Comparison of Estrus Signs and Pregnancy Rate Between Estrus Synchronised Cows Using CIDR Insertion and Modified Herbs Feeding

By

Amirah Fazila Binti Jamaludin

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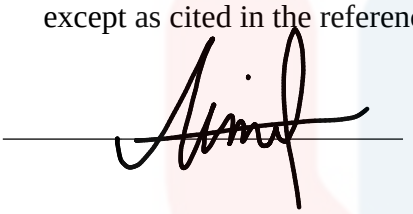
DECLARATION

I hereby declare that the work embodied in here is the result of my own research except as cited in the references.



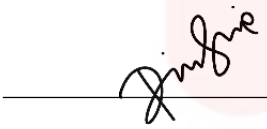
Signature

Student's Name : Amirah Fazila Binti Jamaludin

Matric No : F18A0001

Date : 26 January 2022

Verified by :



Supervisor Signature

Supervisor's Name : Dr Raja Ili Airina bt Raja Khalif

Stamp :

Dr. Raja Ili Airina binti Raja Khalif
Pensyarah Kanan
Fakulti Industri Asas Tani
Universiti Malaysia Kelantan
Kampus Jeli

Date : 26 January 2022

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Comparison of Estrus Signs and Pregnancy Rate Between Estrus Synchronised Cows Using CIDR Insertion and Modified Herbs Feeding

ABSTRACT

Estrus synchronization, which is a reproductive process manipulation, offers a number of advantages. Several estrus synchronization protocols have been developed, each of which employs a combination of drugs and products to modify hormonal changes in the female estrus cycle. Since different reproductive hormones are used in estrus synchronization, having a simple understanding of them will help farmers figure out the protocol would function best for their herd. Controlled Internal Drug Release (CIDR) is a popular method of obtaining estrus in female cattle that is also used commercially. This method, on the other hand, is costly and has many drawbacks especially to small-scale farmers. Therefore, an alternative method of herbal treatment, which is cheaper and easy to obtain will be use as a replacement of CIDR device. The purposes of this study are (1) to compare secondary estrus signs, estrus onset and duration of estrus between CIDR and herbs treatment in cattle and (2) to determine the efficiency of CIDR and herbs on pregnancy rate. 8 cows were divided into two groups in this study, (A) Herbs group (n = 5) and (B) CIDR group (n=3). Estrus sign were observed for 45 minutes at 0700, 0900, 1100, 1300, 1500, 1700, 1900, and 2100. The observation was carried out after three days of continuous feeding of modified herbs (A) and CIDR withdrawal (n=7days)(B). The cattle were mated naturally followed by pregnancy diagnosis with rectal palpation, and urine test (PregnaDrop). Restlessness and sniffs the other vulva in Group A (Herbs) showed significantly higher ($P<0.05$) compared to Group B(CIDR). All primary estrus signs of Group A (Herbs) in the evening showed significantly higher than Group B (CIDR). Secondary signs which vulva swelling and reddening showed 100% (n=8) for both treatments. The outcome of this study showed that Herbs can be a replacement of CIDR in estrus synchronization.

Keywords: Estrous synchronization, Control Internal Drug Release (CIDR), herbs, pregnancy diagnosis

Perbandingan Tanda Estrus dan Kadar Kehamilan Antara Lembu yang Bersinkronisasi Estrus Menggunakan Sisipan CIDR dan Pemberian Herba Ubahsuai

Penyegerakan estrus, yang merupakan manipulasi proses pembiakan, menawarkan beberapa kelebihan. Beberapa protokol penyegerakan estrus telah dibangunkan, setiap satunya menggunakan gabungan ubat dan produk untuk mengubah suai perubahan hormon dalam kitaran estrus betina. Memandangkan hormon pembiakan yang berbeza digunakan dalam penyegerakan estrus, pemahaman yang mudah tentangnya akan membantu petani mengetahui protocol yang akan berfungsi dengan baik untuk kumpulan mereka. Sisipan *Control Internal Drug Release* (CIDR) adalah kaedah popular untuk mendapatkan estrus dalam lembu betina yang juga digunakan secara komersial. Kaedah ini pula memakan kos yang tinggi dan mempunyai banyak kelemahan terutamanya kepada petani berskala kecil. Oleh itu, kaedah alternatif rawatan herba yang lebih murah dan mudah diperolehi akan digunakan sebagai pengganti peranti CIDR. Tujuan kajian ini adalah (1) untuk membandingkan tanda-tanda estrus, permulaan estrus dan tempoh estrus antara rawatan CIDR dan herba dalam lembu dan (2) untuk menentukan kecekapan CIDR dan herba terhadap kadar kehamilan. 8 ekor lembu telah dibahagikan kepada dua kumpulan dalam kajian ini, (A) Kumpulan herba (n = 5) dan (B) kumpulan CIDR (n=3). Tanda estrus diperhatikan selama 45 minit di 0700, 0900, 1100, 1300, 1500, 1700, 1900, dan 2100. Pemerhatian dilakukan selepas tiga hari pemberian berterusan herba ubah suai (A) dan pengeluaran CIDR (n=7hari) (B). Lembu itu dikawinkan secara semula jadi diikuti dengan diagnosis kehamilan dengan palpasi rektum, dan ujian air kencing (PregnaDrop). Kegelisahan dan menghidu vulva lain dalam Kumpulan A (Herba) menunjukkan lebih tinggi secara ketara ($P<0.05$) berbanding Kumpulan B(CIDR). Semua tanda estrus utama Kumpulan A (Herba) pada waktu petang menunjukkan secara ketara lebih tinggi daripada Kumpulan B (CIDR). Tanda-tanda sekunder yang mana vulva bengkak dan kemerahan menunjukkan 100% (n=8) untuk kedua-dua rawatan. Hasil kajian ini menunjukkan bahawa Herba boleh menjadi pengganti CIDR dalam penyegerakan estrus.

Kata kunci: Penyegerakan estrus, *Control Internal Drug Release* (CIDR), herba, diagnosis kehamilan

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LIST OF ABBREVIATIONS

AI	Artificial Insemination
CIDR	Control Internal Drug Release
MH	Modified Herb
FSH	Follicle-stimulating hormone
LH	Luteinizing Hormone
ml	Mililiter
g	Gram
SEM	Standard Error of Mean
h	Hour

LIST OF SYMBOLS

%	percentage

CHAPTER 1

INTRODUCTION

1.1 Research Background

Malaysia's livestock industry is the most significant source of protein for the country's population, however, because of the constant growth of the human population and consumption, the ruminant industry is unable to satisfy local demand. Understanding and assessing the problem is critical for developing a more comprehensive ruminant breeding and management programme. The small-scale farmers are currently dealing with a major problem that is anestrus which is the major contributor to infertility in cattle. In short, only a few reproductive treatments have been identified to address this limitation (Nazhiifah, 2020).

The detection of estrus is one of the most important factors influencing reproductive efficiency in dairy cattle, particularly in farms that use artificial insemination (AI). Reproduction management has a direct impact on the calving-to-conception interval, which affects the calving interval and milk production, both of which have an impact on profit (Reith, 2017).

Herbs are a good source and well-known for their ability to cause timely estrus and ovulation in animals while causing no harm to their uterus (Nazhiifah, 2020). The use of herbs as additives in livestock nutrition in order to lessen the disadvantages of

technology devices, becomes a new goal in animal production. Modified herbs are an alternative replacement of intravaginal devices, CIDR which has lots of advantages to all users especially small-scale farmers as it doesn't require professional skills.

The aim of this study is (1) to prove that modified herbs can be an alternative of CIDR device replacement, an estrus inducer in cattle to treat anestrus which is successful in previous study, treating the herbs on goats. This study also will (2) compare the reproductive performances of cattle with two different treatments, insertion of CIDR and modified herbs.

1.2 Problem Statement

Estrus synchronization guarantees effective breeding control by simultaneous modulation of the estrous cycle in animals, however, ruminant industry especially cattle breeding programs still have problems in fertility, such as anestrus which may cause great economic loss. Other than that, CIDR insertion is not applicable on a small scale husbandman as it is costly and needs experienced labour. This study focuses on the comparison of estrus sign, estrus onset and duration onset between CIDR and modified herbs which is cheaper and doesn't even need a skill to use it. This study also focuses on the determination of the efficiency of conception and birth rate in cattle with CIDR and modified herbs. This study also will use a big sample as the outcome of the previous study on goats varied due to the limited number of samples.

1.3 Hypothesis

This study comprises of two hypotheses:

H_0: Modified herb shows same estrus signs, estrus onset and duration of estrus compared to CIDR in cattle

H_1: Modified herb shows different estrus signs, estrus onset and duration of estrus compared to CIDR in cattle.

H_0: Modified herb shows same conception rate compared to CIDR in cattle

H_1: Modified herb shows different conception rate compared to CIDR in cattle.

1.4 Research Objectives

The objectives of this study are:

1. To compare secondary estrus signs, estrus onset and duration of estrus between CIDR and herbs treatment in cattle.
2. To determine the efficiency of CIDR and herbs on pregnancy rate

1.5 Research Scope

This research mainly aims to compare the reproductive performances of two treatments in order to explain the objectives and hypothesis stated. First, the preferred subjects to be compared on this study is secondary estrus signs, estrus onset and duration of estrus between CIDR and modified herbs treatment in cattle. Second, this study will achieve the result of pregnancy of the cattle by natural mating after the insertion of CIDR and orally given modified herbs to the cattle.

1.6 Research Significance

The uncovering of this study is noteworthy for economic growth of ruminant industry sector in Malaysia as the ingredients in the formulation of modified herb are obtainable and low-cost. In addition, this study also gives farmers especially small scale husbandmen an elementary knowledge in planning breeding management strategy for their livestock. Besides, if the outcome of this study shows modified herb works better in the comparison, then this can be proposed to use modified herbs as a replacement of CIDR in future which doesn't even need experienced labour. In some cases, when CIDR is removed, it may cause vaginal inflammation, resulting in clear, cloudy, or yellow mucus. This is natural and has no bearing on overall effectiveness (Lamb, 2016).

1.7 Limitation of Study

The limitations in this study is less number of female cow use for modified herbs and CIDR as the other female cows are pregnant. Next, these cows were difficult to handle as they were grazing freely. Other than that, the cows were having Lumpy Skin Disease (LSD) and need to be treated first. This makes the starting time of the project was postponed. Lastly, the weather also limited the movement of the project as the farm which located at Kg Tebing, Napoh, Jitra, Kedah was raining heavily and flooding twice.

CHAPTER 2

LITERATURE REVIEW

2.1 Cattle Industry in Malaysia

Malaysia's livestock industry is one of the most important industries in the country's agricultural development which the business includes both ruminants and non-ruminants. It accounts for roughly 18% of the entire Food Sector Agriculture Value Added and export revenues (NAP, 1998). Currently, beef and dairy cattle, dairy buffaloes, sheep, and goats are still raised on a limited basis (Mohamed, 2007). Lack of land resources, high feed prices, cheaper import equivalents, low private sector involvement, and disease prevention and management are all problems that contribute to the ruminant sector's lag (Shanmugavelu, 2014). In recent years, substantial progress has been made, including the modernization of Malaysia's livestock industry in order to meet local demand.

2.2 Productivity of Ruminant Industry

"Further growth of the agricultural sector requires that the nation face the challenge of efficient and optimal usage of current resources in order to promote

competitiveness," according to the Third National Agricultural Policy (NAP3, 1998-2010). Constraints on resources and rapid changes in the global trade environment necessitate the growth of a resilient agriculture industry and the improvement of its worldwide competitiveness. Productivity increases, market development and strengthening, and the removal of market and trade distorting measures are only some of the ways the sector's competitiveness will be improved. Meat output in the country is still low, at 23%. As a result, Malaysia must import more than 70% of its beef. The majority of Malaysia's beef, mutton, and dairy products come from India, Australia, and New Zealand.

2.3 Female Reproductive Physiology and Endocrinology of Cattle

Hormones secreted by the hypothalamus and pituitary glands, as well as the ovary and uterus, govern the estrous cycle in general. The follicular phase (maturation of the preovulatory follicle), estrus, and luteal phase are the three stages of the estrous cycle. In a nonpregnant female, the follicular phase begins when the corpus luteum begins to recede. The pulsatile production of LH rises when plasma progesterone concentrations decrease (due to diminished progesterone negative feedback). Estradiol plasma concentrations rise in tandem with LH levels, resulting in estrous activity (estrus) and induction of the preovulatory gonadotropin surge. The preovulatory surge of LH sets in motion a series of intrafollicular processes that lead to follicular rupture and luteinization.

Following ovulation, the luteal phase begins and concludes with luteolysis. Progesterone concentrations are favourably linked with variations in luteal weight during the luteal phase. During the luteal phase, the pattern of LH secretion alters. The pattern of LH secretion has been described as pulses of high frequency and low amplitude during luteal formation or regression, when plasma progesterone concentrations are low; however, during the mid-luteal phase (high progesterone), the pulsatile pattern of LH secretion has been described as low frequency and high amplitude. During the late luteal phase in cattle, uterine release of prostaglandin F2cx (PGF2cx) increases, causing corpus luteum regression (Allan *et al.*, 1993).

2.4 Estrus signs

Estrus signs can be classified in two signs which are primary sign and secondary signs. Secondary signs of estrus are the cattle showed behaviour such as restless, she rests chin on the back of others and also started to sniff their vulva followed by mounting other cows. In secondary signs, the vulva of the cows started to swell and discharging mucus. A long term treatment is required to await a high estrus synchronization rate if progesterone is used alone without a combination with prostaglandin ($PGF_{2\alpha}$) or estradiol (Yamada, 2005). Synchronization of estrus using progesterone alone often results in poor conception rate. Estrus can be synchronized by injecting $PGF_{2\alpha}$ during luteal phase (Yamada, 2005). In order to improve a synchronization rate of estrus, the cows have to be injected with gonadotropin releasing hormone (G_nRH) a week before $PGF_{2\alpha}$.

2.5 Estrus detection

A successful performance of reproductive in cattle needs precise and structured estrus detection. Low estrus detection rate has been identified as an important factor affecting the reproductive efficiency (Lopez et al., 2004). A clichéd method used for estrual cow detection is visual observation which the accuracy is depending on the experience of the viewer, the frequency, duration and timing of observation period and the intensity of estrus. Estrus detection rates of over 90% have been identified with cows in herds with seasonally concentrated calving systems that can express estrus under ideal conditions while grazing pasture (Macmillan, 2010). The intensity of estrus and its duration have drastically fallen off over the last decades making detection more difficult to farmers (Chanvallion *et al*., 2014). Cows show primary signs at the onset of estrus, however, sniffing and chin resting are not useful as forecasters of estrus because they are not shown by all animals at every estrus and also presented at other stages of the estrus cycle with no apparent consistency (Solano *et al*., 2005).

2.6 CIDR

Controlled Internal Drug Release (CIDR), designed as a T-shaped nylon spine moulded with a progesterone-containing silicone rubber skin is an intravaginal progesterone insert in cattle which is used for the synchronization of estrus. There are several advantages of CIDR such as higher pregnancy rate, profitable to farmers and also can know exact breeding and calving date of cows. Farmers also don't have to observe

cow heat by the use of CIDR. CIDR also has a few disadvantages, including the need for experienced labour, which can result in high costs. A CIDR could potentially be used at least twice in current estrus synchronization protocols as its inserts release progesterone at least 15 days (Macmillan *et al*., 1991; Macmillan and Peterson, 1993). When ovariectomized beef cows were given a once-used CIDR, a concurrent injection of 100mg progesterone was needed to achieve plasma progesterone concentrations (by 24 hours after CIDR insertion) that were comparable to those in cows given a new CIDR (Martnez et al., 2003).

2.7 Herbs

In previous study, local herbs were used as an alternative replacement of CIDR in goats and it gives a successful outcome. Modified herbs had the highest estrus response rate of 80.56 percent, followed by CIDR at 77.78 percent (Nazhiifah, 2020). The herb is a non-hormonal, all-natural supplement that aids in ovarian function coordination. Herbs are well-known for their ability to efficiently induce timely estrus and ovulation while causing no damage to an animal's uterus. It does not necessitate the use of skilled labour to care for the animals. The modified herbs are formulated with local herbs which are cheap and easy to get such as extracts of *Piper nigrum L., Zingiber officinale, Cananga odorata,* and *Cinnamomum verum.*

2.7.1 *Piper nigrum L.*

Piper nigrum Linnis, the black pepper plant, is a member of the piperaceae family. The alkaloid pyrene is responsible for the majority of the effects of black pepper. The findings of investigations suggest that black pepper has anti-inflammatory properties (Yaffe, Power Coombs, Doucette, Walsh, & Hoskin, 2015). For thousands of years, black pepper has been used as a pain reliever in traditional medicine. This effect is attributed to an alkaloid known as pyrene (Takooree et al., 2019). P. nigrum is used for pain alleviation, chills, rheumatism, flu, muscular aches, colds, tiredness, and fevers, as a nerve tonic, to enhance blood circulation, saliva flow, stimulate hunger, and encourage peristalsis (Pruthi, 1993; Ravindran, 2000; Sharon, 2002). Pyrene has an antioxidant effect and prevents lipid peroxidation in carcinogenic materials (Gülçin, 2005; Liu, Yadev, Aggarwal, & Nair, 2010; Prakash & Nikousaleh, 2018). Piperine is the main bioactive compound found in Piper nigrum and Piper longum, and it has been shown to have immunomodulatory, anti-carcinogenic, anti-asthmatic, stimulatory, hepatoprotective, anti-inflammatory (Darshan and Doreswamy, 2004), and antimicrobial properties (Yang et al., 2002) and anti-ulcer activities (Bai and Xu, 2000). P. nigrum were reported to treat menstrual disorders.

2.7.2 *Zingiber officinale*

Ginger was first sold as a dietary supplement in combination with other herbs, vitamins, and minerals for medical purposes (Chang et al., 2012). Piperine can also be found in Zingiber officinale (ginger), which has antioxidant and anti-inflammatory properties (Reddy and Lokesh, 1992). (Jiang, 2005). It also has anti-oxidant and biotransformative properties, and it has been shown to help medications like rifampicin, sulphadiazine, tetracyline, and phenytoin absorb better (Wu, 2007). On mitochondrial bioenergetics and energy metabolism enzymes, piperine has concentration-dependent site-specific effects (Jamwal and Singh, 1993). Ginger also aids in the improvement of bioavailability by increasing intestinal absorption (Prakash and Srinivasan, 2000). The antioxidant chemicals gingerols, zingerone, zingiberene, glucosides-6-gingerdiol, flavonoids, and volatile oil are all abundant in ginger root. These antioxidants protect both the reproductive organs and the body from oxidative stress, lipid peroxidation, and an imbalance of prooxidants and antioxidants in favour of the former. In the testis, prostate, and epididymis, ginger roots were found to increase the activity of antioxidant enzymes such superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Banihani, 2018). Ginger has been shown to be an abortifacient when combined with black pepper in antifertility and abortifacient herbal medications (Chopra et al; 1969).

2.7.3 *Cinnamomum verum*

Cinnamon has been shown to exhibit anti-inflammatory (Kim et al., 2007), antibacterial (Lopez et al., 2007), antifungal (Velluti et al., 2004), antiviral (Premanathan et al., 2000), antihyperglycaemic (Ka et al., 2003; Schoene et al., 2005), antihyperlipidaemic (Kim & Choung Moreover, numerous researchers (Jayaprakasha et al., 2006; Prasad et al., 2009; Ciftci et al., 2010; Azab et al., 2011) have found that extracts from various cinnamomum species have free radical scavenging and significant antioxidant activity. In laboratory animals, ethanolic extracts of C. zeylanicum bark improved reproductive organ weights (Shah et al., 1998), sperm quality parameters (Shah et al., 1998; Hafez, 2010; Shalaby & Mouneir, 2010), and LH, FSH, testosterone concentrations (Modaresi et al., 2009; Hemayatkhah Jahromi et al., 2011). C. zeylanicum boosts LH, FSH, and testosterone levels significantly without changing testicular weight (Modaresi et al., 2009). Menstrual periods can be normalised by using a Cinnamomum cassia supplement for at least six months. Cinnamon, as a bioactive medicine, reduced anti-mullerian hormone levels while also having less negative effects than Metformin. (Moini et al., 2019)

2.8 Pregnancy diagnosis

The cattle industry relies on early pregnancy detection to maximise herd productivity. It's been a long time since ruminants like cattle had a reliable and quick pregnancy test. There are many tests available, including a milk progesterone assay

(Oltenacu et al., 1990; Markusfeld et al., 1990), estrone sulphate analysis (Holdsworth et al., 1982; Warnick et al., 1995), rectal palpation (Hatzidakis et al., 1993), ultrasound (Beal et al., 1992; Cameron and Malmo, 1993), and blood test for pregnancy-specific antigens. To maximise the benefits of breeding by shortening the calving cycle, farmers need prompt and reliable pregnancy diagnosis, which allows them to identify open animals so that they can be treated and rebreed as soon as possible. Pregnancy diagnosis can be performed by day 40 of gestation. A positive pregnancy diagnosis was 100 percent accurate by day 40 of pregnancy, while overall pregnancy and absence of pregnancy diagnoses were right in 94 percent and 90 percent of cases, respectively, from day 25 of pregnancy, Hanzen and Delsaux (1987).

2.8.1 Rectal palpation

Palpation pregnancy diagnosis, a method to feel the texture variations in the pregnant uterus, fetus, and uterine artery with the hand, is an effective method for evaluating the effectiveness of a cattle herd's reproductive management. Rectal palpation of the cow is the most cost-effective method of diagnosing pregnancy in the cow, assessing reproductive physiological status, and diagnosing oviductal, uterine, and ovarian pathologies (Lopes, 2006). Rectal palpation is also the most popular procedure for detecting pregnancy. While palpation is a simple procedure, using breeding records improves the precision of the diagnosis and speeds up the palpation method. Although it is not difficult to establish pregnancy in cattle through palpation technique, it takes practise, experience, and a detailed understanding of the cow's reproductive system to

determine the stages of gestation at 30-day intervals to avoid irritation of the cow's rectum and injuring the fetus.

2.8.2 Ultrasonography

Ultrasonography has a number of advantages over rectal palpation, including fetal sexing and early embryonic detection. An ultrasonography examination of the amniotic vesicle and embryo or foetus size was used to estimate gestational age (Youngquist and Threlfall, 2007). It is also less invasive. Ultrasound has enabled us to visually characterise the uterus, fetus, ovary, corpus luteum, and follicles for research purposes. Ultrasound is a fast way to diagnose a pregnancy, and experienced palpators can easily adjust to it. At the end of a 108- day breeding season, palpation per rectum took 11.3 seconds on average to assess pregnancy in beef heifers, versus 16.1 seconds for ultrasound to assess pregnancy and fetal age (Galland *et al*., 1994). DesCoteaux and Fetrow (1998) found ultrasound to be an economically profitable reproductive control technique for dairy farms. The theoretical basis for detecting fetal fluid in the uterus and blood flow in the uterine artery during early pregnancy diagnosis is based on the physical properties of ultrasound, such as directional changes in its dissemination and reflection (Pieterse *et al*., 1990).

2.8.3 PregnaDrop Test Kit

Figure 2.7.3 PregnaDrop Test Kit

Test Kit PregnaDrop is a chemical reagent created by a unique formulation that detects early pregnancy in a fast way. This kit is an outcome from a researcher, Dr.drh. Mokhamad Fakhrul Ulum, MSi, Faculty of Veterinary Medicine. This test kit is much better in terms of time as diagnoses can be done earlier, starting on day 18 after natural mating or artificial insemination and detection process only need less than 1 seconds to perform which is faster than a laboratory process test or a quick test in general. PregnaDrop works by dropping a reagent in the cow's urine. For non-pregnant cows, a white cloud will appear and the cloud formed will grow larger as more reagent is added, and ending in forming white sand on the tube's bottom. If the urine comes from a pregnant cow, however, neither a cloud nor sediment may develop (IPB University, Bogor Indonesia, 2019).

2.9 Artificial Insemination

Artificial insemination (AI) has proved to be a reliable technology for cattle producers who want to increase their genetic development and monitor venereal diseases in their herds. AI coverage stood at 4.2% in 2009. AI coverage should be increased to at least 10% to boost the genetic makeup of the cattle population in Malaysia quickly (Raymond, 2010). However, if the AI programme is not tailored to the farm's needs, it can reduce reproductive efficiency by lengthening the time between calving and conception and, as a result, lengthening the calving period as compared to natural services (NS) (Baruselli, 2018). The optimal time for artificial insemination (AI) was thought to be about 10 hours before ovulation, 16 to 20 hours after the second injection of GnRH, regardless of the presence of estrus (Yamada, 2005). Conception rate of the cows was at the highest when they were inseminated between 6 and 24 hours before ovulation (Trimberger, 1948). Artificial insemination has many advantages compared with NS (Lima *et al.,* 2010; Lamb and Mercadante, 2016).

CHAPTER 3

METHODOLOGY

3.1 Animals

Figure 3.1: Kedah-Kelantan cattle

A total of 8 Kedah-Kelantan female cattle aged two to three years in a farm located at Kg. Tebing, Napoh, Kedah were used. The cattle were grazing freely. The cattle were separated into 2 groups, A and B. 5 cattle group A and the other 3 in group B. All of them were confirmed that they are not pregnant and free from diseases.

3.2 Modified herbs

Figure 3.2 : Herbs with soy sauce

Commercially available modified herbs containing *Cinnamomum verum, Zingiber officinale,* and *Piper nigrum L* was given 15g each to the 5 female cattle from Group A, orally by syringe and continuously for three days. The herbs were mixed with 10ml of soy sauce. The weighing process was carried out using a balance scale. The expression of the estrus was observed visually.

3.3 CIDR intravaginal device

Figure 3.3: CIDR

CIDR with 1.9g of progesterone device will be inserted into the group B cattle's vaginal using applicator and correct technique and the device will have to remain for 7 days. After that, 2ml of Estrumate will be injected and the estrus signs of the cattle will be observed after the removal of the device.

3.4 Estrus sign

The secondary estrus sign such as restlessness, sniffs the vulva of other cows, rest her chin on the back of others, mounting and vulva swelling and reddening were observed on 0700, 0900, 1100, 1300, 1500, 1700, 1900, 2100 for a duration of 45 minutes. The estrus signs were observed and the allotted number of points was

recorded by hand each time an animal demonstrated a behavioural estrous indicator. It was recorded in the table based on date, time and estrus behavioural after the removal of the CIDR device and on the day after herbs are given continuously for three days. The number of animals in estrus during an observation period was recorded.

3.5 Behaviour in Group B (CIDR)

Cattle in Group B (CIDR) were observed on their behaviour of estrus after 22 days of natural mating. The cattle will be mated again if they showed estrus signs.

3.6 Natural Mating

All the cattle were mated naturally after showing estrus signs. Catlle in Group A (Herbs) were mated after 3 days of herbs feeding. Group B (CIDR) cattle were mated right after they showed estrus signs.

3.7 Pregnancy Diagnosis

The cattle were undergone pregnancy diagnosis by urine test using PregnaDrop, test kit which is a chemical reagent derived from a unique formulation that works quickly to identify early pregnancy. Test Kit PregnaDrop pregnancy tests were performed by dropping 5 drops of reagent into 3 ml of cow urine. The sample produced a white cloud solution from non-pregnant animals. Pregnant cattle, on the other hand, made no modifications to the sample. Cattle in Group A (Herbs) were tested on day 135 to 140 of gestation while cattle in Group B (CIDR) were tested on day 20 to 25.

3.8 Outcome Measure and Analysis

The two-tailed student t-test was used to compare reproductive performance (secondary estrus signs and pregnancy). The analysis was carried out using Microsoft Excel's standard statistical analysis tools. All information gathered will be reported as mean, standard error of the mean (SEM). P values less than 0.05 ($P<0.05$) are considered significant.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Secondary estrus signs of restlessness higher in Group A compared to Group B

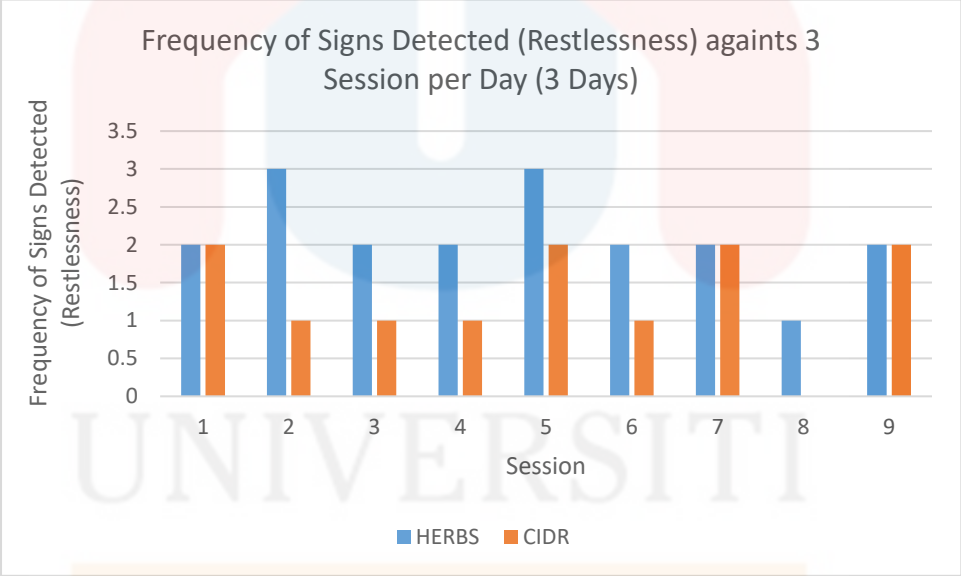

Figure 4.1: The frequency of secondary estrus signs of restlessness on CIDR and Herbs treatments

Indications of restless behaviour were having no interest in grazing, standing up while others were lying, walking along fences and teasing nearby herd mates.

The study showed that restlessness was significantly higher in Group A (herbs) compared to Group B (CIDR) ($P<0.05$) (2.1 ± 0.6 versus 1.3 ± 0.7). This might be due *Piper nigrum L., Zingiber officinale, Cananga odorata,* and *Cinnamomum verum* presence in Group A. This is however, in contrast to Ranasinghe *et al.*, (2009), stated that conception rates for cows demonstrating atypical activity, which was comparable to restlessness in the study of Reimers *et al.*, 1985, were 49.6%. The study found that it was 16.7%. Both studies found no significant differences in conception rates between groups of cows with different secondary estrus sign (restlessness).

In contrast, walking activity in cows with synchronized PGF2α or progesterone + PGF2α showed no differences in the number of steps (Lopez-Gatius *et al.*, 2005). Estrus causes a significant increase in physical activity in cows. A cow in estrus takes around two to four times more steps per hour than a cow in diestrus. Studies found that activity increases significantly in cows approaching estrus, implying a reliable predictor of sexual restlessness (Valenza *et al.*, 2012; Reith *et al.*, 2014a; Madureira *et al.*, 2015). Factors that affect restlessness in cattle are comfortable stalls (Nebel et al. 2000) and time (Reith *et al.*, 2014a). Reith *et al.*, 2014a suggested that restlessness in cows are at most between 0200 and 0800h. and cattle at estrus confined in comfort stalls showed a 2.76 times increase in walking activity (Nebel *et al.*, 2000).

4.2 Secondary estrus signs of sniffing others vulva higher in Group A compared to Group B

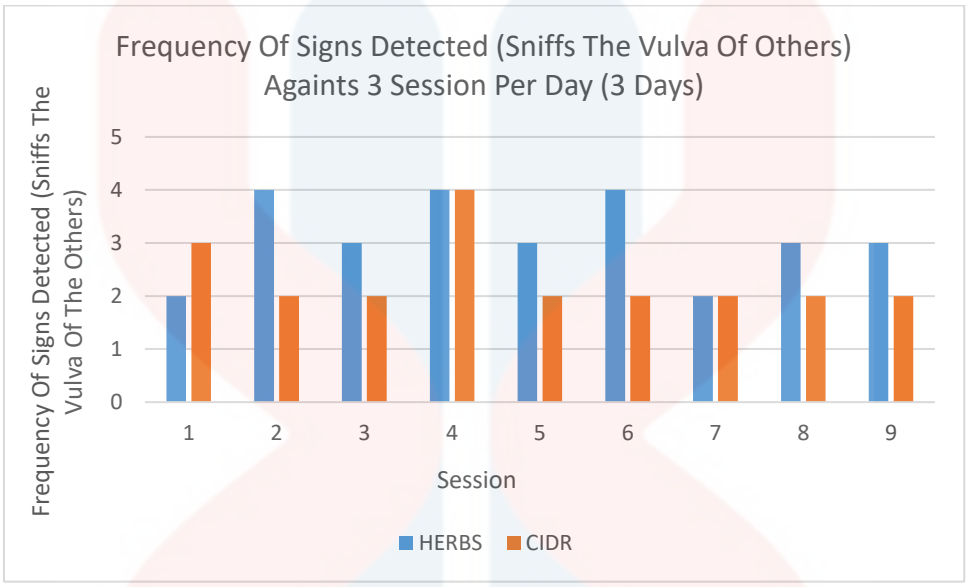

Figure 4.2: The frequency of secondary estrus signs of sniffing others vulva on CIDR and Herbs treatment

The study showed that cows sniffing others vulva was significantly higher in Group A (herbs) compared to Group B (CIDR) ($P<0.05$) (28 vs 21). This is similar to Roelofs *et al.*, 2004 reported that during estrus, primiparous cows sniffed more frequently than multiparous cows (31.6 ± 18.7 versus 22.4 ± 16.3 times; $P < 0.05$), however it also reported during the first 20 days of non-estrus, 87% of the animals who were not in estrus sniffed 5.5 ± 5.2 times (ranging between one and 29 times).

An animal in estrus begins by sniffing and resting its chin, then mounts other animals, and finally exhibits standing heat however, in one study (Eedenburg *et al.*, 2004) sniffing and chin resting were found to be ineffective as predictors of ovulation

time because they were displayed by all animals in every estrus and were not limited to estrus (87% of the animals showed occasional sniffing when they weren't in estrus).

4.3 Secondary estrus sign of mounting was significantly higher in the morning

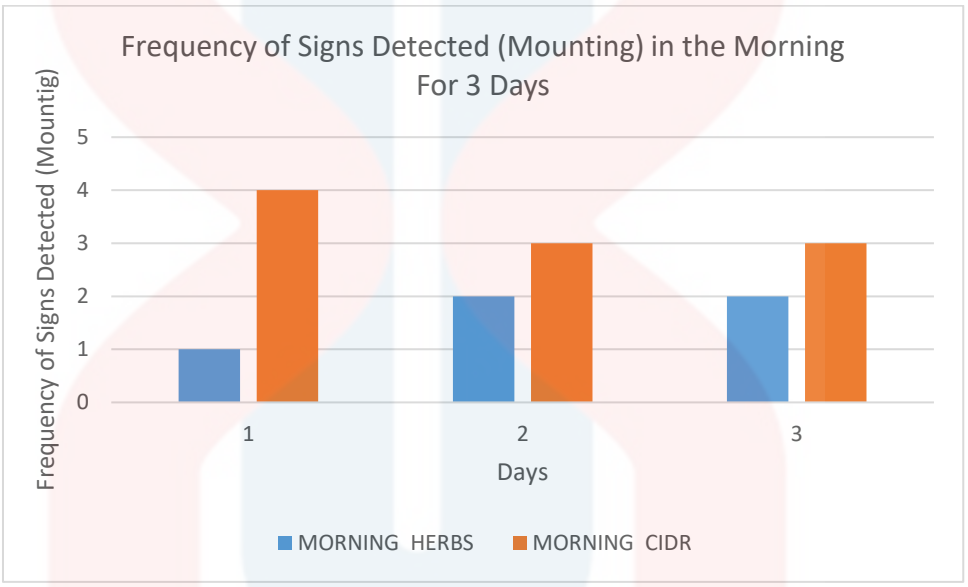

Figure 4.3: The frequency of primary secondary estrus signs of mounting in the morning

Next, the study discovered that mounting sign was significantly higher for group B (CIDR) in the morning (0800 to 1100). This is comparable to Carlos et al., 1982, who found a significant difference ($P<0.05$) in the number of mounts accepted in the two groups (Charolais & Brahman Cross). It was much greater in the Charolais group. The study also stated the percentage of mounts saw throughout the three-hour observation period which is 33% of all mounts between 0600h and 0900h, with the next two biggest numbers occurring between 0900h and 0000h and from 0000h to 0300h.

Another paper also suggested that detection at 0630 and 1530 was 89% as efficient as continuous observation (Berrwinkle *et al*). It would be more efficient to detect early in the morning. Dawn and dusk checks were 94% as effective as thrice/day cheeks for beef cattle, (Beerwinkle). In one study, it was reported that 67% of dairy cows were in estrus in the morning and 45% were no longer in estrus in the evening. Estrus indications exist for a brief period of time, and many mounting-mounted encounters take place in the morning when no one else is around. A study stated that early morning (0600 to 0700 h), early evening (1700 to 1800 h), and casual observation throughout the day appeared to identify about 13% more cows whose beginning of estrus was between 0600h and 1800h than cows whose onset of estrus was between 1800h and 0600 h.

The number of cows in the sexually active group and their social structure have a big impact on mounting behaviour (Hurnik *et al.*, 1975). Mounting on herd mates' behaviour has been reported to be a good predictor of ovulation in Holstein Friesian cows (Roelofs *et al.*, 2005). The overall activities of following a cow and mounting were found to have a highly significant association (PC 0.001) in a correlation test for sexual activities. However, the degree of confinement and connection with calves that are not in heat but willing to mount estrus cattle could explain differences in mounting behaviour.

4.4 Secondary estrus signs were significantly higher in the evening in Group A compared to Group B

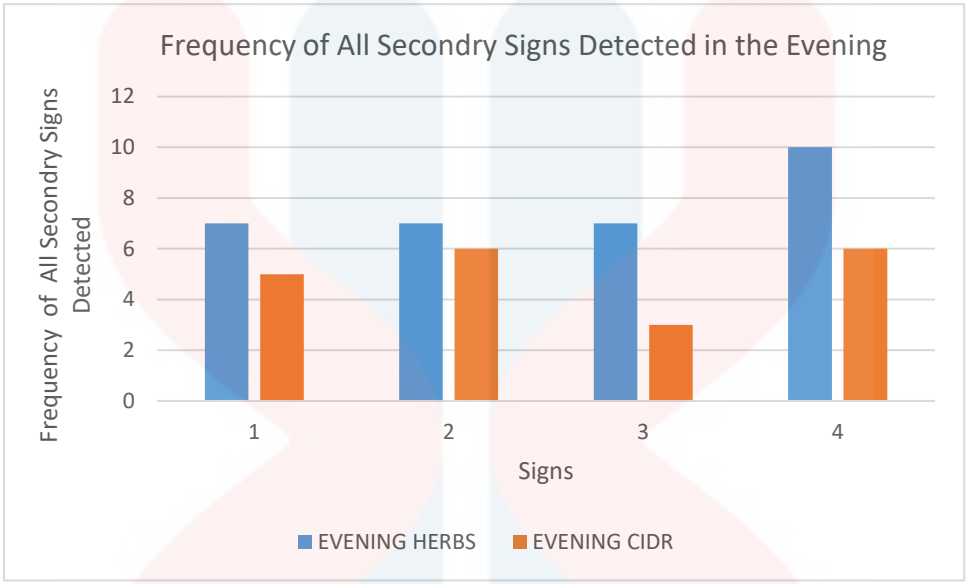

Figure 4.4: The frequency of all secondary estrus signs in the evening

All signs of estrus regardless of method for synchronization were significantly higher (P=0.04) during the evening heat check compared to other sessions. This is similar to Gwazdauskas *et al.*, reported that the evening heat check (7.7 -+.3 m/h, X + SE; n=391) had significantly higher estrus activity than the morning heat check (6.0 -+.2 m/h, n=609). Signs of estrus observed in this study were restlessness, sniffs others vulva, mounting and rest chin on back of others. Factors that may affect the estrus signs were 1) nearer the actual time of onset of estrus (Hurnik *et al*.), 2) reduction of human activities and distractions (Hurnik *et al.*) and 3) environmental temperature (Kalyan De *et al.*, 2020).

It's possible that the increased activity during the initial observation of estrus in the evening is due to the fact that it's getting closer to the actual time of onset of estrus (Hurnik et al.). According to a study, the exact onset of heat occurs about midnight, and most cows show indicators of heat between 2100 and 0400 h. It was recorded 10 to 12

m/h from cows in estrus between 2000 and 0300 h (Esslemont and Bryant). Hurnik *et al.* hypothesised that reducing human activities and distractions could improve estrus activity. The number of mountings per hour (m/h) during the initial observation of estrus is affected by the primary housing area. Cattle kept in barns save for estrus detection showed roughly 3 m/h greater than cattle kept in dry lots or on pasture.

Estrus activity had a substantial curvilinear relationship with maximum environmental temperature on the day of estrus at the first observation of heat (Gwazdauskas *et al.*,). A study reported that temps as low as 32°C had no effect on cyclic activity in beef cattle, but when temperatures reached 38°C, the animals experienced anestrus. One-third of dairy heifers exposed to temperatures above 30°C went into anestrus, while others had shorter estrus periods. The estrus response and lambing percentage on a total ewe basis were significantly greater in the hot-humid with rainfall season, according to the findings of the current study (Kalyan De *et al.*, 2020).

4.5 Urine test using PregnaDrop

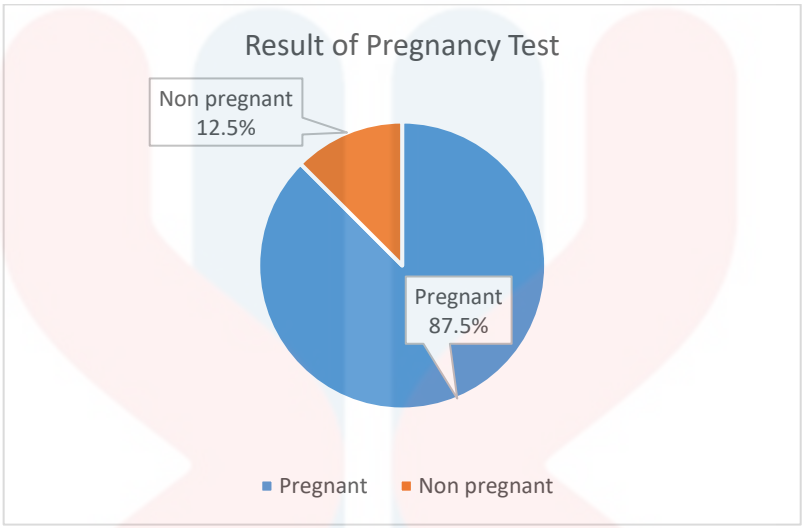

Figure 4.5: The result of urine test (PregnaDrop) of cattle with CIDR and Herbs treatment

Efficiency in conception rate was not significant between Group A and Group B. Pregnancy diagnosis by using PregnaDrop was tested at day 20 to 25 of gestation in Group B, while Group A was tested at day 135 to 140. The figure shows that 87.5% of cattle (7/8) were pregnant. The study showed that CIDR and herbs gave 100% (3/3) and 85% (4/5) of positive pregnancy respectively.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion, the results of the current study show that the primary estrus signs of restlessness, sniffing the vulva of others, resting the chin on the back of others, and mounting, secondary estrus signs of vulva swelling and reddening, and pregnancy rate differed between the CIDR and modified herbs treatments. This could be due to the limited quantity of samples, the environment, or the limitation of time. This research found that there was significant ($P<0.05$) on restlessness and sniffing the vulva of others in Group A with herbs treatment. All estrus signs were significant ($P<0.05$) in the evening for Group A compared to Group B. Herbs are easy to obtain, safe to use and also cheaper compared to CIDR which all farmers can afford. It was found to have similar results to CIDR, implying that additional research into the use of modified herb is promising as there is a lot of benefits on the use of herbs as a replacement of CIDR.

5.2 Recommendation

This research study would prefer some suggestions to improve on current research. First, this research study is about herbs with *Cinnamomum verum, Piper Nigrum L., and Zingiber officinale* used to improve effectiveness of reproduction in cattle. As a result, there is a limitation of data on the efficacy of estrus synchronisation using modified herbs. Observations on this research were performed from 0700 to 2100 manually by herdsman which might be bias for both treatments CIDR and herbs if the cattle have high estrus response at midnight and early morning. Therefore, observation of estrus signs in some period require an aid to detect them accurately. As an example, an accurate result can be obtained by observations from closed-circuit television (CCTV). Because the onset of estrus is so common between 1800 and 0600 h, direct observation during this time or the use of an effective estrus detection device is recommended (Hackett, 1984).

Another recommendation for the study is the pregnancy diagnosis using accurate methods such as rectal palpation and ultrasonography. In this study, I used PregnaDrop, urine test kit to confirm the pregnancy on early pregnancy, day 20 of gestation because of the lack of time on running this project. Ultrasound and rectal palpation at day 100 and 120 of gestation could give accurate confirmation on cattle's pregnancy.

Last but not least, a suggestion on next study would be great to have observations on conception date, litter size and gestation length.

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APPENDIX A

Figure A.1: Mixed herbs

Figure A.2: CIDR Applicator

Figure A.3: CIDR Insertion

Figure A.4: Feeding herbs orally

Figure A.5: Rectal Palpation

APPENDIX B

Table B.1: Secondary sign of estrus synchronisation (Vulva swelling and reddening) (Herbs)

No of cattle	Day / hour
1	Day 3 / 11.00 am
2	Day 2 / 3.15 pm
3	Day 3 / 9.30 am
4	Day 2 / 4.20 pm
5	Day 3 / 12.40 pm

Table B.2: Secondary sign of estrus synchronisation (Vulva swelling and reddening) (CIDR)

No of cattle	Day / hour
6	Day 2 / 9.00 am
7	Day 2 / 12 pm
8	Day 3 / 8.30 am

Table B.3: Pregnancy diagnosis result (Rectal Palpation) on day 72 (Herbs)

No of cattle	Treatment	Pregnant / Not Pregnant
1	Herbs	Pregnant
2	Herbs	Pregnant
3	Herbs	Pregnant
4	Herbs	Not pregnant
5	Herbs	Pregnant

Table B.4: Pregnancy test result (PregnaDrop) on Group A and Group B

No of cattle	Treatment	Clear / Cloudy	Pregnant / Not Pregnant
1	Herbs	Clear	Pregnant
2	Herbs	Clear	Pregnant
3	Herbs	Clear	Pregnant
4	Herbs	Cloudy	Not pregnant
5	Herbs	Clear	Pregnant
6	CIDR	Clear	Pregnant
7	CIDR	Clear	Pregnant
8	CIDR	Clear	Pregnant