UNIVERSITI PUTRA MALAYSIA

HORMONAL AND CYTOGENETIC ANALYSES OF REPRODUCTIVE ACTIVITY OF CATTLE

NURHUSIEN YIMER DEGU

FPV 2011 22

HORMONAL AND CYTOGENETIC ANALYSES OF REPRODUCTIVE ACTIVITY OF CATTLE

By

NURHUSIEN YIMER DEGU

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

July 2011

DEDICATION

To my mother Edugna Ali Ibrahim who took the challenge to bring me up to this level and to my father Yimer Degu Seidu who had great expectations of all his children but failed short of tasting the early fruits of his ambitions.

O Allah! Forgive me, forgive my parents and show mercy on them as they have nourished me when I was little.

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

HORMONAL AND CYTOGENETIC ANALYSES OF REPRODUCTIVE ACTIVITY OF CATTLE

By

NURHUSIEN YIMER DEGU

July 2011

Chair : Rosnina Hj. Yusoff, PhD

Faculty : Veterinary Medicine

The first part of the present study investigated abnormal ovarian cyclicity (AOC) and cystic ovarian diseases (COD) based on plasma progesterone (P_4) profiles and ovarian ultrasonogram in 102 cattle which comprised cows open > 90 days postpartum and heifers with delayed age (>24 months) at first calving. Highest incidence of AOC associated with either COD or inactive ovaries occurred in Brangus (BR; 53.3%) and lowest incidence in Kedah Kelantan (KK) cows (12.5%). The difference in AOC was significant ($P< 0.05$) between KK and the other three breeds. In cows, incidence of COD was highest in FRS (30%), followed by BRF (13.3%), BR (14.3%) and KK (8.7%). Overall, incidence of COD was higher in beef heifers than in cows. Prolonged luteal phase due to luteal cysts was the predominant type of AOC in FRS (66.7%) while cessation of cyclicity due to follicular cysts was the main abnormality in BR (75%) and BRF (76.9%). Thus, this study showed AOC and COD as major causes of reproductive failure in dairy and beef cattle with KK

cows being the least affected. Moreover, the combination of P_4 data and ultrasonograms was able to differentiate the various types of COD.

The study was extended to determine the bulls' fertility status. Breeding soundness evaluation (BSE) revealed 3 of the 8 bulls examined had testicle defects and/or poor semen quality. Testicular lesions included testicular degeneration, hydrocele and fibrotic foci. In addition, cytogenetic investigation on 20 animals (13 females and 7 bulls) showed normal cattle chromosome complement of 60 and confirmed the acrocentric Y in KK bulls as of zebu genotype. Thus, the study ruled out chromosomal aberration as a cause of reproductive failure.

Measurement of faecal progestin levels to monitor reproductive activity in cattle was another aspect of the present study. Evaluation of modified faecal extraction protocol was 76.8% efficient to recover faecal progestins and hence subsequently used in this study. Matched plasma and faecal samples collected twice a week for 3 months, from cycling as well as pregnant KK and from Brangus cows with either regular cycle or ovarian disturbance, were assayed for plasma P_4 and faecal progestin concentrations respectively, by RIA. There was a significant positive correlation ($r= 0.6$, $P< 0.01$) between faecal progestin and plasma P_4 concentrations in the cycling KK cows, indicating physiological validity of the assay method. Mean faecal progestin concentrations during the follicular phase (FP; 212.6 ± 19.3 ng/g) was significantly ($P< 0.01$) lower than the luteal phase (LP; 792.4 ± 66.7 ng/g), indicating that the method can differentiate FP from LP. Comparisons between pregnant and cycling KK cows revealed a clear difference whereby mean pregnant faecal progestin

concentrations (728.6 ± 33.5ng/g) were significantly (P< 0.01) higher than FP but not LP samples. Plasma P_4 level, known to remain >1 ng/ml in pregnant cows, coincided with faecal progestin of >344 ng/g. Hence, faecal progestin concentrations that remain >344 ng/g for >20 days indicate pregnancy in KK cows in the absence of ovarian and/or uterine disorders. In contrast, Brangus cows with ceased ovarian cycle had progestin concentrations of ≤344 ng/g, consistent with plasma P_4 of ≤1 ng/ml. Mean concentration of faecal progestin was significantly (P<0.01) lower in cows with cessation of ovarian cycle (86.6 ± 7.0 ng/g) than with regular cycle (392.3 ± 33.8 ng/g). In conclusion, cessation of ovarian cycle is defined as an AOC characterised by weak luteal activity and faecal progestin concentration of ≤344 ng/g for at least 14 days. Therefore, faecal progestin measurements can be potentially used to monitor regular or irregular reproductive cycle. High performance liquid chromatographic (HPLC) separation of faecal extracts followed by RIA analysis revealed 4 immunoreactive metabolites. Subsequent analysis of HPLC fractions by gas chromatography mass spectrometry to identify metabolites was limited by lack of commercial availability of reference standards. However, intact P_4 was verified to be undetected in faeces. According to mass spectra library search, metabolites which appeared similar to 5α-reduced pregnanes require future research to confirm and test antibodies against these metabolites for more pronounced profiles.

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

ANALISIS HORMON DAN SITOGENETIK TERHADAP AKTIVITI REPRODUKSI LEMBU

Oleh

NURHUSIEN YIMER DEGU

Julai 2011

Pengerusi : Rosnina Hj. Yusoff, PhD

Fakulti : Perubatan Veterinar

Bahagian pertama kajian ini menyiasat keabnormalan kitaran ovari (AOC) dan penyakit ovari sista (COD) berasaskan profil progesteron plasma (P_4) dan ultrasonogram ovari pada 102 lembu yang terdiri daripada lembu terbuka > 90 hari pasca kelahiran dan lembu dara yang lewat (>24 bulan) melahirkan anak pertama. Insiden AOC tertinggi yang dikaitkan dengan COD atau ovari tidak aktif berlaku pada Brangus (BR; 53.3%) sementara insiden terendah berlaku pada lembu Kedah Kelantan (KK) (12.5%). Perbezaan AOC yang signifikan ($P<0.05$) terdapat antara baka KK dan baka lain. Kejadian COD (30%) adalah tertinggi pada FRS, diikuti oleh BRF (13.3%), BR (14.3%) dan KK (8.7%). Keseluruhannya insiden COD lebih tinggi pada lembu dara berbanding lembu dewasa. Fasa luteum yang berpanjangan adalah jenis utama AOC pada FRS (66.7%) yang disebabkan oleh sista luteum manakala pemberhentian kitaran yang disebabkan sista folikel adalah keabnormalan utama pada BR (75%) dan BRF (76.9%). Oleh itu, kajian ini menunjukkan AOC dan

COD sebagai punca utama kegagalan pembiakan lembu tenusu dan pedaging, dengan lembu KK paling kurang terjejas. Selain itu, kombinasi data P_4 dan ultrasonogram dapat membezakan pelbagai jenis COD.

Kajian ini dilanjutkan untuk menentukan status kesuburan lembu jantan berdasarkan penilaian kesempurnaan pembiakan (BSE). Ultrasonograf transkrotum mendedahkan 3 daripada 8 lembu jantan yang diperiksa gagal memenuhi standard BSE. Walau bagaimanapun, siasatan sitogenetik pada 20 ekor haiwan (13 ekor betina dan 7 ekor lembu jantan) menunjukkan lembu normal mempunyai pelengkap kromosom 60 dan pengesahan kromosom Y berbentuk akrosentrik pada lembu jantan KK menunjukkan genotip zebu. Oleh itu, kajian menolak keabnormalan kromosom sebagai penyebab kegagalan pembiakan.

Pengukuran aras progestin tinja untuk memantau aktiviti pembiakan lembu adalah satu lagi aspek dalam kajian ini. Penilaian protokol ekstrak tinja yang diubahsuai adalah 76.8% berkesan untuk mendapatkan semula progestin tinja dan seterusnya, digunak dalam kajian ini. Sampel secocok plasma dan tinja dikumpulkan dua kali seminggu selama 3 bulan dari lembu KK berkitaran serta bunting dan lembu Brangus yang berkitaran biasa atau gangguan ovari diasei untuk masing-masing kepekatan P_4 plasma dan tinja progestin melalui RIA. Terdapat hubungan signifikan yang positif ($r = 0.6$, $P < 0.01$) antara kepekatan tinja progestin dan P_4 plasma pada lembu KK berkitaran, menunjukkan kesahihan fisiologi kaedah asei tesebut. Purata kepekatan tinja progestin semasa fasa folikel (FP; 212.6 ± 19.3 ng/g) nyata lebih rendah ($P<0.01$) daripada fasa luteum (LP; 792.4 ± 66.7 ng/g), menunjukkan kaedah ini

boleh membezakan FP daripada LP. Perbandingan antara lembu KK bunting dan berkitaran menunjukkan perbezaan yang jelas di mana purata kepekatan tinja progestin (728.6 ± 33.5ng/g) nyata lebih tinggi (P<0.01) daripada sampel FP tetapi tidak pada sampel LP. Aras plasma P_4, yang diketahui berada pada aras >1ng/ml pada lembu bunting, bertepatan dengan progestin tinja >344 ng/g. Oleh itu, tinja progestin yang >344 ng/g untuk >20 hari dengan ketiadaan gangguan ovari dan/atau rahim menunjukkan kehamilan pada lembu KK. Sebaliknya, lembu Brangus dengan kitaran ovari terhenti menunjukkan kepekatan progestin ≤ 344 ng/g, selaras dengan plasma P_4 ≤ 1 ng/ml. Purata kepekatan tinja progestin adalah lebih rendah (P<0.01) pada lembu berkitaran ovari terhenti (86.6 ± 7.0 ng/g) berbanding dengan kitaran normal (392.3 ± 33.8 ng/g). Oleh itu, kitaran ovari terhenti ditakrifkan sebagai AOC yang bercirikan aktiviti luteum lemah dan kepekatan progestin tinja ≤ 344 ng/g untuk sekurang-kurangnya 14 hari. Secara umumnya, kajian mengenai pengukuran progestin tinja berpotensi digunakan untuk memantau kitaran pembiakan normal atau sebaliknya. Analisis kromatografi cecair prestasi tinggi (HPLC) terhadap ekstrak tinja dan diikuti analisis RIA mendedahkan 4 metabolit imunoreaktif. Analisa seterusnya terhadap pecahan HPLC oleh spektrometri massa kromatografi gas untuk mengenalpasti metabolit adalah terhad oleh kekurangan ketersediaan komersil standard rujukan. Walau bagaimanapun, P_4 tidak terjejas telah disahkan tidak dapat dikesan di dalam tinja. Mengikut carian perpustakaan spektra jisim, metabolit yang kelihatan serupa dengan 5α-terkurang pregnane perlu dikaji pada masa akan datang bagi mengesah dan menguji antibodi terhadap metabolit tesebut untuk profil lebih ketara.

ACKNOWLEDGEMENT

"In the name of ALLAH, the Most Gracious, the Most Merciful"

All praise be to ALLAH, the All-Knowing, we all are from He and to Him is our final return. I thank Him for all His countless blessings, Alhamdulillah.

My first sincere and heartiest gratitude is to Assoc. Prof. Dr. Rosnina Hj. Yusoff, chairman of the supervisory committee, for her meticulous and rewarding guidance, overall unlimited support and care throughout my study. I better generally say, she was my "ibu" in Malaysia.

My next deepest gratitude goes to my co-supervisors, Assoc. Prof. Dr. Abd Wahid Haron and Prof. Dr. Abdul Aziz Saharee. Beside their crucial academic guidance, valuable comments and advice, they were also involved in buying research materials that I needed. I would also like to acknowledge and thank Dr. Abd Wahid for giving me special graduate research assistance in the mean time that helped me to keep focus on my study. He also helped me to improve my skills and broaden my scope in the field by allowing me to actively participate in his field consultancy services. May Allah reward you all in full in the hereafter for everything that you did for me.

I would also like to thank Dr Baljit Singh and his staff members working in the UPM dairy and beef farm for their cooperation and patience during the study. Mr. Yap Keng Chee, Mr. Ganesamurthi Perumal and Mohd Fahmi bin Mashuri, all from Theriogenology and Cytogenetics lab, had been with me for most of the time with

their friendly hands for technical assistance and hence would like to appreciate and thank for their deeds.

I am also very grateful to Mr. Abdul Halim Abdul Rahaman and Mrs. Suraya Saad from HPLC lab and Mrs. Norlinawati Abd. Halim from GC-MS laboratory of the Faculty of Food Science and Technology as well as Mr Rosli Aslim from Faculty of Biotechnology and Bimolecular Sciences, UPM who technically assisted and taught me how to use HPLC and GC-MS in my research. I would also like to thank Dr Michael Heistermann from Reproductive Biology Unit, German Primate Research Institute, Goettingen, Germany and Assoc. Prof. Dr. Franz Scwarzenberger from University of Veterinary Medicine, Vienna, Austria for sharing me their experience in the area of faecal steroid hormone study through personal communications. Dr Goh Yong Meng's help in data analysis is also highly appreciated and acknowledged.

It is also my great appreciation and thanks to Nuffic (the Netherlands Organization for International Cooperation in Higher Education and Research) who partially supported my study in collaboration with my home University (Faculty of Veterinary Medicine, University of Gondar, Ethiopia). Last but not least, my appreciation goes to my family: my mother-Edugna Ali, brother-Esmail, and sisters: Alya, Khedija, Fatima, Maryam and Zamzam as well as friends back home especially Muhammad Seid and Bilal Tessema for their encouragement and prayers. I am indebted to my dear postgraduate colleagues in Theriogenology lab especially Muhammad Bukar and Akeel Ahmad, and others including Yitbarak and Mammat Hamidin for their contribution along my journey.

APPROVAL

I certify that an Examination Committee has met on 27 July 2011 to conduct the final examination of Nurhusien Yimer Degu on his Doctor of Philosophy thesis entitled "Hormonal and cytogenetic analyses on the reproductive activity of cattle" in accordance with University 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:

Chairperson, PhD
Associate Professor Dr. Md. Zuki Bin Abu Bakar@Zakaria
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(chairman)

Examiner 1, PhD
Professor Dr. Mohamed Ali Rajion
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Examiner 2, PhD
Professor Dr. Mohamed Ariff Bin Omar
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

External Examiner, PhD
Professor Dr. Muhammad Azam Karkar
Faculty of Life Sciences and Informatics
Balochistan University of Information Technology, Engineering and Management Sciences
Pakistan
(External Examiner)

BUJANG KIM HUAT, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 27 July 2011

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 the Supervisory Committee were as follows:

Rosnina Hj. Yusoff, PhD
Assoc. Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(chairman)

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

Abdul Aziz Saharee, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(member)

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

DECLARATION

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

NURHUSIEN YIMER DEGU

Date: 27 July 2011

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

A	Ampere
ACN	Acetonitrile
ACTH	Adrenocorticotropic hormone
AI	Artificial insemination
AOC	Abnormal ovarian cyclicity
$Ba(OH)_2$	Barium hydroxide
BCS	Body condition score
BR	Brangus
BrdU	Bromodeoxyuridine
BRF	Braford
BSE	Breeding soundness evaluation
CI	Calving interval
CASA	Computer assisted semen analysis
CBG	C- band by barium hydroxide using giemsa
CL	Corpus luteum
COD	Cystic ovarian disease
DCP	Dairy cattle pellet
DNA	Deoxyribonucleic acid
DSL	Diagnostic systems laboratories
DF	Dominant follicle
EE	Electro-ejaculator
EIA	Enzyme immunoassay
eV	electron volt
FP	Follicular phase
FR	Friesian
FSH	Follicle stimulating hormone
GC-MS	Gas chromatography mass spectrometry
GnRH	Gonadotropin releasing hormone

H_2O_2	Hydrogen peroxide
HBSS	Hank's balanced salt solution
HPLC	High performance liquid chromatography
ISCNB	International System for Chromosome Nomenclature of Domestic Bovids
IU	International unit
IVF	In vitro fertilization
KCL	Kalium chloride
KK	Kedah Kelantan
LH	Luteinizing hormone
LP	Luteal phase
µg	microgram
µL	microlitre
µm	micrometer
M	molar
mL	millilitre
ng	nanogram
nm	nanometer
P_4	Progesterone
PBS	Phosphate buffer saline
PGF2α	Protaglandin F2 alpha
PKC	Palm kernel cake
PLP	Prolonged luteal phase
pp	postpartum
ppm	parts per million
psi	pounds per square inch
RIA	Radioimmunoassay
rpm	revolution per minutes
RPMI	Roswell park memorial institute
SFT	Society for theriogenology

SLP	Short luteal phase
SPSS	Statistical package for social sciences
TSU	Transscrotal ultrasonography
UPM	Universiti Putra Malaysia
US	Ultrasound
UV	Ultraviolet
V	volt

CHAPTER ONE

INTRODUCTION

In the tropics where smallholder farming predominates, livestock including sheep, goat and cattle provide the well recognized products such as meat and milk for consumption or sale, act as a source of fibre and hides, and their manure can be used as fuel and fertilizer. Export of live animals and animal products make substantial contribution to the foreign exchange of especially many third world countries including Ethiopia while importation causes a loss of foreign exchange in developing countries like Malaysia due to low self-sufficiency and high demand for livestock products such as milk and meat. Despite their economic importance, the productivity of cattle in the tropics is low due to low genetic potential of indigenous breeds, poor husbandry, and other animal and environmental factors. Cross breeding indigenous cattle breeds with *Bos taurus* breeds like Holstein-Friesian and Hereford, has been used as a strategy to improve productivity.

Successful production in dairy and beef farming depends on a year round calving interval in cows and establishment of fertile ovarian cyclicity in heifers following puberty and sexual maturity. In heifers, first calving at 24 months old is generally considered to be economically optimal with regards to productivity and duration of herd life (Hanzen et al., 1994). Calving interval is an important index of cow reproductive performance and, a calving interval of 365 days is desirable for efficient production (Lyimo et al., 2004). The calving interval in cows is influenced by the time lag between calving and the resumption of ovarian activity, between first

observed oestrus and first mating, and between first mating and conception in the postpartum period (Shrestha et al., 2004a).

Generally, an optimum postpartum period of 65 days, which is a voluntary waiting period, is recommended in cows followed by conception by the 85th day postpartum to achieve a year round calving interval (Opsomer et al., 1998; Noakes, 2000). However the postpartum period can be affected by a number of factors such as a prolonged postpartum anestrus period and incidence of abnormal ovarian cyclicity (AOC). A number of studies have been conducted, targeted at the early postpartum period which varies between two and three months in both dairy and beef cows, to investigate ovarian dysfunction (Opsomer et al., 1998), endocrine patterns (Humphrey et al., 1983), risk factors associated with anoestrus (Opsomer et al., 2000; Yavas and Walton, 2000; Santos et al., 2009), and effects of AOC during the early postpartum period on subsequent reproductive performance (Shrestha et al., 2004b). Consequently, much has been understood about the early postpartum ovarian activity and associated disturbances in cattle but studies are rarely carried out in different breeds of cows with a prolonged postpartum period of more than 90 days as well as in heifers with delayed age (> 24 months) at first calving.

Reproductive success is mainly influenced by hormones. Some of the important hormones that regulate female reproductive behavior and functions include progesterone (P_4) and estrogen, which are produced by the ovaries. Analyses of these hormones are used to validate the reproductive activity of animals (Dehnhard et al., 2008). The information obtained can also be used to improve genetics and reproductive performance (Capezzuto et al., 2008) by increasing the understanding

of reproductive cycling and breeding behaviour, improvement of estrus synchronization and induction protocols for successful artificial insemination (AI) as well as treating infertility (Penfold et al., 2005; Asa et al., 2006; Graham et al., 2006; Dehnhard et al., 2008).

The measurement of steroid hormones traditionally involves invasive techniques including blood collection. Accurate investigation requires a repeated collection of samples, which is rather stressful even to docile domestic animals (Capezzuto et al., 2008). Moreover, the use of blood samples to measure stress related hormones like cortisol is also limited as stress caused by the capture and restraining during blood collection, together with venu-puncture may increase their concentrations in the plasma (Palme et al., 1996; Dehnhard et al., 2008). Apart from blood sampling, other body fluids such as milk (Schwarzenberger et al., 1996a) and urine (Evans et al., 1984) can be non-invasively collected from animals to determine concentrations of steroid hormones. However, the milk sample is restricted to lactating cows and can not be collected from heifers and dry cows. Urine sampling on the other hand necessitates restraining the animal in a metabolic cage or following it around to collect midflow samples during urination (Masunda et al., 1999).

The measurement of steroids from non-invasively collected faecal samples has been reported in various species of animals related to reproduction, particularly in free ranging and captive wildlife (Graham, 2004). In domestic ruminants, using the non-invasive methods of measuring of faecal steroid metabolites, a correlation between faecal concentrations of progesterone metabolites and blood plasma level of progesterone in goats (Hirata and Mori, 1995; Capezzuto et al., 2008), ewes (Adams

et al., 1994; Palme et al., 1996), and cows (Masunda et al., 1999; 2002; Isobe et al., 2005b) has been reported. These studies employed various methods of extraction and immunoassay techniques to determine the concentrations of steroids following normal ovarian activity. Extraction procedures based on the availablility of dry faecal samples used by previous studies need further research for modifications as they are time consuming and involve the use of large amounts of solvents. At present, there is no information available on the use of faecal steroid hormone metabolites in cattle related to ovarian disturbances. Despite enormous progress in using faecal hormone analysis for research on reproductive biology, each analytical method needs to be validated each time for a particular animal species and hormone, as steroid metabolism by the liver generates a vast number of faecal steroid metabolites, yet different in even closely related species (Palme et al., 1996; Heistermann et al., 1998; Dehnhard et al., 2008). So far there has not been any research conducted on the detection and measurement of faecal steroid hormone metabolites using commercial assay methods for monitoring ovarian activity and pregnancy in Kedah Kelantan (KK) cows. It is believed that KK cows will also benefit from establishment of a non-invasive method of measuring faecal steroid metabolites for monitoring reproduction.

Although artificial insemination (AI) for cattle breeding has progressively displaced natural service as the preferred method of breeding in the dairy industries of most developed countries of the world, natural breeding is still the most predominant method used in beef cattle operations throughout the world (Parkinson, 2004). Where natural breeding is employed, reproductive statistics on the farm are greatly influenced by the handling and fertility of the bulls. To impregnate a cow by natural

mating, the stipulated bull must produce semen of satisfactory quality and be able to successfully mount and deposit the semen in the vagina of the cow. Failure to meet either criterion results in poor reproductive performance (Kreplin, 1992). The role of the bull in the fertility of both beef and dairy cows is therefore required to be understood in terms of the overall impact on fertility in cattle. Determining the potential fertility of the bull is much more important than determining the fertility of the individual cow (Kreplin, 1992; Chenoweth, 1999; Parkinson, 2004). However, despite frequent grievances from dairy and beef farms of Malaysia for failure to meet the required fertility (including the farm in which this study conducted), the potential effect of the bulls' fertility on the overall reproductive efficiency of cattle herd and their role in the breeding programmes are often underestimated. In addition, the use of standard breeding soundness evaluation (BSE) for bulls before use for breeding is overlooked. Apart from the routine BSE technique, trans-scrotal ultrasonography (TSU) has been reported recently to enhance evaluation of breeding soundness in bulls (Chapwanya et al., 2008). However its application and interpretation of findings in determining fertility status of bulls in relation to the routine BSE technique needs further research.

Cattle, like other domestic species, cannot escape from inherited disorders and congenital malformations which comprise a substantial proportion of economic loss to the cattle breeders. At present, chromosome anomalies which occur occasionally through accidents at either meiosis or mitosis are not preventable since our knowledge on the aetiological mechanisms for these aberrations is limited. In general numerical chromosome anomalies are readily removed by natural selection but structural anomalies may lead to polymorphic systems with pernicious effects on

fertility. Reduced fertility and infertility due to chromosomal anomalies are relatively common in cattle, with a wide range in prevalence rates among breeds or populations. Among the chromosomal anomalies, the Robertsonian translocation, t(1;29) formed by centric fusion between chromosomes 1 and 29, is the most troubling and widely distributed in cattle, mainly of European breeds, and with greatest economic consequences via reductions in fertility (Dyrendahl and Gustavsson, 1979; Schifferli et al., 2003). Following the identification of t(1;29) at higher incidences (Gustavsson and Rockborn, 1964), a national eradication programme which was started in Sweden and later in Brazil, have been initiated in many European countries to bring the translocation under control (Buoen et al., 1988; Popescu, 1996). Subsequently, countries like Sweden have managed to increase fertility by culling AI translocation sires and thus, increase the non-return rate in heifers (Blazak and Eldridge, 1977)

In Malaysia, despite the prevailing problems of reduction in cattle fertility especially in imported breeds, there is no data available on the status of chromosomal anomalies in the country. Moreover, unlike many of the European countries, there has been no routine cytogenetic evaluation of imported or local cattle intended for breeding. This could lead to a risk of rapid distribution of heritable anomalies to subsequent generations. Therefore, owing to the gaps present in the control of chromosomal anomalies, it is likely that chromosomal aberrations do exist in cattle with infertility problems. In this study, cows and heifers with fertility problems as well as the bulls involved in breeding have been subjected to cytogenetic studies with the aim of determining if numerical or structural chromosomal anomaly is a cause of poor fertility in the herd. Furthermore, it has been described that the *taurus*

(temperate) and *indicus* (zebu) breeds of cattle despite having the same diploid number of chromosomes (2n= 60), differ in the morphology of the Y chromosome. The Y chromosome is submetacentric in *taurus* but acrocentric in *indicus* breeds (Halnan and Watson, 1982). Although KK bulls belong to the zebu cattle breeds, there is no scientific evidence that shows the acrocentric nature of the Y chromosome in this breed.

Therefore, the objectives of this study were:

1. To determine the occurence of AOC and associated ovarian disorders in postpartum cows with more than 90 days open and in heifers that fail to conceive by 24 months old.
2. To evaluate the fertility status of breeding bulls.
3. To investigate if structural or numerical chromosomal anomalies are involved in the poor reproductive performance of the herd.
4. To evaluate a modified faecal extraction procedure for P_4 metabolites (progestins) as well as to detect and identify immunoreactive progestins against P_4 antibody RIA in cows.
5. To determine the correlation between faecal progestins and plasma P_4 during the estrous cycle of KK cows as well as to compare the faecal progestin pattern with pregnant KK cows and cows with ovarian disturbance.

REFERENCES

Adams, G., Matteri, R., Kastelic, J., Ko, J., and Ginther, O. (1992). Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. *Reproduction, 94*(1), 177-188.

Adams, N. R., Abordi, J. A., Briegel, J. R., and Sanders, M. R. (1994). Effect of diet on the clearance of estradiol-17 beta in the ewe. *Biology of Reproduction, 51*(4), 668-674.

Ahmad, N., Beam, S. W., Butler, W. R., Deaver, D. R., Duby, R. T., Elder, D. R., et al. (1996). Relationship of fertility to patterns of ovarian follicular development and associated hormonal profiles in dairy cows and heifers. *Journal of Animal Science 74*, 1943-1952.

Ahmad, N., and Noakes, D. E. (1995). A clinical and ultrasonographic study of the testes and related structures of goats and rams after unilateral vasectomy. *The Veterinary Record, 137*(5), 112-117.

Alexander, J. H. (2008). Bull breeding soundness evaluation: a practitioner's perspective. *Theriogenology, 70*(3), 469-472.

Amann, R. P., and Seidel, G. E. (2000). Fertilizing potential in vitro of semen from young beef bulls containing a high or low percentage of sperm with a proximal droplet. *Theriogenology, 54*(9), 1499-1515.

Andersson, M., Vierula, M., and Alanko, M. (1990). Three types of acrosomal aberrations of bull spermatozoa and their relation to fertility. *Acta Veterinaria Scandinavica, 31*(2), 175-179.

Ansari, H., Jung, H., Hediger, R., Fries, R., König, H., and Stranzinger, G. (1993). A balanced autosomal reciprocal translocation in an azoospermic bull. *Cytogenetic and Genome Research, 62*(2-3), 117-123.

Arteaga, A., Baracaldo, M., and Barth, A. D. (2001). The proportion of beef bulls in western Canada with mature spermiograms at 11 to 15 months of age. *The Canadian Veterinary Journal, 42*(10), 783-787.

Asa, C. S., Bauman, K., Callahan, P., Bauman, J., Volkmann, D. H., and Jochle, W. (2006). GnRH-agonist induction of fertile estrus with either natural mating or artificial insemination, followed by birth of pups in gray wolves (Canis lupus). *Theriogenology, 66*(6-7), 1778-1782.

Babu, A., and Verma, R. S. (1987). Chromosome structure: euchromatin and heterochromatin. In G. H. Bourne, K. W. Jeon andM. Friedlander (Eds.), *Intl Rev Cytol: A survey of cell biology* (Vol. 108, pp. 1-60). Orlando, Florida: Academic Press Inc.

Badinga, L., Thatcher, W. W., Diaz, T., Drost, M., and Wolfenson, D. (1993). Effect of environmental heat stress on follicular development and steroidogenesis in lactating Holstein cows. *Theriogenology, 39*(4), 797-810.

Bagley, C. V., and Chapman, C. K. (2005). Breeding soundness evaluation of bulls, 2009, from http://extension.usu.edu/files/publications/publication/AH_Beef_2005-01.pdf

Ball, P. J. H., and Peteres, A. R. (Eds.). (2004). *Reproduction in cattle* (3rd ed.). Oxford, UK: Blackwel Publishing.

Bamberg, E., Möstl, E., Patzl, M., and King, G. (1991). Pregnancy diagnosis by enzyme immunoassay of estrogens in feces from nondomestic species. *Journal of Zoo and Wildlife Medicine, 22*(1), 73-77.

Barham, B., and Pennington, J. A. (2006). Breeding soundness evaluation for beef and dairy bulls. Division of agriculture cooperative extension service. Agriculture and natural resources, September 2009, from http://www.uaex.edu/other_areas/publications/p

Barth, A. D. (2007). Evaluations of potential breeding soundness of the bull. In R. S. Youngquist andW. R. Threlfall (Eds.), *Current Therapy in Large Animal Theriogenology* (2nd ed., pp. 228-240). Saunders: Elsevier.

Barth, A. D., Bowman, P. A., Bo, G. A., and Mapletoft, R. J. (1992). Effect of narrow sperm head shape on fertility in cattle. *The Canadian Veterinary Journal, 33*(1), 31-39.

Barth, A. D., and Waldner, C. L. (2002). Factors affecting breeding soundness classification of beef bulls examined at the Western College of Veterinary Medicine. *The Canadian Veterinary Journal, 43*(4), 274-284.

Bartholomew, A., Caudle, A., and Peroni, J. (2003). Equine-Equine Rounds-Case Presentation: Unusual Clinical Presentation of Scrotal Hydrocele in a Tennessee Walking Stallion. *Compendium on Continuing Education for the Practicing Veterinarian, 25*(8), 632-633.

Bartlett, P. C., Ngategize, P. K., Kaneene, J. B., Kirk, J. H., Anderson, S. M., and Mather, E. C. (1986). Cystic follicular disease in Michigan Holstein-Friesian cattle: incidence, descriptive epidemiology and economic impact. *Preventive Veterinary Medicine, 4*(1), 15-33.

Beal, W. E., Perry, R. C., and Corah, L. R. (1992). The use of ultrasound in monitoring reproductive physiology of beef cattle. *Journal of Animal Science, 70*(3), 924-929.

Beam, S. W., and Butler, W. R. (1999). Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. *Journal of Reproduction and Fertility*, Suppl. 54, 411–424.

Ben Salem, M., Djemali, M., Kayouli, C., and Majdoub, A. (2006). A review of environmental and management factors affecting the reproductive performance of Holstein-Friesian dairy herds in Tunisia. *Livestock Research for Rural Development, 18*(53), http://www.lrrd.org/lrrd18/14/sale18053.htm.

Berger, E., Leus, K., Vercammen, P., and Schwarzenberger, F. (2006). Faecal steroid metabolites for non-invasive assessment of reproduction in common warthogs (Phacochoerus africanus), red river hogs (Potamochoerus porcus) and babirusa (Babyrousa babyrussa). *Animal Reproduction Science, 91*(1-2), 155-171.

Blazak, W. F., and Eldridge, F. E. (1977). A Robertsonian translocation and its effect upon fertility in Brown Swiss cattle. *Journal of Dairy Science, 60*(7), 1133-1142.

Blom, E. (1983). Pathological conditions in the genital organs and in the semen as grounds for rejection of breeding bulls for import and export to or from Denmark. *Nordisk Veterinaermedicin, 35*, 105-130.

Braw-Tal, R., Pen, S., and Roth, Z. (2009). Ovarian cysts in high-yielding dairy cows. *Theriogenology, 72*(5), 690-698.

Bridges, G. A. (2007). *Impact of preovulatory estradiol concentrations on mechanisms affecting fertility in cattle.* Unpublished doctoral dissertation, The Ohio State University, USA.

Brito, L., Silva, A., Rodrigues, L., Vieira, F., Deragon, L., and Kastelic, J. (2002). Effects of environmental factors, age and genotype on sperm production and semen quality in Bos indicus and Bos taurus AI bulls in Brazil. *Animal Reproduction Science, 70*(3-4), 181-190.

Brown, J. L., Wasser, S. K., Wildt, D. E., and Graham, L. H. (1994). Comparative aspects of steroid hormone metabolism and ovarian activity in felids, measured noninvasively in feces. *Biology of Reproduction, 51*(4), 776-786.

Buoen, L., Weber, A., Meiske, J., and Hooker, E. (1988). Cases of 1/29 Robertsonian Tranlocation (centric fusion) in Charolais Cattle. *The Canadian Veterinary Journal, 29*(5), 455-457.

Burkholder, W. J. (2000). Use of body condition scores in clinical assessment of the provision of optimal nutrition. *Journal of the American Veterinary Medical Association, 217*(5), 650.

Butler, W.R. (2003). Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. *Livestock Production Science*, 83, 211-218.

Butler, W.R. (2000). Nutritional interactions with reproductive performance in dairy cattle. *Animal Reproduction Science,* 60–61, 449–457.

Capezzuto, A., Chelini, M. O., Felippe, E. C., and Oliveira, C. A. (2008). Correlation between serum and fecal concentrations of reproductive steroids throughout gestation in goats. *Animal Reproduction Science, 103*(1-2), 78-86.

Cavestany, D., Vinoles, C., Crowe, M. A., La Manna, A., and Mendoza, A. (2009). Effect of prepartum diet on postpartum ovarian activity in Holstein cows in a pasture-based dairy system. *Animal Reproduction Science, 114*(1-3), 1-13.

Chacon, J., Perez, E., Muller, E., Soderquist, L., and Rodriguez-Martinez, H. (1999). Breeding soundness evaluation of extensively managed bulls in Costa Rica. *Theriogenology, 52*(2), 221-231.

Chapwanya, A., Callanan, J., Larkin, H., Keenan, L., and Vaughan, L. (2008). Breeding soundness evaluation of bulls by semen analysis, testicular fine needle aspiration cytology and trans-scrotal ultrasonography. *Irish Veterinary Journal, 61*(5), 315-318.

Chenoweth, P., Farin, P., Mateos, E., Rupp, G., and Pexton, J. (1984). Breeding soundness and sex drive by breed and age in beef bulls used for natural mating. *Theriogenology, 22*(4), 341-349.

Chenoweth, P. J. (1999). Invited paper: Assessment and management of breeding bulls. *Revista Facultad Agronomia, 16*, 677-689.

Christensen, P., Boelling, D., Pedersen, K. M., Korsgaard, I. R., and Jensen, J. (2005). Relationship between sperm viability as determined by flow cytometry and nonreturn rate of dairy bulls. *Journal of Andrology, 26*(1), 98-106.

Citek, J., Rubes, J., and Hajkova, J. (2009). Short communication: Robertsonian translocations, chimerism, and aneuploidy in cattle. *Journal of Dairy Science, 92*(7), 3481-3483.

Coulter, G. H., and Kozub, G. C. (1989). Efficacy of methods used to test fertility of beef bulls used for multiple-sire breeding under range conditions. *Journal of Animal Science, 67*(7), 1757-1766.

Craig, J., and Bickmore, W. (1993). Genes and genomes: Chromosome bands–flavours to savour. *Bioessays, 15*(5), 349-354.

Cribiu, E., Di Berardino, D., Di Meo, G., Eggen, A., Gallagher, D., Gustavsson, I., et al. (2001). International System for Chromosome Nomenclature of Domestic Bovids (ISCNDB 2000). *Cytogenetics and Cell Genetics, 92*(3-4), 283.

Crowe, M. A. (2008). Resumption of ovarian cyclicity in post-partum beef and dairy cows. *Reprod Domest Anim, 43 Suppl 5*, 20-28.

Crowe, M. A., Goulding, D., Baguisi, A., Boland, M. P., and Roche, J. F. (1993). Induced ovulation of the first postpartum dominant follicle in beef suckler cows using a GnRH analogue. *Reproduction, 99*(2), 551-555.

Crowe, M. A., Padmanabhan, V., Mihm, M., Beitins, I. Z., and Roche, J. F. (1998). Resumption of follicular waves in beef cows is not associated with periparturient changes in follicle-stimulating hormone heterogeneity despite major changes in steroid and luteinizing hormone concentrations. *Biology of Reproduction, 58*(6), 1445-1450.

Davis, R. O., and Katz, D. F. (1993). Operational standards for CASA instruments. *Journal of Andrology, 14*(5), 385-394.

Day, M. L. (2004). Hormonal induction of estrous cycles in anestrous Bos taurus beef cows. *Animal Reproduction Science, 82*, 487-494.

De Rensis, F., Bottarelli, E., Battioni, F., Capelli, T., Techakumphu, M., García-Ispierto, I., et al. (2008). Reproductive performance of dairy cows with ovarian cysts after synchronizing ovulation using GnRH or hCG during the warm or cool period of the year. *Theriogenology, 69*(4), 481-484.

Dehnhard, M., Naidenko, S., Frank, A., Braun, B., Goritz, F., and Jewgenow, K. (2008). Non-invasive monitoring of hormones: a tool to improve reproduction in captive breeding of the Eurasian lynx. *Reproduction in Domestic Animals, 43 Suppl 2*, 74-82.

Desaulniers, D. M., Goff, A.K., Betteridge, K.J., Rowell, J.E. and Flood, P.F., (1989). Reproductive hormone concentrations in faeces during the oestrous cycle and pregnancy in cattle (*Bos taurus*) and muskoxen (*Ovibos moschatus*). *Canadian Journal of Zoology, 67*, 1148-1154.

Di Berardino, D., Hayes, H., Fries, R., and Long, S. (1989). International system for cytogenetic nomenclature of domestic animals. *Cytogenetics and Cell Genetics, 53*, 65-79.

Di Berardino, D., Iannuzzi, L., and Lioi, M. (1985). The high-resolution RBA-banding pattern of bovine chromosomes. *Cytogenetics and Cell Genetics, 39*(2), 136.

Diskin, M. G., and Sreenan, J. M. (2000). Expression and detection of oestrus in cattle. *Reproduction Nutrition Development, 40*(5), 481-491.

D'Occhio, M. J., Neish, A., and Broadhurst, L. (1990). Differences in gonadotrophin secretion postpartum between Zebu and European cattle. *Animal Reproduction Science*, 22, 311-317.

Douthwaite, R., and Dobson, H. (2000). Comparison of different methods of diagnosis of cystic ovarian disease in cattle and an assessment of its treatment with a progesterone-releasing intravaginal device. *The Veterinary Record, 147*(13), 355-359.

Ducos, A., Dumont, P., Séguéla, A., Pinton, A., Berland, H., Brun-Baronnat, C., et al. (2000). A new reciprocal translocation in a subfertile bull. *Genetics, Selection, Evolution: GSE, 32*(6), 589-598.

Ducrot, C., Grohn, Y.T., Humblot, P., Bugnard, F., Sulpice, P. and Gilbert, R.O. (1994). Postpartum anestrus in french beef cattle: an epidemiological study. *Theriogenology,* 42, 753-764.

Duffy, P., Crowe, M. A., Boland, M. P., and Roche, J. F. (2000). Effect of exogenous LH pulses on the fate of the first dominant follicle in postpartum beef cows nursing calves. *Journal of Reproduction and Fertility, 118*(1), 9-17.

Dyrendahl, I., and Gustavsson, I. (1979). Sexual functions, semen characteristics and fertility of bulls carrying the 1/29 chromosome translocation. *Hereditas, 90*(2), 281-289.

Echternkamp, S., and Gregory, K. (2002). Reproductive, growth, feedlot, and carcass traits of twin vs single births in cattle. *Journal of Animal Science, 80*(E-Suppl 2), 1-10.

El-Din. Zain, A., Nakao, T., Abdel Raouf, M., Moriyoshi, M., Kawata, K., and Moritsu, Y. (1995). Factors in the resumption of ovarian activity and uterine involution in postpartum dairy cows. *Animal Reproduction Science, 38*(3), 203-214.

Erb, H. N., and White, M. E. (1981). Incidence rates of cystic follicles in Holstein cows according to 15-day and 30-day intervals. *The Cornell veterinarian, 71*(3), 326-331.

Evans, A. C. O., Davies, F. J., Nasser, L. F., Bowman, P., and Rawlings, N. C. (1995). Differences in early patterns of gonadotrophin secretion between early and late maturing bulls, and changes in semen characteristics at puberty. *Theriogenology, 43*(3), 569-578.

Evans, K., Kasman, L., Hughes, J., Couto, M., and Lasley, B. (1984). Pregnancy diagnosis in the domestic horse through direct urinary estrone conjugate analysis. *Theriogenology, 22*(6), 615-620.

Eyestone, W. H., and Ax, R. L. (1984). A review of ovarian follicular cysts in cows, with comparisons to the condition in women, rats and rabbits. *Theriogenology, 22*(2), 109-125.

Farin, P. W., Youngquist, R. S., Parfet, J. R., and Garverick, H. A. (1990). Diagnosis of luteal and follicular ovarian cysts in dairy cows by sector scan ultrasonography. *Theriogenology, 34*(4), 633-642.

Farin, P. W., Youngquist, R. S., Parfet, J. R., and Garverick, H. A. (1992). Diagnosis of luteal and follicular ovarian cysts by palpation per rectum and linear-array ultrasonography in dairy cows. *Journal of the American Veterinary Medical Association, 200*(8), 1085-1089.

Figueiredo, T. R., and Iannuzzi, L. (1993). Frequency and distribution of rob (1; 29) in three Portuguese cattle breeds. *Hereditas, 119*(3), 233-237.

Fissore, R. A., Edmondson, A. J., Pashen, R. L., and Bondurant, R. H. (1986). The use of ultrasonography for the study of the bovine reproductive tract. II. Non-pregnant, pregnant and pathological conditions of the uterus. *Animal Reproduction Science, 12*(3), 167-177.

Fitzpatrick, L., Fordyce, G., McGowan, M., Bertram, J., Doogan, V., De Faveri, J., et al. (2002). Bull selection and use in northern Australia:: Part 2. Semen traits. *Animal Reproduction Science, 71*(1-2), 39-49.

Ford, C., Pollock, D., and Gustavsson, I. (1980). Proceedings of the first international conference for the standardization of banded karyotypes of domestic animals. *Hereditas, 92*, 145-162.

Forde, N., Beltman, M. E., Lonergan, P., Diskin, M., Roche, J. F., and Crowe, M. A. (2010). Oestrous cycles in Bos taurus cattle. *Animal Reproduction Science, doi:10.1016/j.anireprosci.2010.08.025*(Article in Press).

Fordyce, G., Entwistle, K., Norman, S., Perry, V., Gardiner, B., and Fordyce, P. (2006). Standardising bull breeding soundness evaluations and reporting in Australia. *Theriogenology, 66*(5), 1140-1148.

French, J., Koban, T., Rukstalis, M., Ramirez, S., Bardi, M., and Brent, L. (2004). Excretion of urinary steroids in pre-and postpartum female baboons. *General and Comparative Endocrinology, 137*(1), 69-77.

Gallimore, P. H., and Richardson, C. R. (1973). An improved banding technique exemplified in the karyotype analysis of two strains of rat. *Chromosoma, 41*(3), 259-263.

Garverick, H. A. (1997). Ovarian follicular cysts in dairy cows. *Journal of Dairy Science, 80*(5), 995-1004.

Garverick, H. A. (2007). Ovarian follicular cysts. In R. S. Y. a. W. R. Threlfall (Ed.), *Current Therapy in Large Animal Theriogenology* (pp. 379-383): Saunders Elsevier.

Geske, J. M., Schalles, R. R., and Zoellner, K. O. (1995). *Yearling scrotal circumference prediction equation and age adjustment factors for various breeds of beef bulls*. Paper presented at the Cattlemen's Day. from http://hdl.handle.net/2097/4877

Ghanem, M. E., Isobe, N., Kubota, H., Suzuki, T., Kasuga, A., and Nishibori, M. (2008). Ovarian cyclicity and reproductive performance of Holstein cows carrying the mutation of complex vertebral malformation in Japan. *Reproduction in Domestic Animals, 43*(3), 346-350.

Ginther, O. J., Knopf, L., and Kastelic, J. P. (1989). Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. *Journal of Reproduction and Fertility, 87*(1), 223-230.

Ginther, O. J., Kot, K., Kulick, L. J., Martin, S., and Wiltbank, M. C. (1996). Relationships between FSH and ovarian follicular waves during the last six months of pregnancy in cattle. *Reproduction, 108*(2), 271-279.

Goovaerts, I. G. F., Hoflack, G. G., Van Soom, A., Dewulf, J., Nichi, M., de Kruif, A., et al. (2006). Evaluation of epididymal semen quality using the Hamilton-Thorne analyser indicates variation between the two caudae epididymides of the same bull. *Theriogenology, 66*(2), 323-330.

Graham, L. H. (2004). Non-Invasive Monitoring of Reproduction in Zoo and Wildlife Species. *Annual Review of Biomedical Sciences, 6*, 91-98.

Graham, L. H., Byers, A. P., Armstrong, D. L., Loskutoff, N. M., Swanson, W. F., Wildt, D. E., et al. (2006). Natural and gonadotropin-induced ovarian activity in tigers (Panthera tigris) assessed by fecal steroid analyses. *General and Comparative Endocrinology, 147*(3), 362-370.

Gustashaw, K. M. (1991). Chromosome stains. In: The ACT Cytogenetics Laboratory Manual, 2nd edition, by M.J. Barch. the association of cytogenetic technologies, Raven press, Ltd., New York.

Gustavsson, I. (1969). Cytogenetics, distribution and phenotypic effects of a translocation in Swedish cattle. *Hereditas, 63*(1), 68-169.

Gustavsson, I. (1977). Cytogenetic analysis of cattle chromosomes; current utilization and speculation of future applications. *Genetic Selection Evolution, 9*(4), 459-462.

Gustavsson, I. (1979). Distribution and effects of the 1/29 Robertsonian translocation in cattle. *Journal of Dairy Science, 62*(5), 825-835.

Gustavsson, I., and Rockborn, G. (1964). Chromosome Abnormality in Three Cases of Lymphatic Leukaemia in Cattle. *Nature, 203*, 990.

Hafez, E. S. E., Jainudeen, M. R., and Rosnina, Y. (2000). *Hormones, Growth factors, and Reproduction*. In E. S. E. Hafez andB. Hafez (Eds.), *Reproduction in Farm Animals* (pp. 33-54). USA: Lippincott Williams and Wilkins.

Hahn, J., Stouffer, J. R., and Foote, R. H. (1999). Ultrasonographic and other testicular characteristics of Holstein bulls revisited. *Journal of Reproduction and Development, 45*(6), 405-410.

Halnan, C., and Watson, J. (1982). Y chromosome variants in cattle Bos taurus and Bos indicus. *Genetics, Selection, Evolution: GSE, 14*(1), 1-16.

Hamon, M., Fleet, I., Holdsworth, R., and Heap, R. (1981). The time of detection of oestrone sulphate in milk and the diagnosis of pregnancy in cows. *The British Veterinary Journal, 137*(1), 71-77.

Hanada, H., Geshi, M., and Suzuki, O. (1995). Additional evidence of the formation of unbalanced embryos in cattle with the 7/21 Robertsonian translocation. *Theriogenology, 44*(4), 499-505.

Hanzen, C., Laurent, Y., and Ward, W. R. (1994). Comparison of reproductive performance in Belgian dairy and beef cattle. *Theriogenology, 41*(5), 1099-1114.

Hatler, T. B., Hayes, S. H., Laranja da Fonseca, L. F., and Silvia, W. J. (2003a). Relationship between endogenous progesterone and follicular dynamics in lactating dairy cows with ovarian follicular cysts. *Biology of Reproduction, 69*(1), 218-223.

Hatler, T. B., Hayes, S. H., Laranja da Fonseca, L. F., and Silvia, W. J. (2003b). Relationship between endogenous progesterone and follicular dynamics in lactating dairy cows with ovarian follicular cysts. *Biology of Reproduction, 69*(1), 218.

Hayes, H. (2000). Chromosome banding techniques. In P. Popescu, H. Hayes and B. Dutrillaux (Eds.), *Techniques in Animal Cytogenetics* (pp. 25). Verlag Berlin Heidelberg: Springer.

Hayes, H., Rogel-Gaillard, C., Zijlstra, C., De Haan, N., Urien, C., Bourgeaux, N., et al. (2000). Establishment of an R-banded rabbit karyotype nomenclature by FISH localization of 23 chromosome-specific genes on both G-and R-banded chromosomes. *Cytogenetic and Genome Research, 98*(2-3), 199-205.

Heistermann, M., Agil, M., Buthe, A., and Hodges, J. K. (1998). Metabolism and excretion of oestradiol-17beta and progesterone in the Sumatran rhinoceros (Dicerorhinus sumatrensis). *Animal Reproduction Science, 53*(1-4), 157-172.

Heistermann, M., Tari, S., and Hodges, J. (1993). Measurement of faecal steroids for monitoring ovarian function in New World primates, Callitrichidae. *Reproduction, 99*(1), 243-251.

Higdon, H. L., Spitzer, J. C., Hopkins, F. M., and Bridges, W. C. J. (2000). Outcomes of breeding soundness evaluation of 2898 yearling bulls subjected to different classification systems. *Theriogenology, 53*(6), 1321-1332.

Hindle, J. E., and Hodges, J. K. (1990). Metabolism of oestradiol-17b and progesterone in the white rhinoceros (*Ceratotherium simum simum*). *Journal of Reproduction and Fertility, 90*, 571-580.

Hirata, S., and Mori, Y. (1995). Monitoring reproductive status by fecal progesterone analysis in ruminants. *Journal of Veterinary Medical Science, 57*(5), 845-850.

Hodges, J., Van Aarde, R., Heistermann, M., and Hoppen, H. (1994). Progestin content and biosynthetic potential of the corpus luteum of the African elephant (Loxodonta africana). *Reproduction, 102*(1), 163-168.

Hoflack, G., Opsomer, G., Rijsselaere, T., Van Soom, A., Maes, D., de Kruif, A., et al. (2007). Comparison of computer-assisted sperm motility analysis parameters in semen from Belgian blue and Holstein-Friesian bulls. *Reproduction in Domestic Animals, 42*(2), 153-161.

Hoflack, G., Van Den Broeck, W., Maes, D., Van Damme, K., Opsomer, G., Duchateau, L., et al. (2008). Testicular dysfunction is responsible for low sperm quality in Belgian Blue bulls. *Theriogenology, 69*(3), 323-332.

Holroyd, R., Doogan, V., De Faveri, J., Fordyce, G., McGowan, M., Bertram, J., et al. (2002). Bull selection and use in northern Australia:: 4. Calf output and predictors of fertility of bulls in multiple-sire herds. *Animal Reproduction Science, 71*(1-2), 67-79.

Hommeida, A., Nakao, T., and Kubota, H. (2004). Luteal function and conception in lactating cows and some factors influencing luteal function after first insemination. *Theriogenology, 62*(1-2), 217-225.

Hooijer, G. A., van Oijen, M., Frankena, K., and Valks, M. M. H. (2001). Fertility parameters of dairy cows with cystic ovarian disease after treatment with gonadotrophin-releasing hormone. *The Veterinary Record, 149*(13), 383-386.

Hopkins, F. M. (2007). Diseases of the reproductive system of the bull. In R. S. Youngquist andW. R. Threlfall (Eds.), *Current Therapy in Large Animal Theriogenology* (2nd ed., pp. 240-243). Saunders: Elsevier.

Hopkins, F. M., and Spitzer, J. C. (1997). The new Society for Theriogenology breeding soundness evaluation system. *Veterinary Clinics of North American: Food Animal Practice, 13*(2), 283-293.

Humphrey, W. D., Kaltenbach, C. C., Dunn, T. G., Koritnik, D. R., and Niswender, G. D. (1983). Characterization of hormonal patterns in the beef cow during postpartum anestrus. *Journal of Animal Science, 56*(2), 445-453.

Iannuzzi, L. (1996). G-and R-banded prometaphase karyotypes in cattle (Bos taurus L.). *Chromosome Research, 4*(6), 448-456.

Iannuzzi, L., and Di Berardino, D. (2008). Tools of the trade: diagnostics and research in domestic animal cytogenetics. *Journal of Applied Genetics, 49*(4), 357-366.

Iannuzzi, L., Di Meo, G., Perucatti, A., Incarnato, D., Palo, R., and Zicarelli, L. (2004). Reproductive disturbances and sex chromosome abnormalities in two female river buffaloes. *Veterinary Record, 154*(26), 823-824.

Iannuzzi, L., Molteni, L., Di Meo, G., Perucatti, A., Lorenzi, L., Incarnato, D., et al. (2000). A new balanced autosomal reciprocal translocation in cattle revealed by banding techniques and human-painting probes. *Cytogenetic and Genome Research, 94*(3-4), 225-228.

Iannuzzi, L., Rangel Figueiredo, T., Meo, G., and Ferrara, L. (1993). A new centric fusion translocation in cattle, rob (16; 18). *Hereditas, 119*(3), 239-243.

Irons, P. C., No¨thling, J. O., and Bertschinger, H. J. (2007). Bull breeding soundness evaluation in Southern Africa. *Theriogenology, 68*(6), 842-847.

ISCNDB. (2000). International System for Chromosome Nomenclature of Domestic Bovids. Di Berardino D., Di Meo G. P., Gallagher D.S., Hayes H. and Iannuzzi L. (eds.). *Cytogenetics and Cell Genetics (2001). 92,* 283-299.

Isobe, N., Akita, M., Nakao, T., Yamashiro, H., and Kubota, H. (2005a). Pregnancy diagnosis based on the fecal progesterone concentration in beef and dairy heifers and beef cows. *Animal Reproduction Science, 90*(3-4), 211-218.

Isobe, N., and Nakao, T. (2004). Pregnancy diagnosis in miniature pig by direct ELISA of oestrone derivatives in faeces. *Reproduction in Domestic Animals, 39*(1), 48-51.

Isobe, N., Nakao, T., Yamashiro, H., and Shimada, M. (2005b). Enzyme immunoassay of progesterone in the feces from beef cattle to monitor the ovarian cycle. *Animal Reproduction Science, 87*(1-2), 1-10.

Johnson, M. H., and Everitt, B. J. (2000). *Essential reproduction* (5th ed.). Malden, MA: Blackwell Science.

Johnson, K., Dewey, C., Bobo, J., Kelling, C., and Lunstra, D. (1998). Prevalence of morphologic defects in spermatozoa from beef bulls. *Journal of the American Veterinary Medical Association, 213*(10), 1468.

Johnson, W. H. (1997). The significance to bull fertility of morphologically abnormal sperm. *Veterinary Clinics of North American: Food Animal Practice, 13*(2), 255-270.

Jolly, P. D., McDougall, S., Fitzpatrick, L. A., Macmillan, K. L., and Entwistle, K. W. (1995). Physiological effects of undernutrition on postpartum anoestrus in cows. *Journal of Reproduction and Fertility Supplment, 49*, 477-492.

Kahn, W., and Leidl, W. (1989). Ultrasonic characteristics of pathological conditions of the bovine uterus and ovaries. In M. M. Taverne andA. H. Willemse (Eds.), *Diagnostic Ultrasound and Animal Reproduction* (pp. 53–65): Kluwer Acadamic Publisher.

Karg, H. (1981). Physiological impact on fertility in cattle, with special emphasis on assessment of the reproductive function by progesterone assay. *Livestock Production Science, 8*, 233-246.

Kasari, T. R., Wikse, S. E., and Jones, R. (1996). Use of yearling bulls in beef cattle operations. Part 1. Economic analysis and fertility assessment. *The Compendium of Continuing Education for the Practicing Veterinarian 18*, 1244-1253.

Kastelic, J., and Thundathil, J. (2008). Breeding soundness evaluation and semen analysis for predicting bull fertility. *Reproduction in Domestic Animals, 43*(s2), 368-373.

Kastelic, J. P., Curran, S., and Ginther, O. J. (1989). Accuracy of ultrasonography for pregnancy diagnosis on days 10 to 22 in heifers. *Theriogenology, 31*(4), 813-820.

Kesler, D. J. (1999). *Efficacious estrus synchronization programmes in beef cattle.* Paper presented at the Beef Health and Management Conference, 12p.

Kesler, D. J., and Garverick, H. A. (1982). Ovarian cysts in dairy cattle: a review. *Journal of Animal Science, 55*(5), 1147-1159.

Kesler, D. J., Garverick, H. A., Caudle, A. B., Elmore, R. G., Youngquist, R. S., and Bierschwal, C. J. (1980). Reproductive Hormone and Ovarian Changes in Cows with Ovarian Cysts1. *Journal of Dairy Science, 63*(1), 166-170.

Kirk, J. H., Huffman, E. M., and Lane, M. (1982). Bovine cystic ovarian disease: hereditary relationships and case study. *Journal of the American Veterinary Medical Association, 181*(5), 474-476.

Kirkpatrick, J., Shldeler, S., Lasley, B., and Turner Jr, J. (1991). Pregnancy determination in uncaptured feral horses by means of fecal steroid conjugates. *Theriogenology, 35*(4), 753-760.

Kot, K., and Ginther, O. J. (1999). Ultrasonic characterization of ovulatory follicular evacuation and luteal development in heifers. *Journal of Reproduction and Fertility, 115*(1), 39-43.

Kovács, A., Foote, R., and Lein, D. (1990). 1; 16 tandem translocation with trisomy 16 in a Brown Swiss bull. *The Veterinary Record, 127*(8), 205.

Kozumplík, J. (1990). Morphological changes and sperm decapitation as a cause of fertility disorders in breeding stock. *Veterinary Medicine (Praha), 35*(6), 331-336.

Kreplin, C. (1992). Breeding soundness of bulls. Practical information for Alberta's agriculture industry. Alberta Agriculture, food and rural development, from http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex3545/$file/423-15-1.pdf?OpenElement

Kretzschmar, P., Gansloßer, U., and Dehnhard, M. (2004). Relationship between androgens, environmental factors and reproductive behavior in male white rhinoceros (Ceratotherium simum simum). *Hormones and Behavior, 45*(1), 1-9.

Lamming, G. E., and Darwash, A. O. (1998). The use of milk progesterone profiles to characterise components of subfertility in milked dairy cows. *Animal Reproduction Science, 52*(3), 175-190.

Lance, V., Patton, M., and Hagey, L. (2001). Identification of a series of C21O2 pregnanes from fecal extracts of a pregnant black rhinoceros (Diceros bicornis minor). *Steroids, 66*(12), 875-881.

Larsen, R., Littell, R., Rooks, E., Adams, E., Falcon, C., and Warnick, A. (1990). Bull influences on conception percentage and calving date in Angus Hereford, Brahman and Senepol single-sire herds. *Theriogenology, 34*(3), 549-568.

Larter, N., Arcese, P., Rajamahendran, R., and Gates, C. (1993). Measurement of immunoreactive progestins excreted in faeces as a potential indicator of pregnancy. *Wildlife Research, 20*(6), 739-743.

Larter, N., Rajamahendran, R., and Sivakumaran, K. (1994). Immunoreactive faecal progestins as indicators of reproductive status. *Veterinary Record, 134*, 474-475.

Lightner, J. (2008). Karyotype variability within the cattle monobaramin. *Answers Research Journal, 1*, 77-88.

Lioi, M., Scarfi, M., and Di Berardino, D. (1995). An autosomal trisomy in cattle. *Genetics, Selection, Evolution, 27*(5), 473-476.

Long, S. E. (1990). Chromosome methodology. In C. E. Cornelius, R. R. Marshak andE. C. Melby (Eds.), *Advances in Veterinary Science and Comparative Medicine* (Vol. 34). New York: Academic Press.

López-Gatius, F. (2003). Is fertility declining in dairy cattle?: A retrospective study in northeastern Spain. *Theriogenology, 60*(1), 89-99.

López-Gatius, F., Santolaria, P., Yániz, J., Fenech, M., and López-Béjar, M. (2002). Risk factors for postpartum ovarian cysts and their spontaneous recovery or persistence in lactating dairy cows. *Theriogenology, 58*(8), 1623-1632.

Lucy, M. C., Billings, H. J., Butler, W. R., Ehnis, L. R., Fields, M. J., Kesler, D. J., et al. (2001). Efficacy of an intravaginal progesterone insert and an injection of PGF2alpha for synchronizing estrus and shortening the interval to pregnancy in postpartum beef cows, peripubertal beef heifers, and dairy heifers. *Journal of Animal Science 79*(4), 982-995.

Luke, S., Verma, R., Conte, R., and Mathews, T. (1992). Molecular characterization of the secondary constriction region (qh) of human chromosome 9 with pericentric inversion. *Journal of Cell Science, 103*, 919-923.

Lyimo, Z. C., Nkya, R., Schoonman, L., and van Eerdenburg, F. J. (2004). Post-partum reproductive performance of crossbred dairy cattle on smallholder farms in sub-humid coastal Tanzania. *Tropical Animal Health and Production, 36*(3), 269-279.

Madrid, N., Ott, R. S., Veeramachaneni, D. N., Parrett, D. F., Vanderwert, W., and Willms, C. L. (1988). Scrotal circumference, seminal characteristics, and testicular lesions of yearling Angus bulls. *American Journal of Veterinary Research, 49*(4), 579-585.

Makarechian, M., and Farid, A. (1985). The relationship between breeding soundness evaluation and fertility of beef bulls under group mating at pasture. *Theriogenology, 23*(6), 887-898.

Marcum, J. (1974). The Freemartin Syndrome. *Animal Breeding Abstracts, 42*, 227–242.

Mastromonaco, G., Coppola, G., Crawshaw, G., DiBerardino, D., and King, W. (2004). Identification of the homologue of the bovine Rob (1; 29) in a captive gaur (Bos gaurus). *Chromosome Research, 12*(7), 725-731.

Masunda, B., Mutisi, C., Hamudikuwanda, H., and Agumbah, J. (1999). The concentration of faecal progestins during the oestrous cycle in Nkone cows and the effect of duration of storage of faecal samples at room temperature on faecal progestin levels. *Tropical Animal Health and Production, 31*(6), 373-381.

Masunda, B., Mutisi, C., Hamudikuwanda, H., and Agumbah, J. (2002). The Use of Faecal Progestin Measurements to Monitor Reproductive Activity in Mashona Cows in a Smallholder Farming Area of Zimbabwe. *Tropical Animal Health and Production, 34*(4), 309-318.

Matsuda-Motomura, D., Gartley, C., Bellem, A., Liptrap, R., Madison, V., and Goodrowe, K. (1995). Endocrine and behavioral events of estrous cyclicity and synchronization in wood bison (Bison bison athabascae). *Theriogenology, 43*(1), 273-273.

McCosker, T. H., Turner, A. F., McCool, C. J., Post, T. B., and Bell, K. (1989). Brahman bull fertility in a north Australian rangeland herd. *Theriogenology, 32*(2), 285-300.

McDougall, S., Burke, C. R., MacMillan, K. L., and Williamson, N. B. (1995). Patterns of follicular development during periods of anovulation in pasture-fed dairy cows after calving. *Research in Veterinary Science, 58*(3), 212-216.

Miyamoto, A., Shirasuna, K., Hayashi, K. G., Kamada, D., Awashima, C., Kaneko, E., et al. (2006). A potential use of color ultrasound as a tool for reproductive management: New observations using color ultrasound scanning that were not possible with imaging only in black and white. *Journal of Reproduction and Development, 52*(1), 153-160.

Molteni, L., Giovanni-Macchi, A., Succi, G., Cremonesi, F., Stacchezzini, S., Meo, G., et al. (1998). A new centric fusion translocation in cattle: rob (13; 19). *Hereditas, 129*(2), 177-180.

Molteni, L., Meggiolaro, D., and DE Giovanni, M. (2005). Fertility of cryopreserved sperm in three bulls with different Robertsonian translocations. *Animal Reproduction Science, 86*(1-2), 27-36.

Molteni, L., Perucatti, A., Iannuzzi, A., Di Meo, G., De Lorenzi, L., De Giovanni, A., et al. (2007). A new case of reciprocal translocation in a young bull: rcp (11; 21)(q28; q12). *Cytogenetic and Genome Research, 116*(1-2), 80-84.

Montiel, F., and Ahuja, C. (2005). Body condition and suckling as factors influencing the duration of postpartum anestrus in cattle: a review. *Animal Reproduction Science, 85*(1-2), 1-26.

Morrow, C. J., and Monfort, S. L. (1998). Ovarian activity in the scimitar-horned oryx (Oryx dammah) determined by faecal steroid analysis. *Animal Reproduction Science, 53*(1-4), 191-207.

Möstl, E., Choi, H. S., Wurm, W., Ismail, N., and Bamberg, E. (1984). Pregnancy diagnosis in cows and heifers by determination of oestradiole-17α in faeces. *Brazillian Veterinary Journal, 140*, 287-291.

Möstl, E., and Palme, R. (2002). Hormones as indicators of stress. *Domest Animal Endocrinology, 23*(1-2), 67-74.

Möstl, E., Rettenbacher, S., and Palme, R. (2005). Measurement of corticosterone metabolites in birds' droppings: an analytical approach. *Annals of the New York Academy of Sciences, 1046*, 17-34.

Mueller, K. (2007). Cystic ovarian disease in cows- diagnosis and treatment decisions. *UK Vet 13*(1), 46- 51.

Mujuni, P. F., Mgongo, F. O. K., and Kanuya, N. L. (1993). Ovarian cysts, a postpartum ovarian disorder affecting dairy cows in a tropical area. *Animal Reproduction Science, 31*(3-4), 175-186.

Mukasa-Mugerwa, E. (1989). *A review of reproductive performance of female Bos indicus (Zebu) cattle*: ILCA Monograph 6. ILCA, Addis Ababa, Ethiopia.

Muñoz, M., Ocanto, D., Madriz, M., Medina, R., and Vera, O. (1994). Incidence of 1 29 translocation in Venezuelan Creole bulls. *Theriogenology, 41*(2), 379-382.

Murphy, M. G., Boland, M. P., and Roche, J. F. (1990). Pattern of follicular growth and resumption of ovarian activity in post-partum beef suckler cows. *Reproduction, 90*(2), 523-533.

Mwaanga, E. S., Zdunczyk, S., Janowski, T., and Kotowski, K. (2003). Diagnosis and treatment of ovarian afunction disorder with a norgestomet ear-implant (Crestar) in dairy cows. *Bulletin of the Veterinary Institute in Pulawy, 47*(1), 171-176.

Nakao, T., Sugihashi, A., Ishibashi, Y., Tosa, E., Nakagawa, Y., Yuto, H., et al. (1982). Use of milk progesterone enzyme immunoassay for early pregnancy diagnosis in cows. *Theriogenology, 18*(3), 267-274.

Noakes, D. (2000). Fertility and Infertility. In A. H. Andrews (Ed.), *The Health of Dairy Cattle* (pp. 108-148). Oxford, England: Blackwell Science.

Nöthling, J. O., and Arndt, E. P. (1995). Fertility of two bulls with poor sperm morphology. *Journal of the South African Veterinary Association, 66*(2), 74-76.

Opsomer, G., Coryn, M., Deluyker, H., and Kruif, A. (1998). An analysis of ovarian dysfunction in high yielding dairy cows after calving based on progesterone profiles. *Reproduction in Domestic Animals, 33*(3-4), 193-204.

Opsomer, G., Gröhn, Y. T., Hertl, J., Coryn, M., Deluyker, H., and de Kruif, A. (2000). Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study. *Theriogenology, 53*(4), 841-857.

Padula, A. M. (2005). The freemartin syndrome: an update. *Animal Reproduction Science, 87*(1-2), 93-109.

Palme, R. (2005). Measuring fecal steroids: guidelines for practical application. *Annals of the New York Academy of Sciences, 1046*, 75-80.

Palme, R., Fischer, P., Schildorfer, H., and Ismail, M. (1996). Excretion of infused 14C-steroid hormones via faeces and urine in domestic livestock. *Animal Reproduction Science, 43*(1), 43-63.

Palme, R., Möstl, E., Brem, G., Schellander, K., and Bamberg, E. (1997). Faecal metabolites of infused 14C-progesterone in domestic livestock. *Reproduction in Domestic Animals, 32*(4), 199-206.

Palme, R., Rettenbacher, S., Touma, C., El Bahr, S., and Möstl, E. (2005). Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. *Annals of the New York Academy of Sciences, 1040*(1), 162-171.

Palme, C.W., Brito, L.F.C., Arteaga, A.A., Söderquist, Persson, L.Y., Barth, A.D. (2005). Comparison of electroejaculation and transrectal massage for semen collection in range and yearling feedlot beef bulls. *Animal Reproduction Science,* 87, 25–31.

Parkinson, T. J. (2004). Evaluation of fertility and infertility in natural service bulls. *The Veterinary Journal, 168*(3), 215-229.

Parkinson, T. J. (2001). Infertility in male animals. In D. E. Noakes andT. J. Parkinson (Eds.), *Arthur's Veterinary Reproduction and Obstetrics* (8th ed., pp. 695-750). London: Harcourt Brace.

Pechman, R., and Eilts, B. (1987). B-mode ultrasonography of the bull testicle. *Theriogenology, 27*(2), 431-441.

Penfold, L. M., S, L. M., Wolfe, B. A., Citino, S. B., and Wildt, D. E. (2005). Reproductive physiology and artificial insemination studies in wild and captive gerenuk (Litocranius walleri walleri). *Reproduction Fertility and Development, 17*(7), 707-714.

Perea-Ganchou, F., Belloso, E. S., Stagnaro, C. G., Castillo, G. S., and Fonseca, H. H. (2005). Factors Affecting Fertility According to the Postpartum Period in Crossbred Dual-Purpose Suckling Cows in the Tropics. *Tropical Animal Health and Production, 37*(7), 559-572.

Perry, G., Walker, J., and Daly, R. (2008). Reproductive fertility in herd bulls Retrieved 15 October, 2008, from http://www.thebeefsite.com/articles/1491/

Perry, R. C., Corah, L. R., Kiracofe, G. H., Stevenson, J. S., and Beal, W. E. (1991). Endocrine changes and ultrasonography of ovaries in suckled beef cows during resumption of postpartum estrous cycles. *Journal of Animal Science, 69*(6), 2548-2555.

Peters, A. R. (1985). Hormonal control of the bovine oestrous cycle. I. The natural cycle. *Brazillian Veterinary Journal, 141*(6), 564-575.

Petersson, K. J., Gustafsson, H., Strandberg, E., and Berglund, B. (2006). Atypical progesterone profiles and fertility in Swedish dairy cows. *Journal of Dairy Science, 89*(7), 2529-2538.

Pinheiro, L., Carvalho, T., Oliveira, D., Popescu, C., and Basrur, P. (1995). A 4/21 tandem fusion in cattle. *Hereditas, 122*(2), 99-102.

Popescu, C. P. (1996). From chromosome shape to chromosome mapping: 30 years of domestic animal cytogenetics. *Archivos Zootecnia, 45*, 117-124.

Popescu, C. P., and Pech, A. (1991). Une bibliographie sur la translocation 1/29 de bovins dans le monde (1964-1990). *Annales of Zootechnia, 40*, 271-305.

Quina, A., Buschbeck, M., and Di Croce, L. (2006). Chromatin structure and epigenetics. *Biochemical Pharmacology, 72*(11), 1563-1569.

Rabiee, A. R., Macmillan, K. L., and Schwarzenberger, F. (2001). Progesterone metabolism in ovariectomised non-lactating Holstein-Friesian cows treated with progesterone with two levels of feed intake. *Animal Reproduction Science, 66*(1-2), 35-46.

Rajamahendran, R., Ambrose, D.J., and Burton, B. (1994). Clinical and research applications of real-time ultrasonography in bovine reproduction: a review. *Canadian Veterinary Journal*, 35(9), 563-572.

Rajamahendran, R., Burton, B., and Shelford, J. (1993). A field study on the usefulness of milk progesterone determination to confirm estrus and pregnancy of dairy cows in the Fraser Valley area of British Columbia. *The Canadian Veterinary Journal, 34*(6), 349-352.

Randel, R. D. (1976). LH and ovulation in Brahman, Brahman x Hereford and Hereford heifers. *Journal of Animal Science,* 43:300.

Randel, R. D. (1990). Nutrition and postpartum rebreeding in cattle. *Journal of Animal Science, 68*(3), 853-862.

Rao, A. R., Bane, A., and Gustafsson, B. K. (1980). Changes in the morphology of spermatozoa during their passage through the genital tract in dairy bulls with normal and impaired spermat ogenesis. *Theriogenology, 14*(1), 1-12.

Rijsselaere, T., Van Soom, A., Maes, D., and de Kruif, A. (2003). Effect of technical settings on canine semen motility parameters measured by the Hamilton-Thorne analyzer. *Theriogenology, 60*(8), 1553-1568.

Roche, J. F., Mackey, D., and Diskin, M. D. (2000). Reproductive management of postpartum cows. *Animal Reproduction Science, 60-61*, 703-712.

Rosnina, Y. (1994). *Cytogenetic studies on goats with inherited birth defects and reproductive problems.* Unpublished Doctoral dissertation University of Guelph, Canada.

Royal, M. D., Darwash, A. O., Flint, A. P. F., Webb, R., Woolliams, J. A., and Lamming, G. E. (2000). Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. *Animal Science, 70*(3), 487-501.

Rubes, J., Musilova, P., Borkovec, L., Borkovcova, Z., Svecova, D., and Urbanova, J. (1996). A new Robertsonian translocation in cattle, rob(16;20). *Hereditas, 124*(3), 275-279.

Sakaguchi, M., Sasamoto, Y., Suzuki, T., Takahashi, Y., and Yamada, Y. (2004). Postpartum ovarian follicular dynamics and estrous activity in lactating dairy cows. *Journal of Dairy Science, 87*(7), 2114-2121.

Santos, J. E. P., Rutigliano, H. M., and MF Filho, S. (2009). Risk factors for resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows. *Animal Reproduction Science, 110*(3-4), 207-221.

Santos, J. E. P., Thatcher, W. W., Chebel, R. C., Cerri, R. L. A., and Galvao, K. N. (2004). The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. *Animal Reproduction Science, 82*, 513-535.

Sartori, R., Haughian, J. M., Shaver, R. D., Rosa, G. J. M., and Wiltbank, M. C. (2004). Comparison of ovarian function and circulating steroids in estrous

cycles of Holstein heifers and lactating cows. *Journal of Dairy Science, 87*(4), 905-920.

Savio, J. D., Boland, M. P., and Roche, J. F. (1990). Development of dominant follicles and length of ovarian cycles in post-partum dairy cows. *Reproduction, 88*(2), 581-591.

Schifferli, C., Bonelli, A., Wevar, C., Scilingo, A., and Arruga, M. (2003). Presumptive 1/29 Robertsonian translocation observed in the Argentinean Creole cattle breed. *Animal Research, 52*(2), 119-123.

Schmutz, S., Moker, J., Barth, A., and Mapletoft, R. (1991). Embryonic loss in superovulated cattle caused by the 1; 29 Robertsonian translocation. *Theriogenology, 35*(4), 705-714.

Schmutz, S., Moker, J., Pawlyshyn, V., Haugen, B., and Clark, E. (1997). Fertility effects of the 14; 20 Robertsonian translocation in cattle. *Theriogenology, 47*(4), 815-823.

Schumacher, J., and Varner, D. (2007). Surgical correction of abnormalities affecting the reproductive organs of stallions. In R. S. Youngquist andW. R. Threlfall (Eds.), *Current Therapy in Lage Animal Theriogenology* (2nd ed., pp. 23-36). Sounders: Elsevier.

Schwarzenberger, F. (2007). Non-invasive endocrine monitoring using fecal steroid analysis: opportunities and challenges. *Revista Brasileira de Zootecnia, 36*, 87-88.

Schwarzenberger, F., Francke, R., and Goltenboth, R. (1993a). Concentrations of faecal immunoreactive progestagen metabolites during the oestrous cycle and pregnancy in the black rhinoceros (Diceros bicornis michaeli). *Reproduction, 98*(1), 285-291.

Schwarzenberger, F., Möstl, E., Bamberg, E., and Von Hegel, G. (1992). Monitoring of corpus luteum function by measuring progestagens in faeces of non-pregnant mares (Equus caballus) and Przewalski mares (Equus przewalskii). *Animal Reproduction Science, 29*(3-4), 263-273.

Schwarzenberger, F., Möstl, E., Palme, R., and Bamberg, E. (1996a). Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. *Animal Reproduction Science, 42*(1-4), 515-526.

Schwarzenberger, F., Patzl, M., Francke, R., Ochs, A., Buiter, R., Schaftenaar, W., et al. (1993b). Fecal progestagen evaluations to monitor the estrous cycle and pregnancy in the okapi (Okapia johnstoni). *Zoo Biology, 12*(6), 549-559.

Schwarzenberger, F., Son, C. H., Pretting, R., and Arbeiter, K. (1996b). Use of group-specific antibodies to detect fecal progesterone metabolites during the estrous cycle of cows. *Theriogenology, 46*(1), 23-32.

Schwarzenberger, F., Speckbacher, G., and Bamberg, E. (1995). Plasma and fecal progestagen evaluations during and after the breeding season of the female vicuna. *Theriogenology, 43*(3), 625-634.

Schwarzenberger, F., Tomášová, K., Holečková, D., Matern, B., and Möstl, E. (1996). Measurement of fecal steroids in the black rhinoceros (Diceros bicornis) using group-specific enzyme immunoassays for 20-oxo-pregnanes. *Zoo Biology, 15*(2), 159-171.

Seabright, M. (1971). A rapid banding technique for human crhomosomes. *Lancet. 11*, 971-972.

Shaw, H., Green, D., Sainsbury, A., and Holt, W. (1995). Monitoring ovarian function in scimitar-horned oryx (Oryx dammah) by measurement of fecal 20 -progestagen metabolites. *Zoo Biology, 14*(3), 239-250.

Shideler, S., Ortuno, A., Moran, F., Moorman, E., and Lasley, B. (1993). Simple extraction and enzyme immunoassays for estrogen and progesterone metabolites in the feces of Macaca fascicularis during non-conceptive and conceptive ovarian cycles. *Biology of Reproduction, 48*(6), 1290-1298.

Shore, M., Bretzlaff, K., Thompson, J., and Magee, D. (1995). Outcome of scrotal hydrocele in 26 bulls. *Journal of the American Veterinary Medical Association, 207*(6), 757-760.

Short, R., Bellows, R., Staigmiller, R., Berardinelli, J., and Custer, E. (1990). Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. *Journal of Animal Science, 68*(3), 799-816.

Shrestha, H. K., Nakao, T., Higaki, T., Suzuki, T., and Akita, M. (2004a). Resumption of postpartum ovarian cyclicity in high-producing Holstein cows. *Theriogenology, 61*(4), 637-649.

Shrestha, H. K., Nakao, T., Suzuki, T., Higaki, T., and Akita, M. (2004b). Effects of abnormal ovarian cycles during preservice period postpartum on subsequent reproductive performance of high-producing Holstein cows. *Theriogenology, 61*(7-8), 1559-1571.

Silvia, W. J., Hatler, T. B., Nugent, A. M., and Laranja da Fonseca, L. F. (2002). Ovarian follicular cysts in dairy cows: an abnormality in folliculogenesis. *Domestic Animimal Endocrinology, 23*(1-2), 167-177.

Sirois, J., and Fortune, J. E. (1988). Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. *Biology of Reproduction, 39*(2), 308-317.

Smith, M. F., McIntush, E. W., and Smith, G. W. (1994). Mechanisms associated with corpus luteum development. *Journal of Animal Science, 72*(7), 1857-1872.

Söderquist, L., Janson, L., H rd, M., and Einarsson, S. (1996). Influence of season, age, breed and some other factors on the variation in sperm morphological abnormalities in Swedish dairy AI bulls. *Animal Reproduction Science, 44*(2), 91-98.

Söderquist, L., Janson, L., Larsson, K., and Einarsson, S. (1991). Sperm morphology and fertility in AI bulls. *Journal of Veterinary Medicine Series A, 38*(1-10), 534-543.

Spitzer, J. C., Hopkins, F. M., Webster, H. W., Kirkpatrick, F. D., and Hill, H. S. (1988). Breeding soundness examination of yearling beef bulls. *Journal of American Veterinary Medical Association, 193*(9), 1075-1079.

Stahringer, R.C., Byerley, D. J., Kiser, T. E., and Randel, R. D. (1994). Serum luteinizing hormone, 13,14-Dihydro-15-Keto prostaglandin F2α and cortisol profiles during postpartum. *Theriogenology,* 41, 1069-1080.

Staples, C.R., Thatcher, W.W., and Clark, J.H. (1990). Relationship between ovarian activity and energy status during the early postpartum period of high producing dairy cows. *Journal of Dairy Science,* 73, 938-947**.**

Stavisky, R., Russell, E., Stallings, J., Smith, E., Worthman, C., and Whitten, P. (1995). Fecal steroid analysis of ovarian cycles in free ranging baboons. *American Journal of Primatology, 36*(4), 285-297.

Steimer, T. (2007). Reproductive health. Steroid hormone metabolism, September 2008,from www.gfmer.ch/Books/Reproductive_health/Steroid_hormone_metabolism.html

Sullivan, B. A. (2004). Centromeres. *Encyclopedia of Biological Chemistry, 1*, 367-371.

Sumner, A. T. (1972). A simple technique for demonstrating centromeric heterochromatin. *Experimental Cell Research, 75*(1), 304-306.

Sumner, A. T. (1990). *Chromosome banding*. Australia: Allen & Unwin Australia.

Sunderland, S. J., Crowe, M.A., Boland, M.P., Roche, J.F., and Ireland, J.J. (1994). Selection, dominance and atresia of follicles during the oestrus cycle of heifers. *Journal of Reproduction and Fertility, 101*, 547-555.

Tanaka, K., Yamamoto, Y., Amano, T., Yamagata, T., Dang, V. B., Matsuda, Y., et al. (2000). A Robertsonian translocation, rob(2;28), found in Vietnamese cattle. *Hereditas, 133*(1), 19-23.

Tateno, H., and Miyake, Y. (1994). Sperm chromosome study of two bulls heterozygous for different Robertsonian translocations. *Hereditas, 120*(1), 7-11.

Tebble, J. E., O'Donnell, M. J., and Dobson, H. (2001). Ultrasound diagnosis and treatment outcome of cystic ovaries in cattle. *The Veterinary Record, 148*(13), 411-413.

Thundathil, J., Meyer, R., Palasz, A., Barth, A., and Mapletoft, R. (2000). Effect of the knobbed acrosome defect in bovine sperm on IVF and embryo production. *Theriogenology, 54*(6), 921-934.

Thundathil, J., Palasz, A. T., Mapletoft, R. J., and Barth, A. D. (1999). An investigation of the fertilizing characteristics of pyriform-shaped bovine spermatozoa. *Animal Reproduction Science, 57*(1-2), 35-50.

Touma, C., and Palme, R. (2005). Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. *Annals of the New York Academy of Sciences, 1046*, 54-74.

Tronstad, R., Gum, R., Ray, D., and Rice, R. (1993). Range cow culling: herd performance. *Ranch Business Management,* 27-32.

Verstegen, J., Iguer-Ouada, M., and Onclin, K. (2002). Computer assisted semen analyzers in andrology research and veterinary practice. *Theriogenology, 57*(1), 149-179.

Villagomez, D., Andersson, M., Gustavsson, I., and Plöen, L. (1993). Synaptonemal complex analysis of a reciprocal translocation, rcp (20; 24)(q17; q25), in a subfertile bull. *Cytogenetic and Genome Research, 62*(2-3), 124-130.

Washburn, S. P., Silvia, W. J., Brown, C. H., McDaniel, B. T., and McAllister, A. J. (2002). Trends in reproductive performance in southeastern Holstein and Jersey DHI herds. *Journal of Dairy Science, 85*(1), 244-251.

Wasser, S., Hunt, K., Brown, J., Cooper, K., Crockett, C., Bechert, U., et al. (2000). A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. *General and Comparative Endocrinology, 120*(3), 260-275.

Wasser, S., Monfort, S., Southers, J., and Wildt, D. (1994). Excretion rates and metabolites of oestradiol and progesterone in baboon (Papio cynocephalus cynocephalus) faeces. *Reproduction, 101*(1), 213-220.

Wasser, S. K., and Hunt, K. E. (2005). Noninvasive measures of reproductive function and disturbance in the barred owl, great horned owl, and northern spotted owl. *Annals of the New York Academy of Sciences, 1046*, 109-137.

Wildman, E. E., Jones, G. M., Wagner, P. E., Boman, R. L., Troutt Jr, H. F., and Lesch, T. N. (1982). A Dairy Cow Body Condition Scoring System and Its Relationship to Selected Production Characteristics. *Journal of Dairy Science, 65*(3), 495-501.

Wilson, S. J., Marion, R. S., Spain, J. N., Spiers, D. E., Keisler, D. H., and Lucy, M. C. (1998). Effects of controlled heat stress on ovarian function of dairy cattle. 1. Lactating cows. *Journal of Dairy Science, 81*(8), 2124-2131.

Wilson, T. (1990). Identification of the 1/29 Robertsonian translocation chromosome in British Friesian cattle. *The Veterinary Record, 126*(2), 37-39.

Wiltbank, M. C. (2002). Physiological classification of anovulatory conditions in cattle. *Theriogenology, 57*(1), 21-52.

Yavas, Y., and Walton, J. S. (2000). Postpartum acyclicity in suckled beef cows: a review. *Theriogenology, 54*(1), 25-55.

Yoshioka, K., Iwamura, S., and Kamomae, H. (1996). Ultrasonic observations on the turnover of ovarian follicular cysts and associated changes of plasma LH, FSH, progesterone and oestradiol-17 [beta] in cows. *Research in Veterinary Science, 61*(3), 240-244.

You, L. (2004). Steroid hormone biotransformation and xenobiotic induction of hepatic steroid metabolizing enzymes. *Chemico-biological Interactions, 147*(3), 233-246.

Yunis, J. (1976). High resolution of human chromosomes. *Science, 191*, 1268-1270.

Zdu Czyk, S., Mwaanga, E. S., Ma Ecki-Tepicht, J., Bara Ski, W., and Janowski, T. (2002). Plasma progesterone levels and clinical findings in dairy cows with post-partum anoestrus. *Bulletin of the Veterinary Institute in Pulawy, 46*, 79-86.