COMPARISON OF PROGESTERONE, $PGF_{2\alpha}$ & NOVEL *NC SYNCH* GnRH BASED SYNCHRONIZATION PROTOCOLS IN BOER AND VENDA INDIGENOUS GOATS OF SOUTH AFRICA

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A dissertation submitted in fulfillment of the requirements for the degree of Master of Science in Agriculture (Animal Science)

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DECLARATION

I , Onayi Brighton Dara (Student no: 11626969), hereby declare that this dissertation was submitted in fulfillment of the requirements for the degree of MSc in Agriculture (Animal Science) submitted to the Department of Animal science, School of Agriculture University of Venda and has not been submitted previously for any degree at this or any other University. It is based on my original work except for quotations, references and citations which have duly been acknowledged.

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DEDICATION

This work is dedicated to my family: my parents Mr & Mrs R.J Daara, my siblings: Joana, Joyline, Patience, Jonathan, Agie, Ethel, Wonder, Lonsom, my wife: Miriam and my beloved daughter: Charlotte.

ABSTRACT

The objectives of the study were to compare oestrous parameters, fertility parameters and progesterone assays following application of three oestrous synchronization protocols in maiden and multiparous Boer and Venda indigenous breed doelings (n=60). Goat does (n=60) were assigned into four balanced complete randomized block treatment experimental protocols. CIRD-G/eCG protocol goat does (n=15) had (CIDR-G / 0.3 g progesterone, Zoetis SA) intravaginal devices kept in situ for 12 days and 250 IU of eCG (Folligon®,1000IU, Intervet SA) with 10 mg $PGF_{2\alpha}$ (Lutalyse®, Dinoprost 5 mg/ml, Zoetis, SA) injected IM 12 h before device removal. Double $PGF_{2\alpha}$/eCG protocol does (n=15) had two 10 mg $PGF_{2\alpha}$ injections 12 days apart and 250 IU of eCG injected 12 h before second last $PGF_{2\alpha}$ injection. Novel *NC Synch* GnRH based protocol does (n=15) had 10 mg $PGF_{2\alpha}$ injected on day -2 , 0.5 ml GnRH (Receptal®, Buserelin 0.004 mg/ml, Intervet, RSA) injected IM on day 5 and 10 mg $PGF_{2\alpha}$ injected on day 12. Novel *NC Synch* GnRH based protocol goat does also received 0.5 ml GnRH injection at time of AI. Control does (n=15) only received 250 IU of eCG on day of oestrus onset. Trans-cervical deep uterine AI was conducted 24 h after oestrus onset using freshly collected Boer buck semen in responding does. Blood P4 profiles at experiment day 0, 6, 7, 10, 12 and 17 evaluated ovarian function dynamics during protocol application and rectal ultrasound at day 45-55 after AI determined goat doe conception status. Oestrous response, conception rates, oestrus duration and some fertility parameters did not differ significantly (p>0.05) among groups. CIRD-G/eCG protocol ($25.95^{b}\pm3.85$) influenced a significantly shorter (p<0.05) time interval to oestrus onset after treatment withdrawal. Novel *NC Synch* protocol ($147.7^{b}\pm2.5$) recorded significantly shorter (p<0.05) and control goat does ($156.4^{a}\pm2.0$) significantly longer (p<0.05) gestation length. Venda indigenous doelings, CIDR-G/eCG and novel *NC Synch* GnRH based protocol research variables influenced compact oestrus thus giving an opportunity to conduct *fixed*-TAI in goat ART programs.

Key words: Oestrous and ovulation synchronization, goat doelings, Artificial insemination, fertility, progestagen/progesterone.

ACKNOWLEDGEMENTS

First and foremost I want to give credit to the Almighty God who gave me power, strength, motivation, leadership, direction and guidance throughout this research article. Without him I could not have achieved such a milestone when stakes were high.

I would like to take this opportunity to thank the school of Agriculture for earning me admission into the University of Venda MSc in Agriculture specializing in ART animal reproductive programs in 2012. I would also like to thank Research and Innovation Centre at the University of Venda for funding this research and the Department of Higher education led by Minister Dr Blade Nzimande for research initiatives in ART programs at University of Venda.

I feel great pleasure to express my sincere and great appreciation of my supervisor Prof D.M Barry for his enthusiasm, creativity, craftsmanship, motivation, mentorship, financial backing and progressively engineering this ART initiative at University of Venda. His unrelenting fatherly support in all spheres of this research gave me confidence and willpower to succeed. The financial funding of this project had 90% derived from initiatives and motivations generated by him. His desire to succeed saw him tirelessly fought for the establishment of state of the art CEAAR biotechnology laboratory at University of Venda and development of the goat and cattle feedlot to stimulate research not only in Animal Reproduction but other fields of research like Animal Nutrition. His efforts will forever continue to contribute enormously to research in biotechnology in Limpopo, South Africa and Southern Africa as a whole.

I would like to take this opportunity to thank my co-supervisors; Dr J.J Baloyi and Mr F Dondofema for their profound inputs on research outline, literature build up, statistical modeling and contributions towards production of the final product. Sparing part of their productive time on their already busy schedules to input valuable creative suggestions in this research was a commendable feat for research motivation. My special acknowledgements go to Simbarashe Katsande for his unwavering support and statistical evaluations of this research study data converting it into scientific information.

My profound gratitude goes to Moreni Phalanndwa, Steven Ramwedzi, Emmanuel Matshili, Mupfhaliseni Mulaudzi, Collins Mulaudzi, Tendo, Alilali Maphiswana and Innocent Tshikovhi for their contributions rendered during AI, experimental treatments, blood collections, animal care, experimental area preparation, data recording/measurements and maintenance of instruments. I would also like to thank Mighty Ajao, Andrea Raseona and Dakalo Nethengwe for providing a technical cover during experimentation by making necessary reporting, inventory ordering and care of the experimental animals.

Lastly my sincere acknowledgement goes to Mr Mampheu, Mr S Nethengwe, Mr Mgwedi, Mr J Sikhobo, Mr T Nethengwe, Mr T Nengudza, Mr Mugwabana and other farmers who provided animals for this experimentation. Their eagle's eye of a need of research in developing and reinventing newer farming practices was crucial despite the idea of risking losing their animals for the 6 months that they took away from their farming entities. Great acknowledgement goes to Dakalo Nethengwe (Fellow Masters' student) for enticing the local farmers to contribute towards the findings of this research.

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$Y_{ijk} = \mu + B_i + S_j + (BS)_{ij} + e_{ijk}$ 36

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LIST OF ABBREVIATIONS

24 *h*, 48 *h*	: Twenty four hours, Forty eight hours
A/V	: Artificial Vagina for goats,
AI	: Artificial Insemination
AP	: Access to progesterone
AIDE	: AI at detected estrus
ART	: Assisted Reproductive Technique
BCs	: Body Condition Score
Bwt	: Body weight
CIDR-G	: Controlled Intravaginal Drug Release device for Goats
CL	: Corpus luteum
CV	: Coeficient of Variation
E/D	: Estrus duration
eCG	: Equine Chorionic Gonadotropin
ELISA	: Enzyme linked Immunosorbent Assay
ET	: Embryo Transfer
FGA	: Fluorogestrone Acetate
FSH	: Follicle Stimulating Hormone
GnRH	: Gonadotropin Releasing Hormone
GPG	: Gonadotropin Prostaglandin Gonadotropin protocols eg. *Ovsynch* protocol
H	: hour
hCG	: Human Chorionic Gonadotropin
I.M	: Intramuscularly
Hz	: Measure of genetic variation or allelic frequency or heterocigocity
I/H	: Heat Induction
LH	: Luteinizing Hormone
MAP	: Methyl-Acetoxy Progesterone
MD_{50}	: Minimum effective dose
MHz	: Mega Hertz
MOET	: Multiple ovulation and Embryo Transfer
NC Synch	: North California University Synchronization protocol
NCSU	: North Calorina State University
Ovsynch	: Ovulation Synchronization protocol
P4	: Progesterone

$PGF_{2\alpha}$	: Prostaglandin $F_{2\alpha}$
PGPG	: Prostaglandin Gonadotropin protocols eg. *NC Synch*
PMSG	: Pregnant Mare Serum Gonadotropin
RIA	: Radioimmunoassay
Shoats	: Sheep and goat animals regarded as domesticated small ruminant species
Spp	: Species
SYNCH	: Synchronization
TAI	: Timed Artificial Insemination
TC/U	: Trans-cervical uterine AI
USA	: United States of America

CHAPTER ONE: INTRODUCTION

1.1 Background

Oestrous synchronization (*SYNCH*) in animals has been used for decades to predict times of ovulation and Artificial Insemination (AI) in a group of domesticated and wildlife ruminant animal species (Abecia *et al*., 2012; Gordon, 1997; Zanetti, 2010). Controlling the goat reproductive cycle has brought a profitable dimension to commercial goat entities worldwide (Baldassarre and Karatzas, 2004; Fatet *et al*., 2010). Such a tool has improved management aspects of an ever improving goat enterprise leading to much improved feed management, reduced labor costs, early weaning with much improved weaning weights and increased meat, fiber and milk production in times of good product market prices. It has also allowed better and efficient control of genetic selection towards improvement of profitable traits (Avendano-Reyes *et al*., 2007; Fatet et al., 2010; Riaz *et al*., 2012).

Oestrous synchronization is broadly classified into natural and pharmacological methods with preference usually given to much cheaper, less cumbersome and efficient methods (Dogan and Nur, 2006; Rahman *et al*., 2008). Natural methods utilize the buck effect, variation of photoperiod, dietary manipulations and less regarded temporal withdrawal of kids from goat does 28 days into lactation. Pharmacological methods utilize agents that mimic normal endocrine activities linking the hypothalamus, pituitary gland, pineal gland, uterus and ovary (Noakes *et al*., 2009). Goat synchronization methods are often applied empirically in different geographical regions (Whitley and Jackson, 2004). Responses in doelings following ovarian control through oestrous synchronization are often variable and usually depend on synchronization method, age, breed, nutrition and environmental conditions (Amarantidis *et al*., 2004; Avendano-Reyes *et al*., 2007). One of the challenges associated with controlled breeding in goats is prediction of ovulation point and therefore insemination timing (Hashemi *et al*., 2006; Romano, 2004). For longer periods, oestrous synchronization has been used crudely to predict insemination times rather than consideration of more sensitive ovulation synchronization techniques (Farin *et al*., 2013; Romano, 2004), through LH surge timing, progesterone profiling and following up on ovarian function using ultrasound techniques.

AI and MOET are Assisted Reproductive Techniques (ART) used mainly to advance genetic merit of domestic ruminant and sparingly endangered wildlife species (Moradi Kor *et al*., 2011). To improve efficiency of AI and MOET in goats, pharmacological oestrous synchronization improves oestrus synchrony for simultaneous AI in a group of animals (Holtz *et al*., 2008; Whitley and Jackson, 2004. AI promotes propagation of male genetics and

MOET preservation of female genetics in animal populations (Arrebola, 2012; Baldassarre and Karatzas, 2004; Mehmood *et al.*, 2012). Both techniques have invasive and non-invasive methods with technical know how, financial implications and expected level of production determining factors on choice of method (Allison and Hagevoort, 2009). Application of ART procedures in less developed resource limited communities is still poorly implemented due to financial limitations and information gaps existing between these communities (Gordon, 1997).

Optimization of oestrous or ovulation synchronization protocols (Greve *et al.*, 1995; Wiltbank, 2014) to create an ovarian micro-environment necessary for follicular growth and ovulation is paramount to the success of ART programs in goats. Though pharmacological control is an efficient method of better ovarian control, high costs and reduced reproductive proficiency in goat does known to have a history of past exposure to certain treatments pose a challenge to generalized application in different goat raising enterprises (Edmondson *et al.*, 2002). Applications have, therefore, been limited to intensive dairy goat industry and in expensively built breeding stock multiplier herds where strict genetic control through AI and other ART procedures is a prerequisite to success of enterprises (Edmondson *et al.*, 2002; Whitely and Jackson., 2004; Wildeus, 2000). Another limitation of pharmacological ovarian control in meat goat units has been failure to observe withdrawal periods after use of pharmacological agents by farmers leading to hormonal residues in meat (Lopez-Sebastian *et al.*, 2007; Noakes *et al.*, 2009). Therefore pharmacological ovarian control only become handy when used in conjunction with AI to achieve genetic improvement or preservation with better applications in dairy goat industry (Gearheart *et al.,* 1989; Mapletoft and Hasler, 2005).

1.2 Problem statement

Despite extensive research in shoat reproductive endocrinology, pharmacological oestrous synchronization has often generated variable oestrous and fertility results in different shoat breeds, geographical locations, and management set ups (Farin, 2013, Peters and Ball, 1987; Pierson *et al.*, 2003; Rahman *et al.*, 2008; Whitely and Jackson., 2004). Therefore current research should define success of oestrous synchronization in a particular breed and particular geographical area without extrapolations. A goat is also still considered a minor livestock species worldwide (Sönmez *et al.*, 2008; Stanton, 2007; Whitely and Jackson., 2004) thus, procedures utilizing ART techniques in this species such as AI and oestrous synchronization have not advanced greatly over the years (Arrebola *et al.,* 2012; Lopez-Sebastian *et al.*, 2007). Historical progesterone and at times double prostaglandin based protocols often require gonadotropin co-treatments at the time of treatment withdrawal

(Boscos *et al.*, 2002; Omontese *et al.*, 2012). Such co-treatments are usually associated with ovarian refractoriness and diminished ovulatory responses if used repeatedly in same goat subjects (Arrebola *et al.*, 2012; Baldassarre and Karatzas., 2004; Baril *et al.*, 1998; Boscos *et al.*, 2002; Holtz *et al.*, 2008; Lopez-Sebastian *et al.*, 2007). Progestagen based oestrous synchronization have also been associated with aesthetic, animal welfare and animal product residue implications thus often leading to limited use in goat meat and dairy industry (Edmondson *et al.*, 2002; Lopez-Sebastian *et al*, 2007). However, same can not be said of GnRH based ovulation protocols that do not require gonadotropin co-treatments (Martemucci and D' Alessandro., 2011) and without any strong animal product residue, infertility, aesthetic or animal welfare implications.

1.3 Justification

An increase in popularity of goat meat and milk products throughout the world is calling for development of efficient and cost effective oestrous synchronization protocols to meet producer production levels and therefore consumer demands. Such protocols should be able to address the ills of historical pharmacological oestrous synchronization protocols. Historical progestagen and prostaglandin protocols in oestrous synchronization of goats are usually associated with variable oestrous parameters, poor ovulatory responses and therefore asynchronous timing of LH surge to AI in a group of synchronized goats. This often leads to inferior conception rates if AI is not conducted with reference given to a detected oestrus in an individual goat doe. With the discovery of novel GnRH based ovulation protocols for *fixed*-TAI programs in goats, AI can be conducted at predetermined time sets after treatment withdrawal in a group of synchronized goat does without reference given to a detected oestrus in individual goat doelings. Ovulation protocols are also not associated with animal product residue, infertility, aesthetic and animal welfare implications usually associated with historical progestagen protocols. Research in current literature review, a plethora on goat oestrous synchronization protocols has none cited for Venda indigenous breed and therefore, there is a need for research to rediscover most of indigenous breeds especially on factors affecting fertility.

1.4 Objectives

The overall objective of the study was to compare reproductive efficiency of CIRD-G/eCG, double $PGF_{2\alpha}$/eCG and novel *NC Synch* GnRH based oestrous synchronization protocols in Boer and Venda indigenous goat breed does.

The specific objectives were:

1. To evaluate oestrous parameters; oestrous response, oestrus duration, time interval to onset of oestrus after oestrous synchronization treatment withdrawal.
2. To evaluate fertility parameters; kidding rate, litter sizes, prolificacy/fecundity, fertility rate, conception rate, male to female kid ratio and gestation length after deep uterine transcervical AI in responding does.
3. Analyse goat doe blood progesterone profile assays at experiment day 0, 6, 7, 10, 12 and 17.

1.5 Hypothesis

Null hypothesis H_o: Oestrous parameters, fertility parameters and blood progesterone profile assays are not influenced by synchronization protocol, goat doe breed or interaction of the two factors ($p>0.05$).

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

Commercialization of goat farming has evolved greatly in the 20^{th} century with particular emphasis placed towards improving genetics, reproductive efficiency and kid survival rates (Bitaraf *et al.*, 2007; Omontese *et al.*, 2012; Teleb and Ashmawy, 2007). Raising the goat for milk, meat and mohair has improved goat farming through progressive adoption of reinvented farming practices world over. In South Africa, goat farming is mainly for meat and fiber with consideration of the Boer and Angora goat breeds respectively (van Marle-Köster *et al.*, 2004). However, goat farming has been discouraged in other countries such as in Pakistan and Zambia due to their influence on environmental degradation and destabilization of ecological balances (Devendra and Burns, 1983; Ngambi *et al.*, 2012).

Assisted Reproductive Techniques (ART) have developed over the years to improve fertility through manipulations of natural reproductive physiological processes with an outward capacity of bettering reproductive potential of mainly ruminant farm animal species (Hashemi *et al.*, 2006; Paramio and Iziquierdo., 2014). Areas in which reproductive physiology has been improved in goats and other domestic ruminant species are ovarian control (oestrous or ovulation synchronization, super ovulation & ovulation induction), Artificial Insemination (AI), Multiple Ovulation & Embryo Transfer (MOET), cryopreservation of gametes or embryos, cloning and *in vitro* embryo production (Aryes *et al.*, 2012; Baldassarre and Karatzas, 2004; Hafez and Hafez., 2000; Paramio and Iziquierdo., 2014). Ovarian control has facilitated AI and MOET usually through oestrous or ovulation synchronization to improve ovarian responses and augment fertility through program timing (Omontese *et al.*, 2014). It is advantageous to consider ovulation synchronization over oestrous synchronization as the former has applications in *fixed*-TAI programs therefore reduces time spend on oestrus detection (Bowdridge *et al.*, 2013; Holtz *et al.*, 2008; Pierson *et al.*, 2003; Romano, 2004).

Oestrous synchronization methods are often applied empirically in different geographical areas so that a group of animals come to oestrus at once and bred as simultaneously as possible (Farin *et al.*, 2013; Paramio and Iziquierdo, 2014). This has allowed improved production in times of peak product market prices, reduced kidding intervals, improved farm labor management and allowed goat breeding outside breeding seasons (Mehmood *et al.*, 2012; Omentese *et al.,* 2014). Pharmacological oestrous synchronization methods have often employed historical progestagen and prostaglandin based protocols with certain co-treatments superimposed to improve ovarian efficiency (Avendano-Reyes *et al.*, 2007; Lehloenya *et al.*, 2004; Paramio and Iziquierdo, 2014). The turn of the 20th century has seen prostaglandin based protocols invigorated to generate ovulation synchronization protocols through in-cooperation of GnRH analogs for *fixed*-TAI in goats (Holtz *et al.*, 2008; Romano, 2004).

Wide variations in oestrous and fertility parameters in goat does synchronized by historical progestagen and double prostaglandin oestrous synchronization protocols has made prediction of AI times difficult for *fixed*-TAI (Hashemi *et al.*, 2006; Lassala *et al.*, 2004; Souza-Fabjan *et al.*, 2013). Advantages of predicting AI times using ovulation synchronization over oestrous synchronization can not be over emphasized (Farin *et al.*, 2013). Steroid residues in animal products and reduced fertility usually associated with co-treatments used in historical progestagen protocols will always call for research to find better steroid free protocols (Lopez-Sebastian *et al.*, 2007).

The objective of this research was to compare oestrous and fertility parameters following use of CIRD-G devices (Eaze Breed ™, 0.3 g progesterone) left in situ for 12 days and eCG injected 12 hours before device removal; double Prostaglandin injections 12 days apart and eCG injection 12 hours before last prostaglandin analog injection and novel *NC Synch* GnRH based protocol. Trans-cervical deep uterine AI was conducted in all responding goat does 24 hours after onset of overt oestrus signs. Trans-rectal ovarian and uterine ultrasound was used as a core method to determine conception or pregnancy in inseminated doelings.

2.2 The goat

Today's domesticated goat (*Capra hircus*) (Gordon, 1997), originated from wild goat (*Capra aegagrus*) that is subdivided into fertile subspecies *C.a.aegagrus*, *C.a.ibex* and *C.a.falconeri* (Devendra and Burns, 1983). A goat is a short day polyoestral seasonal breeder with an oestrous cycle period ranging between 18 and 21 days (Devendra and Burns, 1983; Fatet *et al.*, 2010; Gordon, 1997). However, in the tropics goats are often regarded as continuous breeders with nutrition the determinant factor and not photoperiod at influencing reproductive cyclicity (Fatet *et al.*, 2010).

Close to 78% (350 million) of estimated world goat population (446 million) is reared in the tropics (Devendra and Burns, 1983). Goats are easily adaptable domesticated animal species that can be reared in harsh environmental conditions worldwide (Bowdridge *et al.*, 2013; Fatet *et al.*, 2010; Hezam, 2008). They are more selective than cattle or any other domesticated animal species on feed quality but have a wide range of preferred plants comparable only to the camel (Payne and Wilson., 1999). Their ability to utilize and digest pastures well (Gordon, 1997) and making the best out of nothing means, in terms of farm turnover, they are a species to consider to provide a rich source of protein and fiber to the ever growing human population (Ngambi *et al.*, 2012). However, when considered with other farm species, goats are usually regarded as environmentally unfriendly through deforestation, soil erosion and land degradation (Devendra and Burns, 1983; Ngambi *et al.*, 2012). Another school of thought is their importance in communities where other ruminant animal species can not be reared due to endemic animal disease epidemiology and other unfavorable animal raising conditions. Its strong involvement in avoiding bush encroachment, especially of invasive alien plant species, makes a goat a very important member of different ecosystems (Devendra and Burns, 1983; Vallecillo *et al.*, 2004).

Generally goat breeding is considered to be seasonal, however, in the tropics goats are regarded as continuous breeders with scarcity of food reserves associated with prolonged anoestrus periods and anovulatory states leading to poor fertility and prolificacy (Fatet *et al.*, 2010; Freitas *et al.*, 2004). There is no evidence of goat doe seasonality in goat farming entities near the equator or in low latitudes (Noakes *et al.*, 2009), as climatic conditions gradually diminish into two magnified fall and summer seasons. Seasonality in goats is influenced by latitude (photoperiod), male goat presence, breed, climate and farm management (Fatet *et al.*, 2010). Seasonality is more apparent with increase in latitude and usually animals adapted to short breeding seasons in lower latitude (temperate) will have extended breeding seasons when moved and adapted to high latitude (tropical/subtropical) regions (Fatet *et al.*, 2010).

In tropical South Africa, shoat breeding season starts in late autumn until late winter with a peak in April and May of each year (Dara *et al.*, current study; Greyling and van Niekerk, 1986). A period of seasonal anoestrus ensue as long tropical daylight hours of spring and summer approach due to biophysical switch down on melatonin release from the pineal gland, a hormone having direct and indirect stimulatory effects on the hypothalamus-hypophysis axis (Noakes *et al.*, 2009). Tropical early autumn months are considered a transition period when a small proportion of does in flocks of most goat breeds ovulate with or without cardinal overt oestrus signs. In temperate regions goats are regarded as seasonal breeders with periods of polyoestral cyclicity interrupted by periods of anoestrus and transition (Fatet *et al.*, 2010).

Compact oestruses of 30-40 h duration are recorded every 21st day during the length of the breeding or mating season in reproductively sound doelings. Goat does, just as other ruminant species, have between 2 to 4 successive waves of follicular recruitment, growth and atresia in the 18-21 day oestrous period (Lassala *et al.*, 2004; Paramio and Iziquierdo, 2014). In shoats, a period of compact oestruses in a short breeding season is normally preceded by a much longer period of seasonal anoestrus. In goat does, ovulation normally occur at the end of each overt oestrus (Allison and Hagevoort, 2009) and this is usually between 12-36 hours after oestrus onset (Noakes *et al.*, 2009).

2.3 Concepts of oestrous synchronization

Control of ovarian activity in domesticated ruminant species has facilitated oestrous synchronization, ovulation induction, AI and MOET programs (Abecia *et al.*, 2012; Edmondson *et al*, 2002). The goal of oestrous synchronization is to allow animals to be in the same reproductive physiological status so that a group of animals come to oestrus at once and all bred as simultaneously as possible (Farin *et al.*, 2013; Holtz *et al.*, 2008). Oestrous synchronization has also allowed increased production and supply of meat, fiber and milk to meet consumer demand throughout the year by influencing and controlling ovarian activity even during anoestrus periods (Fatet *et al.*, 2010; Hazem, 2008). By concentrating kidding, seasonal labor can be correctly procured leading to much improved kid survival up to weaning age therefore adding to farm profitability (Hazem, 2008; Jackson *et al.*, 2014; Omontese *et al.*, 2012). Ovulation synchronization, unlike oestrous synchronization has made *fixed*-TAI possible by predicting ovulation times and reducing over reliance on visual oestrus detection that is usually judged subjectively often leading to poor conception rates following asynchronous timing of ovulation to AI (Holtz, *et al* 2008; Pierson *et al*, 2003; Romano, 2004).

Oestrous synchronization methods are generally classified broadly into natural and pharmacological ovarian control methods. Natural methods with better applications in goats are the buck/male effect, variation of photoperiod, temporal kid withdrawal and manipulative nutritional changes prior to mating (Danko *et al*, 2007; Jackson *et al*, 2006; Kusina *et al*, 2001; Paramio and Iziquierdo, 2014). Pharmacological methods utilize natural progesterone/synthetic progestagen device formulations maintained in situ between 5-18 days, double prostaglandin analog injections 10-12 days apart and novel GnRH based ovulation protocols (Farin *et al*, 2013; Paramio and Iziquierdo, 2014). Though natural methods are cheap, they are less reliable, less efficient and cumbersome when compared to pharmacological methods (Danko *et al*, 2007). Pharmacological methods are expensive but are usually reliable and achieve superior reproductive parameters when applied under controlled conditions (Holtz *et al.*, 2008

2.3.1 Natural control of oestrous synchronization

Natural methods of oestrous or ovulation synchronization utilize the "buck effect", increased feed intake prior to mating season, temporal kid removal and variation of photoperiod. Buck effect utilizes the sense of sight and smell to generate chemosensory stimuli in an anoestrus goat doe kept in isolation at least a mile away from the buck for a period of not less than three weeks. When the buck is suddenly reintroduced the goat doe comes to oestrus at approximately 72-144 h (Fatet *et al*, 2010; Lopez-Sebastian *et al*, 2007). In one experiment 34 out of 40 anoestrus goat does came to detectable oestrus 14-22 days and 47% of them conceived to first service (Devendra and Burns, 1983). Increased feed intake prior to mating season improves ovulation and conception rates in goat doelings following natural mating or AI (Kusina *et al.*, 2001). Temporary kid removal 28 days into lactation can also be employed as a natural method to reduce time to mating through reduction of kidding interval and also improves estrous synchrony in goat does (Jackson *et al.*, 2006; Whitley and Jackson, 2004).

Buck/male effect usually trigger very short 5-7 day ovarian cycles with induction of ovulation bound to happen in 7-9 days after introduction of intact bucks (Lopez-Sebastian *et al.*, 2007). This method can be used during or outside the breeding season. It can also hasten oestrus development in goats that are under oestrous pharmacological control at point of treatment withdrawal (Lopez-Sebastian *et al*, 2007; Mellado and Valdez, 1997, Meilan and Ungerfield, 2014; Whitley and Jackson., 2004). It is postulated that the buck stimulatory effect can overpower the steroid (progesterone) inhibitory effect on the hypothalamus-hypophysis axis (Mellado and Valdez, 1997, Meilan and Ungerfield, 2014). Buck introduction in acyclic anestrous goat does will influence LH pulsatility and cause estradiol release. Estradiol will increase secretion of uterine oxytocin and increase uterine receptor sensitivity to oxytocin. The normal reaction is uterine release of $PGF_{2\alpha}$ a luteolytic agent that will initiate luteolysis of any CL older than 5 days (Meilàn and Ungerfield, 2014). Estradiol may also contribute to oestrus induction through luteolysis of generally young CL (5 days) leading to oestrus development and ovulation (Meilàn and Ungerfield, 2014).

Variation of photoperiod in temperate and subtropical anestrous goat does kept in light-tight barns for longer hours in light (16 hours) per day and then suddenly exposed to longer hours (16 hours) of darkness induce ovulation immediately (Fatet *et al.*, 2010). However, recorded oestrus by this method is not sufficiently fertile for AI (Fatet *et al*, 2010). Photoperiod variation is a common method used in the dairy goat industry, but housing constraints can be impractical for commercial meat goat herds (Whitley and Jackson, 2004). Alternatively melatonin implants or daily oral doses for a month have been suggested as having similar effect as photoperiod at inducing oestrus and ovulation (Fatet *et al*, 2010; Whitley and Jackson, 2004). Oestrous responses of the two methods are comparable, but fertility is much better if melatonin implants are used (Whitley and Jackson, 2004). However, all natural methods cause asynchronous oestruses in a herd of goat does, therefore failure to conduct *fixed*-TAI in a group of does (Lopez-Sebastian *et al.*, 2007).

Increased plane of nutrition or flushing is a known traditional practice to improve reproductive proficiency in sheep, goats and less commonly pigs (Hafez and Hafez, 2000; Stanton, 2008). Dietary manipulation usually through increasing plane of nutrition few weeks before mating has proven to improve ovulatory rates in goat does (Danko *et al*, 2007 ; Fatet *et al*, 2010; Kusina *et al*, 2001). In other instances, indigenous goat doe or ewe breeds have developed nutritive adaptive traits of maintaining reproductive proficiency in face of suboptimal feed sources and still reproduce efficiently (Kusina *et al.*, 2001). The determinant factor has been body fat content (good body condition) that has direct stimulatory or enhancing effect on hypothalamic hormone release (GnRH) and induction of ewe or goat doe cyclicity (Danko *et al*, 2007; Mellado and Valdez, 1997). Feeding ewes an improved diet of Lucerne, rye grass, germinated seeds, lupin grain and oats have traditionally been known to improve hypothalamic–pituitary sensitivity to endocrine hormones with an outward capacity of improving ovulatory and fertility rates (Danko *et al*, 2007; Wildeus, 2000). In ewes with genetic potential, increased plane of nutrition 2-3 months before mating will increase fecundity rates, but such increases should only be maintained 1 month into gestation upon which further increases are withheld until into the last 6-8 weeks before lambing (Noakes *et al*, 2009). However, during some periods of pregnancy increased nutrition is associated with poor fetal survival rates and poor goat doe body structural programming leading to pre-partum conditions like limb edema or arthritis (Dara *et al*, current study; Noakes *et al.*, 2009).

2.3.2 Pharmacological control of oestrous synchronization

Various methods have been employed at different levels of the hypothalamus-hypophysis ovarian axis to mimic endocrine activities influencing folliculogenesis, ovulation and fertilization in domestic farm species (Hafez and Hafez, 2000). This has been achieved through: (1) anterior pituitary hormonal release stimulation; (2) simulation by replacement or supplementation of anterior gonadotropins; (3) use of agents that effects a positive feedback stimuli on anterior pituitary gonadotropin release; and (4) shortening or lengthening the luteal phase by use of luteolytic agents or progesterone preparations respectively (Noakes *et al.*, 2009).

Pharmacological methods either lengthen the luteal phase (progestagen analogs) or shorten it (prostaglandin analogs) (Hafez and Hafez, 2000; Paramio and Iziquierdo, 2014). Natural progesterone impregnated CIRD-G devices, sponges (MAP and FGA), ear implants and daily injections are various formulations available on the market to synchronize goat does during the breeding or outside the breeding season (Danko *et al*, 2007). Double prostaglandin injections 11-14 days apart will achieve oestrous synchronization in cycling does during the breeding season (Abecia *et al*, 2012; Paramio and Iziquierdo, 2014). One of the challenges in using pharmacological methods for oestrous and ovulation synchronization has been the need to reduce over-reliance on eCG use (Fatet *et al*, 2010; Romano, 2004). It is suggested that the long half life of eCG estimated to be around 2-5 days in cattle (Bainbridge *et al*., 1996) will stimulate follicular growth and concomitantly result elevated plasma estradiol concentrations that remain elevated even after natural or induced ovulation. The newly formed young CL becomes susceptible to estradiol luteolysis often leading to fetal resorption and infertility if fertilization had occurred (Bainbridge *et al*., 1996).

Pharmacological synchronization protocols reproductive efficiency is affected apart from other things by breed of does under consideration and for comparative purposes breed lines should be defined prior to experimentation (Boscos *et al*, 2002). Environmental stress and nutrition are well known confounders when comparing pharmacological oestrous synchronization protocols through experimentation (Kusina *et al*, 2001; Whitle and Jackson, 2004). Goat does that are in poor body condition due to reduced feed intakes prior to mating will correspondingly have poor ovulatory responses and conception rates (Kusina *et al*, 2001). In post pubertal goat doelings (6-7 months old), there is continuous follicular growth (recruitment, selection & dominance) as follicles are continuously beign recruited from a resting prophase 1 pool through actions of circulatory GnRH (Noakes *et al*, 2009). In face of stimulatory actions of resultant tonic endogenous gonadotropins, recruited follicles in resting prophase 1 of first meiotic division develop through second meiotic division into oocytes ready for fertilization. Body fat in well fed doelings has direct stimulatory or enhancing effect on GnRH hormone release from the hypothalamus and, therefore, follicular recruitment is improved in well conditioned doelings leading to much improved ovulatory rates (Danko *et al*, 2007).

2.3.2.1 Progestagen based pharmacological methods

These protocols exploit the use of natural progesterone steroids or synthetic progestagen products with the latter being more potent and of longer half-life (Noakes *et al*, 2009).

Synthetic progestagen (MAP/FGA) or natural progesterone (CIDR-G) treatments are administered for a period ranging from 10-16 days in goat does with certain breed specific and geographic variations permitted to achieve better synchrony (Boscos *et al*, 2002). Endogenous progesterone is important for recruitment and succession of follicular waves therefore, if progesterone levels are low the resultant ovarian micro-environment will promote persistence of a dominant follicle that does not ovulate (Lassala *et al*, 2004). Ovarian follicular dynamics and fertility parameters after oestrous synchronization using progestagen protocols are not affected by presence or absence of a CL at the start of progestagen treatments in goats (Lassala *et al*, 2004). Long term progestagen protocols are usually associated with poor fertility due to sub-luteal progesterone concentrations towards treatment withdrawal times as device progesterone levels wanes (Baldassarre and Karatzas, 2004; Fonseca *et al.*, 2005; Martemucci *et al.*, 2011; Teleb and Ashmawy, 2007). Dwindling circulatory progesterone provide futile grounds for development of a dominant follicles that will continue growing without ovulation (Baldassarre and Karatzas, 2004; Lassala *et al.*, 2004; Souza-Fabjan *et al.*, 2011).

To improve compactness of synchrony, quality of oestrous response and ovulatory process that may give chance of twinning in doelings, gonadotropins such as eCG co-treatments are usually superimposed on progestagen protocols on day of progestagen treatment withdrawal (Dogan and Nur*.,* 2006; Fonseca *et al.*, 2005; Omontese *et al.*, 2012; Pierson *et al.*, 2003). Such protocols will allow *fixed*-TAI to be conducted between 30-48 h or at predetermined periods after day of detected onset of oestrus in does (Hafez and Hafez, 2008). In the tropics, the most commonly used method combines progestagen, prostaglandin and gonadotropin analogs. Such a practice is efficient at allowing *fixed*-TAI to be done at precisely predictable times and achievement in excess of 65% fertility rates in goats (Kenfack *et al.*, 2013; Pierson *et al.,* 2003; Romano, 2004). Use of adjunct treatment like eCG at the end of progesterone treatment reduces chances of development of a persistent dominant follicle that does not ovulate (Lassala *et al.*, 2004). Use of gonadotropins with long half-life like eCG (21.1 h ± 1.1) (Boscos *et al.*, 2002), should be limited only when it is unavoidable, usually in seasonal anoestrus breeding (Fonseca *et al.*, 2005; Romano, 2004).

Sponge formulations for goats of synthetic progestagens are available as methyl-acetoxy progesterone (MAP, Repromap® 60mg) available as 60mg strength, fluorogestrone acetate (FGA, Chronogest® 45mg) (Whitley and Jackson., 2004), altrenogest (Intervet), melengestrol acetate (MGA) and norgestomet (Crestar, Intervet) (Farin *et al.*, 2013). Methyl-acetoxy progesterone is used in protocols to synchronize oestrous for does outside the breeding season and FGA on the contrary is a sponge formulation preferred for use during breeding season (Greyling and van Niekerk, 1986). However, sponge formulations cause irritation to the vaginal mucosa causing at times severe purulent vaginitis in goat does (Penna *et al.*, 2013). A more efficient controlled internal drug releasing device for goats (CIRD-G®, 0.3g of progesterone, Pfizer/Zoetis) in the form of silicone impregnated intravaginal slow releasing device was developed in New Zealand (Whitely and Jackson, 2004). CIDR-G contains between 9-12 % (0.3g) of progesterone impregnated in a silicone elastomer (Hafez and Hafez, 2000). CIDR-G progesterone devices are easy on application, do not cause vaginal mucosal irritation and has a 90% retention rates (Omontese *et al*, 2012; Romano *et al.*, 2004). Retention security of sponges up to predetermined withdrawal times is not usually guaranteed when compared to CIDR-G device security (Abdul Muin *et al*, 2013; Omontese *et al.*, 2012). Sponges are not available any more and hence CIDR-G devices are the only probable option of progesterone formulation for progesterone based synchronization protocols in goats and sheep. Vaginitis is caused by flora changes in resident opportunist *coliform spp* and gram positive bacteria like *streptococcus spp* and *staphylococcus spp* (Penna *et al*, 2013). Ear implants of progestagens have also been used with variable success in synchronization protocols for goats.

When MAP-60mg was a choice of progestagen treatment outside the breeding season in indigenous Damascus does and 150 IU of PMSG used in combination at MAP-60mg treatment removal, 100% of does had recorded oestrus and were bred at 30-48 hours after oestrus onset. This protocol achieved 65.8% conception rate, 64.1% kidding rate and a net kid crop of 192.2% (Baldassarre and Karatzas, 2004; Whitley and Jackson, 2000). However, work conducted by Moradi Kor *et al.* (2011) found no significant differences on choice of synchronization protocol with or without combination of a gonadotropin (PMSG/eCG) applied at synchronization treatment withdrawal despite various citations in their work indicating significant differences in reproductive indices (Moradi Kor *et al.*, 2011). However, breed, periods of evaluation and geographical patterns act as confounders in evaluation of synchronization protocols in goats (Moradi Kor *et al.*, 2011).

Despite widespread use of progestagen based protocols in small ruminant, alteration of ovulatory process, an anomalous formation of CL and reduced embryo viability are some of the associated shortfalls of these protocols in AI and embryo transfer programs (Ruiz-Gonzalez *et al.*, 2012). Long-term progestagen protocols have been associated with poor conception rates due to reduced competency of antral follicles and oocyte vitality (Baldassarre and Karatzas, 2004; Zanetti *et al.*, 2010).

2.3.2.2 Prostaglandin based pharmacological methods

Two doses of prostaglandin $F_{2\alpha}$ analog 11-12 days apart in cycling goat does achieves much better synchrony, quality oestrous parameters and higher reproductive indices than any other synchronization method (Amarantidis *et al.*, 2004; Martemucci and D'Alessandro, 2011; Whitley and Jackson, 2004). However, in a study by Martemucci and D'Alessandro, (2011), progestagen devices had better synchronization prowess than $PGF_{2\alpha}$ based protocols. Goats are very sensitive to $PGF_{2\alpha}$ and a normal dose of 15 mg dinoprost is used with great success in most goat synchronization protocols (Farin, 2013). Prostaglandin$F_{2\alpha}$ analogs cause luteolysis and therefore removes endogenous progesterone induced negative feedback stimulus on the hypothalamo-hypophysis ovarian axis thus intiating a preovulatory follicular wave growth that gives birth to a dominant follicle destined for ovulation (Abecia *et al.*, 2012; Hafez and Hafez, 2000; Lopez-Sebastian *et al.*,2007). Prostaglandin$F_{2\alpha}$ shortens the luteal phase by causing luteolysis of a functional corpus luteum (Riaz *et al.*, 2012; Abecia *et al.*, 2012).

Prostaglandin$F_{2\alpha}$ is usually marketed as the product dinoprost 5 mg/ml (Lutalyse®, Pfizer Inc/Zoetis), though other preparations are registered and available on the South African market like cloprostenol 250 µg/ml (Estrumate®, Intervet SA/MSD Animal Health) and luprostinol 7.5 mg/ml (Prosolvin®). Recent ultrasound techniques developed for ovarian studies to monitor ovarian dynamics has shown luteolysis of a day 5-6 old corpus luteum able to induce short oestrous cycles with ovulation occurring quicker with a tighter synchrony than luteolysis of a much older corpus luteum (≥ 11 days) (Lopez-Sebastian *et al.*, 2007). However, day 5 or younger CL are often refractory and insensitive to $PGF_{2\alpha}$ due to the smaller luteal theca interna cells that are still undergoing cellular differentiation and having fewer $PGF_{2\alpha}$ receptors than well differentiated day 7-11 luteal cells (Greyling and van Niekerk., 1986; Levy *et al.*, 2000; Menchaca *et al.*, 2007; Zanetti et al., 2010). The endothelial cells of mature CL has $PGF_{2\alpha}$ receptors, however, endothelial cells of younger than 5 days CL are still immature, undergoing neovascularization and cellular differentiation (Levy *et al.*, 2000). Double $PGF_{2\alpha}$ injections 10-11 days apart will then allow all goats to be at the mid luteal stage of the cycle during which the CL is much more susceptible to $PGF_{2\alpha}$ luteolytic activity (Abecia *et al.*, 2012).

Recent work has found 67% of does treated with a double dose of Prostaglandin analog 11 days apart coming to oestrus and ovulating with 75% kidding rate in responding and inseminated does (Whitley and Jackson, 2004). However, the success of prostaglandin protocols in oestrous synchronization is dose dependent with extremely high doses of $PGF_{2\alpha}$

lowering conception rates though quality of synchrony and oestrous parameters remain favorable (Greyling and van Niekerk, 1986). When compared to progestagen protocols in studies in sheep, prostaglandin protocols have shown to trigger higher post ovulatory P4 levels indicating better ovarian dynamics, an indication of better synchrony, oestrous response and ovulation (Ruiz-Gonzalez *et al.*, 2012). A quality precocious ovarian response of double prostaglandin protocols, however, does not usually translate to fertility superiority over progestagen protocols (Ruiz-Gonzalez *et al.*, 2012).

The major limitation of protocols utilizing prostaglandin analogs is their application only in cycling does during the breeding season (Abecia *et al.*, 2012; Farin *et al.*, 2013; Greyling and van Niekerk, 1990; Riaz *et al.*, 2012). Its use outside the breeding season is doubtful, though it is suggested that in tropical regions, goat does cycle throughout the year with availability of feed reserves as the predominant determinant factor and not photoperiod at influencing goat doe cyclicity (Fatet *et al.,* 2010). Field application of prostaglandin based protocols will yield inconsistent results, especially without consideration of reproductive stage of goats meant for synchronization, and therefore the use of a GnRH analog on day 7 of prostaglandin based protocol such as novel *NC synch* based protocols achieves close to 100% maturity of corpora lutea before the second $PGF_{2\alpha}$ analog injection (Ruiz-Gonzalez *et al.*, 2012). High progesterone concentrations in ewes usually around the second $PGF_{2\alpha}$ injection often creates a micro-environment unfavorable for ovulation usually leading to low fertility often quoted to be around 70% (Albecia *et al.*, 2012).

Prostaglandin analog protocols rarely involve a gonadotropin injection on the day of the second prostaglandin analog treatment, however, using eCG in combination reduces time to oestrus induction (Omontese *et al*, 2014). In the tropics prostaglandin treatments are used with great success in combination with progestagen treatments and male effect to improve quality of synchrony, oestrous parameters and reproductive indices (Abecia *et al.*, 2012; Meilán and Ungerfield, 2014). Does are normally bred naturally or double AI 12 h apart with first insemination conducted within 24-54 h after induction of a synchronized oestrus (Hafez *et al.*, 2000). Prostaglandin$F_{2\alpha}$ based protocols in small ruminants have been associated with poor fertility rates, great variability in ovulatory response due to suboptimal luteal function (Abecia *et al.*, 2012). Very high post-ovulatory progesterone concentrations are usually associated with poor conception rates in ewes (Ruiz-Gonzalez *et al.*, 2012). However, prostaglandin regimes will try to address aesthetic, infertility and animal welfare issues surrounding prostagens use in small ruminants (Abecia *et al.*, 2012; Penna *et al.*, 2013).

2.3.2.3 Ovulation Synchronization GnRH based methods

Ovulation in shoats and other ruminants occurs in response to a combination of physiologic, biophysical and biochemical mechanisms. These mechanisms leads to complex neuroendocrine, neurobiochemical, neurovascular, neuromuscular, and pharmacological mechanisms coupled with enzymatic activity involved in follicular development and ovulation (Hafez and Hafez, 2000). Importance of these mechanisms in explaining the complex ovulatory process in well differentiated mature antral follicles is still considered to be controversial (Hafez and Hafez, 2000). Success of oestrous synchronization protocols in goat fertility depends is measured by occurrence of a good quality ovulatory process (Riaz *et al.*, 2012).

Superimposition of certain co-treatments before withdrawal of oestrous synchronization treatments has allowed ovulation synchronization and much improved ovulatory responses in small and large ruminants (Pierson *et al.*, 2003). Gonadotropin releasing hormone (GnRH) has proven to be potent at control and synchronization of lutenizing hormone (LH) surge in a pre-ovulatory micro-environment moments just before ovulation (Gumen *et al.*, 2002). Gonadotropin releasing hormone analogs are relatively cheap and do not pose a danger of development of immunologic reaction in previously exposed candidates (Martemucci and D'Alessandro, 2011). Human chorionic gonadotrophin can alternatively be used to improve quality of ovulation due to its predominant LH activities (Pierson *et al.*, 2003). In shoats, ovulation and oestrous synchronization protocols have often been used interchangeably to improve quality of oestrus synchrony and ovulatory rates (Moradi Kor *et al.*, 2011). *Fixed*-TAI can be done successfully at predetermined times without the necessity of cumbersome oestrus detection (Romano, 2004). Ovulatory rate is an important function guiding fertility and has applications in assisted reproductive techniques like MOET (Pierson *et al.*, 2003; Riaz *et al.*, 2012).

Two common basic ovulation protocols that have been naturalized for use in shoats are the novel *NC Synch* and *Ovsynch* novel ovulation synchronization protocols (Bowdridge *et al.*, 2013; Farin *et al.*, 2013; Hafez and Hafez., 2000; Yacoub *et al.*, 2011). These protocols utilize a GnRH analog or agonist and $PGF_{2\alpha}$ analogs and were adopted with extrapolations from principles developed for cattle by Pursely *et al.* (1995) (Bowdridge *et al.*, 2013). This has shifted attention from standard oestrus synchronization methods towards ovulation synchronization in modern MOET and AI programs. AI can be done at predetermined times in shoats and other ruminant species (Ashmawy *et al.*, 2012, Farin *et al.*, 2013). Advantage of novel *NC Synch* or *Ovsynch* protocols is the ability to synchronize ovulation and therefore sufficiently conduct *fixed*-TAI in a group of goat does (Holtz *et al.*, 2008). Qualitative and quantitative aspects of ovulation are important to a goat farmer as it determines fertility parameters like fecundity, kidding rates, conception rates and post natal kid survival rates (Pierson *et al.*, 2003; Riaz *et al.*, 2011).

The novel *Ovsynch* or "GPG" protocol was developed for cattle by Pursely *et al* in 1995 (Hafez and Hafez, 2000; Holtz *et al.*, 2008) and the novel protocol comprises of GnRH agonist or analog injection on day 0, $PGF_{2\alpha}$ injection on day 7 and another GnRH treatment 48 hours later (Bowdridge *et al.*, 2013; Hafez and Hafez, 2000; Riaz *et al.*, 2011; Yacoub *et al.*, 2011). Day 7 $PGF_{2\alpha}$ causes regression of a spontaneous corpus luteum or a potential CL induced by GnRH on day 0, or both (Hafez and Hafez, 2000). Pre-requisite of an efficient novel *Ovsynch* protocol is presence of a functional CL or follicles in different stages of development or cystic ovaries and goat does in sound reproductive body condition (Beran *et al*, 2013). However about 50% of cattle synchronized by the novel *Ovsynch* protocol have often been inseminated at wrong times after correrative determination of acetone and urea composition of the cervical plug to ovulation time (Beran *et al.*, 2013). Similar studies of cervical mucus crystal analysis have been used to correctly time insemination times. Presence of ferny-like crystals can be a future cardinal sign in predicting insemination times in ruminant species (Beran *et al.*, 2013). Short cycling in goat does due to luteal insufficiency is a common shortfall of this protocol when used in goats during the breeding season (Bowdridge *et al.*, 2013; Holtz *et al.*, 2008; Yacoub *et al.*, 2011)

A novel *NC Synch* GnRH based *fixed*-TAI protocol for goats incooperates Prostaglandin$F_{2\alpha}$ (35mg) injected on day 0, a Gonadotropin Releasing hormone analog (GnRH/cystorelin®-gonadorelin diacetate tetrahydrate; 100µg) analog injected on day 7, (Farin *et al.*, 2013; Hafez and Hafez, 2000); Prostaglandin$F_{2\alpha}$ (35mg equivalent to 7 ml Lutalyse™ Dinoprost 5 mg/ml, Pfizer) injected on day 14; (GnRH/cystorelin®; 100µg) analog injected on day of AI or *fixed*-TAI at day 17 (Farin *et al.*, 2013). The good fertility recorded by this protocol is derived from two sources, firstly through lutenization properties of the day 7 GnRH injection as it prepares CL which were younger than day 5 and not susceptible to day 0 $PGF_{2\alpha}$ injection (secondary CL) and ovulation thereof, of any dominant follicle available at this point in time. Secondly through stimulation of follicular growth and eventual ovulation by effects of second GnRH analog injection through its minor FSH release properties and predominant LH surge release timing on day of AI (Bowdridge *et al.*, 2013). Therefore, in simple essence, this protocol drives processes of follicular development, luteal regression and ovulation in a simple and well orchestrated stepwise fashion to achieve high fertility rates (Bowdridge *et al.*, 2013).

The short acting GnRH analogs or long acting hCG hormonal analogs (Yacoub *et al.*, 2011) are used to improve precision of oestrus to occur in 70 to 80% of does and to be within 4 days of synchrony without affecting fertility rate that is expected to vary between 65-80% (Hafez and Hafez, 2000). However, CL insufficiency can be encountered in certain goats when using this protocol often leading to poor oestrous synchronization and poor ovulatory responses (Yacoub *et al.*, 2011). Human chorionic gonadotropin has been used in place of short acting GnRH analogs for prolonged follicular stimulation (Yacoub *et al.*, 2011).

Chorionic gonadotropin used in these protocols clusters ovulations with the inhibition of short follicular cycles leading to more purposeful ovulatory responses from mature antral follicles (Kridli *et al.,* 2003; Yacoub *et al.*, 2011). Several modification of this protocol does exist as other protocols are affected by species, environment, breed, season and locality (Wiltbank *et al.*, 2014).

In studies carried out in mixed Boer does at North Calorina State University (NCSU) for 3 consecutive years from 2007 to 2010, the *NC Synch* novel protocol fared better in terms of conception rates and pregnancy rates than the double injection $PGF_{2\alpha}$ novel protocol (Farin *et al.*, 2013). In all the years, novel *NC Synch* GnRH protocol was superior to the $PGF_{2\alpha}$ protocol though pregnancy rates were comparatively different in all the years with differences in weather, management and environment cited as attributed associative factors. However, when the novel *NC Synch* GnRH protocol was compared to progesterone (CIDR-G) protocol by the same workers for three years at the Upper Mountain Research Station USA, pregnancy rates following novel *NC Synch* GnRH protocol were inferior to those recorded following oestrous synchronization using progesterone protocol (Farin *et al.*, 2013). The two studies demonstrated the need to fine tune AI times by properly timing LH surge and consideration of external confounding factorial interactions like weather, management, geographical location, doe parity, age, body condition, semen type, AI method and breed (Farin *et al.*, 2013).

2.4 Adjunct hormonal treatments commonly used in goat synchronization protocols

Certain hormonal treatments are allowed and usually superimposed on oestrous synchronization to improve follicular growth, ovulatory responses and synchrony (Hafez and Hafez, 2000, Noakes *et al.*, 2009; Omontese *et al.*, 2014). Such treatments imitates endogenous endocrine factors to supplement or ameliorate their actions, but within normal homeostatic physiological ranges. Purified and concentrated hormonal animal derivatives are produced commercially from sera, tissue extracts and at times genetic engineering for use in different species (Boscos *et al.*, 2002; Noakes *et al.*, 2009). Gonadotropins commonly used in animal reproduction are eCG/PMSG, hCG, FSH and LH. Human chorionic gonadotropin has predominantly LH activity and eCG/PMSG has predominantly FSH properties in livestock species (Hafez and Hafez, 2000; Noakes *et al.*, 2009). Gonadotropin releasing hormone derivatives can give similar stimulatory response like gonadotropins through an indirect effect on the anterior pituitary gland to produce FSH and LH endogenously. Commercial products of purified FSH and LH are very expensive and recombinant genetic engineering has been employed to produce much cheaper products (Noakes *et al.*, 2009).

2.4.1 Equine chorionic gonadotropin

Gonadotropins like eCG formerly known as PMSG and purified FSH hormonal preparations with predominant FSH activities will stimulate growth of pre-ovulatory antral follicles (Lopez-Sebastian *et al.*, 2007; Moghaddam *et al.*, 2012). Equine chorionic gonadotropin has been used in practice to stimulate ovarian superovulatory responses that allows for twinning in less prolific breeds of goats and sheep (Boscos *et al*, 2002; Devendra and Burns., 1983). When used after prostaglandin or progestagen treatment withdrawal, eCG will stimulate follicular growth, improve ovulatory rates and produce a tighter oestrus (Dogan and Nur, 2006; Moradi Kor *et al.*, 2011). Growth of antral follicles cannot go beyond 2 mm without the presence of gonadotropins (Boscos *et al.*, 2002; Moghaddam *et al.*, 2012). However, limitations of long acting gonadotropins like eCG is continuous recruitment of antral follicles that do not ovulate (Wildeus, 2000). A tighter oestrus following pharmacological oestrous synchronization protocols will synchronize ovulation timing for *fixed*-TAI (Holtz *et al.*, 2008; Romano, 2004).

A recommended dose of eCG in goats and sheep normally ranges from 250-750 IU with 300 IU a preferred dose in goats (Ahmed *et al.*, 1998 Gordon, 1997). Higher doses above this range upper limit can depress conception rates and hence fertility (Ahmed *et al.*, 1998). Such co-treatments have been empirically in-cooperated in synchronization protocols to improve goat reproductive performances (Ahmed *et al.*, 1998). Though used with much success in goats and sheep, there has been some variability in time to oestrus onset, oestrus duration, LH surge and ovulation (Pierson *et al.*, 2003). Therefore proper oestrus detection is still necessary before AI. In short, eCG has been used in small ruminants to improve ovarian response, conception rate and influence multiple pregnancies from induced ovulation (Boscos *et al.*, 2002; Menchaca *et al.*, 2007). However, eCG used in small ruminants especially during anoestrus periods has been associated with development of immunologic protective antibodies with poor fertility parameters associated with repeated future use in goat does with a past history of eCG exposure (Baril *et al.*, 1998; Bodin *et al.*, 1997; Menchaca *et al.*, 2007). Other studies have regarded this immunologic response as negligible with repeated use to the maximum frequency of 17 times in small ruminants having insignificant effect on fertility indices (Devendra *et al.*, 1983). Estradiol has been a drug suggested to replace controversial eCG immunologic limitations through induction of preovulatory LH surge and ovulation (Menchaca *et al.*, 2007).

2.4.2 Gonadotropin releasing hormone (GnRH)

A decapeptide hormone produced endogenously by the hypothalamus has stimulatory actions on the adenohypophysis in stimulating FSH and predominant LH release and therefore stimulate ovarian follicular growth and eventual ovulation (Kridli *et al.*, 2003; Wiltbank *et al.*, 2014). It has been used in synchronization protocols by exploiting its gross LH activities and hence stimulation of ovulation (Yacoub *et al.*, 2011). However, when used exogenously as a sole treatment, it has a transient role due to very short half-life of 2-4 minutes henceforth many researchers use hCG in its' place because the later has a much longer half-life (Yacoub *et al.*, 2011). Gonadotropin releasing hormone has found application in oestrous synchronization protocols by clustering ovulations in $PGF_{2\alpha}$ synchronized goats (Baldassarre *et al.*, 2004; Kridli *et al.*, 2003; Pierson *et al.*, 2003; Yacoub *et al.*, 2011). Its role in maturing young follicles to reach dominant follicle stage that ovulates and luteinization of produced secondary CL compliments well $PGF_{2\alpha}$ luteolytic action (Gumen *et al.*, 2011). Prostaglandin$F_{2\alpha}$ does not have actions on a fairly young (less than 4 days) corpus luteum (Greyling and van Niekerk, 1990). Administration of GnRH at time of breeding has been associated with good fertility in sheep probably through ovulation induction and enhancing the subsequent luteal phase (Riaz *et al*, 2012). Gonadotropin releasing hormone analogs are used in small ruminant species to induce ovulation and hastens early onset of oestrus due to elevated estradiol levels (Kridli *et al.*, 2003; Martemucci and D'Alessandro, 2011). It is a fairly cheap drug analog that does not present immunologic setbacks often associated with eCG use in synchronization protocols (Martemucci and D'Alessandro, 2011).

2.4.3 Follicle stimulating hormone (FSH)

Applicative use of FSH in goat synchronization protocols is limited due to expense of available commercial preparations (Boscos *et al.*, 2002). Exogenous FSH will stimulate follicular growth from primordial to antral follicular stages (Hafez and Hafez, 2000). An advance in molecular biology utilizing genetic engineering has produced a recombinant FSH product thereby substantially reducing the cost of its use in goat reproduction (Boscos *et al.*, 2002). Follicle stimulating hormone, unlike eCG, has a much shorter half-life (110 min ± 8) (Boscos *et al.*, 2002) and is not usually associated with the development of an immunologic response following its repeated use in same subjects.

2.5 Goat breeding systems

Natural mating and artificial breeding systems are at the disposal of today's farmer with the latter being of better applications in goat dairy farming entities where close genetic control is a pre-requisite to the success of the farming practice (Edmondson *et al.*, 2007). Systems can be adapted to localities with level of production, cost, technical expertise, climate and labor factors usually put into perspective on selecting choice of system. Artificial systems consist of an array of AI methods that range from semi non-invasive to invasive techniques (Baldassarre and Karatzas, 2004). Artificial insemination apart from synchronization protocols incorporates any of three other methods of semen preservation (fresh extended, fresh chilled and frozen thawed semen) (Baldassarre, 2004).Three broad methods of AI exist, that is, vaginal, cervical and intra-uterine methods (Baldassarre, 2012).

2.5.1 Natural mating methods

Goats can be allowed to breed naturally especially in extensive and developing countries of the world (Mowlem and Gummer, 1992). Fertile bucks are often made to run with does during the breeding season in a careless and less controlled manner. However, when genetics of a particular buck is required, other bucks are removed and the selected buck allowed to run with does at ratios ranging from 1:20 to 1:50 throughout the breeding season in a partially controlled manner (Mowlem and Gummer, 1992). The common rationale is to place 3 to 4 bucks that are fitted with marking harness per 100 does throughout the breeding season to easily identify does that have been bred (Edmondson *et al.*, 2002). For easy of management during kidding, bucks are allowed to run with goat does for 32 days (1½ reproductive cycles) so that all kids are born within a month of each other (Edmondson *et al.*, 2002). There is no need for oestrus detection in natural mating systems. This is a method commonly used for meat and fiber goat units in temperate and tropical areas, but of less significance in dairy production units where controlled breeding through AI and hand mating are preferred efficient breeding methods (Edmondson *et al.*, 2002).

2.5.2 Artificial Insemination

The term "Artificial" refers to involvement of human hand in achieving a natural process and "Insemination" specifically a well-defined vehicular concept to ensure deposition of an appreciable volume of fertile concentrated semen to achieve fertilization (Allison and Hagevoort, 2009; Holtz *et al.*, 2008). Artificial insemination advances genetic merit through controlled breeding especially for dairy enterprises and is considered as the first generation ART procedure due to its simplicity (Baldassarre, 2012; Holtz *et al.*, 2008). In France alone about 10% of the goat population is bred through AI (Holtz *et al.*, 2008), though this figure could be low in less developed countries. The doe cervix presents a less challenging obstacle than the ewe cervix, as long as semen deposition is done in the midway of the last half of recorded overt oestrus (Greyling and van Niekrerk, 1986; Hafez and Hafez, 2000). Artificial insemination was developed to efficiently promote outstanding sire genes, reduce potential of venereal diseases and goat does can be bred simultaneously using oestrous synchronization methods (Allison and Hagevoort, 2009; Edmondson *et al.*, 2002).

Artificial insemination allows transfer of the male genetic material into the female reproductive tract to allow for fertilization and genetic improvement (Allison and Hagevoort, 2009; Holtz *et al.*, 2008). Invasive (laparoscopic approach) and semi non-invasive techniques (intravaginal, exocervical and transcervical) are used with comparable success results (Loubser *et al.*, 1983). Though pregnancy rates are much higher for laparoscopic technique than the semi non-invasive techniques (Farin *et al.*, 2013), the later techniques are much cheaper, less cumbersome and carries little to no anaesthetic risk to subjects and can be conducted without veterinary supervision (Farin *et al.*, 2013). Invasive laparoscopic insemination is used conservatively when there is a need to improve conception and pregnancy rates using frozen thawed superior buck semen (Steyn *et al.*, 1985).

Artificial insemination brings another breath of maximizing the reproductive capacity of buck genes with outward capacity of improving selective genetic traits of flocks (Baldassarre and Karatzas, 2004; Sohnrey and Holtz, 2005). Controlled goat breeding entities achieve between 8 to 35% genetic progressive efficiency than natural flocks (Gearheart *et al.*, 1989; Yacoub *et al.*, 2011). More goat does at a unit time are better bred using AI often utilizing synchronization protocols (Hazem, 2008). The common recommendation is to do 2 to 3 inseminations at 12 hourly intervals bearing in mind that the process of capacitation has to occur before fertilization (Allison and Hagevoort, 2009).

Oestrus detection in goat does is a challenge to a goat farmer, but with experienced husbandmanship the procedure is fairly easy. A number of methods are used to detect goat does that are in oestrus, but use of a teaser or intact bucks has been employed in most AI, MOET or experimental units with fairly good results (Ahmed *et al.*, 1998). Standing oestrus (mounted by mates) together with other non-specific visual signs like restlessness, tail wagging, vocalization/bleating, frequent urination, edematous vulva swelling, vulva hyperemia, vulva contraction, viscid clear discharge, visual marking of perianal area by discharge and exchange of sexual cues are often observed cardinal signs of overt oestrus (Campbell *et al.*, 2006; Martemucci and D'Alessandro, 2011). Whilst a teaser buck can be made to roam with does during oestrus detection, an intact buck is often allowed to roam up and down passage ails adjacent to does allowing only visual and olfactory contact without mating. A large proportion of goat does start displaying overt signs of oestrus early in the morning (Kenfack *et al.*, 2013; Omontese *et al.*, 2012) (63.13%) and large proportion of oestrus ends late in the afternoon (Fonseca *et al.*, 2005). Oestrus detection in synchronized goats for AI is therefore observed from 0700-1000 h in the morning and 1500-1900 h in the afternoon for four days after synchronization treatment withdrawal (Allison and Hagevoort, 2009; Browning, 2004; Omontese *et al.*, 2012). Development of *fixed*-TAI protocols will allow AI conducted without a need of oestrus detection that is often cumbersome, time consuming and judjed subjectively (Romano, 2004).

2.5.3 Artificial insemination Methods

Various AI methods are at the disposal of a goat farmer with technicalities, cost and number of goat does to be inseminated determint factors at choice of method. Extended fresh semen (34-37°c), fresh chilled semen (5-15°c) and frozen thawed semen can be used with variable success in goat AI. Fresh semen is usually preferred especially during the breeding season when sperm quality is good and A/V should be the preferred method of semen collection. Bucks at the farm should be well trained and not be rushed when A/V is used. Fresh chilled semen can provide options to a group of farmers sharing a particular buck and in farms located within short distances (Baldassarre and Karatzas, 2004).

Cryopreserved semen is when genetics of a particular highly priced buck are marketed over very long distances and are to be preserved even if the buck is already dead (Baldassarre and Karatzas, 2004). Cryopreservation of buck semen is associated with reduction in sperm quality due to bulbourethral seminal plasma phospholipases reacting with phosphatidylcholines of media extenders of egg yolk or milk to produce sperm cytotoxic lysolecithin (Baldassarre, 2012; Noakes *et al.*, 2009; Paramio and Iziquierdo, 2014). Ice crystals and surface area to volume ratio of buck sperm presents a challenge as differential heterogeneity of temperature changes across membranes and inner organelles hinders maintainance of cellular integrity through freezing and thawing (Vallecillo *et al.*, 2004). Extended fresh and chilled semen produce superior results compared to frozen thawed semen, although conception rates are also dependant on site, depth of semen deposition (Arrebola *et al.*, 2012) and receptivity of uterus at embryo implantation.

2.5.3.1 Vaginal insemination

A cow insemination gun is progressed carefully through the dorsal roof of vagina to reach the cranial vagina or cervix and deposit 3-4 x 10^9 of freshly collected semen (Edmondson *et al.*, 2002). The vulva is usually wiped clean using cotton wool and a non spermicidal disinfectant and a duck bill tubular speculum used to direct the pipette to the cranial vagina near the cervix (Edmondson *et al.*, 2002). This method typifies the natural buck mating method though the volume of semen can be reduced to increase the number of goat does that can be inseminated per buck ejaculate. However, it is so uncommon though can be of importance in reproductively naïve and young goat does in their maiden season since young goat doe cervix is usually difficult to transverse by transcervical AI methods. The conception rates by this method ranges between 15 and 30% (Edmondson *et al.*, 2002). The acidic vaginal pH of oestrus caused by estrogen effect kills a large proportion of spermatozoa therefore vaginal insemination is usually not recommended in goat insemination (Alfaris *et al.*, 2012).

2.5.3.2 Cervical insemination

This method is usually conducted in ewes due to limitations of transversing the ewe cervix but of limited use in goat does. The hindquarters of the ewe or doe are raised on a rail and vaginal duckbill speculum lubricated by a non-spermicidal lubricant gently inserted through the vulva with the aid of laparoscopic/pen light. The cervical os is identified as a blue-purplish protrusion into the vaginal vault usually with a whitish plug reminiscent of doe on oestrus (Sohnery and Holtz, 2005). The os is cleared of mucus by raising the head side of the speculum. A loaded goat AI gun/AI pipette (end tapered at 30° angle in sheep) or urinary catheter stiffened by stylet is then probed gently entering the cervical os and depositing an appreciable volume of semen into the cervix. Resultant conception rates are always lower than of trans-cervical and surgical invasive intra-uterine/cornual variants of AI techniques (Gordon, 1997).

2.5.3.3 Laparoscopic insemination

Two invasive approaches to the uterine horns are commonly used in small stock, the endoscopic (6.5 mm, 30° angled light source field and a fiber optic cable) led AI instrumentation (Edmondson *et al.*, 2002; Sohnrey and Holtz, 2005; Steyn *et al.*, 1985) and a complete surgical approach (Farin *et al.*, 2013). Both methods carry the capacity of good fertilization and pregnancy rates with no significant differences recorded following use of diluted frozen thawed semen or freshly collected diluted extended semen (Gordon, 1997).

A surgical procedure is carried out with a goat in a surgical anaesthetic state to gain entry into a limited abdominal cavity approach and deposition of sizable fertile semen volume into both uterine horns (Farin *et al.*, 2013). A fasted goat (feed withdrawn 24 h before procedure) is anaesthetized and placed in dorsal recumbency in a goat cradle at 40° - 45° inclination angle with her rear quarters raised above the head in a *Trendelenberg position* (Edmondson *et al.*, 2002; Hafez and Hafez, 2000; Sohnrey and Holtz, 2005). Abdominal area around the ventral midline is prepared for sterile surgical procedure in which two parallel incisions on either side of the midline are made (Farin *et al.*, 2013).

To achieve a laparoscopic endoscopic approach a low dose of xylazine 2% is required for tranquilization and 2-4 ml local anesthesia (lignocaine 2%) at penetration site 7-10cm ventral to the udder and 5-10 cm on either site of the midline. A trochar and cannula is then passed and an AI instrument loaded endoscope guided to the uterine horns (Alfaris *et al.*, 2012; Sohnrey and Hlotz, 2005; Steyn *et al.*, 1985). In both approaches the abdomen is inflated with CO to avoid damage to abdominal organs, and allow working space between the abdominal wall and the reproductive organs (Alfaris *et al.*, 2012). The uterine horns are located just ventral to the urinary bladder. A slight stab using the AI instrument (aspic) at halfway between the uterine bifurcation and utero-tubal junction is made and deposition of 0.1ml at each horn or 0.2ml in total of frozen thawed semen achieves better fertilization and pregnancy rates than non-invasive means (Steyn *et al.*, 1985). For economics of scale, more goats at a unit time should be inseminated using this method for profitable practice. The limitations of invasive approach despite its superior pregnancy rates (Sohnrey and Holtz, 2005), is the cost, animal welfare issues and the surgical risk(Gordon, 1997), therefore, applications are limited to experimental research work, MOET programs and dairy enterprises (Alfaris *et al.*, 2012).

2.5.3.4 Trans-cervical Insemination

Semen should be deposited in the uterine body or at least into each of the uterine horns using this semi non-invasive technique. However, the cervix of four tight cartilaginous connective tissue rings, thick mucus plug and a relatively small reproductive tract of the goat doe threatens feasibility of this procedure (Farin *et al*, 2013). Oxytocin injections for cervical dilatation at time of AI improved the number of inseminated goats by intrauterine insemination (85%) against controls (25%) (Garcia *et al.*, 1994; Gordon, 1997). Several methods can be used for trans-cervical invasive intrauterine procedure in goat does, but the most documented ones are the standard AI method (tube speculum), deep uterine/cornual insemination (uterine horn catheter-within-catheter) method and North Carolina State University (NCSU) simplified catheter-based method (Farin *et al.*, 2013).

Standard AI (tube speculum) method

In developing countries use of the invasive surgical/laparoscopic intrauterine techniques of AI has economic and technical limitations. On the other hand an application of this technique in developed countries has animal welfare issues to content with (Gordon, 1997). This method is the simplest of them all and utilizes a vaginal speculum (advanced 10-13 cm into vagina) with a detachable light source to identify the external cervix os to direct a goat AI gun (Farin *et al.*, 2013) or a straight pipette method (Steyn *et al*, 1985). Using the straight pipette method, the plunger is drawn to 0.2 ml to have some air behind the semen and an appreciable volume of semen drawn from a tube in a water bath of between 30° to 34°. With the aid of an assistant, the cervix is penetrated without use of force and semen deposited in the body of uterus or the two uterine horns (trans-uterine/deep cornual) (Steyn *et al.*, 1985). The AI gun penetration is the same as penetration by the straight pipette (Allison and Hagevoort, 2009). The major advantage of this method is its simplicity and a resounding success when applied in multiparous older goat does (Farin *et al.*, 2013). However, the difficulties of fully penetrating the cervical rings and smaller size of goat reproductive tract, means that at times intra-cervical deposition of semen can result low pregnancy rates between 20-30% (Sohnrey and Holtz, 2005).

Deep uterine or cornual AI method

The procedure was developed by Sohnrey and Holtz, (2005) (Farin *et al.*, 2013). A soft, small diameter human pediatric urinary catheter stiffened by an AI gun stylet is passed through the cervix, into the uterus or to individual uterine horns. The hindquarter of the goat doe is raised and a forceps (uterine tenaculum forceps, 255 mm long ("Pozzi," Aesculap, Tuttlingen, Germany) used to properly align the cervical rings by grasping the cervix at 11 o'clock and 5 o'clock and twisting the forceps to 90 degrees anticlockwise motion. The catheter is then glided through the cervix to the uterine horn (Sohnery and Holtz, 2005). The stylet is then removed and a small diameter AI tubing threaded in to deposit freshly collected or frozen thawed semen. The urinary catheter is then repositioned into another horn and procedure repeated (Farin *et al.*, 2013). The pregnancy rates following this method are much higher than that of invasive laparoscopic techniques (Sohnery and Holtz, 2005). A single catheter was used with comparable results by Sohnrey and Holtz. (2005).

North Calorina State University simplified catheter-based Method

This is a cocktail technique and still unpublished work with promising results by Farin & Knox, (unpublished) (Farin *et al.*, 2013). The technique was developed from the deep cornual uterine catheter-within-catheter method (Sohnery and Holtz, 2005) and the non-surgical embryo transfer technique described by Kraemer *et al.* (1989) (Farin *et al.*, 2013). The pediatric human urinary catheter, insemination gun stylet and Pozzi tenaculum forceps are used in a fashion that resembles deep cornual uterine catheter within catheter as described by Sohnery and Holtz. (2005) and guiding the catheter into the uterine horns by digital palpation through the vaginal vault just like bovine embryo collection as described by Kraemer *et al.* (1989) (Farin *et al.*, 2013; Hafez and Hafez, 2000). With the catheter in the uterine body, the stylet is removed and semen deposited through the uterine catheter itself (Farin *et al.*, 2013).

2.6 Follow up on ovarian function

Progesterone assaying or profiling using blood collected by veni puncture or fecal progesterone breakdown products (20 –oxo-, 20α-OH-, 20β-OH, and 5α- or 5β-pregnane derivatives) can be used to evaluate ovarian health and luteal function dynamics (Danko *et al.*, 2007; Yacoub *et al.*, 2011). Very high progesterone levels are indicative of a functional CL of pregnancy or luteal phase of the non-pregnant doe. On the contrary low progesterone levels are indicative of oestrus or ovulation times (Boscos *et al.*, 2002). Hormonal assay and especially steroids like progesterone, can help evaluate different ART protocols sensing, and especially steroids like progesterone, can help evaluate different ART protocols like oestrous synchronization and provide useful information on protocol dynamics and fine tuning of reproductive techniques. Different pharmacological treatment preparations can also be evaluated on magnitude of their effects on controlling ovarian activities (Fonseca *et al.*, 2008). Progesterone profiles are mostly determined by commercialized kits like enzyme immunoassay (ELISA) test kit or radio immunoassay test kits (RIA) (Boscos *et al.*, 2002). Inter-assay and intra-assay coefficients are used to determine variations in hormonal concentrations for different protocol stage interpretations.

Apart from progesterone assays, LH assays can also be followed an important factor at determining ovulation times and predetermination insemination times in *fixed*-TAI programs (Pierson *et al.*, 2003). Various tests have been used to generate hormonal assays for interpretation, but RIA and ELISA are easily fine tuned and adaptable to give repeatable results (Boscos *et al.*, 2002; Cengiz *et al.*, 2014; Motlomelo *et al.*, 2002; Vilarino *et al.*, 2011). Computer-assisted ultrasonic echo graphs of ovarian and uterine tissue can be used as pre-screening tool for goat does as cyclical structural integrity and changes of reproductive organ structures can evaluate peripheral endocrine function through correlative associations (Cengiz *et al.*, 2014).

Work by Wentzel *et al.* (1979) on Angora goats found progesterone concentrations at day 0 (oestrus) as 0.4 ng/ml raising exponentially to 6.7 ng/ml by day 13 at which level it remained until 3 days from start of another cycle (Devendra and Burns, 1983). Therefore, progesterone profiles are very useful at predicting the state of ovarian function, though until recently, ultrasound work has made significant milestones at revealing ovarian function better by generating clear non-invasive physical silhouette pictures of the reproductive vat (Souza-Fabjan *et al.*, 2013; Riaz *et al.*, 2012). Ability of tissues of different densities to reflect sound waves transduced by most modern ultrasound machines differently makes it possible to get echo graphic images of ovaries through non-invasive means (Cengiz *et al.*, 2014).

2.7 Reproductive efficiency

Reproductive efficiency is the most important factor of profitability in today's goat and sheep industry (Jackson *et al.*, 2014; Omontese *et al.*, 2012). Fertility is a term normally associated with reproductive prolificacy or efficiency in domestic animals. The ability of an animal to produce normal spermatozoa or ova capable of fertilization and ability to produce more offspring are landmarks that define a fertile animal (Devendra and Burns, 1983; Omontese *et al.*, 2012). Fertility in simple terms is defined as number of does kidding per 100 does bred (Fatet *et al.*, 2010). However, goat fertility is best defined by conception rate, kidding rate, fecundity and weaning rate (Lassala *et al.*, 2004). On the other hand fertility rate in female shoats is directly related to successful ovulations (Riaz *et al.*, 2012). Gonadotropin releasing hormone ovulation inducing properties has been exploited in sheep entities to improve fertility through promotion of ovulation (Riaz *et al.*, 2012). In most instances than not an important aspect of oestrous synchronization is defined by quality of ovulation in a group of animals (Riaz *et al.*, 2012).

The most important aspect of a successful oestrous synchronization protocol is uniformity in timing of onset and duration of a synchronized oestrus from treatment withdrawal (Lassala *et al.*, 2004). The quicker the onset and the shorter the duration of oestrus, the better it is to predetermine the time for *fixed*-TAI (Lassala *et al.*, 2004; Pierson *et al.*, 2003). The words "Tight" and "Compact" are terms used to define a precocious short oestrus in shoat reproduction (Abdul Muin *et al.*, 2013; Dogan and Nur, 2006; Wildeus, 2000). Non return to oestrus after insemination is a very important factor at determining success of AI or potential detrimental side effects of hormonal or drug treatments used during oestrous synchronization in goat does (Freitas *et al.*, 2004).

Oestrus quality following pharmacological ovarian control protocols is defined by oestrous response, oestrus duration and time interval to oestrus after treatment withdrawal. Oestrous response is percentage of animals in oestrus against number of treated female animals, time interval to onset of oestrus is represented by time interval after treatment withdrawal to onset of standing overt oestrus, and oestrus duration the time interval from onset of overt oestrus to end of oestrus. Fertility is also dependent on method of oestrous synchronization apart from other known relative confounders.

Productivity in goat farming is defined by yearly kid crop of a particular farm, and how many of the kids reach weaning age. Farm productivity is dependent on kidding rate, prolificacy rate, fecundity rate, fertility rate, litter size, male kid: female kid ratio, proper selection for traits, weaning percent and rate at which kids are being produced (Edmondson *et al.*, 2002; Hazem, 2008). Fertility rate is defined as a proportion of goats out of 100 mated or artificially inseminated that kid after expected goat doe gestation period (Ahmed *et al.*, 1998). Fecundity rate is defined as a proportion of kids born alive out of 100 does bred/inseminated, whereas prolificacy rate expresses the proportion of kids born alive to 100 kidded does (Ahmed *et al.*, 1998). Litter size is expressed as number of kids born per goat doe. The male kid: female kid ratio expresses the ratio of male kids to female kids born and measures the productivity of the farming entity as more females born will easily and quickly increase the kid crop and profitability of the farm.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study site

The study was carried out at University of Venda Animal Science goat feedlot located in Vhembe district in the Limpopo Province of South Africa **(S 22° 58´ 39.4˝; E 030° 26´ 38.5˝)**. The area has a tropical climate with four very distinct climatic seasons broadly classified as winter, spring, summer and autumn. Summers are very hot with low rainfalls ranging throughout the years to be between 250-750 mm. Winters are cooler as temperatures may drop to 7 °C but are generally warmer with temperatures above 25 °C recorded in some years. The area is generally dry providing most convenient conditions for goat production. Goats were raised in a feedlot with sufficiently good and robust pens providing much needed separation for individual doe parameter recordings. The Metabolic laboratory on site provided shelter during ultrasound and AI procedures. The experiments were carried out at peak tropical goat breeding season (May-July) in tropical South Africa.

3.2 Experiment

3.2.1 Animals and husbandry

Sixty (n=60) goat does that weighed between 35 - 90 kilograms and aged between 1.5 to 4 years of age (dentition) were used in this experiment. Twenty eight (28) indigenous Venda does kept under tropical rangeland sweet veld conditions were collected from four points of at least 7 km radius apart in Mutale municipality from four farmers and the other indigenous Venda goat does were collected from three farmers in Thulamela municipality kept under tropical and mixed veld conditions. Indigenous Venda goats collected were of unknown fertility and there are no current literature citations on fertility of this breed of goats in South Africa. Sixteen (n=16) Boer does used for experimentation belonged to the University of Venda. The body condition score expressed in the mean ± S.E.M of the goat does was 2.9 ± 0.4 (British scale 1-5) at the start of the experimentation. Two weeks prior to experimentation does were vaccinated using Multiclos®+P, dosed with an antihelminthic Prodose® orange and supplemented with Multimin® mineral injections. Ultrasound and fetal ballottement were used for screening to avoid use of pregnant animals in the experiment.

Does were randomly assigned to any of the four experimental treatment groups with each group having a total of fifteen goat does with weight, age, parity and geographical origin used as assignment criteria. For penning purposes the goat does in the four experimental treatment groups were further regrouped into fifteen subgroups of four does each with each subgroup having goat does of all the four treatment protocols and penned in 3 x 6 m^2 pens.

The pens had adequate spacing for watering, two feed troughs, and adequate space for free movement. Goat does maintained visual, auditory and olfactory contacts with Boer goat bucks though physically separated by a robust Bonnox® mesh fence.

Goat does and Boer bucks were fed compressed Driehoek® ewe pellets (supplied by NTK Pty Ltd) and water *ad libitum*. Goat does were conditioned through a dietary pre-adaptation period of 2 weeks during which lucerne, hay and dry pellets were fed. Goats developing diarrhoea or bloat had their diet switched to hay and withdrawn from experimental diet of compressed pellets and lucerne. Four Boer and one Venda bucks were penned separately in well calculated and strategically positioned pens between does in the fifteen pens.

3.2.2 Experimental design

A randomized 4 x 2 factorial design of four oestrous synchronization protocols (CIRD-G/eCG, double $PGF_{2α}$/eCG, novel *NC Synch* GnRH based protocol and control) applied on two indigenous breeds (Venda & Boer) n=60 goat does was used as assignment critea into the four groups of fifteen goat does each and latter fifteen penning subgroups of four goat does each. Goat doe age, breed, Body Condition Scores (BCs), Body Weight (BWt) and geographical origin of goat does were also used as stratification criteria to make a balanced experimental treatment design.

3.2.3 Experimental treatments and calendarized events

Experimental Group 1 Fifteen goat does had CIRD-G (Eazi-Breed™, Zoetis SA) intravaginal devices inserted on day 0; 10 mg prostaglandin$F_{2α}$ (Lutalyse™, dinoprost 5 mg/ml, Zoetis SA) together with 250 IU of eCG (Folligon® 1000 IU/ie, MSD Animal Health SA) injected intramusculary on day 12 and removal of devices 12 h later.

Experimental Group 2 Fifteen goat does had 10 mg prostaglandin$F_{2α}$ (Lutalyse™, dinoprost 5mg/ml, Zoetis SA) injected intramusculary on day 0 and 10 mg prostaglandin$F_{2α}$ (Lutalyse™, dinoprost 5mg/ml, Zoetis SA) together with 250 IU of eCG (Folligon® 1000 IU/ie, MSD Animal Health SA) injected intramusculary on day 12 of the experiment.

Experimental Group 3 Fifteen goat does had 10 mg prostaglandin$F_{2α}$ (Lutalyse™, dinoprost 5 mg/ml, Zoetis SA) injected intramusculary on day -2 , 0.5 ml GnRH (Receptal®, buserelin 0.004 mg/ml, MSD Animal Health SA) injected intramusculary day 5 and 10 mg of prostaglandin$F_{2α}$ (Lutalyse™, dinoprost 5 mg/ml, Zoetis SA) injected intramusculary on day

12 of the experiment. Goat does were then injected intramusculary with 0.5 ml of GnRH (Receptal®, buserelin 0.004 mg/ml, MSD Animal Health SA) at time of AI.

Experimental Group 4 (n=15) the control protocol had 250 IU of eCG (Folligon® 1000 IU/ie, MSD Animal Health SA) injected intramusculary after detected onset of overt oestrus. Does in this control group were teased daily by an intact buck at 0700-1000 h and 1500-1700 h during the whole research period. The intact buck was penned separately and was allowed to roam in the passage adjacent to pens with does during teasing hours. Standing oestrus (mounted by mates) together with other non-specific visual signs like restlessness, tail wagging, vocalization/bleating, frequent urination, vulva edematous swelling, vulva hyperemia, vulva contraction, viscid clear discharge, visual marking of peri-vulva area by discharge and exchange of sexual cues were used as cardinal signs of overt oestrus. Such a goat doe would be injected intramusculary with 250 IU of eCG (Folligon® 1000 IU/ie, Intervet SA) at onset of oestrus and inseminated 24 h later.

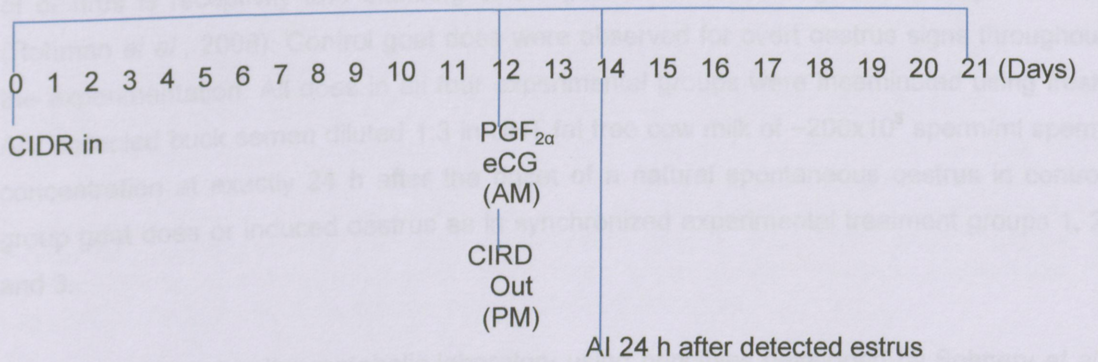

Figure 3.1: Experiment treatment protocol 1 (n=15)

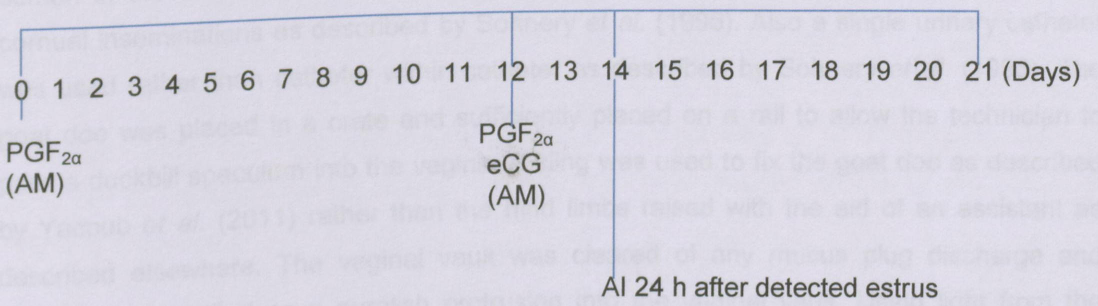

Figure 3.2: Experiment treatment protocol 2 (n=15)

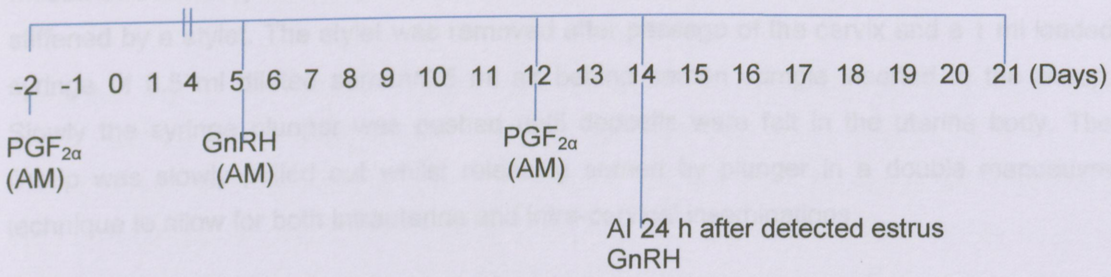

Figure 3.3: Experiment treatment protocol 3 (n=15)

3.2.4 Oestrus Detection and AI

Goat does in experimental groups 1,2 and 3 were observed daily for 4 days after treatment withdrawal for onset of overt oestrus in the morning from 07h00-10h00 and in the afternoon from 15h00-17h00 using an intact buck walking in a passage adjacent to doe pens with adequate visibility, cajoling but of limited physical contact. Oestrus detection in goats is never fairly accomplished in the absence of the buck since the highly regarded definitive sign of oestrus is receptivity and standing of the goat doe whilst being mounted by the buck (Rahman *et al.*, 2008). Control goat does were observed for overt oestrus signs throughout the experimentation. All does in all four experimental groups were inseminated using fresh A/V collected buck semen diluted 1:3 in UHT fat free cow milk of ~200×10^6 sperm/ml sperm concentration at exactly 24 h after the onset of a natural spontaneous oestrus in control group goat does or induced oestrus as in synchronized experimental treatment groups 1, 2 and 3.

AI was carried out in the metabolic laboratory using principles developed by Sohnery *et al.* (1995) of a modified deep cornual catheter within catheter method (Bowdridge *et al*, 2013). The modification was simply a deposition of 0.5ml of diluted freshly collected and extended semen in the uterus by transcervical deep uterine insemination rather than double deep cornual inseminations as described by Sohnery *et al.* (1995). Also a single urinary catheter was used rather than catheter within catheter as described by Sohnery *et al.* (1995). The goat doe was placed in a crate and sufficiently placed on a rail to allow the technician to pass a duckbill speculum into the vagina. A sling was used to fix the goat doe as described by Yacoub *et al.* (2011) rather than the hind limbs raised with the aid of an assistant as described elsewhere. The vaginal vault was cleared of any mucus plug discharge and cervical os identified as a purplish protrusion into the vaginal vault. Using light from the laparoscope, the cervix was grasped at 11 o'clock and 5 o'clock using a Pozzi tenaculum forceps leaving the cervical os unobstructed for passage of the urinary catheter. In a synchronized maneuver, cervical rings were aligned and trans-versed using pull and twist motion of the forceps 90 degrees anticlockwise and forward passage of a urinary catheter stiffened by a stylet. The stylet was removed after passage of the cervix and a 1 ml loaded syringe of 0.5 ml diluted semen/0.5 ml air behind semen sample inserted to the lineup. Slowly the syringe plunger was pushed until deposits were felt in the uterine body. The lineup was slowly pulled out whilst releasing semen by plunger in a double manoeuvre technique to allow for both intrauterine and intra-cervical inseminations.

Figure 3.4: Technique & Instrumentation used during trans-cervical deep uterine AI 24 h after onset of oestrus in responding goat does. 1. duckbill vaginal speculum for large goat, 2. Duckbill vaginal speculum for small goat 3. Graduated 1 ml syringe 4. AI lineup of urinary catheter stiffened by a steel stylet 5. Pozzi tenaculum forceps 6. Presumably sterile 2 ml syringe 7. Graduated 10 ml semen storing plastic tube 8. Laparoscopic light source, can be substituted by pen light

Time interval (h) to onset of oestrus after treatment withdrawal calculations*

Two oestrus recording sessions were done per day, the first at 07h00-10h00 and second at 15h00-17h00. If overt oestrus was seen at 07h00, but not recorded at 15h00/17h00, the previous day then oestrus was presumed to have started at 05h00 of the same day. If oestrus was recorded at 10h00 but not seen at 07h00 then oestrus was presumed to have started at 08h00. If oestrus was seen at 15h00 but was not seen at 10h00 then oestrus was presumed to have started at 13h00. The afternoon oestrus onset recording slot ended at 17h00 of each day.

Oestrus duration (h) calculations*

Time ranged from onset of overt oestrus recorded to overt oestrus end with correction errors added. If oestrus was last seen at 17h00 the previous day and not seen at 07h00 then oestrus was presumed to have ended at 22h00 the previous day. If oestrus was last seen at 07h00 but not seen at 10h00 then oestrus was presumed to have ended at 08h00. If oestrus was last seen at 10h00 but not seen at 15h00 then oestrus was presumed to have ended at 13h00.

3.2.5 Blood sample collection and progesterone profile assays

Blood was collected by jugular vein-puncture using 18 G vacutainer needles and 10 ml plain (without anticoagulant) vacutainer blood collecting tubes. Hair over the jugular vein was clipped and alcohol used to sterilize the site for veni-puncture. Digital pressure over the jugular groove at the distal end of the neck was able to raise the jugular vein outline. Vein-puncture was then done using the vacutainer needle and maintaining the digital pressure over the raised jugular vein. When the needle was anticipated to be in the jugular vein, the other end of vacutainer needle was pushed into the vacutainer blood collecting tube and approximately 10 ml blood collected. Blood samples were collected before each treatment and inbetween successive treatments for P4 profile assays at experimental day 0, 6, 7, 10, 12 and 17. Collected blood samples were placed in a crate in an upside down position overnight and sera poured in aliquot plastic 5 ml tubes and stored at -20°C until evaluation. Access to progesterone (AP) test kit # 43392 QC November 26 2014 (Beckman coulter, Tonnetti, Std 1 A, Growth Point Business Park, Halfway House, 1685, South Africa) of test sensitivity 0.32 - 127 nmol/l equivalent to (0.1 - 40 ng/ml) was used to determine sera progesterone assay profiles.

3.2.6 Pregnancy diagnosis

All inseminated goat does were examined for conception using A5 Sonoscope A-mode ultrasound (Sonoscope Co Ltd, Shenzhen P.R China, 50-60 Hz) using an L561V linear array 3.0-8.0 MHz sonoscope trans-rectal transducer on days 45-55 after AI. Access to progesterone coulter test kit at day 3 post AI blood assays was used to determine ovulation status. Non return to oestrus on day 21-24 post AI also provided crude conception status of inseminated does. Fetal ballottement, milk presence and rectal uterine ultrasound on 90-110 days post AI also gave crude information into pregnancy status of goat doelings.

3.3 Statistical analysis

Analysis of Variance (ANOVA) using General Linear Model package using (SAS® 9.3 version, 2013) compared parametric variables; time interval to oestrus onset after treatment withdrawal, oestrus duration, progesterone assays and gestation length. Intra- and inter-assay Coeficience of Variance (CV) were determined for comparison of progesterone profiles in experimental protocols and in the two breeds. Results of parametric variables were expressed in the mean ± S.E.M format. Non parametric or proportionate variables in percentages or ratios were compared using reverse comparisons of groups using Categorical Data Procedure of (SPSS® Version 21) chi-squared test. Non parametric variables included oestrous response, conception rates, return to oestrus ≥21 days after AI, fecundity rates, fertility rates, kidding rates, male kid: female kid ratio and liter sizes. Pearson's comparison test tested correlation or associations of categorical ranked variables (Buck semen vs conception, non return to oestrus ≥21 days after AI vs conception). Values of $P<0.05$ were considered to be statistically significant.

A linear model used for evaluation of parameters was as follows:

$$Y_{ijk} = \mu + B_i + S_j + (BS)_{ij} + e_{ijk} \quad \text{......equation (1)}$$

Where,

Y_k= variable studied during the period

μ = overall mean

B_i = breed effect on i^{th} term

S_j = synchronization protocol effect on j^{th} term

$(BS)_{ij}$ = breed and synchronization protocol interaction effect and,

e_{ijk} = standard error associated with each variable Y_{ijk}, Normally distributed N $(0,\delta^2_e)$ on k^{th} term. This includes error contributions of factors under consideration.

4.1 Oestrous parameters

4.1.1 Oestrous response

Oestrous response results following hormonal experimental treatments and control groups are tabulated in table 4.1 below.

Table 4.1: Oestrous response, time interval to onset of oestrus (Mean ± S.E.M) and percentage ratio of goat does having oestrus onset falling within experimental time limits after treatment withdrawal in the experimental protocols.

				Oestrus onset Percentage ratio			
Protocol	**N**	***n***	**Oestrous response**	Within 24 h	within 48 h	within 72 h	**Time interval to Oestrus onset (Mean ± S.E.M)**
CIRD®-G/eCG	15	14	93.3 (14/15)	57.1^{a} (8/14)	100^{a} (14/14)	100 (14/14)	25.95^{b}±3.85
Venda does	11	10	90.9 (10/11)	60 (6/10)	100 (10/10)	100 (10/10)	24.9^{b}±4.11
Boer does	4	4	100 (4/4)	50 (2/4)	100 (4/4)	100 (4/4)	27^{b}±6.5
$PGF_{2\alpha}$/eCG	15	14	93.3 (14/15)	14.3^{b} (2/14)	64.3^{b} (9/14)	92.9 (13/14)	47.05^{a}±4.24
Venda does	11	11	100 (11/11)	18.2 (2/11)	63.6 (7/11)	90.9 (10/11)	48.09^{a}±3.92
Boer does	4	3	75 (3/4)	0 (0/3)	66.7 (2/3)	100 (3/3)	46^{ab}±7.51
NC Sych	15	12	80 (12/15)	0^{c} (0/12)	25^{c} (3/12)	100 (12/12)	58^{a}±4.34
Venda does	11	9	81.8 (9/11)	0(0/9)	22.2 (2/9)	100 (9/9)	59.33^{a}±4.33
Boer does	4	3	75 (3/4)	0(0/3)	33.3 (1/3)	100 (3/3)	56.67^{a}±7.51
Total	45	40	89 (40/45)	25 (10/40)	65 (26/40)	96.2 (25/26)	43.9±5.2

^{a,b,c} Values in columns denoted by different superscripts differ significantly (p<0.05)

CIRD®-G/eCG and double $PGF_{2\alpha}$/eCG synchronization protocols both recorded 93.3% oestrous responses compared to 80% of novel *NC Synch* GnRH based protocol. There were no significant statistical differences (p>0.05) on oestrous responses for goat does in all protocols, though goat doe responses in the novel *NC Synch* GnRH based protocol were inferior to the other two hormonal synchronization protocols. Oestrous responses did not differ (p>0.05) between goat breeds, synchronization protocols or interaction of the two research variables. No comparisons were done for oestrous response in control group since oestrus was spontaneous.

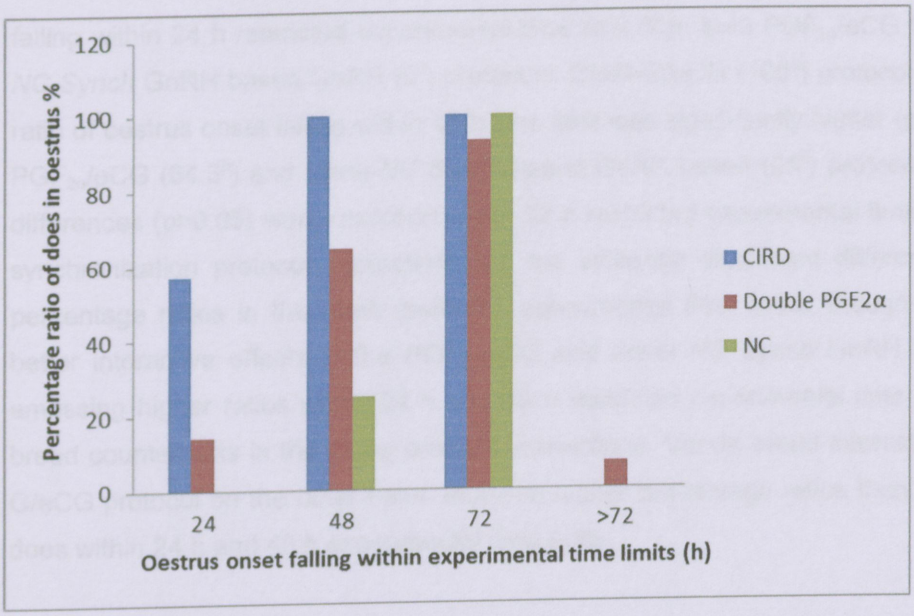

Figure 4.1: Percentage ratio of goat does having onset of oestrus falling within experimental time limits after treatment withdrawal in the experimental protocols.

4.1.2 Time interval to oestrus onset

Time interval to oestrus onset after treatment withdrawal results are tabulated in Table 4.1. Time interval to oestrus onset (*h*) for CIRD®-G/eCG, double $PGF_{2\alpha}$/eCG and novel *NC Synch* GnRH based protocols were $25.95^b \pm 3.85$, $47.05^a \pm 4.24$ and $58^a \pm 4.34$ respectively. CIRD-G/eCG ($25.95^b \pm 3.85$) protocol had a significantly shorter ($p<0.05$) and both $PGF_{2\alpha}$/eCG ($47.05^a \pm 4.24$) and novel *NC Synch* ($58^a \pm 4.34$) GnRH based protocols significantly longer ($p<0.05$) time interval to oestrus onset after treatment withdrawal. There were no statistical differences ($p>0.05$) on time interval to oestrus onset after treatment withdrawal between double $PGF_{2\alpha}$/eCG and novel *NC Synch* GnRH based protocols. Venda indigenous breed ($46^{ab} \pm 7.51$) influenced a significant precocious oestrus ($p<0.05$) in double $PGF_{2\alpha}$/eCG experimental protocols than the Boer breed ($48.09^b \pm 3.92$). No evaluations were done on time interval to oestrus onset in control goat does as the event of oestrus was spontaneous.

Time interval to oestrus onset in experimental groups was compartmentalized as falling within 24 h, 48 h and 72 h restricted experimental time limits. The percentage ratios of goat does having time interval to oestrus onset falling within restricted experimental time limits are tabulated in Table 4.1 and presented in Figure 4.1. Percentage ratios of responding goat does having time interval to oestrus onset falling within the restricted experimental time limits only differed significantly ($p<0.05$) within the 24 h, and 48 h time limits. CIRD-G/eCG protocol achieved the highest (57.1^a) percentage ratio of goat does having oestrus onset falling within 24 h restricted experimental time limit than both $PGF_{2\alpha}$/eCG (14.3^{b}) and novel *NC Synch* GnRH based GnRH (0^{c}) protocols. CIRD-G/eCG (100^{a}) protocol goat percentage ratio of oestrus onset falling within 48 h time limit was significantly higher ($p<0.05$) than both $PGF_{2\alpha}$/eCG (64.3^{b}) and novel *NC Synch* based GnRH based (25^{c}) protocols. No significant differences ($p>0.05$) were recorded within 72 h restricted experimental time limit. Breed and synchronization protocol interactions did not influence significant differences ($p>0.05$) in percentage ratios in the three restricted experimental time limits, though Boer breed had better interactive effects in the $PGF_{2\alpha}$/eCG and novel *NC Synch* GnRH based protocols, amassing higher ratios within 24 h and 48 h restricted experimental time limit than Venda breed counterparts in the same protocol interactions. Venda breed interaction in the CIRD-G/eCG protocol on the other hand amassed higher percentage ratios than Boer breed goat does within 24 h and 48 h experimental time limits.

4.1.3 Oestrus duration

Oestrus duration results for the four treatment protocols are as tabulated in Table 4.2 below.

Table 4.2: Oestrus duration (*h*) (Mean ± S.E.M), conception rates and percentage ratio of goat does having oestrus duration (*h*) falling within experimental time limits in experimental protocols

		Oestrus Duration					
		Percentage ratio Oestrus Duration (h)					
Protocol	*n*	within 24 h	within 48 h	within 72 h	>72 h	Oestrus duration mean ± S.E.M	Conception rate
CIRD®-G/eCG	14	14.3(2/14)	71.4(10/14)	92.9(13/14)	7.1(1/14)	48.53±10.75	71.4
Venda does	10	20(2/10)	90(9/10)	100(10/10)	0(0/10)	38.3±11.5	80
Boer does	4	0(0/4)	25(1/4)	75(3/4)	25(1/4)	58.75±18.18	50
Double $PGF_{2\alpha}$	14	28.6(4/14)	64.3(9/14)	85.7(12/14)	14.3(2/14)	56.65±11.84	78.6
Venda does	11	36.4(4/11)	81.8(9/11)	100(11/11)	9.1(1/11)	36.64±10.96	81.8
Boer does	3	0(0/3)	33.3(1/3)	66.7(2/3)	33.3(1/3)	76.76±20.99	66.7
NC Synch	12	16.7(2/12)	91.7(11/12)	100(12/12)	0(0/12)	39.22±12.11	66.7
Venda does	9	22.2(2/9)	100(9/9)	100(9/9)	0(0/9)0	30.11±12.12	66.7
Boer does	3	0(0/3)	66.7(2/3)	100(3/3)	0(0/3)0	48.33±20.99	66.7
Control	14	14.3(2/14)	64.3(9/14)	78.6(11/14)	21.5(3/14)	55.38±10.75	78.6
Venda does	10	20(2/10)	60(6/10)	80(8/10)	20(2/10)	62.5±11.5	90
Boer does	4	0(0/4)	75(3/4)	75(3/4)	25(1/4)	48.25±18.18	50
Total	54	18.5(44/10)	74.1(14/40)	90.7(5/49)	11.1(48/6)	49.95±11.36	74.1

^{a,b,c} Values in columns denoted by different superscripts differ significantly ($p<0.05$)

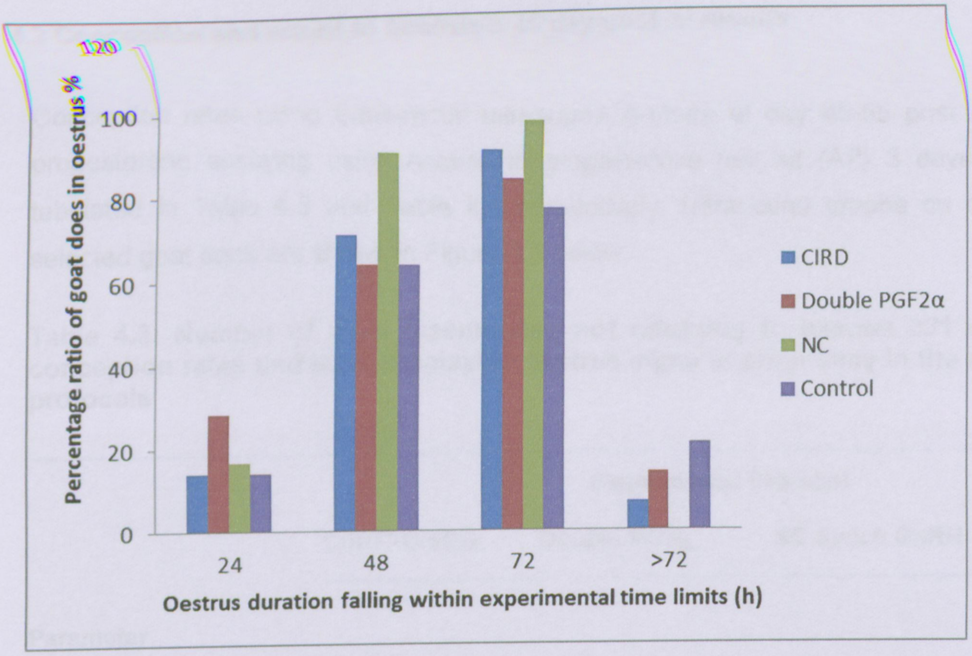

Figure 4.2: Percentage ratio of goat does having oestrus duration (*h*) falling within experimental time limits in the experimental groups.

Oestrus duration (*h*) for CIRD®-G/eCG, double $PGF_{2\alpha}$/eCG, novel *NC Synch* GnRH based protocols and control were 48.53 h ± 10.75, 56.65 h ± 11.84, 39.22 h ± 12.11 and 55.38 h ± 10.75 respectively. Oestrus duration of the four experimental protocols did not differ statistically (p>0.05) and breed, synchronization protocol or breed/synchronization protocol interactions had no effect on influencing the objectives of this parameter. However, despite lack of statistical differences (p>0.05), the Boer breed goat doe interactions in the CIRD-G/eCG, double $PGF_{2\alpha}$/eCG and *NC Synch* GnRH based experimental protocol appeared to influence a much longer oestrus duration than Venda indigenous goat breed in the same experimental protocols. This parameter was highly variable and wide range S.E.M values in oestrus duration did not generate significant statistical differences (p>0.05) resulting from compound effects of breed, synchronization protocol factors or interaction of the two research variables.

Data on percentage ratios of goat does having oestrus duration falling within restricted experimental 24 h, 48 h, 72 h and >72 h time limits after oestrus onset are tabulated in Table 4.2 and presented in Figure 4.2. There were no significant statistical differences (p>0.05) in percentage ratios of goat does having oestrus duration falling within all restricted experimental time limits that could have been attributed to breed, synchronization protocol or breed/synchronization protocol interactive factors. However, there was no Boer breed goat doe having oestrus duration falling within 24 h restricted experimental time limit against 24.4% of Venda indigenous goat does in all four experimental protocols.

4.2 Conception and return to oestrus ≥ 21 day post AI results

Conception rates using trans-rectal ultrasound A-mode at day 45-55 post AI and blood progesterone assaying using Access to progesterone test kit (AP) 3 days post AI are tabulated in Table 4.3 and Table 4.4 respectively. Ultrasound graphs on conception of selected goat does are shown in Figure 4.3 below.

Table 4.3: Number of does inseminated not returning to oestrus ≥21 day after AI, conception rates and does displaying oestrus signs in pregnancy in the experimental protocols

	Experimental Protocol			
	CIRD®-G/eCG	Double $PGF_{2\alpha}$	*NC Synch* GnRH	Control
Parameter				
Number does inseminated	14	14	12	14
Non return to oestrus≥ 21day Post AI	71.4(10)	64.3(9)	75(9)	71.4(10)
Venda does	70(7)	63.4(7)	77.8(7)	80(8)
Boer does	75(3)	66.7(2)	66.7(2)	50(2)
Conception rates % (Day 45-55)	71.4(10)	78.6(11)	66.7(8)	78.6(11)
Venda does	80(8)	81.8(9)	66.7(6)	80(8)
Boer does	50(2)	66.7(2)	66.7(2)	75(3)
Number of does displaying oestrus during pregnancy	0	2	2	1
Venda does	0	1	2	0
Boer does	0	1	0	1

[a,b,c] Values in columns denoted by different superscripts differ significantly ($p<0.05$)

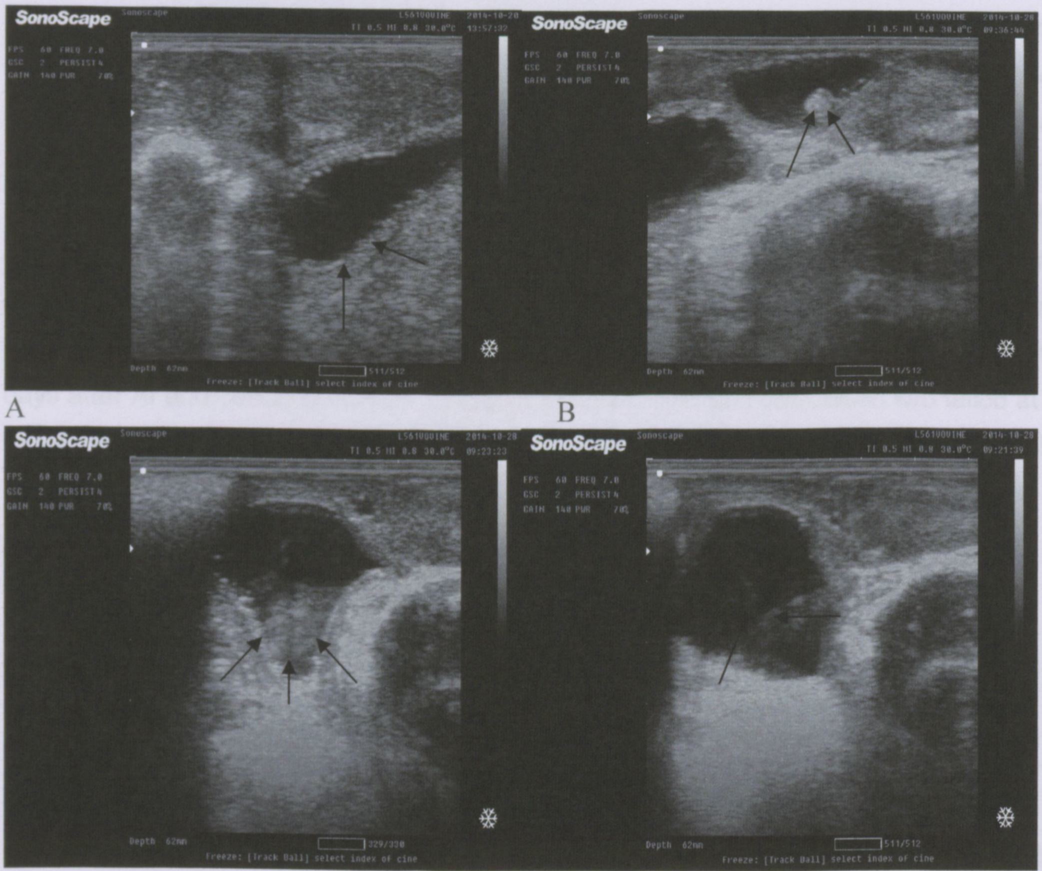

Figure 4.3: Trans-rectal ultrasound images of selected inseminated goat does. (A) Non pregnant Boer doe- note the non-echoic uterus 50 days after AI. (B) Pregnant Boer doe day 55 after AI- note the hyper-echoic area on dorsal uterine horn at 5 o'clock. The uterine horns are enlarged and severely coiled. (C)Pregnant Venda doe day 55 after AI- note the hyper-echoic area on the ventral aspect of the uterus.(D)Pregnant Venda doe 55 day post AI- Note the fetal heart above the hyper echoic area at 5 o'clock.

Conception rate results using trans-rectal A-mode ultrasonography at day 45-55 post insemination were 71.4%, 78.6%, 66.7% and 78.6% for CIRD®-G/eCG, double $PGF_{2\alpha}$/eCG, novel *NC Synch* GnRH based and control experimental protocols, respectively. There were no significant statistical differences ($p>0.05$) on conception rates across all experimental treatment groups, however, double $PGF_{2\alpha}$/ eCG (78.6%) and Control (78.6%) treatment protocols had the highest and the novel *NC Synch* protocol (66.7%) the lowest conception rates. There were no significant statistical differences ($p>0.05$) on conception attributed to breed, synchronization protocol or associative interaction of the two research variables, although Venda indigenous breed had better conception rates in all but novel *NC Synch* GnRH based protocol. There was no correlation between Boer buck used and conception (Pearson correlation- 0.146), (p-Value=0.291).

Non return to oestrus results 21 days after AI were 71.4%, 64.3%, 75% and 71.4% of inseminated goat does in the CIRD-G/eCG, $PGF_{2\alpha}$/eCG, novel *NC Synch* GnRH based and Control experimental groups respectively. Non return to oestrus results 21 days after AI did not differ ($p>0.05$) significantly across all experimental treatment groups, although $PGF_{2\alpha}$/eCG protocol had the lowest and novel *NC Synch* GnRH based protocol the highest non return after experimental treatment group comparisons. There were also no significant experimental group differences ($p>0.05$) on conception attributed to breed, synchronization protocol or associative interaction of the two research variables. Non return to oestrus 21 days after AI and conception were strongly correlated (Pierson correlation two tailed at 0.01) (p-Value=0.00). However some goat does displayed oestrus signs during pregnancy in the $PGF_{2\alpha}$/eCG (2), novel *NC Synch* GnRH based (2) and control (1) experimental groups.

4.3 Progesterone assay results

Table 4.4: Progesterone profile assays (Mean ± S.E.M) at experimental day 0, 6, 7, 10, 12 and 17 in the experimental protocols.

	Experimental protocol			
	CIRD®-G/eCG	**Double $PGF_{2\alpha}$**	***NC Sych***	**Control**
Progesterone profiles nmol/l (Mean ± S.E.M)				
Day 0	$10.97^{bcde} \pm 2.23$	$15.42^{abcd} \pm 2.23$	$11.32^{bcde} \pm 2.23$	-
Day 6	$25.3^{a} \pm 2.3$	$2.63^{e} \pm 2.23$	$2.44^{e} \pm 2.23$	-
Day 7	-	-	$9.7^{cde} \pm 4.06$	-
Day 10	-	-	$15.9^{abcd} \pm 6.2$	-
Day 12	$20.58^{ab} \pm 2.3$	$8.97^{cde} \pm 2.23$	$19.59^{abc} \pm 2.23$	$2.42^{e} \pm 2.23$
Day 17	$12.33^{bcde} \pm 2.3$	$7.92^{de} \pm 2.23$	$8.09^{de} \pm 2.23$	$9.64^{bcde} \pm 2.23$

P4 values having different superscripts $^{a-e}$ in rows or columns differ significantly ($p<0.05$)

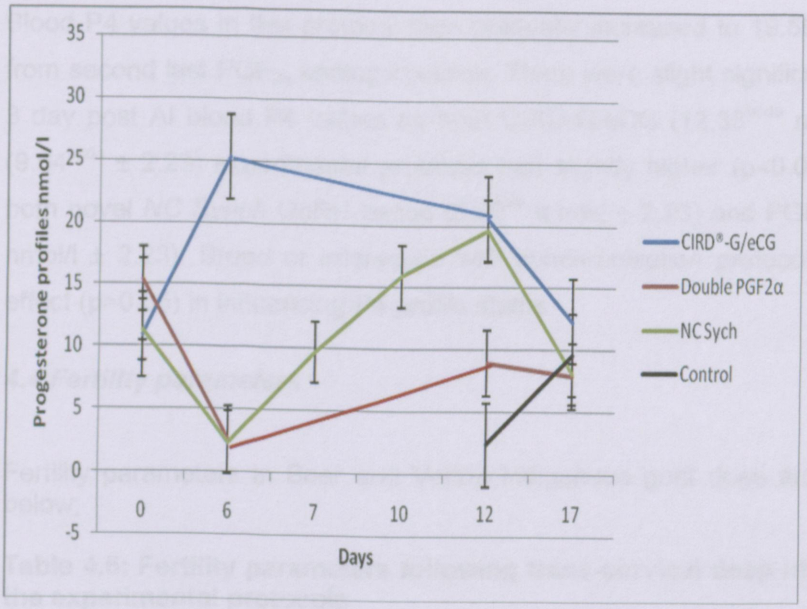

Figure 4.4: Progesterone assay profiles at experimental day 0, 6, 7, 10, 12 and 17 in the experimental protocols.

Progesterone assay profiles in the absence of pregnancy (Ultrasound and fetal ballotment) of goat doelings ranged from 0.59 to 32.94 nmol/l at the start of the experiment. Only five goat does (11.1%) in all experimental groups had subluteal (<2.5 nmol/l) blood P4 value at experiment day 0. CIRD-G devices in the CIRD-G/eCG experimental protocol resulted significant ($p>0.05$) increase in blood P4 values from blood P4 values of 10.97^{bcde} nmol/l ± 2.23 at the start of the experiment to 25.3^{a} nmol/l ± 2.3 at an imaginary experimental mid-luteal stage (Experiment day 6), followed by a gradual fall in blood P4 values to 20.58^{ab} nmol/l ± 2.3 at 12 h from device removal.

In the double $PGF_{2\alpha}$ protocol, there was a significant fall ($p<0.05$) in blood P4 levels after the first $PGF_{2\alpha}$ analog injection (Lutalyse®, Pfizer) as indicated by an imaginary experimental mid-follicular blood P4 values of 2.63^{e} nmol/l ± 2.23 from blood P4 values of 15.42^{abcd} nmol/l ± ($p>0.05$) recorded at experiment day 0. There was a significant ($p<0.05$) gradual blood P4 assays increase at 12 h from second last $PGF_{2\alpha}$ analog injection to blood P4 values of 8.97^{cde} nmol/l ± 2.23 in the same protocol.

The novel *NC Synch* based GnRH protocol had a similar initial P4 assay path to the double $PGF_{2\alpha}$/eCG protocol up to an imaginary experimental mid-follicular stage and a significant increase ($p<0.05$) in blood P4 values following day 7 GnRH (Bureselin®) analog injection from a pre-GnRH analog injection blood P4 values of 9.7^{cde} nmol/l ± 4.1 to 15.9^{abcd} nmol/l ± 6.2 at a second imaginary experimental mid-luteal protocol stage (Experiment day 10).

Blood P4 values in this protocol then gradually increased to 19.59^{abc} nmol/l ± 2.23 at 12 h from second last $PGF_{2\alpha}$ analog injection. There were slight significant differences ($p<0.05$ in 3 day post AI blood P4 values as both CIRD-G/eCG (12.33^{bcde} nmol/l ± 2.23) and control (9.64^{bcde} ± 2.23) experimental protocols had slightly higher ($p<0.05$) blood P4 profiles than both novel *NC Synch* GnRH based (8.09^{de} nmol/l ± 2.23) and $PGF_{2\alpha}$/eCG protocols (7.92^{de} nmol/l ± 2.23). Breed or interaction with synchronization protocol did not have significant effect ($p>0.05$) in influencing P4 profile status.

4.4 Fertility parameters

Fertility parameters in Boer and Venda indigenous goat does are presented in Table 4.5 below;

Table 4.5: Fertility parameters following trans-cervical deep uterine AI in goat does in the experimental protocols

	Experimental protocol			
Parameter	**CIDR-G/eCG**	**Double $PGF_{2\alpha}$/eCG**	***NC Synch* GnRH**	**Control**
Number does inseminated	14	14	12	13
Kidding rate (%)	100	100	100	100
Fertility rate (%)	71.4	78.6	66.7	78.6
Prolificacy/Fecundity	1.3±0.24	1.4±0.24	1.4±0.24	1.05±0.20
Venda G. does	1.0±0.21	1.5±0.43	1.3±0.25	1.1±0.20
Boer G. does	1.5±0.43	0.89±0.20	1.5±0.43	1.0±0.35
Female kids	1.06±0.28	0.75±0.27	0.42±0.29	0.83±0.23
Venda G. does	1.1±0.25	1.0±0.23	0.8±0.29	0.67±0.23
Boer G. does	1.0±0.5	0.5±0.5	0	1.0±0.4
Male kids	0.88^{b}±0.3	0.92^{b}±0.29	1.42^{a}±0.3	0.78±0.25
Venda G. does	0.75^{b}±0.26	0.33^{a}±0.25	0.83^{b}±0.3	0.89±0.25
Boer G. does	1.0^{ab}±0.53	1.5^{ab}±0.53	2.0^{a}±0.52	0.67±0.43
Female kid: male kid ratio	1.1^{a}	1.14^{a}	0.56^{b}	0.9^{a}
Litter size	1.94±0.25	1.67±0.25	1.83±0.26	1.61±0.21
Singleton	3	5	5	5
Twins	5	5	4	7
Triplets	2	-	1	-
Gestation length (Days)	154.4^{ab}±2.4	152.4± 2.3	147.7 ±2.5	156.4^{a}±2.0
Venda G. does	149.4^{b}±2.1	153±2.0	147.8±2.5	153.4^{ab}±2.0
Boer G. does	159.5^{a}±4.3	151±4.2	147.5±4.3	159.3^{a}±3.5

[a,b,c] Values in columns denoted by different superscripts differ significantly ($p<0.05$)

In this current study only 14, 14, 12 and 13 of responding goat does were inseminated in the CIRD-G/eCG, double $PGF_{2\alpha}$/eCG, novel *NC Synch* GnRH based and control experimental protocols, respectively. All experimental groups achieved 100% kidding rates with no significant differences ($p>0.05$) recorded across groups. Fertility rate was highest in double $PGF_{2\alpha}$/eCG (78.6%) and control protocols (78.6%) and least in the novel *NC Synch* GnRH based protocol (66.7%), however, there were no significant statistical differences ($p>0.05$) in all experimental groups on this parameter. Breed, synchronization protocol or breed/synchronization protocol interactions did not have any effect on kidding and fertility rates. Both Novel *NC Synch* GnRH based and double $PGF_{2\alpha}$/eCG protocols recorded highest prolificacy/fecundity (1.4 ± 0.24), though without significant statistical difference ($p>0.05$) across all experimental protocols.

Gestation period in days was significantly shorter ($p<0.05$) for novel *NC Synch* protocol goat does (147.7^{b} ± 2.5) and significantly longer ($p<0.05$) for the control experimental protocol (156.4^{a} ± 2.0). Breed factor on its own could not result significant statistical differences ($p>0.05$) on gestation length. However, the Boer breed interactive effects resulted in significantly longer ($p<0.05$) gestation length in the CIRD/eCG (159.5^{a} ± 4.25) and control (159.33^{a} ± 3.47) protocols. Conversely the Venda indigenous breed interaction influenced significantly shorter gestation length in the CIRD/eCG (149.38^{b} ± 2.13) and control (153.44^{ab} ± 2.0) protocol goat does. There were no discernable breed/synchronization protocol interactions on influencing gestation length in double $PGF_{2\alpha}$/eCG and novel *NC Synch* GnRH based experimental protocols.

Litter sizes were largest, though not statistically significant ($p>0.05$), in the CIDR-G/eCG protocol (1.94 ± 0.25) followed by novel *NC Synch* GnRH based protocol (1.83 ± 0.26) and were least for control experimental protocol (1.61 ± 0.21). Boer breed goat does recorded bigger litter sizes (1.92 ± 0.21), though not statistically significant ($p>0.05$) than Venda indigenous breed goat does (1.61 ± 0.11). There were no significant differences ($p>0.05$) on litter sizes resulting from synchronization protocol, breed or breed/synchronization protocol interactive effects. CIDR-G/eCG and control experimental protocols recorded more multiple pregnancies (seven) than both double $PGF_{2\alpha}$/eCG and novel *NC Synch* GnRH based protocols that recorded five multiple pregnancies apiece. CIDR-G/eCG protocol had least number of singleton pregnancies than the other three experimental protocols which all amassed five singleton pregnancies.

Number of female kids born per goat doe were not significantly different ($p>0.05$) in all experimental protocols though CIDR-G/eCG protocol recorded the most (1.06 ± 0.28) and novel *NC Synch* GnRH based protocol the least number of female kids per kidded doe. There were no statistical differences on number of female kids born caused by breed, synchronization protocol or by breed/synchronization protocol interactive effects.

Novel *NC Synch* GnRH based protocol recorded significantly more ($p<0.05$) male kid pregnancies ($1.42^b \pm 0.3$) and control experimental protocol ($0.78^a \pm 0.25$) the least ($p< 0.05$) male kid pregnancies. Boer breed goat does ($1.29^a \pm 0.25$) carried significantly more ($p< 0.05$) male kid pregnancies than Venda goat does ($0.70^b \pm 0.13$). On breed/synchronization protocol interactive effects on male kid pregnancies, there were significantly more ($p<0.05$) male kid pregnancies by Boer breed/novel *NC Synch* protocol GnRH based protocol ($2.0^a \pm 0.52$) interaction. There was significantly less ($p<0.05$) male kids born from kidded does resulting from the following breed/synchronization protocol interactive effects; Venda indigenous breed/double $PGF_{2\alpha}$/eCG ($0.33^b \pm 0.25$), Boer breed/control experimental protocol ($0.67^{ab} \pm 0.43$) and Venda indigenous breed/CIDR-G/eCG experimental protocol ($0.75^{ab} \pm 0.26$).

The CIDR-G/eCG, double $PGF_{2\alpha}$/eCG and control protocols resulted in an equal chance of getting female to male kids. CIDR-G/eCG, double $PGF_{2\alpha}$/eCG protocols had a slight bias of resulting in more female kids and control protocol slight bias towards male kids. The novel *NC Synch* GnRH protocol resulted in two times chance of getting male to female kids.

CHAPTER 5: DISCUSSION

The three pharmacological synchronization protocols used in this current study were able to induce and synchronize oestrus in both Boer and Venda indigenous goat breed does during peak autumn/winter tropical conditions of South Africa. The oestrous responses above 80% in all experimental groups including controls indicated that experimentation was done during the natural breeding season of goat does in Venda region as suggested by Greyling and van Niekerk, (1986). Seasonality in tropical goats can also be controlled by dietary nutritive manipulations prior to breeding season (Danko *et al.*, 2007; Fatet *et al.*, 2010; Kusina *et al.*, 2001). Current study blood progesterone assays ranging from 0.59 to 32.94 nmol/l prior to experimentation was sound evidence of reproductive cyclicity in experimental animals (Holtz *et al.*, 2008; Menchaca *et al.*, 2007; Yacoub *et al.*, 2011). Goat does in this current study were fed a high plane balanced diet of lucerne, hay and compressed dry ewe pellets thus goat does had very good reproductively sound body condition scores of 2.9 ± 0.4 (British scale 1-5) throughout experimentation. The Control group had 93.3% of goat does spontaneously displaying oestrus albeit in a non-synchronized manner as also suggested by Lopez-Sebastian *et al.*, (2007).

Oestrous responses in the experimental protocols except controls did not differ statistically though both the CIRD-G/eCG and double $PGF_{2\alpha}$/eCG protocols were superior to the novel *NC Synch* GnRH based protocol. CIRD-G/eCG protocol had a good chance of recording a 100% oestrous response, possibly if the non-responding goat doe did not have secretory diarrhoea at day 7 of protocol application. One of the goat does that did not respond in the novel *NC Synch* GnRH based protocol developed a wound on the ventral aspect of the udder during experimentation and fly nuisance stress factor was suggested as a possible cause of suboptimal endocrine activity. A cause of sub-oestrus or silent oestrus in the other two does in the same protocol was not established, but presence of a non responsive CL or infertility was suggested.

After consideration of overall confounding factors on goat doe oestrous responses following application of the three pharmacological oestrous synchronization protocols on the two breeds in this current study, CIRD-G/eCG protocol had better responses than both double $PGF_{2\alpha}$/eCG and novel *NC Synch* GnRH based protocols. Oestrus in domestic and wildlife ruminant species occur spontaneously after pulsatile antral follicle endogenous estradiol hormonal action superceeds progesterone inhibitory effect on the hypothalamus (Meilan and Ungerfield, 2014; Abdul Muin *et al.*, 2013; Pierson *et al.*, 2003). The CIRD-G/eCG protocol maintained supra-optimal blood progesterone levels throughout experimentation as shown in

Table 4.4 & Figure 4.4, thus providing sufficient suppression on pituitary gonadotropin release. Upon treatment withdrawal a rapid follicular growth in dwindling progesterone levels ensures display of a precocious oestrus (Abdul Muin *et al.*, 2013; Hashemi *et al.*, 2006; Pierson *et al.*, 2003; Vilarino *et al.*, 2011). Lassala *et al.*, (2004) & Mencheca *et al.*, (2007) in their studies suggested that progesterone hormone whether from exogenous or endogenous sources is important at influencing follicular turnover until one follicular wave gives birth to an antral follicle responsive to LH and therefore ovulation. The CIDR-G devices in CIDR group influenced better control of follicular turnover than what could have been achieved by both the novel *NC Synch* GnRH based and double $PGF_{2\alpha}$/eCG protocols in this study.

The oestrous responses of goat does in the CIRD-G/eCG (93.3%) was in close agreement with results in Sudanese Nubian goats by Ahmed *et al.*, (1998)(93.3%, CIRD-G devices left in situ for 9 days, 400IU eCG); Hashemi *et al.*, (2006) (93.3%, sheep); Wildeus, (2000) (95%), Menchaca *et al.*, (2007) (86%, CIRD inserted for 5 days); Souza *et al.*, (2011) (87%) and Abdul Muin *et al.*, (2013) (93.3%, without eCG). CIRD-G devices inserted for 14 days in the same study by Ahmed *et al.*, (1998) and other studies by Vilarino *et al.*, (2011), Omontese *et al.*, (2014) (sheep ewes, no eCG) and Motlomelo *et al.* (2002), shoat does had 100% oestrous responses irrespective of breed, age, nutritional status, climate and farm management practices (Whitely and Jackson, 2004). Natural progesterone (CIRD-G) and synthetic progestagen analogs (FGA & MAP formulations) imitates the physiologic luteal phase and upon treatment withdrawal, steroidal negative feedback on the hypothalamic-pituitary-ovarian axis is removed, initiating tonic gonadotropin release that hastens into ovulation (Hafez and Hafez, 2000; Noakes *et al.*, 2009). Estradiol released by growing antral follicles cause a positive feedback stimulation of the anterior pituitary gland to influence pre-ovulatory LH and FSH surge release thus initiating mechanisms of an ovulatory process (Hafez and Hafez, 2000).

Sponge formulations (FGA & MAP) have also often given comparable results to newer CIRD-G devices (Abdul Muin *et al.*, 2013), but animal welfare issues, aesthetic and steroidal animal product residues have limited their use in shoats (Lopez-Sebastian *et al.*, 2007). Progestagen sponge formulation devices cause mucosal irritation, vaginal flora overgrowth leading to fetid vaginal discharges, vaginitis and poor sponge retention up to anticipated sponge withdrawal times (Abdul Muin *et al.*, 2013; Oliveira *et al.*, 2001; Penna *et al.*, 2013; Lopez-Sebastian *et al.*, 2007). It also cause sponge attachment to the vaginal wall causing discomfort at sponge removal usually in nulliporous goat does in their maiden breeding season (Holtz *et al.*, 2008). Oestrous responses following CIRD-G/eCG (93.3%) in this current study was in close agreement to FGA/MAP sponge formulation results by Moradi Kor *et al.*, (2011) (97%, MAP 12 days), Boscos *et al.*, (2002)(92.5%, 12 days, 400IU eCG, sheep), Cengiz *et al.* (2014) (100%, 20 mg FGA, goat does, 12 days, 500IU eCG), Motlomelo *et al.* (2002) (96.7%, 40 mg FGA, 16 days, goat does) and Motlomelo *et al.* (2002) (93.1%, 60 mg MAP, 16 days, goat does).

Success of progestagen synchronization protocols depends on type of progestagen used, dose of analog relative to goat season, duration of progesterone treatment, goat doe breed, co-treatments, age of goat doe, nutritional status and stage of reproductive cycle (Amarantidis *et al.*, 2004; Avendano- Reyes *et al.*, 2007; Kusina *et al.*, 2001; Omontese *et al.*, 2014). Short-term progestagen studies by Menchaca *et al.*, 2007 suggested better synchrony if at point of CIDR insertion, a goat doe has a large follicle that is ready to ovulate as this ensures a fulminant follicular growth which ovulate spontaneously at point of treatment withdrawal. The shortfall of progestagen protocols is when a fully functional CL is present at treatment withdrawal. This suppresses follicular growth through actions of endogenously produced progesterone hormone. Therefore a luteolytic agent co-treatment at synchronization treatment withdrawal abolishes any reminiscent CL actions (Santolaria *et al.*, 2011).

Oestrous responses following double $PGF_{2\alpha}$/eCG (93.3%) protocol in this current study was lower than results by Amarantidis *et al.*, (2004) (95%, without eCG), Moradi Kor *et al.*, (2011) (97%), Riaz *et al.*, (2012) (100%), Greyling and van Niekerk, (1986) (100% at 250μg PGF_2), but was higher than results by Greyling and van Niekerk, (1986) (87.5% at 125μg $PGF_{2\alpha}$). Success of double $PGF_{2\alpha}$ injections is breed or dose dependent and depends on the presence of a CL older than 5 days (Day 5-16 days of oestrous cycle) at start of treatments (Greyling and van Niekerk, (1986; Kenfack *et al.*, 2013; Menchaca *et al.*, 2007; Leite-Browning., 2007). Therefore, two injections 9-14 days apart during breeding season will be able to achieve 100% synchrony and achieve fertility insemination results above 65% during breeding season (Kenfack *et al.*, 2013).

Oestrous responses following novel *NC Synch* GnRH based protocol in this current study was higher than results by Bowdridge *et al.*, (2013) (73%) and lower than results by Yacoub *et al.* (2011) (100%, tail flagging used as sign of overt oestrus) and Yacoub *et al.*, (2011) (85%, posturing used as sign of overt oestrus. Novel *Ovsynch* GnRH based protocol findings by Holtz *et al.*, (2008) (100%) and Ashmawy *et al.*, (2012) (less than 30%, sheep) displayed unreliability of *Ovsynch* protocols especially when prior screening is not conducted. Other literature citations did not document oestrous quality parameters such as studies by Farin *et al.*, (2013). Studies of the novel *Ovsynch and NC Synch* GnRH based protocols by

Bowdridge *et al.*, (2013), Holtz *et al.*, (2008) and Yacoub *et al.*, (2011) documented shortfalls of short cycling in participating goat does, however, the novel *NC Synch* GnRH based protocol of this current study did not encounter such deficiencies. The GnRH analog buserelin (Receptal®, MSD Animal Health SA) dose used for the novel *NC Synch* GnRH based protocol in this current study (0.002 mg/goat) was lower than what was used by Holtz *et al.*, (2008) (0.004 mg/goat, *Ovsynch* protocol); Yacoub *et al.*, (2011) (0.004 mg/goat, novel *NC Synch* variant); Bowdridge *et al.*, (2013) (0.004 mg/goat doe, *NC Synch* protocol). In this current study half the dose of GnRH analog still influenced significant rise in blood P4 values to almost approximating those of the CIDR-G group after day 7 GnRH analog injection. This is an indication of sufficient GnRH induced follicular P4 production or ovulation of a fully grown antral follicle and formation of secondary accessory induced CL. However, more empirical research will determine optimal doses of GnRH that better prepares fully grown antral follicles for ovulation and formation of secondary CL susceptible to day 12 $PGF_{2\alpha}$ injection actions as shown in Figure 4.4.

Time interval to oestrus onset (*h*) of CIRD-G/eCG, double $PGF_{2\alpha}$/eCG and novel *NC Synch* GnRH based protocols were $25.95^{b} \pm 3.85$, $47.05^{a} \pm 4.24$ and $58^{a} \pm 4.34$ respectively. CIRD-G/eCG protocol had significantly shorter ($p<0.05$) time interval to oestrus onset than both the novel *NC Synch* GnRH based and double $PGF_{2\alpha}$/eCG protocols. Time interval to oestrus onset after treatment withdrawal in goats is a highly variable parameter (Hashemi *et al.*, 2006; Romano, 2004; Souza-Fabjan *et al.*, 2013), but is a critical determinant in synchronizing the LH surge and ovulation (Pierson *et al.*, 2003). The most important factor in success of oestrous synchronization is timing uniformity of onset of oestrus after treatment withdrawal and LH surge or ovulation (Hashemi *et al.*, 2006; Lassala *et al*, 2004). Studies by Fonseca *et al.*, (2008) hypothesized that acceptable fertility in goat does is consistent with an earlier occurring precocious oestrus after treatment withdrawal. Studies by Baril *et al.*, (1998) suggested good fertility if oestrus occurred less than 30 hours after treatment withdrawal. This suggests development of a well differentiated and active dominant follicle at the time of treatment withdrawal (Menchaca *et al.*, 2007). Arrebola *et al.*, (2012) suggested that prediction of time interval to onset of oestrus was unachievable in goats following oestrous synchronization by progestagen protocols due to wide variations (32-88 *h*), however, this was without gonadotropin co-treatments at treatment withdrawal. A gonadotropin at progestagen treatment withdrawal hastens oestrus in goats (Arrebola *et al.*, 2012). Breed on its own in this study did not have an effect on time interval to onset of oestrus, however, Venda breed had better interactive effect within the double $PGF_{2\alpha}$/eCG synchronization protocol in which this breed of goat does influenced a slightly shorter time interval to oestrus onset ($46^{ab} \pm 7.5$) than Boer breed goat does ($48.1^{a} \pm 3.9$). Different breed associative sensitivities were shown to exist in response to different dosages of $PGF_{2\alpha}$ analogs used as was also suggested by Greyling and van Niekerk, (1986) & Lehloenya and Greyling, (2010).

Onset to oestrus after treatment withdrawal for the CIRD-G/eCG protocol (25.95 h ± 3.85) was comparable to research studies by Oliviera *et al.*, (2001) (most within 24 hours), Menchaca *et al.*, (2007)(5-7 day protocol,~30 *h*), Hashemi *et al.*, (2006) (30.1 ± 7.6 h, sheep) and Motlomelo *et al.*, (2002) (27.2 h ± 0.40). The time interval to oestrus onset after use of CIRD-G devices is shorter than if progestagen sponge formulation devices (FGA/MAP) are used (Motlomelo *et al.*, 2002). Time interval to oestrus onset in the double $PGF_{2\alpha}$/eCG protocol (47.05[a] ± 4.24) was comparable to results by Ahmed *et al.*, (1998) (52.6 h ± 11.1) and Zanetti *et al.*, (2010) (52.3 h ± 5.60, studies in the Brocket deer), but results of double $PGF_{2\alpha}$/eCG protocol results in the current study deviated strongly from results by Amarantidis *et al.*, (2004) (59.5 h ± 4.2 without eCG) and Riaz *et al.*, (2012) (36.0 h ± 1.2). Time interval to onset of oestrus results of the novel *NC Synch* GnRH based protocol (58[a] ± 4.34) in this current study was in close agreement to those by Bowdridge *et al.*, (2013) (54 h ± 10.0), Yacoub *et al.*, (2011) (42.6 h ± 2.9, a variant novel *NC Synch* GnRH based protocol). The novel *NC Synch* GnRH based protocol is fairly new and some studies could not give oestrous parametric details such as studies by Farin *et al.*, (2013). The control group was not evaluated on this parameter since oestrus was rather spontaneous and not induced like in the other three experimental groups.

Oestrus duration (*h*) in the CIRD-G/eCG, double $PGF_{2\alpha}$/eCG, and novel *NC Synch* GnRH based protocols and control group were 48.53 h ± 10.75, 56.65 h ± 11.84, 39.22 h ± 12.11 and 55.38 h ± 10.75, respectively. There were no significant differences (p>0.05) in oestrus duration attributed to synchronization protocol, breed or their interactions of the two research variables. Wide individual goat doe oestrus duration parameter variation (S.E.M) ensured lack of experimental statistical differences. This current research proved that goat doe oestrus duration is a highly variable parameter that should not be recommended and used as a determinant factor at crudely predicting LH surge timing for *fixed*-TAI programs, however, studies by Hashemi *et al.*, (2006) suggested oestrus duration as an important factor at determining ovulation times.

Oestrus duration of the CIRD-G/eCG protocol was comparable to research results by Motlomelo *et al.* (2002) (35.2 h ± 0.7), Menchaca *et al.* (2007) (31.2 h ± 3.1, CIRD inserted for 5 days), Zanetti *et al.* (2010) (34.7 h ± 4.50, Brocket deer & $PGF_{2\alpha}$ superimposed on CIRD-G) and Hashemi *et al.* (2006) (31.87 h ± 11, sheep). It is interesting to note that responses in wildlife like the Brocket deer (*Muzama gouazobira*), oestrous parameters were comparable to those of domestic ruminant species despite wildlife species low stress susceptibility thresholds (Zanetti *et al.*, (2010).

In the double $PGF_{2\alpha}$/eCG protocol (56.65 ± 11.84), oestrus duration was in close agreement with results by Ahmed *et al.*, (1998) (52.6 h ± 4.8), Riaz *et al.*, (2012) (47.1 h ± 2.9), Zanetti *et al.*, (2010) (37.0 h ± 8.1, brocket deer). Novel *NC Synch* GnRH based protocol is a relatively new protocol in goats and oestrous observations of this protocol are still low to make concrete conclusions. There is still a dearth of information with regard to oestrous objectives of the novel *NC Synch* GnRH based protocol. Oestrus duration of novel *NC Synch* GnRH based protocol was not documented by Bowdridge *et al.* (2013) and Farin *et al.* (2013), in their respective studies. However, other variants of novel *NC Synch* GnRH based protocol like novel *Ovsynch* protocol by Holtz *et al.*, (2008) had oestrus duration of (40.4 h ± 3.8, oestrus by posturing only), (21.0 h ± 4.4, oestrus by flagging only) and (35.7 h ± 3.7, oestrus by posturing & flagging). Oestrus duration of a variant protocol of the novel *NC Synch* GnRH based protocol by Yacoub *et al.*, (2011) (45.1 h ± 3.3) was in close agreement to results of novel *NC Synch* protocol (39.22 h ± 12.11) in this current study. Novel *NC Synch* GnRH based hormonal protocol had 100% of responding does having oestrus duration falling within 72 *h* and this is in agreement to research results by Hafez and Hafez, (2000) and Bowdridge *et al.*, (2013). Compact oestrus duration by this protocol usually compensates for the earlier offset of recording a late occurring oestrus onset. In this current study, the novel *NC Synch* GnRH based protocol reaffirmed a preposition of recommending this protocol in *fixed*-TAI in goat ART programs.

Fixed-TAI ART goat programs are only possible if a precocious oestrous response is associated with a compact synchronous oestrus in a group of goat does. Knowledge of oestrous parameters in different oestrous synchronization protocols is of particular importance in crude predetermination of insemination times (Teleb and Ashmawy, 2007). However, LH profile evaluations provide better precise determination of insemination times in goat does by establishing LH surge timing and therefore ovulation (Pierson *et al.*, 2003). Compactness of oestrus is defined by an earlier occurring oestrus usually below 30 h of treatment withdrawal (Baril *et al.*, 1998; Fonseca *et al.*, 2008; Souza-Fabjan *et al.*, 2013) and predictable oestrus duration.

The CIRD-G/eCG protocol had better qualities of a synchronous compact oestrus than any other protocol under this current study. Re-inventing a CIRD-G/eCG protocol by administering timely GnRH analog injections at strategic times of progestagen application will further improve this protocol for *fixed*-TAI programs in goats (Edmondson *et al.*, 2002; Teleb and Ashmawy, 2007). This will ensure a better control of ovulatory process and influence a compact synchrony of oestruses in a group of goats for *fixed*-TAI (Boscos *et al.*, 2002). The novel *NC Synch* protocol had a good chance of recording predictably shorter oestrus duration however, high S.E.M values per individual goat doe readings ensured that there were no statistical differences recorded in experimental protocols. Though oestrus onset was late in the novel *NC Synch* group ($58^a \pm 4.34$), 75% of goat doelings had onset of oestrus occurring predictably within the 48-72 h time range. The novel *NC Synch* GnRH based protocol performed better than both the double $PGF_{2\alpha}$/eCG and control experimental protocols in having a compact oestrus though of late onset for better crude determination of ovulation and insemination times. Venda indigenous breed associative interactions in all protocols influenced a precocious early occurring oestrus resulting higher proportionate goat does having time interval to onset of oestrus falling within 24 *h* and 72 *h* restricted experimental time limits than Boer breed goat does. This particular aspect should be considered in *fixed*-TAI ART goat programs for insemination time adjustment in this particular breed of goat does.

After consideration of compactness of oestrus and calculated associated errors of measurement, AI following goat doe synchronization by the CIRD-G/eCG, double $PGF_{2\alpha}$/eCG and novel *NC Synch* GnRH based protocols in this current study should have been done within 45-60 h, 70-93 h and 77-93 h time ranges after treatment withdrawal, respectively. However, variability of oestrus duration and time interval to onset of oestrus after treatment withdrawal following double $PGF_{2\alpha}$/eCG protocol and control groups, cannot crudely predict AI times for *fixed*-TAI see Figure 4.4. Leboeuf *et al.*, (2000) suggested pregnancy rates in excess of 65% when AI is done between 43-45 h after treatment withdrawal following 11 day progestagen protocol outside breeding season and using frozen thawed semen (Paramio and Iziquierdo, 2014). A 5-7 day progestagen protocol with $PGF_{2\alpha}$ and eCG at treatment withdrawal by Rubianes & Menchaca, (2003) recorded 64% pregnancy rates when AI was done 54 h after treatment withdrawal (Menchaca *et al.*, 2007). This is in agreement to current findings of this study.

Bowdridge *et al.*, (2013) suggested *fixed*-TAI times at 72 h after treatment withdrawal in goat does following synchronization by novel *NC Synch* GnRH based protocol and this is in close agreement with current study findings of *fixed*-TAI at 77-90 h after treatment withdrawal. Studies by Bowdridge et al., (2013) achieved 68% pregnancy rates and this was in close agreement to the current study (66.7%). A low dose (0.002 mg/goat, Buserelin, Receptal®, MSD Animal Health, SA) of GnRH analog used in this current study was lower than what was used by Bowdridge *et al.*, (2013) and this could explain the lower conception rates in the novel *NC Synch* GnRH based protocol of the current study, however, imaginary experimental mid-luteal progesterone profile assays after a day 7 GnRH injection indicated satisfactory luteal tissue development (Table 4.4 & Figure 4.4) indicating sufficient ovulation and development of secondary CL susceptible to day 12 $PGF_{2\alpha}$ analog injection. This protocol will therefore increase the proportion of goat does that will respond to a second $PGF_{2\alpha}$ analog than what a standard double $PGF_{2\alpha}$ synchronization is capable of achieving. Only goats having a CL older than 4 days will respond to the first $PGF_{2\alpha}$ analog therefore those with an insensitive CL due to age will not be in synchrony to the rest therefore a need for a day 7 GnRH analog injection. GnRH will influence follicular development through imitation of tonic pre- ovulatory gonadotropin environment that culminates into ovulation and formation of induced secondary accessory CL.

Conception rate results at experimental day 45-55 did not differ significantly (p>0.05) among groups, however, double $PGF_{2\alpha}$/eCG and control experimental protocols recorded superior results. This could be due to the buck effect that has superimposed interactive oestrous synchronization effects on double $PGF_{2\alpha}$/eCG protocols (Fatet *et al.*, 2010; Lopez-Sebastian *et al.*, 2007; Whitely and Jackson, 2004). Buck/male effect has minor if any superimposed interactive effect on progestagen protocols (Lopez-Sebastian *et al.*, 2007). Buck effect influences LH pulsatility and estradiol release. Estradiol regulates uterine oxytocin release and increase uterine sensitivity to oxytocin thereby stimulating uterine $PGF_{2\alpha}$ release a luteolytic agent that initiates luteolysis of any CL older than 4 days in goat does (Meilàn and Ungerfield, 2014). It therefore compliments prostaglandin protocols better as hypothalamic steroidal inhibitory effects of progestagen protocols can not be offset by buck effect mechanism (Lopez-Sebastian *et al.*, 2007). Lower conception rates in novel *NC Synch* protocol despite anticipated ovulation prowess due to superimposed buck effect and GnRH analog injections could be related rather to a dissociation of ovulation to insemination timings.

It is usually suggested that long progestagen based protocols achieves lower conception rates than double $PGF_{2\alpha}$/eCG protocols, due to progestagen effects on sperm transport and vitality (Baldassarre and Karatzas, 2004; Jackson *et al.*, 2006; Lopez-Sebastian *et al.*, 2007). Long-term progestagen protocols often leads to development of dominant follicles that continues to grow without ovulation due to subluteal progesterone levels following device waning (Menchaca *et al.*, 2007; Ruiz-González *et al.*, 2012; Santolaria *et al.*, 2011). This has given birth to short-term, usually 5-7 day protocols as suggested by Menchaca *et al.*, (2004). Despite displaying a precocious oestrus of good quality objectives, long-term progestagen protocols often leads to uncharacteristic asynchrony of ovulation to insemination times leading to poor conception rates and outward poor fertility (Santolaria *et al*., 2011). Usually a gonadotropin like eCG and luteolytic agent like $PGF_{2\alpha}$ at treatment withdrawal ameliorates oestrus asynchrony (Santolaria *et al*., 2011). However, a 12 day progestagen protocol and CIDR-G devices in this current study maintained sustained supra-luteal P4 levels until treatment withdrawal. Therefore lower conception rates recorded in this protocol than in the double $PGF_{2\alpha}$ protocol could be explained by differences in quality of ovulatory process rather than lack thereof of ovulation in the two protocols. Fertility in goats following use of progestagen to synchronize oestrus is usually poor especially in cycling goat does (Jackson *et al*., 2006).

Novel *NC Synch* GnRH based protocol recorded lower conception rates than all experimental groups in this study. Comparatively across all experimental groups, a large proportion of goat does in the novel *NC Synch* protocol could have been inseminated late due to short oestrus duration. However, conception results by this protocol were comparable to other prototype studies by Bowdridge *et al*., (2013), Holtz *et al*., (2008) and Yacoub *et al*., (2011). Control goat does recorded comparably good conception rates (78.6%) an indication that natural spontaneous oestrus can be more fertile than a pharmacological induced oestrus as suggested by Arrebola *et al*., (2012) and Teleb and Ashmawy *et al*., (2007).

CIRD-G/eCG protocol conception results (71.4%) in this current study were in agreement to results by Vilariño *et al*., (2011) (75.3%), but higher than results by Wildeus, (2000) (59.5%, laparoscopic AI), Motlomelo *et al*., (2002) (46.7%), Souza-Fabjan *et al*., (2011) (62%, 12d CIRD-G). Conception results in the double $PGF_{2\alpha}$/eCG in this current study (78.6%) was in agreement to results by Riaz *et al*., (2012) (78%), Ahmed *et al*., (1998) (77.8%, fresh semen, trans-cervical deep uterine AI) but were higher than results by Greyling and van Niekerk, (1986) (70.6%), The novel *NC Synch* GnRH based protocol conception rates in the current study (66.7%) was lower than results by Bowdridge *et al*., (2013) (73% novel *NC Synch*, trans-cervical deep uterine AI using frozen thawed semen), but was higher than *Ovsynch* GnRH based protocol results by Riaz *et al*., (2012) (60%), Holtz *et al*., (2008) (58%, trans-cervical deep uterine AI using frozen thawed semen, AI done 43 h into oestrus). Novel *NC Synch* GnRH based protocol is a relatively new protocol with applications in goats still in maiden research years.

Conception rate is a direct function of fertility in domestic ruminant species. Double $PGF_{2\alpha}$/eCG protocol performed better than CIRD-G/eCG protocol, unlike what was suggested by Abecia *et al*., (2012), who proposed higher fertility parameters for the latter protocol than the former. Conception rates following pharmacological oestrous synchronization and AI is a compound function of inseminator proficiency, proper timing of ovulation, doe fertility, uterus receptivity, type of semen used, semen quality, method and depth of insemination (Arrebola *et al.*, 2012; Leite-Browning, 2009; Noakes *et al.*, 2009; Omontese *et al.*, 2014; Paramio and Iziquierdo, 2014; Santolaria *et al.*, 2011).

In this current study semen was collected from four Boer bucks by A/V method and was extended by constant 1:3 dilution in UHT fat free cow milk and placed in water bath at 37 °C prior AI. There was no correlation between buck semen used and conception rate an indication of comparable buck fertility and semen handling techniques. One inseminator did all inseminations using modified trans-cervical deep uterine insemination as described by Sohnery *et al.* (1995) (Bowdridge *et al.*, 2013). Laparoscopic invasive intrauterine insemination offers an opportunity of achieving superior conception rates and this can be achieved using a small volume of frozen thawed semen than if trans-cervical deep uterine insemination was the preferred method of AI (Avendano-Reyes *et al.*, 2007; Noakes *et al.*, 2009; Sohnrey and Hlotz, 2005). However, laparoscopic insemination requires expensive equipment and extra-ordinary skill (Sohnrey and Holtz, 2005). Usually freshly collected semen will give superior conception results than cryopreserved thawed semen (Gordon, 1997; Sohnrey and Holtz, 2005). Semen quality is affected by process of cryopreservation and presence of seminal fluid phospholipases interaction with phosphatidylcholine of media extenders containing egg yolk or milk, thereby producing lysolecithin a cytotoxic principle that affects spermatozoa integrity (Noakes *et al.*, 2009; Paramio and Iziquierdo, 2014). It has been suggested that conception rates following AI after natural spontaneous oestrus is more fertile than pharmacological synchronized oestrus (Arrebola *et al.*, 2012; Teleb and Ashmawy, 2007). Conception rates are also higher following use of fresh semen than frozen thawed semen (Paramio and Iziquierdo, 2014).

The perfect timing of AI is when it is synchronized with or done just few hours before ovulation and this time is usually in the last half of observed oestrus in shoats (Baldassarre and Karatzas, 2004; Hafez and Hafez, 2000; Paramio and Iziquiredo, 2014). Insemination with frozen thawed semen will require close synchrony of ovulation to AI than freshly collected or fresh chilled semen (Edmondson *et al.*, 2002). In general, the more damaged the semen is, the deeper and closer to the oviduct the insemination should be (Baldassarre and Karatzas, 2004). AI in goats or sheep should be done in the second half of overt oestrus, as ovulation in shoats usually occurs towards the end of oestrus (Allison and Hagevoort, 2009). CIRD-G/eCG and novel *NC Synch* GnRH based protocols in this current study offered opportunities of carrying out *fixed*-TAI as oestrus quality was tighter than both double $PGF_{2α}$/eCG and control experimental groups. GnRH analog injection at AI time in novel *NC Synch* GnRH based protocol improved the chances of goat does coming to oestrus late or early coming closer at LH surge and ovulation (Bowdridge *et al.*, 2013; Jackson *et al.*, 2014; Pierson *et al.*, 2003). Therefore GnRH analog clustered and synchronized goat does towards an ovulation point, an important feature of *fixed*-TAI oestrous synchronization protocols (Baldassarre and Karatzas, 2004). Figure 4.4 shows progesterone profiles indicating a significant increase in circulatory blood P4 values after day 7 GnRH injection, a proof of sound development of secondary induced CL in a certain proportion of goat does in this current study.

Non return to oestrus 21 days after AI was strongly correlated to conception rates, thus concurring with various studies relating to a non return as a crude yet robust method of determining pregnancy status in domestic ruminant species following mating season or AI (Hafez and Hafez, 2000; Noakes *et al.*, 2009). However, some pregnant goats in this current study displayed behavioral signs of oestrus 21 days after AI agreeing to a study by (Holtz *et al.*, (2008) where a small proportion of pregnant does displayed oestrus signs throughout pregnancy. Whether this discrepancy in goats could be attributed to goat doe behavior, "buck effect" or rather due to predominating estradiol concentrations in doelings than in other domestic ruminant species is a matter subject to further research. However, there was an increased chance of pregnant goat does showing oestrus signs in double $PGF_{2α}$/eCG and novel *NC Synch* based GnRH protocols than control experimental protocol goat does. None of the CIRD-G/eCG experimental protocol pregnant goat does displayed oestrus signs after day 21 of AI. Display of oestrus in pregnancy could be related purely to $PGF_{2α}$ based protocols though one of control experimental protocol pregnant goat doe also displayed oestrus signs throughout its pregnancy. In other novel ovulation protocol prototype studies by Bowdridge *et al.*, (2013), Holtz *et al.*, (2008) and Yacoub *et al.*, (2011), short cycling was encountered in some goat does during synchronization protocol application and even in goat does that had conceived. Luteal insufficiency was suggested as a possible cause of short cycling in these studies. No short cycling was observed during protocol treatments in this current study.

P4 hormonal profile sensing can give an insight in endocrine dynamics following ovarian control through exogenous hormonal applications and can be an invaluable tool at evaluating synchronization protocols in domestic non ruminant and ruminant animal species. Exogenous progesterone application through historical sponge formulations and recently CIDR devices, promote a fulminant follicular turnover giving birth to development of dominant follicles that will ovulate (Vilarino *et al.*, (2011). Subluteal micro-environment especially of long term progesterone protocols often lead to development of oversized dominant follicles that do not ovulate (Lassala et al., 2004). Progesterone influence on follicular turnover cannot be over emphasized and several studies cited it as a predominating hormonal factor important at influencing follicular turnover (Lassala *et al*., 2004; Vilarino *et al*., 2011).

In this current study, blood P4 profile assays of the CIDR group followed a similar hormonal path as sensed by Wheaton *et al*. (1993) and Selvaraju et al., (1995) (Motlomelo *et al*., 2002). The CIDR group had an opportunity of recording 100% oestrous response which is an indication of proper ovarian follicular dynamics. However, oestrous response does not necessarily translate into ovulation prowess as LH profiles could have given better evidence on ovulatory responses. In double $PGF_{2\alpha}$/eCG protocol, a significant fall in blood P4 from start of experimentation (15.42 ^{abcd} nmol/l ± 2.23) to an imaginary experimental mid-follicular stage (2.63^{e} nmol/l ± 2.23) after first $PGF_{2\alpha}$ injection and a gradual increase to 8.97^{cde} nmol/l ± 2.23, 12 h before last $PGF_{2\alpha}$ injection indicated prowess of luteolytic actions of $PGF_{2\alpha}$ analog injections in goat does with mature CL. Progesterone profiles for double $PGF_{2\alpha}$/eCG protocol did not have significant breed differences after luteolytic action of first and second $PGF_{2\alpha}$ analog dose rate used (10 mg), however comparisons could not be made on differential breed sensitivities as experimental baseline levels were not established at start of experimentation in double $PGF_{2\alpha}$/eCG protocol.

Novel *NC Synch* GnRH based protocol followed an intial P4 profile path of double $PGF_{2\alpha}$/eCG protocol, however, GnRH analog at experimental day 7 influenced significantly higher ($p<0.05$) blood P4 values just before treatment end. Day 7 GnRH stimulates ovulation and development of secondary CL and together with primary CL (CL younger than 4 days at day 0 and not lysed by first $PGF_{2\alpha}$ injection) influence an exponential increase in blood P4 values (19.59^{abc} nmol/l ± 2.23) to almost approximating CIDR group P4 levels (20.58^{ab} nmol/l ± 2.3). High blood P4 levels before treatment withdrawal is an important feature common only to progestagen protocols influencing rapid follicular turnover until one wave culminates into ovulation (Lassala *et al*., 2004). However, very high preovulatory P4 levels interfers with oestrus expression and ovulatory process as very high estradiol concentrations are required to offset the inhibitory steroidal effects of P4 on the hypothalamus (Mellado and Valdez, 1997; Meilàn and Ungerfield, 2014). It should therefore be mentioned that sub-oestrus or silent oestrus in the three goat doelings in the novel *NC Synch* GnRH based protocol could be due to failure to control the hormonal ovarian status quo of low progesterone and high estradiol required for oestrus expression. This research recommendation will be to indiscriminately conduct *fixed*-TAI at 77-90 h after oestrus onset without consideration of absence or presence of oestrus. Very high post ovulatory P4 levels also have a direct negative impact on sperm motility, poor fertilization states and poor oocyte developmental indices (Baldassarre and Karatzas, 2004; Lopez-Sebastian *et al*., 2007; Santolaria et al., 2011; Jackson *et al*., 2006).

Blood P4 values on day of oestrus onset in control does (2.42^{e} nmol/l ± 2.23) indicated does with differing P4 decay status though a nadir (ovulatory point) could have been established by 2 hourly interval blood P4 profiling after oestrus onset. Post ovulatory P4 levels did not differ between CIRD-G/eCG (12.33^{bcde} nmol/l ± 2.3) and control groups (9.64^{bcde} nmol/l ± 2.23), however, these P4 profiles were significantly higher than caused by both double $PGF_{2\alpha}$/eCG (7.92^{de} nmol/l ± 2.23) and *NC Synch* GnRH based (8.09^{de} nmol/l ± 2.23) protocols. This is in contrast to studies by Abecia *et al*., (2012) and Ruiz-Gonzalez *et al*., (2012) who reported very high post ovulatory P4 values in sheep following synchronization by double $PGF_{2\alpha}$ protocols, however same study by Ruiz-Gonzalez et al., (2012) reported post ovulatory P4 values in goat doelings as vague following oestrous synchronization by $PGF_{2\alpha}$ based protocols.

Fertility rate was highest in double $PGF_{2\alpha}$/eCG and control experimental protocols with both achieving a 78.6% fertility rate. This concurred with studies by Arrebola *et al*., (2012) and Teleb and Ashmawy, (2007), who suggested a natural and spontaneous occurring oestrus as being more fertile than pharmacologically induced oestrus. However, fertility features of a natural spontaneous oestrus in this current study did not translate into influencing superiority in prolificacy, fecundity and increased chances of getting more female kids per litter than induced oestrus of pharmacological synchronization protocols. Whether a chance of getting female to male kids is dependent on oestrous synchronization method or just a genetic chance at meiotic metaphase plate should be a subject of future advanced research. However, control protocol achieved more multiple pregnancies than novel *NC Synch* GnRH based protocol and double $PGF_{2\alpha}$/eCG protocol probably supporting earlier suggestions by Arrebola *et al*., (2012) and Teleb and Ashmawy, (2007) of a natural spontaneous oestrus being more fertile than pharmacologically induced oestrus. A gonadotropin at treatment withdrawal stimulates a magnified follicular growth with a resultant improved ovulatory process (Baldassarre and Karatzas, 2004; Boscos *et al*., 2002).

Novel *NC Synch* GnRH based (1.4 ± 0.24) and double $PGF_{2\alpha}$/eCG (1.4 ± 0.20) protocols recorded better prolificacy and fecundity rates than any other experimental protocols, this could be due to a quality ovulatory process resulting in quality embryos leading to improved post natal kid quality life (Bitaraf *et* al., 2007; Omontese *et al*., 2012; Pierson *et al*., 2003;

Teleb and Ashmawy, 2007). However, oestrous synchronization by double prostaglandin protocols in goats has been associated with deficiencies, first by reduced growth of luteal tissue often leading to diminished functionality of luteal tissue and secondly due to unpredictable ovulatory process (Ruiz-González *et al.*, 2012). However, a day 7 GnRH analog injection in a novel *NC Synch* GnRH based protocol will try to correct such deficiencies of double prostaglandin protocols through ovulation induction of dominant follicles and secondary CL development by its strong luteotrophic effects. The superimposed buck effect could have had better interactive effect on these two prostaglandin based protocols than both CIRD-G/eCG and control protocols (Lopez-Sebastian *et al.*, 2007). The second GnRH analog injection at insemination time is crucial for uniformity and synchronization of the LH surge leading to a much improved ovulatory process and rates (Bowdridge *et al.*, 2013; Holtz *et al.*, 2008; Lassala *et al.*, 2004; Pierson *et al.*, 2003; Romano, 2004; Yacoub *et al.*, 2011; Wiltbank *et al.*, 2014).

The novel *NC Synch* GnRH based protocol recorded (1.83 ± 0.26) a second largest to CIDR-G/eCG (1.94 ± 0.25) litter size and this is due to inherent characteristics of the protocol achieved through stepwise development of secondary CL, increased sensitivity of CL to luteolytic agents, rapid follicular growth and ovulation induction driven in a single protocol (Bowdridge *et al.*, 2013; Farin *et al.*, 2013; Holtz *et al.*, 2008). The kidding rate in the novel *NC Synch* GnRH protocol of this current study was higher (100%) than kidding rate in studies by Bowdridge *et al.*, (2013) (68%). No other fertility parameters were documented for the novel *NC Synch* GnRH based protocol by Bowdridge *et al.*, (2013). A double $PGF_{2\alpha}$/eCG protocol in this current study recorded comparable litter sizes (1.67 ± 0.25) to results by Riaz *et al.*, (2011) (1.6 ± 0.2).

CIDR-G/eCG protocol recorded larger litter sizes with increased chance of achieving multiple pregnancies than any other experimental group. $PGF_{2\alpha}$ and eCG analog superimposition on progestagen protocols improved follicular growth, development and differentiation with a concomitant improvement of ovulatory rates (Ahmed *et al.*, (1998); Dogan and Nur, 2006). However, progestagen protocols usually achieve poor fertilization rates due to inadvertent poor sperm transport leading to poor conception rates (Baldassarre and Karatzas, 2004). This is accentuated by using non-penetrative vaginal or exo-cervical semen deposition AI methods. Kidding rate (100%) and litter size (1.94 ± 0.25) following use of progesterone CIDR-G devices in this current study was better than kidding rate (58%) and litter sizes (1.83 ± 0.17) following use of progestagen sponges by Yacoub *et al.*, (2011).

CIDR-G/eCG experimental protocol performed better with respect to oestrous parameters than any other protocol in this study by recording a precocious (93.3%), early onset oestrus (25.95 h ± 3.85) and modest oestrus duration (48.53 h ± 10.75), therefore, such a protocol has attractive features of crudely pre-determining *fixed*-TAI times in goats. The CIDR-G/eCG protocol also recorded better fertility parameters than any other protocol in this study; litter sizes (1.94 ± 0.25), kidding rate (100%) and gave equal chance (1.06 ± 0.28) of carrying female to male kids. Superimposing a GnRH agonist in the middle of a CIDR-G/eCG protocol as suggested by Edmondson *et al*., (2002) or at the end of treatment as suggested by Pierson *et al*., (2003) in shoats, might improve timing of a synchronous ovulation of progestagen based oestrous synchronization protocols for *fixed*-TAI (Baldassarre and Karatzas, 2004). Fecundity and prolificacy of CIDR-G/eCG protocol was lower than novel *NC Synch* GnRH based and double $PGF_{2\alpha}$/eCG protocols, but was higher than controls. Shortfalls of a progestagen based protocol could be related to poor fertilization process possibly through interference with sperm transport and poor oocyte development (Baldassarre and Karatzas, 2004; Mehmood *et al*, 2012; Menchaca *et al*, 2007). Interference of sperm transport is not so important with deep inseminating AI methods such as trans-cervical deep uterine or laparoscopic inseminations. Long-term progestagen protocols are usually associated with luteal insufficiency and poor oocyte development after AI possibly due to waning progesterone towards end of the treatment protocol with repercussions of an anomalous ovulatory process (Baldassarre and Karatzas, 2004; Lassala *et al*, 2004; Mehmood *et al*, 2012). Luteal insufficiency leads to follicular dominance and birth of a dominant follicle that does not ovulate (Lassala *et al*., 2004; Teleb and Ashmawy, 2007). Follicular dominance setback can be circumvented by adoption of short-term progestagen protocols (Menchaca *et al*., 2007; Teleb and Ashmawy, 2007) or P4 profile assaying during progestagen protocol application (Mehmood *et al*., 2012; Vilariño *et al*., 2011).

Despite recording the highest conception rate, double $PGF_{2\alpha}$/eCG protocol did not transfer this superiority into achieving all superior fertility features. This could be explained by perhaps an anomalous ovulatory process that fails to produce quality gametes and therefore poor embryo quality or lower fertility rates. The double $PGF_{2\alpha}$/eCG protocol is still an unattractive protocol for use in goats (Yacoub *et al*., 2011). This is despite being simple and cost effective protocol with no food residue and animal welfare implications (Lopez-Sebastian *et al*., 2007). The double $PGF_{2\alpha}$/eCG protocol or any of its variant protocols can be optional to progestagen protocols due to animal and food residue implications (Bitaraf *et al*., 2007; Lopez-Sebastian *et al*, 2007). However, double $PGF_{2\alpha}$/eCG protocol cannot be used during transition or outside the breeding season in seasonal breeders, therefore, it has less applicative use in temperate regions though it is still of great importance in the tropics

(Mehmood *et al.*, 2012; Omontese *et al.*, 2012). Efficiency of this protocol can be improved by applications only during the breeding season and in goat does in mid-luteal phase of the reproductive cycle (Day 5-16) (Fatet *et al.*, 2010). Goat doe selection using ultrasound or P4 profile assays can also be used prior to oestrous synchronization by double PGF2α based protocols (Mehmood *et al.*, 2012; Vilariño *et al.*, 2011). However, progestagen analog protocols still perform better than double $PGF_{2\alpha}$ based protocols in terms of fertility (Abecia *et al*, 2012).

The novel *NC Synch* GnRH based protocol achieved comparably poorer oestrous parameters than both CIRD-G/eCG and double $PGF_{2\alpha}$/eCG protocols serve for a compact oestrus duration that was better than the other three experimental protocols. Inferior results of the novel *NC Synch* GnRH based protocol compared to the CIRD-G/eCG protocol in this current study was similar to what was found by another prototype study by Farin *et al.* (2013) in which weather patterns and goat doe age and parity were cited as confounders leading to poor results. Time interval to oestrus onset has been documented as the most important determinant parametric factor for *fixed*-TAI through synchronization of LH surge uniformity by oestrous synchronization protocols (Fonseca *et al.*, 2008; Lassala *et al*, 2004; Pierson *et al.*, 2003). The novel *NC Synch* GnRH based protocol recorded the lowest oestrous responses (66.7%) and had the longest time interval to oestrus onset after treatment withdrawal, however, short oestrus duration by this protocol usually compensates for the earlier offset. Despite poor oestrous parameters, this protocol recorded unrealistic high fertility parameters an indication of good quality ovulatory process leading to superior ovulatory rates (Bowdridge *et al*, 2013; Farin *et al.*, 2013). A gestation length (147 ± 2.5) close to 150 ± 5 by Fatet *et al.*, 2010, of limited variation meant that an LH surge was properly synchronized than great variations in gestation period shown by other two experimental protocols. It should be mentioned, however, that the dose of GnRH used in this current study (0.002 mg/goat doe) and $PGF_{2\alpha}$ (10 mg/goat doe) was lower than what was used by Baldassarre et al., (2004); Bowdridge *et al.*, (2013); Farin *et al.*, (2013); Holtz *et al.*, (2008); Pierson *et al.*, (2003) and Yacoub *et al.*, (2011). Lower GnRH analog & $PGF_{2\alpha}$ dose rates and using goat doelings of unknown reproductive status could explain some shortfalls recorded by this protocol in this current study. Ovulation protocols can be improved by proper goat doe selection to include only those displaying reproductive cyclicity. However, studies in the tropics during non-breeding season on goat does in good nutrition should be able to determine whether the novel *NC Synch* GnRH based protocol will be able to offset the ovarian status quo of non-cyclicity or infertility due to ovarian disease and act as an option to progestagen protocols that are efficient even outside breeding season in tropical seasonal goat does.

The natural spontaneous occurring oestrus of control group was more fertile than some of the pharmacological oestrous synchronization, agreeing with what was suggested by Arrebola *et al*., (2012) and Teleb and Ashmawy, (2007). Natural methods of oestrous synchronization, though of archaic value, can still offer cost effective returns in goat farming. Though oestrous parameters using natural methods of oestrous synchronization cannot allow for *fixed*-TAI, superimposition on other protocols like double $PGF_{2\alpha}$/eCG protocols (Whitely and Jackson, 2004) often leads to superior ovulatory and fertility responses in goat does. In this current study single-TAI using freshly collected and extended buck semen following natural spontaneous oestrus, gave comparably good conception rates and modest fertility responses. However, this is in contrast to research findings by Allison and Hagevoort. (2009) who suggested a natural occurring oestrus was not fertile for AI. A gonadotropin injection at onset of estrus or at treatment withdrawal improves oestrous responses, follicular growth, ovulatory responses and conception rates (Boscos *et al*, 2002).

Oestrous parameters in Venda and Boer indigenous local breeds were very comparable in many aspects in all four experimental groups. There was obviously no differences alienated to breed interactive effects though Venda goat does displayed a more precocious oestrus of shorter duration after treatment withdrawal than Boer goat does. In double $PGF_{2\alpha}$/eCG protocol, breed had an interactive effect on time interval to oestrus onset, thus indicating differential breed sensitivities to dose of $PGF_{2\alpha}$ used (Greyling van Niekerk, 1986; Lehloenya *et al*., 2010). Venda indigenous breed had a shorter time interval to oestrus onset than Boer indigenous breed goat does in this experimental protocol. Due to small numbers of Boer goat doelings that displayed an overt oestrus and were eventually inseminated, superior fertility parameters of Venda indigenous goat doelings can be accepted with a pinch of salt. However, due to larger uterine sizes in Boer goat breed to Venda indigenous goat breed, the Boer breed had a better chance of recording multiple pregnancies and better prolificacy and fecundity rates than the Venda breed. Litter sizes are influenced by season, parity, year of mating, body condition and weight of doe (Payne and Wilson, 1999). In well managed natural flocks of South Africa, Boer goat does usually achieve an average litter size of around 1.64 with small litter sizes usually recorded outside South Africa (Payne and Wilson, 1999). This was comparable to litter sizes in this current study (1.92 ± 0.2) though follicular and ovulatory treatments in this study could have magnified fertility responses. Competition for implantation space before day 17 after AI can also result differential litter sizes apart from quality ovulatory responses (Hafez and Hafez, 2000; Noakes *et al*., 2009).

Optimization of synchronization protocols used in goats through P4 or LH profile assays and ovarian echo-graphic ultrasound evaluations will promote development of efficient standardized oestrous or ovulation synchronization methods. Work on the novel *Ovsynch* protocol by Wiltbank & Pursely, (2014) has shown that apart from other ovarian dynamics following oestrous or ovulation protocols in cattle, ovulation of a very small or very large dominant follicle can be a cause of infertility in cattle, however, ovulation of a medium sized dominant follicle gave good fertility results in their work (Wiltbank *et al.,* 2014). Size of follicles is dependent on empirical determination of dosages of gonadotropin in the protocols and there is need for more research in goats before fully adopting the ovulation synchronization protocols. P4 profile assays will assist in determining critical hormonal limits prior to ovulation as luteal insufficiency usually result in follicular dominance (Lassala *et al.*, 2004, Romano, 2004) and this results to large antral follicles associated with infertility (Wiltbank *et al.*, 2014), and short cycling (Holtz *et al.*, 2008).

Evaluation of protocols through hormonal P4 profiling has indicated that a novel *NC Synch* GnRH based protocol is similar to double $PGF_{2\alpha}$/eCG path upto mid protocol thereafter a GnRH analog injection at day 7 will influence P4 profile path that will approximate progestagen protocols towards treatment withdrawal. This experiment has also indicated sufficient ovulatory responses by using half the dose of GnRH, however, lower conception rates by the novel *NC Synch* GnRH based protocol could be attributed to higher doses of GnRH analogs used by Bowdridge *et al.*, (2013), Holtz *et al.*, (2008) and Yacoub *et al.*, (2011). Further empirical research should therefore be able to establish the dose of GnRH that should give excellent and otherwise superior fertility parameters.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

It can be concluded that the CIRD-G devices when used for a period of 12 days will achieve superior oestrous parameter results currently used in ART programs to crudely determine insemination times for *fixed*-TAI in goats. It can also be concluded that time interval to oestrus onset after withdrawal of synchronization treatments is the only probable oestrous parameter that can be used efficiently to crudely determine insemination times in ART *fixed*-TAI programs in goats. The CIDR-G/eCG and novel *NC Synch* GnRH based protocols were the only protocols under this study that achieved close oestrus synchrony in goats therefore gave an opportunity of conducting *fixed*–TAI at 45-60 h and 77-93 h respectively after treatment withdrawal. Shortfall of the CIDR-G/eCG protocol was unexpected poor fertility parameters despite display of superior oestrous parameters. On the other hand novel *NC Synch* GnRH based protocol achieved superior fertility features despite a late occurring oestrus after treatment withdrawal. Inclusion of GnRH injections at strategic stages of progestagen protocol application will therefore improve the ovulatory process for better fertility features as suggested by several related studies. This study was also conducted in well controlled research conditions to limit confounder effect in oestrous synchronization as results of ART procedures in this study were within range of other related research studies. Venda indigenous breed goat does were more sensitive to 10 mg of $PGF_{2\alpha}$ analog used in this study than Boer breed does and this factor should be taken into consideration in ART programs involving the two breeds. The performance of the two breeds in the synchronization protocols in this study was comparable in many aspects except where $PGF_{2\alpha}$ analog injections were involved. After deliberations in this study, goat does in Venda region of Limpopo province can successfully be bred thoughout the winter months of April to July with a peak window in May as suggested by several studies done in tropical South Africa. The overall research recommendation will be to consider the novel *NC Synch* GnRH based protocol as an optional steroid free protocol during breeding season for *fixed*-TAI in goat ART programs. However, when aesthetic, animal welfare and animal product residue implications are not important, the CIDR-G/eCG protocol will be a better option of better efficiency with applications even outside the breeding season.

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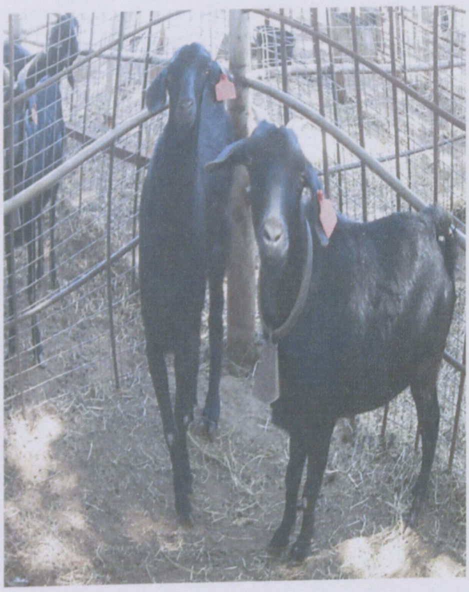
APPENDIXES

APPENDIX 3.1: BOER BUCK AND GOAT DOES USED FOR EXPERIMENTATION

EXPERIMENTAL INDIGENOUS VENDA (A) AND BOER GOAT DOES (B)

A



B

APPENDIX 3.2: OESTRUS RECORDING & AI SHEET

Goat ID	Day 0-Date Treatment Withdrawal	Day 1 7 10 15	Day 2 7 10 15	Day 3 7 10 15	Day 4 7 10 15	Day 5 7 10 15	Day 6 7 10 15	Day 7 7 10 15	Day 8 7 10 15
NS9	2/6/14 AM	x x x	x h h	h AI h	h h h	x x x	x x x	x x x	x x x
NS10	8/6/14 AM	x x x	x h h	h AI h	h h h	x x x	x x x	x x x	x x x
JS4	11/6/14 PM	x x h	h h AI	h h h	h h h	H x x	x x x	x x x	x x x
NKS4	16/6/14 AM	x x x	h h h	AI h h	h h h	H h x	x x x	x x x	x x x
MNS2	18/6/14 PM	x h h	h AI h	h h h	h x x	x x x	x x x	x x x	x x x
2709	25/6/14 PM	x x x	x x x	x x x	x x x	x x h	h h AI	h x x	x x x
2804	26/6/14 AM	x x x	x x x	x x x	x h h	H AI x	x x x	x x x	x x x

Key*

7 10 15 – 0700hrs, 1000hrs and 1500hrs

2/6/14 AM- The second of June 2014 in the morning (0700-1200hrs)

x- Goat doe not showing overt signs of estrus

h- Goat doe displaying cardinal overt signs of estrus

AI^{7} - Artificial insemination at 0700hrs

AI^{10} -Artificial insemination at 1000hrs

AI^{15} -Artificial insemination at 1500hrs

APPENDIX 3.3: TABULATED ONSET OF OESTRUS (IH) AND OESTRUS DURATION (ED) MEASUREMENTS

EXP 1			EXP 2			EXP 3			EXP 4			
Goat ID	**I/H**	**E/D**	**Goat ID**	**I/H**	**E/D**	**Goat ID**	**I/H**	**E/D**	**Goat ID**	**I/H**	**E/D**	**Comments**
7	22	36	6	0	0	17	0	0	11	0	36	6 & 17 suboestrus
12	36	63	9	70	45	18	62	36	20	0	93	
19	22	84	16	34	132	14	70	45	8	0	28	
13	28	52	15	34	53	10	38	64	5	0	36	
NS3	24	46	NS4	38	60	NS13	0	0	NS7	0	62	NS13 suboestrus
NS11	26	36	NS8	38	36	NS1	70	36	NS6	0	216	
NS5	0	0	NS9	96	17	NS2	42	28	MNS5	0	28	NS5 suboestrus
MNS4	23	38	MNS6	19	62	MNS2	62	29	NS14	0	26	
MNS3	30	17	MNS1	46	76	NS10	0	0	NT4	0	168	NS10 suboestrus
NT1	22	36	NT2	62	29	NT3	62	29	NKS4	0	64	
NKS1	22	36	NKS2	65	17	NKS3	58	29	NS12	0	36	
JS1	30	45	JS2	70	28	JS3	46	24	JS4	0	15	
MTJ1	19	24	MTJ2	19	24	MTJ3	62	36	MTJ4	0	0	MTJ4 suboestrus
2692	23	36	2699	38	36	2710	70	24	2698	0	24	
2706	30	69	2707	38	18	2708	62	36	2709	0	48	
Total	357	618	Total	667	633	Total	704	416	Total	0	880	
	25.5	44.1		47.6	45.2		50.3	29.7		0	62.9	
Response %	93.3%		Response %	93.3%		Response %	80%		Response %	93.3%		

APPENDIX 3.4: TABULATED CONCEPTION (C) AND RETURN TO OESTRUS (R) ≥21 DAYS AFTER AI

EXP 1			**EXP 2**			**EXP 3**			**EXP 4**			
Goat ID	**R**	**C**	**Goat ID**	**R**	**C**	**Goat ID**	**R**	**C**	**Goat ID**	**R**	**C**	**Comments**
7	NR	C	6	R	NC	17	R	NC	11	NR	C	
12	NR	NC	9	NR	C	18	NR	C	20	NR	NC	
19	R	C	16	R	NC	14	R	C	8	R	C	
13	R	C	15	NR	C	10	NR	C	5	R	C	
NS3	NR	C	NS4	R	C	NS13	NR	NC	NS7	NR	C	
NS11	R	C	NS8	R	NC	NS1	R	NC	NS6	R	C	
NS5	R	NC	NS9	NR	C	NS2	NR	NC	MNS 5	NR	C	
MNS 4	NR	C	MNS 6	NR	C	MNS 2	NR	C	NS14	NR	C	
MNS 3	R	C	MNS 1	NR	C	NS10	R	NC	NT4	NR	NC	
NT1	R	C	NT2	R	C	NT3	NR	C	NKS4	R	C	
NKS1	NR	C	NKS 2	R	NC	NKS3	R	NC	NS12	NR	C	
JS1	NR	C	JS2	R	C	JS3	NR	C	JS4	R	C	
MTJ1	NR	C	MTJ 2	NR	C	MTJ3	NR	C	MTJ4	R	NC	
2692	NR	NC	2699	NR	C	2710	NR	C	2698	NR	C	
2706	NR	C	2707	R	C	2708	R	C	2709	NR	C	
% Return to estrus	35.5%			57.1 %			33.3%			35.7%		
% Conception		71.4 %			78.6 %			75%			78.6 %	

Key*
R : Return to estrus 21 day post AI
C : Conception
NR: Non –return to estrus 21 day post AI
NC: Doe not conceiving following AI

APPENDIX 3.5: FERTILITY PARAMETER RECORDING FORM

Name of farmer: Mr K.S Nethengwe **Phone Number: 0797753710**

Researcher : Dara O.B **Phone Number: 0719050642**

Goat ID	Kidding date	Number kids born	Sex Kids Born		Comments
			Male	Female	
1. NKS1					
2. NKS2					
3. NKS3					
4. NKS4					
5. JS1					
6. JS2					
7. JS3					
8. JS4					
9. 5					
10. 6					
11. 7					
12. 8					
13. 2706					
14. 2710					
15. 2708					
16. NT1					

APPENDIX 3.6: OWNER CONSENT OR CONTRACT FORM

CONSENT FORM TO USE GOATS FOR AI PROGRAM

DATE: 20 May 2012

Dear sir/Madam,

The Biotechnology Laboratory of the Centre of Excellence in Animal Assisted Reproduction CEAAR of the University of Venda hereby declares that;

1. CEAAR want to use .... goat doelings from ....(owner) for the purpose of a synchronization and Artificial insemination research program until August 2014
2. The CEAAR promises that the goat doelings will be vaccinated, dewormed and supplemented with systemic multimin/vitamin injections during research pre-adaptation period.
3. The goat doelings will be synchronized and inseminated using semen of high genetic quality from feedlot pedigree Boer goat bucks.
4. Ultrasound will be used to diagnose conception/pregnancy between day 45 – 55 weeks after AI.
5. The owner of the animals will be notified of the outcome and the completion date of the program.
6. No fees will be paid for any services or otherwise by either CEAAR or the owner of animals.

The owner promises to keep record of the date of birth, sex of kids, number of kids born and any other conditions recorded and notify CEAAR. The kids will be presented to the master/research official on request.

Regards

....

PROF. DM BARRY ANIMAL OWNER:

HEAD: CEAAR

MASTER STUDENT

O.B DARA

APPENDIX 3.7: AI PROCEDURES AND INSTRUMENTATION

C

D

APPENDIX 3.6: (A) Procedure used to perform deep intra-uterine trans-cervical AI in all does- note the laparoscope, styleted urinary catheter on 1 ml syringe, goat doe positioning & vaginal speculum. (B) Instrumentation used for AI (1. Vaginal speculum large does 2. Vaginal speculum small does 3. Syringe 1ml for semen loading 4.Urinary catheter stiffened by stylet 5. Pozi-tenaculum forceps 6. Syringe 2ml used on loaded urinary catheter 7. Semen/UHT milk mixing tube 7. Laparoscopic light source (C) Venda does used (D) Boer doe used

APPENDIX 4.1: ULTRASOUND GRAPHS USED TO DETERMINE PREGNANT STATUS IN INSEMINATED GOAT DOES