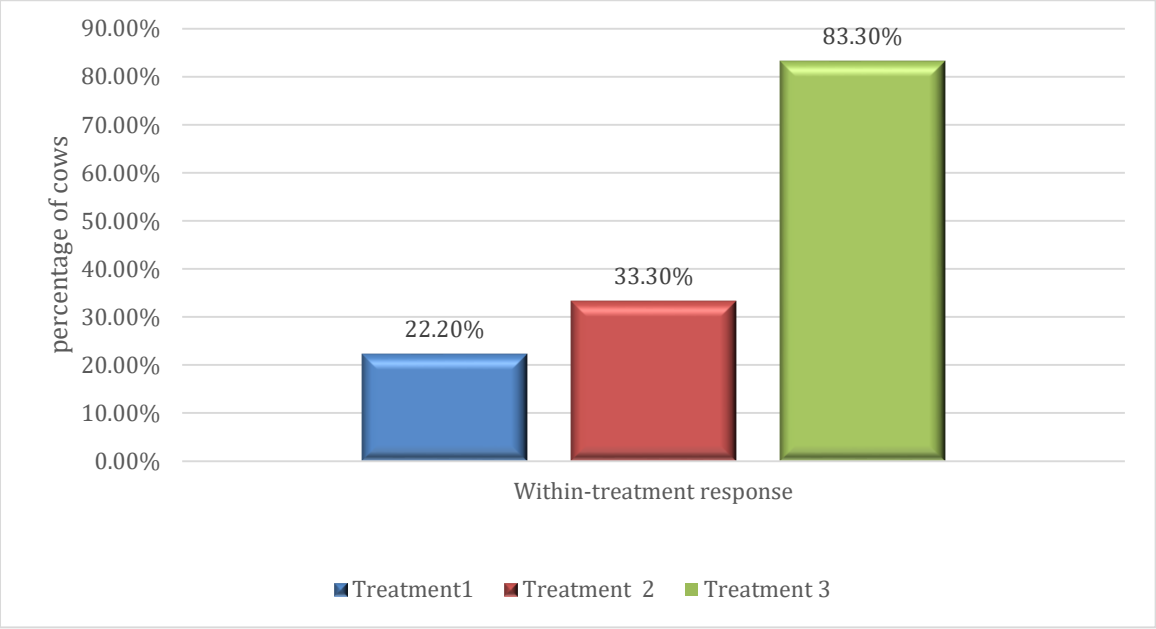


Figure 2: Oestrous onset in cows based on Treatment Protocols

Source: Field Data (2026)

Chi-square tests revealed a statistically significant difference in oestrous onset between cows subjected to Treatment Protocol 1 (Prostaglandin, $PGF_{2\alpha}$) and those under Treatment Protocol

3 (Progesterone [PRID] + Gonabreed [GnRH] + Prostaglandin, $PGF_{2\alpha}$) as well as between cows subjected to Treatment Protocol 2 (Gonabreed [GnRH] + Prostaglandin, $PGF_{2\alpha}$) and those under Treatment Protocol 3 and between cows subjected to Treatment Protocol 1 and those under Treatment Protocol 2 ($\chi^2 = 15.343$, df = 2, p = 0.000). Pairwise comparisons revealed that a statistically significant difference in oestrous onset was observed between cows receiving Treatment Protocol 3 and those receiving Treatment Protocol 2, as well as between cows receiving Treatment Protocol 3 and those receiving Treatment Protocol 1. In contrast, no statistically significant difference in oestrous onset was observed between cows receiving Treatment Protocol 1 and Treatment Protocol 2, as shown in Table 3.

Table 3: Pairwise Comparisons of Treatment Protocols according to oestrous onset

Treatment Protocols	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
Treatment Protocol 3-Treatment Protocol 2	13.500	4.530	2.980	0.003	0.009
Treatment Protocol 3-Treatment Protocol 1	16.500	4.530	3.643	0.000	0.001
Treatment Protocol 2-Treatment Protocol 1	3.000	4.530	0.662	0.508	1.000

Each row tests the null hypothesis that the Treatment Protocol distributions are the same.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

Source: Field Data (2026)

These results indicate that within the study population, the multi-agent Treatment Protocol 3 was associated with a higher observed oestrous response than either of the two treatment protocols containing fewer hormonal components. However, the statistical result should not be interpreted as demonstrating that the additional hormones alone caused the difference. The study was conducted using a selected sample, and not all potentially relevant reproductive

characteristics were incorporated into the treatment comparison. The finding is appropriately interpreted as evidence of a difference in observed response between the tested protocols under the conditions of the experiment.

Treatment Protocol 1 and Treatment Protocol 2 were not significantly different, whereas Treatment Protocol 3 was significantly different, as shown in Table 4.

Table 4: Oestrous onset following Treatment Protocols

	Treatment Protocol 1	Treatment Protocol 2	Treatment Protocol 3
Number of cows that showed oestrous	4[a]	6[a]	15[b]

[a, b] *Figures in the same row with different superscripts were significantly different.*

Source: Field Data (2026)

4.2 Conception in cows based on treatment protocols

There were differences in conception in cows based on different treatment protocols, Treatment Protocols 1, 2, and 3, as shown in Figure 3. Treatment Protocol 1 recorded four conceptions, Treatment Protocol 2 recorded six and Treatment Protocol 3 recorded 14. Treatment Protocol 3 therefore recorded the highest observed number of conceptions.

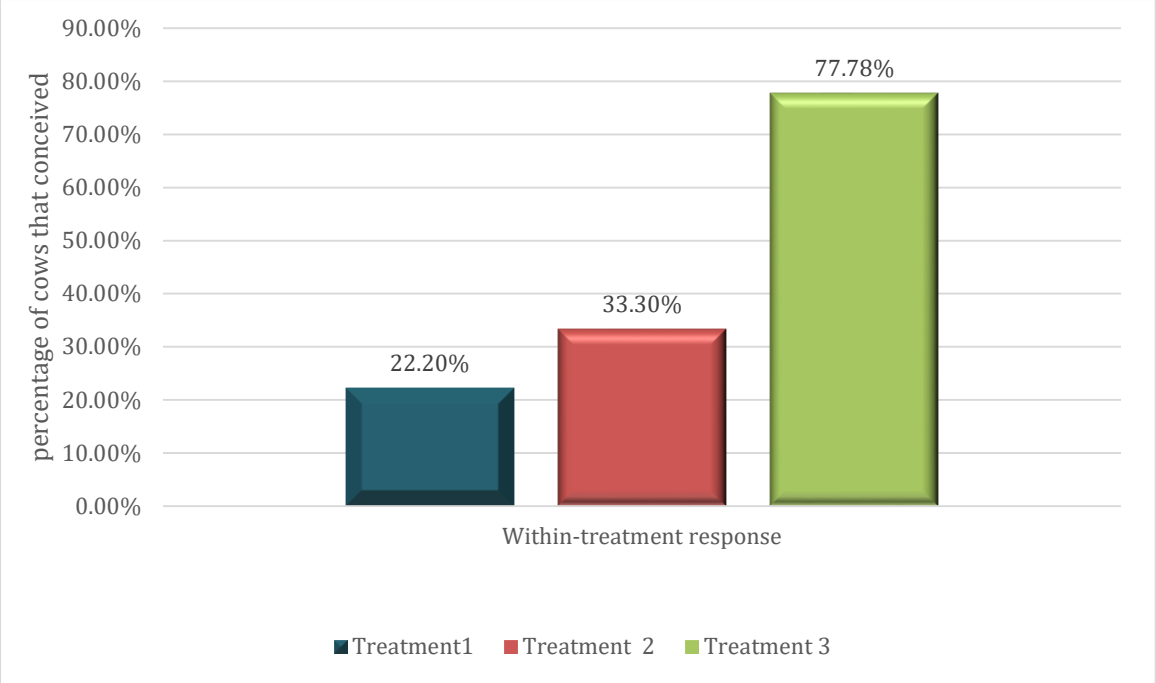


Figure 3: Conception in cows based on Treatment Protocols

Source: Field Data (2026)

Chi-square tests indicated a statistically significant difference in conception between cows subjected to Treatment Protocol 1 (Prostaglandin, $PGF_{2\alpha}$) and those under Treatment Protocol 3 (Progesterone [PRID] + Gonabreed [GnRH] + Prostaglandin, $PGF_{2\alpha}$), as well as between cows subjected to Treatment Protocol 2 (Gonabreed [GnRH] + Prostaglandin, $PGF_{2\alpha}$) and those under Treatment Protocol 3, and between cows subjected to Treatment Protocol 1 and those under Treatment Protocol 2 ($\chi^2 = 12.600$, df = 2, p = 0.002). Subsequent pairwise comparisons showed that a statistically significant difference in conception was observed between cows receiving Treatment Protocol 3 and those receiving Treatment Protocol 2, as well as between cows receiving Treatment Protocol 3 and those receiving Treatment Protocol 1. In contrast, no statistically significant difference in conception was observed between cows receiving Treatment Protocol 1 and Treatment Protocol 2, as shown in Table 5.

Table 5: Pairwise Comparisons of Treatment Protocols According to Conception

Treatment Protocols	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
Treatment Protocol 3-Treatment Protocol 2	12.000	4.514	2.658	0.008	0.024

Treatment Protocol 3-Treatment Protocol 1	15.000	4.514	3.323	0.001	0.003
Treatment Protocol 2-Treatment Protocol 1	3.000	4.514	0.665	0.506	1.000

Each row tests the null hypothesis that the Treatment Protocol distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

[a] *Significance values have been adjusted by the Bonferroni correction for multiple tests.*

Source: Field Data (2026)

The results therefore provide evidence that the observed conception outcome differed across the tested synchronization treatment protocols, with Treatment Protocol 3 producing the highest observed conception frequency. Nevertheless, conception following artificial insemination is a multifactorial reproductive outcome. The statistical association between treatment protocol and conception should not be interpreted as demonstrating that synchronization treatment was the sole determinant of conception.

In particular, factors such as parity, previous reproductive history, ovarian status, semen quality, inseminator competence, timing and technique of artificial insemination, nutritional status, disease status and other management conditions may influence conception. Where these factors were not independently measured or controlled in the present study, their possible contribution was acknowledged when interpreting the treatment differences. Thus, the findings support comparison of the observed performance of the three treatment protocols under the conditions of this study rather than a claim that Treatment Protocol 3 universally determines higher fertility.

Treatment Protocol 1 and Treatment Protocol 2 were not significantly different, while Treatment Protocol 3 was significantly different, as shown in Table 6.

Table 6: Conception following treatment protocols

	Treatment Protocol 1	**Treatment Protocol 2**	**Treatment Protocol 3**
Number of cows that conceived	4[a]	6[a]	14[b]

[a, b] *Figures in the same row with different superscripts were significantly different.*

Source: Field Data (2026)

For cows that received Treatment Protocol 1, 4 cows conceived. For cows that received Treatment Protocol 2, among the 6 cows that conceived, one cow never showed oestrous signs. For cows that received Treatment Protocol 3, among the 14 cows that conceived, 2 cows never showed visual oestrous signs.

CHAPTER FIVE

DISCUSSION

5.1 Discussion

5.1.1 Oestrous onset in cows with single and multi-agent synchronization treatment protocols

The reproductive efficiency is part of a greater system, where the fertility of the herd is a product of the herd baseline factors of energy balance, body condition score (BCS), metabolic status, uterine health, heat detection efficiency, ambient heat stress and AI technician skill (Crowe *et al*., 2018). The basic concept of hormonal synchronization is to play the master train conductor synchronizing all the cows' ovarian clocks, so that each cow can be inseminated at a set time without observing heat.

In this study, multi-agent Treatment Protocol 3 (PRID + GnRH + $PGF_{2\alpha}$) achieved a significantly superior oestrous onset of 83.3% (15/18) within 72 hours compared to 33.3% (6/18) for Treatment Protocol 2 (GnRH + $PGF_{2\alpha}$) and 22.2% (4/18) for Treatment Protocol 1 ($PGF_{2\alpha}$ alone) ($X^2 = 15.343$, df = 2, p = 0.000). The answer to why single-agent Prostaglandin ($PGF_{2\alpha}$) is not a good treatment alone is very simple: if there is a mature Corpus Luteum (CL) then $PGF_{2\alpha}$ will simply remove the CL; it does nothing else. If there is a mature Corpus Luteum (CL), then $PGF_{2\alpha}$ will simply remove the CL (Noguchi *et al*., 2017; Bihon & Assefa, 2021). $PGF_{2\alpha}$ will have no effect if a cow is not responsive with a mature CL on the day of injection. This is because $PGF_{2\alpha}$ does not show uniform and regular heat display (22.2%) as observed in the field by Prajapati *et al*. 2019 and Chanyalew *et al*. 2018.

In Protocol 2, the addition of GnRH is helpful, as it resets the system, triggering ovulation or luteinization of existing large follicles which stimulates a new cohort of follicles to grow at the same time in order to ensure uniformity (Marques *et al*., 2024). Without progesterone support, however, some cows exhibit heat too early before the planned AI day resulting in a slight 33.3% heat synchrony (Kothandaraman *et al*., 2023). All these are treated with Treatment Protocol 3, which involves the use of an intravaginal Progesterone insert (PRID Delta®). Progesterone is an 'emergency brake' or holding gate: it mimics a fake pregnancy and suppresses the release of pulses of luteinizing hormone (LH) and guarantees that all cows don't go into heat prematurely while waiting for the new egg to reach maturity (Morini *et al*., 2019). In this case the brakes are suddenly removed when the PRID is retrieved at the same time as $PGF_{2\alpha}$ injection on Day

7. In response to the last GnRH injection given at AI, there is a sudden release of natural estrogens from the mature dominant follicle that is associated with 83.3% of strong standing heat and to a synchronized ovulation (Tschopp *et al*., 2022; Bogdan *et al*., 2026; Omar *et al*., 2025).

This success was greatly influenced by non-hormonal factors in the ecosystems. The cows were in a deep nutritional energy deficit and/or in disease-induced anestrus if they were not maintained at a Body Condition Score between 3.5 – 4.0, dewormed and treated with trypanocide and had 60+ days postpartum rest, respectively (Butler, 2003; Troxel, 2012; Gizaw *et al*., 2016). In addition, walking activity on 1,099 acres and tropical heat stress increase corticosteroid levels which usually inhibit brain signalling (LH pulses) to display heat. Progesterone priming in Protocol 3 was found to be effective in buffering these environmental stress factors and resulted in a mature and fertile egg at Fixed-Time AI.

5.1.2 Conception in cows with single and multi-agent synchronization treatment protocols

The best criterion for determining the reproductive success of cows is conception, but the initial clue would be whether the cow is in heat. In simple terms, there has to be a healthy egg, which has been fertilized as well as a healthy and fertile uterus that would accommodate the developing embryo (Ayantoye *et al*., 2025). The result of the Chi-Square test indicated that there is a statistical difference (χ^2=15.343, df=2, p=0.00) between the protocols concerning the number of confirmed conceptions at 30/45 days. This is evident based on the empirical evidence provided by Sundar (2024), Haile *et al*. (2023) and Bogdan *et al*. (2026) whereby the conceptions for Multi-agent Protocol 3 are 77.8% while those of Protocol 1 and Protocol 2 are 22.2% and 33.3% respectively.

In a more basic sense, there is a battery charger effect of the PRID insert, as progesterone gives a boost to the developing follicle. During the 7-day incubation period, LH pulses are maintained at a slow and steady frequency, exogenous progesterone increases LH receptor density of the granulosa and theca cells of the follicle (Colazo *et al*., 2013; Morini *et al*., 2019). This yields a well-developed oocyte that is able to undergo complete fertilization and good cleavage (Fricke & Wiltbank, 2022). Importantly, this progesterone-primed follicle then ovulates and transforms into a super-charged corpus luteum that in early pregnancy, produces large amounts of natural progesterone. The endometrium produces ‘uterine milk’ (histotroph) after embryo formation because of the high level of progesterone to support the development of the embryo and prevent early embryonic death (Prajapati *et al*., 2019; Bogdan *et al*., 2026).

The conception in Protocol 1 (22.2%) and Protocol 2 (33.3%) were very low, which may indicate that there are ovulatory problems. Whereas in the single protocols of $PGF_{2\alpha}$, poor-quality eggs with poor fertilization capacity (Bihon & Assefa, 2021) are there in the case of aged and 'stale' follicles. Ovsynch protocols with two agents do not contain progesterone, and this may be in the absence of sufficient luteal phase support, leading to premature LH release that could reduce egg quality and result in weak luteal phases following AI (Kothandaraman *et al*., 2023; Marques *et al*., 2024).

It was one of the most important findings of this study that 2 cows in Protocol 3 became pregnant despite not displaying any visual behaviour that indicated they were in heat (standing heat 'silent heat'). Cows may ovulate quietly (no mounting) during periods of high staking out and hot weather when the cows are extensively grazing the paddocks (Sveberg *et al*., 2015). This will be a clear sign that Fixed-Time AI (FTAI) with progesterone priming (also known as FTAI) is effective in preventing all of the Heat Detection Errors and will guarantee breeding success (Roelofs & Kooij, 2015; Fricke & Wiltbank, 2022).

In the bigger picture of livestock development and policy, adopting multi-hormone kits by individual smallholders can be costly initially, but very cost-effective in nucleus breeding farms such as Njeru Stock Farm (NAGRC&DB) in the state. To enable the public stock farms to increase the number of pregnant high-yielding dairy heifers to be supplied to the smallholder farmers in the country, the number of farms can be multiplied and this will reduce the labour required to detect heat in the heifers and boost dairy commercialization in the country through the Parish Development Model (PDM) (Mbabazi, 2026; NAGRC&DB, 2026; IGC, 2024).

5.2 Limitations of the study

- Because early pregnancy factor test kits were unavailable due to their high procurement and logistical costs, early biochemical confirmation of pregnancy was not possible. Conception was assessed using rectal hand palpation. This limited the study's ability to obtain early objective confirmation of pregnancy, although it did not prevent assessment and comparison of conception outcomes across the three treatment protocol groups.

- The absence of a statistically significant difference in reproductive outcomes between Friesian and Jersey cows in the present study may have been influenced by the

relatively uniform environmental and management conditions under which both breeds were maintained. The cows were kept under the same free-range management system and therefore experienced similar feeding and nutritional conditions, paddock environment, disease-control practices, handling procedures and synchronization management. These common conditions may have reduced environmental variation between the breeds, making breed-related differences in oestrous response or conception less obvious within the study population.

- Although pre-treatment rectal palpation was undertaken to confirm non-pregnancy, the study did not specifically investigate all possible subclinical reproductive disorders such as ovarian cysts. Therefore, undetected reproductive conditions cannot be completely excluded as potential sources of individual variation in reproductive response; however, no evidence from the present study establishes their presence or influence on the observed treatment outcomes.

- The study was based mostly on the visual observation of oestrous signs, which is subjective in nature and may cause misclassification.

- Although the sample size (54 cows) was sufficient for comparing experimental results, it does not bring enough generalizability to the various production systems, agro-ecological regions, and large commercial dairy herds.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The relative effectiveness of the two different hormonal synchronization programs (single agent and multi-agent programs) in improving reproductive efficiency of dairy cattle for open-paddock grazing production was evaluated at Njeru Stock Farm. Based on the empirical findings, the study draws the following specific conclusions in line with the two research objectives:

Treatment Protocol 3 (Progesterone (PRID) + Gonabreed (GnRH) + ($PGF_{2\alpha}$) is significantly better than both two-agent (Gonabreed (GnRH) + Prostaglandin ($PGF_{2\alpha}$) and single-agent (Prostaglandin ($PGF_{2\alpha}$) treatment protocols at synchronizing oestrous onset. Single-agent prostaglandin treatments alone however are biologically inadequate for achieving a predictable heat synchrony while grazing under open paddock conditions as they do not provide a control on follicular wave emergence, while the addition of an exogenous progesterone priming to prostaglandin and a GnRH treatment allows for a control of follicular wave dynamics and guarantees tight, synchronized standing heat.

In addition, the confirmed conception after the application of Fixed-Time Artificial Insemination (FTAI) is very high in treatment protocol 3 versus the other two treatment protocols. Single-agent Prostaglandin ($PGF_{2\alpha}$) and two-agent Gonabreed (GnRH) + Prostaglandin ($PGF_{2\alpha}$) progesterone-primed fixed-time artificial insemination is a tool to synchronise ovulation of fertile oocytes, without depending on visual heat expression and overcomes silent heat bottlenecks and heat detection errors under pasture-grazing conditions.

6.2 Recommendations

The study recommends Treatment Protocol 3, which consists of Progesterone (PRID) + Gonabreed (GnRH) + Prostaglandin ($PGF_{2\alpha}$). This is because multi-hormone synchronization leads to higher oestrous onset and conception.

According to the research, Treatment Protocol 3 can be utilized on all breeds of cows in Uganda because it can produce similar results in terms of oestrous onset and conception.

Appropriate management conditions should be maintained before oestrous synchronization and conception assessment. These conditions include adequate nutrition, provision of mineral supplements and appropriate animal-health management, particularly under a free-range production system. However, these management factors were not independently evaluated in the present study; therefore, their specific contribution to oestrous onset or conception cannot be established from the study findings. They should consequently be regarded as general management considerations rather than factors demonstrated by this study to cause improved synchronization or conception outcomes.

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APPENDICES

Appendix I: Acceptance Letter

National Animal Genetic Resources Center and Data Bank (NAGRC&DB)
P.O.Box 183, Entebbe - Uganda, Plot. 98-106, Nsamizi Road
Tel: 0414-320 831 Email: executivedirector@nagrc.go.ug Website: nagrc.go.ug
THE REPUBLIC OF UGANDA

In any correspondence on this subject
Please quote No. NAGRC&DB/29/OCT/2023

Directorate of Research
And Graduate Training
Makerere University

Dear Sir/Madam,

RE: ACCEPTANCE OF CONDUCTING RESEARCH FOR MS MAGAMBO JUMAH

I am pleased to offer the fore-mentioned graduate student to undertake a research study practicum titled **"Optimization Of Estrus Synchronization Protocol And Conception In Dairy Cattle" at Njeru Stock Farm, Uganda by student No:2000721233 Magambo Jumah 2020/Hd17/21233U** at Njeru Stock Farm, one of the branches of National Animal Genetic Resources Centre and Data Bank (NAGRC&DB). The National Animal Genetic Resources Centre and Data Bank is a semi-autonomous agency of the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF). This farm is engaged in cattle and piggery production.

The study is intended solely for academic purposes and will be conducted in line with ethical research standards. The research will involve 54 (population/sample size), review of relevant institutional records and documents where applicable, and/or administration of questionnaires or interviews as approved.

The findings of the study will be shared with NAGRC&DB upon completion, where relevant, to contribute to knowledge generation and support institutional improvement. The student will ensure flexibility in scheduling to minimize any disruption to staff and ongoing activities.

We thank you for your continued support of academic research and national development.

Sincerely,

Ag. Finance and Administration Manager

National Animal Genetic Resources Center and Data Bank (NAGRC&DB)

NATIONAL ANIMAL GENETIC RESOURCES CENTRE & DATA BANK (NAGRC & DB)
P O. BOX 183. ENTEBBE - UGANDA
TEL. +256 (41) 320831

Appendix II: Study Tool

			Treatment Protocol 1		**Treatment Protocol 2**		**Treatment Protocol 3**		
DAM Tag no.	Breed	Body Condition Score	Oestrous onset	Conception	Oestrous onset	Conception	Oestrous onset	Conception	Number of lactations
23	FR	3.5	NO	NO					2
6356	FR	3.5	NO	NO					2
6421	FR	3.5					YES	YES	3
6351	FR	3.5			NO	NO			2
2006	FR	3.5	NO	NO					2
4037	FR	3.5	NO	NO					2
9671	FR	3.5			YES	YES			2
6076	FR	3.5			NO	NO			3
3197	FR	3.5	YES	YES					3
4189	FR	3.5	NO	NO					3
4013	FR	3.5					YES	YES	3
26	FR	3.5			NO	NO			2
871	FR	3.5					YES	YES	2
4169	FR	3.5			NO	NO			2
9679/2	FR	3.5					YES	YES	2
3988	FR	3.5			YES	YES			2
5753	FR	4	NO	NO					2

0551	FR	4	YES	YES					3
4189	FR	4	NO	NO					3
62534	FR	4					YES	NO	2
4048	FR	4			NO	NO			2
9679/1	FR	4			NO	NO			2
4191	FR	4					NO	NO	2
8204	FR	4					YES	YES	3
4182	FR	4			YES	YES			2
6325/1	FR	4					YES	YES	2
3227	FR	4					YES	YES	2
3228	JER	3.5	NO	NO					2
0859	JER	3.5					YES	YES	2
223	JER	3.5			YES	YES			2
0106	JER	3.5					YES	NO	3
5428	JER	3.5			NO	NO			2
3135	JER	3.5			NO	NO			2
6735	JER	3.5	NO	NO					2
233	JER	3.5	NO	NO					2
090	JER	3.5	NO	NO					3
6206	JER	3.5	NO	NO					2
0554	JER	3.5	NO	NO					2
4028	JER	3.5			NO	NO			3
1104	JER	3.5					NO	NO	2
1004	JER	3.5			NO	NO			2

1110	JER	3.5					YES	YES	3
1122	JER	3.5	YES	YES					2
013	JER	3.5					YES	YES	2
1123	JER	3.5	YES	YES					2
1080	JER	3.5			NO	NO			3
1062	JER	4	NO	NO					2
1019	JER	4			YES	YES			2
1023	JER	4			NO	NO			3
1017	JER	4					YES	YES	2
1003	JER	4					NO	YES	2
1077	JER	4			YES	YES			2
119	JER	4					YES	YES	2
1025	JER	4					YES	YES	3

Appendix III: Experiment Activities In Photos

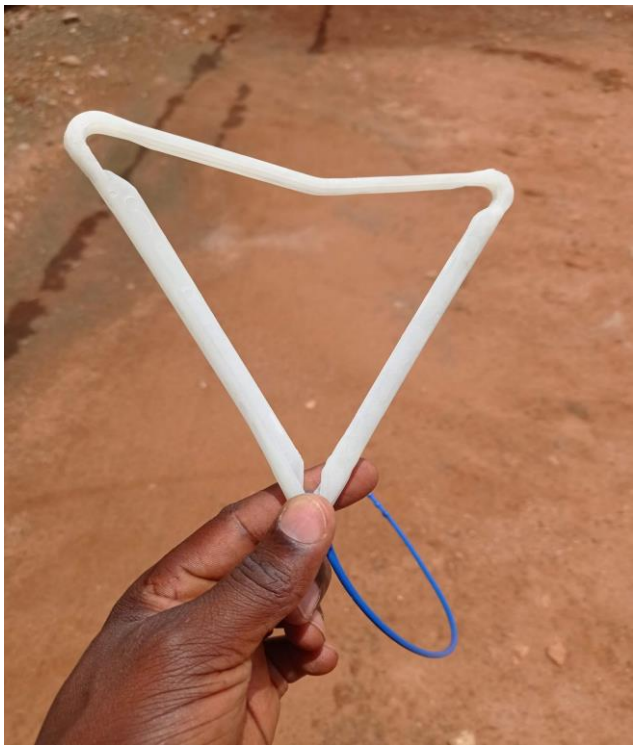


Plate 1. PRID Delta, which was used in the experiment



Plate 2. Van where environmental effects were controlled



Plate 3. Inseminating a Friesian cow in the experiment



Plate 4. **Pregnancy Diagnosis Using Recto Palpation in Jersey Cows**



Plate 5. **Jersey Cows in the Cattle Crush**