UNIVERSITI PUTRA MALAYSIA

EFFECTS OF TIMING PROSTAGLANDIN F2α INJECTION FOLLOWING CONTROLLED INTERNAL DRUG RELEASE INSERT REMOVAL ON OESTRUS RESPONSE, FOLLICULAR DYNAMICS AND PREGNANCY RATE IN NELORE CATTLE

MOHAMED ALI ATTIA HUSSEIN

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By

MOHAMED ALI ATTIA HUSSEIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

DEDICATION

ALLAH YOU ARE MY SUPREME LOVE

ALHAMDULILLAH. …THANK YOU FOR EVERYTHING

MY BELOVED PARENTS, SISTER AND BROTHERS

THANKS FOR YOUR DOA', LOVE AND CARE

FRIENDS

THANKS FOR YOUR HELP AND SUPPORT

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of philosophy

EFFECTS OF TIMING PROSTAGLANDIN $F_{2\alpha}$ INJECTION FOLLOWING CONTROLLED INTERNAL DRUG RELEASE INSERT REMOVAL ON OESTRUS RESPONSE, FOLLICULAR DYNAMICS AND PREGNANCY RATE IN NELORE CATTLE

By

MOHAMED ALI ATTIA HUSSEIN

Chairman: Associate Professor Abd. Wahid Haron, PhD

Faculty: Veterinary Medicine

This research was conducted to evaluate the effect of oestrus synchronization protocol on oestrus response, follicular dynamics, pregnancy rate, and cortisol and progesterone concentrations in Nelore cattle under two types of handlings. In Experiment I (heavy handling), 27 cows were selected and subdivided into four groups, namely the Control G1, G2 and G3. The Control group (n= 8 cows) were treated with only CIDR inserts for 15 days. Meanwhile, G1 (n=5), G2 (n=6) and G3 (n=8) were treated with CIDR inserts for 15 days and given 500 µg of synthetic prostaglandin $F_{2\alpha}$ at 9, 14 and 19 days post CIDR removal, respectively. Forty-eight hours after synchronization treatment, artificial insemination (AI) was done for cows that displayed oestrus, twice at 12 hours interval. Blood samples were collected two times per week on the day of CIDR insertion until after AI and continued for the next 30 days. Ultrasonographic examination was performed to determine follicle dynamic in four groups. In Experiment II (less

handling), 30 cows were subdivided into four groups, Control group (n=7), G1 (n=9), G2 (n=6) and G3 (n=8). The protocol for all the four groups in Experiment II followed that of Experiment I, and the only difference was that blood samples were collected during and after AI, and at weekly intervals until the cows were confirmed to be pregnant by ultrasonography. There is no ultrasonographic examination to determine follicle dynamic in Experiment II. The proportion of cows observed in oestrus was higher in the Control group [62.5% and 100% in Experiments I and II, respectively ($P<0.05$)] than the other groups. The pregnancy rate was higher in G2 (16.6 % in Experiment I; 50 % in Experiment II) than in the other groups. However, the difference was not significant. The total numbers of cows displaying oestrus after treatment was significantly lower in Experiment I (11; 40.7 %) than Experiment II (20; 66.6 %). Similarly, the total number of pregnant cows was not significant in Experiment I (3; 11.1 %) and Experiment II (9; 30 %). There was a significantly difference ($P<0.05$) in the cows which did not display any oestrus after treatment between Experiment I (44.4 %) and Experiment II (13.3 %). The interval from treatment to the onset of oestrus and ovulation time (h) was highest ($P<0.05$) in G2 than other group. However, mean time from standing oestrus to ovulation (h) did not significant among groups. Mean size of dominant follicle at treatment and pre-ovulatory time was larger in control group ($P<0.05$) than other groups. Normal progesterone profile was evident in G1 (Experiment I and II), whereas the other groups showed abnormal progesterone profile throughout the oestrous cycle in both Experiment I and II. The total mean progesterone (P_4) concentrations after AI were higher at Days 7 and 14 in Experiment II compared with Experiment I ($P<0.05$). The total mean cortisol concentration on the day of first

AI, Days 7 and 13-14 post AI were higher in Experiment I than Experiment II but not significant ($P<0.05$). The results from this study showed a negative relationship between the progesterone and cortisol concentrations ($r= -0.267$, $P<0.01$) after first AI in Experiment I. In conclusion, luteal phase of the oestrous cycle and follicular development during the luteal phase may be considered as factors to affect the rate of oestrus synchronization in Nelore cattle. Variability in the interval to oestrus and the distribution of the oestrus response in cows that exhibited oestrus was due primarily to treating cows during the mid stage of the luteal phase (G2). The cause of this variability appears to be related to the manner in which progesterone decreased in cows during the mid stage of the luteal phase after $PGF_{2\alpha}$ treatment and may also be related to ovarian follicular development. However, the greater degree of synchronization among animals was due to treatment with CIDR alone or together with administration of $PGF_{2\alpha}$ 9 days after CIDR removal (G1). The results in the present study indicated that animals treated with $PGF_{2\alpha}$ 9 days after CIDR treatment resulted in acceptable oestrus response and a single timed insemination gave acceptable pregnancy rates. In addition, Heavy handling may have altered the expression of oestrus, reduced the pregnancy rate and altered the progesterone profiles. On the other hand, less handling and gentle interaction with animal could improve the reproductive performance.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN WAKTU SUNTIKAN PROSTAGLANDIN $F_{2\alpha}$ BERKAITAN PENGELUARAN CIDR KE ATAS REAKSI ESTRUS, DINAMIK FOLIKEL DAN KADAR KEBUNTINGAN PADA LEMBU NELORE

Oleh

MOHAMED ALI ATTIA HUSSEIN

Pengerusi: Profesor Madya Abd Wahid Haron, PhD

Fakulti: Perubatan Veterinar

Penyelidikan ini dikendalikan untuk menilai kesan protocol pensinkronian estrus ke atas reaksi estrus, dinamik folikel, kadar kebuntingan dan kepekatan kortisol dan progesterone pada lembu Nelore di bawah dua pengendalian yang berbeza. Dalam Ujikaji I (pengendalian sibuk), 27 lembu dipilih dan dikumpulkan kepada empat sub-kumpulan iaitu kumpulan Kawalan, G1, G2 dan G3. Kumpulan Kawalan (n= 8 lembu) dirawat dengan hanya menggunakan CIDR selama 15 hari. Sementara G1 (n=5), G2 (n=6) dan G3 (n=8) pula dirawat dengan CIDR selama 15 days dan masing-masing disuntik dengan 500 µg prostaglandin $F_{2\alpha}$ pada 9, 14 dan 19 hari selepas CIDR dikeluarkan. Empat puluh lapan jam selepas rawatan pensinkronian, dua kali permanian beradas (AI) selang 12 jam dilakukan ke atas semua lembu yang menunjukkan tanda estrus. Sampel darah diambil dua kali seminggu mulai hari memasukkan CIDR sehingga selepas AI dan berterusan selama 30 hari berikutnya. Pemeriksaan secara ultrabunyi dilakukan untuk menentukan dinamik folikel pada empat kumpulan tersebut.

Dalam Ujikaji II (pengendalian kurang), 30 lembu dibahagikan kepada empat sub-kumpulan, kumpulan Kawalan (n=7), G1 (n=9), G2 (n=6) dan G3 (n=8). Protokol untuk ke semua kumpulan dalam Ujikaji II adalah serupa dengan Ujikaji I. Perbezaannya adalah sampel darah diambil semasa dan selepas AI serta selang seminggu sehingga lembu dipastikan bunting secara ultrabunyi. Tiada pemeriksaan ultrabunyi untuk menentukan dinamik folikel pada Ujikaji II.

Nisbah lembu yang diperhatikan berada dalam estrus adalah lebih tinggi bagi kumpulan Kawalan [masing-masing 62.5% dan 100% pada Ujikaji I dan II ($P<0.05$)] berbanding kumpulan lain. Walaau bagaimanapun, perbezaan ini adalah tidak bererti. Jumlah keseluruhan lembu yang menunjukkan estrus selepas rawatan adalah rendah dan bererti dalam Ujikaji I (11; 40.7 %) berbanding Ujikaji II (20; 66.6 %). Jumlah lembu yang bunting juga menunjukkan perbezaan yang tidak bererti dalam Ujikaji I (3; 11.1%) dan Ujikaji II (9; 30.0%). Terdapat perbezaan bererti ($P<0.05$) di kalangan lembu yang tidak menunjukkan estrus selepas rawatan di antara Ujikaji I (44.4 %) dan Ujikaji II (13.3 %). Selang semasa rawatan hingga tercetus estrus dan ovulasi (h) adalah tinggi ($P<0.05$) dalam G2 berbanding kumpulan lain. Walau bagaimanapun, min masa dari estrus berdiri hingga ovulasi (h) tidak menunjukkan perbezaan bererti di antara kumpulan. Min saiz folikel dominan semasa rawatan dan pre-ovulatori adalah besar dalam kumpulan Kawalan ($P<0.05$) berbanding kumpulan lain. Profil progesteron normal ditunjukkan dalam G1 (Ujikaji I dand II), sementara kumpulan lain menunjukkan profil progesteron yang abnormal keseluruhan kitaran estrus dalam

Ujikaji I dan II. Min jumlah kepekatan progesteron (P_4) selepas AI adalah tinggi pada hari ke-7 dan 14 dalam Ujikaji II berbanding dengan Ujikaji I ($P<0.05$). Min jumlah kepekatan kortisol semasa hari pertama AI, hari ke-7 dan hari ke-13-14 selepas AI adalah tinggi dalam Ujikaji I berbanding Ujikaji II. Namun demikian perbezaan ini tidak bererti ($P<0.05$). Keputusan daripada kajian ini menunjukkan kaitan negatif di antara kepekatan progestron dan kortisol ($r= -0.267$, $P<0.01$) selepas AI pertama dalam Ujikaji I. Sebagai kesimpulan, fasa luteal bagi kitaran estrus dan perkembangan folikel semasa fasa luteal berkemungkinan sebagai faktor yang memberi kesan ke atas kadar pensinkronian estrus pada lembu Nelore. Perbezaan dalam selang tanda estrus dan agihan reaksi estrus pada lembu yang menunjukkan estrus adalah berpunca daripada rawatan lembu semasa peringkat pertengahan fasa luteal (G2). Punca perbezaan ini mungkin berkait dengan penyusutan progesteron pada lembu semasa peringkat pertengahan fasa luteal selepas rawatan $PGF_{2\alpha}$ dan mungkin juga berkaitan dengan perkembangan folikel ovari. Walau bagaimanapun, darjah pensinkronian yang tinggi di kalangan lembu adalah berpunca daripada rawatan CIDR sahaja atau bersama pemberian $PGF_{2\alpha}$, 9 hari selepas CIDR dikeluarkan (G1). Keputusan kajian ini menunjukkan haiwan yang dirawat dengan $PGF_{2\alpha}$ 9 hari selepas CIDR memberikan reaksi estrus yang baik dan sekali permanian yang bermasa memberikan kadar kebunting yang baik. Sebagai tambahan, pengendalian sibuk berkemungkinan akan mengubah gambaran estrus, merendahkan kadar kebuntingan dan mempengaruhi profil progesterone. Sebaliknya pula, pengendalian yang kurang dan interaksi yang mesra terhadap haiwan akan meningkatkan keupayaan reproduksi.

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I certify that an Examination Committee met on August- 27 - 2008 to conduct the final examination of Mohamed Ali Attia Hussein on his Doctor of Philosophy thesis entitled "Effects of Timing Prostaglandin $F_{2\alpha}$ Injection in Relation to CIDR Removal on Oestrus Response, Follicular Dynamics and Pregnancy Rate in Nelore Cattle" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as Follows:

SHEIKH OMAR ABDUL RAHMAN
Professor Dato
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

ABDUL AZIZ SAHAREE
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

MD. ZUKI ABU BAKAR
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

MAURICE P. BOLAND
Professor
Faculty of Agriculture
University College Dublin
Dublin, Ireland
(External Examiner)

HASANAH MOHD. GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of Supervisory Committee were as follows:

Abd Wahid Haron, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Rosina Hj. Yusoff, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Mohd Azam Khan B. Goriman Khan, PhD
Doctor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Abas Mazni Othman, PhD
Doctor
Malaysia Agriculture Research and Development Institute
MARDI
(Member)

HASANAH MOHD. GHAZALI, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 18 December 2008

DECLARATION

I declare that the thesis is my original work except for quotations and citation, which have been duly acknowledged. I also declare that it has not been previously and is not concurrently submitted for any other degree at UPM or at any other institutions.

MOHAMED ALI ATTIA HUSSEIN

Date:17 October 2008

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophin hormone
AI	Artificial Insemination
BCS	Body Condition Score
CIDR	Controlled Internal Drug Release Device
CL	Corpus Luteum
CV	Coefficients of Variation
D	Day
EB	Oestradiol Benzoate
FSH	Follicle Stimulating Hormone
G1	Group 1
G2	Group 2
G3	Group 3
GnRH	Gonadotropin Releasing Hormone
H	Hour
IU	International Unit (s)
kg	Kilogram
LH	Luteinizing Hormone
LHRH	Luteinizing Hormone Releasing Hormone
Mg	Milligram
MGA	Melengestrol Acetate

Ml	Milliliter
Mm	Millimeter
Ng	Nanogram
P_4	Progesterone
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
QC	Quality Control
R	Regression
SEM	Standard Error of the Mean
TAI	Timed Artificial Insemination
Yr	Year

CHAPTER I

INTRODUCTION

Breed can be defined as a variety which is related by descent and has similarity in certain characteristics. These similarities in characteristics are used to distinguish one breed from another breed (Field and Taylor, 2003). There are more than 250 breeds of cattle recognized throughout the world, and several hundred other varieties and types have not been given breed names. At the moment, cattle breed are categorized as either *Bos taurus* or *Bos indicus* (Field and Taylor, 2003).

Most of the world's bovine herds are found in the tropical region. Between the two *Bos*, *B. indicus* predominates, due to their adaptability to the climatic and management conditions. However, *B. indicus* cattle, like the Zebus, usually have lower potential for meat, milk production and lower prolificacy than *B. taurus* breeds (Pinheiro *et al.,* 1998). Originated in Western Asia, *B. indicus* cattle have migrated throughout tropical Asia and, from there they were introduced into the continents of Africa, America and Australia.

Through selection process for many centuries, and also through planned cross-breeding programs with *B. taurus* genotypes, a few well known *B. indicus* pure breeds and cross breeds, with a reputation as dairy animals have been developed in several tropical and sub-tropical countries. Yet, in some South Asian and African countries, *B. indicus* cattle have remained non descript, multi-purpose animals and they form the bulk of the Zebu cattle population in the world. Today, Zebu cattle are found in almost all tropical and sub-tropical environments and they are estimated to account for more than half of the world's cattle population. Nearly half of the world Zebu population is estimated to be in the continent of Asia. The next largest population can be found in South and North America, and Africa, accounting for approximately half of the total estimated world Zebu population (Abeygunawardena and Dematawewa, 2004).

In Latin America, Brazil has become the largest breeder of Nelore cattle (*Bos indicus*), with a population of 80 million heads. Thus, the Nelores represent the major beef cattle breed, and they are exported to other Latin American countries like Argentina, Paraguay, and Venezuela, and to Central America, like Mexico and the United States of America and many other countries of the world including Malaysia. In all those places, the contribution of the Nelore was remarkable, whether through purebred selection within the breed or through crosses with local breeds (Barros *et al*., 2000).

Scientific information with regards to reproduction of Zebu cattle is rather limited compared with that of temperate cattle. However, the information available (Gordon, 1996) does not suggest that there is any great difference in the reproductive physiology between *B. taurus* and *B. indicus* cattle. Zebu cattle, although well adapted to the tropics and sub-tropics, characteristically show delayed puberty and extended postpartum periods (postpartum anoestrus) compared with temperate breeds. It has been reported that there are differences in the hypothalamic, pituitary and ovarian relationships between Zebu and temperate cattle. These differences may account for differences in fertility between the two species even when similarly fed and managed (Abeygunawardena and Dematawewa, 2004).

Reproduction efficiency is a major factor affecting the production and economic efficiency of dairy and beef cow herds. A major reason for a long calving interval in cow is poor oestrus detection. The ideal calving interval of one year can only be achieved if the interval between parturition and successful service is less than 85 days (Stagg *et al*., 1995). This ideal calving interval can only be attained with accurate detection of oestrus and timing of insemination relative to ovulation (Stagg *et al*., 1995).

On the other hand, reduced calving rates and long calving interval are also associated with poor oestrus detection practice in cattle that do not display overt signs of oestrus. In addition, the short duration of oestrus and the tendency to show oestrus during the night, greatly affect the efficiency of artificial insemination programs in *B. indicus* cattle managed in tropical areas (Baruselli *et al*., 2004). The short duration of oestrus behavior (11 h), which is associated with high incidence of oestrus display at night (30 to 50%), makes it difficult to detect oestrus in Nelore females (Barros *et al*., 2000).

In Malaysia, most of the beef herds are pasture-fed and mating is done naturally by mixing bulls with cows at a ratio of 1: 10. Since oestrus detection is not widely practiced. One of the reproductive challenges encountered in pasture-based cattle is repeat breeders. Repeat breeder cows have a long calving interval and are usually not treated. However, with artificial insemination (AI) program, the repeat breeders can be identified and thus treated.

Improvement in fertility must first come from improved breeding management and only then from the use of assisted reproductive technology (ART) such as oestrus synchronization. Oestrus synchronization facilitates the use of genetically superior sires through AI. It may also enhance reproductive efficiency by shortening the breeding and calving seasons. Synchronization of oestrus involves the manipulation of the oestrous cycle in cycling cows by induction of oestrus in anoestrus cows so that a large percentage of females come into oestrus at a predetermined time. Cows will be observed for signs of oestrus and inseminated approximately 12 hours after the observed oestrus signs. Alternatively, cows may not be observed for signs of oestrus but would be inseminated at a fixed predetermined time. This procedure is known as timed breeding, breeding by appointment or mass mating.

For a successful oestrous synchronization, the cows should be closely synchronized with a rapid decline in circulating progesterone concentration as well as synchronous growth and ovulation of a viable follicle. There are two principles of controlling oestrus and ovulation in cattle. The first principle is to prolong the life of the CL, thus delaying oestrus. This can be attainable by administering progestin such as Controlled Internal Drug Release (CIDR) that mimics the function of the CL.the second principle is to shorten the life of the CL hastens the onset of oestrus. This is attainable by adminstering exogenous luteolytic agents such as prostaglandin $F_{2\alpha}$. However, $PGF_{2\alpha}$ is effective only when a fully developed CL is present approximately between D5 and D7 of the oestrous cycle (Hafez and Hafez, 2000).

The success of progestin administration has been limited by reduced fertility of cows that have had their cycle extended (Beal, 1996). However, a longer duration of progestin treatment (more than 14 days) is necessary to allow for the spontaneous occurrence of luteolysis before treatment withdrawal. However, this treatment regime gained the reputation as producing a highly synchronized, but infertile oestrus (Fields *et al*., 2002). Furthermore, it was noted that conception rate is reduced when synchronization with short-term progestin treatments (between 7 and 12 days), combined with $PGF_{2\alpha}$ were initiated in the last third of the oestrous cycle. Therefore, future improvement in oestrus synchronization procedure is most likely to come from achieving more synchrony between the development of a highly fertile ovulatory follicle and controlling of luteal function.

Longer treatment with CIDR for 15 days produces high percentage of synchronized oestrus but low fertility. In the present study, treatment with CIDR for 15 days places all cows in the early, mid and late luteal phase of the oestrous cycle (9, 14, and 19). Our hypothesis is that all cows will have functional CL which will response to $PGF_{2\alpha}$ treatment. Consequentely, the variability in the interval from $PGF_{2\alpha}$ injection to oestrus will reduce and maximum pregnancy rate.

There is factor (stress) during the procedure of our experiment. Handling, restraint and close contact with people cause stress to animals and may result in variable response within animals. Therefore, the second hypothesis is that cows with less handling and restraint will have lower cortisol level and high response to treatment than cows with heavy handling and restraint.