MULTIPLE OVULATION AND EMBRYO TRANSFER IN GOATS

by

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DEDICATION

- *To my late mother, Masehloho and my father, Letele, for their inspiration, guidance and good education. Without your love I could not be this far*

- *To my husband, Mike and my daughter, Tsobotsi, for your patience and understanding throughout my study. Above all thank you for your love and support*

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DECLARATION

I hereby declare that this thesis submitted by me to the University of the Free State for the degree, Philosophae Doctor, has not previously been submitted for a degree to any other university. I further cede copyright of the thesis in favour of the University of the Free State.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

AI	- Artificial Insemination
ANOVA	- Analysis of Variance
CIDR	- Controlled Internal Drug Releasing Device
CL	- Corpus Luteum
DMSO	- Dimethyl sulfoxide
DPBS	- Dulbecco Phosphate Buffered Saline
E_2 -17_β	- Estradiol-17 $_\beta$
eCG	- Equine Chorionic Gonadotrophin
EG	- Ethylene Glycol
FGA	- Flurogestone Acetate
FSH	- Follicle Stimulating Hormone
GLM	- General Linear Model
GnRH	- Gonadotrophin Releasing Hormone
HAP	- Horse Anterior Pituitary extract
hCG	- Human Chorionic Gonadotrophin
H-SOF	- Hepes + Synthetic Oviductal Fluid
ICM	- Inner Cell Mass
IGF-I	- Insulin-like growth factor I
i.m.	- Intramuscular
IU	- International Units
LH	- Luteinizing Hormone
LHRH	- Luteinizing Hormone Releasing Hormone
MAP	- Medroxyprogesterone acetate
MOET	- Multiple Ovulation and Embryo Transfer
oFSH	- Ovine Follicle Stimulating Hormone
OPS	-Open Pulled Straw
pFSH	- Porcine Follicle Stimulating Hormone
$PGF_{2\alpha}$	- Prostaglandin $F_{2\alpha}$
PMSG	- Pregnant Mare Serum Gonadotrophin
s.c.	- Subcutaneous

CHAPTER 1

GENERAL INTRODUCTION

THE SOUTH AFRICAN BOER GOAT HAS MANY PRODUCTIVE ADVANTAGES OVER OTHER GOAT BREEDS WORLD WIDE WHICH HAS LED TO ITS POPULARITY AND DEMAND IN MANY COUNTRIES. AMONG THE ADVANTAGES AND SUPERIOR TRAITS ARE THE QUALITY OF MEAT PRODUCED, ADAPTABILITY OF THE BREED, AND THEIR ABILITY TO PERFORM WELL UNDER EXTENSIVE SEMI-ARID CLIMATIC CONDITIONS, RANGING FROM HOT DRY SEASONS TO SNOW COVERED MOUNTAINS (CASEY & VAN NIEKERK, 1988; BARRY & GODKE, 2001). IN ORDER TO MEET THE HIGH DEMAND FOR THIS BREED ACROSS THE GLOBE, A RAPID REPRODUCTIVE PROGRAMME TO DISSEMINATE SELECTED MALE AND FEMALE GENES, AND ACCELERATE GENETIC PROGRESS FOR GENE TRANSFER IS ESSENTIAL. MULTIPLE OVULATION AND EMBRYO TRANSFER (MOET) COULD THUS BE AN APPROPRIATE ALTERNATIVE TECHNIQUE TO BE USED TO EXPAND THE AVAILABILITY OF THIS BREED.

IN SOUTH AFRICA SPECIFICALLY, THE APPLICATION OF MOET HAS GENERALLY BEEN SLUGGISH, WHEN IT COMES TO THE GENETIC IMPROVEMENT PROGRAMMES OF SMALL RUMINANTS OVER THE YEARS. THIS IS MAINLY DUE TO THE IMPLICATION OF SURGICAL INTERVENTION IN THE RECOVERY AND TRANSFER OF THE EMBRYOS AND THE HIGH COSTS PER EMBRYO (GORDON, 1997). THE INHERENT RISKS INVOLVED IN THE PROCEDURE OF MOET IN SMALL STOCK INCLUDE THE HEALING OF WOUNDS, THE

FORMATION OF THE POST-OPERATION ADHESIONS LEADING TO SUBSEQUENT INFERTILITY OF DONOR ANIMALS AND A REDUCED RESPONSE TO EXOGENOUS GONADOTROPHINS OVER TIME (SCUDAMORE ET AL., 1991; NELLENSCHULTE & NIEMANN, 1992; PEREIRA ET AL., 1998). THE DEVELOPMENT OF THE LAPAROSCOPIC TECHNIQUE OF EMBRYO COLLECTION IN THE 1980'S LED TO REDUCED POST-OPERATION ADHESIONS AND, AS A RESULT AN INCREASE IN THE APPLICATION OF MOET IN SHEEP AND GOATS (FLORES-FOXWORTH ET AL., 1992). DESPITE THE LIMITATIONS OF EMBRYO TRANSFER IN SMALL RUMINANTS, THE POTENTIAL INCREASE IN GENETIC PROGRESS AND THE REPRODUCTIVE EFFICIENCY CONTINUES TO STIMULATE FURTHER RESEARCH. GORDON (1997) SUGGESTED THE RATE OF GENETIC PROGRESS COULD BE INCREASED BY 100% VIA THE USE OF EMBRYO TRANSFER PROGRAMMES TO INCREASE THE SELECTION INTENSITY AND REDUCE THE FEMALE GENERATION INTERVAL.

IN ORDER FOR MOET PROGRAMMES TO FACILITATE GENETIC IMPROVEMENT AND DISSEMINATE SUPERIOR BREEDS IN DEMAND, THE PROGRAMME DEMANDS THE AVAILABILITY AND A SUSTAINABLE YIELD OF TRANSFERABLE EMBRYOS, MORE THAN IS CURRENTLY BEING ACHIEVED (SCUDAMORE ET AL., 1991; BARI ET AL., 2003). MANY FACTORS COULD CONTRIBUTE TO THE VARIATION OBTAINED IN THE RATE OF EMBRYOS RECOVERED, INCLUDING SEASON, FERTILISATION RATE, BREED, AGE OF THE DONOR, PROGESTAGEN DOSE USED DURING THE PRIMING PHASE AND EXOGENOUS GONADOTROPHIC HORMONES USED (BARIL ET AL., 2000). PREVIOUSLY, THE MAJOR CAUSE OF THE VARIATION EXPERIENCED IN OVARIAN RESPONSE HAD BEEN IDENTIFIED AS THE SUPEROVULATION TREATMENT USED (PICAZO

ET AL., 1996; JOYCE ET AL., 1998; COGNIE, 1999; GONZALEZ-BULNES ET AL., 2003C). SUPEROVULATION IN MOET PROGRAMMES DOES REMAIN THE MOST CRUCIAL FACET FOR INCREASING EMBRYO YIELD, AND THUS THE NUMBER OF OFFSPRING FROM FEMALES WITH HIGH GENETIC MERIT AND ACCELERATING GENETIC PROGRESS (D'ALESSANDRO ET AL., 1997).

EXOGENOUS GONADOTROPHINS HAVE BEEN WIDELY USED AS A MEANS OF SUPEROVULATION IN LIVESTOCK EMBRYO TRANSFER PROGRAMMES, RESULTING IN VARYING SUCCESS (JABBOUR & EVANS, 1991A, B). SO FOR EXAMPLE, SUPEROVULATION IN PROGESTERONE-SYNCHRONISED FEMALES HAS GENERALLY LED TO A REDUCED FERTILISATION RATE, DUE TO IMPEDED SPERM TRANSPORT. EQUINE CHORIONIC GONADOTROPHIN (ECG) HAS ALSO BEEN WIDELY USED FOR SUPEROVULATION IN CATTLE, SHEEP AND GOATS WITH LIMITED SUCCESS. IN GOATS THE LONG HALF-LIFE OF ECG HAS BEEN SHOWN TO CAUSE OVER-STIMULATION OF FOLLICULAR GROWTH, EVEN AFTER OVULATION, WHICH INDUCES EARLY LUTEAL REGRESSION. THE END RESULT IS A DECLINE IN CIRCULATING PROGESTERONE CONCENTRATION BEFORE EMBRYO COLLECTION AND HENCE A LOW EMBRYO QUALITY AND EMBRYO SURVIVAL RATE BEING OBTAINED (ESPINOSA-MARQUEZ ET AL., 2004). CURRENTLY THE MOST SUCCESSFUL SUPEROVULATION RESULTS HAVE BEEN ATTAINED WHEN USING PURE FOLLICLE STIMULATING HORMONE (FSH) IN SHEEP AND GOATS (JABBOUR & EVANS 1991B; ESPINOSA-MARQUEZ ET AL., 2004). DESPITE THE HORMONES USED IN A SUPEROVULATION PROTOCOL, FOLLICULAR STATUS AT THE ONSET OF SUPEROVULATION TREATMENT CURRENTLY, SEEMS TO BE THE MAJOR FACTOR

INFLUENCING OVULATION RATE AND EMBRYO OUTPUT (GONZALEZ-BULNES ET AL., 2004A). SO FOR EXAMPLE, PRESENCE OF A LARGE FOLLICLE AT THE TIME OF SUPEROVULATION HAS BEEN REPORTED TO DECREASE THE OVARIAN RESPONSE IN SMALL RUMINANTS (RUBIANES ET AL., 1995; LOPEZ-SEBASTIAN ET AL., 1999; MENCHACA ET AL., 2002; RUBIANES & MENCHACA, 2003). IT IS ASSUMED THAT THE OVULATION RATE CAN BE IMPROVED IF THE NUMBER OF GONADOTROPHIN-RESPONSIVE FOLLICLES PRESENT IS MAXIMAL IN THE OVARIES AT THE BEGINNING OF THE SUPEROVULATION TREATMENT (COGNIE ET AL., 2003; GONZALEZ-BULNES ET AL., 2003A; GONZALEZ-BULNES ET AL., 2004A). A PERIOD DURING WHICH THERE IS HIGH NUMBER OF SMALL FOLLICLES AND THE ABSENCE OF A DOMINANT FOLLICLE COINCIDES WITH THE EMERGENCE OF A FOLLICULAR WAVE. THIS PERIOD CAN THUS BE AFTER OVULATION OR FOLLOWING REGRESSION OF THE DOMINANT FOLLICLES (GINTHER ET AL., 1996; EVANS ET AL., 2002). BETWEEN FOLLICULAR WAVES, THE EMERGENCE OF A FOLLICULAR WAVE CAN BE CREATED BY ABLATION OF A DOMINANT FOLLICLE. THEREFORE SUPEROVULATION COULD BE INITIATED FOLLOWING FOLLICULAR ABLATION OF THE DOMINANT FOLLICLE. IN SHEEP AND CATTLE, SUPEROVULATION TREATMENT FOLLOWING FOLLICULAR ABLATION HAS BEEN REPORTED TO INCREASE THE FERTILISATION RATE AND THE NUMBER OF EMBRYOS RECOVERED (BERGFELT ET AL., 1994; BO ET AL., 1995; DISKIN ET AL., 2002; GONZALEZ-BULNES ET AL., 2002A). FOLLICULAR ABLATION HOWEVER, IS NOT EASY TO PERFORM IN SMALL RUMINANTS, AS IT REQUIRES THE PHYSICAL PUNCTURING OF THE DOMINANT FOLLICLE - WHICH COULD ONLY BE DONE WITH THE AID OF LAPAROSCOPE OR LAPAROTOMY

(SURGICAL OPENING OF THE ABDOMEN TO EXTERIORISE THE OVARY) AND FOLLICULAR PATTERNS ALSO DIFFER IN DIFFERENT ANIMALS (DRIANCOURT, 2001; EVANS ET AL., 2002).

THE PRESENCE OF DOMINANT FOLLICLES IN SMALL RUMINANTS CAN BE AVOIDED BY STARTING SUPEROVULATION SOON AFTER OVULATION (THE SO-CALLED DAY 0 PROTOCOL). IN THIS METHOD EXOGENOUS HORMONES ARE USED TO SYNCHRONISE OESTRUS FOLLOWED BY THE ADMINISTRATION OF A SUPEROVULATION TREATMENT SOON AFTER OESTRUS (RUBIANES & MENCHACA, 2003). IN SHEEP, THERE IS EVIDENCE OF A HIGH OVULATION RATE, IMPROVED EMBRYO QUALITY AND INCREASED NUMBER OF EMBRYOS BEING RECOVERED AFTER USE OF SUCH A 'DAY 0 PROTOCOL' (RUBIANES ET AL., 1997). IN GOATS THE DAY 0 PROTOCOL HAS RESULTED IN A HIGH NUMBER OF OVULATIONS, BUT THE EMBRYO RECOVERY RATE WAS NOT EVALUATED, INDICATING THE NEED FOR FURTHER RESEARCH ON EMBRYO YIELD. THIS ALSO REQUIRES THE UTILISATION OF AN ULTRASONOGRAPHIC SCANNER TO OBSERVE THE TIME OF OVULATION (AS IT IS UNPREDICTABLE IN DIFFERENT ANIMALS) IN ORDER TO INITIATE A SUPEROVULATORY TREATMENT AFTER OVULATION IN INDIVIDUAL ANIMALS. THIS PROCEDURE IS TIME CONSUMING, ESPECIALLY WHERE MANY ANIMALS MUST BE SUPEROVULATED (RUBIANES ET AL., 1997; RUBIANES & MENCHACA, 2003).

ALTERNATIVELY THE ESTABLISHMENT OF A DOMINANT FOLLICLE PRIOR TO INITIATION OF SUPEROVULATORY TREATMENT CAN ALSO BE AVOIDED BY THE INDUCTION OF A LOW BLOOD LUTEINIZING HORMONE (LH) LEVEL. IT HAS BEEN SHOWN THAT FOLLICLES COULD ONLY BE SELECTED (BECOME DOMINANT FOLLICLES) IN THE PRESENCE

OF HIGH BLOOD LH CONCENTRATIONS. THE PATTERN OF LH SECRETION CAN BE MODIFIED BY ADMINISTRATION OF A GONADOTROPHIN-RELEASING HORMONE (GNRH). THEREFORE EXOGENOUS GNRH ANTAGONISTS OR AGONISTS HAVE BEEN USED IN SMALL RUMINANTS TO SUPPRESS THE PRODUCTION OF ENDOGENOUS LH AND GROWTH OF A LARGE DOMINANT FOLLICLE (CAMPBELL ET AL., 1998; OUSSAID ET AL., 1999; DUFOUR ET AL., 2000; GONZALEZ-BULNES ET AL., 2004B). WHEN USED IN A MOET PROGRAMME, THE EXOGENOUS GNRH ANTAGONISTS OR AGONISTS HAVE BEEN APPLIED AS PRE-TREATMENTS, TOGETHER WITH THE PROGESTAGEN TREATMENT, PRIOR TO THE INITIATION OF A SUPEROVULATION TREATMENT (GONZALEZ-ANOVER ET AL., 2004; GONZALEZ-BULNES ET AL., 2004A). FOLLOWING THE UTILISATION OF A GNRH ANTAGONIST AS A PRE-TREATMENT IN A FSH SUPEROVULATION REGIME IN GOATS, AN INCREASE IN THE NUMBER OF SMALL FOLLICLES AND INCREASE IN OVULATION RATE HAS BEEN RECORDED. HOWEVER, A REDUCTION IN THE NUMBER OF TRANSFERABLE EMBRYOS HAS ALSO BEEN OBSERVED (COGNIE ET AL., 2003), ALTHOUGH THERE IS SPARSE INFORMATION REGARDING THE UTILISATION OF GNRH AGONISTS IN SUPEROVULATION PROTOCOLS IN GOATS. IN SHEEP, THE PRE-TREATMENT WITH A GNRH AGONIST IN A SUPEROVULATION PROTOCOL HAS BEEN SHOWN TO INCREASE THE NUMBER OF SMALL FOLLICLES AND INCREASE THE OVULATION RATE (COGNIE, 1999) EMBRYO CRYOPRESERVATION IS AN ESSENTIAL COMPONENT OF A MOET PROGRAMME AIMED AT ACCELERATING GENETIC PROGRESS. IT IS ESSENTIAL IN COMMERCIAL EMBRYO TRANSFER PROGRAMMES FOR STORAGE, CHEAPER TRANSPORTATION OF EMBRYOS AND AVOIDING LOSS OF ANIMALS DURING

TRANSPORTATION. A CONVENTIONAL SLOW FREEZING TECHNIQUE FOR THE CRYOPRESERVATION HAS BEEN WIDELY USED IN GOAT EMBRYOS, WITH VARIABLE SURVIVAL RATES WHEN EVALUATED IN VITRO AND IN VIVO (LI ET AL., 1990; LE GAL ET AL., 1993; EL-GAYAR & HOLTZ, 2001). IN ADDITION, THE TRADITIONAL SLOW FREEZING PROCEDURE OF EMBRYO PRESERVATION HAS BEEN ASSOCIATED WITH DAMAGE TO THE EMBRYO INDUCED BY ICE CRYSTAL FORMATION, AS WELL AS OSMOTIC INJURY AND THE TOXIC EFFECT OF THE CRYOPROTECTANTS (VAJTA, 2000). CURRENTLY, A VITRIFICATION TECHNIQUE OF CRYOPRESERVATION, WHICH INVOLVES THE ADDITION OF A HIGHER CONCENTRATION OF CRYOPROTECTANTS AND VERY RAPID COOLING, HAS BEEN TESTED IN DIFFERENT SPECIES (VAJTA ET AL., 1999; DATTENA ET AL., 2004). THIS TECHNIQUE ELIMINATES THE FORMATION OF ICE CRYSTALS IN THE EMBRYO AND HAS AN ADVANTAGE OVER THE CONVENTIONAL SLOW FREEZING TECHNIQUE WHICH GIVES THE EXPECTATION TO PRODUCE A BETTER EMBRYO SURVIVAL RATE (VAJTA, 2000). HOWEVER, CONFLICTING RESULTS HAVE BEEN OBTAINED WHEN COMPARING THE CONVENTIONAL SLOW FREEZING AND VITRIFICATION METHODS OF EMBRYO CRYOPRESERVATION, REGARDING THE SURVIVAL RATE OF GOAT EMBRYOS. LIMITED RESEARCH FOUND LOWER SURVIVAL RATES FOLLOWING THE TRANSFER OF VITRIFIED GOAT EMBRYOS WHEN COMPARED TO THOSE OBTAINED WITH CONVENTIONAL SLOW FREEZING (KASAI, 1996; EL-GAYAR & HOLTZ, 2001). ON THE OTHER HAND, VITRIFICATION HAS BEEN REPORTED TO LEAD TO HIGHER EMBRYO SURVIVAL RATE COMPARED TO CONVENTIONAL SLOW FREEZING AND IN OTHER REPORTS NO DIFFERENCES WERE OBTAINED

BETWEEN THE TWO METHODS OF PRESERVATION (EL-GAYAR & HOLTZ, 2001; GUIGNOT ET AL., 2006). IT IS THEREFORE OBVIOUS THAT THERE ARE STILL MANY SHORTCOMINGS IN ESTABLISHING AN EFFICIENT AND RELIABLE TECHNIQUE FOR EMBRYO CRYOPRESERVATION AND TRANSFER IN GOATS. THE WORLD-WIDE DEMAND FOR THE BOER GOAT AS SUCH JUSTIFIES THIS STUDY AND IT HOLDS GREAT POTENTIAL FOR GOAT PRODUCTION AS A WHOLE. THIS STUDY WAS THUS CONDUCTED TO EVALUATE, AND DEVELOP A MORE EFFICIENT PROTOCOL FOR SUPEROVULATION AND EMBRYO CRYOPRESERVATION METHOD IN BOER GOATS, WITH THE FOLLOWING SPECIFIC OBJECTIVES:

1. TO REFINE AND ESTABLISH A MORE EFFICIENT AND RELIABLE PROTOCOL FOR SUPEROVULATION AND EMBRYO EVALUATION IN THE BOER GOAT.
2. TO INVESTIGATE WHETHER THE SO-CALLED DAY 0 PROTOCOL CAN BE PERFORMED WITHOUT THE UTILIZATION OF ULTRASONOGRAPHY, AND HOW IT WOULD COMPARE TO OTHER SUPEROVULATION PROTOCOLS IN THE BOER GOAT.
3. TO EVALUATE THE EFFECT OF SEASON, AGE AND REPEATED GONADOTROPHIN TREATMENT ON OVARIAN RESPONSE TO SUPEROVULATION
4. TO EVALUATE A MORE VIABLE METHOD OF EMBRYO CRYOPRESERVATION FOR THE LATER TRANSFER OF BOER GOAT EMBRYOS

CHAPTER 2

LITERATURE REVIEW

2. FactorS affecting the efficiency of MOET

2.1. MOET and the advantages of this reproductive technique

MULTIPLE OVULATION AND EMBRYO TRANSFER (MOET) PROVIDES A USEFUL TOOL IN THE MANAGEMENT OF LIVESTOCK BREEDING AND REPRODUCTION. THIS PROGRAMME CAN BE USED TO GENERATE PROGENY FROM GENETICALLY SUPERIOR DAMS AND ALSO PROVIDE VALUABLE DATA ON THE EMBRYONIC DEVELOPMENT AND VIABILITY FOLLOWING CRYOPRESERVATION. IN ADDITION, THE PROGRAMME CAN INCREASE THE POTENTIAL IN THE MARKETING OF EMBRYOS INTERNATIONALLY FOR ANIMAL BREEDING UPGRADING PURPOSES. MOET HAS BEEN USED RELATIVELY SUCCESSFULLY IN SHEEP AND GOATS TO INCREASE THE NUMBER OF OFFSPRING FROM SUPERIOR FEMALES AND MALES AND TO FACILITATE THE SHORTENING OF THE GENERATION INTERVAL (WULIJI ET AL., 1995; MORAND-FEHR & BOYAZOGLU, 1999).

MOET IN SMALL RUMINANTS AS SUCH INVOLVES THE SYNCHRONISATION OF OESTRUS IN BOTH THE DONORS AND RECIPIENTS, FOLLOWED BY THE ADMINISTRATION OF GONADOTROPHINS (SUPEROVULATION) DURING THE LAST DAYS OF THE SYNCHRONISATION TREATMENT IN THE DONORS (ISHWAR & MEMON, 1996). THE EMBRYOS ARE THEN RECOVERED SURGICALLY, GENERALLY FROM THE UTERINE HORNS ON DAY 6 TO 7 FOLLOWING PROGESTAGEN WITHDRAWAL (PENDLETON ET AL., 1992; PINTADO ET AL., 1998, CORDERIO ET AL., 2003). IN GOATS NON-SURGICAL EMBRYO COLLECTION HAS ALSO BEEN PERFORMED, ALTHOUGH THIS IS NOT COMMON (BONDURANT ET AL., 1984; PEREIRA ET AL., 1998; SUYADI ET AL., 2000). THE RECOVERED EMBRYOS CAN THEN BE TRANSFERRED EITHER AS FRESH (COGNIE, 1999) OR PROCESSED EMBRYOS EITHER VIA CRYOPRESERVATION FOR LATER

TRANSFER OR TRANSPORTATION FOR EXPORT (SUYADI ET AL., 2000), OR MANIPULATED (SPLIT) FRESH OR FOLLOWING CRYOPRESERVATION AND TRANSFERRED TO RECIPIENT DOES (NOWSHARI & HOLTZ, 1993; SZELL ET AL., 1994; OPPENHEIM ET AL., 2000)

ALTHOUGH MOET IN SMALL RUMINANTS HAS BEEN SUCCESSFULLY IMPLEMENTED IN PRACTICE, ITS EFFICIENT UTILIZATION REQUIRES A CONSISTENT PRODUCTION OF VIABLE TRANSFERABLE EMBRYOS RECOVERED MORE THAN IS CURRENTLY BEING ACHIEVED (BARI ET AL., 2003). THERE ARE SEVERAL FACTORS CONTRIBUTING TO THIS VARIATION IN EMBRYO PRODUCTION, AND THESE CAN GENERALLY BE GROUPED INTO INTRINSIC AND EXTRINSIC FACTORS. MANY OF THESE FACTORS ARE CENTRED ON THE RESPONSE TO SUPEROVULATION BY THE DONORS (BARI ET AL., 2000; GONZALEZ-BULNES ET AL., 2004A). THE SURVIVABILITY AND VIABILITY OF THE EMBRYOS ARE ALSO AFFECTED BY WHETHER THE EMBRYOS ARE TRANSFERRED INTACT OR FOLLOWING MANIPULATION (HEYMAN, 1985; WELLS ET AL., 1990), TRANSFERRED FRESH OR FOLLOWING CRYOPRESERVATION (MARTINEZ & MATKOVIC, 1998; BARIL ET AL., 2001). THE VIABILITY OF THE EMBRYO AND PREGNANCY SUCCESS OF IN VIVO OR IN VITRO PRODUCED EMBRYOS ALSO DIFFER FOLLOWING TRANSFER (COGNIE ET AL., 2003). THE MAIN FACTORS AFFECTING THE EFFICIENCY OF A MOET PROGRAMME IN SMALL RUMINANTS ARE DESCRIBED UNDER THE FOLLOWING HEADINGS, WITH SPECIAL EMPHASIS ON THE GOAT:

2.2. Extrinsic factors affecting MOET in goats

2.2.1. Different exogenous gonadotrophins/superovulation techniques used

THE MAJOR DRAWBACK IN THE APPLICATION OF MOET FOR GOAT BREEDING PROGRAMMES IS THE

VARIABILITY IN THE OVULATORY RESPONSE OBTAINED – THIS CAN GENERALLY BE ATTRIBUTED TO THE TYPE AND PREPARATION OF GONADOTROPHIN USED (NOWSHARI ET AL. 1995; PINTADO ET AL. 1998; HOLTZ, 2005). THE THREE MOST EXTENSIVELY USED GONADOTROPHINS IN THE SUPEROVULATION OF GOATS CURRENTLY ARE ECG, FSH AND HORSE ANTERIOR PITUITARY EXTRACT (HAP). INITIALLY, THE FIRST GONADOTROPHIN TO BE USED IN A SUPEROVULATION PROGRAMME FOR GOATS WAS ECG. THIS HORMONE WAS ADMINISTERED AS A SINGLE INJECTION ONE TO TWO DAYS BEFORE OR AT PROGESTAGEN TREATMENT TERMINATION, AS ECG IS KNOWN TO HAVE A LONG HALF-LIFE (AMOAH & GALAYE, 1990; COGNIE, 1999). THIS PROPERTY OF HAVING A LONG HALF-LIFE HOWEVER IS DISADVANTAGEOUS AS IT HAS BEEN REPORTED TO LEAD TO THE PRODUCTION OF A LARGE NUMBER OF OVARIAN FOLLICLES WHICH FAIL TO OVULATE. THESE FOLLICLES OFTEN REMAIN STIMULATED AFTER OVULATION (CYSTIC), MAINTAINING HIGH BLOOD OESTROGEN CONCENTRATIONS FOR AN EXTENDED PERIOD OF TIME (ARMSTRONG ET AL., 1983A; AMOAH & GALAYE, 1990; MAHMOOD ET AL., 1991; SAHARREA ET AL., 1998). THESE UNOVULATORY FOLLICLES ARE THEN OFTEN ASSOCIATED WITH LOWER QUALITY EMBRYOS RECOVERED FOLLOWING OVULATION (BOLAND ET AL., 1978; SAUMANDE ET AL., 1984). THE PRESENCE OF LARGE UNOVULATORY FOLLICLES AT TIME OF EMBRYO RECOVERY HAS ALSO BEEN REPORTED IN OTHER SPECIES SUPEROVULATED WITH ECG (MONNIAUX ET AL., 1983; KAFI ET AL., 1997; NAQVI & GULYANI, 1998). THE ELEVATED CIRCULATING OESTROGEN CONCENTRATION FROM THESE UNOVULATED FOLLICLES IS BELIEVED TO CREATE AN UNFAVOURABLE ENVIRONMENT FOR

THE SPERM, OOCYTES AND EMBRYO SURVIVAL IN THE FEMALE REPRODUCTIVE TRACT - RESULTING IN REDUCED FERTILISATION AND EMBRYO RECOVERY RATES (EVANS & ARMSTRONG, 1984). IN CATTLE THE INCREASE IN UTERINE TONE DETECTED AT EMBRYO COLLECTION TIME HAS BEEN RELATED TO THE PRESENCE OF UNOVULATED FOLLICLES OBSERVED (KAFI ET AL., 1997).

BESIDES THE HIGH INCIDENCE OF FOLLICULAR CYSTS, THE USE OF ECG IN GOATS HAS ALSO BEEN REPORTED TO LEAD TO A HIGH INCIDENCE OF PREMATURE LUTEAL REGRESSION (BATTYE ET AL., 1988; CAMERON ET AL., 1988; SAHARREA ET AL., 1998). THIS PREMATURE LUTEAL REGRESSION BEING THE RESULT OF AN EARLY INCREASE IN THE SECRETION OF ENDOGENOUS PROSTAGLANDIN-F_{2A} (PGF2A), EMANATING FROM THE UTERUS DUE TO THE RESPONSE FOLLOWING STIMULATION FROM ELEVATED OESTROGEN LEVELS FROM THE UNOVULATORY FOLLICLES (ARMSTRONG ET AL., 1983A; BATTYE ET AL., 1988). THE HIGH BLOOD OESTROGEN CONCENTRATION IN RUMINANTS IS KNOWN TO INCREASE ENDOMETRIAL OXYTOCIN RECEPTOR SYNTHESIS AND ACTIVATE THE ENZYMES ASSOCIATED WITH PGF2A SECRETION. IN SHEEP, THE INFUSION OF OESTROGEN (E_2-17_B) RESULTED IN THE FORMATION OF OXYTOCIN RECEPTORS, 6H FOLLOWING TREATMENT, WITH OXYTOCIN STIMULATING THE SECRETION OF PGF2A (MCCRACKEN ET AL., 1984). THE ADMINISTRATION OF EXOGENOUS OXYTOCIN HAS BEEN SUGGESTED TO INCREASE THE SECRETION OF UTERINE PGF2A, WHILE IMMUNISATION AGAINST OXYTOCIN HAS BEEN SHOWN TO DELAY LUTEOLYSIS IN SHEEP AND GOATS (BURGESS ET AL., 1990; GARVERICK ET AL., 1992; SEALS ET AL., 1998; LEMASTER ET AL., 1999). IT WOULD THUS SEEM AS IF THE MORE OXYTOCIN

BINDS TO THE ENDOMETRIUM, THE GREATER STIMULATION OF THE UTERUS TO SECRETE PGF2A, AND INDUCE EARLY LUTEAL REGRESSION (GARVERICK ET AL., 1992). THE END RESULT OF PREMATURE LUTEAL REGRESSION BEING A DECLINE IN PROGESTERONE CONCENTRATION 3 TO 6 DAYS FOLLOWING THE ONSET OF OESTRUS. THIS INHIBITORY EFFECT OF PROGESTERONE ON OESTROGEN RECEPTOR SYNTHESIS THEN DECLINES, LEADING TO AN INCREASE IN ENDOMETRIAL OESTROGEN AND OXYTOCIN RECEPTORS. EVENTUALLY THIS SITUATION WILL LEAD TO A LOSS OF EMBRYOS BEFORE THE SCHEDULED COLLECTION ON DAY 6 OR 7 AFTER MATING OR INSEMINATION (STUBBINGS ET AL., 1986; BURGESS ET AL., 1990; SAHARREA ET AL., 1998).

THE EARLY REGRESSING CORPORA LUTEA DO NOT ONLY LEAD TO A DECLINE IN THE PROGESTERONE CONCENTRATION, BUT HAVE ALSO BEEN INDICATED TO HAVE AN EMBRYO TOXICITY EFFECT (BUFORD ET AL., 1996; HERNANDEZ-FONSECA ET AL., 2000; COSTINE ET AL., 2001). IN CATTLE WITH A SHORT OESTROUS CYCLE (DUE TO SHORT-LIVED CORPORA LUTEA), OOCYTES WERE FERTILISED BUT THE PREGNANCY RATES REPORTED TO BE VERY LOW (CASIDA ET AL., 1968; ODDE ET AL., 1980; RAMIREZ-GODINEZ ET AL., 1982A). THIS CONDITION HAS BEEN CONTEMPLATED TO BE ATTRIBUTED TO THE PREMATURELY REGRESSING CORPUS LUTEUM - AS LUTEAL PROGESTERONE IS ESSENTIAL FOR MAINTAINING PREGNANCY (MCDONALD ET AL., 1952; RAMIREZ-GODINEZ ET AL., 1981; RAMIREZ-GODINEZ ET AL., 1982B; COOPER ET AL., 1991; BREUEL ET AL., 1993). IT COULD THEREFORE BE SPECULATED THAT PROGESTERONE SUPPLEMENTATION COULD SUPPORT PREGNANCY IN CASES OF EARLY REGRESSING CORPORA LUTEA. HOWEVER, IT HAS BEEN REPORTED

THAT WHEN ANIMALS WITH SHORT-LIVED CORPORA LUTEA ARE SUPPLEMENTED WITH EXOGENOUS PROGESTERONE (TO REPLACE THE REGRESSING CORPORA LUTEA), THE PREGNANCY RATE WAS NOT MAINTAINED OR IMPROVED (BUTCHER ET AL., 1992; BREUEL ET AL., 1993). UNLESS ACCOMPANIED BY TREATMENT WITH AN ANTI-PROSTAGLANDIN AGENT OR THE REMOVAL OF THE REGRESSING CORPORA LUTEA. THIS OBSERVATION INDICATES THE DECLINE IN BLOOD PROGESTERONE CONCENTRATION FOLLOWING EARLY LUTEAL REGRESSION NOT ONLY TO BE THE CAUSE OF EMBRYONIC DEATH, BUT THE REGRESSING CORPORA LUTEA ALSO RELEASE FACTORS CONTRIBUTING TO EMBRYONIC DEATH (BUFORD ET AL., 1996).

THERE IS EVIDENCE OF A HIGH CONCENTRATION OF PGF2A IN SHEEP AND CATTLE WITH SHORT-LIVED CORPORA LUTEA, COMPARED TO ANIMALS WITH A NORMAL LUTEAL PHASE. A DECLINE IN EMBRYO QUALITY WAS REPORTED WITH AN INCREASE IN PGF2A CONCENTRATION ON DAY 6 FOLLOWING OESTRUS (HU ET AL., 1990; COOPER ET AL., 1991; SCHRICK ET AL., 1993). THIS FACT INDICATES THE REGRESSING CORPUS LUTEUM TO SECRETE PGF2A, WITH THIS PGF2A HAVING A DIRECT EMBRYO TOXIC EFFECT (GARVERICK ET AL., 1992; COSTINE ET AL., 2001). THE DIRECT EMBRYO TOXICITY EFFECT OF PGF2A HAS ALSO BEEN DEMONSTRATED IN OTHER SPECIES. IN VIVO AND IN VITRO TREATMENT OF COWS WITH PGF2A HAS BEEN REPORTED TO REDUCE THE EMBRYO SURVIVAL AND RECOVERY RATES (BUFORD ET AL., 1996; SEALS ET AL., 1998). IN ADDITION, THE INCUBATION OF BOVINE EMBRYOS WITH PGF2A HAS BEEN REPORTED TO DELAY EMBRYONIC DEVELOPMENT (FAZIO ET AL., 1997). BESIDES SECRETING PGF2A, THE EARLY REGRESSING CORPUS LUTEUM HAS BEEN INDICATED TO SECRETE

OXYTOCIN, INCREASE MACROPHAGES AND INFLAMMATORY CELLS WHICH ACT TOGETHER WITH PGF2A TO REDUCE THE EMBRYONIC SURVIVAL RATE (BAGAVANDOSS ET AL., 1988; BAGAVANDOSS ET AL., 1990; BRANNSTROM ET AL., 1994; SHAW & BRITT, 1995; BUFORD ET AL., 1996; LEMASTER ET AL., 1999; COSTINE ET AL., 2001).

THE ADVERSE SIDE-EFFECTS OBSERVED FOLLOWING THE UTILISATION OF ECG IN SUPEROVULATION PROTOCOLS CAN BE MINIMISED BY THE ADMINISTRATION OF E.G. ANTI-ECG, HCG OR GNRH AT THE ONSET OF OESTRUS IN SHEEP AND CATTLE (MONNIAUX ET AL., 1983; SAUMANDE ET AL., 1984; MOYAERT ET AL., 1985; MARTEMUCCI ET AL., 1995; COGNIE, 1999). IN SHEEP AND CATTLE, THE TREATMENT WITH ANTI-ECG AT OESTRUS HAS RESULTED IN A DECREASE IN THE CONCENTRATION OF OESTRADIOL AFTER OVULATION. THE NUMBER OF LARGE FOLLICLES AT THE TIME OF EMBRYO RECOVERY, IMPROVED OVULATION AND FERTILISATION RATES AND THE QUALITY OF EMBRYOS COLLECTED WERE ALSO OBSERVED FOLLOWING TREATMENT WITH ANTI-ECG (KUMMER ET AL., 1980; SAUMANDE ET AL., 1984; MARTEMUCCI ET AL., 1995). HOWEVER, IN GOATS SUPEROVULATED WITH ECG, THE TREATMENT WITH HCG OR GNRH FAILED TO IMPROVE THE OVULATION RATE, OR TO REDUCE THE NUMBER OF LARGE FOLLICLES WHICH FAILED TO OVULATE (ARMSTRONG ET AL., 1982; SAHARREA ET AL., 1998). THE ADMINISTRATION OF ANTI-ECG IN GOATS SUPEROVULATED WITH ECG HAS LED TO VARIABLE OVARIAN RESPONSES AND FAILED TO REDUCE THE INCIDENCE OF PREMATURE LUTEAL REGRESSION IN SUPEROVULATED DAIRY AND MURCIANA GOATS (STUBBINGS ET AL., 1986). ON THE OTHER HAND, PINTADO ET AL. (1998) REPORTED AN INCREASE IN THE NUMBER OF VIABLE GOAT

EMBRYOS WHEN TREATED WITH ANTI-ECG, COMPARED TO THE CONTROLS.

THE PROBLEMS EMANATING FROM THE USE OF ECG IN GOATS HAVE LED TO SEVERAL INVESTIGATIONS TO DETERMINE AN ALTERNATIVE GONADOTROPHIN TO BE USED FOR SUPEROVULATION. INITIALLY, HAP WAS TESTED ON ANGORA GOATS AND ADMINISTERED ONCE A DAY SUBCUTANEOUSLY OVER A PERIOD OF 3 DAYS, BEGINNING ON THE LAST DAY OF PROGESTAGEN TREATMENT, WITH SATISFACTORY FERTILISATION AND EMBRYO RECOVERY RATES (MOORE, 1974). WHEN COMPARING THE RESPONSE TO SUPEROVULATION IN GOATS, BOTH ECG AND HAP WERE EFFECTIVE IN INDUCING MULTIPLE OVULATIONS. THE NUMBER OF THE CORPORA LUTEA RECORDED DID NOT DIFFER SIGNIFICANTLY BETWEEN THE TWO GONADOTROPHIN TYPES. MOREOVER, THE NUMBER OF PERSISTENT FOLLICLES INDUCED WAS ALSO SIMILAR IN THE HAP AND ECG TREATED DOES. EVEN THOUGH HAP FAILED TO REDUCE THE NUMBER OF LARGE UNOVULATORY FOLLICLES IN GOATS, THIS GONADOTROPHIN HAD THE ADVANTAGE OF HAVING A HIGH EMBRYO RECOVERY AND FERTILISATION RATE, WHEN COMPARED TO ECG (MOORE & EPPLESTON, 1979). HOWEVER, A LIMITATION WAS THAT HAP AS SUCH IS NOT FREELY AVAILABLE (PENDLETON ET AL., 1992).

DUE TO THE FACT THAT HAP FAILED TO REDUCE THE NUMBER OF UNOVULATORY FOLLICLES AND IMPROVE THE OVARIAN RESPONSE TO SUPEROVULATION, PURE FSH WAS IMPLEMENTED AS AN ALTERNATIVE GONADOTROPHIN TO ECG IN MOST ANIMAL SPECIES (NUTI ET AL., 1987; JABBOUR & EVANS, 1991A; KELLY ET AL., 1997; D'ALESSANDRO ET AL., 2005). CURRENTLY THE MOST COMMONLY USED FSH PREPARATIONS IN CAPRINE MOET PROGRAMMES

ARE OVINE (OFSH) AND PORCINE (PFSH) PRODUCED FSH (BARIL & VALLET, 1990; COGNIE, 1999). THESE FSH PREPARATIONS BEING NORMALLY ADMINISTERED TWICE DAILY AT 12H INTERVALS, OVER A PERIOD OF 3 TO 4 DAYS, IN GOATS (PENDLETON ET AL., 1992; ROSNINA ET AL., 1992; GORDON, 1997; HOLTZ, 2005), DUE TO THEIR SHORT HALF-LIFE WHEN COMPARED TO E.G. ECG (DEMOUSTIER ET AL., 1988).

SATISFACTORY OVULATION RATES RANGING BETWEEN 8.4±0.9 AND 28.7±2.3 HAVE BEEN RECORDED IN GOATS SUPEROVULATED WITH FSH (SENN & RICHARDSON, 1992; ROSNINA ET AL., 1992; ISHWAR & MEMON, 1996; GREYLING ET AL., 2002). THERE IS HOWEVER STILL LARGE VARIATION IN THE OVARIAN RESPONSE TO SUPEROVULATION FOLLOWING THE UTILISATION OF FSH IN MOST SPECIES. THIS IS BELIEVED TO BE ATTRIBUTED TO THE FSH/LH RATIO IN THE GONADOTROPHIN PREPARATION (LINDSELL ET AL., 1986; DONALDSON, 1990; HENDERSON ET AL., 1990).

IN OTHER RUMINANTS, FSH PREPARATIONS WITH A HIGH LH CONTENT HAVE BEEN FOUND TO RESULT IN LOWER OVULATION AND FERTILISATION RATES AND POOR QUALITY EMBRYOS (MURPHY ET AL., 1984; DONALDSON ET AL., 1986). THE HIGH BLOOD LH LEVELS CAUSE PREMATURE OVULATION OF THE LARGE FOLLICLES WHICH ARE PRESENT AT THE ONSET OF THE SUPEROVULATION REGIME. THIS OVULATION LEADS TO THE PRODUCTION OF PROGESTERONE DURING THE PRE-OVULATORY PERIOD OF THE NEWLY INDUCED FOLLICLES. THE PROGESTERONE AND OESTROGEN RATIO OF THE PREMATURELY STIMULATED FOLLICLES ARE THEN ALTERED, LEADING TO DISTURBANCES IN THE PROCESS OF MATURATION OF THESE FOLLICLES, HENCE THE PRODUCTION OF POOR QUALITY EMBRYOS (CALLESEN ET AL., 1986; CALLESEN ET AL.,

1987). WHEN LH WAS REMOVED FROM THE PFSH, THE EFFECTIVENESS OF THE FSH INCREASED AS THE LOWER DOSES OF LH IN PFSH GAVE THE HIGHEST OVULATORY RATE AND NUMBER OF TRANSFERABLE EMBRYOS (DONALDSON ET AL., 1986). IN GOATS, AS THE RATIO OF FSH/LH WAS REDUCED IN A SUPEROVULATION TREATMENT, MORE CORPORA LUTEA AND TRANSFERABLE EMBRYOS WERE OBTAINED, COMPARED TO WHEN A CONSTANT FSH/LH RATIO WAS UTILISED (BARIL ET AL., 1989). THIS OBSERVATION AND THE ADVERSE EFFECTS DEMONSTRATED BY THE FSH PREPARATIONS WITH LH, LED TO THE PRODUCTION AND UTILISATION OF A MORE PURIFIED COMMERCIAL PREPARATION OF FSH WITH A LOW LH CONTENT (OFSH: OVAGEN AND PFSH: FOLLTROPIN AND STIMAFOL) (KELLY ET AL., 1997). WHEN THE EFFICIENCY OF OFSH AND PFSH WERE EVALUATED IN GOATS, THE OVULATION RATE AND NUMBER OF TRANSFERABLE EMBRYOS INDUCED WERE SIMILAR (MCNATTY ET AL., 1989). HOWEVER, SUPEROVULATION WITH A HIGHLY PURIFIED FSH PREPARATION HAS LED TO A LOWER SUPEROVULATORY RESPONSE THAN THE LH-SUPPLEMENTED FSH PREPARATIONS (HERRLER ET AL., 1991; COGNIE, 1999). MOREOVER THERE HAS BEEN AN INCREASE IN THE FREQUENCY OF OVULATION ABNORMALITIES OBSERVED FOLLOWING SUPEROVULATION WITH THESE COMMERCIALLY PURIFIED FSH BATCHES, E.G. PREMATURE OVULATION AND UNOVULATED FOLLICLES (COGNIE ET AL., 2003). THIS OCCURRENCE INDICATES A MINIMUM AMOUNT OF LH BEING REQUIRED FOLLOWING PROGESTAGEN TREATMENT TERMINATION. IN GOATS, THE SUPPLEMENTATION OF FSH WITH 40% PURIFIED LH HAS BEEN SUGGESTED TO BE THE OPTIMAL DOSE, AND HAS BEEN PROVEN TO PRODUCE SATISFACTORY OVULATION RATES AND A

HIGH NUMBER OF TRANSFERABLE EMBRYOS (PULS-KLEINGELD ET AL., 1992; NOWSHARI ET AL., 1995).
THE EFFICIENCY OF FSH AND ECG AS SUPEROVULATION AGENTS HAS BEEN COMPARED IN GOATS AND OTHER SPECIES. SUPEROVULATION WITH FSH PRODUCED HIGHER OVULATION AND EMBRYO RECOVERY RATES, WHEN COMPARED TO ECG TREATED GOATS (GOEL & AGRAWAL, 1990; SELGRATH ET AL., 1990; MAHMOOD ET AL., 1991). THE HIGHER NUMBER OF ABNORMAL CORPORA LUTEA (WHICH IS AN INDICATION OF PREMATURE LUTEAL REGRESSION) WAS OBSERVED IN ECG TREATED DOES, COMPARED TO FSH TREATED DOES (PENDLETON ET AL, 1992). MOREOVER, THE INCIDENCE OF A LARGE NUMBER OF UNOVULATORY FOLLICLES WAS HIGHER IN ECG STIMULATED DOES, COMPARED TO FOLLOWING FSH TREATMENT (ARMSTRONG ET AL., 1983A). THE DIFFERENCE IN OVARIAN RESPONSE BETWEEN THESE TWO GONADOTROPHINS CAN LARGELY BE ATTRIBUTED TO THEIR DIFFERENCES IN BIOLOGICAL HALF-LIFE ACTIVITY (APPROXIMATELY 5H FOR FSH, AS OPPOSED TO 20H FOR ECG). THIS MEANS THAT FSH CAN BE CLEARED FROM THE CIRCULATION MORE QUICKLY, COMPARED TO ECG (ISHWAR & MEMON, 1996; HOLTZ, 2005).
EVEN THOUGH THE UTILISATION OF FSH IN THE SUPEROVULATION PROGRAMME OF GOATS HAS DEMONSTRATED BETTER RESULTS, THIS REGIME IS MORE LABOUR INTENSIVE AND IMPOSES MORE STRESS TO THE ANIMALS, DUE TO EXCESSIVE HANDLING - AS IT MUST BE ADMINISTERED OVER A 4 DAY PERIOD, TWICE DAILY. SEVERAL ATTEMPTS TO SIMPLIFY THE ADMINISTRATION PROCEDURE OF FSH TREATMENT WITHOUT COMPROMISING THE SUPEROVULATORY RESPONSE HAVE BEEN MADE OVER TIME (BATT ET AL., 1993; GORDON, 1997). SOME

OF THE ATTEMPTS WERE THE ADMINISTRATION OF A SINGLE INJECTION OF FSH COMBINED WITH ECG, OR REPLACING THE LAST INJECTIONS OF FSH AT PROGESTAGEN TREATMENT WITHDRAWAL WITH ECG. A SINGE DOSE OF FSH AND ECG HOWEVER LED TO A LOWER OVULATION RATE, WHEN COMPARED TO THE MULTIPLE FSH REGIME (HOLTZ, 2005).
ON THE OTHER HAND, BALDASSARRE ET AL. (2002) RECORDED NO DIFFERENCE IN SUPEROVULATION RESPONSE, BASED ON THE NUMBER OF FOLLICLES ASPIRATED AND OOCYTES COLLECTED BETWEEN A MULTIPLE FSH REGIME AND A COMBINATION OF FSH AND ECG. THE REPLACEMENT OF CERTAIN FSH INJECTIONS WITH ECG ALSO GAVE SIMILAR RESULTS WITH RESPECT TO THE OVULATION RATE, THE NUMBER OF FOLLICLES AND THE TOTAL NUMBER OF EMBRYOS RECOVERED, COMPARED TO A FSH TREATMENT ALONE (PINTADO ET AL., 1998). EMBRYO RECOVERY AND QUALITY CAN BE IMPROVED BY THE ADMINISTRATION OF LH, HCG OR GNRH AT THE ONSET OF OESTRUS FOLLOWING SUPEROVULATION WITH FSH, AS OPPOSED TO THE USE OF ECG (ISHWAR & MEMON, 1996; HOLTZ, 2005). HIGH OVULATION RATES INCREASED THE TOTAL NUMBER OF EMBRYOS RECOVERED AND A HIGHER NUMBER OF GOOD QUALITY EMBRYOS FOLLOWING GNRH ADMINISTRATION IN FSH SYNCHRONISED GOATS HAVE BEEN REPORTED (BARIL & VALLET, 1990; AKINLOSOTU & WILDER, 1993; KRISHER ET AL., 1994). ALTHOUGH ECG HAS THE ADVANTAGE OF BEING ADMINISTERED AS A SINGLE INJECTION, WHICH IS SIMPLER AND MORE PRACTICAL THAN 6 TO 8 INJECTIONS GIVEN WHEN USING FSH, ITS USE IN GOATS IS CURRENTLY NOT PREFERRED. THE MAIN REASON BEING THE HIGH INCIDENCE OF PREMATURE LUTEAL REGRESSION, LOW EMBRYO RECOVERY RATES AND POOR QUALITY EMBRYOS COLLECTED IN

THE ECG SUPEROVULATED GOATS, COMPARED TO FSH TREATED GOATS. THE IMPROVEMENT OF EMBRYO RECOVERY AND EMBRYO QUALITY FOLLOWING THE SUPPLEMENTATION WITH LH, HCG OR GNRH IN THE FSH PROTOCOLS WHICH FAILED IN THE ECG REGIME, ALSO SUPPORT THE USE OF FSH FOR SUPEROVULATION IN GOATS AS OPPOSED TO ECG.

2.2.2. Variation in superovulation response associated with the use of progestagens

IN GOATS, THE MOET PROGRAMME USUALLY ENTAILS THE STIMULATION OF THE OVARIES BY ADMINISTRATION OF GONADOTROPHINS DURING THE LAST DAYS OF PROGESTERONE PRIMING. EXOGENOUS PROGESTAGENS SUCH AS INTRAVAGINAL SPONGES CONTAINING 45MG FLUOROGESTONE ACETATE (FGA) OR 60MG MEDROXYPROGESTERONE ACETATE (MAP) AND CONTROLLED INTERNAL DRUG RELEASE DEVICES (CIDR) HAVE BEEN WIDELY USED TO SYNCHRONISE THE TIME OF OESTRUS AND OVULATION IN GOATS (MOTLOMELO ET AL., 2002; ESPINOSA-MARQUEZ ET AL., 2004; LEHLOENYA ET AL., 2005). THE PROGESTAGEN TREATMENT IS USUALLY APPLIED OVER A PERIOD OF 16 TO 21 DAYS (GORDON, 1997). DURING PROGESTAGEN TREATMENT, THE NATURAL CORPUS LUTEUM WILL REGRESS, BUT NEITHER OESTRUS NOR OVULATION OCCURS UNTIL AFTER REMOVAL OF THE EXOGENOUS PROGESTERONE. THE ADMINISTRATION OF THESE PROGESTAGENS SUPPRESSES THE LH SECRETION AND IN TURN ASSURES THE SPONTANEOUS OCCURRENCE OF OESTRUS AND OVULATION IN A CONTROLLED

MANNER FOLLOWING PROGESTAGEN REMOVAL. HOWEVER, THE ADMINISTRATION OF PROGESTAGENS HAS BEEN REPORTED TO ALTER THE PATTERN OF LH SECRETION, AS WELL AS THE PATTERN OF FOLLICULAR GROWTH AND DOMINANCE (NOEL ET AL., 1994; GONZALEZ-BULNES ET AL., 2004A). IN GOATS AND OTHER RUMINANTS, A HIGH LEVEL OF PROGESTERONE FOLLOWING THE ADMINISTRATION OF EXOGENOUS PROGESTERONE SUPPRESSED BOTH THE LH PULSE AND OESTRADIOL-INDUCED LH SURGE. HENCE THE FOLLICULAR DEVELOPMENT AND GROWTH IS STIMULATED BY AN INCREASED LH PULSE (MARTIN, 1984; KASTELIC ET AL., 1990; KIM ET AL., 2003).

HIGH BLOOD PROGESTERONE CONCENTRATIONS WILL COINCIDE WITH THE ONSET OF THE PROGESTAGEN TREATMENT, AND TOWARDS THE END OF THE TREATMENT THE PROGESTERONE LEVELS ARE GENERALLY LOW AND SOMETIMES LOWER THAN THE BASAL LEVELS, WHICH CANNOT MIMIC THE LUTEAL PHASE (LEYVA ET AL., 1998). THIS TENDENCY HAS BEEN OBSERVED IN GOATS, WHERE LOW PROGESTERONE LEVELS COMPLETELY SUPPRESS THE LH SURGE, WITHOUT ANY EFFECT ON THE LH PULSATILE SECRETION (DE CASTRO ET AL., 1999; KIM ET AL., 2003). IN SHEEP AND CATTLE, THE INDUCTION OF A LOW PROGESTERONE ENVIRONMENT HAS BEEN INDICATED TO EXTEND THE LIFESPAN AND INCREASE THE SIZE OF THE DOMINANT FOLLICLE (SAVIO ET AL., 1993A, B; VINOLES ET AL., 1999). THESE PERSISTENT LARGE FOLLICLES LEAD TO AN INCREASED SECRETION OF OESTROGEN TOWARDS THE END OF THE PROGESTERONE TREATMENT AND THE SUBSEQUENT FOLLICULAR PHASE. THE HIGH LEVEL OF OESTROGEN INDUCED BY A LOW PROGESTERONE CONCENTRATION ALSO ALTERS THE SPERM

TRANSPORT AND COULD REDUCE THE FERTILISATION RATE (JOHNSON ET AL., 1996). MOREOVER, A HIGH CONCENTRATION OF OESTROGEN HAS ALSO BEEN REPORTED TO ALTER THE DEVELOPMENTAL COMPETENCE OF THE OOCYTES. IT HAS BEEN SUGGESTED THAT OOCYTES FROM PERSISTENT OVARIAN FOLLICLES ARE NORMALLY AT A MORE ADVANCE STAGE OF NUCLEAR MATURATION BEFORE THE ONSET OF THE PRE-OVULATORY LH SURGE. THIS ADVANCEMENT OF MEIOSIS LEADS TO THE PRODUCTION OF ABNORMAL OOCYTES WHICH WOULD BE LESS FERTILE AND RESULT IN INCREASED EMBRYONIC MORTALITY RATES, IF FERTILISED. OVULATION OF THESE ABNORMAL FOLLICLES MAY ALSO ALTER THE FUNCTION OF THE SUBSEQUENT CORPUS LUTEUM. THE OTHER SUBSEQUENT EFFECT OF A HIGH OESTROGEN ENVIRONMENT HAS BEEN REPORTED AS AN ALTERATION IN THE OVIDUCTAL OR UTERINE ENVIRONMENT (KOJIMA ET AL., 1992; REVAH & BUTLER, 1996; WEHRMAN ET AL., 1997; BINELLI ET AL., 1999).

ALL EFFECTS ORIGINATING FROM LOW BLOOD PROGESTERONE CONCENTRATIONS AT THE END OF SYNCHRONISATION TREATMENT ARE FURTHER INCREASED FOLLOWING SUPEROVULATION AND RESULT IN THE VARIABILITY REGARDING THE ONSET OF OESTRUS AND LH PEAK. HENCE THE MIS-TIMING OF OVULATION, WHICH ENDS UP IN REDUCED FERTILITY, AS INDICATED BY THE RECOVERY OF MORE UNFERTILISED OVA, ESPECIALLY FOLLOWING FIXED-TIME AI. DUE TO A HIGH VARIATION REGARDING THE ONSET OF OESTRUS AND OVULATION RECORDED IN DAIRY GOATS, BARIL AND VALLET (1990) SUGGESTED THAT THE TIME OF OVULATION CANNOT BE PREDICTED FROM EITHER THE TIME OF SPONGE REMOVAL OR THE

OCCURRENCE OF OESTRUS FOLLOWING PROGESTAGEN TREATMENT AND SUPEROVULATION. THE DISTURBANCE ON OOCYTE MATURATION AND ALTERATION OF THE UTERINE ENVIRONMENT THEN ALSO LEADS TO THE INCREASED INCIDENCE OF ABNORMAL EMBRYOS, REDUCING THE NUMBER OF TRANSFERABLE EMBRYOS (SCUDAMORE ET AL., 1992, 1993A, B).

THE LOW LEVELS OF CIRCULATING PROGESTERONE RECORDED TOWARDS THE END OF SYNCHRONISATION TREATMENT, SUGGESTS AN INADEQUACY OF THE PROGESTAGEN USED TO BLOCK THE LH PULSE SECRETION. THIS MAY THUS BE ATTRIBUTED TO THE DOSE OR TYPE OF THE PROGESTAGEN USED. IN SHEEP WHEN USING DIFFERENT PROGESTAGEN TREATMENTS FOLLOWING SUPEROVULATION (FSH, PMSG OR HAP), THE SPONGE PESSARIES LED TO A HIGHER OVULATION RATE AND THUS A HIGHER NUMBER OF OVA RECOVERED, COMPARED TO SYNCHRONISATION WITH CIDR’S (BOLAND ET AL., 1983; THOMPSON ET AL., 1990). IN A STUDY COMPARING THE EFFECT OF THE DOSE OF PROGESTAGEN ON THE RESPONSE TO SUPEROVULATION, OVULATION AND EMBRYO RECOVERY RATES, THE RESPONSES WERE NOT AFFECTED BY THE DIFFERENT DOSAGES OF PROGESTERONE PRIMING USED. HOWEVER, THE NUMBER OF TRANSFERABLE EMBRYOS RECORDED WAS LOWER IN THE LOWER DOSAGES OF FGA, COMPARED TO THE HIGHER DOSAGES. THIS INDICATES THAT THE LOW PROGESTERONE AT THE BEGINNING OF SUPEROVULATION LEADS TO A REDUCTION IN EMBRYO QUALITY (SCUDAMORE ET AL., 1992; WALLACE, 1992). WHEN TWO CIDR’S WERE SUCCESSIVELY USED IN THE SUPEROVULATION PROGRAMME (FIRST CIDR REPLACED ON DAY 9 BY A NEW CIDR THEN REMOVED ON DAY 12), IN ATTEMPT

TO MAINTAIN HIGHER PROGESTERONE CONCENTRATIONS, THE SUPEROVULATION RESPONSE WAS HIGHER, COMPARED TO WHEN A SINGLE CIDR WAS USED THROUGHOUT THE SYNCHRONISATION TREATMENT (THOMPSON ET AL., 1990). ON THE OTHER HAND, WHEN TWO INDIVIDUAL INTRAVAGINAL SPONGES WERE USED CONSECUTIVELY TO SYNCHRONISE OESTRUS IN A SUPEROVULATION PROGRAMME (COMPARED TO A SINGLE SPONGE UTILISATION), THE OVULATION RATE AND THE NUMBER OF RECOVERED EMBRYOS DID NOT DIFFER. HOWEVER THE UTILISATION OF TWO INTRAVAGINAL SPONGES IMPROVED THE NUMBER OF EMBRYOS RECOVERED. THE BEST SUPEROVULATORY RESPONSE, OVULATION RATE, TOTAL NUMBER OF EMBRYOS RECOVERED AND VIABLE EMBRYOS WERE ATTAINED WHEN THE TWO CONSECUTIVE INTRAVAGINAL SPONGES WERE UTILISED TOGETHER WITH A SINGLE INJECTION OF PROSTAGLANDIN, COINCIDING WITH THE FIRST SUPEROVULATION INJECTION (GONZALEZ-BULNES ET AL., 2004A)

2.2.3. Repeated superovulation and embryo recovery

THE POTENTIAL OF MOET TO ACCELERATE THE GENETIC PROGRESS IN GOATS CAN BE ACCOMPLISHED THROUGH REPEATED SUPEROVULATION AND RECOVERY OF EMBRYOS FROM SUPERIOR DONORS. SUCCESSIVE SUPEROVULATION AND THE COLLECTION OF EMBRYOS IN LESS SEASONAL BREEDERS SUCH AS THE BOER GOAT COULD LEAD TO FASTER PROGRESS, ABOVE THAT ACHIEVED IN A NATURAL BREEDING MANAGEMENT SYSTEM. ALTHOUGH THE IDEA IS FEASIBLE, REPEATED SUPEROVULATION IN SMALL RUMINANTS IN THE PAST HAS LED TO UNDESIRABLE SIDE-EFFECTS. REPEATED SUPEROVULATION WITH PFSH IN GOATS HAS BEEN REPORTED TO REDUCE THE

NUMBER OF OVULATIONS AND EMBRYOS RECOVERED, AS WELL THE NUMBER OF TRANSFERABLE EMBRYOS (NUTI ET AL., 1987; BARIL ET AL., 1989; BECKERS ET AL., 1990). SIMILAR OBSERVATIONS HAVE BEEN REPORTED IN OTHER SPECIES (CHUPIN & SAUMANDE, 1979; AL-KAMALI ET AL., 1985; BAVISTER ET AL., 1986). IN ADDITION TO REDUCED OVULATION RATES IN SHEEP, THE OESTROUS RESPONSE AND THE NUMBER OF EWES OVULATING WERE REDUCED (AL-KAMALI ET AL., 1985; FUKI ET AL., 1985).

THIS REDUCTION IN SUPEROVULATION RESPONSE IN THE PAST WAS SUGGESTED TO BE ATTRIBUTED TO THE REFRACTORINESS OF THE OVARIES IF SUPEROVULATION WAS REPEATED WITHIN AN INTERVAL OF 2 TO 6 MONTHS (WILLETT & BUCKNER, 1953; AL-KAMALI ET AL., 1985; NUTI ET AL., 1987; BREBION ET AL., 1992). OTHER RESEARCHERS HAVE STATED THAT SUPEROVULATION AT AN INTERVAL OF 2 MONTHS SHOULD NOT LEAD TO A REDUCTION IN OVARIAN RESPONSE TO SUPEROVULATION. THIS ARGUMENT WAS BASED ON THE FACT THAT IN SHEEP 20 TO 40 GONADOTROPHIN-RESPONSIVE FOLLICLES HAVE BEEN RECORDED ON THE OVARY OF AN EWE DURING EACH OESTROUS CYCLE, AND ALSO THAT THE PRIMORDIAL FOLLICLES TAKE ABOUT 40 DAYS TO REACH THE OVULATORY STAGE. THESE RESEARCHERS FOUND NO SIGNIFICANT DIFFERENCE WITH RESPECT TO OVULATION RATE IN SHEEP BETWEEN THE FIRST AND THE SECOND SUPEROVULATION TREATMENT (CORDEIRO ET AL., 2003).

ANOTHER EXPLANATION REGARDING THE REDUCTION IN OVULATION RATE AND THE NUMBER OF FEMALES RESPONDING TO REPEATED SUPEROVULATION WAS THAT GONADOTROPHIN ANTIBODIES ARE FORMED FOLLOWING SUCCESSIVE

SUPEROVULATION (HOLTZ, 2005). IN CATTLE AND RABBITS ANTI-GONADOTROPHINS FOLLOWING REPEATED SUPEROVULATION HAVE BEEN INDICATED TO NEUTRALISE THE FOLLICULAR STIMULATORY EFFECT OF THE HORMONE USED FOR SUPEROVULATION (JAINUDEEN ET AL., 1966; MAURER ET AL., 1968). MOREOVER, AN INCREASE IN THE IMMUNE RESPONSE TO ECG FOLLOWING REPEATED TREATMENT WAS OBSERVED IN GOATS AND CATTLE (DRION ET AL., 2001A, B). THE ANTI-ECG ANTIBODIES PRODUCED IN GOATS HAVE BEEN INDICATED TO HAVE A NEGATIVE EFFECT ON REPRODUCTION, ESPECIALLY WHEN FIXED-TIME AI IS PERFORMED. THIS WAS CONFIRMED BY ROY ET AL. (1999), WHERE THE HIGH CONCENTRATION OF ANTI-ECG ANTIBODIES WAS CORRELATED WITH A DECREASE IN FERTILITY. THE REDUCTION IN FERTILITY IS BELIEVED TO ARISE FROM THE ALTERATION IN THE TIME OF THE OCCURRENCE OF THE EXPECTED OESTRUS. IT HAS BEEN OBSERVED THAT THE PROPORTION OF GOATS EXHIBITING A DELAYED ONSET OF INDUCED OESTROUS BEHAVIOUR, INCREASED WITH THE NUMBER OF ECG TREATMENTS (BARIL ET AL., 1993). THE DELAY IN THE PRE-OVULATORY LH SURGE AND TIME OF OVULATION OBSERVED FOLLOWING REPEATED ECG TREATMENT HAS ALSO BEEN ASSOCIATED WITH THE FORMATION OF ANTI-ECG ANTIBODIES (HERVE ET AL., 2004). HOWEVER, IT HAS ALSO BEEN REPORTED THAT WHEN PFSH WAS UTILISED TO REPEATEDLY SUPEROVULATE COWS, NO ANTIBODIES WERE DETECTED IN THE PLASMA (REMY ET AL., 1991). YEARS AGO, RESEARCH INDICATED NO EVIDENCE OF A REDUCTION IN OVULATORY RESPONSE FOLLOWING REPEATED SUPEROVULATION PROCEDURE - WHETHER UTILISING ECG OR PFSH IN SHEEP AND CATTLE (CHRISTIE ET AL., 1979; TERVIT

ET AL., 1991). IN CONTRAST, MORE ANTI-GONADOTROPHINS HAVE BEEN DETECTED IN POOR AND NON-RESPONDER GOATS, FOLLOWING REPEATED SUPEROVULATION WITH PFSH (BECKERS ET AL., 1990; REMY ET AL., 1991). IT HAS ALSO BEEN SUGGESTED THAT CAPRINE OR OVINE FSH MUST BE UTILISED IF EMBRYOS ARE TO BE COLLECTED FROM A DONOR SEVERAL TIMES (CHEMINEAU ET AL., 1999). REPEATED EMBRYO COLLECTION IN GOATS IS MOSTLY INFLUENCED BY THE METHOD USED TO RECOVER EMBRYOS. GOAT EMBRYOS IN A MOET PROGRAMME ARE USUALLY COLLECTED SURGICALLY (ARMSTRONG ET AL., 1983B; BESSOUDO ET AL., 1988; NOWSHARI ET AL., 1995A; BARIL ET AL., 1996) AND TRANSFERRED WITH THE AID OF A LAPAROSCOPE (BESENFELDER ET AL., 1994). HOWEVER, SURGICAL EMBRYO FLUSHING IMPOSES SEVERAL DISADVANTAGES TO THE DONOR ANIMAL, SUCH AS SURGICAL TRAUMA DURING EXTERIORSATION OF THE REPRODUCTIVE TRACT THROUGH LAPARATOMY AND THE FORMATION OF POST-OPERATIVE ADHESIONS. THIS LIMITS THE NUMBER OF TIMES SURGICAL FLUSHINGS MAY BE PERFORMED ON THE SAME ANIMAL (MCKELVEY ET AL., 1985; ISHWAR & MEMON, 1996; PEREIRA ET AL., 1998; SUYADI ET AL., 2000). IN PAST TRIALS IT HAS BEEN INDICATED THAT GOATS OFTEN HAVE TO BE ELIMINATED FROM A MOET PROGRAMME AFTER 2 OR 3 SURGICAL PROCEDURES (REMY ET AL., 1991). THE POST-OPERATIVE ADHESIONS HAVE BEEN IDENTIFIED TO BE THE MAIN FACTOR LEADING TO A REDUCTION IN OVULATION RATE AND EMBRYO YIELD FOLLOWING REPEATED SUPEROVULATION TREATMENT (AL-KAMALI ET AL., 1985; COGNIE, 1999). NON-SURGICAL METHODS OF EMBRYO COLLECTION SUCH AS THE USE OF LAPAROSCOPY AND THE TRANSCERVICAL PASSAGE OF A CATHETER BY

MECHANICAL DILATION OF THE CERVIX OR RIPENING OF THE CERVIX WITH PROSTAGLANDIN E_2, OR OESTRADIOL HAVE BEEN PERFORMED WITH SUCCESS IN SHEEP AND GOATS (MCKELVEY ET AL., 1985; PEREIRA ET AL., 1998; WULSTER-RADCLIFFE ET AL., 1999). LAPAROSCOPIC EMBRYO COLLECTION HAS THE ADVANTAGE OF LEADING TO FEWER ADHESIONS AND PUTTING LESS STRAIN ON THE ANIMAL, WHEN COMPARED TO SURGICAL COLLECTION, BUT THE APPROACH REQUIRES SPECIAL INSTRUMENTS AND SKILLED PERSONNEL (PEREIRA ET AL., 1998; SUYADI ET AL., 2000). WHEN EMBRYOS ARE COLLECTED VIA LAPAROSCOPY, THE DONOR CAN BE COLLECTED MORE THAN 7 TIMES WITHOUT SEVERE ADHESIONS BEING FORMED, WHEREAS FOLLOWING SURGICAL COLLECTION A DECREASE IN RECOVERY RATE HAS BEEN OBSERVED AFTER THE SECOND COLLECTION, DUE TO THE FORMATION OF ADHESIONS (BARIL ET AL., 1989).

THE EMBRYO RECOVERY RATES RECORDED RANGE FROM 60 TO 78.7% FOLLOWING LAPAROSCOPIC EMBRYO FLUSHING (BARIL ET AL., 1989, FLORES-FOXWORTH ET AL., 1992). THIS IS COMPARABLE TO RECOVERY RATES REPORTED IN GOATS RANGING FROM 60 TO 90% FOLLOWING SURGICAL EMBRYO RECOVERY. AS LAPAROSCOPIC EMBRYO COLLECTION ALSO LEADS TO THE FORMATION OF ADHESIONS AND LIMITS THE NUMBER OF TIMES A DONOR CAN BE COLLECTED (ALTHOUGH LESS THAN WHEN COMPARED TO SURGICAL EMBRYO COLLECTION), MORE ATTEMPTS ARE BEING MADE REGARDING TRANSCERVICAL EMBRYO COLLECTION IN GOATS (ARMSTRONG ET AL., 1983B; NOWSHARI ET AL., 1994).

TRANSCERVICAL EMBRYO COLLECTION IN SHEEP AND GOATS HAS BEEN LIMITED IN THE PAST BECAUSE OF THE DIFFICULTY OF PASSING THE

CATHETER THROUGH THE CERVIX. HENCE THIS TECHNIQUE WAS PERFORMED UNDER ANAESTHESIA, EITHER IN VENTRAL OR DORSAL RECUMBENCY (NAGASHIMA ET AL., 1987; ISHWAR & MEMON, 1996; SUYADI ET AL., 2000). IN SHEEP, BARRY ET AL. (1990) ACHIEVED A 65% EMBRYO RECOVERY RATE VIA TRANSCERVICAL EMBRYO COLLECTION FOLLOWING THE RIPENING OF THE CERVIX OF THE DONOR ANIMAL, WITH PROSTAGLANDIN E2 AND OESTRADIOL. IN GOATS HOWEVER, EARLY RESEARCH ON TRANSCERVICAL EMBRYO COLLECTION HAS DEMONSTRATED A LOWER EMBRYO RECOVERY RATE (36.9%) (FLORES-FOXWORTH ET AL., 1992). THE DISCOVERY OF THE INDUCTION OF LUTEOLYSIS AND CONTRACTILITY OF THE UTERUS DURING FLUSHING BY INJECTING THE ANIMALS WITH PGF_{2A} BEFORE COLLECTION OF THE EMBRYOS HAS CONTRIBUTED TO AN INCREASED SUCCESS OF TRANSCERVICAL EMBRYO COLLECTION IN GOATS. USING THIS TECHNIQUE, TRANSCERVICAL EMBRYO COLLECTION CAN BE PERFORMED WITHOUT SEDATING THE DONOR ANIMAL (ISHWAR & MEMON, 1996; PEREIRA ET AL., 1998; HOLTZ, 2005). TREATING OF GOATS WITH PGF_{2A} 8 OR 16 H BEFORE EMBRYO FLUSHING HAS LED TO HIGHER NUMBER OF EMBRYOS OR OVA BEING RECOVERED, AS WELL AS A HIGHER RECOVERY RATE (PEREIRA ET AL., 1998; SUYADI ET AL., 2000). WITH THIS APPROACH EMBRYO RECOVERY RATES RANGING FROM 60 TO 80% HAVE BEEN OBTAINED IN GOATS, COMPARABLE WITH THE EMBRYO RECOVERY RATES (RANGING FROM 60 TO 90%) RECORDED FOLLOWING THE SURGICAL EMBRYO RECOVERY PROCEDURE (NOWSHARI ET AL., 1995A; SUYADI ET AL., 2000; HOLTZ, 2005). AS PREVIOUSLY STATED, TRANSCERVICAL EMBRYO COLLECTION HAS SEVERAL ADVANTAGES OVER SURGICAL AND LAPAROSCOPIC EMBRYO

COLLECTION PROCEDURES E.G. LESS TRAUMA TO THE ANIMAL, NO NEED FOR SEDATING THE ANIMAL AND NO LIMITATION TO THE NUMBER OF TIMES A DONOR CAN BE FLUSHED. THIS PROCEDURE HENCE HOLDS THE POTENTIAL FOR MORE POPULAR UTILISATION IN GOATS, IF FURTHER INVESTIGATION COULD IMPROVE THE EFFICIENCY THIS PROCEDURE. HOLTZ ET AL. (2000) REPORTED GOATS TO BE FLUSHED 10 TIMES WITHIN ONE YEAR WHEN TRANSCERVICAL EMBRYO COLLECTIONS WERE PERFORMED.

ALTHOUGH THE TREATMENT OF DONORS WITH PGF2A BEFORE THE TRANSCERVICAL FLUSHING OF EMBRYOS HAS BEEN A BREAKTHROUGH IN GOAT MOET PROGRAMMES, THIS PROCEDURE STILL HAS A MAJOR CONSTRAINT IN THE TIME REQUIRED TO RECOVER THE EMBRYOS FROM AN INDIVIDUAL ANIMAL. PEREIRA ET AL. (1998) REPORTED 24 EMBRYO FLUSHES TO BE REQUIRED, OF WHICH 12 FLUSHES TOOK ABOUT 45 MIN PLUS A 2H PAUSE BETWEEN THE FIRST AND THE SECOND 12 EMBRYO FLUSHES. THE WHOLE PROCEDURE WAS RECORDED TO TAKE ABOUT 4H. IN AN ATTEMPT TO REDUCE THE TIME SPENT FLUSHING EMBRYOS, SUYADI ET AL. (2000) EVALUATED THE EFFECT OF INJECTING THE DONOR ANIMALS WITH PGF2A, 24H PRIOR TO FLUSHING, PLUS AN ADDITIONAL OXYTOCIN INJECTION. THESE RESEARCHERS OBSERVED A REDUCTION IN THE DURATION OF THE FLUSHING PERIOD (63 MIN), BUT NO EFFECT OF BOTH TREATMENTS ON EMBRYO RECOVERY RATE. HOLTZ ET AL. (2000) ALSO REPORTED A DURATION PERIOD OF 30 TO 40 MIN FLUSHING PER DOE, WHEN THE FLUSHINGS WERE REDUCED TO 20. HOWEVER, THE TIME OF THE FLUSHING PROCEDURE WHEN THE GOATS ARE TREATED WITH PGF_{2A} AND OXYTOCIN 24H BEFORE FLUSHING FOR COMMERCIAL EMBRYO

RECOVERY PURPOSES IS STILL THE MAJOR CONSTRAINT FACING TRANSCERVICAL EMBRYO COLLECTION IN GOATS. THUS AT THE END OF THE DAY, SURGICAL EMBRYO COLLECTION STILL REMAINS THE MOST COMMONLY UTILISED METHOD IN GOAT MOET PROGRAMMES.

2.2.4. Fertilisation failure in goats

THE YIELD OF FERTILISED OVA/EMBRYOS FOLLOWING SUPEROVULATION IS MAINLY RELATED TO THE POOR SYNCHRONISATION OF OESTRUS AND OVULATION, ESPECIALLY FOLLOWING FIXED-TIME AI (BARIL ET AL., 1989; BARIL & VALLET, 1990; KAFI & MCGOWAN, 1997; COGNIE ET AL., 2003). IN GOATS, ASYNCHRONY OF OESTRUS HAS BEEN COMMONLY OBSERVED FOLLOWING PROGESTAGEN WITHDRAWAL IN FSH TREATED FEMALES. THE ONSET OF OESTRUS IN ANGORA, ALPINE AND SAANEN GOATS HAS BEEN REPORTED TO RANGE FROM 24 TO 54 H FOLLOWING INTRAVAGINAL PROGESTAGEN WITHDRAWAL. THE DOES WITH THE SHORTEST INTERVAL FROM INTRAVAGINAL SPONGE WITHDRAWAL TO THE OCCURRENCE OF OESTRUS RECORDED A HIGHER OVULATION RATE, COMPARED TO DOES TAKING A LONGER TIME TO EXHIBIT BEHAVIOURAL SIGNS OF OESTRUS. THIS LARGE VARIATION IN THE TIME INTERVAL FROM PROGESTAGEN WITHDRAWAL TO THE ONSET OF THE INDUCED OESTROUS PERIOD INDICATES THAT THE TIME OF OVULATION CANNOT BE PREDICTED BASED ONLY ON THE ONSET OF OESTRUS. POOR FERTILISATION RATES RESULT, ESPECIALLY IN FIXED-TIME AI, AS A RESULT OF POOR SYNCHRONISATION OF OESTRUS AND OVULATION (BARIL ET AL., 1989; BARIL & VALLET, 1990). POORER SYNCHRONISATION EFFICIENCY OF OVULATION IS USUALLY OBSERVED FOLLOWING SUPEROVULATION IN GOATS, COMPARED TO SHEEP.

IT HAS BEEN REPORTED THAT IN GOATS ONLY 8.8% OVULATIONS OCCURRED 50H FOLLOWING INTRAVAGINAL SPONGE WITHDRAWAL, WHILE 88% OF THE OVULATIONS OCCURRED FROM BETWEEN 50 TO 80H, A CLEAR INDICATION THAT MOST GOATS IN A FIXED-TIME AI PROGRAMME (E.G. 36 H AND 48 H) WILL BE INSEMINATED TOO EARLY WITH RESPECT TO THE TIME OF OVULATION. IN GOATS, IT HAS ALSO BEEN REPORTED THAT THE DISTRIBUTION OF OVULATION WITHIN FEMALES CAN BE ATTRIBUTED TO THE TIME INTERVAL FROM THE ONSET OF OESTRUS TO THE ONSET OF THE PRE-OVULATORY LH SURGE, AS WELL AS THE INTERVAL FROM THE FIRST TO THE LAST OVULATION (COGNIE ET AL., 2003; GONZALEZ-BULNES ET AL., 2004A).

IN SHEEP, EFFICIENT SYNCHRONISATION OF THE LH SURGE HAS BEEN REPORTED FOLLOWING PROGESTAGEN REMOVAL (MEAN OF 56±6H) AND THE AVERAGE TIME FROM THE FIRST TO THE LAST OVULATION REPORTED AS 6H, WHICH IS RELATIVELY SHORT. HOWEVER, IN GOATS THE DISTRIBUTION IN THE TIME OF THE LH SURGE HAS BEEN REPORTED TO RANGE FROM 24 TO 64H FOLLOWING PROGESTAGEN TERMINATION (MEAN OF 63±9H) AND THE AVERAGE TIME FROM THE FIRST TO THE LAST OVULATION TO BE 12H - WHICH IS LONG, COMPARED TO THE 6 H IN SHEEP (COGNIE ET AL., 2003).

IN A MOET PROGRAMME INVOLVING FIXED-TIME AI, THE TIMING OF OVULATION IS CRITICAL FOR OVERALL FERTILITY RESULTS AND THEREFORE THE INDUCTION OF THE LH PEAK WITH EXOGENOUS GNRH AND LH MAY GIVE BETTER RESULTS. IN SHEEP SUPEROVULATED WITH EITHER FSH OR ECG, THE NUMBER OF FERTILISED OVA RECOVERED WAS INCREASED BY TREATING THE EWES WITH GNRH (WALKER ET AL., 1989; NAQVI & GULYANI, 1998). IN GOATS, NOT ONLY WAS THE OVULATION RATE

INCREASED BY GNRH TREATMENT FOLLOWING SUPEROVULATION WITH ECG, BUT ALSO THE OVULATION TIME WAS SYNCHRONISED - LEADING TO 91% OF THE OVULATIONS OCCURRING BETWEEN 36 AND 48H FOLLOWING SPONGE REMOVAL. IN GOATS TREATED WITH ONLY ECG, OVULATIONS CONTINUED TO OCCUR UNTIL UP TO 77H FOLLOWING INTRAVAGINAL SPONGE WITHDRAWAL. THIS OBSERVATION INDICATES THAT THE FERTILISATION RATE COULD BE IMPROVED IN GOATS SUPEROVULATED WITH FSH BY TREATING THE ANIMALS WITH GNRH, AS THE OCCURRENCE OF OVULATION COULD BE SYNCHRONISED (CAMERON ET AL., 1988).

LOW EMBRYO RECOVERY RATES DUE TO FERTILISATION FAILURE FOLLOWING SUPEROVULATION HAVE BEEN REPORTED IN MOST SPECIES (EVANS & ARMSTRONG, 1984; HAWK, 1988). THIS HAS BEEN LARGELY ASCRIBED TO THE FAILURE OF SPERM TO CAPACITATE AND IMPEDED SPERM TRANSPORT THROUGH THE UTERUS TO THE FALLOPIAN TUBE WHERE FERTILISATION TAKES PLACES (ARMSTRONG & EVANS, 1983; EVANS & ARMSTRONG, 1984; HAWK, 1988; KAFI & MCGOWAN, 1997; COGNIE, 2003). IMPEDED SPERM TRANSPORT HAS BEEN LARGELY ASSOCIATED WITH THE METHOD OF MATING, WHERE NATURAL MATING AND CERVICAL INSEMINATION LED TO HIGH INCIDENCES OF FERTILISATION FAILURE AND INTRAUTERINE INSEMINATIONS RESULTED IN IMPROVED FERTILITY IN EWES FOLLOWING SUPEROVULATION (BOLAND, ET AL. 1983; REXROAD & POWELL, 1990; ISHWAR & MEMON, 1996). THERE IS HOWEVER, AMPLE RESEARCH INDICATING THAT SUPEROVULATION ALSO LEADS TO THE IMPAIRMENT OF SPERM TRANSPORT (MOORE & EPPLESTON, 1979; BARIL ET AL., 1989; BREBION ET AL., 1992). IN MOST SPECIES,

FERTILISATION FAILURE IS NORMALLY INDICATED BY A LACK OF SPERM RECOVERY AND SPERM NUMBERS IN THE UTERUS AND OVIDUCTS (SITE OF FERTILISATION) FOLLOWING SUPEROVULATION, WITH EITHER FSH OR ECG (EVANS & ARMSTRONG, 1984; HAWK, 1988). THIS IMPAIRMENT OF SPERM TRANSPORT FOLLOWING SUPEROVULATION HAS BEEN SUGGESTED TO RESULT FROM PERI-OVULATORY ELEVATED OESTROGEN LEVELS (ARMSTRONG AT AL., 1983B; GREVE ET AL., 1995). IN GOATS IMPEDED SPERM TRANSPORT FOLLOWING SUPEROVULATION HAS ALSO BEEN A RESULT OF PREMATURE OVULATION, AS EARLY OVULATIONS LEAD TO AN INCREASE IN CIRCULATING PROGESTERONE CONCENTRATIONS DURING THE PRE-OVULATORY PERIOD (CAMERON ET AL., 1988). LASTLY, THIS PHENOMENON OF SPERM IMPAIRMENT REDUCES THE FERTILISATION RATE, AS THOSE OVA RELEASED EARLY WILL NEVER BE FERTILISED BY THE TIME THE SPERM REACH THE OVIDUCT (MOOR ET AL., 1984).

FERTILISATION FAILURE IN GOATS COULD ALSO BE ATTRIBUTED TO THE ABNORMAL MATURATION OF THE OOCYTES FOLLOWING SUPEROVULATION (KUMAR ET AL., 1990, 1991). ABNORMALITIES IN OOCYTE MATURATION ARE GENERALLY REFLECTED BY THE PREMATURE CONDENSATION OF CHROMATIN IN THE GOAT OOCYTES, WHICH IS A SIGN OF PREMATURE ACTIVATION OF THE INITIAL STAGES OF MEIOTIC MATURATION (CAMERON ET AL., 1988; KUMAR ET AL., 1990). PREMATURE ACTIVATION OF OOCYTE MATURATION HAS ALSO BEEN ALSO REPORTED IN OTHER SPECIES FOLLOWING SUPEROVULATION. THE OCCURRENCE OF THIS PHENOMENON HAS BEEN REPORTED TO BE HIGHER IN ANIMALS SUPEROVULATED WITH ECG THAN IN FSH TREATED ANIMALS (MOOR ET AL., 1984; MOORE,

1985; CALLESEN ET AL., 1987; KUMAR ET AL., 1990; HYTTEL ET AL., 1991). THIS PHENOMENON IS THE RESULT OF INCREASED BLOOD LH CONCENTRATIONS, WHICH IS PREDOMINANT FOLLOWING ECG TREATMENT WITH LH-LIKE BIOACTIVITY INHERENT TO THIS GONADOTROPHIN (MOORE, 1985, CAMERON ET AL., 1988, MCNATTY ET AL., 1989; KUMAR ET AL., 1991). THIS CONDITION CONTRIBUTES TO MAJOR EMBRYONIC LOSS FOLLOWING SUPEROVULATION, AS SOME OF THESE ACTIVATED OOCYTES WILL BE LEFT AS LUTEINIZED FOLLICLES, WHILE OTHERS WILL BE OVULATED AS OLD EGGS AND WILL END UP BEING ABNORMAL EMBRYOS (MOOR ET AL., 1984).

2.2.5. Nutritional effect on reproduction

NUTRITION HAS BEEN KNOWN TO PLAY A FUNDAMENTAL ROLE IN CONTROLLING SEVERAL REPRODUCTIVE EVENTS INCLUDING, HORMONE PRODUCTION, GAMETOGENESIS, FERTILISATION AND EARLY EMBRYONIC DEVELOPMENT IN FARM ANIMALS (ASHWORTH, 1995; COX, 1997; BUTLER, 2000; BOLAND ET AL., 2001; LUCY, 2003; PEURA ET AL., 2003; PAULA ET AL., 2005). IN GOATS, A LOW LEVEL OF NUTRITION FOR EXAMPLE, CAN LEAD TO A LOSS IN BODY WEIGHT, BODY CONDITION AND A REDUCTION IN OVULATION RATE (MANI ET AL., 1992). IN SHEEP, LOW FEED INTAKE LEVELS ALSO REDUCE THE OVULATION RATE, WHILE DIETS SUPPLEMENTED WITH HIGH ENERGY AND PROTEIN CONCENTRATES CAN INCREASE THE OVULATION RATE IN EWES WITH A LOW BODY CONDITION. IN ALL THESE FINDINGS, NUTRITION HAS A DEFINED INFLUENCE ON OVULATION RATE, SUGGESTING THAT NUTRITION MAY PLAY A ROLE IN OOCYTE DEVELOPMENT, THE COMPETENCE AND MORPHOLOGY THEREOF (SMITH, 1991; DOWNING ET AL., 1995).

WHILE TRYING TO DETERMINE THE INTERACTION OF NUTRITION ON OVULATION RATE, IT HAS ALSO BEEN SHOWN THAT THE NUTRITIONAL EFFECTS ARE MEDIATED AT THE HYPOTHALAMO-PITUITARY LEVEL. BASED ON THIS ASPECT, SEVERAL NUTRITIONAL EFFECTS HAVE BEEN RECOGNISED WHEN ANIMALS ARE IN A NEGATIVE ENERGY BALANCE E.G. THE INHIBITION OF GNRH SECRETION BY THE HYPOTHALAMUS, ABSENCE OF LH PULSES, LOW FSH CONCENTRATIONS, INHIBITION OF FOLLICULOGENESIS AND A HIGH NEGATIVE FEEDBACK SENSITIVITY (DOWNING & SCARAMUZZI, 1991; GONG, 2002; WADE & JONES, 2005; SCARAMUZZI ET AL., 2006). MOREOVER, THE FEEDING OF THE ANIMALS WITH DIETS ABOVE MAINTENANCE HAS BEEN ASSOCIATED WITH AN INCREASE IN BLOOD GLUCOSE, INSULIN AND LEPTIN, LEADING TO INCREASED FOLLICULOGENESIS AND OVULATION RATES (ABECIA ET AL., 2006; SCARAMUZZI ET AL., 2006). DIETARY ENERGY HAS BEEN INDICATED TO ALTER THE FOLLICULAR DYNAMICS IN SUPEROVULATED RUMINANTS (GONG, 2002). HOWEVER THE RESPONSE TO THE NUTRITIONAL INFLUENCE FOLLOWING SUPEROVULATION AND EMBRYO TRANSFER HAS BEEN VARIABLE AND INCONSISTENT. THERE IS A LACK OF INFORMATION REGARDING THE EFFECTS OF DIETARY ENERGY ON THE RESPONSE TO SUPEROVULATION IN GOATS. SOME STUDIES INDICATED THAT SHEEP NUTRITION HAS FAILED TO AFFECT THE RESPONSE TO SUPEROVULATION, WITH NO DIFFERENCES BEING RECORDED IN THE NUMBER OF FOLLICLES ASPIRATED AND OOCYTES RECOVERED BETWEEN HIGH AND LOW ENERGY DIET GROUPS (MCEVOY ET AL., 1995; PEURA ET AL., 2003; KAKAR ET AL., 2005; BOROWCZYK ET AL., 2006). ON THE OTHER HAND, SUPEROVULATED EWES ON A LOWER ENERGY DIET

HAVE BEEN SHOWN TO PRODUCE FEWER FOLLICLES (O'CALLAGHAN ET AL., 2000).

IN TRIALS DESIGNED TO DETERMINE THE EFFECT OF ENERGY RESTRICTION OR NUTRITIONAL INTAKE BEFORE AND DURING SUPEROVULATION IN CATTLE, AN INCREASE IN THE NUMBER OF FOLLICLES AND IMPROVEMENT IN THE QUALITY OF EMBRYOS HAS BEEN REPORTED IN ANIMALS FED A LOW ENERGY DIET, COMPARED TO THOSE ON A HIGH ENERGY DIET (NOLAN ET AL., 1998). HOWEVER, THE QUALITY OF EMBRYOS MATURED IN VITRO WAS INFLUENCED BY THE RESTRICTION OF FEED. IN THIS CASE OOCYTES DERIVED FROM UNDERFED ANIMALS YIELDED LOWER CLEAVAGE AND BLASTOCYST RATES (BOROWCZYK ET AL., 2006).

THE EFFECT OF NUTRITION AND METABOLIC HORMONES ON THE RESPONSE TO SUPEROVULATION HAS BEEN WIDELY DOCUMENTED IN SHEEP AND CATTLE AND LESS IN GOATS. WHILE EXPECTING AN INCREASE IN THE RESPONSE TO SUPEROVULATION FOLLOWING SUPPLEMENTATION WITH HIGHER ENERGY DIETS, THE CONTRARY WAS TRUE. IN SHEEP AND CATTLE LOWER SUPEROVULATORY RESPONSE, IN TERMS OF ANIMALS OVULATING, OVULATION RATE PER ANIMAL OVULATING AND THE QUALITY OF EMBRYOS RECOVERED HAVE BEEN OBSERVED IN ANIMALS FED DIETS OF HIGH ENERGY CONCENTRATES (BLANCHARD ET AL., 1990; YAAKUB ET AL., 1997; LOZANO ET AL., 2003). POOR SUPEROVULATORY RESPONSES HAVE BEEN ASSOCIATED WITH AN INCREASE IN INSULIN OR IGF-I (INSULIN-LIKE GROWTH FACTOR I), OBSERVED IN ANIMALS ON HIGH ENERGY INTAKES. IN SUPPORTING THIS OBSERVATION, IT HAS BEEN FOUND THAT UNDER-NUTRITION CAN REDUCE THE CONCENTRATION OF IGF-I IN SHEEP AND CATTLE (GONG, 2002; LOZANO ET AL., 2003).

HIGH CONCENTRATIONS OF INSULIN AND IGF-I HAVE BEEN REPORTED TO REDUCE THE AMOUNT OF FSH NEEDED TO SUPPORT THE GONADOTROPHIN-DEPENDENT FOLLICLES (ADASHI ET AL., 1985; DOWNING & SCARAMUZZI, 1991; YAAKUB ET AL., 1997).THIS EXPLAINS THE LOW SUPEROVULATORY RESPONSE INDUCED FOLLOWING HIGH ENERGY INTAKE DIETS. IN OTHER TRIALS, DIETS WITH A HIGH UREA CONCENTRATION INCREASED THE EMBRYO MORTALITY AND REDUCED THE PREGNANCY RATES FOLLOWING EMBRYO TRANSFER IN SHEEP (MCEVOY ET AL., 1997). OTHER STUDIES INDICATED THAT, DIETS SUPPLEMENTED WITH CRUDE PROTEIN HAVE BEEN SHOWN TO HAVE NO EFFECT ON THE OVULATION RATE AND THE NUMBER OF EMBRYOS RECOVERED FOLLOWING SUPEROVULATION IN CATTLE (GARCIA-BOJALIL ET AL., 1994; GATH ET AL., 1999; MIKKOLA ET AL., 2005). ALTHOUGH THESE RESULTS ARE INCONSISTENT, IT CAN BE SUGGESTED FROM THESE OBSERVATIONS THAT THE TYPE OF NUTRIENTS HAS DIFFERENT EFFECTS ON THE RESPONSE TO SUPEROVULATION. HOWEVER, IN CATTLE DIFFERENT TYPES OF CONCENTRATES FED TO AN ANIMAL FAILED TO HAVE ANY EFFECT ON THE RESPONSE TO SUPEROVULATION, ALTHOUGH THE TYPE OF CONCENTRATES INFLUENCED THE QUALITY OF EMBRYOS RECOVERED (YAAKUB ET AL., 1997). BLANCHARD ET AL. (1990) SUPPORTED THIS FINDING BY REPORTING AN INFERIOR EMBRYO QUALITY FOLLOWING THE FEEDING OF EXCESS CRUDE PROTEIN. THE EFFECT OF SUPPLEMENTARY FEEDING WITH ENERGY OR PROTEIN CONCENTRATES ON SUPEROVULATED ANIMALS IS ALSO HIGHLY DEPENDENT ON THE BODY CONDITION OF THESE TREATED ANIMALS. THIS HAS BEEN DEMONSTRATED BY AN OBSERVATION IN GOATS WHERE DOES IN A POOR BODY CONDITION RECORDED A HIGHER

SUPEROVULATORY RESPONSE AS INDICATED BY THE INCREASE IN OVULATION RATE AND HIGH NUMBER OF OOCYTES COLLECTED. NUTRITIONAL PRIMING HOWEVER REDUCED THE NUMBER OF OOCYTES PRODUCED IN OVER-CONDITIONED DOES (BUZZEL ET AL., 2003).

OVERFEEDING AND UNDERFEEDING CAN DELAY EMBRYONIC DEVELOPMENT AND INCREASE THE EMBRYO MORTALITY RATE FOLLOWING FERTILISATION (PARR ET AL., 1987; MANI ET AL., 1992; RHIND, 1992; ABECIA ET AL., 1996, 1997). FOLLOWING SUPEROVULATION, AD LIBITUM DIETS MAY ALSO REDUCE THE QUALITY AND QUANTITY OF EMBRYOS RECOVERED. THE HIGH ENERGY DIET INTAKE DOES NOT ONLY HAVE A DETRIMENTAL EFFECT ON THE MORPHOLOGICAL CLASSIFICATION AND CLEAVAGE RATE OF THE EMBRYOS PRODUCED IN VIVO, BUT ALSO EXERTS NEGATIVE EFFECTS ON THE DEVELOPMENT OF THE IN VITRO EMBRYO COLLECTED AS EARLY AS DAY 2 OF PREGNANCY (CREED ET AL., 1994; LOZANO ET AL., 2003). IN SUPPORT OF THESE FINDINGS IT HAS ALSO BEEN FOUND THAT DIETARY RESTRICTION CAN INCREASE THE NUMBER OF FOLLICLES AND OVULATION RATE IN SUPEROVULATED HEIFERS (NOLAN ET AL., 1998). THESE PHENOMENA HAVE LED TO THE CONCLUSION THAT A HIGH ENERGY INTAKE CAN NEGATIVELY AFFECT THE EMBRYONIC DEVELOPMENT, PARTIALLY BEFORE FERTILISATION AND DURING THE ATTAINMENT OF OOCYTE DEVELOPMENTAL COMPETENCE. THE EFFECT OF DIETARY INTAKE ON EMBRYONIC DEVELOPMENT HAS ALWAYS BEEN RELATED TO THE CIRCULATING PROGESTERONE CONCENTRATION, AS THIS HORMONE IS RESPONSIBLE FOR THE MAINTENANCE OF PREGNANCY (LOZANO ET AL., 2003).

THERE ARE MANY FINDINGS CONSISTENTLY REPORTING AN INVERSE RELATIONSHIP BETWEEN THE DIETARY INTAKE AND BLOOD PROGESTERONE CONCENTRATION IN RUMINANTS (PARR ET AL., 1987; CREED ET AL., 1994; MCEVOY ET AL., 1995; ABECIA ET AL., 2006). ANIMALS ON A HIGH ENERGY DIET HAVE BEEN REPORTED TO HAVE LOWER PROGESTERONE CONCENTRATIONS, COMPARED TO THOSE ON LOWER DIETARY ENERGY INTAKES OR ON RESTRICTED DIETS (NOLAN ET AL., 1998; YAAKUB ET AL., 1997; O'CALLAGHAN ET AL., 2000; LOZANO ET AL., 2003). THE DECREASE IN BLOOD PROGESTERONE CONCENTRATION OF ANIMALS ON A HIGH PLANE OF NUTRITION IS THE RESULT OF THE INCREASED METABOLISM OF PROGESTERONE BY THE LIVER (SYMONDS & PRIME, 1989; PARR, 1992; PARR ET AL., 1993; ABECIA ET AL., 2006). THE FINDINGS OF PARR ET AL. (1993) CONFIRMED THAT THE MEAN BLOOD FLOW IN THE PORTAL VEINS OF EWES FED HIGH ENERGY DIETS WAS HIGHER, WHEN COMPARED TO EWES ON LOW ENERGY DIETS AND 90% OF THE PROGESTERONE PASSING THROUGH THE GUT WAS METABOLISED. THE REDUCED CIRCULATING PROGESTERONE CONCENTRATION DUE TO A HIGH ENERGY INTAKE IN SUPEROVULATED EWES HAS BEEN REPORTED TO REDUCE THE VIABILITY OF THE EMBRYOS PRODUCED IN VIVO AND CULTURED IN VITRO. THIS HAS BEEN EMPHASISED BY THE REDUCTION IN NUMBER OF EMBRYOS REACHING THE BLASTOCYST STAGE IN EWES FED HIGH ENERGY DIETS. THIS CONFIRMS THAT A LOWER CONCENTRATION OF PROGESTERONE, AS A RESULT OF FEEDING HIGHER ENERGY DIETS DURING PRE-OVULATORY PERIOD (SUPEROVULATION), CAN AFFECT EMBRYO SURVIVAL RATE BY ALTERING THE OOCYTE DEVELOPMENT AND OVIDUCTAL ENVIRONMENT (MCEVOY ET AL., 1995).

2.3. Intrinsic factors affecting MOET in goats

2.3.1. Breed effect

BREED OR GENOTYPE HAS BEEN INDICATED IN SEVERAL STUDIES TO BE A FACTOR TO BE TAKEN INTO CONSIDERATION IN MOET PROGRAMMES (HOLNESS ET AL., 1980; DONALDSON, 1984; BINDON ET AL., 1986; NUTI ET AL., 1987; BARIL ET AL., 1989; DUFOUR ET AL., 2000). IT SHOULD BE NOTED THAT IN THE FIRST STEP (OESTROUS SYNCHRONISATION) OF A MOET PROGRAMME, DIFFERENT BREEDS RESPOND DIFFERENTLY. THIS REQUIRES DIFFERENT TIMINGS OF AI WITH RESPECT TO OVULATION. WHEN ALPINE AND NUBIAN DOES WERE COMPARED, 87% OF THE NUBIAN GOATS CAME INTO OESTRUS 36H FOLLOWING SPONGE REMOVAL, WHILE ONLY 50% OF THE ALPINE GOATS SHOWED SIGNS OF OESTRUS (NUTI ET AL., 1987). THE ONSET OF OESTRUS HAS A BIG INFLUENCE ON THE OVULATION RATE FOLLOWING SUPEROVULATION. WHEN THIS PARAMETER WAS EVALUATED, A HIGHER OVULATION RATE WAS RECORDED IN ALPINE GOATS THAT EXHIBITED SIGNS OF OESTRUS WITHIN 24H AFTER PROGESTAGEN WITHDRAWAL, COMPARED TO DOES THAT SHOWED OVERT OESTRUS 24H FOLLOWING PROGESTAGEN REMOVAL. TO THE CONTRARY, NO DIFFERENCES WERE OBSERVED WITH RESPECT TO THE OVULATION RATE BETWEEN SAANEN DOES WHICH EXHIBITED SIGNS OF OESTRUS BEFORE OR 24H FOLLOWING PROGESTAGEN TREATMENT TERMINATION. THIS OBSERVATION INDICATES THAT THE RESPONSE TO OESTROUS SYNCHRONISATION AND SUPEROVULATION TO BE BREED SPECIFIC (BARIL ET AL., 1989).

BESIDES THE DIFFERENT TIMES TO THE ONSET OF OESTRUS RECORDED IN DIFFERENT RUMINANT BREEDS, BREED HAS BEEN INDICATED AS A MAJOR FACTOR CONTRIBUTING TO THE VARIATION

RECORDED IN THE OVARIAN RESPONSE TO SUPEROVULATION (DONALDSON, 1984; TORRES ET AL., 1987; VIVANCO ET AL., 1994; AMMOUN ET AL., 2006). IN CATTLE, SHEEP AND GOATS THE NUMBER OF CORPORA LUTEA RECORDED AND NUMBER OF TRANSFERABLE EMBRYOS RECOVERED FOLLOWING SUPEROVULATION DIFFER BETWEEN BREEDS (DONALDSON, 1984; BARIL ET AL., 1989; GOEL & AGRAWAL, 2005; AMMOUN ET AL., 2006). IN GOATS FOR EXAMPLE, A HIGHER NUMBER OF EMBRYOS (AVERAGE OF 10.1) WERE RECOVERED IN ALPINE DOES, COMPARED TO ANGORA DOES (AVERAGE OF 7.5) (BARIL ET AL., 1989). IN ADDITION, A HIGHER REFRACTORINESS WAS RECORDED IN ALPINE GOATS, COMPARED TO NUBIAN GOATS FOLLOWING SUPEROVULATION (NUTI ET AL., 1987). HOWEVER, THE BREED EFFECT HAS BEEN ASSOCIATED WITH THE DIFFERENT PROLIFICACY OF THE BREEDS, WHERE A HIGH PROLIFIC BREED HAS BEEN REPORTED TO RESPOND BETTER TO EXOGENOUS GONADOTROPHINS (BINDON ET AL., 1986). IT HAS ALSO BEEN FOUND THAT SHEEP SELECTED FOR PROLIFICACY TEND TO BE MORE SENSITIVE TO GONADOTROPHIN TREATMENT (KELLY ET AL., 1983; BINDON ET AL., 1986). IN CONTRAST, PICAZO ET AL. (1996) FAILED TO ESTABLISH A CLEAR BREED DIFFERENCE IN OVARIAN RESPONSE IN THREE SHEEP BREEDS SUPEROVULATED WITH FSH.

GENOTYPE HAS BEEN RECOGNISED TO HAVE A MAJOR EFFECT ON OVARIAN FOLLICULAR DEVELOPMENT. EVEN THOUGH GONADOTROPHIN TREATMENT INCREASES THE FOLLICULAR DEVELOPMENT IN ALL BREEDS, THE NUMBERS OF OVARIAN FOLLICLES WHICH ARE RECRUITED TO OVULATE DIFFER IN THE DIFFERENT BREEDS (DUFOUR ET AL., 2000; AMMOUN ET AL., 2006). IN SHEEP FOR EXAMPLE, IT HAS BEEN OBSERVED THAT

EWES CARRYING THE *FEC* GENE HAVE A HIGHER OVARIAN FOLLICLE SELECTION RATE. THEREFORE, A LOWER OVULATION RATE FOLLOWING SUPEROVULATION HAS BEEN RECORDED IN THIS BREED, DUE TO THE HIGH ATRETIC RATE RECORDED AMONG RECRUITED FOLLICLES. ON THE OTHER HAND, EWES WITHOUT THE *FEC* GENE HAVE SHOWN LESS FOLLICULAR ATRESIA, MAKING MORE FOLLICLES AVAILABLE TO BE RECRUITED AND PROCEEDING TO THE OVULATION STAGE, HENCE LEADING TO A HIGH OVULATION RATE FOLLOWING SUPEROVULATION (DUFOUR ET AL., 2000).

2.3.2. Seasonal effects on MOET

SMALL RUMINANTS ARE SEASONAL BREEDERS THAT BREED SPONTANEOUSLY DURING AUTUMN TO WINTER AND IN SPRING (SHORT DAYLIGHT LENGTH). THUS MOET IS MORE FREQUENTLY CONDUCTED DURING THE BREEDING SEASON. HOWEVER, IT WOULD BE A GREAT ADVANTAGE TO INCREASE THE NUMBER OF OFFSPRING BORN PER DONOR PER YEAR BY BEING ABLE TO CONDUCT THIS PROGRAMME THROUGHOUT THE YEAR. HOWEVER, CONFLICTING RESULTS HAVE BEEN REPORTED IN STUDIES DESIGNED TO DETERMINE THE EFFECT OF SEASON ON MOET. THERE IS GENERAL AGREEMENT THAT SEASON HAS AN EFFECT ON THE OVARIAN RESPONSE TO SUPEROVULATION TREATMENT. DIFFERENCES HAVE BEEN OBSERVED IN OVARIAN RESPONSE CONCERNING CORPUS LUTEUM FORMATION AND FUNCTION, LH PEAK AND PROGESTERONE CONCENTRATION, AS WELL AS THE NUMBER AND QUALITY OF EMBRYOS RECOVERED DURING THE DIFFERENT SEASONS. A HIGHER OVULATION RATE AND THE NUMBER OF EMBRYOS RECOVERED HAVE ALSO BEEN RECORDED EARLY IN THE BREEDING SEASON, COMPARED TO LATE IN THE BREEDING SEASON IN GOATS (PENDLETON ET AL., 1986; WALKER

ET AL., 1989; SEBASTIAN-LOPEZ ET AL., 1990; MITCHELL ET AL., 2002).

THE NUMBER OF EMBRYOS RECOVERED AND VIABLE EMBRYOS HAS BEEN SHOWN TO BE HIGHEST DURING THE BREEDING SEASON. HOWEVER, THE DIFFERENCES RECORDED HAVE NOT BEEN THAT GREAT BETWEEN THE BREEDING SEASON AND THE OTHER MONTHS OF THE YEAR. THIS OBSERVATION HAS PRODUCED MORE DOUBT REGARDING THE EFFECT OF SEASON ON THE OVARIAN RESPONSE TO SUPEROVULATION IN GOATS. IT HAS ALSO BEEN OBSERVED THAT IN DOES, THE NUMBER OF LARGE UNOVULATORY FOLLICLES FOLLOWING SUPEROVULATION TO BE MORE PROMINENT DURING THE SEASONAL ANOESTROUS PERIOD (BARIL ET AL., 1989; SENN & RICHARDSON, 1992; GONZALEZ-BULNES ET AL., 2003A). IN SHEEP HOWEVER, THE NUMBER OF UNOVULATORY FOLLICLES AT EMBRYO RECOVERY FOLLOWING SUPEROVULATION WITH ECG WAS RECORDED TO BE HIGHER IN AUTUMN THAN IN SPRING. THIS LED TO A LOWER NUMBER OF EMBRYOS BEING RECOVERED IN AUTUMN, THAN IN SPRING (CHAGAS E SILVA ET AL., 2003).

IN CATTLE THE NUMBER OF TRANSFERABLE EMBRYOS RECOVERED WAS SHOWN TO BE GREATLY INFLUENCED BY SEASON (TEGEGNE ET AL., 1997). CONTRARY, SEASON IN BOTH SHEEP AND GOATS HAVE BEEN REPORTED TO HAVE NO EFFECT ON THE NUMBER OF CORPORA LUTEA, LARGE UNOVULATORY FOLLICLES, FERTILISATION RATE, OVA AND EMBRYO RECOVERED, QUALITY OF TRANSFERABLE EMBRYOS, AS WELL AS THE EMBRYONIC SURVIVAL RATES OBSERVED FOLLOWING TRANSFER. THESE CONTRADICTORY RESULTS WITH RESPECT TO THE EFFECT OF SEASON ON OVARIAN RESPONSE TO SUPEROVULATION, MAY INDICATE THAT MOET PROGRAMMES IN SMALL

STOCK CAN BE PERFORMED THROUGHOUT THE YEAR, WITHOUT A REDUCTION IN OVARIAN RESPONSE TO SUPEROVULATION AND THE QUALITY OF EMBRYOS RECOVERED (LOPEZ-SEBASTIAN ET AL., 1990; GREANEY ET AL., 1991; SAMARTZI ET AL., 1995; MITCHELL ET AL., 2002; GONZALEZ-BULNES ET AL., 2003B).

TO HIGHLIGHT THE USE OF MOET THROUGHOUT THE YEAR, GREANEY ET AL. (1991) RECORDED A HIGHER OVULATION RATE OUTSIDE THE BREEDING SEASON IN SHEEP, COMPARED TO DURING THE BREEDING SEASON. THE LOW OVULATION RATE OBSERVED DURING THE BREEDING SEASON COULD LARGELY BE ATTRIBUTED TO THE PRESENCE OF A LARGE FOLLICLE AT THE ONSET OF THE SUPEROVULATION TREATMENT. IN SHEEP THE OCCURRENCE OF A LARGE OVARIAN FOLLICLE AT THE TIME THE FIRST SUPEROVULATION TREATMENT WAS MORE PROMINENT DURING THE BREEDING SEASON. ALTHOUGH THE PRESENCE OF LARGE FOLLICLES AT THE ONSET OF SUPEROVULATION DID NOT HAVE ANY EFFECT ON THE OVULATION RATE, THE NUMBER AND QUALITY OF EMBRYOS RECOVERED WAS REDUCED DURING THE BREEDING SEASON. THIS THEREFORE, EMPHASISES THAT FOLLICULAR DOMINANCE IS MORE PROFOUND DURING THE BREEDING SEASON THAN DURING THE ANOESTROUS PERIOD (GONZALEZ-BULNES ET AL., 2003B).

2.3.3. Donor age effect on MOET

FOR MOET TO BE EFFICIENT IN ACCELERATING GENETIC IMPROVEMENT, ONE METHOD IS BY SHORTENING THE GENERATION INTERVAL. THIS COULD BE ACHIEVED BY UTILISING YOUNG ANIMALS IN THE PROGRAMME. SEVERAL STUDIES HAVE BEEN CONDUCTED TO EVALUATE THE EFFECT OF AGE ON THE OVARIAN RESPONSE TO SUPEROVULATION. THERE IS GENERAL AGREEMENT THAT JUVENILES

CAN BE SUPEROVULATED AND THE FOLLICLES ARE SENSITIVE TO GONADOTROPHIN STIMULATION (DONALDSON, 1984; DRIANCOURT ET AL., 1990; RANGEL-SANTOS ET AL., 1991; HASLER, 1992; KUHHOLZER & BREM, 1999). HOWEVER, CONTRADICTORY RESULTS HAVE BEEN REPORTED REGARDING THE EFFECT OF AGE FOLLOWING SUPEROVULATION IN YOUNG ANIMALS. A POORER RESPONSE TO SUPEROVULATION IN TERMS OF LOWER OVULATION AND FERTILISATION RATES, AS WELL AS LOWER EMBRYO RECOVERY AND SURVIVAL RATES HAVE BEEN RECORDED IN YOUNG FEMALES, COMPARED TO ADULT FEMALES (QUIRKE & HANRAHAN, 1977; RANGEL-SANTOS ET AL., 1991; DRIANCOURT & AVDI, 1993).

MORE TRIALS EVALUATING THE EFFECT OF AGE ON THE OVARIAN RESPONSE TO SUPEROVULATION HAVE BEEN CONDUCTED IN SHEEP AND CATTLE THAN IN GOATS. OVA AND EMBRYOS RECOVERED FROM YOUNG EWES HAVE BEEN REPORTED TO HAVE A LOWER POTENTIAL FOR DEVELOPMENT, WHEN COMPARED TO OVA PRODUCED BY ADULT EWES (QUIRKE & HANRAHAN, 1977; WRIGHT ET AL., 1981; MCMILLAN & MCDONALD, 1985). BARIL ET AL. (2000) REPORTED ADULT EWES TO HAVE HIGHER OVULATION RATES. IN CATTLE, THE EFFECT OF AGE ON THE SUPEROVULATORY RESPONSE IS NOT ALWAYS TAKEN AS A DECIDING FACTOR, AS THERE IS EITHER A TOO SMALL DIFFERENCE IN SUPEROVULATORY RESPONSE DUE TO AGE OR NO EFFECT OF AGE ON THE TOTAL NUMBER OF EMBRYOS RECOVERED (DONALDSON, 1984; HASLER, 1992).

2.3.4. Reproductive status

2.3.4.1. Ovarian response following superovulation in goats

THE OVULATION RATE AND THE YIELD OF TRANSFERABLE EMBRYOS FOLLOWING SUPEROVULATION CONTINUES TO VARY BETWEEN TREATMENTS AND ANIMALS IN THE SAME TREATMENT, IRRESPECTIVE OF THE ADVANCES IN ANIMAL MANAGEMENT, GONADOTROPHIN PREPARATIONS AND ADMINISTRATION PROTOCOLS ACHIEVED. THIS HAS LED TO THE SEEKING OF AN ALTERNATIVE WAY OF APPROACHING RESEARCH IN MOET TRIALS. PREVIOUSLY, RESEARCHERS APPLYING MOET HAVE FOCUSED ON THE VARIATION IN OVARIAN RESPONSE TO SUPEROVULATION BEING ACHIEVED IN A GROUP OF ANIMALS. AN ALTERNATIVE WAS HOWEVER TO FOCUS ON THE RESPONSE OF AN INDIVIDUAL ANIMAL WITH REGARD TO SUPEROVULATION TREATMENT. WITH THE AID OF ENDOSCOPY AND ULTRASONOGRAPHY VIA REGULAR MONITORING OF THE OVARY IN ANIMALS IN A MOET PROGRAMME, IT WAS REVEALED THAT THE OVULATION RATE AND QUALITY OF EMBRYOS RECOVERED ARE GREATLY INFLUENCED BY THE STATE OF THE OVARY AT THE ONSET OF THE SUPEROVULATORY TREATMENT. THROUGH THESE TECHNOLOGIES IT WAS OBSERVED THAT AT THE BEGINNING OF THE SUPEROVULATORY TREATMENT, DONOR ANIMALS HAVE A DIFFERENT OVARIAN STATUS. THIS COULD HENCE ACCOUNT FOR THE LARGE VARIATION IN OVULATION RATE AND EMBRYO YIELD FOLLOWING SUPEROVULATION BEING RECORDED BETWEEN INDIVIDUAL ANIMALS (COGNIE ET AL., 2003; GONZALEZ-BULNES ET AL., 2003B, 2004A; HOLTZ, 2005).

IT IS WELL DOCUMENTED THAT THE TREATMENT OF SHEEP AND GOATS WITH HIGH DOSES OF EXOGENOUS FSH WILL STIMULATE THE FOLLICULAR GROWTH OF THE SO-CALLED GONADOTROPHIN-RESPONSIVE FOLLICLES FOLLOWING THE FIRST FSH

INJECTION, TO REACH A PRE-OVULATORY PHASE. THESE ARE SMALL FOLLICLES WITH A DIAMETER OF APPROXIMATELY 2 TO 3 MM IN SHEEP AND 2 TO 6 MM IN GOATS. IN ALL RUMINANTS IT HAS BEEN OBSERVED THAT THE NUMBER OF CORPORA LUTEA IS POSITIVELY CORRELATED WITH THE NUMBER OF GONADOTROPHIN RESPONSIVE OVARIAN FOLLICLES PRESENT AT THE ONSET OF THE SUPEROVULATORY REGIME. HOWEVER, IN GOATS THE NUMBER OF VIABLE AND TRANSFERABLE EMBRYOS IS POSITIVELY CORRELATED TO A NARROWER GROUP OF OVARIAN FOLLICLES (4-6 MM). THIS OBSERVATION INDICATES THAT IN GOATS, ALTHOUGH ALL SMALL FOLLICLES (2-6 MM) CAN BE STIMULATED TO GROW WITH THE AID OF EXOGENOUS GONADOTROPHINS, VERY SMALL FOLLICLES (2-3 MM) SELDOMLY ATTAIN MATURITY. THIS IS SUPPORTED BY THE FINDINGS THAT THE ATTAINMENT OF DEVELOPMENTAL COMPETENCE IN GOAT OOCYTES IS ACQUIRED BY FOLLICLES >3 MM. ON THE OTHER HAND, BASED ON THE SUGGESTION THAT FULL DEVELOPMENTAL COMPETENCE OF OOCYTES IS ALSO INDICATED BY THE SECRETION OF INHIBIN, THE FOLLICLES CAPABLE OF SECRETING INHIBIN IN GOATS ARE REPORTED TO BE FOLLICLES OF A SIZE ≥ 4 MM (BREBION AND COGNIE, 1989; DE SMEDT ET AL., 1994; GONZALEZ-BULNES ET AL., 2000, 2003A, 2004B, D).

ALTHOUGH THE ADMINISTRATION OF EXOGENOUS GONADOTROPHINS STIMULATE THE GROWTH OF SMALL OVARIAN FOLLICLES UNTIL THE PRE-OVULATORY AND OVULATION STAGES, WITH THE AID OF ULTRASONOGRAPHY, IT HAS BEEN OBSERVED THAT THE NUMBER OF OVULATIONS AND EMBRYOS RECOVERED IS NEGATIVELY INFLUENCED BY THE PRESENCE OR ABSENCE OF A DOMINANT FOLLICLE AT THE ONSET OF THE SUPEROVULATORY

TREATMENT. IN SMALL RUMINANTS, IT HAS BEEN OBSERVED THAT THERE ARE A HIGHER NUMBER OF GONADOTROPHIN-RESPONSIVE FOLLICLES WHEN NO DOMINANT FOLLICLE IS PRESENT. THE PRESENCE OF A DOMINANT FOLLICLE AT THE ONSET OF SUPEROVULATION REDUCES FOLLICULAR RECRUITMENT, OVULATION RATE, YIELD AND THE NUMBER OF TRANSFERABLE EMBRYOS. MOREOVER, THE PRESENCE OF A DOMINANT FOLLICLE AT THE START OF A SUPEROVULATION TREATMENT NEGATIVELY AFFECTS THE OVARIAN FOLLICLES MATURING AND EVENTUALLY THE QUALITY OF EMBRYOS RECOVERED (RUBIANES ET AL., 1995, 1997; LOPEZ-SEBASTIAN ET AL., 1999; MENCHACA ET AL., 2002; COGNIE ET AL., 2003; GONZALEZ-BULNES ET AL., 2004D; HOLTZ, 2005).

IN GOATS IT HAS BEEN FOUND THAT THE PRESENCE OF A DOMINANT FOLLICLE AT THE BEGINNING OF SUPEROVULATION TREATMENT LEADS TO DELAYED FOLLICULAR RECRUITMENT AND HENCE A SMALLER NUMBER OF LARGE FOLLICLES DURING THE OESTROUS PERIOD. THIS ALSO SUGGESTS THAT THE PRESENCE OF A DOMINANT FOLLICLE IN SMALL RUMINANTS DOES NOT COMPLETELY INHIBIT THE GROWTH OF NEW FOLLICLES. HOWEVER, IT COULD REDUCE THE NUMBER OF NEW EMERGING FOLLICLES AND SUPPRESS THEIR SUBSEQUENT GROWTH. IT WAS ALSO OBSERVED THAT THE OVULATION RATE IN GOATS WAS REDUCED IF SUPEROVULATION TREATMENT BEGAN WHEN A HIGH NUMBER OF FOLLICLES >7 MM (DOMINANT FOLLICLES) WERE PRESENT (MENCHACA ET AL., 2002; GONZALEZ-BULNES ET AL., 2003A, 2004D).

THE NUMBER OF QUALITY EMBRYOS OBTAINED FOLLOWING SUPEROVULATION IS NOT ONLY INFLUENCED BY THE PRESENCE OR ABSENCE OF A DOMINANT FOLLICLE AT THE ONSET OF

SUPEROVULATION TREATMENT BUT, ALSO BY THE PRESENCE OR ABSENCE OF A CORPUS LUTEUM. IT HAS BEEN OBSERVED THAT WHEN SUPEROVULATION TREATMENT WAS INITIATED IN THE ABSENCE OF A CORPUS LUTEUM, IT LED TO HIGH INCIDENCES OF DEGENERATED EMBRYOS BEING PRODUCED. THEREFORE, REDUCING THE NUMBER OF VIABLE EMBRYOS AT THE TIME OF RECOVERY. THESE RESULTS WERE ALSO SUPPORTED BY AN INCREASE IN EMBRYO VIABILITY AND DECREASED INCIDENCES OF DEGENERATED EMBRYOS OBSERVED IN EWES WITH A CORPUS LUTEUM PRESENT. THIS EMPHASISES THE IMPORTANCE OF THE PRESENCE OF A CORPUS LUTEUM FOR GROWTH OF OVARIAN FOLLICLES (GONZALEZ-BULNES ET AL., 2002B; GONZALEZ-BULNES ET AL., 2004A, 2005; VEIGA-LOPEZ ET AL., 2005).

IN CATTLE IT HAS BEEN FOUND THAT A LOW CONCENTRATION OF PROGESTERONE AT THE BEGINNING OF SUPEROVULATION TREATMENT LEADS TO A REDUCED NUMBER OF QUALITY EMBRYOS BEING PRODUCED (CALLESEN ET AL., 1988). IT HAS ALSO BEEN REPORTED THAT THE NUMBER OF VIABLE EMBRYOS IN SHEEP COULD BE IMPROVED BY STARTING SUPEROVULATION TREATMENT DURING THE EARLY LUTEAL PHASE (GONZALEZ-BULNES ET AL., 2005). IT HAS THEREFORE BEEN HYPOTHESISED THAT IF SUPEROVULATION TREATMENT BEGAN IN THE ABSENCE OF A LARGE FOLLICLE AND THE PRESENCE OF A HIGH NUMBER OF GONADOTROPHIN-RESPONSIVE FOLLICLES IN THE EARLY LUTEAL PHASE (WHEN THERE IS A PRESENCE OF A CORPUS LUTEUM), THE RESPONSE (OVULATION RATE AND YIELD OF TRANSFERABLE EMBRYOS) TO SUPEROVULATION COULD BE INCREASED. THIS SITUATION COULD BE CREATED IN DIFFERENT WAYS, INCLUDING THE ABLATION OF THE DOMINANT

FOLLICLE, INDUCTION OF HIGH ENOUGH PROGESTERONE LEVELS TO SUPPRESS FOLLICULAR DEVELOPMENT BEFORE SUPEROVULATION, THE PRE-TREATMENT WITH A GNRH AGONIST OR ANTAGONIST BEFORE SUPEROVULATION TO PREVENT EMERGENCE OF A DOMINANT FOLLICLE OR THE INITIATION OF SUPEROVULATION DURING THE FIRST FOLLICULAR EMERGENCE AFTER OVULATION. BEFORE CREATING AN IDEAL TIME FOR STARTING SUPEROVULATION TREATMENT IT IS IMPORTANT HOWEVER TO UNDERSTAND THE PATTERN OF OVARIAN FOLLICULAR DEVELOPMENT AND HORMONAL FLUCTUATIONS (BUNGARTS & NIEMMAN, 1994; COGNIE, 1999; MENCHACA ET AL., 2002; RUBIANES & MENCHACA, 2003; HOLTZ, 2005).

2.3.4.2. Ovarian follicular development and patterns

IN GOATS, THE SAME AS IN OTHER RUMINANTS, ULTRASONOGRAPHIC STUDIES HAVE SHOWN OVARIAN FOLLICULAR DEVELOPMENT AND GROWTH TO OCCUR IN A WAVE-LIKE PATTERN DURING THE OESTROUS CYCLE (GINTHER & KOT, 1994; BO ET AL., 1995; DE CASTRO ET AL. 1998; ROCHE ET AL., 1998; GONZALEZ-BULNES ET AL., 1999; DRIANCOURT, 2000; DISKIN ET AL., 2002; RUBIANES & MENCHACA, 2003). A FOLLICULAR WAVE IS CHARACTERISED BY THE SYNCHRONISED EMERGENCE (RECRUITMENT) OF A GROUP OF SMALL ANTRIAL FOLLICLES FROM WHICH ONE OR TWO FOLLICLES WILL BE SELECTED AND GROW TO BECOME A DOMINANT FOLLICLE OF >5 MM IN DIAMETER AND EVENTUALLY OVULATE, WHILE THE OTHER COHORT OF FOLLICLES WILL REGRESS (FIGURE 2.1). WHEN A DOMINANT FOLLICLE APPEARS, THE OTHER OVARIAN FOLLICLES REGRESS AND DURING THIS DOMINANCE PERIOD THERE IS NO NEW FOLLICULAR RECRUITMENT. ONLY GONADOTROPHIN-DEPENDENT FOLLICLES WILL BE RECRUITED.

RECRUITMENT DEPENDS ON THE SIZE OF THE FOLLICLE AT WHICH IT COULD BECOME A GONADOTROPHIN-DEPENDENT FOLLICLE. THE NUMBER OF WAVES WITHIN AN OESTROUS CYCLE RANGES FROM 2 TO 6. MOST GOATS WITH A NORMAL OESTROUS CYCLE LENGTH RANGING BETWEEN 19 AND 22 DAYS, DEMONSTRATE 4 FOLLICULAR WAVES (GINTHER & KOT, 1994; DE CASTRO ET AL., 1998; DE CASTRO ET AL., 1999; SCHWARZ & WIERZCHOS, 2000; MENCHACA & RUBIANES, 2002; EVANS, 2003; CRUZ ET AL., 2005). THE OCCURRENCE OF FOLLICULAR WAVES SEEMS TO DIFFER DEPENDING ON THE DURATION OF THE OESTROUS CYCLE. HOWEVER, IN GOATS IT COULD BE SUMMARISED AS WAVE 1, 2, 3, AND 4 (OVULATORY WAVE), OCCURRING AROUND DAY 0-1, 4-7, 8-11, AND 13-15 POST OVULATION, RESPECTIVELY (FIGURE 2.2) (GINTHER & KOT, 1994; DE CASTO ET AL., 1999; RUBIANES & MENCHACA, 2003; TENORIO FILHO ET AL., 2007).

THERE ARE SEVERAL OTHER FEATURES THAT HAVE BEEN OBSERVED DURING A FOLLICULAR WAVE E.G. THE DIAMETER OF A DOMINANT FOLLICLE DIFFERS WITH THE FOLLICULAR WAVE. THE DIAMETER OF THE LARGEST FOLLICLE IN WAVE 1 AND THE OVULATORY WAVE IN GOATS IS USUALLY LARGER THAN THE DIAMETER OF WAVE 2 AND 3. THE MAXIMUM DIAMETER IN THE DOE WHICH COULD BE ATTAINED BY THE LARGEST FOLLICLE IN WAVE 1 WAS REPORTED TO BE 8.7±0.3 MM, WHILE THE MAXIMUM DIAMETER THAT THE DOMINANT FOLLICLE IN THE OVULATORY WAVE COULD ATTAIN WAS A MEAN OF 9.7±0.3 MM. THE DIAMETER OF A DOMINANT FOLLICLE IN WAVES 1 AND 3 AVERAGED 7.2±0.2 MM AND 7.3±0.2 MM, RESPECTIVELY. THIS COULD SUGGEST THAT FOLLICULAR DOMINANCE IN GOATS COULD ALSO BE MORE DURING WAVE 1 AND

THE OVULATORY WAVE, COMPARED TO THE MIDDLE FOLLICULAR WAVES.

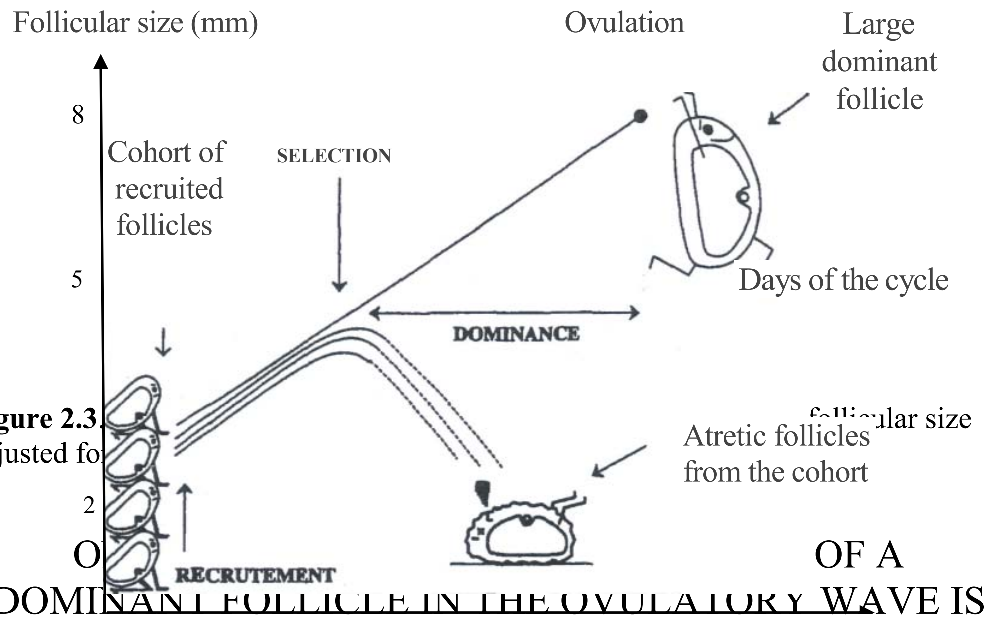

Figure 2.3 … follicular size
adjusted fo…

O… OF A DOMINANT FOLLICLE IN THE OVULATORY WAVE IS USUALLY MORE THAN THE DIAMETER OF THE LARGEST FOLLICLE IN THE UNOVULATORY FOLLICULAR WAVES. MOREOVER, THE TIME INTERVAL BETWEEN THE OCCURRENCE OF WAVE 1 AND 2 IS ALSO USUALLY LONGER THAN THE TIME INTERVAL BETWEEN THE OCCURRENCE OF WAVE 2 AND 3 OR WAVE 3 AND THE OVULATORY FOLLICULAR WAVE (GINTHER & KOT, 1994; DE CASTRO ET AL., 1999; MENCHACA & RUBIANES, 2002). THE NUMBER OF DAYS BETWEEN THE EMERGENCE OF WAVE 1 AND 2 HAS BEEN REPORTED TO BE 7.3±0.9D, WHILE THAT BETWEEN WAVE 2 AND 3 4.0±0.4D AND 3.8±1.1D BETWEEN THE EMERGENCE OF WAVE 3 AND THE OVULATORY FOLLICULAR WAVE (DE CASTO ET AL., 1999). IT HAS ALSO BEEN DEMONSTRATED THAT THE NUMBER OF FOLLICLES RECRUITED INCREASES AS THE LUTEAL PHASE PROGRESSES, WHILE THE TIME PERIOD BETWEEN FOLLICULAR WAVES SHORTENS AS THE LUTEAL PHASE ADVANCES (GINTHER & KOT, 1994; DE CASTRO ET AL., 1999). THE MEAN NUMBER OF FOLLICLES

RECRUITED AND REACHING A DIAMETER OF >4 MM HAS BEEN REPORTED AS BEING 5.8±1.0, WITH 29.8±4.9 OVARIAN FOLLICLES BEING RECRUITED IN AN OESTROUS CYCLE (SCHWARZ & WIERZCHOS, 2000)

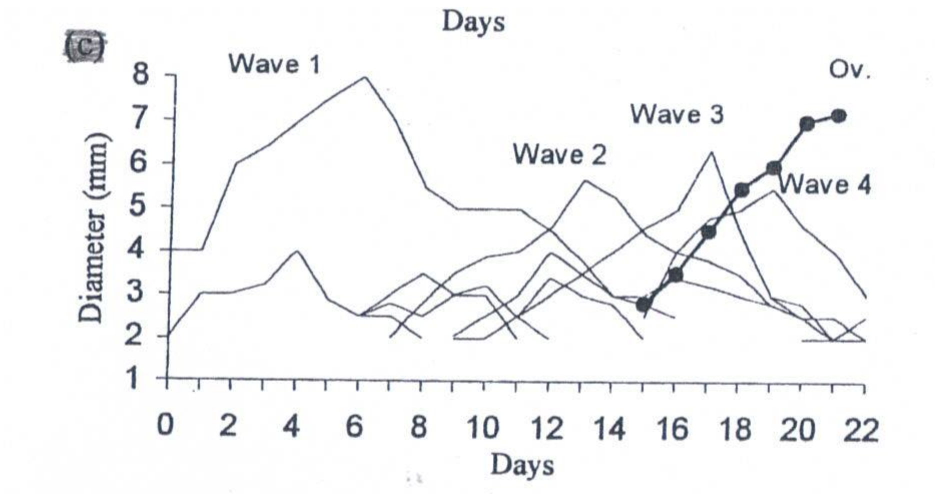

Figure 2.4. Follicular growth profiles in an oestrous cycle of goats with 4 follicular waves (de Castro et al., 1999)

2.3.4.3. The relationship between reproduction hormonal (FSH, oestrogen, LH and progesterone) profiles and ovarian follicular waves

IN GOATS, THERE IS LACK OF RESEARCH CONCERNING THE RELATIONSHIP BETWEEN FSH AND FOLLICULAR DYNAMICS (RUBIANES AND MENCHACA, 2003; GAAFAR ET AL., 2005). IT HAS BEEN OBSERVED THAT BLOOD FSH CONCENTRATIONS FLUCTUATE DURING THE OESTROUS CYCLE AND THESE FLUCTUATIONS ARE NORMALLY CORRELATED WITH THE EMERGENCE OF FOLLICULAR WAVES IN RUMINANTS. IN GOATS FSH FLUCTUATIONS HAVE ALSO BEEN RECORDED. THE EMERGENCE OF A FOLLICULAR WAVE IN THE DOE IS USUALLY PRECEDED BY AN INCREASE IN THE CIRCULATING FSH CONCENTRATION. THIS FSH CONCENTRATION DECLINES AS THE LARGE FOLLICLE GROWS IN SIZE

(ADAMS ET AL., 1992; GINTHER ET AL., 1995; LEYVA-OCARIZ ET AL., 1995; SOUZA ET AL., 1998; BARTLEWSKI ET AL., 1999; SCHWARZ & WIERZCHOS, 2000; DISKIN ET AL., 2002; EVANS ET AL., 2002; RUBIANES AND MENCHACA, 2003). HOWEVER, IN SHEEP, THE MIDDLE GROUP OF FOLLICULAR WAVES WERE FOUND NOT TO BE PRECEDED BY A DETECTABLE QUANTITY OF FSH AND SOME FSH FLUCTUATIONS WERE NOT PRECEDING FOLLICULAR EMERGENCE (GIBBONS ET AL., 1999; VINOLES ET AL., 2002). IN GOATS ON THE OTHER HAND, ONE OR TWO FSH PEAKS HAVE BEEN REPORTED. WHERE ONE FSH PEAK WAS RECORDED, IT WAS GENERALLY OBSERVED AROUND THE TIME OF OESTRUS AND THIS INCREASE IN FSH LEVEL WAS NORMALLY ASSOCIATED WITH A PRE-OVULATORY LH SURGE (LEYVA-OCARIZ ET AL., 1995; SCHWARZ & WIERZCHOS, 2000; MEDAN ET AL., 2003). WHERE TWO FSH FLUCTUATIONS WERE OBSERVED, ONE WAS STILL ASSOCIATED WITH THE PRE-OVULATORY SURGE WHILE, THE OTHER PEAK WAS REPORTED TO OCCUR 48 H FOLLOWING THE PRE-OVULATORY LH SURGE. THE LATTER WAVE HAS ALSO BEEN OBSERVED IN CATTLE AND IS NORMALLY ASSOCIATED WITH THE OVARIAN FOLLICULAR WAVE 1 (CHEMINEAU ET AL., 1982; ADAMS ET AL., 1992; GINTHER & KOT, 1994)

IN GOATS, 2 PEAKS OF OESTROGEN DURING THE OESTROUS CYCLE HAVE BEEN REPORTED. ONE PEAK OCCURS DURING THE FOLLICULAR PHASE AND SPECIFICALLY 2 DAYS BEFORE OVULATION AND THE OTHER PEAK DURING THE EARLY LUTEAL PHASE. THE CONCENTRATION DECREASES RAPIDLY FROM DAY 5 POST OVULATION FOR THE ENTIRE LUTEAL PHASE. THESE 2 PEAKS CORRELATE WELL WITH THE 2 FOLLICULAR WAVES. THE FIRST PEAK OCCURRING DURING OESTROUS PERIOD, COINCIDING WITH THE

OVULATORY WAVE, WITH THE SECOND PEAK OCCURRING DURING THE EARLY LUTEAL PHASE AND COINCIDING WITH WAVE 1 (LEYVA-OCARIZ ET AL., 1995; DE CASTRO ET AL., 1999; RUBIANES AND MENCHACA, 2003; GAAFAR ET AL., 2005). AS HAS BEEN PREVIOUSLY DEMONSTRATED, A DOMINANT FOLLICLE IS RESPONSIBLE FOR THE PRODUCTION OF OVARIAN OESTROGENS, WHILE THE SUBORDINATE FOLLICLES ARE RESPONSIBLE FOR THE PRODUCTION OF ABOUT 10% OF THE OVARIAN OESTROGENS (BADINGA ET AL., 1992; MANN ET AL., 1992).

THE HIGHEST CONCENTRATIONS OF OESTROGEN COINCIDE WITH FOLLICULAR WAVE 1 AND THE OVULATORY WAVE IN THE OESTROUS CYCLE, DUE TO THE FACT THAT THE LARGEST DOMINANT FOLLICLE OCCURS DURING THESE TWO WAVES (GINTHER & KOT, 1994; DE CASTRO ET AL., 1999; MENCHACA & RUBIANES, 2002). THE DOMINANT OVARIAN FOLLICLES GROWING DURING THE MID-LUTEAL PHASE DO NOT PRODUCE HIGH OESTROGEN LEVELS AND THIS MAY BE ATTRIBUTED TO THE INHIBITORY FACTOR FROM THE INCREASING PROGESTERONE CONCENTRATION WHICH SUPPRESSES THE PULSATILE LH SECRETION (BAUERNFEIND & HOLTZ, 1991; RUBIANES AND MENCHACA, 2003; GAAFAR ET AL., 2005).

THE FREQUENCY OF THE LH PULSES IN GOATS IS REPORTED TO BE HIGH DURING THE FIRST HALF OF THE LUTEAL PHASE. THIS INCREASE IN LH CONCENTRATION AND PULSES HAS ALSO BEEN SHOWN TO COINCIDE WITH THE GROWING PHASE OF THE DOMINANT FOLLICLE, AS WELL AS THE TIME WHEN THE CONCENTRATION OF PROGESTERONE IS LOW (ADAMS, 1999; KAWATE ET AL., 2000; MENCHACA & RUBIANES, 2002). THIS THUS JUSTIFIES THE SUGGESTION THAT A DOMINANT FOLLICLE ESCAPES ITS REGRESSION BY CHANGING ITS RELIANCE FROM

FSH TO LH. IT ALSO IMPLIES THAT IF THE CONCENTRATION OF LH CAN REMAIN LOW, LARGE FOLLICLES WILL CONTINUE TO DEPEND ON FSH AND IT WILL NOT EXPERIENCE THE DETRIMENTAL EFFECT OF A DOMINANT FOLLICLE. THIS HAS BEEN OBSERVED IN SHEEP AND GOATS, WHERE OUT OF SEASON AND DURING THE LUTEAL PHASE (WHEN THE CONCENTRATION OF LH IS LOW), THERE IS A LACK OF DOMINANCE (BAIRD ET AL., 1983; CAMPBELL ET AL., 1995, 1998; ADAMS, 1999; MENCHACA & RUBIANES, 2002). DURING THE MID-LUTEAL PHASE AND THE REST OF THE LUTEAL PHASE, BOTH THE CONCENTRATION AND PULSES OF LH DECLINE, DUE TO AN INCREASE IN THE PROGESTERONE CONCENTRATION. THEREAFTER, THE LH CONCENTRATION AND FREQUENCY OF PULSES INCREASE FOLLOWING LUTEOLYSIS (DECLINE IN PROGESTERONE CONCENTRATION), COINCIDING WITH THE ONSET OF OESTRUS AND THE OVULATORY WAVE (BAIRD ET AL., 1983; CAMPBELL ET AL., 1995, 1998; DE CASTRO ET AL., 1999; GAAFAR ET AL., 2005). THIS INDICATES THAT PROGESTERONE LEVELS CONTROL FOLLICULAR DEVELOPMENT AND GROWTH, POSSIBLY MEDIATED THROUGH THE CONTROL OF THE LH PULSES. IN GOATS SMALL FOLLICLES HAVE BEEN OBSERVED IN WAVES DEVELOPING UNDER HIGH LEVELS OF PROGESTERONE. IT WAS OBSERVED THAT HIGH CIRCULATING PROGESTERONE CONCENTRATIONS DURING THE EARLY MID-LUTEAL PHASE IN GOATS LEADS TO 4 FOLLICULAR WAVES, WHILE LOW PROGESTERONE LEVELS DURING THE MID-LUTEAL PHASE LEAD TO 2 OR 3 WAVES. MOREOVER, CIRCULATING OESTROGEN LEVELS DECLINE EARLIER IN GOATS WITH 4 WAVES, PROBABLY DUE TO THE EARLY INCREASE IN PROGESTERONE CONCENTRATION WHICH SUPPRESSES THE LH

PULSES, HENCE LEADING TO THE EARLY OR QUICK EMERGENCE OF FOLLICULAR WAVE 2. IN CONTRAST, GOATS WITH 3 WAVES HAVE A DELAYED WAVE 2 OCCURRENCE, WHICH HAPPENS WITH THE DECLINE IN OESTROGEN CONCENTRATION OCCURRING LATER. WHEN INDUCING A HIGH PROGESTERONE CONCENTRATION WITH THE AID OF INTRAVAGINAL PROGESTAGEN PESSARIES EARLY DURING THE LUTEAL PHASE, THE LIFESPAN OF A DOMINANT FOLLICLE IS REDUCED, HENCE ADVANCING THE EMERGENCE OF FOLLICULAR WAVE 1 (GINTHER & KOT, 1994; DE CASTRO ET AL., 1999; RUBIANES & MENCHACA, 2003).

2.3.4.4. Creating an ideal time for the onset of superovulation/synchronisation of follicular wave development

IT IS CLEAR THAT SYNCHRONISATION OF A COHORT OF FOLLICULAR EMERGENCE, OR IN OTHER WORDS THE SYNCHRONISATION OF FOLLICULAR WAVES IS NECESSARY FOR INITIATING A SUPEROVULATION TREATMENT. IN CATTLE MANY SUPEROVULATION PROTOCOLS HAVE BEEN DESIGNED TO SYNCHRONISE FOLLICULAR WAVE EMERGENCE AND THESE PROGRAMMES HAVE BEEN REPORTED TO IMPROVE THE SUPEROVULATORY RESPONSE. SO FOR EXAMPLE, IT HAS BEEN OBSERVED THAT THE REMOVAL OF A DOMINANT FOLLICLE THROUGH ASPIRATION CAN LEAD TO A BRIEF FSH CONCENTRATION INCREASE AND THE EMERGENCE OF A NEW FOLLICULAR WAVE WITHIN 1 TO 2 DAYS (BERGFELT ET AL., 1994; DISKIN ET AL., 2002). HOWEVER, THIS APPROACH MAY SEEM IMPRACTICAL AT THE FARM LEVEL AND THEREFORE, THE UTILISATION OF EXOGENOUS HORMONES WOULD BE MORE APPROPRIATE. FOR THIS REASON, PROGESTERONE AND OESTROGEN HAVE BEEN USED TO SYNCHRONISE FOLLICULAR EMERGENCE AS THE TWO HORMONES CAN SUPPRESS FSH AND LH

SECRETION AND TERMINATE THE EXISTING FOLLICULAR WAVE, HENCE STIMULATING THE BEGINNING OF A NEW FOLLICULAR WAVE. ANOTHER APPROACH INVOLVES THE ADMINISTRATION OF GNRH TO INDUCE LUTEINIZATION OR OVULATION OF THE DOMINANT FOLLICLE, LEADING TO THE EMERGENCE OF A NEW FOLLICULAR WAVE (BO ET AL., 1995; TWAGIRAMUNGU ET AL., 1995; DISKIN ET AL., 2002). IN SHEEP AND GOATS METHODS FOR INDUCING THE SYNCHRONISATION OF FOLLICULAR WAVE IN SUPEROVULATORY PROTOCOLS ARE CURRENTLY BEING INCLUDED. THIS INVOLVES STARTING SUPEROVULATION SOON AFTER OVULATION WHICH COINCIDES WITH EMERGENCE OF A FOLLICULAR WAVE, KNOWN AS A DAY 0 PROTOCOL OR THE PRE-TREATMENT WITH A GNRH AGONISTS OR ANTAGONISTS TO AVOID THE DEVELOPMENT OF A DOMINANT FOLLICLE (COGNIE, 1999; RUBIANES & MENCHACA, 2003).

2.3.4.4.1. The effect of follicular ablation in response to superovulation

FOLLICULAR ABLATION IS A PROCESS INVOLVING THE PHYSICAL REMOVAL OF A DOMINANT FOLLICLE, MOSTLY THROUGH THE USE OF ULTRASOUND-GUIDED TRANSVAGINAL FOLLICULAR ASPIRATIONS IN CATTLE OR LAPAROSCOPIC FOLLICULACENTESIS IN SMALL ANIMALS (BURNGARTS & NIEMANN, 1994; BO ET AL., 1995). THERE IS CURRENTLY LIMITED INFORMATION AVAILABLE REGARDING THE REMOVAL OF A DOMINANT OVARIAN FOLLICLE IN SHEEP AND GOATS. IN CATTLE FOLLICULAR ABLATION HAS BEEN UTILISED TO SYNCHRONISE THE EMERGENCE OF FOLLICULAR DEVELOPMENT (ADAMS ET AL., 1993A; BUNGARTS & NIEMANN, 1994; BO ET AL., 1995). THIS IS DUE TO THE FACT THAT IT HAS BEEN OBSERVED THAT THE REMOVAL OF A DOMINANT FOLLICLE DURING THE EARLY LUTEAL

PHASE DELAYS THE REGRESSION OF THE SECOND LARGEST FOLLICLE AND LEADS TO A PREMATURE INCREASE IN FSH CONCENTRATION. A SHORTENED TIME PERIOD BETWEEN FOLLICULAR WAVE 1 AND WAVE 2 IS INDUCED AND HENCE AN INCREASE IN THE OCCURRENCE OF ANIMALS WITH 3 FOLLICULAR WAVES, COMPARED TO 2 FOLLICULAR WAVES. THIS INDICATES THAT FOLLICULAR ABLATION CAN HASTEN THE EMERGENCE OF SMALL FOLLICLES, WHICH CAN RESPOND TO EXOGENOUS GONADOTROPHIN TREATMENT (KO ET AL., 1991; ADAMS ET AL., 1993B; EVANS ET AL., 2002).
THUS, FOLLICULAR ABLATION CAN BE PERFORMED PRIOR TO COMMENCEMENT OF A SUPEROVULATORY TREATMENT TO SYNCHRONISE FOLLICULAR EMERGENCE AND HENCE CREATE AN IDEAL TIME FOR INITIATING SUPEROVULATORY TREATMENT. IN CATTLE, SUPEROVULATION FOLLOWING SYNCHRONISED FOLLICULAR EMERGENCE BY FOLLICULAR ABLATION HAS ALSO BEEN REPORTED TO INCREASE THE FERTILISATION RATE. IN SHEEP, THE NUMBER OF EMBRYOS RECOVERED WAS INCREASED AFTER SUPEROVULATING EWES FOLLOWING FOLLICULAR ABLATION (BO ET AL., 1995; GONZALEZ-BULNES ET AL., 2002A). THE UTILISATION OF FOLLICULAR ABLATION IN SMALL RUMINANTS HOWEVER, IS NOT PRACTICAL AS IT REQUIRES THE USE OF LAPAROSCOPIC TECHNIQUES OR LAPAROTOMY FOR THE PHYSICAL PUNCTURING OF THE DOMINANT FOLLICLE. THEREFORE, THE UTILISATION OF EXOGENOUS HORMONES IN SYNCHRONISING FOLLICULAR EMERGENCE MAY STILL REMAIN A MORE SUITABLE OPTION (GONZALEZ-BULNES ET AL., 2004A, B).

2.3.4.4.2. Effect of GnRH agonists or antagonists in the response to superovulation

IT IS WELL KNOWN THAT A BETTER SUPEROVULATION OVARIAN RESPONSE COULD BE

OBTAINED IF THE SUPEROVULATION TREATMENT IS STARTED AT THE STAGE OF EMERGENCE OF A FOLLICULAR WAVE 2 WHICH COINCIDES WITH THE ABSENCE OF A DOMINANT FOLLICLE. MOREOVER, THE NUMBER OF OVULATIONS AND EMBRYOS RECOVERED FOLLOWING SUPEROVULATION IS POSITIVELY CORRELATED TO THE HIGH NUMBER OF SMALL FOLLICLES PRESENT ON THE OVARY AT THE ONSET OF A SUPEROVULATION TREATMENT (GONZALEZ-BULNES ET AL., 2002C, 2003A). THUS, IN SMALL RUMINANTS, DUE TO THEIR SMALL BODY SIZE, THE UTILISATION OF PHARMACOLOGICAL TREATMENT WOULD BE MORE APPROPRIATE FOR CREATING THIS IDEAL TIME OF STARTING SUPEROVULATION TREATMENT (GONZALEZ-BULNES ET AL., 2004A, B). IT HAS BEEN OBSERVED THAT OVARIAN FOLLICLES COULD CHANGE FROM BEING FSH DEPENDENT AND BECOME DOMINANT FOLLICLES, DEPENDING ON THE CONCENTRATION OF LH. THUS, IT COULD BE HYPOTHESIZED THAT IF THE CONCENTRATION OF LH COULD REMAIN LOW, THERE WOULD BE NO DEVELOPMENT OF A DOMINANT FOLLICLE AND HENCE ALL FOLLICLES GROWING UNDER THIS CONDITION WOULD BE DEPENDENT ON FSH AND REMAIN GONADOTROPHIN-RESPONSIVE FOLLICLES. THIS WOULD BE AN IDEAL ENVIRONMENT FOR STARTING A SUPEROVULATION TREATMENT (JOSEPH ET AL., 1992; DRIANCOURT ET AL., 2000; GONZALEZ-BULNES ET AL., 2004A).

GNRH COULD BE UTILISED TO ADJUST THE LH CONCENTRATION IN DONORS, AS THE RELEASING HORMONES CAN CONTROL THE SECRETION OF THE GONADOTROPHINS FROM THE ANTERIOR PITUITARY GLAND (SCARAMUZZI ET AL., 1993; D’OCCHIO ET AL., 2000; CRAWFORD ET AL., 2004; PADULA, 2005; SCHNEIDER ET AL., 2006). GNRH AGONIST OR ANTAGONIST ANALOGUES HAVE BEEN UTILISED TO

REDUCE THE LH SECRETION AND HAVE ALSO BEEN INCLUDED IN SUPEROVULATION PROTOCOLS. WHEN THESE AGENTS ARE INCLUDED IN A SUPEROVULATION PROGRAMME, THE AGENTS ARE ADMINISTERED TOGETHER WITH A PROGESTAGEN TREATMENT, TO SERVE AS A PRE-TREATMENT PRIOR TO THE ONSET OF SUPEROVULATION TREATMENT OF SHEEP AND GOATS IN ORDER TO SUPPRESS THE ENDOGENOUS GONADOTROPHIN SECRETION. IN THIS WAY THE LH CONCENTRATION WILL BE LOW (E.G. 0.4±0.1 NG/ML) AND THE DEVELOPMENT OF A DOMINANT FOLLICLE WILL ALSO BE BYPASSED - AS DOMINANCE CAN ONLY BE ESTABLISHED WHEN HIGH LH CONCENTRATIONS (E.G. 0.6±0.2 NG/ML) ARE PRESENT. IN HUMANS FOR EXAMPLE, IT HAS BEEN SHOWN THAT THE ADMINISTRATION OF A GNRH AGONIST ALSO HELPS TO PREVENT THE PREMATURE LH SURGES (JOSEPH ET AL., 1992; CAMPBELL ET AL., 1995; D'OCCHIO ET AL., 1997; DAL PRATO ET AL., 2004; GONZALEZ-BULNES ET AL., 2004B).

THE GNRH AGONIST AND ANTAGONIST ANALOGUES ACT DIFFERENTLY. GNRH AGONIST INJECTIONS INITIALLY INDUCE A 'FLARE EFFECT' ON THE GONADOTROPHINS I.E. AN ACUTE INCREASE IN LH AND FSH LEVELS WHICH COULD LAST FOR A FEW DAYS AND THEN RETURN TO LOWER LEVELS. THEREFORE IN ORDER TO REDUCE THE GONADOTROPHIN SECRETION, THE GNRH AGONIST MUST BE ADMINISTERED OVER A PERIOD OF TIME. IF THE GNRH TREATMENT IS CONTINUED, IT WOULD LEAD TO A DOWN REGULATION AND DESENSITISATION OF THE GNRH RECEPTORS IN THE PITUITARY. HENCE, A REDUCTION IN THE LH SYNTHESIS AND PULSES (MELSON ET AL., 1986; GONG ET AL., 1995, 1996; D'OCCHIO & ASPEDEN, 1996; ASPEDEN ET AL., 1997; D'OCCHIO ET AL., 2000; HERBERT & TRIGG, 2005; PADULA, 2005). THE GNRH

AGONIST TREATMENT IS GENERALLY ADMINISTERED OVER A 2 WEEK PERIOD; HOWEVER, IN CATTLE IT HAS BEEN FOUND THAT THE EFFECT ON LH WAS SIMILAR FOLLOWING 7, 14 OR 21 DAY TREATMENT WITH A GNRH AGONIST (PADULA & MACMILLAN, 2005).

TREATMENT WITH GNRH ANTAGONISTS ON THE OTHER HAND, BLOCKS THE GNRH RECEPTORS AND LEADS TO AN IMMEDIATE DECLINE IN GONADOTROPHIN LEVELS (DIEDRICH ET AL., 1994; CAMPBELL ET AL., 1998; GONZALEZ-BULNES ET AL., 2004B; HWANG ET AL., 2004). CONSEQUENTLY, A REDUCTION IN THE LH LEVELS IS ESTABLISHED AND THIS LEADS TO A HOMOGENEOUS POOL OF SMALL FOLLICLES ON THE OVARY. THESE HOMOGENEOUS GONADOTROPHIN-RESPONSIVE FOLLICLES COULD LEAD TO A CONSISTENT AND IMPROVED SUPEROVULATION RESPONSE. IN GOATS AND SHEEP, THE PRE-TREATMENT WITH GNRH AGONISTS OR ANTAGONISTS HAS BEEN SHOWN TO SUPPRESS THE SECRETION OF LH AND FSH (CAMPBELL ET AL., 1998; GONZALEZ-BULNES ET AL., 2004BC).

THERE IS VERY LITTLE INFORMATION AVAILABLE REGARDING THE UTILISATION OF GNRH AGONISTS AS A PRE-TREATMENT BEFORE SUPEROVULATION TREATMENT IN GOATS. IN SHEEP, A PRE-TREATMENT WITH A GNRH AGONIST HAS BEEN SHOWN TO INCREASE THE NUMBER OF SMALL FOLLICLES AND OVULATION RATE, AS WELL AS PREVENTING THE ESTABLISHMENT OF LARGE FOLLICLES. THE LATTER COULD ALSO REDUCE THE VARIATION IN RESPONSE OBTAINED WITH SUPEROVULATION, AS ALL FOLLICLES FOR RECRUITMENT WOULD FALL WITHIN A NARROW SIZE RANGE (1-2 MM DIAMETER) (COGNIE, 1999). PRE-TREATMENT WITH GNRH ANTAGONISTS THEN AGAIN, COULD INCREASE THE NUMBER OF SMALL FOLLICLES (2-3 MM IN SIZE) AND REDUCE THE

NUMBER OF LARGE FOLLICLES AS WELL AS THE SECRETION OF INHIBIN IN BOTH SHEEP AND GOATS. THE INCREASE OF SMALL FOLLICLES IN THE LATTER TREATMENT INDICATES THE ESTABLISHMENT OF FOLLICULAR DOMINANCE (CAMPBELL ET AL., 1998; GONZALEZ-BULNES ET AL., 2004B, C; LOPEZ-ALONSO ET AL., 2004). IN SHEEP, THE NUMBER OF OVA AND TRANSFERABLE EMBRYOS RECOVERED WAS IMPROVED WHEN A PRE-TREATMENT WITH A GNRH ANTAGONIST WAS INCLUDED IN THE SUPEROVULATION PROTOCOL (COGNIE ET AL., 2003). IN GOATS, THE PRE-TREATMENT WITH A GNRH ANTAGONIST CAN INCREASE THE OVULATION RATE BY ALMOST 50%. HOWEVER, THIS HIGH SUPEROVULATORY RESPONSE HAS BEEN REPORTED TO BE COUNTERACTED BY A REDUCTION IN THE NUMBER OF TRANSFERABLE EMBRYOS RECOVERED AS A RESULT OF AN INCREASE IN THE NUMBER OF UNFERTILISED OVA AND DEGENERATED EMBRYOS (COGNIE ET AL., 2003; GONZALEZ-BULNES ET AL., 2004A, B). THE INCREASE IN THE NUMBER OF UNFERTILISED OVA AND DEGENERATED EMBRYOS MAY BE LINKED TO A LACK IN MATURITY OF THE SMALL FOLLICLES RECRUITED IN GOATS AT THE PRE-OVULATORY STAGES. IT HAS BEEN FOUND THAT IN GOATS, SMALL FOLLICLES WITH A DIAMETER OF 2 TO 3 MM DO NOT SHOW ANY CORRELATION WITH THE EMBRYO RECOVERY RATE. LOWER EMBRYO RECOVERY RATES HAVE BEEN OBTAINED WHEN SUPEROVULATION TREATMENT WAS INITIATED WITH A HIGH NUMBER OF FOLLICLES HAVING A DIAMETER OF 2 MM. THE NUMBER AND VIABILITY OF EMBRYOS RECOVERED WAS THEN FINALLY, POSITIVELY CORRELATED TO THE LIMITED CATEGORY OF FOLLICLES WITH A DIAMETER OF 4 TO 6 MM (GONZALEZ-BULNES ET AL., 2003A)

2.3.4.4.3. Day 0 protocol

IT IS CLEAR THAT THE PHARMACOLOGICAL APPROACH OF SUPPRESSING THE SECRETION OF LH TO AVOID THE DEVELOPMENT OF A DOMINANT FOLLICLE AND THUS CREATING A HOMOGENOUSLY POOL OF SMALL FOLLICLES, WILL HAVE A DETRIMENTAL EFFECT ON THE YIELD AND QUALITY OF EMBRYOS PRODUCED IN GOATS. THE UTILISATION OF A ‘DAY 0 PROTOCOL’ MAY POSSIBLY LEAD TO BETTER RESULTS IN GOAT MOET PROGRAMMES. THE DAY 0 PROTOCOL INVOLVES THE INITIATION OF THE SUPEROVULATION TREATMENT ON THE DAY OF OVULATION (DAY 0) OR SOON AFTER OVULATION. IN A WAY THIS PROGRAMME ATTEMPTS TO TARGET THE PRE-WAVE FSH INCREASE OR EMERGENCE OF A FOLLICULAR WAVE AND THIS TIME SHOULD BE BEFORE THE SELECTION OF A DOMINANT FOLLICLE (RUBIANES ET AL., 1997; RUBIANES & MENCHACA, 2003; GONZALEZ-BULNES ET AL., 2004A; HOLTZ, 2005). IN GOATS, IT HAS BEEN OBSERVED THAT ON DAY 0 OF THE INTER-OVULATORY INTERVAL, ALL GOATS HAVE NO LARGE FOLLICLES AND A RESULTANT UNIFORM POOL OF SMALL FOLLICLES. THEREFORE, IF THE SUPEROVULATION TREATMENT IN THE DOE COULD BE INITIATED UNDER THESE CIRCUMSTANCES THE VARIATION IN THE OVARIAN RESPONSE COULD BE MINIMISED. SO FOR EXAMPLE, THE NUMBER OF LARGE FOLLICLES RECRUITED AND THE NUMBER OF OVULATIONS RECORDED FOLLOWING SUPEROVULATION HAVE BEEN INCREASED IN GOATS USING A DAY 0 PROTOCOL AT THE ONSET OF OESTRUS, WHEN COMPARED TO A SUPEROVULATION TREATMENT INITIATED ON DAY 3 AFTER OVULATION. THE NUMBER AND QUALITY OF THE EMBRYOS WERE HOWEVER NOT EVALUATED (MENCHACA ET AL., 2002; RUBIANES & MENCHACA, 2003). IN SHEEP AGAIN, THE NUMBER OF OVULATIONS AND TRANSFERABLE EMBRYOS WERE HIGHER WHEN

IMPLEMENTING A DAY 0 PROTOCOL, COMPARED TO INITIATING A SUPEROVULATION TREATMENT ON DAY 3 AFTER OVULATION. FROM THE LITERATURE IT IS CLEAR THAT MORE RESEARCH IS NEEDED IN GOATS WITH RESPECT TO THE UTILISATION OF THIS DAY 0 PROTOCOL - AS THE HIGH OVULATION RATE ACHIEVED DOES NOT NECESSARILY LEAD TO HIGH FERTILISATION RATE AND EMBRYO VIABILITY (RUBIANES ET AL., 1997).

2.5. Embryo manipulation in goats

2.5.1. Embryo cryopreservation in goats

EMBRYO CRYOPRESERVATION IS AN ESSENTIAL PHASE IN ANY MOET PROGRAMME AIMED AT IMPROVING GENETIC PROGRESS OR THE EMBRYO TRANSFER INDUSTRY. SO FOR EXAMPLE, FROZEN EMBRYOS CAN EASILY AND CHEAPLY BE TRANSPORTED FROM ONE PLACE TO ANOTHER, WITHOUT THE RISK OF HEALTH RISKS AND ANIMAL LOSSES. THE TECHNIQUE OF CRYOPRESERVATION ALSO HELPS TO STORE EMBRYOS PRODUCED IN A MOET PROGRAMME WHEN THE NUMBER OF RECIPIENT ANIMALS ARE NOT SUFFICIENT FOR TRANSFER OF THE COLLECTED FRESH EMBRYOS. IN ADDITION, THIS TECHNIQUE ALSO HAS THE ADVANTAGE, AS A MANAGEMENT TOOL, BY ALLOWING ARTIFICIAL BREEDING OUTSIDE THE NATURAL BREEDING SEASON OR WHEN FARM ACTIVITIES ARE LESS HECTIC. (LEIBO, 1989; FAHNING & GARCIA, 1992; LEONI ET AL., 2001; DOBRINSKY, 2002; GARCIA-GARCIA ET AL., 2006; GUIGNOT ET AL., 2006). GOAT EMBRYOS ARE CURRENTLY SUCCESSFULLY CRYOPRESERVED EITHER BY USING THE CONVENTIONAL SLOW FREEZING TECHNIQUE, OR THE VITRIFICATION METHOD (LE GAL ET AL., 1993; TRALDI ET AL., 1999; EL-GAYAR & HOLTZ, 2001; BEGIN ET AL., 2003; GUIGNOT ET AL., 2006).

2.5.1.1. Conventional slow freezing technique of goat embryos

SINCE THE FIRST SUCCESSFUL CRYOPRESERVATION OF MAMMALIAN EMBRYOS IN THE MOUSE, THE CONVENTIONAL SLOW FREEZING TECHNIQUE OF EMBRYOS HAS BEEN APPLIED ON OTHER FARM ANIMALS E.G. CATTLE, SHEEP AND GOATS WITH RELATIVE SUCCESS (WHITTINGHAM ET AL., 1972; WILMUT & ROWSON, 1973; BILTON & MOORE, 1976; WILLADSEN, 1977). THIS SLOW FREEZING TECHNOLOGY WAS DESIGNED TO MAINTAIN THE FRAGILE EQUILIBRIUM BETWEEN THE CRYOPROTECTANTS AND DIFFERENT PARTS OF THE EMBRYO WHICH, IF NOT PROPERLY PERFORMED, COULD LEAD TO INJURY OF THE EMBRYO I.E. ICE CRYSTAL FORMATION, OSMOTIC DAMAGE, CRYOPROTECTANT TOXICITY, CONCENTRATED INTRACELLULAR ELECTROLYTES, ZONA AND EMBRYO CRACKS, THE ALTERATION OF INTRACELLULAR ORGANELLES AND DAMAGE TO THE CYTOSKELETON OF THE EMBRYOS (NIEMANN, 1991; FAIR ET AL., 2001; DOBRINSKY, 2002; GUIGNOT ET AL., 2006).

THE FIRST CRYOPROTECTANTS USED IN THE PRESERVATION OF MAMMALIAN EMBRYOS (INCLUDING THAT OF SHEEP AND GOATS) VIA THE SLOW FREEZING METHOD WERE PREDOMINANTLY DIMETHYL SULPHOXIDE (DMSO) OR GLYCEROL, COMBINED WITH SUCROSE. THE LATTER HELPS TO DEHYDRATE THE EMBRYOS IN ORDER TO REDUCE THE TIME IN WHICH THE EMBRYO IS EXPOSED TO THE MORE TOXIC INFUSING CRYOPROTECTANTS (BILTON & MOORE, 1976; MASSIP ET AL., 1987, RAO ET AL., 1988; MASSIP, 2001; MOORE & BONILLA, 2006). IN GOATS THE EMBRYO SURVIVAL RATE RECORDED RANGED FROM 26.4% TO 54% FOLLOWING THIS CONVENTIONAL FREEZING WITH GLYCEROL OR DMSO (BARIL ET AL., 1989; LI ET AL., 1990; LE GAL ET AL., 1993). BOTH THESE CRYOPROTECTANTS WHEN

USED IN GOAT EMBRYO CRYOPRESERVATION MOSTLY PRODUCED SIMILAR KIDDING RATES OR EMBRYO SURVIVAL RATES (RAO ET AL., 1988; LI ET AL., 1990).

LATER, ETHYLENE GLYCOL WAS IDENTIFIED AND USED AS A CRYOPROTECTANT AND WAS REPORTED TO HAVE AN ADVANTAGE OF A SMALLER MOLECULAR WEIGHT, COMPARED TO DMSO AND GLYCEROL AND BEING HIGHLY PERMEABLE TO THE EMBRYOS. IN ADDITION, ETHYLENE GLYCOL WAS ALSO FOUND TO RESULT IN A BETTER SURVIVAL RATE OF THE EMBRYOS POST THAWING AND HAVING A LOW TOXICITY EFFECT ON THE EMBRYOS (BRACKE & NIEMANN, 1995; SOMMERFELD & NIEMANN, 1999). ETHYLENE GLYCOL AS A CRYOPROTECTANT FOR THE CRYOPRESERVATION OF RUMINANT EMBRYOS BECAME POPULAR NOT ONLY DUE TO ITS PROPERTIES BUT, ALSO AS A RESULT OF THE BETTER PERFORMANCE OBTAINED - BASED ON SURVIVAL RATES OF EMBRYOS OBTAINED FOLLOWING IN VITRO CULTURE OR AFTER TRANSFER WHEN COMPARED TO GLYCEROL AND DMSO (VOELKEL & HU, 1992; LEIBO & LOSKUTOFF, 1993; COCERO ET AL., 1996; MARTINEZ & MATKOVIC, 1998).

SIMILARLY IN GOATS, WHEN COMPARING ETHYLENE GLYCOL AND GLYCEROL AS CRYOPROTECTANTS FOLLOWING CONVENTIONAL SLOW FREEZING, HIGHER SURVIVAL RATES HAVE BEEN OBTAINED WHEN USING ETHYLENE GLYCOL AS A CRYOPROTECTANT (LE GAL ET AL., 1993).

THEREFORE, LIKE IN OTHER RUMINANTS THE SLOW EMBRYO FREEZING METHOD WAS CONTINUED TO BE EXTENSIVELY USED TO FREEZE GOAT EMBRYOS, WITH ETHYLENE GLYCOL BEING THE FAVOURED CRYOPROTECTANT - FOLLOWED BY GLYCEROL AND THEN DMSO OR A COMBINATION OF ETHYLENE GLYCOL AND DMSO OR GLYCEROL AND DMSO.

HOWEVER, THE EMBRYO SURVIVAL RATES OBTAINED FOLLOWING THE CONVENTIONAL SLOW FROZEN THAWED TRANSFERRED GOAT EMBRYOS WERE STILL GENERALLY ACCEPTED TO BE UNSATISFACTORY (BARIL ET AL., 1989; HOLM ET AL., 1990; BARIL ET AL., 2000; GUIGNOT ET AL., 2006).

2.5.1.2. Vitrification of goat embryos

THE VITRIFICATION METHOD OF CRYOPRESERVATION BECAME AN ALTERNATIVE FORM OF MAMMALIAN EMBRYO CRYOPRESERVATION IN THE 1990'S. VITRIFICATION INVOLVES THE INTRODUCTION OF EMBRYOS TO HIGH CONCENTRATIONS OF A CRYOPROTECTANT AND RAPIDLY COOLING (BY BEING PLUNGED INTO LIQUID NITROGEN). THIS LEADS TO FORMATION OF A GLASS-LIKE SOLIDIFICATION, BYPASSING THE POSSIBLE ICE CRYSTAL FORMATION DURING COOLING. THUS, AVOIDING THE CRYSTALLISATION OF THE EXTRACELLULAR AND INTRACELLULAR CONTENTS OF THE EMBRYO (FAHY ET AL., 1984; RALL & FAHY, 1985; NIEMANN, 1991; DOBRINSKY, 2002; KULESHOVA & LOPATA, 2002). THIS METHOD OF VITRIFICATION HAS BEEN REPORTED TO LEAD TO A HIGHER EMBRYO SURVIVAL RATE FOLLOWING TRANSFER IN SHEEP AND GOATS - APPROACHING THE VALUES RECORDED FOLLOWING THE TRANSFER OF FRESH EMBRYOS. IN SHEEP, SIMILAR SURVIVAL RATES HAVE BEEN RECORDED AFTER THE TRANSFERRING OF VITRIFIED AND FRESH EMBRYOS (MERMILLOD ET AL., 1999; BARIL ET AL., 2001). GOAT EMBRYOS HAVE GENERALLY BEEN SUCCESSFULLY VITRIFIED IN NORMAL STRAWS, AS WELL AS USING THE LATEST TECHNIQUE OF OPEN PULLED STRAWS (OPS) - WITH SURVIVAL RATES FOLLOWING TRANSFER RANGING BETWEEN 9% AND 64% (YUSWIATI & HOLTZ, 1990; TRALDI ET AL., 1999; TRALDI, 2000; EL-GAYAR & HOLTZ, 2001; BEGIN ET AL., 2003).

ALTHOUGH RUMINANT EMBRYOS HAVE BEEN SUCCESSFULLY VITRIFIED IT MUST BE BORNE IN MIND THAT EXPOSURE OF EMBRYOS TO THESE HIGH CONCENTRATION CRYOPROTECTANTS MAY LEAD TO TOXICITY AND OSMOTIC DAMAGE TO THE EMBRYO. HOWEVER, THESE EFFECTS COULD BE REDUCED VIA RAPID COOLING AND THE SELECTION OF THE MOST APPROPRIATE CRYOPROTECTANT RELATED TO ITS PERMEABILITY. THIS COULD IMPLY THAT CRYOPROTECTANTS WHICH ARE MORE PERMEABLE HAVE A HIGHER RISK OF TOXIC DAMAGE TO THE EMBRYO. CONSEQUENTLY, A MIXTURE OF LOW AND HIGH PERMEABLE CRYOPROTECTANTS MAY REDUCE THE OSMOTIC DAMAGE (DATTENA ET AL., 2004; GUIGNOT ET AL., 2006). IN SHEEP A COMBINATION OF ETHYLENE GLYCOL AND GLYCEROL OR DMSO WITH ETHYLENE GLYCOL PRODUCED SIMILAR SURVIVAL RATES (DATTENA ET AL., 2004). DESPITE THIS OBSERVATION, ETHYLENE GLYCOL HAS BEEN PREFERRED AS A CRYOPROTECTANT WHEN COMPARED TO GLYCEROL AND DMSO FOR THE VITRIFICATION OF RUMINANT EMBRYOS (ARAV ET AL., 1993; KASSAI, 1996; MASSIP, 2001; BEGIN ET AL., 2003). ALTHOUGH, ETHYLENE GLYCOL IS CURRENTLY THE POPULAR CRYOPROTECTANT FOR THE VITRIFICATION OF GOAT EMBRYOS, ACCEPTABLE RESULTS HAVE ALSO BEEN OBTAINED WHEN ETHYLENE GLYCOL WAS COMBINED WITH DMSO, UTILISING THE OPS METHOD (BRANCA ET AL., 2000; EL-GAYAR & HOLTZ, 2001).

2.5.1.3. Factors influencing the cryopreservation of goat embryos

2.5.1.3.1. Cryopreservation technique

THE CRYOPRESERVATION METHOD USED MAY INFLUENCE THE EFFICIENCY OF CRYOPRESERVATION AND WHEN COMPARING CONVENTIONAL SLOW FREEZING TO VITRIFICATION, VARIABLE RESULTS HAVE BEEN REPORTED. SO FOR EXAMPLE IN CERTAIN

TRIALS THE VITRIFICATION METHOD RECORDED A HIGHER EMBRYO SURVIVAL RATE THAN USING THE CONVENTIONAL SLOW FREEZING METHOD OF EMBRYOS. ON THE OTHER HAND, VITRIFICATION HAS ALSO LED TO LOWER EMBRYONIC SURVIVAL RATES THAN WITH SLOW FREEZING AND IN CERTAIN INSTANCES, THE TWO METHODS OF CRYOPRESERVATION GAVE SIMILAR RESULTS (VAN WAGTENDONK-DE LEEUW ET AL., 1994; EL-GAYAR & HOLTZ, 2001; COGNIE ET AL., 2003; GUIGNOT ET AL., 2006). HOWEVER, FEW TRIALS HAVE BEEN CONDUCTED COMPARING THE EFFICIENCY OF VITRIFICATION WITH THE SLOW FREEZING OF EMBRYOS IN GOATS. NO MATTER WHICH CRYOPROTECTANT AND METHOD OF CRYOPRESERVATION IS USED, THERE HAS ALWAYS BEEN A 10% HIGHER IN VIVO OR IN VITRO SURVIVAL RATE WITH FRESH EMBRYOS, WHEN COMPARED TO CRYOPRESERVED EMBRYOS (NIEMANN, 1991; COCERO ET AL., 1996; PALASZ & MAPLETOFT, 1996; MARTINEZ & MATKOVIC, 1998; VAJTA, 2000; BARIL ET AL., 2001).

2.5.1.3.2. Origin of the embryos

THE EFFICIENCY OF CRYOPRESERVATION IS NOT ONLY INFLUENCED BY THE METHOD USED, BUT ALSO OTHER FACTORS SUCH AS THE ORIGIN OF EMBRYOS AND THE EMBRYONIC STAGE OF DEVELOPMENT AT FREEZING (RAO ET AL., 1988; MASSIP, 2001). THE IN VITRO PRODUCED EMBRYOS ARE ALSO REPORTED TO BE MORE SENSITIVE TO THE PROCESS OF FREEZING, THAN IN VIVO DERIVED EMBRYOS. THIS SENSITIVITY TO FREEZING IS DUE TO SEVERAL FACTORS, INCLUDING LESS COMPACT MORULAE, THE MEDIUM AND GASEOUS ATMOSPHERE IN WHICH THE EMBRYOS DEVELOP, AS WELL AS THE HIGH LIPID: PROTEIN RATIO (IWASAKI ET AL., 1990; GREVE ET AL., 1993; LEIBO & LOSKUTOFF, 1993; VOJTA ET AL., 1996;

VOJTA ET AL, 1999; MASSIP, 2001; COGNIE ET AL., 2003). THIS CONDITION OF BEING SENSITIVE TO FREEZING THEREFORE REDUCES THE EMBRYO SURVIVAL RATE FOLLOWING CRYOPRESERVATION. THIS ASPECT HAS BEEN PROVEN IN SHEEP, WHERE THE SURVIVAL RATE OF IN VITRO PRODUCED EMBRYOS WAS RECORDED TO BE LOWER THAN THAT OF IN VIVO PRODUCED EMBRYOS (COGNIE, 1999). A VERY HIGH SURVIVAL RATE OF IN VITRO PRODUCED EMBRYOS HAS BEEN OBTAINED IN GOATS FOLLOWING VITRIFICATION, COMPARED TO IN VIVO EMBRYOS IN A STUDY COMPARING THE EFFICIENCY OF EMBRYO CRYOPRESERVATION IN SHEEP AND GOATS. HOWEVER, IN ANOTHER TRIAL, NO SIGNIFICANT DIFFERENCES IN EMBRYO SURVIVAL RATE WERE RECORDED BETWEEN IN VIVO AND IN VITRO PRODUCED GOAT EMBRYOS FOLLOWING VITRIFICATION (COGNIE, 1999; TRALDI ET AL., 2000).

2.5.1.3.3. Stage of development of the goat embryo

THE STAGE OF DEVELOPMENT OF GOAT EMBRYOS AT THE TIME OF CRYOPRESERVATION CAN DETERMINE THE SURVIVAL RATE THEREOF (LI ET AL., 1990; LE GAL ET AL., 1992). SIMILAR OBSERVATIONS HAVE ALSO BEEN REPORTED IN SHEEP AND CATTLE (FAHNING & GARCIA, 1992; COCERO ET AL., 1996; MARTINEZ & MATKOVIC, 1998; MASSIP, 2001). HIGHER SURVIVAL RATES FOLLOWING SLOW FREEZING AND VITRIFICATION OF EMBRYOS HAVE BEEN OBTAINED AT LATER STAGES OF EMBRYONIC DEVELOPMENT (EARLY, EXPANDED, HATCHING AND HATCHED BLASTOCYSTS), COMPARED TO THE MORULA STAGE OF EMBRYO DEVELOPMENT. THIS WAS ALSO EVIDENT WHEN THE EMBRYOS WERE EVALUATED IN VITRO (CHEMINEAU ET AL., 1986; LI ET AL., 1990; NOWSHARI & HOLTZ, 1995B; BARIL ET AL., 2000; BRANCA ET AL., 2000).

DUE TO THE LOW GOAT MORULAE SURVIVAL RATE (±11%), IT IS EVIDENT THAT GOAT MORULAE ARE VERY SENSITIVE TO THE FREEZING AND THAWING PROCESSES. CAPRINE MORULAE SHOULD THEREFORE BE CULTURED IN VITRO TO ATTAIN THE BLASTOCYST STAGE BEFORE BEING CRYOPRESERVED (PULS-KLEINGELD, ET AL., 1992; GORDON, 1997). HOWEVER, THE EFFECTS OF THE STAGE OF DEVELOPMENT OF EMBRYOS IN MOST CASES HAVE BEEN EVALUATED OF THOSE RECOVERED ON THE SAME DAY, BUT AT DIFFERENT STAGES. EMBRYOS THAT WERE AT EARLY STAGES OF DEVELOPMENT DURING COLLECTION (AND WERE SUPPOSED TO BE AT FURTHER STAGES OF DEVELOPMENT) ARE ALREADY DEMONSTRATING SLOW DEVELOPMENT. HENCE, THE SURVIVAL RATE OF THESE EMBRYOS WILL BE REDUCED. THIS ASPECT REGARDING THE IMPORTANCE OF THE STAGE OF DEVELOPMENT HAS BEEN DEMONSTRATED IN BOVINE IN VITRO STUDIES WHERE DAY 8 BLASTOCYSTS RECORDED LOWER HATCHING RATES, COMPARED TO DAY 7 BLASTOCYSTS FOLLOWING VITRIFICATION (SOMMERFELD & NIEMANN, 1999).

2.5.2. Embryo splitting

EMBRYO BISECTION CAN BE USED AS A METHOD TO INCREASE THE NUMBER OF TRANSFERABLE EMBRYOS FROM GENETICALLY SUPERIOR EMBRYOS AND PROVIDE GENETICALLY IDENTICAL ANIMALS FOR EXPERIMENTAL PURPOSES (ROWSON, 1971; WILLIAMS ET AL., 1984; GRAY ET AL., 1991; LOSKUTOFF ET AL., 1993; AMOAH & GELAYE, 1997; COGNIE, 1999; HOLTZ, 2005). IF EXCEPTIONALLY GOOD QUALITY EMBRYOS ARE UTILISED, EMBRYO SPLITTING HAS THE POTENTIAL TO INCREASE THE NUMBER OF OFFSPRING OBTAINED FROM SELECTED

FEMALES. IT HAS BEEN REPORTED THAT THE PREGNANCY RATE FOLLOWING THE TRANSFER OF DEMI-EMBRYOS IS COMPARABLE TO THE TRANSFER OF THE WHOLE EMBRYO. IN SHEEP THE EFFICIENCY OF DUPLICATING OFFSPRING BORN PER WHOLE EMBRYO (DOUBLE DEMI-EMBRYOS) TRANSFERRED HAS BEEN REPORTED TO BE CLOSE TO 100%. IN GOATS THE SURVIVAL RATES OF EMBRYOS FOLLOWING TRANSFER OF DEMI-EMBRYOS RANGED FROM 29% TO 73% (TSUNODA ET AL., 1985; VIVANCO ET AL., 1991, 1992; NOWSHARI & HOLTZ, 1993).

THE STAGE OF DEVELOPMENT OF AN EMBRYO AT SPLITTING HOWEVER, SEEMS TO PLAY A MAJOR ROLE ON THE SURVIVAL RATE OF THE DEMI-EMBRYOS FOLLOWING TRANSFER. IT IS WELL DOCUMENTED THAT EMBRYOS FOR BISECTION MUST AT LEAST BE IN THE COMPACT MORULA STAGE (AMOAH & GELAYE, 1997; COGNIE, 1999). NEVERTHELESS, IN GOATS THE SURVIVAL RATE OF DEMI-MORULAE HAS BEEN UNSATISFACTORY, OR WITH NO DEMI-EMBRYOS SURVIVING TRANSFER. IN GOATS, TSUNODA ET AL. (1985) REPORTED SURVIVAL RATES OF 0%, 33% AND 55% FOR MORULAE, BLASTOCYSTS AND HATCHED BLASTOCYSTS, RESPECTIVELY. THESE RESULTS INDICATE THAT IN GOATS HATCHED BLASTOCYSTS SEEM TO BE MORE SUITABLE FOR SPLITTING, WHEN COMPARED TO THE MORULAE STAGE. NONETHELESS, EVEN WHEN BLASTOCYSTS ARE UTILISED FOR BISECTION, GOAT EMBRYOS ARE STILL REPORTED TO BE MORE SENSITIVE TO SPLITTING THAN E.G. SHEEP AND CATTLE EMBRYOS. THERE ARE SEVERAL REASONS FOR THIS HIGHER SENSITIVITY OF GOAT EMBRYOS THAT HAVE BEEN DOCUMENTED, E.G. THE ZONA PELLUCIDA OF GOAT EMBRYOS SEEM TO BE MORE FRAGILE AND THUS LEADING TO A HIGHER INCIDENCE OF SQUASHING DURING BISECTION. BESIDES BEING FRAGILE, GOAT

EMBRYOS ALSO HAVE LOOSE JUNCTIONS BETWEEN THE BLASTOMERES, AND THEREFORE MAY DISSOCIATE DURING THE SPLITTING PROCESS (UDY, 1987; AMOAH & GELAYE, 1997).

GOAT EMBRYOS ARE ALREADY SENSITIVE TO SPLITTING, WHEN COMPARED TO OTHER RUMINANT EMBRYOS THEREFORE, WHEN GOAT DEMI-EMBRYOS ARE CRYOPRESERVED, THE SCENARIO IS EVEN COMPOUNDED DUE TO DOUBLE INJURY (DAMAGE FROM BISECTION AND CRYOPRESERVATION) OF THE CELLS. IN CATTLE, IT HAS BEEN OBSERVED THAT THE FREEZING AND THAWING PROCEDURES LEAD TO A DEGENERATION OF THE EMBRYONIC AND TROPHOBLASTIC CELLS. WHEN COMPARING FRESH AND FROZEN GOAT DEMI-EMBRYOS, THE SURVIVAL RATES OBTAINED FOLLOWING THE TRANSFER OF FROZEN-THAWED DEMI-EMBRYOS HAVE GENERALLY BEEN VERY LOW OR EVEN NO KIDDING IN SOME CASES HAS BEEN REPORTED. THIS HAS BEEN CONFIRMED BY A 19% AND 59% SURVIVAL RATE RECORDED FOLLOWING THE TRANSFER OF FRESH GOAT DEMI-EMBRYOS COMPARED TO 0% AND 9% SURVIVAL AND PREGNANCY RATES FOLLOWING TRANSFER OF FROZEN-THAWED DEMI-EMBRYOS. THE POOR RESPONSE OF FROZEN GOAT DEMI-EMBRYOS IS NOT ONLY OBSERVED AFTER KIDDING, BUT ALSO INDICATED EARLY DURING PREGNANCY ESTABLISHMENT. A MUCH LOWER PREGNANCY SUCCESS RATE (4%) (OBSERVED VIA ULTRASONOGRAPHY) FOLLOWING FROZEN THAWED-DEMI-EMBRYOS HAS BEEN RECORDED IN GOATS, COMPARED TO A 27% SUCCESS RATE WITH FRESH DEMI-EMBRYO TRANSFERS. POOR SURVIVAL RATES OF DEMI-EMBRYOS FOLLOWING CRYOPRESERVATION HAS ALSO BEEN OBSERVED IN SHEEP AND CATTLE (HEYMAN, 1985; SHELTON &

SZELL, 1988; NOWSHARI & HOLTZ, 1993, BECKETT ET AL., 1999; OPPENHEIM ET AL., 2000).

THE LOW SURVIVAL RATE OF FROZEN DEMI-GOAT EMBRYOS OBTAINED MAY INDICATE THAT THERE IS A NEED FOR THE ESTABLISHMENT OF CRYOPRESERVATION AND THAWING PROCEDURES, SPECIFICALLY FOR BISECTED EMBRYOS. RESEARCHERS HAVE EMPHASIZED THE NECESSITY FOR AN INTACT ZONA PELLUCIDA WHEN FREEZING DEMI-EMBRYOS - AS FREEZING WITHOUT A ZONA PELLUCIDA MAY LEAD TO AN OSMOTIC STRAIN ON THE BLASTOMERES. IN MOST CASES FOR GOATS AND OTHER RUMINANTS THE DEMI-EMBRYOS ARE PLACED INTO AN EMPTY ZONA PELLUCIDA AND EMBEDDED IN AGAR TO SEAL THE EMBRYO (PICARD ET AL., 1985; TSUNODA ET AL., 1987; UDY, 1987; YONG & WANG, 1990; NOWSHARI & HOLTZ, 1993). IN SHEEP AND CATTLE IT HAS BEEN SHOWN THAT THE SURVIVAL RATE OF SPLIT EMBRYOS FRESH OR FROZEN IS NOT INFLUENCED BY THE REPLACEMENT INTO THE ZONA PELLUCIDA (BLAKEWOOD ET AL., 1986; TAKADA ET AL., 1987; SHELTON & SZELL, 1988; SZELL AND HUDSON, 1991).

BESIDES THE STAGE OF DEVELOPMENT OF THE EMBRYO AT SPLITTING AND CRYOPRESERVATION, THE SURVIVAL RATE OF DEMI-EMBRYOS IS ALSO INFLUENCED BY SEVERAL OTHER FACTORS, SUCH AS THE CONDITION AND LENGTH OF CULTURE AFTER SPLITTING, THE ORIGIN OF THE SPLIT EMBRYOS AND WHETHER THE DEMY-EMBRYOS WERE TRANSFERRED AS SINGLE OR PAIRED HALVES. IT HAS BEEN SUGGESTED THAT IN ORDER TO OBTAIN GOOD FERTILITY RESULTS FOLLOWING TRANSFER OF DEMI-EMBRYOS, THE TRANSFER PROCEDURE MUST BE PERFORMED AS SOON AS POSSIBLE AFTER BISECTION. IN GOATS THERE HAS NOT BEEN MUCH RESEARCH REGARDING THIS ASPECT, BUT IN CATTLE

IT HAS BEEN REPORTED THAT THE CULTURING OF SPLIT EMBRYOS IN VITRO BEFORE TRANSFER IS DETRIMENTAL TO THE EMBRYO SURVIVAL RATE. SIMILAR PREGNANCY RATES HAVE ALSO BEEN OBTAINED FOLLOWING THE TRANSFER OF DEMI-BLASTOCYSTS PRODUCED FROM IN VIVO AND IN VITRO DERIVED EMBRYOS (HEYMAN, 1984; BAKER & SHEA, 1985; CHESNE ET AL., 1987; SEIKE ET AL., 1990; LOSKUTOFF ET AL., 1993).

THE MEDIUM FOR CULTURING OF SPLIT EMBRYOS HAS BEEN INDICATED ALSO TO AFFECT THEIR SURVIVAL RATE, ALTHOUGH THERE IS LIMITED RESEARCH IN THIS REGARD IN GOATS. IN SHEEP, IT WAS FOUND THAT NEITHER DULBECCO PHOSPHATE BUFFERED SALINE (DPBS) NOR WHITTEN'S MEDIA OFFERED BETTER SURVIVAL RATES IN DEMI-EMBRYOS. WHEN THE SUCROSE CONCENTRATION WAS MODIFIED BY REDUCING THE CONCENTRATION IN THE CRYOPROTECTANT, THE EMBRYO SURVIVAL RATE IMPROVED. THEN AGAIN, IN SHEEP AND CATTLE, CO-CULTURES WITH OVIDUCTAL EPITHELIAL CELLS HAVE LED TO INCREASED PREGNANCY AND EMBRYO SURVIVAL RATES IN DEMI-EMBRYOS (VOELKEL ET AL., 1985; SHELTON & SZELL, 1988; HERR ET AL., 1990; LOSKUTOFF ET AL., 1993)

EVEN WITH A PROVEN CULTURE MEDIA, THE SURVIVAL RATE OF DEMI-EMBRYOS, WHETHER FRESH OR FROZEN, IS USUALLY LOWER, WHEN COMPARED TO THAT OF INTACT EMBRYOS. IN MOST CASES THERE IS THE IDENTIFICATION OF PREGNANCIES (POSITIVE PREGNANCIES DURING DIAGNOSIS) BUT, VERY LOW EMBRYO SURVIVAL RATE OR NO SURVIVAL RATE AT ALL FOLLOWING SPLITTING AND CRYOPRESERVATION. SO FOR EXAMPLE, PREGNANCY RATES OF 82% AND 55% HAVE BEEN OBTAINED WITH FRESH AND FROZEN EMBRYOS

RESPECTIVELY - WHILE THE SURVIVAL RATES OF FRESH AND FROZEN DEMI-EMBRYOS RECORDED WERE 59% AND 9%. SIMILAR TRENDS HAVE ALSO BEEN OBSERVED IN SHEEP (NOWSHARI & HOLTZ, 1993; BECKETT ET AL., 1999). THE LOWER SURVIVAL RATE OF THE SPLIT EMBRYOS MAY INDICATE THAT DEMI-EMBRYOS LOOSE THEIR VIABILITY, AS PREGNANCY PROGRESSES. IN ADDITION THE DEMI-EMBRYOS MAY NOT INDUCE ENOUGH MATERNAL RECOGNITION OF PREGNANCY, WITH RESULT THAT THE RECIPIENTS FAIL TO SUSTAIN A CORPUS LUTEUM FOR PREGNANCY. THIS PHENOMENON IS DEMONSTRATED BY A HIGHER INCIDENCE OF PREGNANCY AND SURVIVAL RATES AFTER THE TRANSFER OF TWO OR MORE INTACT OR DEMI-EMBRYOS, COMPARED TO TRANSFER OF ONLY ONE DEMI-EMBRYO OR A SINGLE WHOLE EMBRYO IN CATTLE (OZIL ET AL., 1982; LAMBETH ET AL., 1983; HEYMAN, 1985; HOLM ET AL., 1991).

TO THE CONTRARY, SIMILAR PREGNANCY RATES IN CATTLE FOLLOWING THE TRANSFER OF WHOLE AND SINGLE OR DOUBLE DEMI-EMBRYOS HAVE BEEN RECORDED (WILLIAMS ET AL., 1984; LOSKUTOFF ET AL., 1993). THESE RESULTS COULD SUGGEST THAT THE OVARIAN AND UTERINE SIGNALS ALSO NEED TO BE REVISED. CONCERNING THIS ASPECT, SOME RESEARCHERS HAVE EVALUATED THE TRANSFER OF DEMI-EMBRYOS WITH THE ADDITION OF TROPHOBLASTIC VESICLES - AS THESE HAVE BEEN REPORTED TO MAINTAIN THE CORPUS LUTEUM WHEN PLACED INTO THE UTERUS WITHOUT AN EMBRYO IN SHEEP AND CATTLE OR IN THE TRANSFER OF DEMI-EMBRYOS CONCURRENTLY WITH EXOGENOUS PROGESTAGENS (HEYMAN, 1985; HEYMAN ET AL., 1987; BECKETT ET AL., 1999). IN GOATS, THE TRANSFER OF FRESH DEMI-EMBRYOS, TOGETHER WITH PROGESTAGEN IMPLANTS

INCREASED THE EMBRYONIC RECOGNITION AND THIS WAS REFLECTED BY A HIGHER PREGNANCY AND EMBRYO SURVIVAL RATE. THE USE OF PROGESTIN IMPLANTS ALSO IMPROVED THE PREGNANCY RATES FROM 0% TO 13% FOLLOWING THE TRANSFER OF FROZEN DEMI-EMBRYOS IN GOATS (BECKETT ET AL., 1999).

2.5.3. Factors influencing the survivability of transferred goat embryos

EMBRYO TRANSFER IS THE LAST STAGE IN THE MOET PROGRAMME. NORMALLY EMBRYOS ARE TRANSFERRED TO RECIPIENTS WHICH HAVE SHOWN SIGNS OF OVERT OESTRUS, CONCURRENTLY WITH THEIR RESPECTIVE DONORS (MOORE, 1974; BESSOUDO ET AL., 1988; WALLACE, 1992). IT IS CLEAR THAT THE SYNCHRONISATION OF THE DONOR AND THE RECIPIENT'S OESTROUS PERIOD IS IMPORTANT TO ACHIEVE A FAVOURABLE ENVIRONMENT FOR THE DEVELOPMENT OF THE TRANSFERRED EMBRYOS. TO DEMONSTRATE THE EFFECT OF AN ASYNCHRONOUS ONSET OF OESTRUS IN THE EWE BETWEEN THE DONOR AND THE RECIPIENT, EMBRYOS RECOVERED ON DAY 4 FOLLOWING OESTRUS FAILED TO SURVIVE WHEN TRANSFERRED TO RECIPIENTS ON DAY 7 OF THE CYCLE (72H ASYNCHRONY) (WILMUT & SALES, 1981; WALLACE, 1992). OPTIMAL EMBRYO SURVIVAL RATES HAVE BEEN OBTAINED WHEN THE ONSET OF OESTRUS OF THE DONOR AND THE RECIPIENTS IS WITHIN 24 H OF EACH OTHER. HOWEVER, SATISFACTORY EMBRYO SURVIVAL RATES HAVE ALSO BEEN OBTAINED EVEN WHEN THE RECIPIENTS SHOW OESTROUS SIGNS BETWEEN 12H AND 48H BEFORE OR AFTER THEIR RESPECTIVE DONORS (MOORE, 1974; MOORE & EPPLESTON, 1979; ISHWAR & MEMON, 1996; OPPENHEIM ET AL., 2000; HOLTZ, 2005). BESIDES THE SYNCHRONY OF OESTRUS BETWEEN THE DONOR AND RECIPIENTS, LIKE IN OTHER RUMINANTS, THERE ARE OTHER FUNDAMENTAL

PARAMETERS OF THE GOAT RECIPIENTS INFLUENCING THE SURVIVAL RATE OF THE EMBRYOS TRANSFERRED E.G. HEALTH, THE REPRODUCTIVE STATUS, NUTRITION STATUS, BREED, AS WELL AS THE AGE AND PARITY OF THE RECIPIENT. SO FOR EXAMPLE, A LOW EMBRYO SURVIVAL RATE HAS BEEN REPORTED IN GOATS UNDERNOURISHED BEFORE AND AFTER EMBRYO TRANSFER, COMPARED TO RECIPIENTS ON A MAINTENANCE DIET. IN ADDITION, POOR PREGNANCY RATES HAVE BEEN REPORTED IN RECIPIENT GOATS IN WHICH EMBRYOS WERE TRANSFERRED OUTSIDE THE NATURAL BREEDING SEASON (MANI ET AL., 1994; GONZALEZ-BULNES ET AL., 2004; HOLTZ, 2005).

IN SHEEP, THE SURVIVAL RATE OF EMBRYOS HAS BEEN REPORTED TO BE 52% IN YOUNG RECIPIENTS, COMPARED TO 73% IN ADULT EWES. IN CONTRAST, OTHER FINDINGS HAVE REPORTED THE EMBRYO SURVIVAL RATE TO BE HIGHER IN YEARLING RECIPIENTS, COMPARED TO MULTIPAROUS DAMS (2 TO 6 YEARS OF AGE), AS WELL AS SIMILAR SURVIVAL RATES IN ALL AGE GROUPS. (WALLACE, 1992; MANI ET AL., 1994; GORDON, 1997; BARI ET AL., 1999, 2003). THERE ARE SEVERAL OTHER FACTORS INFLUENCING THE SURVIVABILITY OF EMBRYOS FOLLOWING TRANSFER, BESIDES THE STATUS OF THE RECIPIENT. THESE INCLUDE THE STAGE OF DEVELOPMENT OF THE EMBRYO TRANSFERRED, THE NUMBER AND QUALITY OF EMBRYOS TRANSFERRED, THE EMBRYO ORIGIN, THE PRESENCE AND THE NUMBER OF CORPORA LUTEA PRESENT (ARMSTRONG ET AL., 1983C; ISHWAR & MEMON, 1996; BARI ET AL., 2003).

2.5.3.1. Site of embryo transfer

EMBRYOS ARE NORMALLY TRANSFERRED INTO THE OVIDUCT OR UTERINE HORN, DEPENDING ON THE AGE OR STAGE OF DEVELOPMENT OF THE EMBRYO BEING TRANSFERRED. EARLY EMBRYONIC STAGES

I.E. EMBRYOS AT 8-16 CELL STAGE OR EMBRYOS COLLECTED FROM DAY 3 TO 5 FOLLOWING MATING ARE USUALLY TRANSFERRED TO THE OVIDUCT WITH THE AID OF A FINE PIPETTE, IN ORDER TO TRANSFER A MINIMUM AMOUNT OF THE CULTURE OR HOLDING MEDIUM (ARMSTRONG ET AL., 1983C; BESSOUDO ET AL., 1988; WALLACE, 1992; HOLTZ, 2005). WHEN THE SITE OF TRANSFER WAS EVALUATED DURING EARLY EMBRYONIC STAGES IN SHEEP AND GOATS IT WAS FOUND THAT A HIGHER THE SURVIVAL RATE WAS OBTAINED IF 4 TO 8-CELL EMBRYOS ARE TRANSFERRED INTO THE OVIDUCT - THAN E.G. WHEN THE EMBRYO IS TRANSFERRED INTO THE UTERINE HORN. THE POOR SURVIVAL RATE OF AN EARLY STAGE EMBRYO TRANSFERRED INTO THE UTERINE HORN IS INDICATIVE OF AN UNFAVOURABLE ENVIRONMENT FOR THE DEVELOPMENT AND SURVIVAL OF THE EMBRYO. THIS IS UNDERSTANDABLE, AS DURING THE IN VIVO DEVELOPMENT OF THE EMBRYOS AT THIS STAGE THE EMBRYOS ARE STILL IN THE OVIDUCT (MOORE & SHELTON, 1964; ARMSTRONG ET AL., 1983B; ISHWAR & MEMON, 1996). EMBRYOS AT A MORE ADVANCED STAGE OF DEVELOPMENT I.E. FROM DAY 5 AND LATER AFTER MATING ARE GENERALLY TRANSFERRED INTO THE LUMEN OF THE UTERINE HORN. THE EMBRYOS ARE DEPOSITED BY PUNCTURING THE UTERINE WALL WITH A BLUNT NEEDLE CLOSE TO THE UTEROTUBAL JUNCTION (WALLACE, 1992; ISHWAR & MEMON, 1996; HOLTZ, 2005). IN GOAT MOET PROGRAMMES EMBRYOS ARE GENERALLY TRANSFERRED INTO THE UTERINE HORN OF RECIPIENTS, AT DAY 6 TO 7 FOLLOWING OESTRUS. THIS IS TO ACCOMMODATE THE TRANSFER OF FROZEN-THAWED EMBRYOS NORMALLY AT THE MORULA TO HATCHED BLASTOCYST STAGE AND ALSO BECAUSE OF A HIGHER SURVIVAL RATE BEING

OBTAINED AT THIS EMBRYONIC STAGE (TRALDI ET AL., 1999; EL-GAYER & HOLTZ, 2001; GUIGNOT ET AL., 2006).

IN GOATS, EMBRYOS ARE MOSTLY TRANSFERRED INTO THE OVIDUCT OR UTERINE HORN IPSILATERAL TO THE OVARY, WITH AT LEAST ONE NORMAL CORPUS LUTEUM (MOORE & EPPLESTON, 1979; BESSOUDO ET AL., 1988; STEFANI ET AL., 1990; BESENFELDER ET AL., 1994; EL-GAYER & HOLTZ, 2001; HOLTZ, 2005; GUIGNOT ET AL., 2006). IN SHEEP AND CATTLE IF EMBRYOS ARE TRANSFERRED TO THE CONTRALATERAL HORN TO THE CORPUS LUTEUM, THE EMBRYOS NORMALLY DIE AND THE CORPUS LUTEUM ON THE OTHER SIDE WILL ALSO REGRESS DUE TO ABSENCE OF AN EMBRYO. THIS OBSERVATION INDICATES THAT THE UTERUS HAS A DIRECT LUTEOLYTIC EFFECT. IN GOATS THE EMBRYO SURVIVAL RATE WAS NOT AFFECTED WHEN TRANSFERRING ONE EMBRYO TO EACH UTERINE HORN OR BY TRANSFERRING BOTH EMBRYOS TO THE UTERINE HORN IPSILATERAL TO THE OVULATION POINT IN UNILATERALLY OVULATED GOATS (ROWSON, 1971; TERVIT, 1987).

BESIDES TRANSFERRING EMBRYOS TO THE UTERINE HORN IPSILATERAL TO THE OVARY THAT OVULATED, THE NUMBER OF CORPORA LUTEA AT TRANSFER CAN ALSO INFLUENCE THE EMBRYO SURVIVAL RATE. IN GOATS, THE EMBRYO SURVIVAL RATE HAS BEEN POSITIVELY CORRELATED WITH THE NUMBER OF NORMAL CORPORA LUTEA PRESENT IN THE RECIPIENT DURING TRANSFER. THIS HAS BEEN DEMONSTRATED WHERE THE EMBRYO SURVIVAL RATE IN A RECIPIENT WITH ONE OVULATION WAS LOWER WHEN COMPARED TO A DOE RECIPIENT WITH 2 OR 3 CORPORA LUTEA. HOWEVER, THE EMBRYO SURVIVAL RATE BETWEEN RECIPIENTS WITH 2 OR 3 OVULATIONS DID NOT DIFFER. IN SHEEP ON THE

OTHER HAND, THE EMBRYO SURVIVAL RATE WAS LOWER IN RECIPIENTS WITH ONE CORPUS LUTEUM, COMPARED TO RECIPIENTS WITH 2 CORPORA LUTEA WHEN GOOD QUALITY EMBRYOS (GRADE 1) WERE TRANSFERRED. HOWEVER, THE EMBRYO SURVIVAL RATE OF EWES WITH ONE AND 3 OR MORE CORPORA LUTEA DID NOT DIFFER SIGNIFICANTLY.

THUS, IN ORDER TO INCREASE THE OVULATION RATE OF THE RECIPIENTS, ALTHOUGH SYNCHRONISED THE SAME WAY AS THE DONORS, ECG SHOULD BE INJECTED 48H BEFORE OR AT PROGESTAGEN TREATMENT CESSATION (MOORE, 1974; ARMSTRONG ET AL., 1983B; BARI ET AL., 2003). IN ADDITION TO THE TRANSFERRING OF EMBRYOS TO THE SIDE WITH 2 OR MORE ACTIVE CORPORA LUTEA, EMBRYO SURVIVABILITY CAN GENERALLY BE IMPROVED BY THE UTILISATION OF RECTAL ULTRASONOGRAPHY. THIS COULD HELP IN EVALUATING THE OVULATION SITE OF THE RECIPIENT AND GIVING A CLEAR INDICATION OF THE DEVELOPMENT OF THE CORPUS LUTEUM (BY THE MEASURING OF THE DIAMETER). THIS TECHNIQUE COULD ALSO HELP TO EVALUATE THE STATE OF THE UTERUS, ESPECIALLY FOR PATHOLOGICAL SYMPTOMS (COX ET AL., 1998; SANTIAGO-MORENO ET AL., 2001; GONZALEZ-BULNES ET AL., 2004).

2.5.3.2. Method of embryo transfer in goats

IN THE PAST EMBRYOS HAVE BEEN TRANSFERRED IN GOATS BY SURGICAL, LAPAROSCOPIC AND TRANSCERVICAL METHODS WITH SUCCESS (BESSOUDO ET AL., 1988; FLORES-FOXWORTH ET AL., 1992; BESENFELDER ET AL., 1994). IN GOATS, SIMILAR TO SHEEP, EMBRYOS ARE MOSTLY TRANSFERRED SURGICALLY INTO THE OVIDUCT OR UTERINE HORN. THE REPRODUCTIVE TRACT BEING EXTERIORISED VIA A MID-VENTRAL LAPAROTOMY, WHICH ALLOWS FOR THE INSPECTION OF THE OVARIES FOR

PRESENCE OF CORPORA LUTEA (MOORE, 1974; ARMSTRONG AT AL., 1983; KIESSLING ET AL., 1986; BESSOUDO ET AL., 1988; LI ET AL., 1990; SELGRATH ET AL., 1990; YUSWIATI & HOLTZ, 1990; WALLACE, 1992; HOLTZ, 2005; GUIGNOT ET AL., 2006). HOWEVER, THE LAPAROSCOPIC METHOD OF EMBRYO TRANSFER HAS LATELY GAINED POPULARITY IN SMALL RUMINANTS - THE REASONS BEING THAT IT IS SAFE, EASY AND QUICK TO PERFORM AND IT CREATES THE OPPORTUNITY TO VISUALLY CONFIRM THE PRESENCE AND QUALITY OF CORPORA LUTEA BEFORE TRANSFER. THIS LEADS TO SURVIVAL RATES SIMILAR TO SURGICAL EMBRYO TRANSFER. IN LAPAROSCOPIC EMBRYO TRANSFER A SMALL INCISION IS MADE AS OPPOSED TO SURGICAL TRANSFER WHICH INVOLVES THE EXTERIORATION OF THE TIP OF THE UTERINE HORN IN WHICH EMBRYO TRANSFER IS GOING TO BE MADE (KRAEMER, 1989; STEFANI ET AL., 1990; BREBION ET AL., 1992; FLORES-FOXWORTH ET AL., 1992; BESENFELDER ET AL., 1994; ISHWAR & MEMON, 1996; COGNIE, 1999).

TRANSCERVICAL EMBRYO TRANSFER IN GOATS HAS BEEN TRIED WITH SUCCESS. WHEN COMPARING THE PREGNANCY RATES FOLLOWING TRANSCERVICAL AND LAPAROSCOPIC EMBRYO TRANSFER NO SIGNIFICANT DIFFERENCES WERE FOUND. HOWEVER, THE TRANSCERVICAL EMBRYO TRANSFER METHOD IS NOT POPULAR DUE TO THE LOW EMBRYO SURVIVAL RATES GENERALLY OBTAINED. THIS IS DUE TO THE FACT THAT THIS METHOD DOES NOT ALLOW FOR THE CONFIRMATION OF THE PRESENCE OF CORPORA LUTEA. MOREOVER, TRANCERVICAL TRANSFERS HAVE BEEN REPORTED TO INDUCE CONTRACTIONS OF THE CERVIX AND THE UTERUS, WHICH RESULT IN THE REJECTION OF THE TRANSFERRED EMBRYOS. IN CATTLE, EMBRYOS

WERE FOUND IN THE VAGINA A FEW HOURS FOLLOWING TRANSFER (ROWSON, 1971; FLORES-FOXWORTH ET AL., 1992; WALLACE, 1992; COGNIE, 1999).

2.5.3.3. Number of embryos transferred

THE NUMBER OF EMBRYOS TRANSFERRED HAS AN EFFECT ON THE SURVIVAL RATE OF EMBRYOS IN GOATS. TWO EMBRYOS ARE USUALLY TRANSFERRED PER RECIPIENT IN SMALL RUMINANTS. THIS IS COMMON PRACTICE AS IT HAS BEEN REPEATEDLY REPORTED THAT THE SURVIVABILITY OF THE EMBRYOS IS HIGHER WHEN TWO EMBRYOS ARE TRANSFERRED, COMPARED TO THE TRANSFER OF ONE OR 3 EMBRYOS (MOORE, 1974; ARMSTRONG ET AL, 1983; TERVIT ET AL., 1986; ISHWAR & MEMON, 1996; GOOTWINE ET AL., 1997; EL-GAYAR & HOLTZ, 2001, 2005). THE SURVIVAL RATES FOLLOWING A TWIN EMBRYO TRANSFER HAS BEEN REPORTED TO EVEN IMPROVE WHEN THE 2 EMBRYOS ARE TRANSFERRED TO THE SAME OVIDUCT (UNILATERAL TRANSFER), COMPARED TO BILATERAL TRANSFER (ONE EMBRYO TRANSFER TO EACH OVIDUCT). THIS MAY SUGGEST THAT UNILATERAL EMBRYO TRANSFER INDUCES A STRONGER MATERNAL SIGNAL OF RECOGNITION OF PREGNANCY TO THE ENDOMETRIUM (ARMSTRONG ET AL., 1983; ISHWAR & MEMON, 1996). ALTHOUGH THERE IS GENERAL AGREEMENT REGARDING A HIGHER EMBRYO SURVIVAL RATE FOLLOWING TWO EMBRYOS BEING TRANSFERRED, OTHER STUDIES HAVE SHOWN NO DIFFERENCE WITH RESPECT TO THE SURVIVAL RATE FOLLOWING A SINGLE OR TWIN EMBRYO TRANSFER. THE SURVIVABILITY OF EMBRYOS HAS ALSO BEEN SHOWN TO DECLINE (OR SOMETIMES NO EMBRYOS SURVIVED), WHEN 3 OR MORE EMBRYOS WERE TRANSFERRED PER RECIPIENT (MOORE, 1974;

BESSOUDO ET AL., 1988; STEFANI ET AL., 1990; ISHWAR & MEMON, 1996).

2.5.3.4. The origin of the embryos transferred

THE NUMBER OF EMBRYOS FOR TRANSFER IN A MOET PROGRAMME CAN BE INCREASED THROUGH THE UTILISATION OF NOT ONLY IN VIVO PRODUCED EMBRYOS, BUT ALSO IN VITRO PRODUCED EMBRYOS. PRODUCTION OF GOAT EMBRYOS IN VITRO IS A RAPIDLY ADVANCING FIELD OF RESEARCH, AS IT PROVIDES AN ALTERNATIVE TO SUPEROVULATION AS A SOURCE OF EMBRYOS FOR TRANSFER IN A MOET PROGRAMME - ESPECIALLY WHEN CONSIDERING THE UNPREDICTABILITY OF THE RESPONSE TO SUPEROVULATION. FOR THE PRODUCTION OF IN VITRO EMBRYOS, MATURE OOCYTES MAY BE RECOVERED FROM THE OVIDUCT OF THE DONOR AND THE EMBRYOS COLLECTED WITHIN 5H FOLLOWING OVULATION. HOWEVER, THIS PROCEDURE IS IMPRACTICAL DUE TO DIFFICULTY OF DETECTING THE TIME OF OVULATION. IMMATURE OOCYTES IN GOATS ARE GENERALLY RECOVERED FROM THE OVARIES OBTAINED EITHER FROM AN ABATTOIR, FROM LIFE ANIMALS THROUGH LAPAROTOMY, ULTRASOUND GUIDED TRANSVAGINAL ASPIRATION OR LAPAROSCOPY (BALDASSARRE ET AL., 1994; PAWSHE ET AL., 1994; GRAFF ET AL., 1999; CROZET ET AL., 2000; HAN ET AL., 2001; REGGIO ET AL., 2001; BALDASSARRE ET AL., 2003; BALDASSARRE & KARATZAS, 2004).

THE EMBRYO SURVIVAL RATE FOLLOWING FRESH IN VITRO PRODUCED EMBRYOS HAS BEEN REPORTED TO RANGE BETWEEN 25% AND 61% (KESKINTEPE ET AL., 1994; AMOAH & GELAYE, 1997; COGNIE, 1999; COGNIE ET AL., 2003). THE SURVIVAL RATE OF IN VITRO PRODUCED EMBRYOS WERE HOWEVER BEEN REPORTED TO BE LOWER FOLLOWING TRANSFER, WHEN COMPARED TO IN VIVO PRODUCED EMBRYOS

(GREVE ET AL., 1993; COGNIE, 1999). IN GOATS, IN VITRO PRODUCED EMBRYO SURVIVAL RATES OF 47% HAVE BEEN RECORDED COMPARED TO 71% FOR IN VIVO PRODUCED EMBRYOS. SEVERAL FACTORS HAVE BEEN INDICATED TO CONTRIBUTE TO THE LOW SURVIVABILITY OF IN VITRO PRODUCED EMBRYOS, WHEN COMPARED TO THE IN VIVO DERIVED EMBRYOS (COGNIE ET AL., 2003).

LOW SURVIVABILITY OF EMBRYOS HAS BEEN COUPLED WITH DIFFERENCES OBSERVED BETWEEN IN VIVO AND IN VITRO PRODUCED EMBRYOS. FIRSTLY, ARE THE DIFFERENCES IN THE GENE EXPRESSION OBSERVED BETWEEN THE IN VIVO AND IN VITRO PRODUCED EMBRYOS. SO FOR EXAMPLE, THE CELL-CELL COUPLING HAS BEEN PROMINENT IN THE IN VIVO PRODUCED BLASTOCYSTS, YET NOT TOO EVIDENT IN BLASTOCYSTS PRODUCED IN VITRO. THE CELL NUMBER OF IN VITRO PRODUCE EMBRYOS HAS ALSO BEEN OBSERVED TO BE SLIGHTLY LOWER, WHEN COMPARED TO THE IN VIVO DERIVED EMBRYOS. FURTHERMORE, THE INNER CELL MASS (ICM) OF CELLS FROM IN VITRO PRODUCED EMBRYOS ARE ALSO LOWER AND LESS COMPACT DUE TO A HIGHER DEGREE OF VACUOLIZATION AND HAVE FEWER AND SHORTER JUNCTION COMPLEXES BETWEEN THE CELLS. THESE DIFFERENCES BETWEEN IN VITRO AND IN VIVO DERIVED EMBRYOS IN GOATS ARE SIMILAR TO THOSE OBSERVED IN OTHER RUMINANT ANIMALS - WHICH CAN MOSTLY BE ASCRIBED TO THE STRUCTURAL AND BIOCHEMICAL COMPONENTS. IN VITRO PRODUCED EMBRYOS HAVE BEEN REPORTED TO HAVE A DARKENED APPEARANCE, RELATIVELY MORE INTRACELLULAR LIPIDS IN RELATION TO PROTEIN AND SMALL INNER CELL MASS AND A MORE FRAGILE ZONA PELLUCIDA, COMPARED TO IN VIVO DERIVED EMBRYOS (IWASAKI ET AL., 1990; GREVE ET AL., 1993; POLLARD & LEIBO,

1994; THOMPSON, 1997; LONERGAN ET AL., 2001; PAPADOPOULOS ET AL., 2002).

THE SIZE OF THE FOLLICLES USED TO PRODUCE AN EMBRYO MAY ALSO REDUCE THE SURVIVAL RATE FOLLOWING EMBRYO TRANSFER. THIS IS INDICATED BY THE LOW BLASTOCYST DEVELOPMENT RATE OBSERVED IN OOCYTES FROM SMALL FOLLICLES, COMPARED TO LARGE FOLLICLES. BLASTOCYSTS FROM SMALL AND MEDIUM SIZED GOAT OOCYTES POSSESSED A SMALLER NUMBER OF CELLS, AS WELL AS ICM - WHICH DEMONSTRATES A POOR DEVELOPMENTAL CAPACITY. IT IS THUS, IMPORTANT TO UTILISE SELECTED OOCYTES FROM LARGE FOLLICLES FOR PRODUCING EMBRYOS IN VITRO. IN GOATS, THE SURVIVAL RATE TO BLASTOCYST OF EMBRYOS EVALUATED IN VITRO FROM FOLLICLES LARGER >5 MM WAS HIGHER THAN THE SURVIVAL RATE OF EMBRYOS FROM OOCYTES FROM SMALLER FOLLICLES (2-3MM) (CROZET ET AL., 1995; COGNIE, 1999).

2.5.3.5. Quality and stage of development of embryos transferred

THE QUALITY AND STAGE OF DEVELOPMENT OF THE EMBRYO HAS A SIGNIFICANT EFFECT ON THE SUBSEQUENT SURVIVAL RATE. IN GOATS, EMBRYOS ARE GENERALLY COLLECTED ON DAY 7 TO 8 FOLLOWING OVULATION. AT THIS TIME THE RECOVERED EMBRYOS ARE EXPECTED TO BE AT THE MORULA TO BLASTOCYST STAGE. HOWEVER, GOAT EMBRYOS COLLECTED AT THIS TIME ARE ALWAYS AT DIFFERENT STAGES OF DEVELOPMENT, EVEN FOLLOWING THE SYNCHRONISATION OF THE ONSET OF OESTRUS AND EVEN FOR THE SAME INDIVIDUAL (BARIL ET AL., 1988; YANG ET AL., 1991; GORDON, 1997). NORMALLY THE STAGE OF DEVELOPMENT INFLUENCES THE EMBRYO QUALITY, AS IT IS BASED ON THE MORPHOLOGICAL APPEARANCE AND THE STAGE OF DEVELOPMENT. AS QUALITY IS

SUBJECTIVE, IT IS NOT EASY TO ALWAYS EXPLAIN THE DIFFERENCES IN SURVIVAL RATE BETWEEN GRADE 1 AND 2 EMBRYOS. HOWEVER, A LOWER EMBRYO SURVIVAL RATE HAS BEEN REPORTED FOLLOWING THE TRANSFER OF GRADE 3 AND 4 EMBRYOS WHEN COMPARED TO GRADE 1 AND 2 EMBRYOS. WHEN LOOKING AT THE STAGE OF DEVELOPMENT, THE TRANSFER OF BLASTOCYSTS ALWAYS LEADS TO A HIGHER SURVIVAL RATE COMPARED TO THE MORULA STAGE IN RUMINANTS. CONVERSELY, RESEARCHERS HAVE REPORTED NO SIGNIFICANT DIFFERENCE IN EMBRYO SURVIVAL RATE BETWEEN THE TRANSFERS OF MORULA OR BLASTOCYST EMBRYOS. HOWEVER, WHEN ADVANCED BLASTOCYSTS COLLECTED ON DAY 5 ARE TRANSFERRED, A HIGHER SURVIVAL RATE WAS OBTAINED, COMPARED TO THE TRANSFER OF RETARDED MORULA EMBRYOS COLLECTED ON DAY 6. FOR THIS REASON, THE TRANSFER OF EMBRYOS WITH RETARDED GROWTH MUST BE MINIMISED IN ORDER TO HAVE GOOD EMBRYO SURVIVAL RATES (DONALDSON, 1985; BREUEL ET AL., 1991; WALLACE, 1992; BARI ET AL., 2003).

2.6. Summary

THE BOER GOAT IS A SOUTH AFRICAN INDIGENOUS BREED HIGHLY RECOGNISED AND RENOWNED INTERNATIONALLY FOR THE QUALITY AND QUANTITY OF MEAT PRODUCED. DUE TO THE POPULARITY OF THE BREED, THE DEMAND IS ALSO HIGH. THEREFORE, A REPRODUCTIVE PROGRAMME WHICH CAN SPEED UP THE GENETIC PROGRESS, INCREASE THE GENETIC MATERIAL TRANSFER AND DISTRIBUTION ACROSS THE WORLD IS ESSENTIAL. A RELIABLE AND SUSTAINABLE MOET PROGRAMME COULD THEREFORE, IMPROVE AND DISSEMINATE THE GENETIC MATERIAL OF THIS VALUABLE BREED ACROSS THE GLOBE. ALTHOUGH MOET IS AN

APPROPRIATE TOOL, THERE IS LIMITED RESEARCH UNDERTAKEN WITH RESPECT TO THE IMPROVEMENT OF THE PROGRAMME IN GOATS. BESIDES BEING LIMITED, MANY PHASES OR COMPONENTS OF THIS PROGRAMME STILL WARRANT RESEARCH.

THE MOST IMPORTANT COMPONENT OF MOET I.E. SUPEROVULATION, STILL PROVIDES VARIABLE RESULTS IN GOATS - DUE TO MANY TO INTRINSIC AND EXTRINSIC FACTORS. FIRSTLY, IT HAS BEEN SHOWN THAT THE GONADOTROPHINS USED FOR THE SUPERSTIMULATION TO HAVE AN INFLUENCE ON THE OVARIAN RESPONSE, DUE TO THE PREPARATION AND THE COMPOSITION OF THE GONADOTROPHIN USED. THE THREE EXOGENOUS GONADOTROPHINS USED FOR SUPEROVULATION IN FARM ANIMALS ARE HAP, ECG AND FSH. PRESENTLY THE MOST COMMONLY USED HORMONES ARE FSH AND ECG, WITH HAP NOT GENERALLY BEING AVAILABLE. THE ECG HORMONE IS USUALLY ADMINISTERED AS A SINGLE INJECTION DUE TO ITS LONG HALF-LIFE. HOWEVER, THIS LONG HALF-LIFE PROPERTY OF ECG COULD LEAD TO THE PRODUCTION OF LARGE UNOVULATORY FOLLICLES FOLLOWING SUPEROVULATION, WHICH ARE OBSERVED DURING EMBRYO RECOVERY. THESE UNOVULATORY OR CYSTIC FOLLICLES PRODUCE ELEVATED OESTROGEN LEVELS AND THIS STIMULATES THE EARLY INCREASE IN THE SECRETION OF ENDOGENOUS PGF2A. THIS INCREASE IN PGF2A THEN LEADS TO HIGH INCIDENCES OF PREMATURE LUTEAL REGRESSION. THE END RESULT OF PREMATURE LUTEAL REGRESSION IS THUS A DECLINE IN PROGESTERONE CONCENTRATION BEFORE EMBRYO COLLECTION, LEADING TO A LOSS IN EMBRYOS. PREMATURE LUTEAL REGRESSION NOT ONLY REDUCES THE PROGESTERONE CONCENTRATION, BUT ALSO HAS AN EMBRYO TOXICITY EFFECT. THESE DETRIMENTAL EFFECTS

FOLLOWING SUPEROVULATION WITH THE USE OF ECG CAN BE MINIMISED BY THE ADMINISTRATION OF ANTI-ECG, HCG OR GNRH.
IN CAPRINE MOET PROGRAMMES, OFSH AND PFSH ARE THE MOST COMMONLY UTILISED FSH PREPARATIONS. THESE FSH PREPARATIONS GIVE SATISFACTORY SUPEROVULATORY RESULTS, ALTHOUGH LARGE VARIATION IN OVARIAN RESPONSE ATTRIBUTED TO THE FSH:LH RATIO IN THE GONADOTROPHIN CONTENT IS EXPERIENCED. A HIGH LH CONTENT IN THE FSH PREPARATION GENERALLY LEADS TO PREMATURE OVULATION OF THE LARGE FOLLICLES AND THIS EVENTUALLY LEADS TO THE PRODUCTION OF PROGESTERONE DURING THE PRE-OVULATORY PERIOD OF THE NEWLY STIMULATED FOLLICLES. ALTERING THE PROGESTERONE AND OESTROGEN RATIO OF THESE FOLLICLES EVENTUALLY DISTURBS THE MATURATION PROCESS WITH A RESULTANT PRODUCTION OF POOR QUALITY EMBRYOS. THUS, MORE PURIFIED FSH PREPARATIONS (E.G. OVAGEN, FOLLTROPIN AND STIMAFOL) WITH LOW A LH CONCENTRATION ARE PRESENTLY USED. THESE PURIFIED FSH PREPARATIONS LEAD TO HIGHER INCIDENCES OF ABNORMAL OVULATIONS AND UNOVULATED FOLLICLES. THIS PHENOMENON CAN HOWEVER BE REDUCED BY THE SUPPLEMENTATION OF LH, HCG AND GNRH. EVEN THOUGH, PURIFIED FSH STILL HAS RESTRICTIONS REGARDING THE RESPONSE TO SUPEROVULATION AND IS LABORIOUS TO IMPLEMENT, IT IS STILL PREFERRED TO THE USE OF ECG IN GOATS. THIS IS DUE TO BETTER OVARIAN RESPONSE AND THE LOW INCIDENCE OF PREMATURE LUTEAL REGRESSION. THE VARIATION IN RESPONSE TO SUPEROVULATION IS ALSO INDUCED BY INADEQUATE PROGESTERONE LEVELS FROM THE PROGESTAGEN TREATMENT TO BLOCK THE LH PULSES TOWARDS THE END OF THE

OESTROUS SYNCHRONISATION PERIOD. HENCE EXTENDING THE LIFE SPAN OF A DOMINANT FOLLICLE. THIS LARGE FOLLICLE WILL INCREASE THE OESTROGEN SECRETION AND ALTER THE COMPETENCE OF THE NEWLY STIMULATED OOCYTES THROUGH SUPEROVULATION, THUS THE PRODUCTION OF OOCYTES LEADING TO INCREASED EMBRYONIC MORTALITIES. A LOW PROGESTERONE CONCENTRATION AT THE END OF OESTROUS SYNCHRONISATION ALSO LEADS TO VARIABILITY IN THE ONSET OF OESTRUS AND OVULATION. THE END RESULT BEING THE MIS-TIMING OF OVULATION, WHICH LEADS TO A HIGH RECOVERY OF UNFERTILISED OVA, ESPECIALLY FOLLOWING FIXED-TIME AI. THE VIABILITY OF EMBRYOS COULD BE IMPROVED BY UTILISING TWO VAGINAL PESSARIES CONSECUTIVELY WITHIN A SYNCHRONISATION PERIOD. THE OVERALL RESPONSE TO SUPEROVULATION BEING INCREASED WHEN THE TWO PESSARIES ARE USED TOGETHER, WITH AN INJECTION OF PGF2A COINCIDING WITH THE FIRST FSH TREATMENT.

REPEATED SUPEROVULATION CAN REDUCE THE OVULATION RATE, EMBRYO YIELD AND VIABILITY OF THE EMBRYOS RECOVERED. IT HAS BEEN FOUND THAT SUCCESSIVE SUPEROVULATION PROCEDURES LEAD TO THE FORMATION OF GONADOTROPHIN ANTI-BODIES. REPEATED EMBRYO COLLECTION IN GOATS ON THE OTHER HAND, ALSO LEADS TO THE FORMATION OF POST-OPERATIVE ADHESIONS, WHEN DONE SURGICALLY. THE FORMATION OF THE ADHESIONS LIMITS THE NUMBER OF TIMES THAT A DONOR CAN BE FLUSHED TO 2 TO 3 TIMES. NON-SURGICAL METHODS OF EMBRYO COLLECTION IN GOATS (LAPAROSCOPY AND TRANSCERVICAL PASSAGE OF THE CATHETER THROUGH DILATION OF

THE CERVIX WITH PGF2A OR OESTROGEN) COULD ALSO BE PERFORMED.

POOR EMBRYO RECOVERY YIELDS FOLLOWING SUPEROVULATION IS ALSO A RESULT OF FERTILISATION FAILURE DUE TO POOR SYNCHRONISATION OF OESTRUS AND OVULATION, ESPECIALLY FOLLOWING FIXED-TIME AI. THE ONSET OF OVULATION CAN BE SYNCHRONISED BY THE TREATMENT OF EXOGENOUS LH, GNRH OR ECG. THIS CAN INCREASE THE OVULATION RATE, AS WELL AS THE EMBRYO RECOVERY RATE. FERTILISATION FAILURE FOLLOWING SUPEROVULATION IS ALSO THE CONSEQUENCE OF IMPEDED SPERM TRANSPORT, GENERALLY ASSOCIATED WITH NATURAL AND CERVICAL INSEMINATIONS. INTRA-UTERINE INSEMINATIONS CAN THEREFORE IMPROVE THE FERTILISATION RATE. THE IMPEDED SPERM TRANSPORT IS DUE TO ELEVATED PERI-OVULATORY OESTROGEN LEVELS (DUE TO MANY FOLLICLES REACHING THE PRE-OVULATORY STAGE) AND PREMATURE OVULATION LEADING TO INCREASED PROGESTERONE LEVELS DURING THE PRE-OVULATORY PERIOD. FERTILISATION FAILURE IN GOATS COULD ALSO BE ATTRIBUTED TO THE ABNORMAL MATURATION OF OOCYTES FOLLOWING SUPEROVULATION.

THE FEEDING OF CONCENTRATES AD LIBITUM TO SUPEROVULATED ANIMALS MAY REDUCE THE OVULATION RATE AND THE QUANTITY AND QUALITY OF EMBRYOS RECOVERED. THE EFFECT OF SUPPLEMENTARY NUTRITION IN RESPONSE TO SUPEROVULATION IS GREATLY INFLUENCED BY THE BODY CONDITION OF THE DONOR. FEED SUPPLEMENTATION OF ANIMALS IN GOOD BODY CONDITION HAS A DETRIMENTAL EFFECT ON THE QUALITY OF EMBRYOS RECOVERED. HOWEVER, SUPPLEMENTATION OF ANIMALS IN POOR BODY

CONDITION COULD INCREASE THE OVULATION RATE, YIELD AND QUALITY OF EMBRYOS RECOVERED. THE DETRIMENTAL EFFECT OF FEEDING CONCENTRATES TO ANIMALS IS RELATED TO THE PROGESTERONE CONCENTRATION. ANIMALS ON HIGH ENERGY DIETS USUALLY HAVE LOW BLOOD PROGESTERONE CONCENTRATIONS, HENCE LOWER EMBRYO SURVIVAL RATES.

INTRINSIC FACTORS SUCH AS BREED, SEASON AND AGE ALSO HAVE A BIG IMPACT ON THE OVARIAN RESPONSE TO SUPEROVULATION. THE NUMBER OF CORPORA LUTEA, RECOVERED AND TRANSFERABLE EMBRYOS DIFFER WITH BREEDS. IN GOATS, MORE UNOVULATORY LARGE FOLLICLES HAVE BEEN OBSERVED DURING SEASONAL ANOESTRUS THAN DURING THE NATURAL BREEDING SEASON. THE EFFECT OF AGE OF THE DAM IN MOET PROGRAMMES DEMONSTRATES MINIMUM DIFFERENCES BETWEEN AGES. OF ALL THE INTRINSIC FACTORS, THE EFFECT OF THE STATUS OF THE OVARY AT THE ONSET OF SUPEROVULATION TREATMENT IS CURRENTLY RECEIVING THE MOST ATTENTION. IT IS BELIEVED THAT THE STATE OF THE OVARY AT THE ONSET OF A SUPEROVULATION IS THE MAJOR FACTOR ACCOUNTABLE FOR THE VARIATION IN RESPONSE TO SUPEROVULATION. AT THE ONSET OF A SUPEROVULATORY TREATMENT DONORS GENERALLY HAVE A DIFFERENT OVARIAN FOLLICULAR STATUS.

THE ADMINISTRATION OF EXOGENOUS GONADOTROPHINS IN GOATS CAN STIMULATE GONADOTROPHIN-RESPONSIVE FOLLICLES (2-6 MM IN SIZE) TO GROW AND REACH THE PRE-OVULATORY PHASE. IF SUPEROVULATION TREATMENT BEGINS WHEN THERE ARE HIGH NUMBERS OF THESE FOLLICLES, THE OVULATION RATE WILL INCREASE. HOWEVER, THE YIELD AND NUMBER OF

TRANSFERABLE EMBRYOS IS POSITIVELY ASSOCIATED WITH A LIMITED RANGE OF FOLLICLE SIZE (4-6 MM). THE NUMBER OF GONADOTROPHIN-RESPONSIVE FOLLICLES IS HIGH WHEN NO DOMINANT FOLLICLE IS PRESENT. HENCE, THE PRESENCE OF A DOMINANT FOLLICLE AT THE START OF A SUPEROVULATION TREATMENT REDUCES THE FOLLICULAR RECRUITMENT, THE NUMBER OF OVULATIONS, YIELD AND NUMBER OF TRANSFERABLE EMBRYOS. THE PRESENCE OF A CORPUS LUTEUM AT THE START OF A SUPEROVULATORY TREATMENT ALSO INCREASES THE NUMBER OF VIABLE EMBRYOS. THEREFORE, THE CREATION OF AN ENVIRONMENT WHERE THERE IS A HIGH NUMBER OF GONADOTROPHIN-RESPONSIVE FOLLICLES, NO DOMINANT FOLLICLE AND THE PRESENCE OF A CORPUS LUTEUM AT THE ONSET OF A SUPEROVULATORY TREATMENT IS ESSENTIAL. THIS CONDITION CAN BE ACHIEVED BY THE SYNCHRONISATION OF THE OVARIAN FOLLICULAR EMERGENCE THROUGH FOLLICULAR ABLATION, AVOIDING THE DEVELOPMENT OF A DOMINANT FOLLICLE BY LOWERING LH CONCENTRATION OR BY STARTING SUPEROVULATION SOON AFTER OVULATION (AT FOLLICULAR WAVE EMERGENCE). THERE IS VERY LITTLE RESEARCH DONE ON FOLLICULAR ABLATION IN GOATS. HOWEVER, THE UTILISATION OF GNRH AGONISTS OR ANTAGONISTS INCREASE THE OVULATION RATE, BUT LEAD TO HIGHER NUMBERS OF UNFERTILISED OVA AND DEGENERATED EMBRYOS BEING PRODUCED. HENCE A REDUCTION IN THE NUMBER OF TRANSFERABLE EMBRYOS. MORE RESEARCH IS NEEDED IN GOATS, WITH RESPECT TO THE IMPLEMENTATION OF A DAY 0 PROTOCOL, AS THE NUMBER OF OVULATIONS CAN BE INCREASED BY IMPLEMENTING THIS PROGRAMME. THE EMBRYO YIELD AND VIABILITY ARE ASPECTS

YET TO BE REPORTED ON FOLLOWING THE USE OF THIS PROTOCOL.

CRYOPRESERVATION IS AN IMPORTANT ASPECT IN A MOET PROGRAMME AS IT ALLOWS AN EASY AND CHEAP WAY OF TRANSPORTING EMBRYOS (GENETIC MATERIAL) AND FACILITATES THE STORING OF EMBRYOS UNTIL SUCH TIME WHEN SUITABLE RECIPIENTS ARE AVAILABLE. GOAT EMBRYOS ARE MOSTLY STORED FOLLOWING THE CONVENTIONAL SLOW FREEZING METHOD WITH ETHYLENE GLYCOL, OR A COMBINATION OF ETHYLENE GLYCOL AND DMSO. ALTERNATIVELY EMBRYOS CAN BE VITRIFIED WITH SIMILAR CRYOPROTECTANTS BEING UTILISED AS FOR THE CONVENTIONAL SLOW EMBRYO FREEZING. GOAT EMBRYOS COULD ALSO BE BISECTED (SPLIT) TO INCREASE THE NUMBER OF TRANSFERABLE EMBRYOS. ALTHOUGH THE SURVIVAL OF DEMI-EMBRYOS IS LOW, IT COULD POSSIBLY BE IMPROVED BY THE SPLITTING AT THE HATCHED BLASTOCYST DEVELOPMENTAL STAGE.

THE LAST STAGE OF MOET I.E. EMBRYO TRANSFER, REQUIRES THE SYNCHRONY OF OESTRUS BETWEEN THE DONOR AND THE RECIPIENTS. TWO MORULAE OR BLASTOCYST EMBRYOS ARE SURGICALLY TRANSFERRED TO THE OVIDUCT OR UTERINE HORN IPSILATERAL TO THE CORPUS LUTEUM OF THE RECIPIENT - WITH AT LEAST ONE NORMAL CORPUS LUTEUM BEING PRESENT ON DAY 6 TO 7 FOLLOWING OESTRUS.

CHAPTER 3

3. Effect of the pre-treatment with a GNRH agonist in superovulation of Boer goats

3.1. Introduction

THE SOUTH AFRICAN BOER GOAT ENJOYS POPULARITY AND IS IN DEMAND IN MANY COUNTRIES WORLDWIDE. IN ORDER TO MEET THIS HIGH DEMAND FOR THE BREED ACROSS THE GLOBE, A RAPID REPRODUCTIVE PROGRAMME SUCH AS MULTIPLE OVULATION AND EMBRYO TRANSFER (MOET), WHICH CAN DISSEMINATE SELECTED FEMALE AND MALE GENES TO ACCELERATE THE GENETIC PROGRESS, IS ESSENTIAL. HOWEVER, A VERY IMPORTANT ASPECT OR PHASE OF THE MOET PROGRAMME IN GOATS, SUPEROVULATION, HAS BEEN PRODUCING VARIABLE OVARIAN RESPONSES. THE MAJOR CAUSE OF THIS VARIATION IN RESPONSE TO SUPEROVULATION HAS BEEN ASCRIBED TO THE OVARIAN FOLLICULAR STATUS AT THE ONSET OF SUPEROVULATION TREATMENT (GONZALEZ-BULNES ET AL., 2004A).

SO FOR EXAMPLE, THE PRESENCE OF A LARGE OVARIAN FOLLICLE AT THE TIME OF SUPEROVULATION HAS BEEN REPORTED TO DECREASE THE OVARIAN RESPONSE IN SMALL RUMINANTS (RUBIANES ET AL., 1995; LOPEZ-SEBASTIAN ET AL., 1999; RUBIANES & MENCHACA, 2003). THE EFFECT OF THIS DOMINANT FOLLICLE CAN BE EXPLAINED BY SEVERAL EVENTS OCCURRING DURING FOLLICULAR DEVELOPMENT. DURING THE OESTROUS CYCLE, A COHORT OF FOLLICLES IS RECRUITED TO GROW IN WAVES IN A FSH ENVIRONMENT. THE BIGGEST FOLLICLE IN A GROUP

WILL SECRETE LARGE QUANTITIES OF OESTRADIOL AND INHIBIN, LEADING TO A DECLINE IN THE FSH LEVEL AND ATRESIA OF THE OTHER SUBORDINATE FOLLICLES (BAIRD, 1983; TSONIS ET AL., 1988; MCNEILLY ET AL., 1991). THIS DOMINANT FOLLICLE THUS INHIBITS THE GROWTH OF OTHER FOLLICLES IN A GROUP AND PREVENTS THE DEVELOPMENT OF NEW FOLLICLES (FORTUNE, 1994). THE DOMINANT FOLLICLE PREVENTS ITS ATRESIA BY CHANGING ITS DEPENDENCY FROM FSH TO LH. IT HAS BEEN OBSERVED THAT WHEN THE LH CONCENTRATION REMAINS LOW NO DOMINANCE IS ESTABLISHED, AS ALL FOLLICLES ARE DEPENDENT ON FSH ((BAIRD ET AL., 1983; CAMPBELL ET AL., 1995, 1998; ADAMS, 1999; MENCHACA & RUBIANES, 2002).

A DOMINANT OVARIAN FOLLICLE REDUCES THE NUMBER OF SMALL FOLLICLES OR THE SO CALLED GONADOTROPHIN-RESPONSIVE FOLLICLES. THE NUMBER OF THESE SMALL FOLLICLES IS IMPORTANT AT THE ONSET OF THE SUPEROVULATORY TREATMENT, AS THESE ARE THE FOLLICLES WHICH ARE RESPONSIVE TO EXOGENOUS GONADOTROPHIN APPLICATION. HIGHER NUMBERS OF GONADOTROPHIN-RESPONSIVE FOLLICLES HAVE BEEN OBSERVED AT THE EMERGENCE OF A FOLLICULAR WAVE, WHEN THERE IS NO DOMINANT FOLLICLE AND A LOW LH CONCENTRATION PRESENT (GINTHER & KOT, 1994; DE CASTO ET AL., 1998; ADAMS, 1999; MENCHACA & RUBIANES, 2002) THUS, IT IS PREDICTED THAT THE OVULATION RATE CAN BE IMPROVED IF THE NUMBER OF SMALL FOLLICLES IS MAXIMAL ON THE OVARIES AT THE ONSET OF SUPEROVULATION TREATMENT (THE ABSENCE OF A DOMINANT FOLLICLE) (COGNIE ET AL., 2003; GONZALEZ-BULNES *ET AL.*, 2004A). HOWEVER, TREATMENT IN THE ABSENCE OF A DOMINANT FOLLICLE IS DIFFICULT TO PERFORM IN PRACTICE

DUE TO THE UNPREDICTABILITY OF THESE OVARIAN FOLLICULAR PATTERNS OR WAVES (DRIANCOURT, 2001). THUS, THE INDUCTION OF A LOW BLOOD LH CONCENTRATION COULD BE AN OPTION FOR CREATING AN ENVIRONMENT SUITABLE FOR STARTING A SUPEROVULATION TREATMENT IN ORDER TO AVOID DOMINANCE (CAMPBELL ET AL., 1995).

LOW CIRCULATING LH LEVELS HAVE BEEN ACHIEVED BY THE ADMINISTRATION OF EXOGENOUS GNRH ANTAGONISTS OR AGONISTS. THE GNRH ANTAGONISTS INHIBIT GONADOTROPHIN RELEASE WITHIN HOURS FOLLOWING ADMINISTRATION, BY BLOCKING THE PITUITARY RECEPTORS. THE AGONISTS ON THE OTHER HAND, PRODUCE A ‘FLARE UP’ EFFECT AFTER ADMINISTRATION. OVER TIME THE GNRH AGONISTS DOWN REGULATE THE GNRH RECEPTORS AND DESENSITISE THE ANTERIOR PITUITARY GLAND TO ENDOGENOUS GNRH AND ABOLISH THE PULSATILE RELEASE OF LH (CHEUNG, ET AL., 2005). THE ADMINISTRATION OF GNRH ANTAGONISTS OR AGONISTS THUS NOT ONLY REDUCES THE CONCENTRATION OF CIRCULATING LH, BUT ALSO LEADS TO A DECLINE IN THE NUMBER OF LARGER FOLLICLES (MCNEILLY & FRASER, 1987; DIEDRICH ET AL., 1994). THE USE OF GNRH AGONISTS OR ANTAGONISTS AS A PRE-TREATMENT PRIOR TO SUPEROVULATION IN A MOET PROGRAMME FOR SHEEP HAS BEEN RECORDED, WITH A RESULTANT INCREASE IN THE NUMBER OF SMALL FOLLICLES AND OVULATION RATE. IN GOATS ON THE OTHER HAND, THERE IS VERY LITTLE INFORMATION REGARDING THE RESPONSE FOLLOWING THE UTILISATION OF GNRH AGONISTS AS A PRE-TREATMENT IN MOET PROGRAMMES (COGNIE, 1999). HOWEVER, WHEN A GNRH ANTAGONIST WAS UTILISED IN DOES, THERE WAS AN INCREASE IN THE

NUMBER OF SMALL FOLLICLES AND OVULATION RATE FOLLOWING SUPEROVULATION. THIS GOOD SUPEROVULATORY RESPONSE WAS HOWEVER COUNTERACTED BY AN INCREASE IN THE NUMBER OF UNFERTILISED OVA AND DEGENERATED EMBRYOS (COGNIE ET AL., 2003). THE AIM OF THIS STUDY WAS THUS TO EVALUATE THE OVARIAN RESPONSE OF BOER GOATS TO SUPEROVULATION AND EMBRYO RECOVERY PROCEDURES FOLLOWING A PRE-TREATMENT WITH A GNRH AGONIST DURING AND OUTSIDE THE NATURAL BREEDING SEASON.

3.2. Materials and methods

3.2.1. Study location

THE STUDY WAS CONDUCTED DURING THE AUTUMN AND SPRING SEASONS OF 2005 AT THE UNIVERSITY OF THE FREE STATE. THE UNIVERSITY IS SITUATED IN BLOEMFONTEIN, SOUTH AFRICA - LOCATED AT 28.57º SOUTH LONGITUDE AND 25.89º EAST LATITUDE, AT AN ALTITUDE OF 1304 M ABOVE SEA LEVEL. THE HIGHEST MEAN MAXIMUM AMBIENT TEMPERATURE RECORDED IN WINTER IS 22.8ºC AND 37.9ºC IN SUMMER, WITH THE MINIMUM TEMPERATURE BEING -5.5ºC AND 7.7ºC IN WINTER AND SUMMER, RESPECTIVELY. THE AVERAGE ANNUAL RAINFALL IN OF THIS AREA IS 559 MM WHICH FALLS PREDOMINATELY DURING THE SUMMER MONTHS (DECEMBER TO FEBRUARY), WITH THE MEAN DAYLIGHT LENGTH RANGING FROM 9.8 H IN WINTER TO 13.2 H IN SUMMER.

3.2.2. Animals

FORTY-THREE MULTIPAROUS MATURE BOER GOAT DOES (PLATE 3.1) WERE USED AS DONORS DURING THE NATURAL BREEDING SEASON (AUTUMN; N=21) AND OUTSIDE THE BREEDING SEASON (SPRING; N=22) IN THIS TRIAL. DOES WERE KEPT IN OPEN PENS AND FED A MAINTENANCE DIET OF PELLETS AND

LUCERNE HAY. FOR THE ENTIRE PERIOD OF THE EXPERIMENT WATER WAS PROVIDED *AD LIBITUM*. AT THE BEGINNING OF THE TRIAL, BODY WEIGHT, AGE AND PARITY OF THE DOES WERE RECORDED AND USED TO BALANCE THE ANIMALS WITH REGARD TO THE TREATMENT GROUPS. IN AUTUMN THE BODY WEIGHT OF THE DOES VARIED BETWEEN 40.1 KG AND 69.0 KG, THE AGE FROM 2 TO 8 TEETH AND PARITY BETWEEN 2 AND 3. THE AVERAGE BODY WEIGHT IN AUTUMN WAS THUS 55.5 KG. IN SPRING THE BODY WEIGHT VARIED BETWEEN 30.9 KG AND 76.4 KG, THE AGE FROM 2 TO 8 TEETH AND PARITY VARYING BETWEEN 2 TO 4. THE AVERAGE BODY WEIGHT OF THE DOES IN SPRING WAS 54.5 KG.

Plate 3.13 Boer goat does in open pens with free access to feed and water

3.2.3. Measurements and experimental protocols

3.2.3.1. Treatments

IN ALL DOES, THE ONSET OF THE OESTRUS WAS SYNCHRONISED WITH THE AID OF CONTROLLED INTERNAL DRUG RELEASE DISPENSERS (CIDR; PHAMACICA & UPJOHN, AUCKLAND, NEW ZEALAND)

INSERTED INTRAVAGINALLY FOR 17 DAYS. THE CIDR WAS ADMINISTERED WITH AN APPLICATOR AND A SMALL AMOUNT OF AN ANTISEPTIC CREAM WAS APPLIED TO THE CIDR TO ACT AS A LUBRICANT AND TO PREVENT INFECTIONS. THE APPLICATOR WAS RINSED WITH CLEAN WATER BETWEEN CIDR ADMINISTRATIONS. DOES WERE THEN DIVIDED INTO THE TREATMENT AND CONTROL GROUPS.

ALL DOES WERE SUPEROVULATED WITH PFSH (FOLLTROPIN, VETREPHARM). THE SUPEROVULATORY TREATMENT CONSISTED OF A TOTAL DOSE OF 200 MG FSH/DOE, ADMINISTERED I.M. IN 7 DOSAGES, AT 12 H INTERVALS PER DAY, STARTING 48 H PRIOR TO CIDR REMOVAL (THE FIRST DOSE BEING 50 MG AND ALL OTHERS 25 MG). THE TREATMENT GROUP RECEIVED A GNRH AGONIST (GNRHA) (LEUPROLIDE, LUCRIN, NL, CH) (40µG/DAY/DOE) TREATMENT, ADMINISTERED AS 2 INJECTIONS (S.C.) PER DAY FOR 7 DAYS (PADULA & MACMILLAN, 2005), STARTING ON DAY 8 FOLLOWING CIDR INSERTION. THE CONTROL DOES WERE SYNCHRONISED AND SUPEROVULATED BUT RECEIVED NO GNRHA.

3.2.3.2. Oestrous detection

OESTROUS DETECTION DURING BOTH SEASONS COMMENCED 2 MONTHS PRIOR TO TREATMENT, TO DETERMINE THE CYCLIC ACTIVITY AND THE STAGE OF THE OESTROUS CYCLE OF THE ANIMALS WITH THE AID OF VASECTOMISED BUCKS (PLATE 3.2). OESTROUS DETECTION WAS PERFORMED TWICE DAILY (08H00 AND 15H00) FOR APPROXIMATELY 30 MIN UNTIL THE DAY OF CIDR INSERTION. STARTING AT CIDR WITHDRAWAL, ALL DOES WERE TESTED FOR OVERT SIGNS OF OESTRUS THREE TIMES A DAY, AT 8 H INTERVALS FOR A PERIOD OF THREE DAYS (72 H) TO DETERMINE THE RESPONSE AND THE ONSET AND DURATION OF THE INDUCED OESTROUS PERIOD.

Plate 3.14 Oestrous detection with the aid of vasectomised bucks

3.2.3.3. AI procedure

IN BOTH TRIALS ALL DOES WERE LAPAROSCOPICALLY INSEMINATED WITH 0.1ML FRESH DILUTED BOER SEMEN (150 X 10^6 SPERM/INSEMINATION) (PLATE 3.3) AT A FIXED TIME (36H AND 48H) FOLLOWING CIDR WITHDRAWAL. THE SEMEN USED FOR AI WAS COLLECTED WITH THE AID OF AN ARTIFICIAL VAGINA FROM 3 BUCKS OF PROVEN FERTILITY, USING A DOE IN OESTRUS. THE SEMEN WAS THEN EVALUATED MICROSCOPICALLY FOR PROGRESSIVE MOTILITY AND DILUTED AT A RATIO OF 1:2 WITH STERILIZED SKIMMED MILK. ONLY SEMEN SAMPLES WITH A 3+ MOTILITY SCORE WERE UTILISED FOR AI.

Plate 3.15 Laparoscopic AI in goats

3.2.3.4. Blood sampling during the breeding season

BLOOD SAMPLES WERE COLLECTED FROM 5 ANIMALS SELECTED IN EACH GROUP VIA JUGULAR VENI-PUNCTURE INTO 10 ML VACUTAINER TUBES WITH NO ANTI-COAGULANT (PLATE 3.4). DURING PROGESTAGEN TREATMENT THE BLOOD SAMPLES WERE COLLECTED AT 4 DAYS INTERVALS (FROM PROGESTAGEN INSERTION TO DAY 12). ONE BLOOD SAMPLE WAS ALSO TAKEN ON DAY 14 OF PROGESTAGEN TREATMENT TO EVALUATE THE HORMONAL EFFECT OF GNRHA TREATMENT. FROM FIRST SUPEROVULATION (PFSH) TREATMENT UNTIL CIDR REMOVAL, BLOOD SAMPLES WERE TAKEN TWICE DAILY (08H00 AND 20H00). A FURTHER THREE BLOOD SAMPLES WERE TAKEN FROM CIDR REMOVAL FOR A PERIOD OF 3 DAYS. FROM ONE DAY FOLLOWING THE SECOND AI, BLOOD SAMPLES WERE TAKEN AT 24H INTERVALS UNTIL EMBRYO FLUSHING (DAY 6 FOLLOWING AI). AFTER BLOOD SAMPLE COLLECTION, SERUM WAS SEPARATED FOLLOWING CENTRIFUGATION AT 1500 RPM FOR 15 MINUTES. THE SERUM WAS RECOVERED AND STORED AT -20ºC, UNTIL ASSAYED FOR PROGESTERONE AND OESTROGEN CONCENTRATIONS.

Plate 3.16 Blood sampling for progesterone and oestrogen assays

3.2.3.4.1. Serum progesterone and oestrogen radioimmunoassay (RIA) during the breeding season

THE SERUM PROGESTERONE AND OESTROGEN CONCENTRATIONS WERE EVALUATED USING A RADIOIMMUNOASSAY (RIA) TECHNIQUE. THE RIA FOR PROGESTERONE AND OESTROGEN CONCENTRATION DETERMINATION WAS BASED ON THE PRINCIPLE OF COMPETITIVE BINDING. FIRSTLY 100 µL OF CALIBRATOR, CONTROL, OR SAMPLE OF PROGESTERONE OR OESTROGEN WERE ADDED TO ANTIBODY-COATED TUBES. A 500 µL ^{125}I-LABELED PROGESTERONE (FOR PROGESTERONE ASSAY) OR OESTROGEN (OESTROGEN ASSAY) WAS THEN ADDED TO EACH TUBE AS A TRACER, MIXED AND INCUBATED FOR 3H (18-25 °C) IN A SHAKING MODE (350 RPM). AFTER INCUBATION THE CONTENTS OF THE TUBES WERE ASPIRATED AND THE RADIOACTIVITY BOUND TO THE ANTIBODY–COATED TUBE COUNTED. RESULTS WERE OBTAINED BY

ESTABLISHING A CALIBRATED STANDARD CURVE AND UNKNOWN VALUES WERE DETERMINED BY INTERPOLATION FROM THE CURVE. THE SENSITIVITY OF THE ASSAY DURING PROGESTERONE AND OESTROGEN DETERMINATIONS WAS 0.05 NG/ML AND <11 PG/ML, RESPECTIVELY. THE INTRA- AND INTER-ASSAY COEFFICIENTS OF VARIATION FOR PROGESTERONE WERE 5.8% AND 9.0%, RESPECTIVELY, WHILE FOR OESTROGEN THE INTRA- AND INTER-ASSAY COEFFICIENTS OF VARIATION WERE 12.1% AND 11.2%, RESPECTIVELY.

3.2.3.4.2. Blood sampling and progesterone assay outside the breeding season

THE BLOOD SAMPLING PROCEDURES WERE SIMILAR TO THAT PERFORMED IN AUTUMN (PARAGRAPH 3.2.3.4). HOWEVER, THE SERUM PROGESTERONE CONCENTRATION WAS DETERMINED USING THE AUTOMATED CHEMILUMINESCENCE SYSTEM (CHIRON DIAGNOSTICS ACS: 180, USA). THE CHIRON DIAGNOSTICS ACS: 180 PROGESTERONE ASSAY WAS BASED ON A COMPETITIVE IMMUNOASSAY, USING DIRECT CHEMILUMINESCENT TECHNOLOGY. PROGESTERONE IN THE SAMPLE BINDS TO AN ACRIDINIUM ESTER-LABELED MOUSE MONOCLONAL ANTI-PROGESTERONE ANTIBODY IN THE LIGHT REAGENT. UNBOUND ANTIBODY BINDS TO A PROGESTERONE DERIVATIVE COVALENTLY COUPLED TO PARAMAGNETIC PARTICLES IN THE SOLID PHASE. THE AMOUNT OF PROGESTERONE PRESENT IN THE SAMPLE IS INVERSELY RELATED TO THE AMOUNT OF RELATIVE LIGHT UNITS DETECTED BY THE SYSTEM. THE ACS: 180 PROGESTERONE ASSAY SENSITIVITY IS 0.11NG/ML. THE ANALYTICAL SENSITIVITY BEING DEFINED AS THE CONCENTRATION OF SERUM PROGESTERONE THAT CORRESPONDS TO THE RELATIVE LIGHT UNITS (RLUS) OF 20 REPLICATE DETERMINATIONS OF THE PROGESTERONE ZERO STANDARDS. THE INTER- AND INTRA-ASSAY

COEFFICIENTS OF VARIATION WERE 9.1% AND 14.6%, RESPECTIVELY.

3.2.3.5. Ovary inspection and embryo collection

ON DAY 5 FOLLOWING AI ALL DOES WERE DEPRIVED OF FEED AND WATER FOR 24H AND THE FOLLOWING DAY (DAY 6 AFTER AI), A LAPAROSCOPIC EVALUATION (PLATE 3.5) OF THE OVARIES WAS PERFORMED TO DETERMINE THE PRESENCE, ABSENCE AND QUALITY OF THE CORPORA LUTEA (CL'S). DOES WITH NO CL'S AND ABNORMAL CL'S (SMALL CL'S APPEARING WHITE OR LIGHT PINK), AS CLASSIFIED BY ESPINOSA-MARQUEZ ET AL. (2004), WERE NOT FLUSHED. EMBRYOS WERE THEN SURGICALLY RECOVERED (DAY 6) UNDER GENERAL ANAESTHESIA.

Plate 3.17 Laparoscopic evaluation of the ovaries prior to embryo flushing

BEFORE SURGERY, DOES WERE PRE-TREATED WITH ATROPINE AND ANAESTHETISED AND MAINTAINED AT THIS STAGE WITH HALOTHANE COMBINED WITH NITROUSOXIDE. ONCE ANAESTHETISED, A MID-VENTRAL SURGICAL INCISION WAS MADE CRANIAL TO THE UDDER TO EXTERIORISE THE REPRODUCTIVE TRACT (PLATE 3.6 AND 3.7) AND THE NUMBER OF CL'S RECORDED. THEREAFTER, A TWO-WAY FOLEY'S CATHETER WAS INSERTED AT THE BASE OF THE UTERINE HORN AND THE CUFF INFLATED WITH FLUSHING MEDIA, WHILE AN INTRAVENOUS 18G CATHETER WAS INSERTED AT THE UTERO-TUBAL JUNCTION. THE EMBRYOS WERE FLUSHED (PLATE 3.8) USING EMCARE^{TM} FLUSHING MEDIA AND TRANSFERRED INTO EMCARE^{TM} HOLDING MEDIA. THE TOTAL NUMBER OF RECOVERED STRUCTURES (UNFERTILISED OVA AND EMBRYOS) WERE EVALUATED MICROSCOPICALLY (PLATE 3.9) FOR THE STAGE OF DEVELOPMENT AND QUALITY USING

MORPHOLOGICAL CRITERIA. THE EMBRYOS WERE CLASSIFIED AS UNFERTILISED OVA (IF THERE IS NO CLEAVAGE), DEGENERATE EMBRYOS (EMBRYOS AT 8-CELL STAGE AND EARLIER STAGE) OR AS

TRANSFERABLE EMBRYOS GRADE 1, 2 AND 3 (MORPHOLOGICALLY INTACT COMPACTED MORULAE, EARLY BLASTOCYSTS AND EXPANDED BLASTOCYST) (PLATE 3.10 TO 3.12). GRADE 1 EMBRYOS WERE MORPHOLOGICALLY INTACT AND HAD AN EVEN GRANULATION AND CELL DISTRIBUTION, GRADE 2 WERE EMBRYOS WITH SMALL DEVIATIONS, LIKE FEW EXCLUDED BLASTOMERES WHILE GRADE 3 EMBRYOS HAD AN UNEVEN CELL ORGANISATION, LOOSE STRUCTURES, WITH NUMEROUS FREE BLASTOMERES (LINDNER & WRIGHT, 1983; NUTI ET AL., 1987)

Plate 3.18 Exteriorised reproductive tract of the doe showing ovulation points

Plate 3.19 Sutured mid-ventral incision following laparotomy and the exteriorisation of the reproductive tract

Plate 3.20 Embryo flushing of the donor does on day 6 after AI

Plate 3.21 Evaluation of goat embryos following flushing

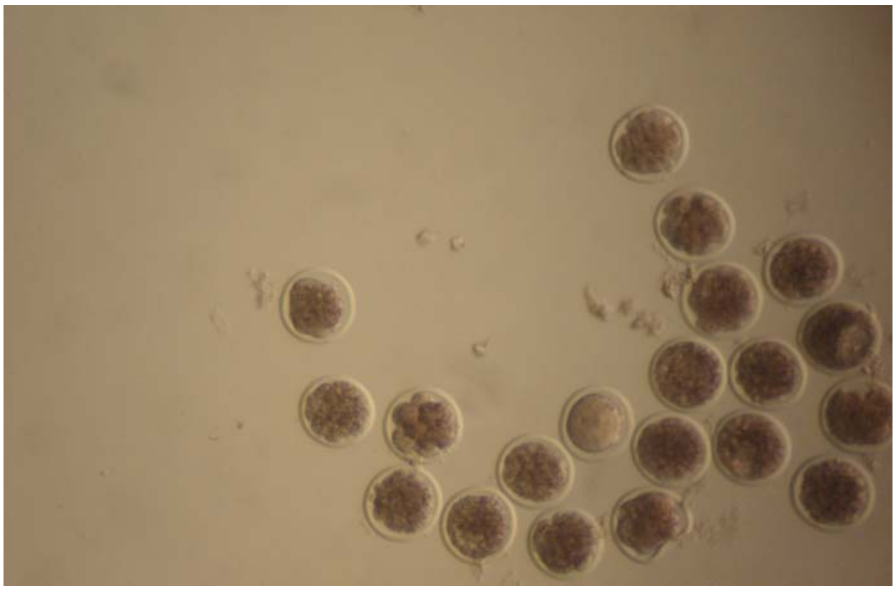

Plate 3.22 Boer goat morulae recovered on day 6 after AI

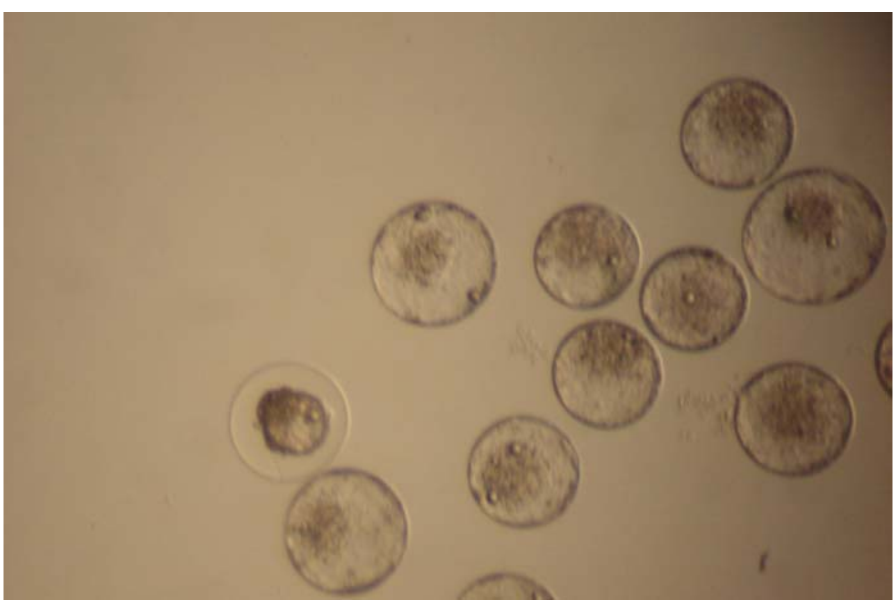

Plate 3.23 Boer goat expanded blastocysts flushed

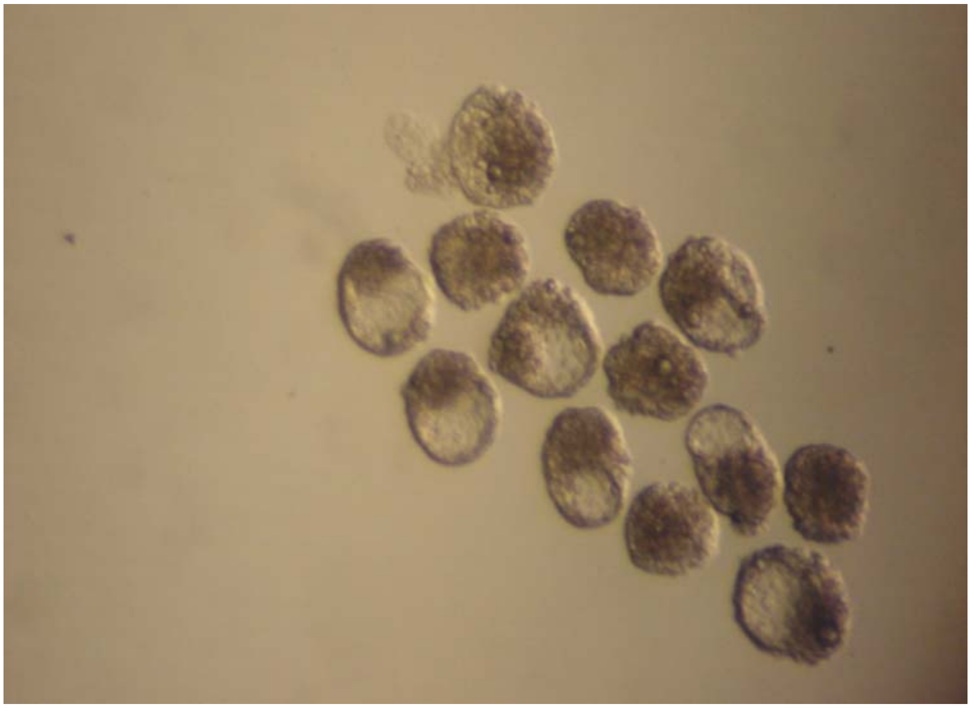

Plate 3.24 Boer goat hatched blastocysts recovered from a donor

3.2.4. Statistical analyses

DATA REGARDING THE ONSET OF OESTRUS FOLLOWING THE CESSATION OF TREATMENT AND THE DURATION OF THE INDUCED OESTROUS PERIOD, THE TOTAL NUMBER OF OVULATIONS, THE TOTAL STRUCTURES RECOVERED, UNFERTILISED OVA AND EMBRYO YIELD AND RECOVERY RATES WERE ANALYSED USING THE GENERAL LINEAR MODEL (GLM) WHILE, DATA PERTAINING TO THE OESTROUS RESPONSE WERE ANALYSED WITH THE AID OF THE CHI-SQUARE TEST (SAS, 2003).

3.3. Effect of pre-treatment with a GnRH agonist in a superovulation regime during the breeding season

3.3.1. Results

3.3.1.1. Oestrous response

BEFORE THE ONSET OF OESTROUS SYNCHRONISATION AND SUPEROVULATION TREATMENT, THE PERCENTAGE OF DOES RECORDED CYCLIC WAS 81%, WITH ONLY 4 DOES (19%) THAT DID NOT EXHIBIT OESTRUS. THE MEAN DURATION OF THESE OESTROUS CYCLES WAS 20.1±3.5D WHILE THE NATURAL OESTROUS PERIOD LASTED 37.7±11.9H. THE OESTROUS RESPONSE FOLLOWING OESTROUS SYNCHRONISATION IS SUMMARISED IN TABLE 3.1. ALL DOES THAT DID NOT DEMONSTRATE OESTRUS BEFORE SYNCHRONISATION RESPONDED TO TREATMENT AND EXHIBITED AN INDUCED OESTRUS. ALL DOES IN THE TREATMENT GROUP (FSH/GNRHA) DEMONSTRATED SIGNS OF OESTRUS FOLLOWING SYNCHRONISATION, WHILE OESTRUS WAS NOT OBSERVED IN 2 DOES IN THE CONTROL GROUP (TABLE 3.1). THERE WAS NO SIGNIFICANT DIFFERENCE BETWEEN THE TWO GROUPS REGARDING OESTROUS RESPONSE AND TREATMENT HAD NO EFFECT ON THE TIME TO ONSET OF OESTRUS. THE MEAN OVERALL INTERVAL FROM SPONGE WITHDRAWAL TO ONSET OF OESTRUS FOR BOTH GROUPS WAS 26.3±3.6H. THE DURATION OF THE INDUCED OESTRUS TENDED TO BE SHORTER IN THE GNRHA TREATED DOES, COMPARED TO THE CONTROL, WITHOUT A SIGNIFICANCE DIFFERENCE. THE OVERALL DURATION OF THE INDUCED OESTROUS PERIOD FOR ALL DOES WAS 21.5±6.4H. THE DURATION OF THE NATURAL OESTROUS PERIOD (37.7±11.9H) WAS SIGNIFICANTLY ($P<0.01$) LONGER COMPARED TO THE INDUCED OESTRUS (21.5±6.4H).

Table 3.5 The mean (±SD) oestrous response in Boer goat does pre-treated with a GnRH agonist and superovulated with FSH

Treatment	n	Oestrous response (%)	Time to onset of oestrus (h)	Duration of induced oestrus (h)

FSH (control)	10	80	25.5±3.0	25.0±7.4
FSH/GnRHa	11	100	26.9±4.0	19.6±5.5

No significant difference

3.3.1.2. Serum progesterone concentration

THREE DOES FROM WHICH BLOOD WAS SAMPLED WERE REMOVED FROM THE TRIAL. THUS ONLY 7 DOES (4 FROM THE FSH/GNRHA AND 3 FROM THE FSH GROUP) WERE SAMPLED FOR THE DURATION OF THE TREATMENT PERIOD. THE SERUM PROGESTERONE LEVELS FROM CIDR INSERTION TO REMOVAL ARE ILLUSTRATED IN FIGURE 3.1. THE CONCENTRATIONS OF SERUM PROGESTERONE FOR BOTH GROUPS WERE HIGH AT CIDR INSERTION (DUE TO STAGE OF OESTROUS CYCLE), EXCEPT FOR ONE ANIMAL FROM THE FSH/GNRHA GROUP WITH A LEVEL OF 1.1 NG/ML SERUM PROGESTERONE AT CIDR INSERTION. THE MEAN SERUM PROGESTERONE CONCENTRATIONS DECLINED IN BOTH GROUPS FROM CIDR INSERTION UNTIL DAY 8 FOLLOWING PROGESTAGEN ADMINISTRATIONS AND THEN STABILISED UNTIL DAY 14 OF PROGESTAGEN TREATMENT. THE MEAN SERUM PROGESTERONE LEVELS TENDED TO DECLINE FOLLOWING THE INITIATION OF THE SUPEROVULATION TREATMENT (DAY 15 OF PROGESTAGEN TREATMENT) TO A MEAN LEVEL OF 1.0±0.4 NG/ML PROGESTERONE IN TREATMENT DOES VS. 6.7±7.8 NG/ML IN THE CONTROL DOES ON THE DAY OF CIDR REMOVAL (DAY 17). A LARGE VARIATION IN SERUM PROGESTERONE CONCENTRATION WAS RECORDED BETWEEN ANIMALS IN THE FSH-TREATED DOES

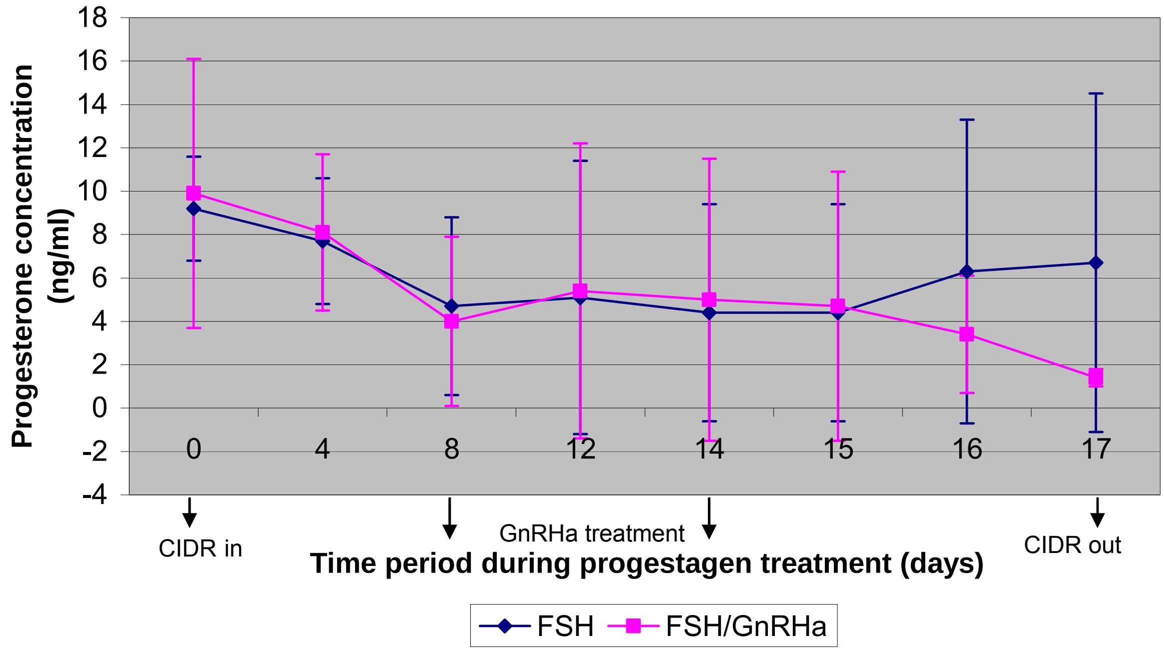

Figure 3.7 Mean (±SD) serum progesterone concentrations from CIDR insertion to withdrawal in Boer goats superovulated with FSH or FSH/GnRHa

THE MEAN SERUM PROGESTERONE CONCENTRATIONS TENDED TO BE LOWER IN THE FSH/GNRHA-TREATED DOES, COMPARED THE CONTROL DOES (FROM CIDR WITHDRAWAL TO EMBRYO FLUSHING), ALTHOUGH NO SIGNIFICANT DIFFERENCE WAS RECORDED BETWEEN GROUPS. THE MEAN PROGESTERONE LEVELS WERE AT A BASAL LEVEL IN THE FSH/GNRHA GROUP, WHILE BEING >5 NG/ML IN THE CONTROL DOES AT THE FIRST AND SECOND AI - WITHOUT SIGNIFICANT DIFFERENCES BETWEEN THE RESPECTIVE GROUPS. AT 48H FOLLOWING THE SECOND AI (96H AFTER CIDR REMOVAL) THE CONTROL DOES RECORDED A SIGNIFICANTLY ($P<0.05$) HIGHER MEAN PROGESTERONE CONCENTRATION (11.3±4.9 NG/ML) THAN IN THE TREATMENT (GNRHA) DOES (4.3±2.1 NG/ML). THE SERUM PROGESTERONE CONCENTRATIONS INCREASED FOLLOWING THE SECOND AI AND WERE HIGHEST ON THE DAY OF

EMBRYO FLUSHING IN BOTH GROUPS - WITHOUT ANY SIGNIFICANT DIFFERENCES (FIGURE 3.2).

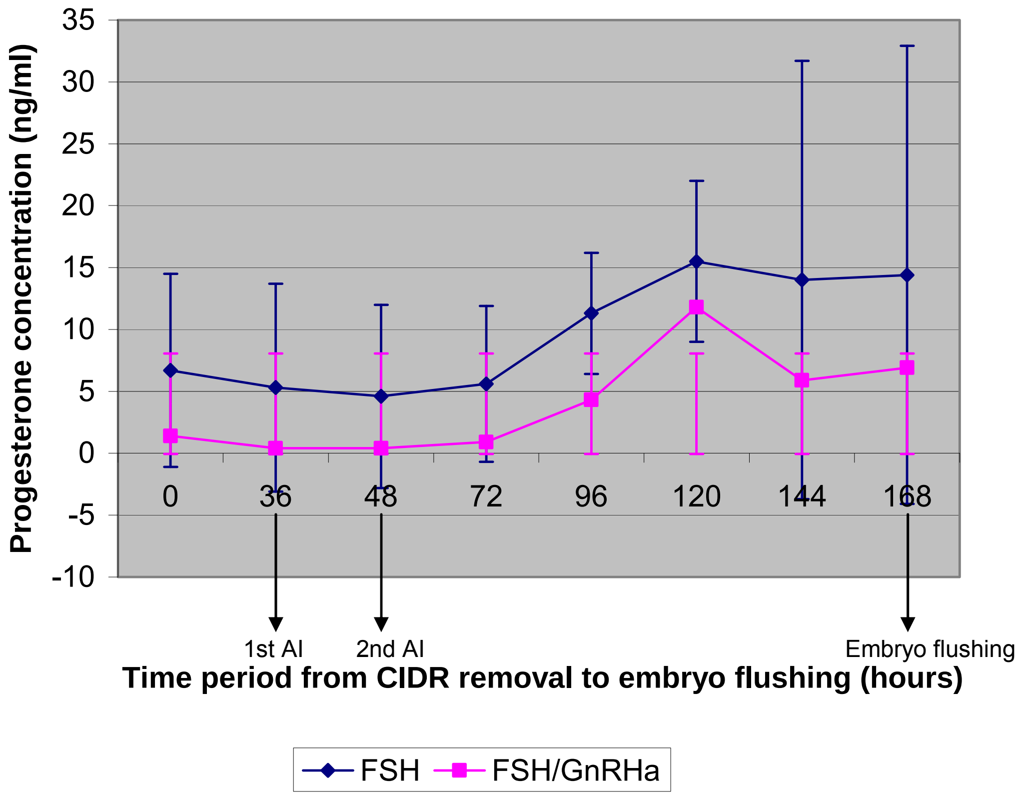

Figure 3. 8 Mean (±SD) serum progesterone concentration from CIDR withdrawal to embryo flushing in Boer goats superovulated using different protocols

3.3.1.3. Serum oestrogen concentration

FIGURE 3.3 ILLUSTRATES THE MEAN SERUM OESTROGEN LEVELS RECORDED FROM CIDR INSERTION TO WITHDRAWAL. THE MEAN SERUM OESTROGEN CONCENTRATION WAS HIGHER IN THE FSH/GNRHA TREATED DOES AT CIDR INSERTION, ALTHOUGH NOT SIGNIFICANT (LARGE INDIVIDUAL VARIATION). THE SERUM OESTROGEN LEVELS DECLINED IN BOTH GROUPS FROM CIDR INSERTION AND WERE SIMILAR ON DAY 8 OF PROGESTAGEN TREATMENT. ON DAY 12 OF SYNCHRONISATION (5TH DAY OF GNRHA TREATMENT), THE MEAN SERUM OESTROGEN CONCENTRATION WAS SIGNIFICANTLY

(P<0.05) HIGHER IN THE FSH/GNRHA TREATED DOES (10.4±2.9 PG/ML), COMPARED TO THE CONTROL DOES (4.2±2.3 PG/ML). THE SERUM OESTROGEN CONCENTRATION IN THE FSH/GNRHA GROUP DECLINED TO BELOW THAT OF THE CONTROL GROUP DURING THE LAST DAY OF GNRHA TREATMENT (DAY 14 OF SYNCHRONISATION TREATMENT).

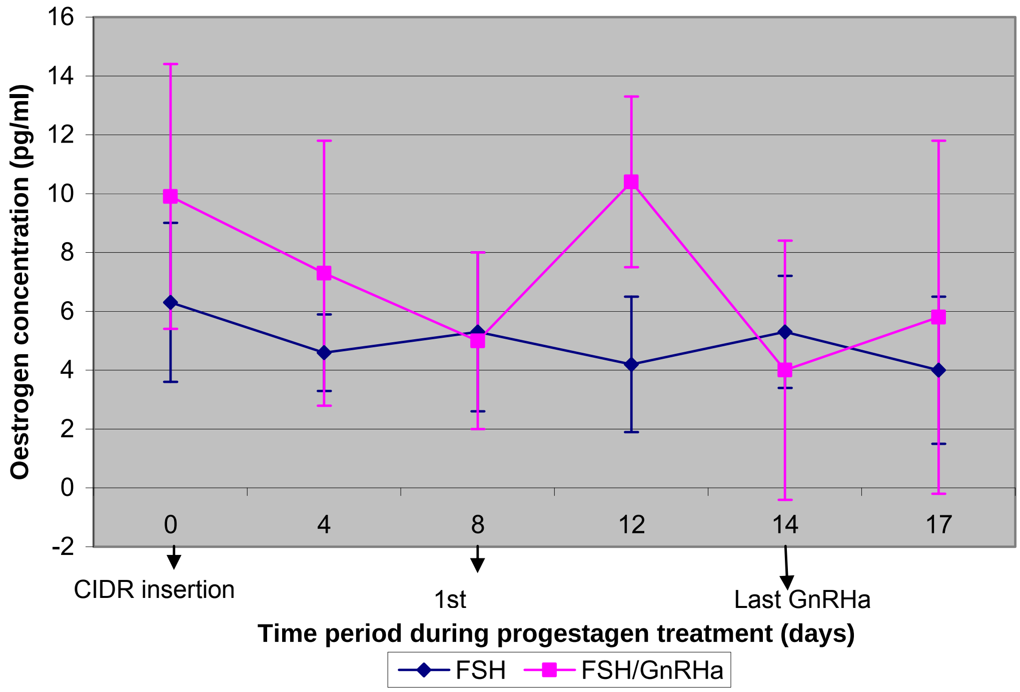

Figure 3.9 Mean (±SD) serum oestrogen concentrations from CIDR insertion to withdrawal in Boer goats superovulated with FSH or FSH/GnRHa

THE MEAN SERUM OESTROGEN LEVELS DURING SUPEROVULATION TREATMENT ARE DEMONSTRATED IN FIGURE 3.4 AND STARTED TO INCREASE FOLLOWING SUPEROVULATION TREATMENT IN BOTH TREATMENT GROUPS. AT THE 4^{TH} PFSH (SUPEROVULATION) INJECTION, THE MEAN OESTROGEN LEVEL WAS SIGNIFICANTLY (P<0.05) HIGHER IN FSH/GNRHA-TREATED DOES (9.9±1.2 PG/ML), COMPARED TO THE CONTROL DOES (6.4±1.7 PG/ML). AT THE LAST PFSH INJECTION THE MEAN SERUM OESTROGEN CONCENTRATION WAS AGAIN

ABOVE 30 NG/ML IN CONTROL DOES, ALTHOUGH THE MEAN FOR BOTH GROUPS DID NOT DIFFER SIGNIFICANTLY (FIGURE 3.4). A LARGE VARIATION IN SERUM OESTROGEN LEVELS WAS ALSO RECORDED BETWEEN ANIMALS IN THE CONTROL GROUP AT THE LAST PFSH TREATMENT.

THE MEAN SERUM OESTROGEN CONCENTRATIONS FOLLOWING CIDR REMOVAL AND DURING THE TIME WHEN DOES WERE IN OESTRUS (INDUCED) ARE DEMONSTRATED IN FIGURE 3.5. THE MEAN SERUM OESTROGEN CONCENTRATION IN BOTH GROUPS WAS BELOW 10 PG/ML FROM CIDR REMOVAL AND THIS LEVEL TENDED TO INCREASE EARLIER IN THE CONTROL DOES. AT FIRST AI (36H FOLLOWING CIDR REMOVAL) THE MEAN SERUM OESTROGEN CONCENTRATION WAS SIGNIFICANTLY ($P<0.05$) HIGHER (58.3 ± 26.9 PG/ML) IN THE CONTROL (FSH-STIMULATED) DOES, COMPARED TO THE FSH/GNRHA-TREATED DOES (15.7 ± 17.4 PG/ML). THE MEAN SERUM OESTROGEN CONCENTRATION IN FSH/GNRHA TREATED DOES REACHED A PEAK (48.8 ± 27.3 PG/ML) LEVEL 6H FOLLOWING THE FIRST AI. AT THE SECOND AI (48H FOLLOWING CIDR REMOVAL) THE MEAN SERUM OESTROGEN CONCENTRATION FOR BOTH GROUPS WAS LOWER THAN 5 PG/ML.

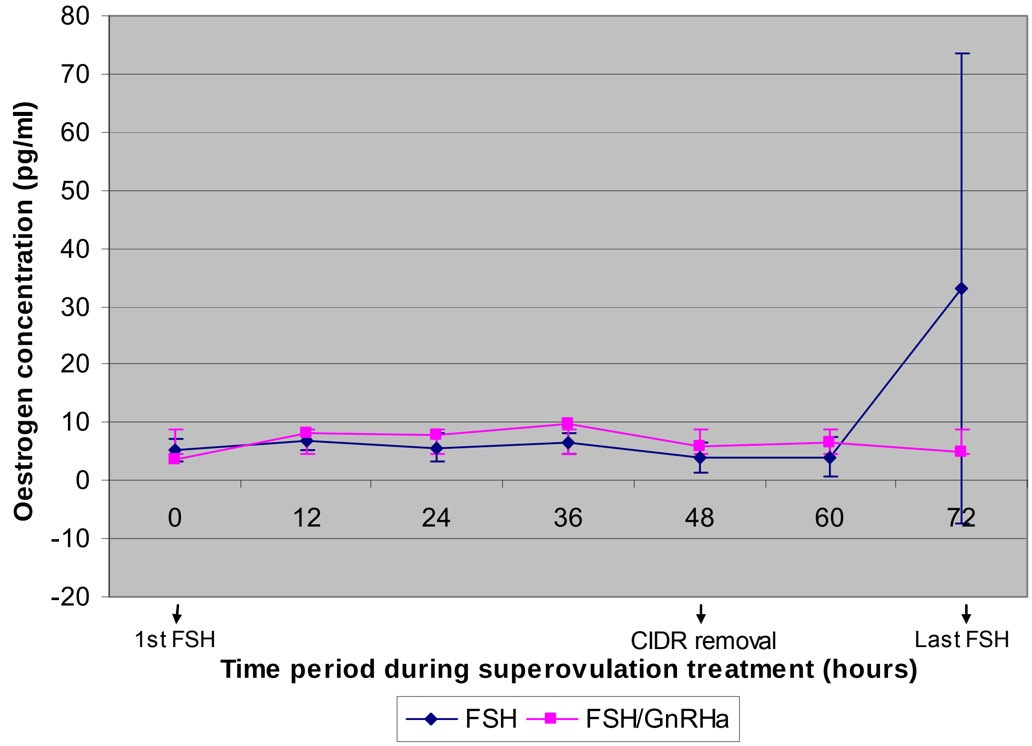

Figure 3.10 Mean (±SD) serum oestrogen concentration of Boer goat does during superovulation treatment using different protocols

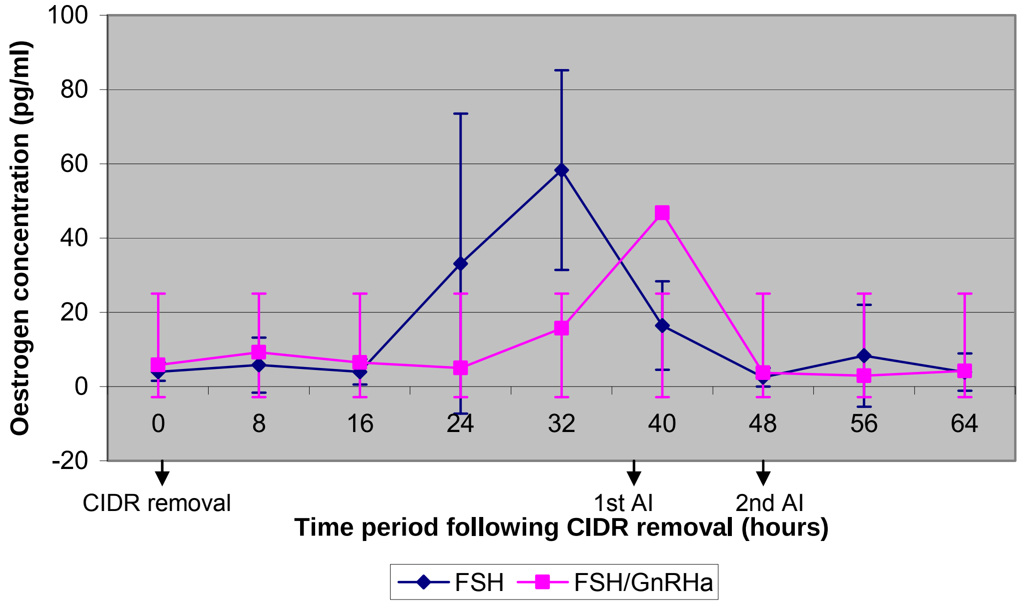

Figure 3. 11 Mean oestrogen concentration of Boer goat does over a period of 3 days from CIDR removal

3.3.1.4. Ovarian response and embryo yield

DURING LAPAROSCOPIC INSPECTION OF THE OVARIES PRIOR TO FLUSHING, 8 DOES (38.1%) (3 IN THE CONTROL GROUP AND 5 DOES IN THE TREATMENT GROUP) HAD ABNORMAL CL'S, WHILE 5 DOES (23.8%) (4 FROM THE CONTROL GROUP AND 1 FROM THE TREATMENT GROUP) HAD NO CL'S. THE ABNORMAL CL'S WERE SMALL, PALE AND AVASCULAR, AS OPPOSED TO THE NATURAL, HIGHLY VASCULAR AND RED FUNCTIONAL CL'S. ALL DOES WITH ABNORMAL CL'S AND WITHOUT CL'S PRESENT WERE NOT FLUSHED. ONLY 8 DOES (38.1%) (3 FROM THE CONTROL GROUP AND 5 FROM THE TREATMENT GROUP) WERE THUS FLUSHED. THE TOTAL STRUCTURES AND EMBRYO RECOVERY RATES OBTAINED ARE SET OUT IN TABLE 3.2. THERE WERE NO SIGNIFICANT DIFFERENCES IN ALL EMBRYO YIELD PARAMETERS. HOWEVER, IN DOES PRE-TREATED WITH GNRHA THE NUMBER OF DEGENERATED EMBRYOS TENDED TO BE HIGHER,

WHILE THE NUMBER OF TRANSFERABLE EMBRYOS TENDED TO BE LOWER WHEN COMPARED TO THE CONTROL DOES.

Table 3.6 Mean (±SD) structures yields and embryo recovery rate in Boer goat does pre-treated with GnRHa

	Overall	**FSH (control)**	**FSH/GnRHa**
No. of goats	8	3	5
No. of structures recovered/donor	14.3±5.8	15.0±6.2	13.8±5.5
Fertilised ova/donor (embryos/donor)	11.0±5.7	11.0±7.5	11.0±4.1
Fertilised ova /collected ova (%)	74.4±26.6	72.4±36.7	75.6±19.8
Unfertilised ova/donor	3.9±4.2	4.0±4.5	3.8±4.0
Degenerate embryos/donor	4.8±3.5	1.7±1.5	6.6±4.2
Transferable embryos/donor	5.6±4.2	9.3±6.1	3.4±2.7
Transferable embryos /fertilised ova (%)	39.7±25.1	61.2±26.6	26.8±24.4

No significant differences

3.3.2. Discussion

3.3.2. 1. Oestrous response

THE INCIDENCE OF DOES NOT CYCLIC IN THE PRESENT STUDY IS A COMMON PHENOMENON IN GOATS, ESPECIALLY AT THE ONSET OF THE NATURAL BREEDING SEASON (DEVENDRA & BURNS, 1983; EVANS & MAXWELL, 1987). THE MEAN DURATION OF THE OESTROUS CYCLE (20.1±3.5D) AND NATURAL OESTROUS PERIOD (37.7±11.9H) RECORDED WAS

SIMILAR TO THE 20.7±0.7D AND 21.5±0.8D DURATION OF THE OESTROUS CYCLE AND THE 37.4±8.6H LENGTH OF THE OESTROUS PERIOD, REPORTED FOR BOER GOATS AND NUBIAN GOATS (CAMP ET AL., 1983; GREYLING, 2000). THE OESTROUS RESPONSE OBTAINED IS ALSO IN LINE WITH PREVIOUS REPORTS FOR GOATS SYNCHRONISED WITH EITHER INTRAVAGINAL PROGESTAGEN SPONGES OR CIDR'S AND SUPEROVULATED WITH FSH (ARMSTRONG ET AL., 1983A; PENDLETON ET AL., 1992; ESPINOSA-MARQUEZ ET AL., 2004). THE OVERALL MEAN TIME FROM CIDR REMOVAL TO THE ONSET OF OESTRUS (26.3±3.6H) WAS ALSO IN LINE WITH FINDINGS ON GOATS SYNCHRONISED WITH CIDR OR INTRAVAGINAL SPONGES, AS WELL AS IN GOATS SUPEROVULATED WITH A FSH/GNRH ANTAGONIST (BARIL ET AL., 1996; MOTLOMELO ET AL., 2002; ESPINOSA-MARQUEZ ET AL., 2004). THESE RESULTS INDICATE THE EFFICIENT OESTROUS SYNCHRONISATION WITH THE AID OF CIDR'S IN BOER GOAT DOES.

THE TIME TO ONSET OF THE OESTRUS WAS HOWEVER EARLIER, WHEN COMPARED TO THE 32.0±3.5H RECORDED IN OTHER GOAT BREEDS AND THE 42.0±18.0H IN BOER GOATS SYNCHRONISED WITH THE AID OF CIDR'S AND SUPEROVULATED WITH FSH (PENDLETON ET AL., 1992, GREYLING ET AL., 2002). THE DIFFERENCES OBSERVED WITH RESPECT TO THE ONSET OF THE INDUCED OESTROUS PERIOD MAY BE RELATED TO SEVERAL FACTORS LIKE E.G. BREED DIFFERENCES, DIFFERENT INTRAVAGINAL PROGESTAGEN TREATMENT, DOSE OF THE SUPEROVULATORY TREATMENT AND DIFFERENT SUPEROVULATORY PREPARATIONS. WHERE THE ONSET OF OESTRUS OCCURRED AT 42.0±18.0H IN BOER GOATS, THE DOSE OF A SUPEROVULATORY TREATMENT WAS 20MG PFSH/ DOE, WHILE IN THE

PRESENT STUDY THE TOTAL DOSAGE WAS 10MG PFSH/DOE. IT HAS BEEN OBSERVED THAT DOES SUPEROVULATED WITH A HIGHER DOSE OF FSH DEMONSTRATED A DELAYED TIME TO ONSET OF THE INDUCED OESTRUS, COMPARED TO DOES TREATED WITH LOWER DOSAGES (SELGRATH ET AL., 1990). THE EARLIER TIME TO THE ONSET OF OESTRUS RECORDED IN THE BOER GOAT DOES MAY ALSO BE DUE TO A HIGHER RESPONSE TO SUPEROVULATION IN THIS BREED, WITH MORE FOLLICLES PRODUCING MORE OESTROGEN, LEADING TO THE EARLY ONSET OF OESTRUS. THE MEAN DURATION OF THE INDUCED OESTROUS PERIOD ALSO TENDED TO BE SHORTER IN THE FSH/GNRHA TREATED DOES THAN IN THE CONTROL GROUP. THE PRESENT DURATION OF THE INDUCED OESTRUS PERIOD WAS SHORTER WHEN COMPARED TO EARLIER RESULTS OF THE 38.0±17.0H IN BOER GOAT SUPEROVULATED WITH PFSH. THE SHORTER DURATION OF THE INDUCED OESTROUS PERIOD IN THIS TRIAL MAY BE ASCRIBED TO THE UTILISATION OF MORE YOUNG DOES, COMPARED TO THE PREVIOUS STUDY. HOWEVER, THE SHORTER DURATION OF THE INDUCED OESTROUS PERIOD, COMPARED TO THE NATURAL DURATION OF OESTRUS MAY CLEARLY INDICATE THE EFFECT OF EXOGENOUS SUPEROVULATORY HORMONES ADMINISTERED, WHICH STIMULATED MORE FOLLICLES TO GROW - HENCE MORE OESTROGEN PRODUCED, LEADING TO SHORTER DURATION OF OESTRUS. THIS OBSERVATION DEMONSTRATES THAT WHEN MORE OVARIAN FOLLICLES ARE STIMULATED TO GROW TO THE PRE-OVULATORY STAGE, THIS WILL LEAD TO A REDUCED DURATION OF THE INDUCED OESTROUS PERIOD.

3.3.2.2. Serum progesterone concentration

THE SERUM PROGESTERONE CONCENTRATION RECORDED AT THE INITIATION OF

SYNCHRONISATION TREATMENT WAS HIGHER IN THIS STUDY FOR BOTH GROUPS - COMPARED TO PREVIOUS REPORTS IN BOER GOATS AND TELLICHERRY GOATS (GREYLING ET AL. 2000; MOTLOMELO ET AL., 2000; SENTHIL KUMAR ET AL., 2003). THE HIGHER SERUM PROGESTERONE CONCENTRATION AT CIDR INSERTION IN THIS STUDY MAY BE ATTRIBUTED TO THE FACT THAT ALL DOES HAD BEEN INJECTED WITH PGF2A PRIOR TO THE INITIATION OF THE TRIAL TO BE ABLE TO DETERMINE THE STAGE OF THE OESTROUS CYCLE, WHEN TREATED. AS A RESULT MOST DOES WERE ON DAY 13 TO 14 OF THE OESTROUS CYCLE (MID-LUTEAL PHASE) AT THE ONSET OF TREATMENT. SIMILAR HIGH BLOOD PROGESTERONE LEVELS HAVE ALSO BEEN REPORTED IN GOATS BETWEEN DAYS 5 TO 14 OF THE OESTROUS CYCLE (GAAFAR ET AL., 2005). THE DECLINE IN SERUM PROGESTERONE CONCENTRATION FOLLOWING SYNCHRONISATION TREATMENT AND SUBSEQUENT INDUCED OESTRUS IS A COMMON PHENOMENON IN GOATS (GREYLING ET AL. 2000; SENTHIL KUMAR ET AL., 2003).

THE LOW SERUM PROGESTERONE CONCENTRATION FOLLOWING PROGESTAGEN TERMINATION AND DURING THE INDUCED OESTROUS PERIOD HAS ALSO BEEN REPORTED PREVIOUSLY. IN THIS STUDY HOWEVER, THE LEVEL OF PROGESTERONE WAS >5 NG/ML IN THE CONTROL DOES FOLLOWING CIDR WITHDRAWAL AND WHEN DOES WERE IN OESTRUS – WHICH IS UNACCEPTABLY HIGH AND COULD POSSIBLY BE RELATED TO THE SENSITIVITY OF THE ASSAY. THIS OBSERVATION MAY HAVE BEEN A MAJOR FACTOR LEADING TO THE REDUCED NUMBER OF DOES RESPONDING AND FLUSHED IN THIS GROUP. IT HAS BEEN REPORTED THAT THE CIRCULATING PROGESTERONE CONCENTRATION ON THE DAY OF OESTRUS HAS A NEGATIVE CORRELATION WITH THE

SUPEROVULATORY RESPONSE (GREYLING ET AL. 2000; SENTHIL KUMAR ET AL., 2003). THE HIGH PROGESTERONE CONCENTRATION DURING THE EARLY LUTEAL PHASE (96H FOLLOWING CIDR REMOVAL) HAS ALSO BEEN PREVIOUSLY OBSERVED IN SUPEROVULATED DOES AND IS REPORTED TO BE BENEFICIAL FOR THE EMBRYO RECOVERY AND THE NUMBER OF TRANSFERABLE EMBRYOS – INDICATIVE OF THE NUMBER OF CL'S (GREYLING & VAN DER NEST, 2000; SENTHIL KUMAR ET AL., 2003).

IN THIS STUDY THE CONTROL DOES RECORDED HIGHER SERUM PROGESTERONE CONCENTRATIONS 96H FOLLOWING CIDR REMOVAL (CL FORMATION) AND THIS COULD EXPLAIN THE HIGHER NUMBER OF TRANSFERABLE EMBRYOS OBTAINED IN THIS GROUP. THESE DIFFERENCES BETWEEN GROUPS WERE HOWEVER NOT SIGNIFICANT, DUE THE LOW NUMBER OF DOES FLUSHED. IT HAS ALSO PREVIOUSLY BEEN OBSERVED THAT DOES WITH LOW SERUM PROGESTERONE CONCENTRATIONS DURING THE EARLY LUTEAL PHASE SHOW PREMATURE LUTEAL REGRESSION (ESPINOSA-MARQUEZ ET AL., 2004). IN THE CURRENT STUDY THE HIGH OCCURRENCE OF PREMATURE LUTEAL REGRESSION COULD BE ASSUMED TO HAD LED TO THE REDUCTION IN THE NUMBER OF DOES FLUSHED.

3.3.2.3. Serum oestrogen concentration

THE HIGHER SERUM OESTROGEN CONCENTRATIONS RECORDED IN FSH/GNRHA-TREATED DOES COULD BE ATTRIBUTED TO THE VALUES OF ONE ANIMAL WHICH WAS ON DAY 2 OF ITS OESTROUS CYCLE AT THE INITIATION OF PROGESTAGEN TREATMENT. THE HIGH LEVELS OF SERUM OESTROGEN RECORDED IN THE FSH/GNRHA-TREATED DOES ON DAY 12 OF PROGESTAGEN TREATMENT (DAY 5 OF GNRHA TREATMENT) MAY DUE TO THE INCREASE IN THE NUMBER OF FOLLICLES STIMULATED BY INCREASED

PRODUCTION OF FSH INDUCED BY GNRHA TREATMENT. GNRH AGONISTS ARE REPORTED TO INDUCE AN INCREASE IN FSH AND LH AT THE ONSET OF TREATMENT. WHEN THE GNRH AGONIST TREATMENT IS CONTINUED, IT DOWNREGULATES THE HYPOTHALAMUS AND LEADS TO A DECLINE IN GONADOTROPHIN PRODUCTION (D'OCCHIO ET AL., 2000; HERBERT & TRIGG, 2005). AT THE LAST GNRHA TREATMENT, THE OESTROGEN LEVEL IN THE FSH/GNRHA GROUP TENDED TO BE LOWER THAN IN THE CONTROLS. THIS SUGGESTS THAT THE GNRHA WAS ABLE TO DOWN REGULATE THE HYPOTHALAMUS WITHIN THE 7 DAYS TREATMENT PERIOD AND IT IS THEREFORE NOT NECESSARY TO APPLY THE TREATMENT FOR A TOTAL PERIOD OF 14 DAYS. THIS OBSERVATION HAS ALSO BEEN REPORTED IN CATTLE. HOWEVER, IRRESPECTIVE OF WHETHER THE GNRHA TREATMENT IS APPLIED FOR 7, 14 OR 21 DAYS, FOLLOWING THE TERMINATION OF TREATMENT, ANIMALS WERE FOUND TO RESPOND SIMILARLY (PADULA & MACMILLAN, 2005).

THE SUPEROVULATORY TREATMENT WORKED EFFICIENTLY IN THIS STUDY, AS INDICATED BY THE INCREASE IN OESTROGEN CONCENTRATION (MORE FOLLICLES RECRUITED LEAD TO HIGHER OESTROGEN LEVELS) FOLLOWING THE INITIATION OF THE SUPEROVULATION TREATMENT. AN INCREASE IN SERUM OESTROGEN LEVELS FOLLOWING SUPEROVULATION HAS ALSO BEEN OBSERVED IN OTHER GOAT BREEDS (SENTHIL KUMAR ET AL., 2003). THE HIGHER SERUM CONCENTRATION OF OESTROGEN IN THE FSH/GNRHA AT THE 4TH SUPEROVULATION TREATMENT MAY HAVE BEEN DUE TO AN INCREASE IN THE NUMBER OF FOLLICLES RECRUITED TO GROW IN THE ABSENCE OF A DOMINANT FOLLICLE. HOWEVER, THE RECRUITED FOLLICLES MAY HAVE EXPERIENCED LIMITED

FOLLICULAR GROWTH - AS INDICATED BY THE HIGHER OESTROGEN LEVEL AT THE LAST PFSH INJECTION IN THE CONTROL DOES, COMPARED TO THE FSH/GNRHA-TREATED DOES (ALTHOUGH NO SIGNIFICANT DIFFERENCE WAS RECORDED). THUS, THE DEVELOPMENT OF COMPETENT OOCYTES FROM THESE FOLLICLES MAY ALSO BE REDUCED. THIS COULD HAVE CONTRIBUTED TO THE HIGH NUMBER OF DEGENERATED EMBRYOS RECOVERED IN THE FSH/GNRHA GROUP.

THE HIGH SERUM OESTROGEN CONCENTRATION RECORDED IN THE CONTROL (FSH-TREATED) DOES ON THE DAY OF OESTRUS MAY HAVE CONTRIBUTED TO THE TENDENCY OBSERVED IN THIS GROUP OF EXHIBITING A LONGER DURATION OF OESTRUS THAN IN THE FSH/GNRHA DOES. THE HIGH SERUM OESTROGEN LEVEL RECORDED ON DAY OF OESTRUS HAS ALSO BEEN REPORTED IN OTHER GOAT BREEDS ((SENTHIL KUMAR ET AL., 2003). THIS IS ALSO AN INDICATION OF THE PRESENCE OF HIGH NUMBER OF MEDIUM-SIZED TO LARGE OVARIAN FOLLICLES, WHICH ARE NORMALLY OBSERVED AT THIS TIME OF THE CYCLE (RIESENBERG ET AL., 2001).

3.3.2.4. Ovarian response

THE HIGH OCCURRENCE OF ABNORMAL CL’S RECORDED IN THIS TRIAL CONTRIBUTED TO A REDUCTION IN THE NUMBER OF ANIMALS FLUSHED. THIS CONDITION MAY BE ATTRIBUTED TO THE FACT THAT THE DOES WERE SUPEROVULATED DURING THE NATURAL BREEDING SEASON - WHICH MAY LEAD TO HIGHER OVARIAN RESPONSE (OVER STIMULATION), TO SUPEROVULATION AND HENCE EXCEEDING THE CAPABILITY OF THE ANIMALS TO ACCOMMODATE THE MULTIPLE OVULATIONS. THIS PHENOMENON CAN ALSO BE RELATED TO AN LH DEFICIENCY, AS POOR STIMULATION OF OOCYTES DUE TO A LOWER LH PULSE HAS BEEN GENERALLY ASSOCIATED WITH

SHORT-LIVED CL'S (PREMATURE LUTEAL REGRESSION) (CAMP ET AL., 1983; GREYLING, 1988). HOWEVER, THE APPEARANCE OF ABNORMAL CL'S IS A COMMON TREND OBSERVED IN GOATS FOLLOWING SUPEROVULATION AND THE CONDITION IS REPORTED TO BE ATTRIBUTED TO PREMATURE LUTEAL REGRESSION, EMANATING FROM ELEVATED OESTROGEN LEVELS FOLLOWING SUPEROVULATION (ARMSTRONG ET AL., 1983A, B; PENDLETON ET AL., 1992).

THE 38.1% OCCURRENCE OF PREMATURE LUTEAL REGRESSION IN THIS STUDY WAS HOWEVER LOWER, WHEN COMPARED TO 47% ABNORMAL CL'S RECORDED IN MURCIANA GOATS SUPEROVULATED WITH FSH (PINTADO ET AL., 1998). THE OCCURRENCE OF PREMATURE LUTEAL REGRESSION IS A CONDITION GENERALLY OBSERVED IN GOATS SUPEROVULATED WITH ECG. HIGHER INCIDENCES OF ABNORMAL CL DEVELOPMENT, RANGING FROM 61.5% TO 78.0% HAVE ALSO BEEN OBSERVED IN DOES SUPEROVULATED WITH ECG, COMPARED TO FSH TREATED DOES (22.0%) (PENDLETON ET AL., 1992; ESPINOSA-MARQUEZ ET AL., 2004).

THE MEAN TOTAL STRUCTURES SURGICALLY FLUSHED WAS ACCEPTABLE, INDICATING THAT THE FLUSHING TECHNIQUE USED WAS EFFICIENT AND DID NOT DIFFER SIGNIFICANTLY BETWEEN THE TWO TREATMENT GROUPS. THE OVERALL MEAN OVA RECOVERED (14.3±5.8) PER DOE WAS HIGHER WHEN COMPARED TO 6.7±1.4, 7.1±1.4 AND 8.3±2.4 REPORTED FOR DIFFERENT GOAT BREEDS SUPEROVULATED WITH FSH (PENDLETON ET AL., 1992, SELVARAJU ET AL., 2003; SENTHIL KUMAR ET AL., 2003). THE CURRENT RESULTS INDICATE BOER GOAT DOES TO RESPOND BETTER TO SUPEROVULATION TREATMENT, COMPARED TO MOST OTHER BREEDS. THE MEAN NUMBER OF EMBRYOS RECORDED AND

THE FERTILISATION RATE WAS HOWEVER SIMILAR FOR BOTH TREATMENTS IN THE TRIAL. THIS WAS NOT EXPECTED AS IN ANOTHER TRIAL WHERE GOATS WERE SUPEROVULATED WITH FSH COMBINED WITH A PRE-TREATMENT OF A GNRH ANTAGONIST (WITH SIMILAR ACTIVITY AS GNRH AGONIST), THE FERTILISATION RATE RECORDED WAS HIGHER IN THE FSH TREATED GOATS, COMPARED TO THE FSH/GNRH ANTAGONIST GROUP (COGNIE ET AL., 2003).
THE OVERALL FERTILISATION RATE (74.4±26.6%) OBTAINED WAS LOWER, COMPARED TO THE 94% PREVIOUSLY REPORTED IN BOER GOATS. THE POSSIBLE REASON BEING HIGHER NUMBER OF UNFERTILISED OOCYTES BEING RECOVERED IN THE PRESENT STUDY (GREYLING ET AL., 2002). SIMILARLY, THE OVERALL MEAN FERTILISED OVA WAS COMPARABLE TO THE FINDINGS OF GONZALEZ-BULNES ET AL. (2003A) WITH MURCIANO-GRANADIANA DOES. THE PRESENT MEAN OF 11.0±5.7 FERTILISED OVA (EMBRYOS/DONOR) WAS HIGHER THAN THE 7.1±1.4 RECORDED IN OTHER GOAT BREEDS SUPEROVULATED WITH FSH (PENDLETON ET AL., 1992). THE DIFFERENCES IN OVARIAN RESPONSE TO SUPEROVULATION MAY BE ATTRIBUTED TO DIFFERENCES IN GENOTYPE. THE MEAN NUMBER OF UNFERTILISED OVA RECORDED IN THIS TRIAL DID NOT DIFFER SIGNIFICANTLY BETWEEN THE TWO TREATMENT GROUPS AND THESE RESULTS SUGGEST THAT A PRE-TREATMENT WITH GNRH AGONIST DOES NOT AFFECT THE FERTILISATION RATE OF SUPEROVULATED OOCYTES. IT SHOULD BE NOTED THAT THE FERTILISATION RATE OBTAINED IS A DELICATE BALANCE BETWEEN THE NUMBER OF OVULATIONS, THE SPERM DOSE AND THE HORMONAL ENVIRONMENT (COGNIE ET AL., 2003; GONZALEZ-BULNES ET AL., 2004A). WITH A HORMONAL IMBALANCE, THE TIME OF OVULATION COULD

ALTER, ALSO AFFECTING THE FERTILISATION RATE (COGNIE, 1999).

THE MEAN OVERALL NUMBER OF UNFERTILISED OVA (3.9±4.2) RECORDED WAS HIGHER COMPARED TO 0.3±0.21 OVA PER DOE RECORDED IN TELLICHERRY GOATS SUPEROVULATED USING THE SAME FSH PRODUCT AS UTILISED IN THE PRESENT TRIAL (SENTHIL KUMAR ET AL., 2003). THE RECOVERY OF A HIGH NUMBER OF UNFERTILISED OVA MAY BE ASCRIBED TO OOCYTES MATURING WITH GENETIC ABNORMALITIES FOLLOWING SUPEROVULATION (NOWSHARI ET AL., 1995; ARMSTRONG, 2001). THIS SHOWS THAT ALTHOUGH THE BOER GOAT DOES DEMONSTRATED A HIGHER OVARIAN RESPONSE (NUMBER OF CL'S) COMPARED TO OTHER BREEDS, NOT ALL OOCYTES OVULATED WERE FERTILISED.

THE MEAN NUMBER OF DEGENERATE EMBRYOS IN THE FSH/GNRHA GROUP (6.6±4.2 PER DOE) TENDED TO BE HIGHER THAN IN THE FSH GROUP (1.7±1.5 PER DOE) ALTHOUGH, THESE DIFFERENCES WERE NOT STATISTICALLY SIGNIFICANT. THE LACK OF DIFFERENCES MAY BE ATTRIBUTED TO LESS DOES BEING FLUSHED PER GROUP (3 IN THE FSH AND 5 IN THE FSH/GNRHA GROUP) AND THIS LIMITS FURTHER CONCLUSIONS AND RECOMMENDATIONS.

THE NUMBER OF TRANSFERABLE EMBRYOS IS AN INDICATOR OF THE ECONOMIC VIABILITY OF THE MOET PROGRAMME. IN THIS STUDY THE MEAN NUMBER OF TRANSFERABLE EMBRYOS AND EMBRYO RECOVERY RATE WAS NOT SIGNIFICANTLY DIFFERENT BETWEEN THE TWO TREATMENT GROUPS. HOWEVER, THESE MEANS TENDED TO BE LOWER IN THE FSH/GNRHA GROUP, COMPARED TO THE FSH GROUP. THE LOWER NUMBER OF TRANSFERABLE AND RECOVERED EMBRYOS MAY BE DUE TO THE LOW LH LEVEL FOLLOWING PRE-TREATMENT WITH A GNRH AGONIST AND THUS THE OOCYTES NOT BEING

COMPETENTLY MATURE (DUE TO LACK OF LH). THESE RESULTS ARE CONTRADICTORY TO OTHER FINDINGS WHERE A LOWER NUMBER OF TRANSFERABLE EMBRYOS AND RECOVERY RATE HAS BEEN REPORTED IN GOATS TREATED WITH ONLY FSH, COMPARED WITH FSH/GNRH ANTAGONIST TREATED GOATS, (8.1 VS. 2.3 AND 74.0% VS. 47.5%, RESPECTIVELY) (COGNIE ET AL., 2003). THE LOWER NUMBER OF TRANSFERABLE EMBRYOS IN THE FHS/GNRH TREATED DOES CAN BE ATTRIBUTED TO THE HIGH NUMBER OF DEGENERATE EMBRYOS. THE PRESENT OVERALL MEAN TRANSFERABLE EMBRYOS HOWEVER, WAS COMPARABLE TO OTHER STUDIES IN GOATS FOLLOWING SUPEROVULATION (PENDLETON ET AL., 1992; SELVARAJU ET AL., 2003; SENTHIL KUMAR ET AL., 2003).

3.3.3. Conclusions

THE OESTROUS RESPONSE AND ONSET OF OESTRUS FOLLOWING THE CESSATION OF TREATMENT DID NOT SEEM TO BE AFFECTED BY THE GNRH AGONIST TREATMENT. HOWEVER, THE DURATION OF THE INDUCED OESTROUS PERIOD IN THE DOES TENDED TO BE SHORTER IN THE GNRHA GROUP WHICH COULD ULTIMATELY AFFECT THE FERTILISATION RATE. THE OCCURRENCE OF ABNORMAL CL DEVELOPMENT OBSERVED WAS A MAJOR FACTOR THAT CONTRIBUTED TO THE REDUCED NUMBER OF DOES FLUSHED. ALTHOUGH THE SMALL NUMBER OF DOES FLUSHED MAY HAVE INFLUENCED THE SIGNIFICANCE, IT WAS OBSERVED THAT THE USE OF A GNRHA HAD NO ECONOMIC ADVANTAGE ON THE TOTAL NUMBER OF STRUCTURES AND EMBRYOS RECOVERED. INSTEAD, THE USE OF A GNRH AGONIST REDUCED THE NUMBER OF TRANSFERABLE EMBRYOS BY INCREASING THE TOTAL NUMBER OF DEGENERATE EMBRYOS – A FACT THAT MUST BE BORNE IN MIND WHEN CONSIDERING THE

IMPLEMENTATION OF THIS SUPEROVULATION TREATMENT IN GOATS.
THE DOWN REGULATION OF THE HYPOTHALAMUS IN THE SUPPRESSION OF GONADOTROPHIN PRODUCTION CAN BE ACHIEVED WITH A GNRH AGONIST WITHIN THE 7-DAY TREATMENT. THE PROGESTERONE CONCENTRATION DURING OESTRUS AND DURING THE EARLY LUTEAL PHASE IS IMPORTANT FOR THE OVARIAN RESPONSE TO SUPEROVULATION AND EMBRYO VIABILITY. THE BLOOD HORMONAL LEVELS INDUCED DURING SUPEROVULATION ARE DEPENDENT ON VARIOUS OVARIAN FACTORS AND THUS ULTIMATELY DETERMINE THE COMPETENCE OF THE OOCYTES AND EVENTUALLY VIABILITY OF THE EMBRYOS.

3.4. Effect of pre-treatment with a GnRH agonist outside the breeding season

3.4.1.Results

3.4.1.1. Oestrous response

ONLY 10 DOES (45.5%) SHOWED OVERT SIGNS OF OESTRUS PRIOR TO THE APPLICATION OF THE OESTROUS SYNCHRONISATION TREATMENT FOR A PERIOD OF ONE OESTROUS CYCLE - THEREFORE, HINDERING THE DETERMINATION OF THE LENGTH OF THE NATURAL OESTROUS CYCLE OUTSIDE THE BREEDING SEASON.

Table 3.7 The mean (±SD) oestrous response in Boer goats superovulated with FSH or FSH plus GnRHa

Treatment	n	Oestrous response (%)	Time to oestrus (h)	Duration of oestrus (h)
FSH	11	100	30.6±9.1	18.2±3.7
FSH/GnRHa	11	100	31.1±8.8	18.9±4.0

No significant differences

THE INDUCED OESTROUS RESPONSE IN TREATED DOES IS SET OUT IN TABLE 3.3 AND IT CAN BE SEEN

THAT ALL GOATS RESPONDED. FOLLOWING OESTROUS SYNCHRONISATION THE MEAN TIME TO THE ONSET OF OESTRUS WAS 30.8±8.9H FOLLOWING CIDR REMOVAL AND OESTRUS LASTED FOR 18.6±3.9H, WITH NO SIGNIFICANT DIFFERENCES BETWEEN THE TREATMENT AND THE CONTROL GROUPS.

3.4.1.2. Ovarian response

THE OVULATION RESPONSE AND EMBRYO RECOVERY RATE OBTAINED ARE SET OUT IN TABLE 3.4. THE MEAN NUMBER OF OVULATIONS PER DOE WAS 18.8±6.5, WITH NO SIGNIFICANT DIFFERENCE BETWEEN THE TREATMENT AND THE CONTROL GROUPS - ALTHOUGH THE MEAN NUMBER OF OVULATIONS/DONOR TENDED TO BE HIGHER IN THE CONTROL GROUP (21.3±5.9), COMPARED TO THE TREATMENT GROUP (16.1±2.9). THE OVERALL MEAN TOTAL NUMBER OF STRUCTURES RECOVERED PER DONOR WAS 15.0±5.5, WITH THE TOTAL MEAN STRUCTURES FLUSHED BEING SIGNIFICANTLY ($P<0.05$) LOWER IN THE DOES PRE-TREATED WITH GNRHA (12.6±6.0), COMPARED TO THE CONTROLS (17.6±4.9).

THE MEAN NUMBER OF EMBRYOS COLLECTED PER DONOR AND THE FERTILISATION RATE WERE SIGNIFICANTLY ($P<0.05$) LOWER IN DOES PRE-TREATED WITH GNRHA, WITH THE MEAN NUMBER OF EMBRYOS FLUSHED PER DONOR AND FERTILISATION RATE IN BOTH GROUPS BEING 14.0±5.7 AND 87.1±26.6%, RESPECTIVELY. TREATMENT AS SUCH HAD NO EFFECT ON THE MEAN NUMBER OF UNFERTILISED OVA/DONOR, WITH AN OVERALL MEAN OF 1.0±2.5 PER DONOR. PRE-TREATMENT OF DOES WITH GNRHA RESULTED IN A SIGNIFICANTLY ($P<0.01$) HIGHER MEAN NUMBER OF DEGENERATIVE EMBRYOS WHEN COMPARED TO THE CONTROLS (6.9±4.5 VS. 3.2±4.2). CONSEQUENTLY, THE NUMBER OF TRANSFERABLE EMBRYOS AND TRANSFERABLE

RATE (4.3±4.0 AND 32.7±26.9%) WAS SIGNIFICANTLY (P<0.01) LOWER IN DOES PRE-TREATED WITH GNRHA, COMPARED TO THE CONTROL DOES (13.1±5.3 AND 75.2±26.8%, RESPECTIVELY).

Table 3.8 The effect of a pre-treatment with GnRHa on the superovulatory response in Boer goat does

	Overall	FSH	FSH/GnRH
No. of goats	22	11	11
No. of ovulations (total CL's/donor)	18.8±6.5	21.3±5.9^{a}	16.1±7.0^{a}
No. of CL's (left ovary)	8.1±3.4	9.0±3.4^{a}	7.2±2.9^{a}
No. of CL's (right ovary)	10.7±3.4	12.3±2.8^{a}	8.9±4.8^{a}
Total structures flushed/donor	15.0±5.5	17.5±4.9^{a}	12.6±6.0^{b}
No. of embryos /donor	14.0±5.7	16.5±6.1^{a}	11.5±5.3^{b}
Fertilised ova /collected ova (%)	87.1±26.6	92.6±19.5^{a}	81.6±32.2^{b}
Unfertilised ova/donor	1.0±2.5	0.9±2.4^{a}	1.2±2.5^{a}
Degenerated embryos/donor	5.0±4.4	3.2±4.2^{a}	6.9±4.5^{b}
Transferable embryos/donor	8.7±4.7	13.1±5.3^{c}	4.3±4.0^{d}
Transferable embryos /fertilised ova (%)	87.1±26.6	75.2±26.8^{c}	32.7±36.9^{d}

^{a,b} Values with different superscripts within the same row differ significantly (P<0.05)

^{c,d} Values with different superscripts within the same row differ significantly (P<0.01)

3.4.1.3. Serum progesterone concentrations

FIGURE 3.6 ILLUSTRATES THE CONCENTRATION OF SERUM PROGESTERONE DURING TREATMENT PERIOD

TO EMBRYO FLUSHING. THE MEAN SERUM PROGESTERONE CONCENTRATION WAS SIMILAR IN BOTH GROUPS THROUGHOUT THE OESTROUS SYNCHRONIZATION TREATMENT. THE FSH/GNRHA-TREATED DOES TENDED TO RECORD A LOWER PROGESTERONE CONCENTRATION FOLLOWING OESTRUS AND THE MEAN SERUM PROGESTERONE CONCENTRATION WAS SIGNIFICANTLY (P<0.01) LOWER TWO DAYS BEFORE EMBRYO FLUSHING THAN IN THE FSH-TREATED DOES. IN BOTH GROUPS THE HIGHEST PROGESTERONE CONCENTRATION WAS OBSERVED ON THE DAY OF FLUSHING.

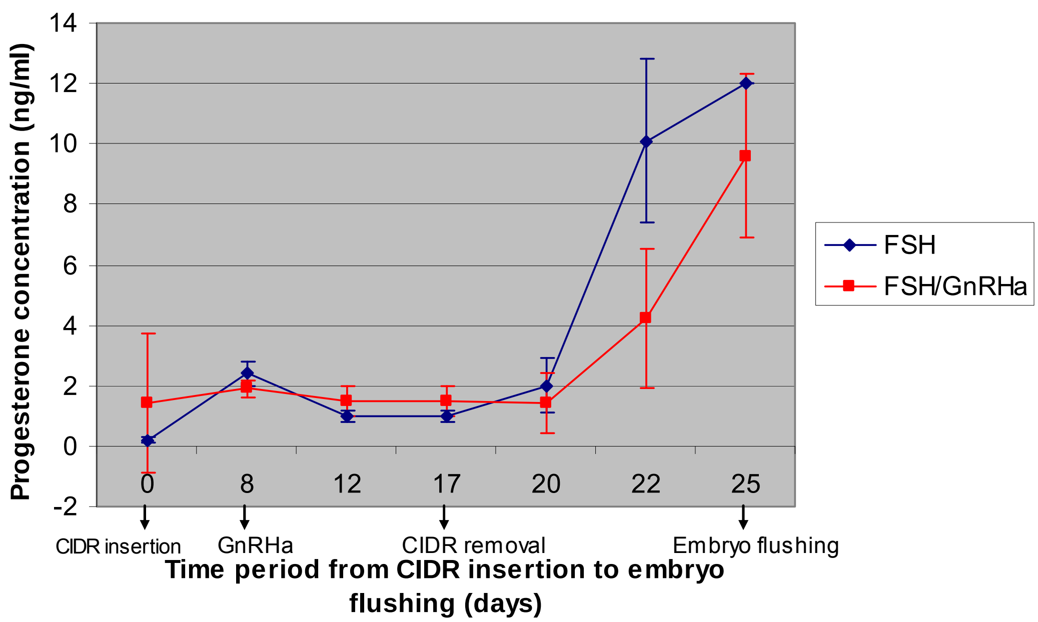

Figure 3. 12 Mean (±SD) serum progesterone concentrations in Boer goats from CIDR insertion to embryo flushing

3.4.2. Discussion

3.4.2.1. Oestrous response

LESS THAN HALF THE NUMBER OF THE DOES EXHIBITED SIGNS OF OESTRUS (CYCLIC ACTIVITY) BEFORE COMMENCEMENT OF THE

SYNCHRONISATION TREATMENT. THIS COULD MAINLY BE ATTRIBUTED TO THE SEASONAL EFFECT (PHOTOPERIOD), AS THE SEXUAL ACTIVITY IN GOATS IS REPORTED TO PEAK IN AUTUMN (SHORT DAYLIGHT LENGTH) (GREYLING, 2000). THIS TRIAL WAS CONDUCTED IN SPRING (LONG DAYLIGHT LENGTH), WHICH IS A PERIOD OUTSIDE THE NATURAL BREEDING SEASON (DEVENDRA & MCLEROY, 1982; GORDON, 1997). IT HAS BEEN REPORTED THAT ALTHOUGH BOER GOATS DO NOT EXHIBIT A COMPLETE ANOESTROUS PERIOD, THEY DEMONSTRATE LOWER CYCLIC ACTIVITY OUTSIDE THE NATURAL BREEDING SEASON (AUTUMN) (GREYLING, 2000).

ALL DOES SHOWED OVERT SIGNS OF OESTRUS FOLLOWING OESTROUS SYNCHRONISATION – DEMONSTRATING INDUCED OESTROUS ACTIVITY FROM A HIGHER THAN THRESHOLD OESTROGEN LEVEL FOLLOWING SUPEROVULATION. A SIMILAR 100% OESTROUS RESPONSE HAS ALSO BEEN REPORTED IN DIFFERENT GOAT BREEDS OUT OF SEASON FOLLOWING CONTROLLED BREEDING (SELVARAJU ET AL., 2003, ESPINOSA-MARQUEZ ET AL., 2004). THUS, DEMONSTRATING THE ACHIEVEMENT OF EFFICIENT SYNCHRONISATION OF OESTRUS IN BOER GOAT DOES OUTSIDE THE BREEDING SEASON, WITH THE AID OF CIDR'S AND EXOGENOUS GONADOTROPHINS.

PRE-TREATMENT WITH GNRHA HAD NO EFFECT ON THE TIME TO ONSET AND DURATION OF THE INDUCED OESTROUS PERIOD, INDICATING NO REAL GONADOTROPHIC HORMONAL ADVANTAGE OR IMBALANCE FOLLOWING THIS TREATMENT. THE OVERALL MEAN ONSET OF OESTRUS FOR BOTH GROUPS OF 30.8±8.9H WAS IN LINE WITH 32.0±3.5H AND 33.40±4.7H PREVIOUSLY REPORTED IN OTHER GOAT BREEDS (PENDLETON ET AL., 1992; SELVARAJU

ET AL., 2003). HOWEVER, THE MEAN DURATION OF THE INDUCED OESTROUS PERIOD (18.6±3.9H) TENDED TO BE SHORTER, WHEN COMPARED TO 36.60±3.5H AND 38.0±17.0H REPORTED IN OTHER GOAT BREEDS AND BOER GOAT DOES FOLLOWING SUPEROVULATION WITH PFSH (GREYLING ET AL., 2002; SELVARAJU ET AL., 2003). A LONGER DURATION OF THE INDUCED OESTROUS PERIOD HAS BEEN REPORTED DURING THE BREEDING SEASON, SUGGESTING THAT THE SHORT OESTROUS LENGTH IN THIS TRIAL COULD BE ATTRIBUTED TO A SEASONAL EFFECT (PHOTOPERIOD), DUE TO LOWER LEVELS OF FSH AND LH.

3.4.2.2. Ovarian response and embryo recovery rates

IT WAS EXPECTED THAT THE PRE-TREATMENT WITH A GNRH AGONIST WOULD INCREASE THE OVULATION RATE, AS IT HAS THE POTENTIAL TO REDUCE THE LH CONCENTRATION AND HENCE REDUCE THE NUMBER OF LARGE FOLLICLES DEVELOPING PRIOR TO SUPEROVULATORY TREATMENT. HOWEVER, IN THIS STUDY THE PRE-TREATMENT WITH GNRHA HAD NO SIGNIFICANT EFFECT ON THE NUMBER OF OVULATIONS FOLLOWING SUPEROVULATION IN BOER GOAT DOES. THE FACT THAT THE STAGE OF THE OESTROUS CYCLE IN THE DONOR AT THE ONSET OF THE SUPEROVULATORY TREATMENT WAS GENERALLY NOT KNOWN IN ALL DOES, MAKING IT DIFFICULT TO SPECULATE REGARDING THE STAGE OF THE OESTROUS CYCLE AT THE ONSET OF GONADOTROPHIN TREATMENT. IT IS HYPOTHESIZED THAT IF A SUPEROVULATORY TREATMENT COULD BE INITIATED WHEN THERE ARE NO DOMINANT FOLLICLES PRESENT AND THERE ARE HIGHER NUMBER OF SMALL FOLLICLES, THE SUPEROVULATORY RESPONSE (OVULATION RATE AND EMBRYO RECOVERY YIELDS) COULD BE IMPROVED AND THE VARIATION IN RESPONSE ALSO

BE REDUCED (MENCHACA & RUBIANES, 2002; GONZALEZ-BULNES ET AL., 2004A). IN SHEEP, PRE-TREATMENT OF EWES WITH A GNRH AGONIST FOLLOWED BY A SUPEROVULATION TREATMENT HAS LED TO THE TOTAL ELIMINATION OF LARGE FOLLICLES AT THE ONSET OF THE SUPEROVULATION TREATMENT. THE NUMBER OF OVULATIONS WERE ALSO FOUND TO BE SIGNIFICANTLY HIGHER IN GNRHA PRE-TREATED EWES (19.2±4.1), COMPARED TO EWES SUPEROVULATED WITH FSH ALONE (13.2±5.5) (COGNIE, 1999). THIS OBSERVATION SEEMS TO INDICATE THAT THERE ARE DIFFERENCES IN THE RESPONSE TO PRE-TREATMENT WITH A GNRH AGONIST IN DIFFERENT SPECIES.

WHEN A GNRH ANTAGONIST, WITH THE SIMILAR EFFECT AS A GNRH AGONIST, WAS UTILISED AS A PRE-TREATMENT IN GOATS SUPEROVULATED WITH FSH, THE RESPONSE WITH RESPECT TO THE NUMBER OF CL'S INDUCED ALSO IMPROVED BY ALMOST 50% - WHEN COMPARED TO FSH TREATMENT ALONE (COGNIE ET AL., 2003). THE OVERALL MEAN OVULATION RATE PER DOE (18.8±6.5) FOR BOTH GROUPS IS IN LINE WITH THE 18.0 PER DOE RECORDED IN BOER GOATS AND 17.6±5.5 PER DOE IN FERAL GOATS PREVIOUSLY REPORTED (ARMSTRONG ET AL., 1983; GREYLING ET AL. 2002). HOWEVER, THIS OVERALL MEAN OVULATION RATE IS HIGHER COMPARED TO THE 8.3±1.8 AND 10.2±3.1 PER DONOR IN OTHER GOAT BREEDS (SELVARAJU ET AL., 2003; SENTHIL KUMAR ET AL., 2003). THESE DIFFERENCES IN OVULATION RATE OBSERVED MAY BE ATTRIBUTED TO AMONGST OTHERS, BREED DIFFERENCES - THUS DEMONSTRATING THE BOER GOAT DOES TO HAVE A HIGHER RESPONSE TO SUPEROVULATION – PROBABLY ATTRIBUTED TO ITS HIGH PROLIFICACY. HIGH OVARIAN RESPONSE TO SUPEROVULATION MAY THUS BE CORRELATED TO

HIGHER FECUNDITY OBSERVED IN BOER GOATS, COMPARED TO OTHER BREEDS - INDICATING THAT HIGHLY FERTILE GOATS RESPOND BETTER TO SUPEROVULATION (AMOAH & GELAYE, 1990). IT IS ALSO IMPORTANT THAT THE BCS (NUTRITIONAL STATUS) AND EVEN AGE OF THE DOE – IRRESPECTIVE OF THE SEASON SHOULD BE TAKEN INTO CONSIDERATION.

THE MEAN TOTAL NUMBER OF STRUCTURES FLUSHED, EMBRYOS RECOVERED AND FERTILISATION RATE WAS SIGNIFICANTLY LOWER IN THE FSH/GNRHA TREATED DOES, COMPARED TO THE CONTROL GOATS. THESE RESULTS INDICATE THAT THE PRE-TREATMENT WITH A GNRH AGONIST HAS A NEGATIVE EFFECT ON THE TOTAL RECOVERY RATE (OVA/EMBRYOS RECOVERED) AND THE FERTILISATION RATE IN BOER GOATS. SIMILAR TRENDS HAVE BEEN REPORTED IN GOATS TREATED WITH GNRH ANTAGONISTS, WHERE A LOW FERTILISATION RATE (28.5%) IN THE FSH/GNRH ANTAGONIST TREATED GOATS WAS RECORDED, WHEN COMPARED TO FSH TREATED GOATS (93.6%) (COGNIE ET AL., 2003). THE IMPLICATION OF THESE RESULTS MAY BE THAT IN GOATS THE SMALL OVARIAN FOLLICLES (AND SUPPRESSION OF LH) INDUCED BY THE PRE-TREATMENT WITH GNRH AGONISTS OR ANTAGONISTS, MAY NOT BE CAPABLE OR NOT BE SELECTED TO GROW TO A PRE-OVULATORY STAGE, AS INDICATED BY THE LOW RECOVERY RATE. THE NUMBER OF UNFERTILISED OVA PER DONOR WAS FOUND NOT TO DIFFER BETWEEN TREATMENTS - INDICATIVE OF A SATISFACTORY AI TECHNIQUE AND TIMING. FSH/GNRHA TREATMENT HOWEVER, RESULTED IN A HIGHER NUMBER OF DEGENERATE EMBRYOS. THIS OBSERVATION COULD BE HORMONE RELATED, AS WELL AS THE OVULATION OF GENETICALLY

ABNORMAL OOCYTES. SIMILAR TENDENCIES HAVE BEEN REPORTED IN GOATS WHERE THE HIGHER OVULATION RATE OBSERVED FOLLOWING A PRE-TREATMENT WITH A GNRH ANTAGONIST, WAS NEGATED BY THE HIGH RECOVERY OF UNFERTILISED OVA AND DEGENERATE EMBRYOS (COGNIE ET AL., 2003; GONZALEZ-BULNES ET AL., 2004A). THE HIGHER RECOVERY OF DEGENERATE EMBRYOS IMPLIES A REDUCTION IN THE CIRCULATING LH CONCENTRATION THROUGH THE UTILISATION OF THE GNRH ANTAGONISTS OR AGONISTS. THESE AFFECT THE MATURATION PROCESS OF THE OOCYTES AND HENCE THESE OOCYTES MAY BE FERTILISED, BUT NOT DEVELOP FURTHER. THIS TENDENCY WAS CLEARLY ILLUSTRATED IN THIS STUDY AS THE DEGENERATE EMBRYOS WHICH ON THE DAY OF COLLECTION (DAY 6 FOLLOWING AI), WERE AT A 2 TO 16-CELL STAGE. IMPLICATING THAT THESE EMBRYOS HAD CEASED TO DEVELOP, AS AT THIS STAGE EMBRYOS SHOULD HAVE BEEN AT THE MORULAE TO BLASTOCYST STAGES. IN GOATS, IT HAS BEEN REPORTED THAT THE NUMBER AND VIABILITY OF EMBRYOS ARE POSITIVELY CORRELATED TO FOLLICLES OF 4 TO 6 MM IN DIAMETER. NO CORRELATION WAS HOWEVER BEEN RECORDED FOR THE SMALL FOLLICLES OF 2 TO 3 MM (GONZALEZ-BULNES ET AL., 2003A).

THIS PHENOMENON SUGGESTS THAT IN GOATS, A PRE-TREATMENT WITH A GNRH AGONIST LEADS TO HIGH NUMBERS OF SMALL FOLLICLES OF LESS THAN 4 MM IN SIZE - WHICH IF STIMULATED TO GROW, COULD LEAD TO OVULATION AND THE RELEASE OF IMMATURE OOCYTES. THE MEAN NUMBER OF TRANSFERABLE EMBRYOS AND TRANSFERABLE EMBRYOS/FERTILISED OVA WAS SIGNIFICANTLY HIGHER IN THE CONTROL GOATS, COMPARED TO FSH/GNRHA TREATED DONORS. THE LOW

TRANSFERABLE EMBRYO RATE OBTAINED IN FSH/GNRHA TREATED GOATS CAN MOSTLY BE ATTRIBUTED TO THE HIGHER NUMBER OF DEGENERATE EMBRYOS RECOVERED IN THIS GROUP. SIMILAR TRENDS OF A LOW TRANSFERABLE EMBRYO RATE HAVE BEEN REPORTED IN GOATS TREATED WITH A FSH/GNRH ANTAGONIST, WHEN COMPARED TO GOATS TREATED WITH ONLY FSH (COGNIE ET AL., 2003).

THE RESULTS IN THIS STUDY DEMONSTRATE THE PRE-TREATMENT WITH A GNRH AGONIST IN A FSH SUPEROVULATION PROGRAMME OF BOER GOAT DOES, TO HAVE A DETRIMENTAL EFFECT ON THE RESPONSE TO SUPEROVULATION. THIS MAY IMPLY THAT IF THIS PROTOCOL IS USED IN GOATS, THE INITIATION OF A SUPEROVULATION TREATMENT MUST BE DELAYED - IN ORDER TO ALLOW THE OVARIAN FOLLICLES TO ACQUIRE A SIZE (E.G. 4-6 MM IN DIAMETER) WHICH WOULD LEAD TO VIABLE EMBRYOS AT FLUSHING. ALTERNATIVELY BEFORE SUPEROVULATION TREATMENT IS INITIATED, THE ANIMALS SHOULD BE TREATED WITH GROWTH HORMONE (GH) IN ORDER TO STIMULATE THE OVARIAN FOLLICLES TO ACQUIRE A LARGER SIZE.

3.4.2.3. Serum progesterone concentrations

THE LEVEL OF CIRCULATING BLOOD PROGESTERONE DURING OESTRUS, AFTER FERTILISATION AND AT THE TIME OF EMBRYO FLUSHING IS A VERY IMPORTANT FACTOR DETERMINING THE QUANTITY AND QUALITY OF EMBRYOS RECOVERED IN A MOET PROGRAMME. THE LOW SERUM PROGESTERONE CONCENTRATION BEFORE FLUSHING IN FSH/GNRHA-TREATED DOES MAY BE ASCRIBED TO LOW NUMBER OF CL'S PRODUCED IN THIS GROUP FOLLOWING SUPEROVULATION – AS PROGESTERONE IS PRINCIPALLY SECRETED BY THE CL'S. HOWEVER, THE NUMBER OF OVULATIONS TENDED TO BE LOW IN

THE GNRHA PRE-TREATED GROUP. THIS OBSERVATION INDICATES THAT THE CL'S PRODUCED FOLLOWING A PRE-TREATMENT WITH A GNRHA MAY NOT BE CAPABLE OF PRODUCING SUFFICIENT PROGESTERONE. THE NEGATIVE EFFECT OF THE LOW PROGESTERONE CONCENTRATIONS FOLLOWING THIS REGIME OBSERVED IN THIS TRIAL COULD BE THE REASON FOR THE PRODUCTION OF A HIGH NUMBER OF DEGENERATING EMBRYOS AND THE LOW NUMBER OF EMBRYOS AND TRANSFERABLE EMBRYOS (GOOD QUALITY EMBRYOS) RECOVERED. IT HAS BEEN OBSERVED THAT IN GOATS THE CONCENTRATION OF PROGESTERONE AROUND THE TIME OF EMBRYO FLUSHING IS POSITIVELY CORRELATED TO THE NUMBER OF OVULATIONS, TOTAL NUMBER OF EMBRYO RECOVERED AND THE NUMBER OF TRANSFERABLE EMBRYOS (SENTHIL KUMAR ET AL., 2003).

3.4.3. Conclusions

THE OESTROUS RESPONSE, TIME TO ONSET AND DURATION OF THE INDUCED OESTROUS PERIOD WERE NOT AFFECTED BY THE ADMINISTRATION OF A GNRH AGONIST PRE-TREATMENT IN A NORMAL FSH SUPEROVULATION PROTOCOL. THE NUMBER OF CL'S WAS ALSO NOT AFFECTED BY A PRE-TREATMENT WITH THE GNRH AGONIST. THE ADDITION OF THE GNRHA IN THE FSH GOAT SUPEROVULATION PROTOCOL REDUCED THE NUMBER OF STRUCTURES RECOVERED, EMBRYO YIELD, AND THE FERTILISATION RATE (THE NUMBER OF EMBRYOS PER STRUCTURES). ALTHOUGH THE NUMBER OF UNFERTILISED OVA WAS NOT AFFECTED BY THE TREATMENT. THE PRE-TREATMENT WITH GNRHA ALSO INCREASED THE NUMBER OF DEGENERATE EMBRYOS, WHICH AGAIN REDUCED THE TOTAL NUMBER OF TRANSFERABLE EMBRYOS. IT WOULD THEREFORE, SEEM AS IF A PRE-TREATMENT WITH

GNRH AGONIST IN A BOER GOAT MOET PROGRAMME IS NOT WARRANTED AS IT LEADS TO POOR CL DEVELOPMENT SECRETING LOW PROGESTERONE CONCENTRATIONS, WHICH EVENTUALLY REDUCE THE QUALITY AND YIELD OF THE EMBRYOS RECOVERED.

CHAPTER 4

4. effect of season on the response to superovulation

4.1. Introduction

IN ORDER TO REALISE THE COMMERCIAL APPLICATION OF MOET PROGRAMMES IN SMALL STOCK, A CONTINUOUS HIGH SUPPLY OF EMBRYOS THROUGHOUT THE YEAR IS CRUCIAL. HOWEVER, IN MOST COUNTRIES, MOET IN SMALL RUMINANTS IS LIMITED TO THE NATURAL BREEDING SEASON, DUE TO THE SEASONAL CYCLIC ACTIVITY OF SHEEP AND GOATS (CHAGAS E SILVA ET AL., 2003). IN SOUTH AFRICA, FOR EXAMPLE, IT HAS BEEN REPORTED THAT THE BOER GOATS SHOW PEAK SEXUAL ACTIVITY IN AUTUMN (SHORT DAYLIGHT LENGTH) AND LOWEST ACTIVITY IN SPRING (GREYLING & VAN NIEKERK, 1987). IN OTHER GOAT BREEDS, THE HIGHEST OVULATION RATE AND EMBRYO YIELDS HAVE BEEN RECORDED DURING THE NATURAL BREEDING SEASON AND THE LOWEST OUTPUT RECORDED DURING THE ANOESTROUS PERIOD (GONZALEZ-BULNES ET AL., 2003A). THIS TRIAL WAS THUS INITIATED TO EVALUATE THE EFFECT OF SEASON ON THE OVARIAN RESPONSE TO SUPEROVULATION IN BOER GOAT DOES.

4.2. Materials and methods

TWENTY MATURE MULTIPAROUS BOER GOAT DOES WERE SUPEROVULATED DURING THE NATURAL BREEDING SEASON (N=9; AUTUMN) AND OUTSIDE THE NATURAL BREEDING SEASON (N=11; SPRING) IN THIS TRIAL. THE DONOR DOES RECORDED AN AVERAGE BODY WEIGHT OF 55.5KG AND 55KG DURING AND OUTSIDE THE BREEDING SEASON, RESPECTIVELY. OESTROUS SYNCHRONISATION OF ALL THE DOES

WAS PERFORMED USING CONTROLLED INTERNAL DRUG RELEASE DISPENSERS (CIDR; PHARMACIA & UPJOHN, AUCKLAND, NEW ZEALAND), INSERTED FOR A 17 DAY PERIOD. DOES WERE SUPEROVULATED WITH A TOTAL OF 200MG FSH/DOE (PFSH) (FOLLTROPIN®-VETREPHARM). THE SUPEROVULATION TREATMENT (PFSH) WAS ADMINISTERED I.M. IN 7 DOSAGES, AT 12H INTERVALS, STARTING 48H PRIOR TO CIDR REMOVAL (THE FIRST DOSE BEING 50 MG AND ALL OTHERS BEING 25 MG).

OESTROUS DETECTION WAS PERFORMED 3 TIMES DAILY AT 8H INTERVALS FOLLOWING CIDR WITHDRAWAL WITH THE AID OF TEASER BUCKS TO DETERMINE THE OESTROUS RESPONSE TO SYNCHRONISATION AND THE ONSET AND DURATION OF THE INDUCED OESTROUS PERIOD. FIXED TIME CERVICAL INSEMINATIONS (0.01ML FRESH undiluted semen) were performed 36h and 48h following CIDR withdrawal. The semen used for AI was collected from bucks of proven fertility with the aid of an artificial vagina. Thereafter, the semen was evaluated for progressive motility and only semen samples with a 3+ motility score was utilised for AI. Embryos were surgically flushed on day 6 following the second AI and evaluated and classified according to their morphological appearance as described by Nuti et al. (1987) and Lindner and Wright (1983). The total numbers of recovered, degenerate and transferable embryos (grades 1, 2 and 3) per doe flushed were RECORDED. BLOOD SAMPLING PROCEDURES AND PROGESTERONE CONCENTRATION DETERMINATION WERE PERFORMED AS DESCRIBED IN CHAPTER 3 (PARAGRAPH 3.2.3.4 AND 3.2.3.4.2)

DATA REGARDING THE ONSET AND DURATION OF THE INDUCED OESTROUS PERIOD, TOTAL CL'S, TOTAL

STRUCTURES FLUSHED, UNFERTILISED OVA AND EMBRYOS COLLECTED WERE ANALYSED USING THE ANOVA PROCEDURES. THE OESTROUS RESPONSE, EMBRYO RECOVERY RATES AND FERTILISATION RATE WERE ANALYSED USING THE CHI-SQUARE TEST (SAS, 2003).

4.3. Results

4.3.1. Ovarian response

RESULTS REGARDING THE OESTROUS RESPONSE, TIME TO ONSET OF OESTRUS FOLLOWING CIDR WITHDRAWAL AND DURATION OF THE INDUCED OESTROUS PERIOD ARE SET OUT IN TABLE 4.1. ALL DOES RESPONDED TO OESTROUS SYNCHRONISATION AND ON AVERAGE, OESTRUS WAS DEMONSTRATED 28.0±7.5H FOLLOWING CIDR REMOVAL AND THIS OESTROUS PERIOD LASTED FOR 20.8±4.7H. THE MEAN TIME TO ONSET OF OESTRUS WAS SIGNIFICANTLY ($P<0.05$) EARLIER DURING THE NATURAL BREEDING SEASON, COMPARED TO OUTSIDE THE BREEDING SEASON IN DOES. SIMILARLY, THE MEAN DURATION OF THE INDUCED OESTRUS WAS SIGNIFICANTLY ($P<0.05$) LONGER DURING THE NATURAL BREEDING SEASON.

THE MEAN NUMBER OF CL'S (OVULATION RATE) AND STRUCTURE RECOVERY RATES ARE SET OUT IN TABLE 4.2. SEASON DID NOT HAVE ANY SIGNIFICANT EFFECT ON THE TOTAL NUMBER OF CL'S, THE NUMBER OF CL'S ON THE LEFT OVARY, STRUCTURES RECOVERED, AND EMBRYOS, DEGENERATE AND TRANSFERABLE EMBRYOS PER DONOR. THE MEAN NUMBER OF OVULATIONS ON THE RIGHT OVARY WAS HOWEVER SIGNIFICANTLY ($P<0.05$) HIGHER OUTSIDE THE NATURAL BREEDING SEASON, COMPARED TO IN THE NATURAL BREEDING SEASON. THE MEAN NUMBER OF UNFERTILISED OVA PER DONOR WAS SIGNIFICANTLY ($P<0.05$) HIGHER IN

GOATS TREATED OUTSIDE THE NATURAL BREEDING SEASON.

Table 4.1 The effect of season on oestrous response in Boer goat does superovulated with pFSH during and outside the natural breeding season

Season	**n**	**Oestrous response (%)**	**Time to oestrus (h)**	**Duration of oestrus (h)**
During	9	100	24.9 ± 4.8^{a}	24.0 ± 5.7^{b}
Outside	11	100	30.5 ± 9.1^{a}	18.2 ± 3.7^{b}

^{ab} values in the same column with different superscripts are significantly different ($P<0.05$)

Table 4.2 The mean (±SD) ovulation rate and structures (ova and embryos) recovery rate in Boer goat does superovulated with pFSH during and outside the breeding season

Parameters	**Season**	
	During	**Outside**
No. of does	8	11
Total number of CL's (right ovary)	9.8 ± 2.7^{a}	12.3 ± 2.8^{b}
Total number of CL's (left ovary)	7.8 ± 4.2^{a}	9.0 ± 3.4^{a}
Ovulation rate (total CL's/donor)	17.5 ± 6.3^{a}	21.3 ± 5.9^{a}
Total number of structures (unfertilised ova & embryos per doe)	18.0 ± 5.8^{a}	17.5 ± 4.8^{a}
Total number of embryos recovered/donor	16.4 ± 7.0^{a}	16.5 ± 6.1^{a}
Total number of unfertilised ova recovered/donor	0.9 ± 2.4^{a}	3.3 ± 2.8^{b}
Total number of degenerate embryos/donor	3.6 ± 3.4^{a}	3.2 ± 4.2^{a}
Total number of transferable embryos/donor	12.3 ± 9.4^{a}	13.1 ± 5.3^{a}

^{a,b} Values with different superscripts within the same row differ significantly ($P<0.05$)

4.3.2. Serum progesterone profiles

THE CONCENTRATION OF SERUM PROGESTERONE IN BOER GOAT DOES SUPEROVULATED WITH PFSH

DURING OR OUTSIDE THE NATURAL BREEDING SEASON IS ILLUSTRATED IN FIGURE 4.1. THE MEAN SERUM PROGESTERONE CONCENTRATION WAS ELEVATED IN DOES SUPEROVULATED OUTSIDE THE BREEDING SEASON, WHILE MAINTAINED AT A BASAL LEVEL IN DOES SUPEROVULATED DURING THE BREEDING SEASON FOLLOWING THE INITIATION OF SYNCHRONISATION TREATMENT. AS A CONSEQUENCE THE MEAN LEVEL OF SERUM PROGESTERONE CONCENTRATION WAS SIGNIFICANTLY ($P<0.01$) HIGHER ON DAY 8 OF SYNCHRONISATION TREATMENT IN DOES TREATED OUTSIDE THE NATURAL BREEDING SEASON. THE MEAN SERUM PROGESTERONE CONCENTRATION IN BOTH SEASONS WAS MAINTAINED AT A BASAL LEVEL FROM DAY 12 OF SYNCHRONISATION TREATMENT UNTIL CIDR REMOVAL. THE SERUM PROGESTERONE CONCENTRATION STARTED TO INCREASE FOLLOWING OESTRUS UNTIL EMBRYO FLUSHING, IN BOTH SEASONS. DOES TREATED IN SPRING (OUTSIDE THE BREEDING SEASON) HAD A SIGNIFICANTLY HIGHER ($P<0.05$) SERUM PROGESTERONE CONCENTRATION 2 DAYS BEFORE AND AT EMBRYO FLUSHING THAN IN DOES TREATED IN AUTUMN (DURING THE NATURAL BREEDING SEASON).

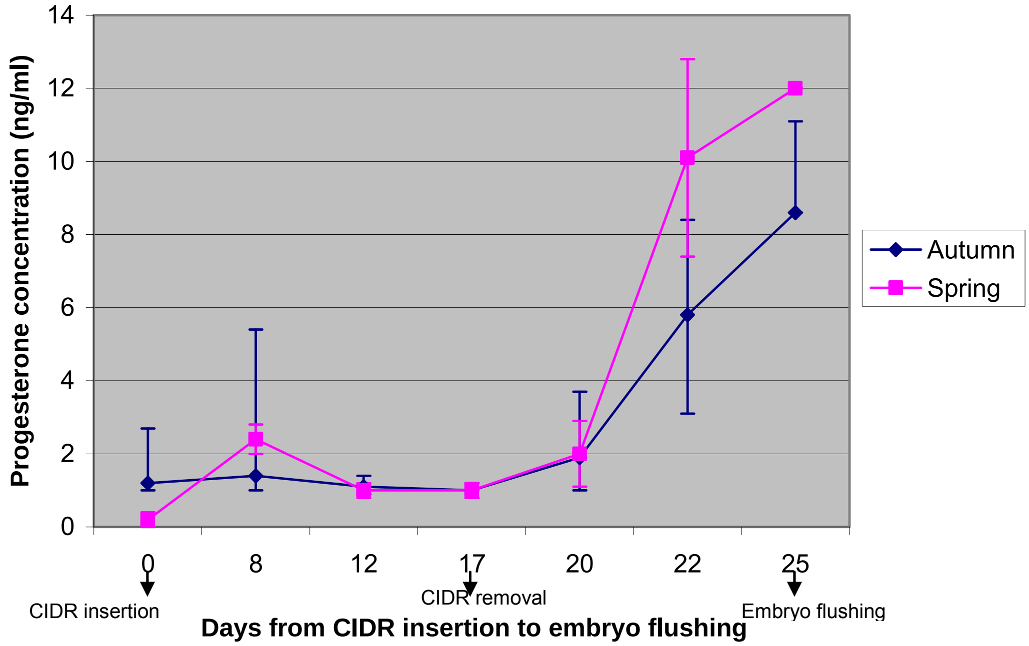

Figure 4.1 Mean (±SD) serum progesterone concentration from CIDR insertion to embryo flushing

4.4. Discussion

THE 100% OESTROUS RESPONSE OBSERVED IN THIS STUDY HAS ALSO BEEN REPORTED IN DIFFERENT GOAT BREEDS SUPEROVULATED, ESPECIALLY DURING THE NATURAL BREEDING SEASON (SENN & RICHARDSON, 1991; AKINLOSOTU & WILDER, 1993; SELVARAJU ET AL., 2003, ESPINOSA-MARQUEZ ET AL., 2004) AND EVEN OUTSIDE THE BREEDING SEASON (BARIL & VALLET, 1990). HOWEVER, SENN AND RICHARDSON (1991) REPORTED A NEGATIVE EFFECT OF SEASON ON THE OVARIAN RESPONSE TO SYNCHRONISATION AS NOT ALL DOES RESPONDED DURING THE LATE BREEDING SEASON COMPARED TO EARLY IN THE BREEDING SEASON. ALTHOUGH BOER GOATS EXHIBIT REDUCED CYCLIC ACTIVITY OUTSIDE THE BREEDING SEASON, THEY SEEM TO RESPOND WELL TO INDUCED OESTRUS. INDICATING

THAT THE OVARIES ARE NOT ENTIRELY DORMANT. THE OVERALL MEAN TIME TO THE ONSET OF OESTRUS (28.0±7.5H) FOR BOTH SEASONS OBTAINED IN THIS TRIAL, SEEM TO BE EARLIER, WHEN COMPARED WITH 32.0±3.5H AND 33.40±4.7H REPORTED IN OTHER GOAT BREEDS FOLLOWING SUPEROVULATION. THE EARLIER ONSET OF OESTRUS IN BOER GOAT DOES MAY BE ASCRIBED TO THE HIGH OESTROGEN LEVELS PRODUCED AS A RESULT OF HIGH RESPONSE TO SUPEROVULATION. (PENDLETON ET AL., 1992; SELVARAJU ET AL., 2003). HOWEVER, THIS MEAN TIME TO ONSET OF OESTRUS IS COMPARABLE TO THE 27.2±0.4H REPORTED FOR BOER GOATS SYNCHRONISED WITH CIDR'S WITHOUT BEING SUPEROVULATED. CONFIRMING PREVIOUS REPORTS THAT THE TIME TO ONSET OF OESTRUS WHEN GOATS ARE SYNCHRONISED WITH CIDR'S IS EARLIER THAN WHEN GOATS ARE SYNCHRONISED WITH INTRAVAGINAL PROGESTAGEN SPONGES (WITHOUT SUPEROVULATION), DURING THE BREEDING SEASON (MOTLOMELO ET AL., 2002).

DOES TOOK A LONGER TIME FROM CIDR REMOVAL TO THE ONSET OF OESTRUS OUTSIDE THE BREEDING SEASON. THESE RESULTS ARE IN AGREEMENT WITH THE OBSERVATION IN SHEEP AND GOATS THAT ANIMALS SHOW SIGNS OF OESTRUS EARLIER DURING THE NATURAL BREEDING SEASON AND TAKE A LONGER TIME TO RESPOND FROM PROGESTAGEN REMOVAL TO THE ONSET OF OESTRUS OUTSIDE THE BREEDING SEASON. THIS IS AS EXPECTED DURING THE BREEDING SEASON AS THE OVARIES NEED LESS STIMULATION AND REACT FASTER TO EXOGENOUS HORMONES THAN OUTSIDE THE NATURAL BREEDING SEASON. THE EARLY ONSET OF OESTRUS DURING THE BREEDING SEASON THUS INDICATES THAT THE BOER GOAT DOES ARE HIGHLY SENSITIVE TO EXOGENOUS HORMONES (SUPEROVULATION TREATMENT)

DURING THE BREEDING SEASON, LEADING TO BETTER FOLLICULAR DEVELOPMENT. THE HIGH NUMBERS OF INDUCED FOLLICLES WILL THEN SYNTHESIZE AND SECRETE MORE OESTROGEN, LEADING TO DOES EXHIBITING OESTRUS EARLIER. IN ADDITION, THE VARIATION (WITHIN AND BETWEEN GROUPS) REGARDING THE TIME TO ONSET OESTRUS IN GOATS TREATED DURING THE NON-BREEDING SEASON WAS LARGE, COMPARED TO THE NATURAL BREEDING SEASON – DUE TO INDIVIDUAL DIFFERENCES (BARIL & VALLET, 1990; SAMARTZI ET AL., 1995). THIS BIG VARIATION WITH RESPECT TO THE ONSET OF OESTRUS WAS ALSO OBSERVED IN THE PRESENT STUDY IN THE GOATS TREATED OUTSIDE THE NATURAL BREEDING SEASON, INDICATING THAT MORE HORMONAL STIMULATION IS POSSIBLY NEEDED. THE MEAN DURATION OF THE INDUCED OESTROUS PERIOD FOLLOWING TREATMENT WAS SHORTER OUTSIDE THE BREEDING SEASON THAN DURING THE NATURAL BREEDING SEASON – ONCE AGAIN AN INDICATION OF POSSIBLE LOWER STEROID (OESTROGEN) LEVELS. THIS IS EMPHASIZED BY THE LONGER TIME TO THE ONSET OF OESTRUS TAKEN BY DOES OUTSIDE THE BREEDING SEASON.

ALTHOUGH, THE TOTAL NUMBER OF CL’S ON THE LEFT OVARIES WERE SIMILAR, THE AVERAGE NUMBER OF CL’S PER DOE TREATED TENDED TO BE HIGHER OUTSIDE THE BREEDING SEASON. THE LOWER TOTAL NUMBER OF CL’S RECORDED DURING THE BREEDING SEASON MAY BE ATTRIBUTED TO THE PRESENCE OF AN ACTIVE DOMINANT FOLLICLE, AS DOES WERE CYCLIC WHICH IS REPORTED TO SUPPRESS THE OVARIAN RESPONSE TO SUPEROVULATION (STAGE OF FOLLICULAR WAVES UNKNOWN). THE DETRIMENTAL EFFECTS OF THE DOMINANT FOLLICLE ARE SAID TO BE MORE

PROMINENT DURING THE NATURAL BREEDING SEASON (GONZALEZ-BULNES ET AL., 2003B). THUS A HIGHER RESPONSE TO SUPEROVULATION WITH RESPECT TO OVULATION RATE OUTSIDE THE NATURAL BREEDING SEASON COULD BE EXPECTED IN GOATS AS THIS WAS SIMILAR TO THE TENDENCY OBSERVED IN SHEEP (GREANEY ET AL., 1991). THIS HIGHER OVULATION RATE COULD WARRANT THE CONDUCTION OF MOET PROGRAMMES OUTSIDE THE NATURAL BREEDING SEASON IN GOATS.

THE PRESENT RESULTS INDICATE NO SIGNIFICANT EFFECT OF SEASON ON THE TOTAL NUMBER OF CL'S INDUCED AND, CONTRADICT PREVIOUS FINDINGS IN NUBIAN GOATS FOLLOWING SUPEROVULATION, WHERE SEASON WAS FOUND TO HAVE A DEFINITE EFFECT ON THE MEAN NUMBER OF CL'S PRODUCED, BEING HIGHER DURING THE EARLY BREEDING SEASON. HOWEVER, CURRENT RESULTS ARE IN AGREEMENT WITH THE FINDINGS OF RESEARCHERS IN OTHER GOAT BREEDS, WHERE SEASON DID NOT HAVE AN EFFECT ON THE OVULATION RATE (BARIL ET AL., 1989; BARIL & VALLET, 1991). THE OVULATION RATE RECORDED DURING THE NATURAL BREEDING SEASON IN THIS STUDY (17.5±6.3) WAS LOWER COMPARED TO THE MEAN OF 28.7±2.3 IN NUBIAN GOATS DURING THE BREEDING SEASON (SENN & RICHARDSON, 1991). THERE ARE CERTAIN ENVIRONMENTAL AND GENOTYPE TRAITS THAT COULD BE RESPONSIBLE FOR THIS DIFFERENCE. THIS OVULATION RATE OBTAINED WAS COMPARABLE TO THE 16.1 AND 15 OVULATIONS PER DOE OBSERVED IN ANGORA AND NUBIAN GOATS, RESPECTIVELY (ARMSTRONG ET AL., 1983; KIESSLING ET AL., 1986). THE OVULATION RATE RECORDED WAS HOWEVER HIGHER THAN THE 8.8 OVULATIONS PER DOE RECORDED IN JAMNAPARI GOATS (GOEL & AGRAWAL, 1990). THE OBSERVED DIFFERENCES ALSO

DEMONSTRATE THAT DIFFERENT BREEDS RESPOND DIFFERENTLY TO SUPEROVULATION TREATMENT AND THE DIFFERENCES MAY ALSO BE ASCRIBED TO DIFFERENT DURATIONS OF PROGESTAGEN TREATMENT.

THE NUMBER OF OVULATIONS WAS HIGHEST (28.7±2.3) WHERE THE SYNCHRONISATION PERIOD LASTED FOR 9 DAYS AS OPPOSED TO THE PRESENT STUDY AND IN MURCIANO-GRANADINA GOATS, WHERE THE OESTROUS SYNCHRONISATION TREATMENT LASTED FOR 16 TO 17 DAYS. LONG-TERM PROGESTAGEN PRIMING HAS BEEN ASSOCIATED WITH SUB-LUTEAL PROGESTERONE CONCENTRATIONS TOWARDS THE END OF THE SYNCHRONISATION PERIOD. THESE LOW PROGESTERONE CONCENTRATIONS FAIL TO SUPPRESS THE LH SURGE AND PULSES, HENCE PROMOTING THE DEVELOPMENT OF A DOMINANT FOLLICLE WHICH WILL SUPPRESS THE OVARIAN RESPONSE TO SUPEROVULATION AND THE OVULATION RATE. IN SHEEP, IT HAS BEEN OBSERVED THAT THE OVULATORY FOLLICLE EMERGES 3 DAYS PRIOR TO PROGESTAGEN REMOVAL IN A LONG-TERM (12D) PROGESTERONE PRIMING, WHILE IN A SHORT-TERM (6D) PROGESTAGEN TREATMENT THE OVULATORY FOLLICLE EMERGED APPROXIMATELY AT INTRAVAGINAL SPONGE REMOVAL (VINOLES ET AL., 2002).

THE NUMBER OF STRUCTURES (UNFERTILISED OVA AND EMBRYOS), EMBRYOS, DEGENERATED AND TRANSFERABLE EMBRYOS WAS NOT AFFECTED BY SEASON. THEREFORE IN BOER GOAT DOES SUPEROVULATION CAN BE PERFORMED IN EITHER SEASON, WITHOUT THE FEAR OF A REDUCED RECOVERY RATE. THESE RESULTS HOWEVER CONTRADICT PREVIOUS FINDINGS IN OTHER GOAT BREEDS WHERE SEASON HAS BEEN SHOWN TO HAVE

A BIG EFFECT ON THE EMBRYO RECOVERY RATE. IT HAS ALSO BEEN DEMONSTRATED BY A HIGHER TOTAL NUMBER OF EMBRYOS RECOVERED DURING THE NATURAL BREEDING SEASON (SENN & RICHARDSON, 1991; GONZALEZ-BULNES ET AL., 2003A). A HIGHER NUMBER OF UNFERTILISED OVA WERE RECOVERED OUTSIDE THE NATURAL BREEDING SEASON – POSSIBLY INDICATING A DEFICIENCY IN FSH AND LH SECRETION FOR GROWTH AND MATURATION OF THE RECRUITED FOLLICLES. IN SHEEP, SIMILAR FINDINGS HAVE ALSO BEEN OBSERVED (MITCHELL ET AL., 2002).

THE POOR FERTILISATION RATE OBTAINED OUTSIDE THE BREEDING SEASON COULD ALSO BE ATTRIBUTED TO AN INFERIOR SEMEN QUALITY AND IMPEDED SPERM TRANSPORT. IN GOATS, POORER QUALITY SEMEN HAS BEEN RECORDED OUTSIDE THE NATURAL BREEDING SEASON (KARATZAS ET AL., 1997; WEBB ET AL., 2004). THE LOW FERTILISATION RATE MAY ALSO BE ASCRIBED TO POOR TIMING OF INSEMINATION, AS AI WAS PERFORMED AT THE SAME TIME PERIOD AS DURING THE NATURAL BREEDING SEASON. IN THE CURRENT STUDY IT WAS OBSERVED THAT THE BOER GOAT DOES TAKE A LONGER TIME FROM PROGESTAGEN WITHDRAWAL TO THE ONSET OF OESTRUS OUTSIDE THE BREEDING SEASON AND ALSO THE DURATION OF THE INDUCED OESTRUS PERIOD IS SHORTER OUTSIDE THE BREEDING SEASON – PROBABLY DUE TO LOWER HORMONAL LEVELS. THE OBSERVED INCREASE IN SERUM PROGESTERONE CONCENTRATION LEADING TO HIGH CONCENTRATIONS ON DAY 8 OF OESTROUS SYNCHRONISATION TREATMENT OUTSIDE THE BREEDING SEASON INDICATES DOES TO BE MORE RESPONSIVE TO EXOGENOUS PROGESTAGEN ADMINISTRATION. THE SHARP INCREASE IN PROGESTERONE CONCENTRATION OUTSIDE THE

BREEDING SEASON IS A COMMON PHENOMENON IN GOATS (RUBIANES & MENCHACA, 2003). THE HIGH LEVEL OF SERUM PROGESTERONE LEVELS BEFORE EMBRYO FLUSHING IN DOES TREATED OUTSIDE THE BREEDING SEASON COULD HAVE BEEN INDUCED BY MORE CL'S PRODUCED FOLLOWING SUPEROVULATION (SENTHIL KUMAR ET AL., 2003). IN THIS STUDY THE HIGH NUMBER OF CL'S WERE ONLY RECORDED ON THE RIGHT OVARY OUTSIDE THE BREEDING SEASON (THE REASON BEING UNCLEAR), WHILE THE TOTAL NUMBER OF OVULATIONS WERE SIMILAR IN BOTH SEASONS.

4.5. Conclusions

FROM THE PRESENT STUDY, IT IS EVIDENT THAT SEASON HAS AN EFFECT ON THE TIME TO ONSET AND THE DURATION OF THE INDUCED OESTROUS PERIOD FOLLOWING SUPEROVULATION USING EXOGENOUS FSH. THE TIME INTERVAL FROM CIDR REMOVAL TO ONSET OF OESTRUS WAS UNDERSTANDABLY SHORTER DURING THE NATURAL BREEDING SEASON AND LONGER OUTSIDE THE BREEDING SEASON. THE DURATION OF THE INDUCED OESTROUS PERIOD WAS ALSO LONGER (INDICATION OF HIGHER AND EXTENDED OESTROGEN LEVELS) DURING THE BREEDING SEASON. THESE FACTS NEED TO BE TAKEN INTO CONSIDERATION, ESPECIALLY WHEN FIXED-TIME AI IS PERFORMED AND THE TIME OF OVULATION ESTIMATED. ALTHOUGH, THE RIGHT OVARY PRODUCED MORE OVULATIONS OUTSIDE THE BREEDING SEASON, THE OVERALL OVULATION RATE PER DONOR DID NOT DIFFER DURING AND OUTSIDE THE BREEDING SEASON. THE NUMBER OF UNFERTILISED OVA OUTSIDE THE BREEDING SEASON WAS HIGHER (POSSIBLY DUE TO INFERIOR SEMEN QUALITY) HOWEVER, THIS DID NOT REFLECT ON THE NUMBER OF TRANSFERABLE EMBRYOS PRODUCED. THUS, A SUPEROVULATION REGIME IS WARRANTED

OUTSIDE THE NATURAL BREEDING SEASON IN THE BOER GOAT, AS THE OVULATION RATE, TOTAL NUMBER OF EMBRYOS AND NUMBER OF TRANSFERABLE EMBRYOS WERE FOUND TO BE SIMILAR IN AND OUTSIDE THE NATURAL BREEDING SEASON. THIS RECOMMENDATION HOLDS GREAT POTENTIAL FOR THE PRODUCTION OF GOAT EMBRYOS THROUGHOUT THE YEAR.

CHAPTER 5

5. effect of route of superovulatory gonadotrophin administration on embryo recovery and transfer following cryopreservation

5.1. Introduction

SUPEROVULATION IS THE MOST IMPORTANT STEP IN A MOET PROGRAMME, HOWEVER, ITS RESPONSE CONTINUES TO VARY BETWEEN AND WITHIN TREATMENTS, AS WELL AS BETWEEN ANIMALS IN THE SAME TREATMENT GROUP. THE MAJOR REASON FOR THIS VARIATION IN RESPONSE BEING ATTRIBUTED TO THE STATUS OF THE OVARY AT THE ONSET OF THE SUPEROVULATORY TREATMENT. THE HYPOTHESIS BEING THAT THE VARIATION IN SUPEROVULATORY RESPONSE COULD BE MINIMIZED IF THE SUPEROVULATORY TREATMENT COULD BE INITIATED WHEN THERE IS A HOMOGENOUS POOL OF SMALL FOLLICLES PRESENT ON THE OVARIES - HENCE AN IMPROVEMENT IN THE OVARIAN RESPONSE.

IN A PREVIOUS TRIAL (CHAPTER 3), A GNRH AGONIST WAS INCLUDED IN THE SUPEROVULATORY PROGRAMME AS A PRE-TREATMENT, ATTEMPTING TO CREATE AN IDEAL TIME TO START A SUPEROVULATION TREATMENT AND OBTAIN MAXIMUM RESULTS. THE GNRH AGONIST WAS ADDED TO THE PROTOCOL, AS IT IS KNOWN TO REDUCE THE LH CONCENTRATION WHEN GIVEN OVER A PERIOD OF TIME BEFORE THE ONSET OF THE SUPEROVULATION TREATMENT, HENCE PREVENTING THE ESTABLISHMENT OF A DOMINANT FOLLICLE. ALL THE FOLLICLES WHICH ARE ON THE OVARY AT THE TIME OF GNRH AGONIST TREATMENT WOULD DEPEND ON BLOOD FSH LEVEL AND FORM A

HOMOGENOUS POOL OF SMALL OVARIAN FOLLICLES (GONZALEZ-BULNES ET AL., 2004BC; LOPEZ-ALONSO ET AL., 2004).

THE RESULTS IN THE PREVIOUS TRIAL (CHAPTER 3) HOWEVER, INDICATED THAT A PRE-TREATMENT WITH A GNRH AGONIST REDUCES THE NUMBER AND VIABILITY OF THE EMBRYOS RECOVERED AND INCREASES THE NUMBER OF DEGENERATE EMBRYOS. SUPEROVULATION WITH FSH ALONE (NO GNRH AGONIST) GAVE BETTER EMBRYO QUALITY RESULTS. IN MOST TRIALS CONDUCTED IN GOATS THE SUPEROVULATORY TREATMENT (FSH) IS GENERALLY ADMINISTERED INTRAMUSCULARLY (I.M.). THERE IS VERY LITTLE INFORMATION ON GOATS, REGARDING THE ROUTE OF THE SUPEROVULATORY GONADOTROPHIN TREATMENT ADMINISTRATION (MAHMOOD ET AL., 1991; PENDLETON ET AL., 1992; SELVARAJU ET AL., 2003; GOEL & AGRAWAL, 2005). IN HUMANS IT HAS BEEN OBSERVED THAT THE ABSORPTION OF FSH IS HIGHER FOLLOWING AN I.M. INJECTION, COMPARED TO THE SUBCUTANEOUS (S.C.) ROUTE OF ADMINISTRATION. HOWEVER, THE S.C. ADMINISTRATION OF FSH HAS BEEN SHOWN TO LEAD TO A HIGHER NUMBER OF GROWING FOLLICLES, WHEN COMPARED TO THE I.M. ROUTE OF ADMINISTRATION. IN GOATS, THERE IS LITTLE INFORMATION ON THE RESPONSE TO SUPEROVULATION FOLLOWING A S.C. ADMINISTRATION OF SUPEROVULATORY TREATMENT, ALTHOUGH IT IS GENERALLY ACCEPTED THAT THE UPTAKE OF GONADOTROPHIN STIMULATION IS SLOWER FOLLOWING S.C. ADMINISTRATION. THUS THE ROUTE OF GONADOTROPHIN ADMINISTRATION COULD BE CRUCIAL IN TIMING OF THE SUPEROVULATORY TREATMENT, POSITIONING OF THE TIME OF

OVULATION AND DETERMINING ITS EFFICIENCY (DOBBS ET AL., 1994; VOORTMAN ET AL., 2000). CRYOPRESERVATION IS ANOTHER IMPORTANT TECHNIQUE IN THE WHOLE MOET PROGRAMME WHICH COULD ASSIST IN PERFECTING THE EMBRYO PRODUCTION AND STORAGE CHAIN, AS WELL AS HELPING IN THE IMPROVEMENT OF THE TRANSPORTATION OF GENETIC MATERIAL ACROSS THE GLOBE. GOAT EMBRYOS HAVE GENERALLY BEEN CRYOPRESERVED USING THE CONVENTIONAL SLOW FREEZING METHOD. DIFFERENT CRYOPROTECTANTS INCLUDING GLYCEROL, ETHYLENE GLYCOL AND DMSO OR A COMBINATION OF EITHER OF THE TWO CRYOPROTECTANTS HAVE GENERALLY BEEN USED FOR THIS SLOW FREEZING OF GOAT EMBRYOS. ETHYLENE GLYCOL AS A CRYOPROTECTANT HAS BEEN PREFERRED FOR SLOW FREEZING OF GOAT EMBRYOS, DUE TO THE HIGHER EMBRYO SURVIVAL RATE OBTAINED FOLLOWING ITS UTILISATION (COMPARED TO GLYCEROL OR DMSO). HOWEVER, THE EMBRYO SURVIVAL RATE FOLLOWING THE CONVENTIONAL SLOW FREEZING TECHNIQUE HAS GENERALLY BEEN UNSATISFACTORY (BARIL ET AL., 1989; LI ET AL., 1990; LE GAL ET AL., 1993; GUIGNOT ET AL., 2006). CURRENTLY VITRIFICATION OF GOAT EMBRYOS HAS BEEN ATTEMPTED WITH SUCCESS AND BETTER RESULTS HAVE BEEN OBTAINED WITH GOAT EMBRYOS VITRIFIED WITH A COMBINATION OF ETHYLENE GLYCOL AND DMSO (TRALDI ET AL., 1999, 2000; BRANCA ET AL., 2000; EL-GAYAR & HOLTZ, 2001). WHEN COMPARING THESE TWO METHODS OF EMBRYO CRYOPRESERVATION THERE HAS BEEN CONTRADICTORY RESULTS REGARDING THE SURVIVAL RATE FOLLOWING EMBRYO TRANSFER, ALTHOUGH ONLY A FEW TRIALS HAVE BEEN CONDUCTED IN GOATS COMPARING THESE METHODS

OF CRYOPRESERVATION. THE VITRIFICATION METHOD HAS BEEN SHOWN TO LEAD TO A HIGHER KIDDING RATE AND HIGHER EMBRYO SURVIVAL RATE FOLLOWING EMBRYO TRANSFER. HOWEVER, CONVENTIONAL SLOW FREEZING AND VITRIFICATION METHODS OF CRYOPRESERVATION FOLLOWING EMBRYO TRANSFER HAVE ALSO BEEN SHOWN TO LEAD TO SIMILAR EMBRYO SURVIVAL RATES (EL-GAYAR & HOLTZ, 2001; GUIGNOT ET AL., 2006).

THE AIM OF THIS STUDY WAS TO EVALUATE AND REFINE THE FSH SUPEROVULATORY TREATMENT BY COMPARING TWO ROUTES OF GONADOTROPHIN (FSH) ADMINISTRATION FOR A BETTER OVULATORY RESPONSE, WHILE ALSO EVALUATING THE SURVIVAL RATE OF GOAT EMBRYOS THUS PRODUCED FOLLOWING THE CONVENTIONAL SLOW FREEZING OR VITRIFICATION METHOD FOR IN VIVO PRODUCED EMBRYOS.

5.2. Materials and methods

5.2.1. Study location and experimental animals

THE STUDY WAS CONDUCTED IN AUTUMN, NATURAL BREEDING SEASON IN 2006, AT THE EXPERIMENTAL FARM OF THE UNIVERSITY OF THE FREE STATE. THE FARM IS SITUATED APPROXIMATELY 20 KM SOUTH OF BLOEMFONTEIN, SOUTH AFRICA. THE VEGETATION IS CHARACTERISED AS SEMI-ARID, WITH A TEMPERATE CLIMATE. THE TEMPERATURE, RAINFALL AND ALTITUDE ARE SIMILAR TO THAT EXPRESSED IN CHAPTER 3 (PARAGRAPH 3.2.1). FORTY-FOUR MULTIPAROUS MATURE BOER GOAT DOES (PLATE 5.1) WITH A BODY WEIGHT RANGING BETWEEN 36.1 KG AND 64.1 KG WERE USED IN THIS TRIAL - EITHER AS DONORS (N=17) OR RECIPIENTS (N=27). ALL DOES WERE ALLOWED TO GRAZE ON NATURAL PASTURES DURING THE DAY, INITIALLY

IMPROVED BY HAND SOWING OF SMUTS FINGER GRASS (*DIGITARIA ERIANTHA*). THE PASTURE COMPOSITION ALSO INCLUDED RED GRASS (*THEMEDA TRIANDRA*) AS A DOMINANT SPECIE, WEEPING GRASS (*ERAGROSTIS LEHMANNIANA*), DROP SEED GRASS (*SPOROBOLAS FIMBRIATUS*) AND PRICKLE GRASS (*ARISTIDA CONGESTA*). AT NIGHT ALL THE ANIMALS WERE SUPPLEMENTED WITH MILLED LUCERNE WHILE KRAALED IN COVERED PENS. FOR THE WHOLE PERIOD OF THE EXPERIMENT WATER AND A MINERAL LICK WERE AVAILABLE *AD LIBITUM*.

Plate 5. 2 Mature multiparous Boer goat does used in the trial

5.2.2. Treatments

5.2.2.1. Treatment of the donor animals

THE OESTROUS CYCLES OF 17 DONORS WERE SYNCHRONISED USING INTRAVAGINAL PROGESTAGEN DEVICES (CIDR’S) AS SET OUT IN CHAPTER 3 (PARAGRAPH 3.2.3.1), AND THEN DIVIDED INTO TWO GROUPS. NINE OF THE DOES RECEIVED THE SUPEROVULATORY PFSH TREATMENT

INTRAMUSCULARLY (I.M.), WHILE 8 DOES (FORMING THE SECOND GROUP) RECEIVED A SUPEROVULATORY PFSH TREATMENT SUBCUTANEOUSLY (S.C.). THE SUPEROVULATION DOSE, TIME OF TREATMENT AND GONADOTROPHIN USED WERE SIMILAR TO THAT SET OUT IN PARAGRAPH 3.2.3.1. BLOOD SAMPLING PROCEDURES AND SERUM PROGESTERONE CONCENTRATION DETERMINATION WERE PERFORMED AS DESCRIBED IN CHAPTER 3 (PARAGRAPHS 3.2.3.4 AND 3.2.3.4.2)

ALL DOES WERE CERVICALLY INSEMINATED (PLATE 5.2) WITH 0.1ML FRESH UNDILUTED SEMEN (APPROXIMATELY 350 X 10^6 SPERM/INSEMINATION) PERFORMED AT A FIXED TIME (36H AND 48H) FOLLOWING CIDR WITHDRAWAL. SEMEN WAS DEPOSITED AT THE MOUTH OF THE CERVIX, LOCATED WITH THE AID OF A SPECULUM. THE SEMEN USED FOR AI WAS COLLECTED IN A SIMILAR MANNER AS THAT DESCRIBED IN PARAGRAPH 3.3.3.3. THE SEMEN COLLECTED WAS MICROSCOPICALLY EVALUATED FOR PROGRESSIVE MOTILITY AND ONLY SEMEN SAMPLES WITH A MOTILITY SCORE OF 3+ (OUT OF A SCORE OF 5) WERE UTILISED FOR AI. OESTROUS DETECTION, SEMEN COLLECTION, AI, EMBRYO COLLECTION AND EVALUATION WERE PERFORMED THE SAME WAY AS DESCRIBED IN PARAGRAPH 3.2.3.2 AND 3.2.3.4.

Plate 5. 3 Cervical AI of donor does with fresh semen

5.2.2.2. Embryo cryopreservation

FOLLOWING EMBRYO COLLECTION AND GRADING, THE EMBRYOS WERE PLACED IN EMCARE HOLDING MEDIA (ICPBIO LTD, AUCKLAND, NEW ZEALAND), UNTIL THE INITIATION OF THE CRYOPRESERVATION PROCESS AND EMBRYO TRANSFER. EMBRYOS SELECTED FOR CRYOPRESERVATION WERE ALL AT THE BLASTOCYST STAGE (DAY 7 FOLLOWING AI). THE EMBRYOS SELECTED FOR CRYOPRESERVATION WERE EITHER CRYOPRESERVED VIA THE CONVENTIONAL SLOW FREEZING OR THE VITRIFICATION METHOD (LEWIS, 1992; OBERSTEIN ET AL., 2001).

ETHYLENE GLYCOL WAS USED AS THE CRYOPROTECTANT FOR THE CONVENTIONAL SLOW FREEZING METHOD OF EMBRYOS (LEWIS, 1992; DUPRAS ET AL., 2007). INITIALLY THE EMBRYOS WERE WASHED IN EMCARE HOLDING MEDIA AND PLACED INTO A 1.5 M EMCARE ETHYLENE GLYCOL (ICPBIO LTD, AUCKLAND, NEW ZEALAND) SOLUTION FOR A PERIOD OF 5 MIN AT ROOM TEMPERATURE TO EQUILIBRATE. THEREAFTER, THE EMBRYOS WERE

LOADED IN GROUPS OF 2 OR 4 INTO 0.25 ML PLASTIC STRAWS (IMV, L'AIGLE, FRANCE). THE STRAWS FOR THE LOADING OF THE EMBRYOS WERE FIRST FILLED WITH A COLUMN OF CRYOPROTECTANT MEDIA FOLLOWED BY AIR BUBBLES. THE EMBRYOS WERE INSERTED, THEN FOLLOWED BY ANOTHER SET OF AIR BUBBLES AND LASTLY A COLUMN OF MEDIA. ALL STRAWS WERE SEALED WITH A COLOUR WAX AND PLACED IN A CONTROLLED FREEZER (FREEZE CONTROL, MODEL CL5500; BIOGENICS, NAPA, CA) AT -6 °C FOR AT LEAST 1 MIN TO ALLOW EACH STRAW TO EQUILIBRATE TO THE TEMPERATURE. STRAWS WERE THEN SEEDED AND LEFT IN THE CONTROLLED FREEZER FOR A PERIOD OF 5 MIN TO ALLOW THE ICE CRYSTAL INDUCED BY SEEDING TO DISTRIBUTE THROUGHOUT THE ENTIRE STRAW. THE TEMPERATURE SETTING OF THE FREEZER WAS THEN DECREASED TO -35 °C, AT A COOLING RATE OF 0.5 °C/MIN AND THE EQUILIBRATION TIME SET AT -35 ºC FOR 10 MIN. FROZEN STRAWS WERE THEN PLUNGED INTO LIQUID NITROGEN (LN_2) AND STORED (-196 °C) FOR AT LEAST 2H BEFORE THAWING AND TRANSFERRING THE EMBRYOS.

EMBRYOS FOR CRYOPRESERVATION USING THE VITRIFICATION METHOD ON THE OTHER HAND WERE REMOVED FROM THE EMCARE HOLDING MEDIA AND EQUILIBRATED IN 4 STEPS AT ROOM TEMPERATURE (OBERSTEIN ET AL., 2001). FIRSTLY, THE EMBRYOS WERE PLACED IN A V_1 MEDIUM (HEPES + SYNTHETIC OVIDUCTAL FLUID [H-SOF]; SIGMA, ST LOUIS, MO, USA) AND EQUILIBRATED FOR 5 MIN. THEN THE EMBRYOS WERE PLACED INTO THE SAME MEDIUM IN ANOTHER PETRI DISH (V_2) FOR 5 TO10 SEC. THE EMBRYOS WERE THEN TRANSFERRED INTO A V_3 DISH (H-SOF + 7.5% ETHYLENE GLYCOL (EG) + 7.5% DIMETHYL SULFOXIDE [DMSO]; SIGMA, ST LOUIS, MO, USA) FOR 2.5 SEC AND LASTLY, THE EMBRYOS WERE

PLACED IN A V_4 SOLUTION (H-SOF + 16.5% EG + 16.5% DMSO +10 MG/ML FICOLL; FISHER SCIENTIFIC, OTTAWA, ONTARIO, CANADA + 0.5 M SUCROSE) FOR 8 SEC. THE 20 EMBRYOS IN A COLUMN OF 3μL V_4 SOLUTION WERE ASPIRATED WITH A MODIFIED PASTEUR PIPETTE (PASTEUR PIPETTE SLIGHTLY MELTED OVER FLAME AND HAND PULLED TO ACHIEVE A DIAMETER HALF OF ITS ORIGINAL DIAMETER, THEN COOLED IN AIR FOR A FEW SECONDS PRIOR TO CUTTING AT THE NARROWEST POINT) AND RELEASED AS A DROP ONTO THE CRYOTIPS (IRVINE SCIENTIFIC) TO FORM A GLASS BEAD IN WHICH THE EMBRYOS WERE TRANSFERRED INTO HALF-SEALED AND PRE-COOLED STRAWS. THESE STRAWS WERE SEALED AND TRANSFERRED INTO LIQUID NITROGEN FOR AT LEAST 2 H BEFORE THAWING.

5.2.2.3. Embryo thawing

CRYOPRESERVED EMBRYOS USING THE CONVENTIONAL SLOW FREEZING METHOD, WERE THAWED USING A SUCROSE SOLUTION IN THREE STAGES (LEWIS, 1992). THREE PETRI DISHES WERE PREPARED FOR THAWING, THE FIRST CONTAINING 0.75 M EMCARE ETHYLENE GLYCOL AND 0.5 M EMCARE SUCROSE, WHILE THE SECOND DISH CONTAINED 0.5 M EMCARE SUCROSE AND EMCARE HOLDING MEDIUM AND THE LAST PETRI DISH ONLY EMCARE HOLDING MEDIUM. A SINGLE STRAW WAS TAKEN FROM THE LIQUID NITROGEN FLASK, HELD IN THE AIR FOR 7 SEC AND THEN PLUNGED INTO A WATER BATH AT 35 ºC FOR 30 SEC. THEREAFTER THE STRAW WAS DRIED TO REMOVE ALL WATER AND HELD IN A HORIZONTAL POSITION AND THE SEAL WAXED END CUT.

THE STRAW WAS THEN CONNECTED TO A 1 ML SYRINGE (AB TECHNOLOGY, BIONICHE, USA) WITH

AN ADAPTOR, AND HELD AT AN ANGLE TO CUT THE PLUNGER END OF THE STRAW OVER A DRY PETRI DISH. THE CONTENTS WERE EMPTIED INTO THE EMPTY PETRI DISH AND THE STRAW FLUSHED WITH EMCARE HOLDING MEDIUM TO MAKE SURE THAT ALL EMBRYOS WERE RECOVERED. THE EMBRYOS WERE LOCATED UNDER A STEREO MICROSCOPE AND THEN PLACED INTO THE FIRST PETRI DISH FOR A PERIOD OF 5 MIN AND THEN INTO THE SECOND DISH FOR ANOTHER 5 MIN AND FINALLY INTO THE LAST PETRI DISH FOR A PERIOD OF 5 MIN AT ROOM TEMPERATURE. TWO EMBRYOS WERE LOADED AT A TIME INTO A TOM CAT CATHETER (AB TECHNOLOGY, BIONICHE, USA) CONNECTED TO A 1 ML SYRINGE FOR TRANSFER. FOR VITRIFIED EMBRYOS, THE THAWING PROCESS WAS PERFORMED BY INTRODUCING THE CRYOTIP IN A WATER-BATH AT 37°C. EMBRYOS WERE THEN EXPELLED INTO A THAWING MEDIUM (H-SOF + 1 M SUCROSE) FOR 2 MIN. AFTER BEING KEPT IN THE FRESH SOLUTION FOR 2 MIN, THE EMBRYOS WERE MOVED AND STORED IN A HOLDING MEDIUM (H-SOF + AMINO ACIDS), UNTIL TRANSFERRED TO RECIPIENT DOES.

5.2.2.4. Treatment of recipient does and embryo transfer

THE OESTROUS CYCLES OF ALL 27 RECIPIENT BOER GOAT DOES WERE SYNCHRONISED USING A SIMILAR PROCEDURE FOR THE DONORS AS SET OUT IN CHAPTER 3 (PARAGRAPH 3.2.3.1). HOWEVER, THE INTRAVAGINAL PROGESTAGEN DEVICES WERE REMOVED 12H PRIOR TO THAT OF THE DONOR DOES. AT PROGESTAGEN WITHDRAWAL EACH RECIPIENT DOE WAS INJECTED I.M. WITH 300IU ECG (FOSTIM; UPJOHN) AND OESTROUS DETECTION WITH THE AID OF VASECTOMISED BUCKS, WAS INITIATED 12H FOLLOWING DEVICE WITHDRAWAL. THE RECIPIENT DOES WERE ALLOCATED TO THREE GROUPS (N=9 PER GROUP) DEPENDING ON THE TYPE OF EMBRYOS

TRANSFERRED. THE FIRST GROUP RECEIVED FRESH EMBRYOS (NO CRYOPRESERVATION), THE SECOND GROUP RECEIVED THE CONVENTIONAL SLOW FROZEN-THAWED EMBRYOS, WHILE THE LAST GROUP RECEIVED VITRIFIED-THAWED EMBRYOS.
BEFORE EMBRYO TRANSFER, THE OVARIES OF THE RECIPIENT DOES WERE INSPECTED BY LAPAROSCOPY FOR THE PRESENCE OR ABSENCE OF CL'S (DOES WITH NO CL'S DID NOT RECEIVE EMBRYOS).
LAPAROSCOPIC EMBRYO TRANSFER WAS PERFORMED FOLLOWING SEDATION OF DOES WITH 0.2 ML/DOE ROMPUN (XYLOZINE, BAYER, AUSTRALIA). BRIEFLY, THE ABDOMEN OF A RECIPIENT WAS INFLATED WITH AIR (CARBON DIOXIDE), TWO INCISIONS MADE, ONE FOR THE LAPAROSCOPE AND ANOTHER FOR THE TROCAR (ACCOMMODATING THE FORCEPS) INSERTION. THE WOUND FOR THE TROCAR WAS EXTENDED USING A SURGICAL BLADE AND THE TIP OF THE UTERINE HORN EXTERIORISED WITH THE FORCEPS. TWO BLASTOCYST EMBRYOS WERE TRANSFERRED INTO THE UTERINE HORN OF EACH RECIPIENT, IPSILATERAL TO THE FUNCTIONAL CL WITH THE AID OF A TOM CAT CATHETER.

5.2.3. Pregnancy diagnosis and kidding performance

FORTY DAYS FOLLOWING EMBRYO TRANSFER, ALL THE RECIPIENTS WERE TESTED FOR PREGNANCY WITH THE AID OF A SONOGRAPHIC SCANNER (ALOKA, SSD 500, TOKYO, JAPAN), USING A TRANSRECTAL 5 MHZ LINEAR ARRAY PROBE. AT PARTURITION, THE GESTATION LENGTH, PREGNANCY RATE, EMBRYO SURVIVAL RATE AND SEX OF KIDS WERE RECORDED.

5.2.4. Statistical analyses

DATA REGARDING THE ONSET AND DURATION OF THE INDUCED OESTROUS PERIOD, TOTAL NUMBER OF CL'S, TOTAL FLUSHED STRUCTURES, UNFERTILISED

OVA AND EMBRYOS COLLECTED, NUMBER OF TRANSFERABLE EMBRYOS, GESTATION LENGTH AND LITTER SIZE WERE ANALYSED USING THE ANOVA PROCEDURES (SAS, 2003). DATA CONCERNING THE OESTROUS RESPONSE, EMBRYO RECOVERY RATES, FERTILISATION RATES, PREGNANCY RATE, RECIPIENTS KIDDING AND EMBRYO SURVIVAL RATES WERE ANALYSED AND COMPARED WITH THE AID OF THE CHI-SQUARE TEST (SAS, 2003).

5.3. Results

5.3.1. Oestrous response of the donors

THE OCCURRENCE OF OESTRUS, TIME FROM CIDR WITHDRAWAL TO THE ONSET OF OESTRUS AND INDUCED OESTROUS PERIOD DURATION ARE SET OUT IN TABLE 5.1. ALL DONOR DOES SHOWED OVERT SIGNS OF OESTRUS FOLLOWING DEVICE REMOVAL. THE OVERALL TIME FROM INTRAVAGINAL CIDR WITHDRAWAL TO THE ONSET OF OESTRUS WAS 24.5±4.6H, WITH NO DIFFERENCE BEING RECORDED BETWEEN THE ROUTES OF FSH ADMINISTRATION. SIMILARLY, THE DURATION OF THE INDUCED OESTROUS PERIOD DID NOT DIFFER BETWEEN THE GROUPS, WITH THE OVERALL TIME BEING 24.0±5.1H.

Table 5. 1 Mean (±SD) oestrous response in Boer goats synchronised and superovulated intramuscularly or subcutaneously with exogenous FSH

Treatment	**n**	**Oestrous response (%)**	**Time to oestrus (h)**	**Duration of oestrus (h)**
Intramuscular	9	100	24.8±4.8	24.0±5.7
Subcutaneous	8	100	24.0±4.3	24.0±2.7

No significant differences

5.3.2. Response to superovulation treatment and serum progesterone concentration

DURING LAPAROSCOPIC INSPECTION OF THE OVARIES PRIOR TO EMBRYO FLUSHING , ONLY 2 (12%) OF THE DOES (ONE FROM EACH GROUP) HAD ABNORMAL CL'S (AVASCULAR AND PALE CL'S) WITHOUT ANY ACTIVE CL (HIGHLY VASCULAR AND RED) BEING OBSERVED ON THE OVARIES. THESE DOES WERE NOT FLUSHED. FROM THE FLUSHED DOES, THE OVERALL OVULATION RATE RECORDED PER DONOR WAS 18.9±5.3. DOES RECEIVING THE SUPEROVULATION TREATMENT (FSH) S.C. TENDED TO RECORD A HIGHER OVULATION RATE. HOWEVER, THE DIFFERENCES BETWEEN THE TWO GROUPS WERE NOT SIGNIFICANTLY DIFFERENT AND THE VARIATION WITH RESPECT TO OVULATION RATE BETWEEN ANIMALS IN THE I.M. GROUP WAS GENERALLY HIGH.

THE OVERALL MEAN OVA (TOTAL STRUCTURES COLLECTED) WAS 18.9±5.2 WITHOUT ANY SIGNIFICANT DIFFERENCES BETWEEN THE TWO GROUPS (TABLE 5.2). SIMILARLY, THE MEAN TOTAL NUMBER OF EMBRYOS PER DONOR AND FERTILISATION RATE WERE NOT DIFFERENT BETWEEN TREATMENT GROUPS. HOWEVER, THE VARIATION BETWEEN ANIMALS IN THE INTRAMUSCULAR (I.M.) TREATED GROUP REGARDING THE NUMBER OF EMBRYOS RECOVERED AND FERTILISATION RATE WAS HIGHER. THE COEFFICIENT OF VARIATION FOR BOTH GROUPS REGARDING THE TOTAL NUMBER OF EMBRYOS RETRIEVED PER DONOR AND FERTILISATION RATE WAS 51% AND 57%, RESPECTIVELY, WITH THE OVERALL MEAN TOTAL NUMBER OF UNFERTILISED OVA BEING 1.9±3.5. THE TOTAL MEAN NUMBER OF UNFERTILISED OVA IN THE I.M. GROUP (3.3±4.8) WAS SIGNIFICANTLY ($P<0.05$) HIGHER, COMPARED TO THE SUBCUTANEOUS (S.C.) GROUP (0.3±0.8). THE MEAN NUMBER OF DEGENERATE EMBRYOS PER DONOR IN

THE DOES ADMINISTERED THE FSH S.C. (5.9±4.5) WAS SIGNIFICANTLY ($P<0.05$) HIGHER, COMPARED TO THE I.M. GROUP (2.6±2.3). THE OVERALL MEAN NUMBER OF DEGENERATE EMBRYOS WAS 4.1±3.5. THE MEAN TOTAL NUMBER OF TRANSFERABLE EMBRYOS WAS NOT AFFECTED BY THE ROUTE OF SUPEROVULATORY ADMINISTRATION, WITH THE OVERALL MEAN BEING 12.9±7.9. HOWEVER THE VARIATION FOR DOES IN THE I.M. GROUP WAS VERY HIGH - AS REFLECTED BY THE STANDARD DEVIATION. THE COEFFICIENT OF VARIANCE WAS 62%.

Table 5. 2 The effect of route of gonadotrophin administration on the mean (±SD) response to superovulation treatment in Boer goat does

Parameters	**Overall**	**Intramuscular**	**Subcutaneous**
No. of does flushed	15	8	7
Ovulation rate (total CL's/donor)	18.9±5.3	17.5±6.3[a]	20.6±3.8[a]
Number of structures recovered per doe flushed (unfertilised ova & embryos)	18.9±5.2	18.0±5.8[a]	19.9±4.3[a]
Number of embryos recovered /donor	17.0±7.4	14.8±9.1[a]	19.6±4.5[a]
Fertilisation rate (%)	86.8±27.5	87.6±22.5[a]	98.4±4.2[a]
Number of unfertilised ova/donor	1.9±3.5	3.3±4.8[a]	0.3±0.8[b]
Number of degenerate embryos/donor	4.1±3.5	2.6±2.3[a]	5.9±4.5[b]
Number of transferable embryos/donor	12.9±7.5	12.1±9.3[a]	13.7±4.8[a]

[ab] Values within the same row with different superscripts differ significantly ($P<0.05$)

THE ROUTE OF GONADOTROPHIN ADMINISTRATION HAD NO EFFECT ON THE SERUM PROGESTERONE CONCENTRATIONS. THE MEAN SERUM PROGESTERONE CONCENTRATION WAS SIMILAR THROUGHOUT THE SYNCHRONISATION PERIOD AND INCREASED ON DAY 4 FOLLOWING OESTRUS, BEING

HIGHEST ON THE DAY OF FLUSHING IN BOTH GROUPS.

5.3.3. Ovarian response of the recipients

ONLY ONE DOE FROM EACH GROUP (11.1%) DID NOT SHOW ANY SIGNS OF OVERT OESTRUS FOLLOWING OESTROUS SYNCHRONISATION. THE OVERALL OESTROUS RESPONSE BEING 88.9%. DURING LAPAROSCOPIC INSPECTION (BEFORE EMBRYO TRANSFER), ALL THE DOES WHICH EXHIBITED OVERT SIGNS OF OESTRUS HAD ACTIVE CL'S WITH NUMBERS RANGING BETWEEN 1 AND 3 PER DOE.

5.3.4. Pregnancy diagnosis and kidding performance

ONE DOE FROM THE FRESH EMBRYO TRANSFERRED GROUP BECAME ILL BEFORE PREGNANCY DIAGNOSIS, AND THIS ANIMAL WAS REMOVED FROM THE TRIAL. THE PREGNANCY AND EMBRYO SURVIVAL RATES ARE SET OUT IN TABLE 5.3. DURING PREGNANCY DIAGNOSES (40 DAYS FOLLOWING EMBRYO TRANSFER), ONLY ONE DOE WAS DIAGNOSED AS BEING NOT PREGNANT, IN THE DOES RECEIVING FRESH EMBRYOS. THUS 85.7% DOES (N=7) WERE PREGNANT FOLLOWING THE TRANSFER OF FRESH EMBRYOS. THIS TENDED TO BE BETTER THAN IN THE DOES RECEIVING FROZEN AND VITRIFIED EMBRYOS, (N=4; 50.0% AND N=3; 37.5% DOES PREGNANT, RESPECTIVELY). THESE DIFFERENCES WERE HOWEVER NOT STATISTICALLY DIFFERENT. HOWEVER, NOT ALL DOES CONFIRMED PREGNANT 40 DAYS AFTER EMBRYO TRANSFER, KIDDED FOLLOWING FRESH AND SLOW FROZEN-THAWED EMBRYO TRANSFER - THE NUMBER OF RECIPIENTS KIDDING DECLINING TO N= 4 (57.0%) AND N=2 (25.0%) FOR FRESH AND SLOW FROZEN TRANSFER GROUPS, RESPECTIVELY. ALL RECIPIENTS (N=3) CONFIRMED PREGNANT (37.5%) IN THE VITRIFIED GROUP HOWEVER, KIDDED. THUS EMBRYO SURVIVAL RATES

OF 35.7%, 25.0% AND 31.3% WERE RECORDED FOLLOWING FRESH, SLOW FROZEN AND VITRIFIED EMBRYO TRANSFER, RESPECTIVELY. THE SURVIVAL RATE WAS NOT AFFECTED BY THE NUMBER OF CL'S PRESENT AT THE TIME OF TRANSFER OF THE EMBRYOS. THE OVERALL GESTATION LENGTH RECORDED WAS 146.3±3.0D WITH AN OVERALL LITTER SIZE OF 1.7±0.5. LITTER SIZE AS SUCH HAD NO EFFECT ON THE GESTATION LENGTH. THERE WAS ALSO A TENDENCY OF MORE FEMALE KIDS (10) BEING BORN THAN MALE KIDS (5) (RATIO1:2, MALE: FEMALE), ALTHOUGH THIS CAN BE SEEN AS A COINCIDENTAL OBSERVATION.

Table 5. 3 Pregnancy and embryo survival rate following the transfer of cryopreserved Boer goat embryos

Parameters	**Embryos transferred**		
	Fresh	**Slow frozen**	**Vitrified**
Number of embryos transferred	14	16	16
Number of recipients	7	8	8
Recipients pregnant (%)	6 (85.7)	4 (50.0)	3 (37.5)
Mean gestation length (d)	147.5±3.3	145.0±2.8	146.3±1.6
Recipients kidding (%)	4 (57.0)	2 (25.0)	3 (37.5)
Mean litter size	1.3±0.5	2.0±0	1.7±0.6
Embryo survival rate (%)	5 (35.7)	4 (25.0)	5 (31.3)

No significant differences

5.4. Discussions

5.4.1. Oestrous response of donor does

ALL DONOR DOES SHOWED SIGNS OF OESTRUS FOLLOWING A 17-DAY SYNCHRONISATION TREATMENT WITH INTRAVAGINAL CIDR'S. THE

RESULTS THUS DEMONSTRATED CIDR'S TO BE EFFICIENT IN SYNCHRONISING OESTRUS OF BOER GOATS DURING THE BREEDING SEASON – AS SHOULD BE EXPECTED. SIMILAR RESULTS OF A 100% RESPONSE TO SYNCHRONISATION HAS ALSO BEEN REPORTED IN OTHER GOAT BREEDS, PARTICULARLY WHEN TREATMENTS WERE ADMINISTERED DURING THE NATURAL BREEDING SEASON (SELVARAJU ET AL., 2003; ESPINOSA-MARQUEZ ET AL., 2004). THIS OESTROUS RESPONSE WAS HIGHER WHEN COMPARED TO 90% AND 88% OBSERVED IN JAKHRANA AND ALPINE GOATS WHICH HAVE BEEN REPORTED TO DEMONSTRATE A LOWER OESTROUS RESPONSE FOLLOWING SUPEROVULATION, BUT HERE NUTRITION, AGE AND PARITY COULD ALSO PLAY A ROLE (PENDLETON ET AL., 1992; GOEL & AGRAWAL, 2005).

THE MEAN TIME TO THE ONSET OF THE INDUCED OESTRUS WAS SIMILAR BETWEEN THE I.M. AND S.C. TREATMENT GROUPS WITH AN OVERALL MEAN OF 24.5±4.6H. THIS IS SIMILAR TO 24H PREVIOUSLY REPORTED IN GOATS SYNCHRONISED WITH NORGESTOMET EAR IMPLANTS (SELGRATH ET AL., 1990). HOWEVER, THE OVERALL MEAN TIME FROM CIDR REMOVAL TO THE ONSET OF OESTRUS RECORDED WAS SHORTER WHEN COMPARED TO THAT OF 32.0±3.5H AND 36.0±8.2H IN JAKHRANA AND ALPINE GOATS – DEMONSTRATING SOME VARIATION (PENDLETON ET AL., 1992; GOEL & AGRAWAL, 2005). A LONGER TIME TO THE ONSET OF OESTRUS IS USUALLY OBSERVED IN GOATS SUPEROVULATED WITH ECG, DUE TO ITS LONG HALF-LIFE, WHEN COMPARED TO PFSH (SELGRATH ET AL., 1990; PENDLETON ET AL., 1992). THE MEAN DURATION OF THE INDUCED OESTROUS PERIOD WAS ALSO SIMILAR BETWEEN TREATMENT GROUPS, WITH THE OVERALL MEAN OF 24.0±5.1H BEING COMPARABLE TO A

DURATION OF 22.7±1.3H RECORDED IN CERTAIN GOAT BREEDS. THE OVERALL DURATION OF THE INDUCED OESTROUS PERIOD WAS HOWEVER AGAIN SHORTER WHEN COMPARED TO THE 33.6±7.1H AND 38.0±17.0H REPORTED IN OTHER GOAT BREEDS AND BOER GOATS FOLLOWING SUPEROVULATION (GREYLING ET AL., 2002; GOEL & AGRAWAL, 2005). WITH A LONGER FSH STIMULATION AND SUBSEQUENT OESTROGEN PRODUCTION, A LONGER OESTROUS PERIOD COULD BE EXPECTED. GENERALLY, DURING THIS TRIAL THE BOER GOAT DOES SEEMED TO DEMONSTRATE A HIGH OESTROUS RESPONSE, A SHORTER TIME INTERVAL FROM CIDR REMOVAL TO THE ONSET OF OESTROUS, AS WELL AS EXHIBITING A SHORTER DURATION OF THE INDUCED OESTROUS PERIOD, IRRESPECTIVE OF THE ROUTE OF GONADOTROPHIN TREATMENT USED. THESE CHARACTERISTICS MAY BE ASCRIBED TO THEIR HIGHER SENSITIVITY TO EXOGENOUS HORMONES AND HIGH FERTILITY, ESPECIALLY DURING THE NATURAL BREEDING CYCLE WHEN THE OVARIES ARE VERY SENSITIVE TO STIMULATION.

5.4.2. Superovulatory response

THE OCCURRENCE OF ABNORMAL CL DEVELOPMENT, WHICH IS AN INDICATION OF PREMATURE LUTEAL REGRESSION IS A PHENOMENON COMMON IN GOATS FOLLOWING SUPEROVULATION. IN THIS STUDY THE INCIDENCE OF ABNORMAL CL'S WAS LOWER, COMPARED TO THE 38.1% OBSERVED IN THE PREVIOUS TRIAL (PARAGRAPH 3.2.3.1), AND WHICH IS IN AGREEMENT WITH PREVIOUS FINDINGS FOLLOWING SUPEROVULATION WITH THE AID OF PFSH (SEVARAJU ET AL., 2003). HIGHER INCIDENCES OF ABNORMAL CL'S HAVE BEEN COMMONLY OBSERVED IN GOATS SUPEROVULATED WITH EXOGENOUS ECG COMPARED TO FSH. THIS COULD POSSIBLY BE ASCRIBED TO HORMONAL IMBALANCES DUE TO THE DEFICIENCY IN CIRCULATING LH.

HOWEVER, THIS COULD NOT BE VERIFIED BY SERUM FSH OR LH PROFILES (ARMSTRONG ET AL., 1983A; PENDLELTON ET AL., 1992; SAHARREA ET AL, 1998). THE OVULATION RATE AS DETERMINED BY THE NUMBER OF OVULATIONS IS AN IMPORTANT PARAMETER UTILISED TO DETERMINE THE RESPONSE OF DOES TO SUPEROVULATION AND THE SUCCESS OF THE SUPEROVULATORY TREATMENT. THIS PARAMETER ALSO GIVES AN INDICATION OF THE NUMBER OF FOLLICLES WHICH WERE RECRUITED OR INDUCED BY THE ADMINISTRATION OF THE EXOGENOUS FSH GONADOTROPHIN AND WERE ABLE TO MATURE - AS DESIGNATED BY OVULATION. MATURE OOCYTES ARE FURTHERMORE, EXPECTED TO BE FERTILISED AFTER AI OR NATURAL MATING AND COULD GIVE AN INDICATION OF THE NUMBER OF EMBRYOS TO BE RECOVERED LATER DURING EMBRYO FLUSHING. THE OVULATION RATE IN THE DOES WAS NOT AFFECTED BY THE ROUTE OF SUPEROVULATORY ADMINISTRATION, AS WAS VERIFIED BY NO SIGNIFICANT DIFFERENCE BETWEEN THE TREATMENT GROUPS REGARDING THE OVULATION RATE. HOWEVER, THE SUBCUTANEOUS STIMULATED GROUP TENDED TO INDUCE A HIGHER OVULATION RATE, WITH LESS VARIATION BETWEEN THE DONORS. HERE THE SLOW RELEASE OF THE EXOGENOUS FSH VIA S.C ADMINISTRATION COULD HAVE PLAYED A ROLE. THE INTRAMUSCULAR TREATED GROUP TENDED TO HAVE A LOWER OVULATION RATE WITH A HIGHER VARIATION BETWEEN THE ANIMALS WITHIN THE GROUP. A HIGHER VARIATION IN OVARIAN RESPONSE TO SUPEROVULATION HAS BEEN REPORTED IN GOATS AND REPORTED TO BE A MAJOR FACTOR, LIMITING THE USE OF MOET PROGRAMMES IN GOATS (ARMSTRONG ET AL., 1983B; SELGRATH ET AL., 1990; MAHMOOD ET AL., 1991).

IN MOST TRIALS IN GOATS, WHEN THE SUPEROVULATORY TREATMENT WAS ADMINISTERED INTRAMUSCULARLY, THERE HAS BEEN AN INDICATION OF A HIGH VARIATION IN THE OVULATION RATE. WHEN USING THIS VARIABILITY IN OVARIAN RESPONSE RECORDED FOLLOWING SUPEROVULATION (OVULATION RATE) AS A REFERENCE OBSERVED IN THIS STUDY, THE SUBCUTANEOUS ROUTE OF ADMINISTRATION OF SUPEROVULATORY TREATMENT SEEMED TO REDUCE THIS VARIATION. THE OVERALL MEAN NUMBER OF OVULATIONS (18.9±5.3) RECORDED FOR BOTH TREATMENT GROUPS WAS HOWEVER STILL IN LINE WITH VALUES OF 16.5, 16.8±7.4, 17.6±5.5 AND 19.8±9.0 REPORTED IN OTHER GOAT BREEDS FOLLOWING SUPEROVULATION WITH PFSH (ARMSTRONG ET AL., 1983A; SELGRATH ET AL., 1990; MAHMOOD ET AL., 1991; COGNIE ET AL., 2003). THE OVERALL MEAN OVULATION RATE IN THE PRESENT STUDY WAS ALSO HIGHER, WHEN AGAIN COMPARED TO THE OVULATION RATES OF 14.7±2.5, 14.3±0.5 AND 11.8±2.9 RECORDED IN DAIRY GOATS, MURCIANO-GRANADINA AND JAKHRANA GOATS, RESPECTIVELY (PENDLETON ET AL., 1992; GONZALEZ-BULNES ET AL., 2003A; GOEL & AGRAWAL, 2005). THESE DIFFERENCES IN OVULATION RATE MAY BE ATTRIBUTED TO THE DURATION AND TYPE OF PROGESTAGEN, TREATMENT PROTOCOL, CLIMATE, AS WELL AS DIFFERENT TECHNIQUES OF OESTROUS SYNCHRONISATION USED - WHICH DIFFERED FROM THIS STUDY.

THE MEAN NUMBER OF STRUCTURES RECOVERED FOLLOWING FLUSHING WAS NOT AFFECTED BY THE ROUTE OF FSH ADMINISTRATION. THE OVERALL MEAN NUMBER OF STRUCTURES – OVA AND EMBRYOS RECOVERED (18.9±5.3) IN THE PRESENT STUDY, WAS HIGHER, WHEN COMPARED TO 13.6±8.3 AND 7.1±1.4 RECORDED IN ALPINE AND DAIRY GOATS,

RESPECTIVELY, SUPEROVULATED WITH FSH (SELGRATH ET AL., 1990; PENDLETON ET AL., 1992). THESE DIFFERENCES MAY BE ATTRIBUTED TO BREED DIFFERENCES WHICH HAVE BEEN PREVIOUSLY ASSOCIATED WITH THE PROLIFICACY OF A BREED, WHILE THE FLUSHING TECHNIQUE AND NUTRITIONAL STATUS OF THE DONORS COULD ALSO PLAY A ROLE (GONZALEZ-BULNES ET AL., 2003A). IT HAS BEEN INDICATED IN OTHER RUMINANTS THAT HIGHLY PROLIFIC BREEDS ARE MORE SENSITIVE TO GONADOTROPHIN STIMULATION AND HENCE RESPOND BETTER THAN THE LESS PROLIFIC BREEDS (KELLY ET AL., 1983; BINDON ET AL., 1986).

THE MEAN NUMBER OF EMBRYOS AND FERTILISATION RATE TENDED TO BE HIGHER IN DOES TREATED WITH FSH ADMINISTERED S.C. (19.6±4.5 AND 98.4±4.2%, RESPECTIVELY), COMPARED TO THE I.M. TREATED GROUP (14.8±9.1 AND 87.6±22.5%), ALTHOUGH THIS DIFFERENCE WAS NOT SIGNIFICANT. A HIGH VARIATION IN RESPONSE BETWEEN THE DOES REGARDING THESE TWO PARAMETERS WAS RECORDED, ESPECIALLY IN THE INTRAMUSCULAR TREATED GROUP. THE HIGH INITIAL PRODUCTION OF OESTROGEN FOLLOWING STIMULATION (FSH) COULD HAVE AFFECTED THE UTERUS CONTRACTIONS AND UTERINE ENVIRONMENT AND HENCE FERTILISATION RATE (LEONI ET AL., 2001). THE OVERALL MEAN NUMBER OF EMBRYOS RECOVERED PER DONOR (17.0±7.4) IN THE PRESENT EXPERIMENT WAS HIGH AND SATISFACTORY, WHEN COMPARED TO THE 12.6±1.9, 11.3±0.5 AND 10.7±1.1 PREVIOUSLY RECORDED IN FERAL , DAIRY AND MURCIANO-GRANADINA GOATS, RESPECTIVELY (MCNATTY ET AL., 1989; GONZALEZ-BULNES ET AL., 2003A, 2004D). BREED DIFFERENCE, TYPE OF FSH UTILISED AND SUPEROVULATION PROTOCOL MAY HAVE CONTRIBUTED TO THE ULTIMATE FERTILISATION

RATE, AS REPORTED IN OTHER TRIALS (GONZALEZ-BULNES ET AL., 2003A; GONZALEZ-BULNES ET AL., 2004A). SO FOR EXAMPLE, OVAGEN (OFSH) HAS BEEN USED AS THE SUPEROVULATORY TREATMENT IN CERTAIN TRIALS, WHILE FOLLTROPIN (PFSH) WAS USED IN THE CURRENT STUDY. HOWEVER, THE CURRENT MEAN NUMBER OF EMBRYOS RECOVERED WAS STILL HIGHER IN THIS TRIAL, EVEN WHEN COMPARED TO THE 10.2±1.6 MEAN NUMBER OF EMBRYOS ALSO OBTAINED WITH PFSH ((MCNATTY ET AL., 1989). THEREFORE, BREED COULD BE A MAJOR LIMITING FACTOR CONTRIBUTING TO THESE DIFFERENCES IN EMBRYO RECOVERY RATE. SEASON OF ADMINISTRATION IS A FACTOR NOT TO BE IGNORED, ESPECIALLY IN THE SEASONAL BREEDERS. THE MEAN NUMBER OF UNFERTILISED OVA RECOVERED WAS SIGNIFICANTLY HIGHER IN THE I.M. TREATED GROUP, COMPARED TO THE S.C GROUP. THIS COULD POSSIBLY BE ASCRIBED TO OVERSTIMULATION AND HIGH CIRCULATING OESTROGEN LEVELS WORKING NEGATIVELY ON THE FERTILISATION RATE. THIS HIGHER NUMBER OF UNFERTILISED OVA TENDED TO REDUCE THE NUMBER OF EMBRYOS RECOVERED IN THE I.M. GROUP, ALTHOUGH THE DIFFERENCES WITH RESPECT TO EMBRYOS RECOVERED WERE NOT STATISTICALLY DIFFERENT. THE PRESENT TRIAL DEMONSTRATED THAT THE ADMINISTRATION OF A SUPEROVULATORY TREATMENT SUBCUTANEOUSLY ENHANCES FERTILITY - AS ILLUSTRATED BY THE LOWER NUMBER OF UNFERTILISED EMBRYOS RECORDED IN THIS GROUP. THE HIGHER NUMBER OF UNFERTILISED OVA IN THE I.M. TREATED GROUP MAY HAVE BEEN CAUSED BY FACTORS INDUCIVE TO A POOR FERTILISATION RATE (E.G. UTERINE ENVIRONMENT) (LEONI ET AL., 2001). THE OCCURRENCE OF LOW FERTILISATION FOLLOWING

SUPEROVULATION IS OFTEN CAUSED BY POOR SYNCHRONISATION AND THE TIMING OF OVULATION AS SUCH (COGNIE, 1999; COGNIE ET AL., 2003). THIS HAS BEEN PROVEN BY AN INCREASE IN THE FERTILISATION RATE (AS INDICATED BY HIGHER NUMBER OF EMBRYOS RECOVERED) FOLLOWING SUPEROVULATION WITH FSH, WHEN OVULATION WAS SYNCHRONISED WITH LHRH IN GOATS (AKINLOSOTU & WILDER, 1993). THE MEAN NUMBER OF UNFERTILISED OVA (0.3±0.8) IN THE S.C. GROUP IS IN AGREEMENT WITH A VALUE OF 0.3±0.2 UNFERTILISED OVA REPORTED IN TELLICHERRY GOATS SUPEROVULATED USING A SIMILAR GONADOTROPHIN VIA A SIMILAR ROUTE TO THE PRESENT STUDY (S.C.). THE OVERALL MEAN NUMBER OF UNFERTILISED OVA (1.9±3.5) ON THE OTHER HAND, WAS SIMILAR TO THE 1.8±0.9 RECORDED IN A STUDY FOLLOWING SUPEROVULATION WITH OVINE FSH (SENTHIL KUMAR ET AL., 2003).

THE SUBCUTANEOUS ROUTE OF FSH ADMINISTRATION RESULTED IN A HIGHER NUMBER OF DEGENERATE EMBRYOS. THIS OBSERVATION SUGGESTS THAT, ALTHOUGH THE S.C. ROUTE OF FSH ADMINISTRATION CAN INDUCE FOLLICLES TO GROW AND OVULATE, THE RECRUITED OOCYTES DO NOT ATTAIN COMPLETE MATURATION. THE PROLONGED PERIOD OF STIMULATION AND LOWER FSH LEVEL COULD CONTRIBUTE TO THIS PHENOMENON. THEREFORE, THE OOCYTES COULD BE FERTILISED, BUT CANNOT SUSTAIN THE VIABILITY OF THE EMBRYO. THESE OOCYTES THAT DO NOT ATTAIN COMPLETE MATURATION ARE GENERALLY RECRUITED FROM THE FOLLICLES OF SMALLER THAN 2 TO 3 MM IN SIZE. USUALLY WHEN SMALL FOLLICLES (2-3 MM IN DIAMETER) ARE RECRUITED THROUGH SUPEROVULATION, THE OVA FAIL TO ATTAIN COMPLETE MATURATION. LARGER

FOLLICLES INDUCED HAVE BEEN INDICATED TO INDUCE A LOW INHIBIN CONCENTRATION, ALTHOUGH INHIBIN IS AN INDICATION OF A FULLY MATURE FOLLICLE. FURTHERMORE, IN GOATS IT HAS BEEN FOUND THAT VIABLE EMBRYOS ARE ONLY ASSOCIATED WITH THE RECRUITMENT OF FOLLICLES OF 4 TO 6 MM IN DIAMETER (GONZALEZ-BULNES ET AL., 2003A, 2004B). THE OCCURRENCE OF A HIGHER NUMBER OF DEGENERATE EMBRYOS HAS BEEN REPORTED IN GOATS PRE-TREATED WITH A GNRH ANTAGONIST PRIOR TO ADMINISTRATION OF A SUPEROVULATORY TREATMENT , ALSO POSSIBLY RELATED TO THE SIZE OF OVARIAN FOLLICLES INDUCED (MCNATTY ET AL., 1989; COGNIE ET AL., 2003).

THE HIGHER NUMBER OF DEGENERATE EMBRYOS IN THE S.C. TREATED GROUP HAD NO EFFECT ON THE NUMBER OF TRANSFERABLE EMBRYOS PRODUCED PER DONOR, AS INDICATED BY A SIMILAR MEAN NUMBER OF TRANSFERABLE EMBRYOS FOR THE TWO TREATMENT GROUPS. NEVERTHELESS, THE MEAN NUMBER TRANSFERABLE EMBRYOS IN THE S.C. GROUP (13.7±4.8) WAS HIGHER, WHEN COMPARED TO THE 7.16±1.96 REPORTED FOR TELLICHERRY GOATS ADMINISTERED FSH (S.C.) USING THE SAME TYPE OF PFSH (FOLLTROPIN) (SENTHIL KUMAR ET AL., 2003). CONVERSELY, THE MEAN NUMBER OF TRANSFERABLE EMBRYOS RECORDED WAS IN LINE WITH THAT OF 12.6±1.9 RECORDED IN GOATS ADMINISTERED OFSH (S.C.) (MCNATTY ET AL., 1989). THE OVERALL TRANSFERABLE EMBRYOS (12.9±9.3) RECORDED IN THIS STUDY WAS HIGHER WHEN COMPARED TO THE MEAN NUMBER OF TRANSFERABLE EMBRYOS WITHIN A RANGE OF 5.8±1.3 TO11.1±1.8 RECORDED IN OTHER GOAT BREEDS SUPEROVULATED WITH THE AID OF FSH (MCNATTY ET AL., 1989; PENDLETON ET AL., 1992; COGNIE ET AL.,

2003; SELVARAJU ET AL., 2003; GONZALEZ-BULNES ET AL., 2004D). THESE DIFFERENCES IN THE NUMBER OF TRANSFERABLE EMBRYOS MAY BE ATTRIBUTED NOT ONLY TO BREED, BUT ALSO TO ENVIRONMENTAL FACTORS (NUTRITIONAL STATUS, DIET, ETC) (GONZALEZ-BULNES ET AL., 2003A), SUGGESTING THAT BOER GOAT DOES DO EXHIBIT A MORE SUPERIOR OVARIAN RESPONSE FOLLOWING SUPEROVULATION WHEN COMPARED TO MOST OTHER GOAT BREEDS. THESE RESULTS OBTAINED ALSO DEMONSTRATED THE EFFICIENCY OF SUPEROVULATION IN BOER GOAT DOES WHEN USING FSH AND COULD ALSO BE AN INDICATIVE OF AN ACCEPTABLE TECHNIQUE OF EMBRYO FLUSHING USED.

5.4.3. Oestrous response of recipient does

THE OVERALL RESPONSE TO SYNCHRONISATION OF 88.9% OBTAINED IS COMPARABLE TO THE 97% AND 95.5% REPORTED PREVIOUSLY IN BOER GOATS DURING THE BREEDING SEASON (MOTLOMELO ET AL., 2002; LEHLOENYA ET AL., 2005). THE OESTROUS RESPONSE IS ALSO IN LINE WITH THE RESPONSE (80.0 TO 94.4%) RECORDED WHEN THE SYNCHRONISATION TREATMENT IS ADMINISTERED FOR A SHORTER PERIOD OF TIME THAN IN THE PRESENT STUDY (6 TO 11 DAYS) (FREITAS ET AL., 1997; FONSECA ET AL., 2005). THE PRESENT RESULTS THEREFORE, SUGGEST THAT THE PROGESTAGEN (CIDR) USED IN THIS STUDY TO BE AN EFFICIENT SYNCHRONISING AGENT, ALTHOUGH IT DID NOT LEAD TO 100% OESTROUS RESPONSE AS HAS BEEN OBSERVED IN OTHER GOAT TRIALS DURING THE BREEDING SEASON OR EVEN WHEN OTHER PROGESTAGEN DEVICES WERE UTILISED (REQUEIRO ET AL., 1999; ROMANO, 2004; WHITLEY & JACKSON, 2004). THE OESTROUS RESPONSE OF THE RECIPIENT DOES OBSERVED IN THIS STUDY WAS HIGHER WHEN COMPARED TO THE

74% AND 70% RESPONSE REPORTED IN THE SAME BREED (GREYLING & VAN DER NEST, 2000). THESE DIFFERENCES MAY BE ATTRIBUTED TO THE TYPE AND DOSE OF PROGESTAGEN USED OR THE BODY CONDITION OF THE DOES. THESE 74% AND 70% OESTROUS RESPONSES RECORDED WERE OBTAINED WHEN WHOLE AND HALVED PROGESTAGEN MAP SPONGES WERE UTILISED TO SYNCHRONISE OESTRUS. A LOWER OESTROUS RESPONSE IN GOATS HAS ALSO BEEN OBSERVED FOLLOWING THE OESTROUS SYNCHRONISATION OF YOUNG DOES (DRION ET AL., 2001) OR WHEN CIDR'S ARE UTILISED WITHOUT THE SUPPLEMENTATION OF ECG (OLIVEIRA ET AL., 2001).

5.4.4. Pregnancy, kidding and embryo survival rates

MORE RECIPIENTS (6 OF THE 7 DOES RECEIVING EMBRYOS) WERE DIAGNOSED PREGNANT 40 DAYS FOLLOWING EMBRYO TRANSFER IN THE FRESH TRANSFERRED EMBRYO GROUP. ALTHOUGH THESE DIFFERENCES WERE NOT SIGNIFICANT DUE TO THE LOW NUMBERS OF RECIPIENTS ANIMALS. THE PREGNANCY RATE OF 85.7% FOLLOWING THE TRANSFER OF FRESH EMBRYOS WAS HIGHLY ACCEPTABLE AND COMPARABLE TO A PREGNANCY RATE OF 79% RECORDED IN DAIRY GOATS (KIESSLING ET AL., 1986). THE PREGNANCY RATE OBTAINED FROM THIS TRIAL WAS HOWEVER HIGHER, WHEN COMPARED TO A RANGE OF 27.0 TO 60.4% QUOTED FOLLOWING LAPAROSCOPIC, SURGICAL AND NON-SURGICAL TRANSFER OF GOAT EMBRYOS (BESSOUDO ET AL., 1988; LI ET AL., 1990; FLORES-FOXWORTH ET AL., 1992). THESE PREGNANCY RATES OBTAINED ARE ALSO INDICATIVE OF AN ACCEPTABLE PROCEDURE OF EMBRYO TRANSFER USED IN THIS TRIAL. THE DIFFERENCES OBTAINED COULD ALSO BE ATTRIBUTED TO THE STAGE OF DEVELOPMENT OF THE EMBRYOS AND THE NUMBER

OF EMBRYOS TRANSFERRED PER DONOR. IT HAS BEEN REPORTED THAT THE TRANSFER OF MORE THAN TWO EMBRYOS AND EMBRYOS AT AN EARLIER STAGE OF DEVELOPMENT, REDUCE THE SURVIVAL RATE OF THE TRANSFERRED EMBRYOS (ARMSTRONG ET AL., 1983B; LI ET AL., 1990; ISHWAR & MEMON, 1996; EL-GAYAR, & HOLTZ, 2005).

THE PREGNANCY RATE FOLLOWING THE TRANSFER OF CONVENTIONAL SLOW FROZEN-THAWED EMBRYOS IN THIS STUDY WAS ALSO IN LINE WITH 58% THAT WAS OBTAINED FOLLOWING THE TRANSFER OF 7-DAY OLD GOAT BLASTOCYSTS CRYOPRESERVED WITH GLYCEROL AS A CRYOPROTECTANT (EL-GAYAR & HOLTZ, 2001). IT WAS HOWEVER LOWER (50.0%) COMPARED TO THE 73% AND 83% QUOTED FOLLOWING THE TRANSFER OF CONVENTIONAL SLOW FROZEN GOAT EMBRYOS (NOWSHARI & HOLTZ, 1995; GUIGNOT ET AL., 2006). THE REASONS FOR THE DIFFERENCES IN PREGNANCY RATES OBTAINED FOLLOWING CONVENTIONAL SLOW FROZEN-THAWED EMBRYO TRANSFER IS NOT EASY TO EXPLAIN, AS THERE ARE TOO MANY VARIABLES THAT COULD PLAY A ROLE. THE DIFFERENCES OCCURRED EVEN WHEN EMBRYOS WERE AT THE SAME STAGE OF DEVELOPMENT, OF A SIMILAR BREED OR SIMILAR CRYOPROTECTANTS USED. THIS OBSERVATION DEMONSTRATES THE UNPREDICTABILITY OF THE RESULTS FOLLOWING THE TRANSFER OF SLOW FROZEN-THAWED GOAT EMBRYOS.

FOLLOWING THE TRANSFER OF VITRIFIED-THAWED EMBRYOS, THE PREGNANCY RATE (37.5%) IN THE PRESENT STUDY WAS SIMILAR TO THE 39% TO 52% REPORTED FOLLOWING THE TRANSFER OF STANDARD VITRIFIED AND CONVENTIONAL STANDARD VITRIFIED GOAT EMBRYOS (GUIGNOT ET AL., 2006). THE PREGNANCY RATE HOWEVER, WAS

LOWER WHEN COMPARED TO THE 100% OBTAINED BY EL-GAYAR AND HOLTZ (2001) FOLLOWING THE TRANSFER OF VITRIFIED-THAWED EMBRYOS. THE HIGHER PREGNANCY RATE FROM THE LATTER STUDY MAY BE DUE TO THE USE OF THE OPEN PULLED STRAW (OPS) METHOD OF VITRIFICATION, WHICH IS CURRENTLY REPORTED TO GIVE BETTER RESULTS (YUSWIATI & HOLTZ, 1990; BEGIN ET AL., 2003).
THE NUMBER OF RECIPIENTS KIDDING DECLINED FROM 6 (87.5%) CONFIRMED PREGNANT TO 4 (57.0%) KIDDING FOLLOWING THE TRANSFER OF FRESH EMBRYOS. THIS OBSERVATION MAY HAVE BEEN CULMINATION OF MANY FACTORS AFFECTING THE RECIPIENTS OR THE EMBRYOS. FROM THE RECIPIENT SIDE, A LOW SERUM PROGESTERONE CONCENTRATION FROM A SINGLE CL AT TRANSFER MAY HAVE REDUCED THE NUMBER OF DOES KIDDING. IT HAS ALSO BEEN OBSERVED THAT THE SURVIVAL RATE OF EMBRYOS, IS INCREASED BY TRANSFERRING EMBRYOS TO RECIPIENTS WITH 2, 3 OR MORE CL'S AND A HIGH CIRCULATING PROGESTERONE CONCENTRATION (ARMSTRONG ET AL., 1983B). HOWEVER, IN THE PRESENT STUDY IT WOULD SEEM THAT THE NUMBER OF CL'S PRESENT AT TRANSFER DID NOT HAVE ANY EFFECT ON THE EVENTUAL PREGNANCY RATE, THE NUMBER OF RECIPIENTS KIDDING AND EMBRYO SURVIVAL RATE, PRESUMABLY DUE TO THE LOW NUMBER OF RECIPIENTS INVOLVED. THE DECLINE IN THE RECIPIENTS KIDDING FROM THE RECIPIENTS CONFIRMED PREGNANT (SONOGRAPHY) ALSO OCCURRED FOLLOWING THE TRANSFER OF FROZEN-THAWED EMBRYOS IN THE PRESENT TRIAL. SIMILAR TENDENCIES FOLLOWING TRANSFER OF FROZEN-THAWED EMBRYOS HAVE BEEN RECORDED IN GOATS AND SHEEP (NOWSHARI & HOLTZ, 1995; BARIL ET AL., 2001; EL-GAYAR & HOLTZ, 2001). SEVERAL FACTORS

HAVE BEEN QUOTED AS BEING RESPONSIBLE FOR EMBRYONIC RESORPTION AND AS ANIMALS WERE NOT MONITORED 24H A DAY E.G. FOR FEVER OR METABOLIC DISTURBANCES.

THE KIDDING RATE OF 25.0% WAS LOWER WHEN COMPARED TO 50%, 69%, 71% AND 75% RECORDED FOLLOWING THE TRANSFER OF CONVENTIONAL SLOW FROZEN-THAWED GOAT EMBRYOS IN THE LITERATURE (NOWSHARI & HOLTZ, 1995; BARIL ET AL., 2000; EL-GAYAR & HOLTZ, 2001; GUIGNOT ET AL., 2006). ALL DOES DIAGNOSED PREGNANT KIDDED, IN THE GROUP OF DOES WHICH RECEIVED THE VITRIFIED-THAWED EMBRYOS – WHICH COULD POINT TO THIS METHOD OF EMBRYO CRYOPRESERVATION AS BEING SUPERIOR IN TERMS OF THE VIABILITY OF THE THAWED EMBRYOS. SIMILAR TENDENCIES REGARDING PREGNANCY SUCCESS FOLLOWING STANDARD AND THE OPS VITRIFICATION METHODS HAVE BEEN REPORTED IN SHEEP AND GOATS (BARIL ET AL., 2001; EL-GAYAR & HOLTZ, 2001; GUIGNOT ET AL., 2006). THE PERCENTAGE OF DOES KIDDING (37.5%) OBTAINED IN THIS STUDY IS STILL WITHIN THE RANGE OF 22 TO 48% OBTAINED PREVIOUSLY IN GOATS FOLLOWING THE TRANSFER OF VITRIFIED-THAWED EMBRYOS (YUSWIATI & HOLTZ, 1990; GUIGNOT ET AL., 2006). THE VARIATION RECORDED IN THE LITERATURE REGARDING THE SUCCESS RATES INDICATES THAT THERE IS STILL MUCH RESEARCH NEEDED TO PERFECT THE FREEZING AND/OR TRANSFER TECHNIQUES OF EMBRYOS IN GOATS.

THE EMBRYO SURVIVAL RATE FOLLOWING THE TRANSFER OF FRESH, FROZEN-THAWED AND VITRIFIED-THAWED EMBRYOS IN THIS STUDY SHOWED A RELATIVE SMALL DIFFERENCE AND THE 35.7% RECORDED IN THIS STUDY WAS IN LINE WITH THE 35.7%, 36.7% AND 46.5% PREVIOUSLY RECORDED FOLLOWING FRESH EMBRYO TRANSFER (UDY, 1987;

BESSOUDO ET AL., 1988). THE CURRENT EMBRYO SURVIVAL RATE OBTAINED WAS HOWEVER, LOWER THAN THE 51.6% AND 65.2% FOLLOWING TRANSFER OF SINGLE OR TWIN EMBRYO TRANSFERS (ISHWAR & MEMON, 1996). THESE DIFFERENCES MAY BE ATTRIBUTED TO BREED, OR QUALITY DIFFERENCES IN THE EMBRYOS PRODUCED BY DIFFERENT DONORS. THE SURVIVAL RATE FOLLOWING TRANSFER OF CONVENTIONAL SLOW FROZEN-THAWED EMBRYOS (25.0%), WAS LOWER AND UNSATISFACTORY. SIMILAR LOWER SURVIVAL RATES OF 37.5%, 41%, 42%,45% AND 47.3% HAVE ALSO BEEN REPORTED USING SIMILAR CRYOPROTECTANTS TO THOSE IN THE PRESENT STUDY AND OTHER CRYOPROTECTANTS ALSO DOCUMENTED IN OTHER GOAT BREEDS (RAO ET AL., 1988; BARIL ET AL., 2000; EL-GAYAR & HOLTZ, 2001; GUIGNOT ET AL., 2006). THE SUCCESS OF EMBRYO SURVIVAL, IRRESPECTIVE OF THE METHOD OF CRYOPRESERVATION USED COULD ALSO BE ATTRIBUTED TO HORMONAL IMBALANCES AFFECTING THE MAINTENANCE OF PREGNANCY AND ASCRIBED WITH STRESSFUL CONDITIONS. RESULTS DEMONSTRATE THE LOW SURVIVABILITY AND VIABILITY OF EMBRYOS FOLLOWING CONVENTIONAL SLOW FREEZING OF GOAT EMBRYOS - WHICH IS OFTEN ATTRIBUTED TO THE SENSITIVITY OF THE EMBRYOS TO CHILLING AND THE FORMATION OF ICE CRYSTALS DURING THE FREEZING PROCESS (NIEMANN, 1991; DOBRINSKY, 2002). THE EMBRYO SURVIVAL RATE FOLLOWING TRANSFER OF VITRIFIED-THAWED EMBRYOS (31.3%) WAS COMPARABLE TO THE 35 TO 44% REPORTED IN OTHER GOAT BREEDS (BRANCA ET AL., 2000; TRALDI, 2000; GUIGNOT ET AL., 2006). THE LOW EMBRYO SURVIVAL RATE HOWEVER FOLLOWING VITRIFICATION IN THIS STUDY MAY BE ATTRIBUTED TO THE METHOD USED (CRYOTIPS). A HIGHER EMBRYO SURVIVAL RATE OF

64% HAS BEEN RECORDED IN THE LITERATURE WHEN THE OPS METHOD OF VITRIFICATION WAS USED (EL-GAYAR & HOLTZ, 2001). HOWEVER, THERE IS A LACK IN CONSISTENCY AND LARGE VARIATION REGARDING THE SUCCESS FOLLOWING THE TRANSFER OF EMBRYOS VITRIFIED USING THE OPS METHOD. OTHER FINDINGS WITH THE OPS METHOD HAVE RESULTED IN A SURVIVAL RATE AS LOW AS 14%. THE DIFFERENCE IN EMBRYO SURVIVAL RATE WHEN UTILISING THE OPS METHOD MAY BE EXPLAINED BY DIFFERENT COMPONENTS (AGENTS) USED FOR THE VITRIFICATION PROCESS IN THE DIFFERENT TRIALS AND A DIFFERENCE IN COMPATIBILITY OF THE EMBRYOS TO THE CRYOPROTECTANTS.

THE MEAN GESTATION LENGTH (147.5±3.3 D) FOLLOWING THE TRANSFER OF FRESH EMBRYOS WAS RELATIVELY SHORTER, WHEN COMPARED TO THE 151 D RECORDED IN OTHER GOAT BREEDS FOLLOWING EMBRYO TRANSFER (YUSWIATI & HOLTZ, 1990). THESE OBSERVATIONS INDICATE SMALL DIFFERENCES IN THE GESTATION LENGTHS OF DIFFERENT GOAT BREEDS DUE TO EXTENSIVE FACTORS, BUT ALL WITHIN AN ACCEPTABLE RANGE. THE MEAN GESTATION LENGTH IN THIS STUDY FOLLOWING THE TRANSFER OF FRESH EMBRYOS IS IN AGREEMENT WITH THE 148.2±3.7 D AND 148.6±0.9 D REPORTED IN BOER GOATS. THE GESTATION PERIOD AFTER THE TRANSFER OF CRYOPRESERVED EMBRYOS (SLOW FROZEN AND VITRIFIED), WAS 1 TO 2 DAYS EARLIER THAN FRESH TRANSFERRED EMBRYOS AND EARLIER BY APPROXIMATELY 3 DAYS FOLLOWING NATURAL AND INDUCED OESTRUS PREGNANCIES PREVIOUSLY REPORTED (GREYLING, 1988; LEHLOENYA ET AL., 2005). THIS PERIOD IS MINIMAL WHEN MEASURED OVER A PERIOD OF APPROXIMATELY 150D.

THE SIMILAR TREND AND MEAN SERUM PROGESTERONE CONCENTRATIONS THROUGHOUT THE OBSERVATION PERIOD CONFIRMS THAT BOTH METHODS OF EITHER ADMINISTERING GONADOTROPHINS INTRAMUSCULARLY OR SUBCUTANEOUSLY CAN BE USED IN BOER GOAT MOET PROGRAMMES.

5.5. Conclusions

THE ROUTE OF SUPEROVULATORY AGENT ADMINISTRATION HAD NO EFFECT ON THE INITIAL OESTROUS RESPONSE, FOLLOWING THE INDUCED OESTROUS PERIOD (OCCURRENCE, ONSET AND DURATION OF OESTRUS). THE APPEARANCE OF ABNORMAL CL'S WAS LOW FOR BOTH ROUTES OF ADMINISTRATION. THE RESPONSE TO SUPEROVULATION TENDED TO FAVOUR THE SUBCUTANEOUS ROUTE OF GONADOTROPHIN ADMINISTRATION, WITH RESPECT TO OVULATION RATE, TOTAL NUMBER OF OVA AND EMBRYOS RECOVERED - ALTHOUGH NO STATISTICAL DIFFERENCES WERE RECORDED. THE VARIATION BETWEEN ANIMALS TREATED INTRAMUSCULARLY WAS HIGH WITH RESPECT TO THESE THREE PARAMETERS MEASURED. THE SUBCUTANEOUS ROUTE ALSO HAD AN ADVANTAGE OF RESULTING IN A LOWER NUMBER OF UNFERTILISED OVA RECOVERED. HOWEVER, THIS ADVANTAGE WAS COUNTERACTED BY HIGHER NUMBER OF DEGENERATE EMBRYOS OBSERVED IN THE S.C. GROUP - WHEN COMPARED TO THE I.M. GROUP. THIS AT THE END RESULTED IN A SIMILAR NUMBER OF TRANSFERABLE EMBRYOS OBTAINED IN BOTH GROUPS. BASED ON THE PRESENT RESULTS, EITHER OF THESE TWO ROUTES CAN BE USED FOR THE ADMINISTRATION OF A SUPEROVULATION TREATMENT IN BOER GOAT DOES DURING THE BREEDING SEASON.

THE TRANSFER OF FRESH GOAT BLASTOCYSTS RESULTED IN HIGHER PREGNANCY RATES BEING OBTAINED, WHILE BOTH CRYOPRESERVATION METHODS (CONVENTIONAL SLOW FREEZING AND VITRIFICATION) RESULTED IN UNSATISFACTORY LOW PREGNANCY RATES. ALL RECIPIENTS CONFIRMED PREGNANT FOLLOWING PREGNANCY DIAGNOSIS IN THE VITRIFICATION GROUP KIDDED, WHILE NOT ALL DOES FROM THE FRESH AND SLOW FROZEN-THAWED EMBRYO GROUPS CONFIRMED PREGNANT AFTER DIAGNOSIS, KIDDED. THUS, REDUCING THE NUMBER OF RECIPIENTS KIDDING IN BOTH GROUPS. HOWEVER, THIS TENDENCY WAS NOT SIGNIFICANT AS THE RECIPIENTS KIDDING IN THE 3 GROUPS WAS SIMILAR. THE PREGNANCY RATE RECORDED WAS ALSO VERY LOW AND THE NUMBER OF ANIMALS TOO LIMITED TO MAKE REAL RECOMMENDATIONS. THE SURVIVAL RATE OF EMBRYOS FOLLOWING FRESH, SLOW FROZEN-THAWED AND VITRIFIED-THAWED EMBRYOS WAS LOWER, ALTHOUGH COMPARABLE TO PREVIOUS FINDINGS. IT COULD THEREFORE BE CONCLUDED THAT MORE RESEARCH IS WARRANTED WITH LARGER NUMBERS, DIRECTED AT IMPROVING THE SURVIVABILITY OF EMBRYOS FOLLOWING FRESH AND CRYOPRESERVED GOAT EMBRYO TRANSFER.

CHAPTER 6

6. COMPARISON OF THREE SUPEROVULATION PROTOCOLS AND SURVIVAL RATES FOLLOWING the TRANSFER OF FRESH AND FROZEn goat EMBRYOS

6.1. Introduction

THE VARIATION IN RESPONSE TO SUPEROVULATION AND RELATIVELY LOW SURVIVAL RATES FOLLOWING EMBRYO TRANSFER ARE MAJOR FACTORS LIMITING THE SUCCESSFUL USE OF A MOET PROGRAMME IN GOATS AND OTHER FARM ANIMAL ENTERPRISES. THE SUPEROVULATORY PROTOCOL IN GOATS TRADITIONALLY INCLUDES THE ADMINISTRATION OF AN INTRAVAGINAL PROGESTAGEN PRE-TREATMENT OVER AN 11 TO 21D PERIOD, FOLLOWED BY THE SUPEROVULATORY TREATMENT WITH GONADOTROPHINS, WHICH BEGINS 72 TO 48H BEFORE PROGESTAGEN REMOVAL (MCNATTY ET AL., 1989; SELGRATH ET AL., 1990; GONZALEZ-BULNES ET AL., 2003A). WHEN INTRAVAGINAL PROGESTAGEN TREATMENT IS PERFORMED OVER A SHORT PERIOD (9-11D), A PROSTAGLANDIN-F2A (PGF2A) INJECTION IS GENERALLY ADMINISTERED, COINCIDING WITH THE FIRST SUPEROVULATORY TREATMENT – 24 TO 48 H PRIOR TO OR AT PROGESTAGEN WITHDRAWAL, IN ORDER TO ALLOW FOR A PRECISE TIMING OF THE ONSET OF OESTRUS (KRISHER ET AL., 1994; SENTHIL KUMAR ET AL., 2003).

EVEN WHEN LONG PROGESTAGEN TREATMENT PROTOCOLS ARE USED, IT HAS BECOME THE NORM TO ADMINISTER AN INJECTION OF PGF2A (ESPECIALLY DURING THE BREEDING SEASON), BEFORE OR AT THE INITIATION OF

SUPEROVULATORY TREATMENT TO ENSURE COMPLETE LUTEOLYSIS AND REMOVAL OF ANY POSSIBLE CL'S. IN PAST STUDIES, THE SUPEROVULATION PROTOCOL INVOLVED THE UTILISATION OF A LONG SYNCHRONISATION PROGRAMME (17D) WITHOUT ANY PGF2A TREATMENT. CURRENTLY THERE IS LIMITED INFORMATION, REGARDING THE ADMINISTRATION OF PGF2A DURING THE SUPEROVULATORY PROTOCOL IN BOER GOATS AND WHETHER IT HAS ANY BENEFIT. THE FIRST AIM OF THIS STUDY WAS THUS TO EVALUATE THE EFFICIENCY OF SUPEROVULATION FOLLOWING A LONG PROGESTAGEN SYNCHRONISATION PROTOCOL, WITH OR WITHOUT THE USE OF PGF2A.

THE VARIATION IN OVARIAN RESPONSE TO SUPEROVULATION TREATMENT IS HYPOTHESISED TO BE REDUCED WHEN INITIATING THE SUPEROVULATORY TREATMENT WHEN THERE IS A HOMOGENOUS POOL OF SMALL OVARIAN FOLLICLES PRESENT. THIS PERIOD COINCIDES WITH THE EMERGENCE OF A FOLLICULAR WAVE AND IN THE ABSENCE OF A DOMINANT FOLLICLE (GINTHER & KOT, 1994; DE CASTO ET AL., 1998; COGNIE ET AL., 2003; GONZALEZ-BULNES ET AL., 2004A). IN GOATS THE EMERGENCE OF A FIRST FOLLICULAR WAVE HAS BEEN FOUND TO OCCUR ON DAY 0 OF THE INTEROVULATORY INTERVAL. THEREFORE, IF A SUPEROVULATORY TREATMENT COULD BE INITIATED DURING THIS PERIOD, IT IS HYPOTHESIZED THAT THE VARIATION IN OVARIAN RESPONSE WILL BE REDUCED AND THE OVARIAN RESPONSE BE INCREASED. IN GOATS THE SO-CALLED DAY 0 PROTOCOL (IN WHICH SUPEROVULATION IS INITIATED JUST AFTER OVULATION – DAY 0 BEING DAY OF OVULATION) WAS UTILISED, WITH PROMISING RESULTS, BASED ON THE NUMBER OF

OVULATIONS INDUCED WITHOUT THE EVALUATION OF EMBRYO RECOVERY RATE (MENCHACA ET AL., 2002). THIS PROTOCOL HOWEVER, REQUIRES THE USE OF AN ULTRASONOGRAPHIC SCANNER TO VISUALLY CONFIRM OVULATION AND LIMITS THE UTILISATION OF THE DAY 0 PROTOCOL, ESPECIALLY IN A COMMERCIAL SITUATION WHERE AN EXTENSIVE MOET PROGRAMME IS TO BE PERFORMED. A LIMITING FACTOR WHEN UTILISING THIS PROTOCOL IS NOT ONLY LARGE MOET PROGRAMMES, BUT ALSO AT THE FARM LEVEL - NOT EVERY FARMER OWNS AN ULTRASONOGRAPHIC SCANNER OR HAS THE KNOWLEDGE TO UTILISE THIS TYPE OF SOPHISTICATED EQUIPMENT FOR OVARIAN EVALUATIONS. IF THIS PROTOCOL WAS TO BE UTILISED WITHOUT THE USE OF AN ULTRASONOGRAPHIC APPARATUS, IT WOULD REQUIRE AN ESTIMATION OF THE OCCURRENCE OF OVULATION (HORMONAL ASSAY) FOLLOWING OESTROUS SYNCHRONISATION TERMINATION. THUS, THE SECOND AIM OF THIS STUDY WAS TO COMPARE THE SUPEROVULATION RESPONSE FOLLOWING THE DAY 0 PROTOCOL, USING THE PREDETERMINED TIME OF OVULATION WITHOUT THE USE OF A SONOGRAPHIC SCANNER AS PREVIOUSLY USED IN SUPEROVULATORY PROTOCOLS.

THE SUCCESS IN ANY MOET PROGRAMME DOES NOT ONLY DEPEND ON THE SUPEROVULATORY OVARIAN RESPONSE, BUT ALSO ON THE SURVIVAL RATE OF THE EMBRYOS TRANSFERRED. THE LATTER IS GREATLY INFLUENCED BY THE CAPACITY OF THE RECIPIENT TO SUPPORT PREGNANCY TO TERM - AN ASPECT WHERE OESTROUS SYNCHRONISATION OF THE RECIPIENT AND TIMING OF OESTRUS BECOMES A CRUCIAL FACTOR. AT THE INSTITUTION WHERE THE RESEARCH WAS CONDUCTED, THE BOER GOAT DOES WERE SYNCHRONISED MOSTLY WITH THE AID OF

INTRAVAGINAL PROGESTAGENS FOLLOWED BY THE ADMINISTRATION OF ECG FOR A COMPACT OESTROUS RESPONSE. THE ADDITION OF ECG OR PGF2A IN THE PROTOCOL HAS BEEN INDICATED TO IMPROVE THE OESTROUS RESPONSE (REGUEIRO ET AL., 1999; ROMANO, 2004, HOLTZ, 2005). THE LAST AIM OF THIS STUDY WAS THUS TO EVALUATE THE VIABILITY AND SURVIVABILITY OF FROZEN-THAWED AND FRESH BOER GOAT EMBRYOS WHEN TRANSFERRED INTO DOES AFTER OESTROUS SYNCHRONISATION OF RECIPIENTS USING DIFFERENT PROTOCOLS.

6.2. Materials and methods

6.2.1. Animals

THIS STUDY WAS CONDUCTED DURING THE NATURAL BREEDING SEASON (AUTUMN, 2007) AT THE UNIVERSITY OF THE FREE STATE'S EXPERIMENTAL FARM AND ALL DOES WERE MAINTAINED IN A SIMILAR MANNER AS DESCRIBED IN PARAGRAPH 5.2.1. THE DOES UTILISED HAD 2 TO 8 PERMANENT INCISORS (1-4 YEARS OF AGE) AND THIS CRITERIA WAS USED AS AN INDICATOR OF THE AGE OF THE EXPERIMENTAL ANIMALS. DOES UTILISED THUS INCLUDED MAIDEN DOES (ALSO SUPEROVULATED FOR THE FIRST TIME) AND DOES WHICH HAD PREVIOUSLY BEEN SUPEROVULATED. THE BODY WEIGHT OF THE DOES RANGED BETWEEN 28.7 AND 68.6 KG.

6.2.2. Donor treatments

TWENTY-FOUR BOER GOAT DONOR DOES WERE ALLOCATED TO 3 TREATMENT GROUPS. THE GROUPS WERE STRATIFIED BASED ON AGE, PREVIOUS SUPEROVULATION TREATMENTS AND BODY WEIGHT - SO THAT EACH GROUP CONSISTED OF AN EQUAL NUMBER OF YOUNG AND ADULT DOES, AS WELL AS HAVING A SIMILAR AVERAGE BODY WEIGHT PER

GROUP OF APPROXIMATELY 48.4 KG. IN THE FIRST GROUP OF DOES (GROUP 1; N=8; DAY 0 PROTOCOL GROUP), THE OESTROUS CYCLES WERE SYNCHRONISED WITH THE AID OF INTRAVAGINAL CIDR'S FOR 7 DAYS. AT CIDR INSERTION DOES WERE ALSO TREATED (I.M.) WITH 0.05 MG/DOE PGF2A (ESTRUMATE: CLOPROSTENOL). FOR THE PURPOSE OF DETERMINING DAY 0 (DAY OF OVULATION) IN THIS SPECIFIC GROUP THE OVULATION TIME WAS ESTIMATED BASED ON PREVIOUS FINDINGS AND PREDETERMINED TO OCCUR APPROXIMATELY 72 TO 80H FOLLOWING PROGESTAGEN WITHDRAWAL.

THE SUPEROVULATORY TREATMENT (PFSH) WAS THEN INITIATED 88H FOLLOWING CIDR WITHDRAWAL AND ADMINISTERED OVER A PERIOD OF TIME IN 7 DOSAGES AT 12H INTERVALS (PARAGRAPH 3.2.3.1). CONCURRENTLY WITH THE 6^{TH} DOSAGE, DOES WERE ADMINISTERED AN I.M. INJECTION OF 0.05 MG/DOE PGF2A. FIXED TIME AI USING 0.1ML FRESH UNDILUTED BOER GOAT SEMEN (SPERM CONCENTRATION OF APPROXIMATELY 350 X 10^6/ML) WAS PERFORMED 24H AND 36H FOLLOWING THE LAST SUPEROVULATORY INJECTION. OESTROUS DETECTION WAS PERFORMED WITH THE AID OF VASECTOMISED BUCKS AT 8H INTERVALS FOLLOWING CIDR REMOVAL, FOR A PERIOD OF 96H FOLLOWING THE LAST FSH TREATMENT.

IN THE SECOND GROUP (GROUP 2, N=8), THE OESTROUS CYCLES OF THE DOES WERE SYNCHRONISED USING THE INTRAVAGINAL CIDR'S FOR A PERIOD OF 17 DAYS. ON DAY 14 (TAKING THE DAY 0 AS THE DAY OF CIDR INSERTION) FOLLOWING CIDR INSERTION, ALL DOES IN THIS GROUP WERE INJECTED WITH 0.05 MG/DOE PGF2A AND THE SUPEROVULATORY FSH TREATMENT INITIATED ON DAY 15. IN THE LAST GROUP (GROUP 3; CONTROL; N=8), THE SYNCHRONISATION AND

SUPEROVULATION TREATMENTS WERE PERFORMED USING A SIMILAR APPROACH AS THAT IN THE SECOND GROUP. HOWEVER, DOES DID NOT RECEIVE A PROSTAGLANDIN-F2A TREATMENT. THE SUPEROVULATORY PFSH TREATMENT WAS ADMINISTERED I.M. OVER TIME IN 7 TREATMENTS AS DESCRIBED IN CHAPTER 3 (PARAGRAPH 3.2.3.1).

Group 2 and 3 does were cervically inseminated with 0.1ml fresh undiluted Boer goat SEMEN (SPERM CONCENTRATION OF APPROXIMATELY 350 X 10^6 SPERM/ML) PERFORMED AT A FIXED TIME (36H AND 48H) FOLLOWING CIDR WITHDRAWAL. DOES FROM GROUP 2 AND 3 WERE ALSO CHECKED FOR OESTRUS AT 8H INTERVALS FROM CIDR REMOVAL FOR A TOTAL PERIOD OF 96H. COLLECTION OF EMBRYOS, AS WELL AS THE EVALUATION OF THE EMBRYOS WERE PERFORMED AS PREVIOUSLY REPORTED IN PARAGRAPH 3.2.3.4. BLOOD SAMPLING PROCEDURES AND PROGESTERONE CONCENTRATION DETERMINATIONS WERE PERFORMED AS DESCRIBED IN CHAPTER 3 (PARAGRAPH 3.2.3.4 AND 3.2.3.4.2)

FOLLOWING EMBRYO EVALUATION, A GROUP OF EARLY BLASTOCYSTS (GRADE 1 EMBRYOS) WERE CHOSEN AT RANDOM AND HALF WERE SLOW FROZEN, AND THEN THAWED AFTER A 2H FREEZING PERIOD. THE CONVENTIONAL SLOW FREEZING AND THAWING PROCEDURES OF EMBRYOS PERFORMED WERE SIMILAR TO THOSE DESCRIBED IN PARAGRAPH 4.2.2.2 AND 4.2.2.3.

6.2.3. Recipient treatments

TWENTY-FIVE RECIPIENT BOER GOAT DOES WERE ALLOCATED TO THE 3 TREATMENT GROUPS AND THESE GROUPS WERE ALSO STRATIFIED IN A SIMILAR MANNER TO THE DONOR DOES (PARAGRAPH 5.2.1). IN GROUP A (N=9) DOES WERE SYNCHRONISED FOR OESTRUS USING INTRAVAGINAL CIDR'S AND ADMINISTERED 0.05 MG/DOE PGF2A (I.M.) 24 H BEFORE CIDR REMOVAL, FOLLOWED BY AN I.M. INJECTION OF 300IU ECG (CHRONOGEST, INTERVET) AT CIDR WITHDRAWAL. DOES IN GROUP B (N=8) WERE SYNCHRONISED WITH THE AID OF CIDR'S AND INJECTED I.M. WITH 300IU ECG (FOLLIGON, INTERVET) (A DIFFERENT ECG PRODUCT FROM THE FIRST GROUP) AT CIDR REMOVAL. THE LAST (GROUP C; N=8), WERE SYNCHRONISED WITH CIDR'S FOLLOWED BY AN INJECTION (I.M.) OF 300IU ECG (CHRONOGEST, INTERVET) AT THE TIME OF CIDR REMOVAL. THIS GROUP WAS USED AS THE CONTROL BECAUSE OF FOLLOWING A SIMILAR OESTROUS SYNCHRONISATION TECHNIQUE OF THE RECIPIENTS. IN ALL DOES THE PROGESTAGEN TREATMENT WAS ADMINISTERED FOR A PERIOD OF 17 DAYS.

THE SYNCHRONISATION TREATMENT OF THE RECIPIENTS WAS TERMINATED 12H PRIOR TO THE REMOVAL OF CIDR'S FROM THE DONOR DOES. ALL DOES WERE CHECKED FOR OCCURRENCE OF OESTRUS AT 8H INTERVALS FOR A PERIOD OF 96H, BEGINNING FROM CIDR REMOVAL. FOLLOWING LAPAROSCOPIC INSPECTION OF THE OVARIES TO CONFIRM THE PRESENCE OR ABSENCE OF ACTIVE CL'S ON THE OVARIES, TWO BLASTOCYSTS (EITHER SLOW FROZEN-THAWED OR FRESH) WERE TRANSFERRED TO THE UTERINE HORNS IPSILATERAL TO THE ACTIVE CL VIA LAPAROSCOPIC TRANSFER TO EACH RECIPIENT. PREGNANCY DIAGNOSIS FOR DETERMINING SUCCESSFUL PREGNANCIES WAS PERFORMED BY LAPAROSCOPIC INSPECTION OF THE

REPRODUCTIVE TRACT 40 DAYS FOLLOWING THE EMBRYO TRANSFER. BLOOD SAMPLES WERE COLLECTED FROM 5 SELECTED RECIPIENTS FROM EACH GROUP ON DAY OF EMBRYO COLLECTION. BLOOD SAMPLING PROCEDURES AND PROGESTERONE CONCENTRATION DETERMINATION WERE PERFORMED AS DESCRIBED IN CHAPTER 3 (PARAGRAPH 3.2.3.4 AND 3.2.3.4.2).

6.2.4. Statistical analyses

ALL DATA WERE ANALYSED USING THE ANOVA PROCEDURES OF SAS, EXCEPT FOR DATA INCORPORATING THE OCCURRENCE OF OESTRUS, PREGNANCY RATE AND FERTILISATION RATE, WHICH WERE ANALYSED USING THE CHI SQUARE TEST (SAS, 2003).

6.3. Results

6.3.1. Oestrous response of donors

FROM THE DAY 0 GROUP (GROUP 1) ONE BOER GOAT DOE BECAME ILL AND WAS REMOVED FROM THE TRIAL. OF THE REMAINING 7 DOES, ONLY 5 (71.4%) EXHIBITED OVERT SIGNS OF OESTRUS FOLLOWING CIDR REMOVAL AFTER A 7 DAY PROGESTAGEN TREATMENT PRIOR TO THE ADMINISTRATION OF THE SUPEROVULATORY TREATMENT, WHILE 2 (28.6%) DOES DID NOT RESPOND. THE TIME TO THE ONSET OF OESTRUS RECORDED IN THIS FIRST INDUCED OESTRUS FOLLOWING CIDR REMOVAL WAS 37.2±5.2H AND THE OESTROUS PERIOD LASTED FOR 36.4±3.8H. ONE DOE FROM GROUP 3 HAD AN ABSCESS AND WAS ALSO REMOVED FROM THE TRIAL. THE OESTROUS RESPONSE WAS 100% FOLLOWING OESTROUS SYNCHRONISATION AND SUPEROVULATION IN GROUP 2 AND 3, WHILE ONLY ONE DOE EXHIBITED SIGNS OF OESTRUS IN THE DAY 0 (GROUP 1) GROUP FOLLOWING THE SUPEROVULATION TREATMENT. THE TIME TO THE ONSET OF OESTRUS (33.0±7.9H VS.

28.6±4.3H) AND DURATION OF THE INDUCED OESTROUS PERIOD (26.0±7.1H VS. 21.7±12.8H) FOR GROUP 2 AND 3 DID NOT DIFFER SIGNIFICANTLY.

6.3.2. Donor superovulatory response

THE SUPEROVULATION RESPONSE OF THE BOER GOAT DOES FOLLOWING THE 3 DIFFERENT SUPEROVULATORY REGIMES IS SET OUT IN TABLE 6.1. THE DAY 0 PROTOCOL (GROUP 1) RESULTED INTO A SIGNIFICANTLY ($P<0.01$) LOWER TOTAL NUMBER OF CL'S (4.0±3.5) BEING PRODUCED PER DOE, COMPARED TO GROUP 2 AND 3. THE DIFFERENCES IN THE MEAN NUMBER OF CL'S BETWEEN GROUP 2 (14.5±4.6) AND GROUP 3 (16.5±5.9) WERE NOT STATISTICALLY DIFFERENT.

THE MEAN TOTAL NUMBER OF STRUCTURES (FERTILISED AND UNFERTILISED OVA) RECOVERED FROM GROUP 2 (8.4±7.7) AND GROUP 3 (11.4±7.3) WERE ALSO RELATIVELY SIMILAR. THE MEAN TOTAL NUMBER OF STRUCTURES RECOVERED FROM GROUP 1 (1.4±0.5) WAS HOWEVER SIGNIFICANTLY ($P<0.05$) LOWER, COMPARED TO GROUP 3 (11.4±7.3). THIS MEAN FROM THE DAY 0 GROUP, HOWEVER DID NOT DIFFER SIGNIFICANTLY WHEN COMPARED TO GROUP 2 (8.4±7.7). THE VARIATION RECORDED FOR ANIMALS IN THE GROUP 2 WAS VERY HIGH.

CONCERNING THE TOTAL NUMBER OF EMBRYOS RECOVERED, THE DAY 0 PROTOCOL RESULTED IN A SIGNIFICANTLY ($P<0.01$) LOWER MEAN NUMBER OF EMBRYOS BEING RECORDED, COMPARED TO GROUP 2 AND 3. ALTHOUGH THE GROUP 1 DOES DEMONSTRATED A LOWER RESPONSE TO SUPEROVULATION TREATMENT, THE MEAN NUMBER OF UNFERTILISED OVA WAS SIMILAR FOR ALL THE TREATMENT GROUPS. WITH RESPECT TO THE DEGENERATE EMBRYOS, GROUP 1 DID NOT SHOW ANY OCCURRENCE IN TERMS OF DEGENERATING EMBRYOS. THE MEAN NUMBER OF DEGENERATING

EMBRYOS WAS THUS SIMILAR FOR ALL 3 TREATMENT GROUPS. REGARDING THE FERTILISATION RATE AND THE NUMBER OF TRANSFERABLE EMBRYOS RECORDED, THE DAY 0 (GROUP 1) GROUP RESULTED IN THE COLLECTION OF ONLY ONE EMBRYO, WHICH WAS ALSO TRANSFERABLE, BUT DUE TO THE SINGLE VALUE THIS DATA WAS NOT ANALYSED. THERE WERE NO SIGNIFICANT DIFFERENCES WITH RESPECT TO THE FERTILISATION RATE AND THE MEAN NUMBER OF TRANSFERABLE EMBRYOS RECORDED BETWEEN GROUP 2 AND 3.

Table 6. 5 The mean (±SD) ovarian response in Boer goat does following different superovulatory regimes

Parameters	**Day 0 (Group 1)**	**CIDR/PGF2α/FSH (Group 2)**	**CIDR/FSH (Group 3)**
No. of does flushed	5	8	7
No. of ovulations (total CL's/donor)	4.0 ± 3.5^{a}	14.5 ± 4.6^{b}	16.5 ± 5.9^{b}
Total number of structures recovered per doe flushed (unfertilised ova & embryos)	1.4 ± 0.5^{c}	8.4 ± 7.7^{cd}	11.4 ± 7.3^{d}
Total number of embryos recovered /donor	0.2 ± 0.4^{a}	13.2 ± 4.1^{b}	11.5 ± 7.9^{b}
Fertilisation rate (%)	-	98.9 ± 2.5^{a}	83.3 ± 40.8^{a}
Total number of unfertilised ova/donor	1.2 ± 0.8^{a}	0.2 ± 0.4^{a}	1.8 ± 4.5^{a}
Total number of degenerate embryos/donor	0^{a}	1.2 ± 1.6^{a}	2.3 ± 3.5^{a}
Total number of transferable embryos/donor	1.0^{a}	12.0 ± 3.3^{a}	9.2 ± 6.9^{a}

^{ab} Values within the same row with different superscripts differ significantly (P<0.01

^{cd} Values within the same row with different superscripts differ significantly P<0.05)

6.3.3. The effect of age in response to superovulation

Table 6.2 depicts the oestrous and ovarian response of the young and adult Boer goat does following superovulation. The time interval from CIDR removal to the onset of oestrus in adult does was significantly ($P<0.01$) shorter than that recorded in the young does. However, age did not have any effect on the duration of the induced oestrous period. The mean duration of oestrus was 19.4±11.2h and 24.9±4.8h for young and adult does respectively. The variation between animals with respect to the duration of the induced oestrous period was also higher in the young does. The mean number of CL's, structures and embryos recovered were significantly ($P<0.01$) higher in the adult does, compared to the young does. No recovery of unfertilised ova was recorded in the young does and the fertilisation rate and mean number of unfertilised ova did not differ between the young and adult does. Thus, age as such had no effect on the mean number of degenerate embryos per donor recorded - with a mean of 2.1±3.2 and 5.1±4.3 for the young and adult does respectively. The mean number of transferable embryos in the adult does (15.8±6.4) was however significantly ($P<0.01$) higher, than in the young does (9.5±3.7).

Table 6. 6 The mean (±SD) effect of age on the response of Boer goat does to superovulation treatment

Parameters	**Young**	**Adult**
No. of does	7	9
Time to onset of oestrus (h)	$32.0±4.6^{a}$	$24.0±4.0^{b}$
Duration of oestrus (h)	$19.4±11.2^{a}$	$24.9±4.8^{a}$
No. of ovulations (total CL's/donor)	$13.7±3.8^{a}$	$19.8±4.8^{b}$
Total number of structures recovered per doe flushed (unfertilised ova & embryos)	$11.7±5.0^{a}$	$21.3±3.9^{b}$
Total number of embryos recovered /donor	$11.7±±5.0^{a}$	$20.9±4.5^{b}$
Fertilisation rate (%)	$100.0±0^{a}$	$97.4±5.2^{a}$
Total number of unfertilised ova/donor	0^{a}	$0.4±0.8^{a}$
Total number of degenerate embryos/donor	$2.1±2.3^{a}$	$5.1±4.3^{a}$
Total number of transferable embryos/donor	$9.6±3.7^{a}$	$15.7±6.4^{b}$

[ab] Values within the same row with different superscripts differ significantly ($P<0.01$)

6.3.4. The effect of repeated superovulation treatment

THE EFFECT OF REPEATED SUPEROVULATION TREATMENT ON OVARIAN RESPONSE IS SET OUT IN TABLE 6.3. THE TIME FROM CIDR REMOVAL TO ONSET OF OESTRUS WAS NOT AFFECTED BY REPEATED SUPEROVULATION TREATMENT IN THE ADULT DOES, WITH AN OVERALL MEAN OF 30.9±6.3H IN TERMS OF OESTROUS RESPONSE TIME. THE DURATION OF THE INDUCED OESTROUS PERIOD ON THE OTHER HAND, WAS SIGNIFICANTLY (P<0.05) AFFECTED (LONGER) BY REPEATED TREATMENT. DOES SUPEROVULATED FOR THE FIRST TIME SHOWED A SHORTER MEAN DURATION OF OESTRUS (20.8±10.1H), WHEN COMPARED TO THOSE REPEATEDLY SUPEROVULATED (30.4±6.7 H). REPEATED SUPEROVULATION DID NOT DEMONSTRATE AN EFFECT ON THE TOTAL NUMBER OF CL'S PRODUCED PER DOE. HOWEVER, THE MEAN NUMBER OF STRUCTURES RECOVERED WAS SIGNIFICANTLY (P<0.05) LOWER IN THE REPEAT-TREATED DOES (6.0±8.7), COMPARED TO DOES SUPEROVULATED FOR THE FIRST TIME (11.7±5.0). THE VARIATION BETWEEN ANIMALS CONCERNING THE TOTAL NUMBER OF STRUCTURES RECOVERED PER DOE WAS VERY HIGH, RANGING BETWEEN 0 AND 19, WITH A COEFFICIENT OF VARIATION OF 72%. SIMILARLY, THE MEAN NUMBER OF EMBRYOS RECOVERED PER DOE WAS SIGNIFICANTLY (P<0.05) HIGHER (12.9±5.0) IN DOES SUPEROVULATED FOR THE FIRST TIME, COMPARED TO THOSE HAVING BEEN SUPEROVULATED REPEATEDLY (3.8±8.4). THE VARIATION IN THE EMBRYO RECOVERY RATE RECORDED WAS ALSO HIGHER BETWEEN ANIMALS IN THE GROUP TREATED REPEATEDLY.

THE MEAN NUMBER OF UNFERTILISED OVA PER DONOR WAS ALSO SIGNIFICANTLY (P<0.05) HIGHER IN REPEATEDLY-TREATED DOES (5.5±7.8), COMPARED TO THE 0.1±0.3 FOR DOES SUPEROVULATED FOR THE

FIRST TIME. THIS RESULTED IN A SIGNIFICANTLY ($P<0.05$) LOWER FERTILISATION RATE BEING OBTAINED IN THE REPEATEDLY-TREATED DOES (50.0±70.7%), COMPARED TO DOES SUPEROVULATED FOR THE FIRST TIME (99.4±1.9%) MOREOVER, THE VARIATION RECORDED WAS HIGHER BETWEEN ANIMALS IN THE REPEATEDLY-TREATED GROUP. ALTHOUGH DOES WHICH HAD BEEN REPEATEDLY SUPEROVULATED DID NOT LEAD TO THE HARVEST OF DEGENERATE EMBRYOS, THE MEAN NUMBER OF DEGENERATE EMBRYOS DID NOT DIFFER SIGNIFICANTLY BETWEEN THE FIRST AND REPEATEDLY SUPEROVULATED DOES. THE NUMBER OF TRANSFERABLE EMBRYOS RECORDED WAS SIGNIFICANTLY ($P<0.05$) LOWER IN THE REPEATEDLY TREATED DOES, COMPARED TO THEIR COUNTERPARTS.

Table 6. 7 The mean (±SD) effect of repeated superovulation treatment on ovarian activity in Boer goat does

Parameters	**First time superovulation**	**Repeated superovulation**
No. of does	10	5
Time to onset of oestrus (h)	32.8 ± 7.0^{a}	27.2 ± 4.4^{a}
Duration of oestrus (h)	20.8 ± 10.1^{a}	30.4 ± 6.9^{b}
No. of ovulations (total CL's/donor)	14.8 ± 4.5^{a}	16.8 ± 7.0^{a}
Total number of structures recovered per doe flushed (unfertilised ova & embryos)	11.7 ± 5.0^{a}	6.0 ± 8.7^{b}
Total number of embryos recovered /donor	12.9 ± 4.9^{a}	3.8 ± 8.5^{b}

Total number of unfertilised ova/donor	0.1±0.3[a]	5.5±7.8[b]
Fertilisation rate (%)	99.4±1.9[a]	50.0±70.7[b]
Total number of degenerate embryos/donor	2.2±2.9[a]	0±0[a]
Total number of transferable embryos/donor	10.7±4.0[a]	3.8±8.5[b]

[ab] Values within the same row with different superscripts differ significantly ($P<0.05$)

6.3.5. Serum progesterone concentration in donor does

THE MEAN SERUM PROGESTERONE CONCENTRATIONS FOLLOWING 3 DIFFERENT SUPEROVULATION PROTOCOLS FROM SYNCHRONISATION PERIOD TO FLUSHING ARE SET OUT IN FIGURE 6.1. THE MEAN SERUM PROGESTERONE CONCENTRATION FOR THE 3 GROUPS FOLLOWED A SIMILAR TREND FROM CIDR INSERTION TO EMBRYO FLUSHING. THE MEAN SERUM PROGESTERONE CONCENTRATION FOR THE 3 GROUPS INCREASED AFTER CIDR INSERTION AND WAS SIGNIFICANTLY ($P<0.01$) LOWER IN THE DAY 0-TREATED DOES (GROUP 1) ON DAY 4 FOLLOWING CIDR INSERTION COMPARED TO THE OTHER 2 GROUPS. AT CIDR REMOVAL (DAY 7 IN DAY 0 GROUP AND DAY 17 IN GROUP 2 AND 3 FOLLOWING CIDR INSERTION) THE MEAN SERUM PROGESTERONE CONCENTRATION WAS SIMILAR IN ALL TREATMENT GROUPS. THE MEAN PROGESTERONE CONCENTRATIONS INCREASED IN THE 3 GROUPS POST OESTRUS AND WERE HIGHEST ON THE DAY OF FLUSHING (DAY 6 FOLLOWING AI). NEITHER AGE NOR REPEATED SUPEROVULATION TREATMENT HAD AN EFFECT ON THE SERUM PROGESTERONE CONCENTRATION FROM CIDR INSERTION TO EMBRYO FLUSHING.

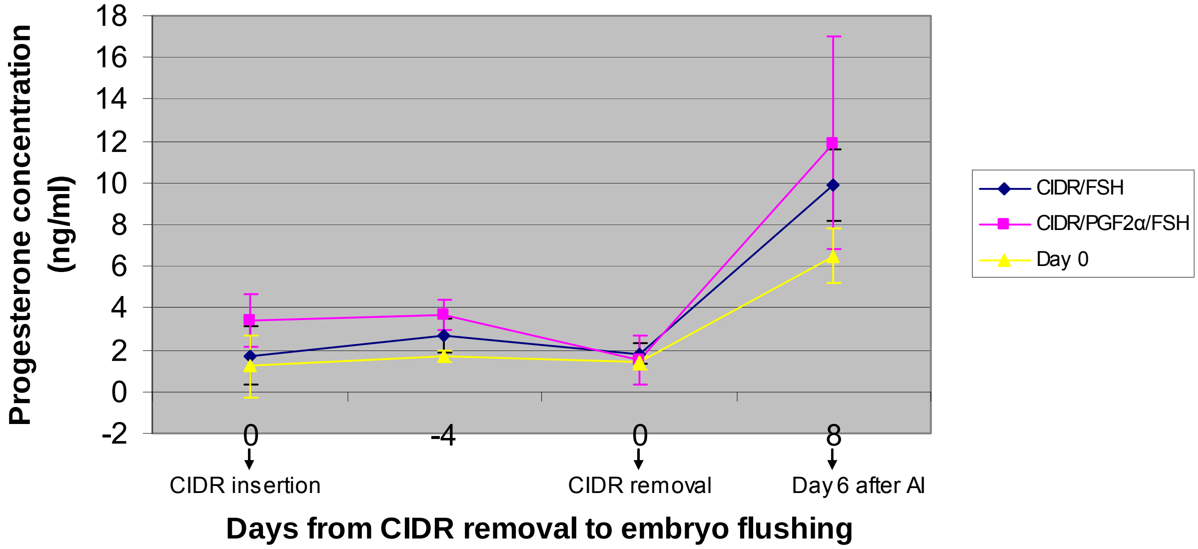

Figure 6. 3 Mean serum progesterone concentration following 3 superovulation protocols in Boer goat does

6.3.6. Recipient doe response

6.3.6.1. Ovarian response

THE OESTROUS RESPONSE OF THE RECIPIENT DOES FOLLOWING THE DIFFERENT SYNCHRONISATION REGIMES IS SET OUT IN TABLE 6.4, WITH THE DISTRIBUTION OF THE ONSET OF OESTRUS FOLLOWING CIDR REMOVAL BEING ILLUSTRATED IN FIGURE 6.2. ONLY ONE (11.1%) OF THE DOES FROM GROUP A DID NOT SHOW OVERT SIGNS OF OESTRUS, WHILE 8 (88.9%) RESPONDED TO OESTROUS SYNCHRONISATION. THIS DOE NOT DEMONSTRATING OESTRUS DID HOWEVER HAVE AN ACTIVE CL FOLLOWING LAPAROSCOPIC INSPECTION OF THE OVARIES BEFORE EMBRYO TRANSFER. ALL DOES (100.0%) FROM GROUP B AND C EXHIBITED SIGNS OF OESTRUS FOLLOWING OESTROUS SYNCHRONISATION.

ALTHOUGH THE OCCURRENCE OF OESTRUS WAS NOT AFFECTED BY THE SPECIFIC SYNCHRONISATION TREATMENT IMPLEMENTED, THE ONSET AND DURATION OF THE INDUCED OESTROUS PERIOD WAS SIGNIFICANTLY (P<0.05) AFFECTED BY TREATMENT. THE FIRST DOES TO SHOW OESTRUS WERE FROM GROUP C - 24H FOLLOWING CIDR WITHDRAWAL. DOES FROM GROUP B STARTED EXHIBITING SIGNS OF OESTRUS 8H LATER, WHILE THE DOES FROM GROUP A ON THE OTHER HAND, ONLY STARTED SHOWING SIGNS OF OESTRUS 40H FOLLOWING CIDR REMOVAL (FIGURE 5.2). DOES FROM GROUP A (42.0±3.7H) THUS EXHIBITED A LONGER (P<0.05) TIME INTERVAL FROM CIDR WITHDRAWAL TO THE ONSET OF OESTRUS, COMPARED TO GROUP C DOES (32.0±8.6H). THERE WAS HOWEVER, NO SIGNIFICANT DIFFERENCE WITH RESPECT TO THE TIME INTERVAL FROM CIDR REMOVAL TO THE ONSET OF OESTRUS BETWEEN GROUP A AND B, WHILE GROUP B AND C DOES RECORDED SIMILAR TIMES FROM CIDR WITHDRAWAL TO THE ONSET OF OESTRUS. THE DURATION OF THE INDUCED OESTROUS PERIOD WAS SIGNIFICANTLY (P<0.05) SHORTER IN GROUP B (19.0±13.5H), WHEN COMPARED TO GROUP A (39.0±15.1H), WITH NO SIGNIFICANT DIFFERENCE BEING OBSERVED BETWEEN GROUP B AND C, REGARDING THE DURATION OF THE INDUCED OESTROUS PERIOD.

Table 6. 8 Mean (±SD) oestrous response in Boer goats following different synchronisation treatments

Treatment	n	Oestrous response (%)	Time to oestrus (h)	Duration of oestrus (h)
Group A	9	88.9	42.0 ± 3.7^{a}	39.0 ± 15.1^{a}
Group B	8	100	39.0 ± 6.7^{ab}	19.0 ± 13.5^{b}
Group C	8	100	32.0 ± 8.6^{b}	29.0 ± 11.3^{ab}

Overall	25	96.0	37.7±6.6	29.0±13.4

[ab] Values within the same column with different superscripts differ significantly (P<0.05)

The mean serum progesterone concentration in recipient does did not differ significantly in the 3 groups on the day of flushing (7.2±3.1, 10.1±5.4 and 11.5±7.4ng/ml for Group A, B and C , respectively).

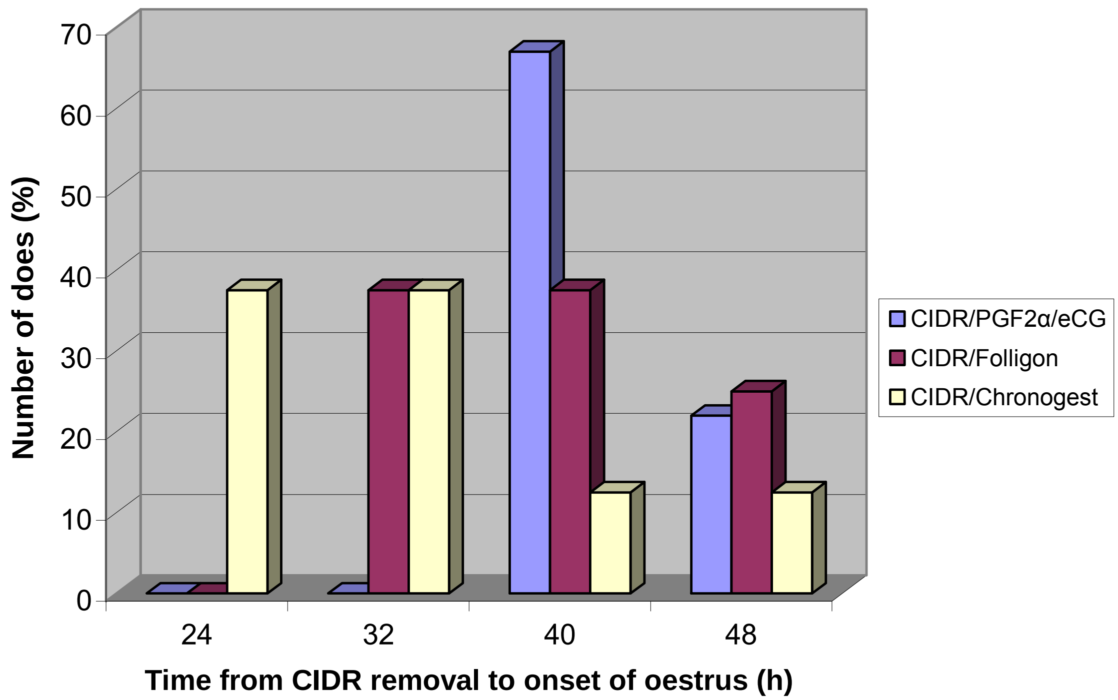

Figure 6. 4 Distribution of time from CIDR removal to onset of oestrus in recipient Boer goat does following different synchronisation protocols

6.3.4.2. Pregnancy rate

DURING LAPAROSCOPIC OVARIAN INSPECTION BEFORE EMBRYO TRANSFER, 6 DOES (3, 2 AND 1 FROM GROUP A, GROUP B, AND GROUP C, RESPECTIVELY) HAD NO CL'S VISIBLE - AS A RESULT NO EMBRYOS WERE TRANSFERRED TO THESE DOES. REGARDLESS OF THE EMBRYOS TRANSFERRED, THE PREGNANCY RATE RECORDED 40 DAYS FOLLOWING EMBRYO

TRANSFER FOR THE 3 SYNCHRONISATION GROUPS WAS 42.9%, 33.3% AND 33.3% FOR GROUP C, GROUP B AND GROUP A, RESPECTIVELY. WHEN DATA WAS POOLED FOR THE TYPE OF EMBRYOS TRANSFERRED, OF THE 9 RECIPIENTS WHICH RECEIVED FRESH EMBRYOS, ONLY 4 (44.4%) DOES WERE DIAGNOSED AS PREGNANT, WHILE ONLY 3 (30.0%) RECIPIENT DOES WERE PREGNANT FOLLOWING THE TRANSFER OF FROZEN-THAWED EMBRYOS - OUT OF A TOTAL OF 10 RECIPIENTS.

6.4. Discussion

6.4.1. Donor oestrous response

THE PROGESTAGEN SYNCHRONISATION TREATMENT PRECEDING SUPEROVULATION IN GROUP 1 (DAY 0 PROTOCOL) DOES WAS PERFORMED FOR A SHORT PERIOD OF TIME (7 DAYS), WITH EFFICIENT SYNCHRONISATION IN THE DOES, AS INDICATED BY THE OCCURRENCE OF OESTRUS. IT HAS PREVIOUSLY BEEN OBSERVED THAT WHEN GOATS ARE PRIMED WITH PROGESTERONE FOR A SHORTER PERIOD OF TIME THEY GENERALLY EXHIBIT A MORE COMPACT OESTRUS (OLIVEIRA ET AL., 2001). THE OESTROUS RESPONSE HAS BEEN REPORTED TO BE EVEN HIGHER (ALMOST 100%) FOLLOWING SHORT PROGESTAGEN PRIMING WHEN PGF2A WAS ADMINISTRATED AT THE TIME OF INTRAVAGINAL PROGESTAGEN DEVICE INSERTION. THIS TREATMENT (PGF2A) BEING AIMED AT ENSURING LUTEOLYSIS OF ALL CL’S (RUBIANES & MENCHACA, 2003).

IN THE CURRENT TRIAL THE OESTROUS RESPONSE IN THE DAY 0 (GROUP 1) DONORS WAS 71.4%, WHICH WAS LOWER TO THAN 100% FOR SYNCHRONISATION WITH CIDR’S (SHORT AND LONG OESTROUS SYNCHRONISATION PERIODS) PROTOCOLS PREVIOUSLY RECORDED IN GOATS (OLIVEIRA ET AL., 2001; MOTLOMELO ET AL., 2002; ROMANO, 2004). THUS,

FOR A SHORT SYNCHRONISATION TREATMENT TO RESULT IN A HIGH OESTROUS RESPONSE, IT REQUIRES THE ADMINISTRATION OF PGF2A WHICH ENSURES LUTEOLYSIS. HOWEVER, THE OESTROUS RESPONSE IN THIS TRIAL IS IN LINE WITH THE 81% AND 70% REPORTED IN GOATS FOLLOWING A SHORT PROGESTAGEN SYNCHRONISATION PROTOCOL (OLIVEIRA ET AL., 2001; RUBIANES & MENCHACA, 2003). THE LOWER OESTROUS RESPONSE OBTAINED MAY BE ASCRIBED TO THE OMISSION OF AN ECG TREATMENT AT PROGESTAGEN REMOVAL IN THIS SHORT PROGESTAGEN TREATMENT PROTOCOL. IN A TRIAL WHERE A 70% OESTROUS RESPONSE WAS ALSO RECORDED, THE DOES WERE NOT INJECTED WITH ECG AT CIDR REMOVAL, SIMILAR TO THE PRESENT STUDY (OLIVEIRA ET AL., 2001). IT WAS ASSUMED THAT EXOGENOUS ECG TREATMENT WOULD BE SUPEROVULATORY INSIDE THE NATURAL BREEDING SEASON – AS THE BOER GOATS ARE HIGHLY RECEPTIVE AT THIS TIME OF THE YEAR.

THE TIME INTERVAL FROM CIDR REMOVAL TO THE ONSET OF OESTRUS RECORDED IN THE DAY 0 (GROUP 1) GROUP PROCEEDING SUPEROVULATION TREATMENT, WAS IN LINE WITH THE 39.8±6.3H AND 40.2±10.5H FOLLOWING A SHORT PROGESTERONE PRIMING TREATMENT REPORTED WHEN ALSO USING INTRAVAGINAL CIDR'S (MENCHACA ET AL., 2002; ROMANO, 2004). IN ADDITION, THE DURATION OF THE INDUCED OESTROUS PERIOD FOR THIS DAY 0 GROUP WAS SIMILAR TO THE 36.0±0.7H REPORTED FOLLOWING UTILISATION OF A SHORT PROGESTAGEN PROTOCOL, WITHOUT THE ADMINISTRATION OF A PGF2A TREATMENT, AS WELL AS 35.2±0.7H AND 39.2±10.9H FOLLOWING LONG PROGESTERONE PRIMING PROTOCOLS IN GOATS (OLIVEIRA ET AL., 2001; MOTLOMELO ET AL., 2002; ROMANO, 2004). THESE OBSERVATIONS SHOW THAT

SHORT PROGESTAGEN PRIMING DOES NOT REALLY AFFECT THE DURATION OF THE INDUCED OESTROUS PERIOD.

THE OESTROUS RESPONSE OF 100% RECORDED IN GROUP 2 AND 3 WAS SIMILAR TO THAT PREVIOUSLY REPORTED FOLLOWING OESTROUS SYNCHRONISATION AND SUPEROVULATION IN GOATS (SELVARAJU ET AL., 2003; ESPINOSA-MARQUEZ ET AL., 2004). THE OESTROUS RESPONSE, THE TIME INTERVAL FROM CIDR WITHDRAWAL TO THE ONSET OF OESTRUS AND THE DURATION OF THE INDUCED OESTROUS PERIOD WERE NOT AFFECTED BY THE TREATMENT WITH A PGF2A. THESE RESULTS THUS INDICATE NO BENEFICIAL ADVANTAGE FOR THE INCLUSION PROSTAGLANDIN IN A SUPEROVULATION PROTOCOL WHEN IMPLEMENTING A LONG PROGESTAGEN SYNCHRONISATION TREATMENT FOR BOER GOAT DOES DURING THE NATURAL BREEDING SEASON. THE TIME FROM CIDR WITHDRAWAL TO THE ONSET OF OESTRUS FOR GROUP 2 WAS LONGER WHEN COMPARED TO THE 27.6±3.5H REPORTED IN ALPINE GOATS FOLLOWING SYNCHRONISATION WITH PROGESTAGEN IN COMBINATION WITH PGF2A AND SUPEROVULATED WITH PFSH (BARIL & VALLET 1990).

THE LONGER TIME FROM CIDR REMOVAL TO THE ONSET OF OESTRUS IN THIS GROUP MAY BE ASCRIBED TO THE LONG PROGESTAGEN SYNCHRONISATION PROGRAMME. HOWEVER, THE EFFECT OF BREED AND NUTRITIONAL STATUS OF THE ANIMALS ARE FACTORS TO BE BORNE IN MIND. A SHORTER REACTION TIME FROM THE TERMINATION OF SYNCHRONISATION TO ONSET OF OESTRUS HAS BEEN RECORDED WHEN THE SYNCHRONISATION TREATMENT WAS PERFORMED FOR A PERIOD OF 9 DAYS (BARIL & VALLET 1990). THE TIME INTERVAL FROM CIDR REMOVAL TO THE ONSET OF OESTRUS IN

GROUP 3 (17 DAY CIDR + PFSH TREATMENT) WAS ALSO LONGER, COMPARED TO 24 TO 25.3H REPORTED IN ALPINE GOATS SYNCHRONISED WITH PROGESTAGEN AND SUPEROVULATED WITH FSH (SELGRATH ET AL., 1990). THE DIFFERENCES OBSERVED MAY BE ATTRIBUTED TO BREED AND SEASONAL DIFFERENCES. THE DURATION OF THE INDUCED OESTROUS PERIOD OF DOES IN GROUP 2 AND 3 ON THE OTHER HAND, WAS SHORTER, WHEN COMPARED TO THE 38.0±17.0H REPORTED IN BOER GOATS FOLLOWING OESTROUS SYNCHRONISATION WITH PROGESTAGENS AND SUPEROVULATED WITH PFSH WITHOUT THE ADDITION OF PGF2A IN THE PROTOCOL (GREYLING ET AL., 2002). THE DURATION OF THE INDUCED OESTROUS PERIOD RECORDED IN GROUP 2 AND 3 WAS SIMILAR TO THE OVERALL MEAN DURATION OF 24.5±4.6H REPORTED IN CHAPTER 5 (PARAGRAPH 5.3.1). THE SHORTER DURATION OF THE INDUCED OESTROUS PERIOD IN THIS STUDY MAY BE ATTRIBUTED TO THE UTILISATION OF A NUMBER OF YOUNG DOES IN THE TRIAL. MOREOVER, THE DURATION OF OESTRUS IN THE TWO TREATMENT GROUPS WAS ALSO SHORTER, EVEN WHEN COMPARED TO 35.2±0.7H AND 39.2±10.9H FOLLOWING OESTROUS SYNCHRONISATION WITHOUT SUPEROVULATION TREATMENT (MOTLOMELO ET AL., 2002; ROMANO, 2004). HOWEVER, IN THIS CASE THE SHORTER DURATION OF THE INDUCED OESTROUS PERIOD MAY DEMONSTRATE THE REDUCTION BEING INDUCED BY THE SUPEROVULATION TREATMENT AND THE HORMONAL INTERVENTION.

6.4.2. Donor superovulatory response

UNEXPECTEDLY, THE DAY 0 PROTOCOL (GROUP 1) RESULTED IN A LOWER NUMBER OF OVULATIONS PER DOE BEING INDUCED, WHEN COMPARED TO GROUP 2 AND 3, AS WELL AS THE 13.6±1.9 CL’S

RECORDED FOLLOWING A DAY 0 PROTOCOL IN GOATS (MENCHACA ET AL., 2002). THE LOWER OVULATION RATE OBTAINED FOLLOWING THE DAY 0 SUPEROVULATION PROTOCOL MAY BE ASCRIBED TO THE POOR RECRUITMENT AND DEVELOPMENT OF OVARIAN FOLLICLES TO THE OVULATORY STAGE OR INSUFFICIENT STIMULATION. THIS POOR RECRUITMENT OR STIMULATION OF OVARIAN FOLLICLES USUALLY OCCUR WHEN A DOMINANT FOLLICLE IS PRESENT AT THE TIME OF SUPEROVULATION INITIATION – THE DOMINANT FOLLICLE IS KNOWN TO SUPPRESS GROWTH AND RECRUITMENT OF NEW OVARIAN FOLLICLES (DRIANCOURT, 2001; SENGER, 2003). THIS OBSERVATION MAY THEREFORE, MEAN THAT THE SUPEROVULATORY TREATMENT IN THIS TRIAL MAY HAVE STARTED PRIOR TO OVULATION, AS THE TIME OF OVULATION WAS ESTIMATED. TO EMPHASIZE THIS ARGUMENT IT WAS ALSO OBSERVED VIA THE ULTROSONOGRAPHIC SCANNER, THAT THE NUMBER OF SMALL FOLLICLES TENDED TO INCREASE FROM 0 TO 24H AFTER OVULATION, WITH THE MAXIMUM NUMBER OF SMALL FOLLICLES BEING OBSERVED 36H FOLLOWING OVULATION (MENCHACA ET AL., 2002). IT MUST ALSO BE POINTED OUT THAT THE OCCURRENCE OF THE FIRST FOLLICULAR WAVE MAY DIFFER WITH DIFFERENT BREEDS, AS THESE ARE DETERMINED BY THE DURATION OF THE OESTROUS CYCLE. BASED ON THIS INFORMATION AND THE FACT THAT OVULATION AS SUCH WAS NOT SYNCHRONISED AND THE APPLICATION OF ECG AT CIDR REMOVAL WAS NOT PERFORMED, THESE RESULTS MAY HAVE BEEN ATTRIBUTED TO POOR TIMING OF OVULATION AND HENCE THE INITIATION OF THE SUPEROVULATORY TREATMENT. IT IS ASSUMED THAT IF THE SUPEROVULATORY TREATMENT COULD BE INITIATED 24H FOLLOWING

THE EXPECTED OVULATION TIME IT WOULD BE POSSIBLE TO CAPTURE ALL THE ANIMALS IMMEDIATELY AFTER OVULATION, AND THUS COINCIDING WITH THE PERIOD DURING WHICH THERE IS A HIGHER NUMBER OF SMALL FOLLICLES PRESENT. NONETHELESS, THE NUMBER OF CL'S FOLLOWING THE DAY 0 PROTOCOL (GROUP 1) WAS COMPARABLE TO THE 5.6±0.8 PER ANIMAL RECORDED IN SHEEP FOLLOWING A SIMILAR DAY 0 PROTOCOL (RUBIANES ET AL., 1997).

THE NUMBER OF OVULATIONS RECORDED IN GROUP 2 AND 3 DID NOT DIFFER MUCH AND WAS LOWER, WHEN COMPARED TO THE 21.3±6.5 AND 18.9±5.2 PER DOE RECORDED IN THE PREVIOUS CHAPTERS FOLLOWING A SUPEROVULATORY PROTOCOL AND SIMILAR TO THE ONE USED FOR GROUP 3 IN THIS TRIAL (TABLE 3.4 AND TABLE 5.2). THESE DIFFERENCES MAY BE ATTRIBUTED TO THE AGE OF THE DONOR DOES USED IN THE DIFFERENT TRIALS. IN THE CURRENT TRIAL MORE YOUNG MAIDEN DOES WERE MATED FOR THE FIRST TIME, COMPARED TO THE PREVIOUS TRIALS (WHERE MULTIPAROUS BOER GOAT DOES WERE GENERALLY USED). THIS PHENOMENON WAS BEEN CONFIRMED BY A SIMILAR SUPEROVULATORY RESPONSE OF 15.0±6.2 PER DONOR IN THE PREVIOUS TRIAL (TABLE 3.2) - WHERE THE MAJORITY OF DONORS WERE IN THEIR 2ND PARITY. HOWEVER, THE MEAN OVERALL OVULATION RATE OBTAINED IN THESE TWO PROTOCOLS ARE IN LINE WITH THE OVULATION RATE OBTAINED IN MOST GOAT BREEDS AND IS HIGHLY ACCEPTABLE (ARMSTRONG ET AL., 1983A; MAHMOOD ET AL., 1991; COGNIE ET AL., 2003; GONZALEZ-BULNES ET AL., 2003A).

THE DAY 0 PROTOCOL (GROUP 1) RESULTED IN A LOWER NUMBER OF STRUCTURES BEING RECOVERED, ALTHOUGH THE MEAN WAS

COMPARABLE WITH THAT (2.1±0.6) OBTAINED PER DONOR IN SHEEP FOLLOWING A SIMILAR DAY 0 PROTOCOL. IN GOATS THERE ARE LIMITED TRIALS INVOLVING THE EMBRYO RECOVERY SUCCESS RATES FOLLOWING A DAY 0 PROTOCOL. MORE RESEARCH IS WARRANTED TO FURTHER PERFECT THIS PARTICULAR ASPECT OF SUPEROVULATION AT A TIME JUST AFTER OVULATION. EVEN THOUGH THE NUMBER OF STRUCTURES RECOVERED FROM GROUP 1 WAS LOWER, THAN IN GROUP 3, IT WAS SIMILAR WHEN COMPARED TO GROUP 2. EVIDENT IS THE HIGH VARIATION IN THE NUMBER OF STRUCTURES RECORDED FOR THE INDIVIDUAL ANIMALS WITHIN GROUP 2 AND 3. THIS TENDS TO INDICATE THE IMPORTANCE OF MONITORING THE FOLLICULAR WAVES WHEN ADMINISTERING GONADOTROPHINS FOR THE PURPOSE SUPEROVULATION. THE HIGH VARIATION EXPRESSED COULD IMPLY THE VARIOUS ANIMALS WITHIN A TREATMENT GROUP BEING AT DIFFERENT STAGES OF THEIR OESTROUS CYCLES. WITH REGARD TO THE HIGH VARIATION OBSERVED IN THE NUMBER OF STRUCTURES RECORDED, A SUPEROVULATION PROTOCOL WITH AN ADDITIONAL PGF2A (GROUP 2) TO A LONG SYNCHRONISATION PROGESTAGEN TREATMENT IN GOATS SEEMED TO HAVE NO ADVANTAGE. THIS IS IN CONTRAST TO CATTLE WHERE PGF2A IS INCLUDED IN ALL PROTOCOLS AS IT IS KNOWN TO IMPROVE THE RECOVERY RATE (MAPLETOFT ET AL., 2002). HOWEVER, THE TIME OF PROSTAGLANDIN ADMINISTRATION AND THE DOSAGE USED COULD ALSO FACILITATE AND AFFECT THE RESPONSE INDUCED.

THE LOW NUMBER OF EMBRYOS RECOVERED IN GROUP 1 WAS EXPECTED, AS ONLY ONE DOE EXHIBITED OESTRUS FOLLOWING SUPEROVULATION. THIS OBSERVATION ALSO DEMONSTRATED A NEED

FOR FURTHER STUDIES IN PERFECTING THIS DAY 0 PROTOCOL, ESPECIALLY WHERE ULTRASONOGRAPHY EQUIPMENT IS NOT AVAILABLE. ON THE OTHER HAND, THE MEAN NUMBER OF EMBRYOS PER DONOR RECOVERED FROM THE GROUP 2 AND 3 ARE SIMILAR WITH A MEAN NUMBER OF EMBRYOS RECORDED OF 14.7±2.5, 14.3±0.5 AND 11.8±2.9 FOLLOWING SUPEROVULATION IN DAIRY GOATS, MURCIANO-GRANADINA AND JAKHRANA GOATS, RESPECTIVELY (PENDLETON ET AL., 1992; GONZALEZ-BULNES ET AL., 2003A; GOEL & AGRAWAL, 2005). THE MEAN NUMBER OF EMBRYOS RECOVERED IN THE CURRENT TRIAL IN GROUP 2 AND GROUP 3 WAS LOWER, WHEN COMPARED TO THE PREVIOUS TRIALS IN CHAPTER 3 AND 4, WHERE THE NUMBER OF EMBRYOS RECOVERED PER DOE WERE 16.5±6.1 (TABLE 3.4) AND 17.0±7.4 (TABLE 5.2). THESE DIFFERENCES MAY BE DUE TO A HIGHER NUMBER OF YOUNG DOES UTILISED IN THIS TRIAL AND THUS WEAKER OVARIAN RESPONSE OBTAINED, COMPARED TO THE PREVIOUS TRIALS. THIS ASPECT IS FURTHER EMPHASIZED BY THE MEAN NUMBER OF EMBRYOS (11.0±7.5) RECOVERED IN A PREVIOUS TRIAL (TABLE 3.2) FOLLOWING A PROTOCOL SIMILAR TO THAT IN GROUP 3 IN THIS STUDY AND WHERE MORE YOUNG DOES WERE USED - WHICH WAS COMPARABLE TO THE 13.2±4.1 AND 11.5±7.9 EMBRYOS OBTAINED IN THIS STUDY (FOR GROUP 2 AND 3, RESPECTIVELY). THE MEAN NUMBER OF TRANSFERABLE EMBRYOS OF ACCEPTABLE QUALITY IN GROUP 2 AND GROUP 3 WAS HIGHER, WHEN COMPARED TO THAT REPORTED IN OTHER GOAT BREEDS, WITH OR WITHOUT AN INJECTION OF PGF2A IN THE FSH SUPEROVULATION REGIME (MCNATTY ET AL., 1989; PENDLETON ET AL., 1992; GONZALEZ-BULNES ET AL., 2003A). THESE DIFFERENCES IN EMBRYO QUALITY MAY BE DUE TO DIFFERENCES IN THE RESPONSE TO

SUPEROVULATION AND THE HORMONAL ENVIRONMENT CREATED IN THE DIFFERENT BREEDS AND COULD ALSO INDICATE BOER GOAT DOES TO RESPOND BETTER THAN OTHER GOAT BREEDS TO EXOGENOUS HORMONAL SUPEROVULATION TREATMENTS. WHEN CONSIDERING THE OVARIAN RESPONSE INDUCED BY SUPEROVULATORY TREATMENT, THE DAY 0 PROTOCOL PERFORMED POORLY. HOWEVER, IT SHOULD BE POINTED OUT THAT THIS TRIAL WAS AIMED AT EVALUATING IF THIS DAY 0 PROTOCOL COULD BE USED WITHOUT PRIOR VISUAL OVARIAN INSPECTION USING THE ULTRASOUND TO DETERMINE THE EXACT TIME OF OVULATION. THUS, THE TIME OF OVULATION WAS ASSUMED TO BEGIN AT SUPEROVULATORY TREATMENT. IN ORDER TO BE ABLE TO UTILISE THIS PROTOCOL WITHOUT THE USE OF ULTRASONOGRAPHY, MORE TIME INTERVALS FOR INITIATING SUPEROVULATION TREATMENT NEED TO BE EVALUATED FOR AN ACCURATE ESTIMATE OF THE OVULATION TIME FOR SPECIFIC GOAT BREEDS. MOREOVER, THE OVULATION TIME NEEDS TO BE SYNCHRONISED MORE PRECISE TO ALLOW A BETTER PREDICTION OF OVULATION AND HENCE THE TIME TO START SUPEROVULATORY TREATMENT. HERE THE TREATMENT WITH GNRH TO INDUCE OVULATION COULD BE AN ALTERNATIVE, WITH OR WITHOUT THE USE OF ULTRASONOGRAPHY.

6.4.3. Effect of age on donor response

THE EFFECT OF DONOR AGE ON THE OVARIAN RESPONSE TO SUPEROVULATION IS AN ASPECT ALMOST ALWAYS UNDERESTIMATED IN MOST FARM ANIMAL SPECIES. HOWEVER, AGE COULD CONTRIBUTE GREATLY TO THE VARIATION IN OVARIAN RESPONSE TO SUPEROVULATION BETWEEN ANIMALS WITHIN THE SAME GROUP - AS ANIMALS OF DIFFERENT AGES HAVE BEEN SHOWN TO HAVE

DIFFERENT PHYSIOLOGICAL NEEDS AND RESPOND DIFFERENTLY (JAINUDEEN ET AL., 2000). IN GOATS THERE IS LIMITED INFORMATION RELATING TO THE EFFECT OF AGE ON OVARIAN RESPONSE TO SUPEROVULATION, COMPARED TO OTHER RUMINANTS. WHEN EVALUATING THE EFFECT OF AGE ON OESTROUS RESPONSE FOLLOWING SUPEROVULATION, YOUNGER DOES RECORDED A LONGER TIME INTERVAL FROM CIDR REMOVAL TO THE ONSET OF OESTRUS. SIMILAR RESULTS HAVE BEEN REPORTED IN SHEEP AND GOATS (DRION ET AL., 2001A; BARI ET AL. 2000).

FOR THE YOUNG DOES IT WAS THE FIRST TIME TO BE TREATED WITH EXOGENOUS HORMONES - THUS THEY MAY HAVE RESPONDED SLOWER. IT COULD IMPLY THAT THE HORMONAL THRESHOLD LEVEL IS HIGHER AND A GREATER STIMULUS IS NEEDED TO ELICIT AN OVARIAN RESPONSE. THE DURATION OF THE INDUCED OESTROUS PERIOD ALSO TENDED TO BE 5.5H SHORTER IN THE YOUNG DOES. HOWEVER, DUE TO A HIGH VARIATION BETWEEN ANIMALS WITHIN GROUPS, THESE DIFFERENCES WERE NOT SIGNIFICANT. IT WOULD THUS BE PURE SPECULATION TO COMMENT ON THIS PHENOMENON. THE RESULTS REGARDING THE OESTROUS RESPONSE IN THIS TRIAL ARE IMPORTANT, ESPECIALLY WHERE FIXED-TIME AI IS PERFORMED AS PART OF A MOET PROGRAMME.

THE RESPONSE TO SUPEROVULATION AS REFLECTED BY OVULATION RATE, TOTAL NUMBER OF STRUCTURES AND EMBRYOS COLLECTED WAS LOWER IN THE MAIDEN DOES. IN BOTH SHEEP AND GOATS POORER SUPEROVULATORY RESPONSES HAVE BEEN RECORDED IN YOUNGER FEMALES (DINGWALL ET AL., 1993; TORRES ET AL., 1987, MAHMOOD ET AL., 1991). SEVERAL FACTORS MAY CONTRIBUTE TO THIS TENDENCY. FIRSTLY, THE OVARIES OF YOUNG DOES

MAY NOT BE MATURE ENOUGH TO BE SENSITIZED BY EXOGENOUS GONADOTROPHIN TREATMENT, WHILE ON THE OTHER HAND, THE LOWER RESPONSE TO SUPEROVULATION MAY INDICATE A POOR RECRUITMENT OF FOLLICLES TO THE OVULATORY STAGE ON THE OVARY OF THE YOUNG DOES. POOR RECRUITMENT OR STIMULATION OF FOLLICLES COULD BE ATTRIBUTED TO THE SUPPRESSIVE EFFECT OF A DOMINANT FOLLICLE, WHICH IS KNOWN TO SECRETE STEROIDS (TOGETHER WITH INHIBIN), WHICH EVENTUALLY SUPPRESSES THE FOLLICULAR RECRUITMENT (DRIANCOURT, 2001; SENGER, 2003). THEREFORE MAIDEN DOES MAY BE MORE SENSITIVE TO THIS INHIBITORY EFFECT OF THE STEROIDS AND INHIBIN, COMPARED TO ADULT DOES.

THE FERTILISATION RATE OBTAINED WAS NOT AFFECTED BY AGE, WHICH IS IN CONTRAST TO AN OBSERVATION IN SHEEP, WHERE YOUNG EWES RECORDED A LOWER FERTILISATION RATE, COMPARED TO ADULT EWES (RANGEL-SANTOS ET AL., 1991). THESE RESULTS MAY INDICATE DIFFERENCES BEING SPECIE-SPECIFIC. ALTHOUGH YOUNG DOES IN THIS TRIAL RECORDED A HIGHER FERTILISATION RATE AND HARVESTED GOOD QUALITY EMBRYOS (BASED ON THE DEGENERATION RATE OF THE EMBRYOS) THE NUMBER OF TRANSFERABLE EMBRYOS WAS LOWER, WHEN COMPARED TO THE OLDER DOES. THE LOWER NUMBER OF ACCEPTABLE QUALITY EMBRYOS WAS MAINLY DUE TO A LOWER NUMBER OF EMBRYOS RECOVERED FROM THE YOUNG DOES.

6.4.4. Effect of repeated superovulation

THE TIME RESPONSE FROM CIDR REMOVAL TO THE ONSET OF OESTRUS FOR DOES SUPEROVULATED FOR THE FIRST TIME (32.8±7.0H) AND FOR THE 4TH TIME (27.2±4.4H), IS IN LINE WITH A TIME OF 32.0±3.5H AND 27.6±3.5H PREVIOUSLY REPORTED IN OTHER GOAT

BREEDS FOLLOWING SUPEROVULATION (BARIL & VALLET, 1990, PENDLETON ET AL., 1992). THE SHORTER DURATION OF THE INDUCED OESTROUS PERIOD FOR DOES SUPEROVULATED FOR THE FIRST TIME COMPARED TO DOES REPEATEDLY TREATED, MAY BE DUE TO AN AGE FACTOR IN THIS TRIAL, AS THE MAJORITY OF DOES SUPEROVULATED FOR THE FIRST TIME WERE MAIDEN DOES. FROM TABLE 6.2, IT IS EVIDENT THAT YOUNGER DOES RECORDED A SHORTER DURATION OF THE INDUCED OESTROUS PERIOD. THE NUMBER OF OVULATIONS RECORDED WAS NOT AFFECTED BY THE REPEATED SUPEROVULATION TREATMENT. THIS OBSERVATION CONTRADICTS PREVIOUS FINDINGS IN GOATS WHERE REPEATED SUPEROVULATION HAS BEEN OBSERVED TO REDUCE THE NUMBER OF CL'S OBTAINED (NUTI ET AL., 1987; BARIL ET AL., 1989) - ESPECIALLY FOLLOWING THE 4TH AND 5TH SUPEROVULATION TREATMENT AS WAS THE CASE IN THIS STUDY. LACK OF DIFFERENCES IN THE NUMBER OF CL'S INDUCED IN THIS TRIAL MAY MAINLY BE ATTRIBUTED TO AN AGE EFFECT. THERE WERE MORE YOUNG DOES AVAILABLE IN THIS TRIAL WHICH WAS SHOWN TO LEAD TO A LOWER SUPEROVULATORY RESPONSE. THE REDUCED NUMBER OF CL'S FROM REPEATEDLY-TREATED DOES WAS ALSO OBSCURED BY THE LOWER RESPONSE IN THE YOUNG DOES. FROM THE PREVIOUS STUDIES UTILISING MULTIPAROUS BOER GOAT DOES, THE AVERAGE MEAN NUMBER OF CL'S FOR THE 1ST AND 3RD SUPEROVULATORY TREATMENT WAS 18.8±6.5 AND 18.9±5.3 PER DOE, RESPECTIVELY (TABLE 3.4 AND TABLE 5.2). THESE MEANS WERE HIGHER WHEN COMPARED TO THIS TRIAL, INDICATING A POSSIBLE REDUCED RESPONSE FROM REPEATED TREATMENT OF DOES BY APPROXIMATELY TWO CL'S PER DOE.

THE NUMBER OF STRUCTURES RECOVERED (RECOVERY RATE), EMBRYOS AND TRANSFERABLE EMBRYOS COLLECTED FOLLOWING REPEATED SUPEROVULATION TREATMENT WERE ALSO LOWER. THE LOWER RESPONSE TO SUPEROVULATION TREATMENT FOLLOWING REPEATED TREATMENT MAY BE DUE TO REDUCED ACTIVITY OF THE EXOGENOUS GONADOTROPHIN APPLIED DUE TO IT BEING NEUTRALISED BY ANTIBODIES GENERATED FROM PREVIOUS GONADOTROPHIN TREATMENTS. THE HIGHER OCCURRENCE OF ANTIBODIES FOLLOWING REPEATED SUPEROVULATION TREATMENT WITH FSH IN GOATS GENERALLY OCCURS AFTER THE 3^{RD} TREATMENT. THESE FSH ANTIBODIES FOLLOWING REPEATED SUPEROVULATION TREATMENT IN GOATS HAS BEEN HIGHLY CORRELATED WITH A REDUCED SUPEROVULATORY RESPONSE (BECKERS ET AL., 1990; REMY ET AL., 1991). THE REDUCTION IN SUPEROVULATORY RESPONSE CAN ALSO BE DUE TO THE FORMATION OF POST OPERATIVE ADHESIONS FOLLOWING REPEATED SURGICAL COLLECTION OF EMBRYOS. THIS FACTOR HAS ALSO BEEN SHOWN TO REDUCE THE OVULATION RATE AND THE NUMBER OF EMBRYOS RECOVERED (AL-KAMALI ET AL., 1985; COGNIE, 1999).

THE HIGHER NUMBER OF UNFERTILISED OVA OBTAINED FROM REPEATEDLY TREATED DOES HAS ALSO BEEN EMPHASIZED BY A POOR FERTILISATION RATE. THIS PHENOMENON MAY ALSO BE ATTRIBUTED TO THE FORMATION OF FSH ANTIBODIES FOLLOWING REPEATED SUPEROVULATION TREATMENT. THE ANTIBODIES FORMED CAN INFLUENCE THE HORMONAL BALANCE AND HENCE AFFECT THE DEVELOPMENTAL COMPETENCE OF THE OOCYTES. THUS, IF THE OOCYTES HAVE IMPROPER DEVELOPMENTAL

COMPETENCE, THE END RESULT WOULD EITHER BE REDUCED FERTILISATION CAPACITY OR IMPROPER DEVELOPMENT OF THE EMBRYOS FOLLOWING FERTILISATION. THE RESULTS FROM THE CURRENT STUDY INDICATE THAT DONOR GOATS CAN BE REPETITIVELY TREATED WITH GONADOTROPHINS FOR AT LEAST THREE MOET PROGRAMMES.

6.4.5. Serum progesterone levels in donor does

THE LOW SERUM PROGESTERONE CONCENTRATION RECORDED ON DAY 4 OF PROGESTAGEN TREATMENT IN DAY 0 GROUP COULD BE INDUCED BY DEGENERATION OF THE ENDOGENOUS CL'S, DUE TO THE PROSTAGLANDIN TREATMENT APPLIED AT CIDR INSERTION – AS PGF2A IS A LUTEOLYTIC AGENT (SENGER, 2003). THE POOR RESPONSE OF THE DAY 0 PROTOCOL CANNOT BE LINKED TO THE CONCENTRATION OF PROGESTERONE, AS ALL GROUPS HAD SIMILAR MEAN PROGESTERONE CONCENTRATION AT CIDR REMOVAL. THE MEAN SERUM PROGESTERONE CONCENTRATION IN GOATS DO NOT SEEM TO BE AFFECTED BY AGE OR REPEATED SUPEROVULATION.

6.4.6. Recipient response

THE OVERALL OESTROUS RESPONSE OF 96.0% FOR THE RECIPIENT DOES SHOW THE OESTROUS SYNCHRONISATION PROTOCOLS USED TO BE EFFICIENT IN SYNCHRONISING OESTRUS. THE OCCURRENCE OF SILENT OESTRUS IS A PHENOMENON IN SHEEP AND GOATS THAT IS USUALLY OBSERVED IN YOUNG ANIMALS OR AT THE BEGINNING OR END OF THE BREEDING SEASON (GORDON, 1997; RIVERA ET AL., 2003). IN THIS STUDY, AGE MAY BE A FACTOR, AS A DOE THAT EXHIBITED THIS BEHAVIOUR, WAS GENERALLY A MAIDEN DOE. THE DOES IN GROUP C OR CONTROLS RECORDED A SHORTER TIME FROM CIDR REMOVAL TO THE ONSET

OF OESTRUS, COMPARED TO THE OTHER TWO PROGESTAGEN TREATMENT GROUPS. THIS INTERVAL IS SIMILAR TO THAT (27.2±0.4H) REPORTED IN BOER GOATS FOLLOWING A SIMILAR PROTOCOL OR WHEN INTRAVAGINAL PROGESTAGEN SPONGES WERE USED TO SYNCHRONISE OESTRUS, COMBINED WITH ECG AND A PGF2A INJECTION - OR THE TREATMENT WITH EACH OF THESE TWO HORMONES 24 TO 48H BEFORE OR AT PROGESTAGEN REMOVAL IN GOATS (FREITAS ET AL., 1997; REGUEIRO ET AL., 1999; MOTLOMELO ET AL., 2002; ROMANO, 2004).

THE SHORTER TIME FROM PROGESTAGEN REMOVAL TO THE ONSET OF OESTRUS HAS BEEN SUGGESTED TO BE INDUCED BY A HIGHER DOSE OF ECG USED AT PROGESTAGEN REMOVAL, WHICH WILL ULTIMATELY INDUCE GREATER OVARIAN ACTIVITY (MOSTLY MULTIPLE OVULATIONS) IN GOATS. THE ECG USED IN GROUP B (FOLLIGON) MAY HAVE A LOWER POTENCY OR EFFICIENCY COMPARED TO THE TYPE OF ECG USED IN GROUP C (CHRONOGEST). HENCE THE GROUP B DOES TOOK A LONGER TIME FROM CIDR REMOVAL TO THE ONSET OF OESTRUS THAN THE GROUP C DOES. THE ONSET OF OESTRUS IN GROUP A WAS IN AGREEMENT WITH PREVIOUS FINDINGS WHERE CIDR'S WERE USED TOGETHER WITH PGF2A OR INTRAVAGINAL SPONGES WITH ECG AND PGF2A TREATMENT FOLLOWING A SHORT PROGESTAGEN PROTOCOL (REGUEIRO ET AL., 1999; FONSECA ET AL., 2005). THESE RESULTS SUGGEST THAT THE ADDITION OF PGF2A IN SYNCHRONISATION PROTOCOLS COULD LEAD TO DOES TAKING A LONGER REACTION TIME FROM PROGESTAGEN REMOVAL TO THE ONSET OF OESTRUS. THIS COULD BE DUE TO EXCESS PROSTAGLANDIN BEING PRESENT FOR A PERIOD OF TIME – THUS DELAYING CL FORMATION AND THE OCCURRENCE OF OESTRUS.

THE DURATION OF THE INDUCED OESTROUS PERIOD TENDED TO BE SHORTER WHEN THE SYNCHRONISATION PROTOCOL ENTAILED THE USE OF CIDR'S AND GONADOTROPHINS AND LONGER WITH THE INCLUSION OF PGF2A TREATMENT. THE ADDITION OF PGF2A WAS DONE TO ENSURE COMPLETE LUTEOLYSIS AT PROGESTAGEN WITHDRAWAL, ESPECIALLY WHERE A SHORT PROGESTAGEN PROTOCOL IS USED (CORTEEL ET AL., 1988; FREITAS ET AL., 1997; FONSECA ET AL., 2005).THE DURATION OF THE INDUCED OESTROUS PERIOD IN GROUP A OBTAINED IN THIS STUDY WAS SIMILAR TO A PERIOD OF 39.2±10.9H REPORTED FOR NUBIAN GOATS SYNCHRONISED WITH CIDR, FOLLOWED BY A PGF2A INJECTION AT CIDR REMOVAL (ROMANO, 2004). THE LONGER DURATION OF THE INDUCED OESTROUS PERIOD WHEN PGF2A TREATMENT WAS INCLUDED IN A SYNCHRONISATION PROTOCOL MAY IMPLY THAT ACCESS PROSTAGLANDIN PREVENTS OR RETARDS THE FORMATION OF A NEW CL. THE LONGER DURATION OF THE OESTROUS PERIOD MAY BE BENEFICIAL, ESPECIALLY WHERE NATURAL MATING IS UTILISED, TO ALLOW ALL DOES SYNCHRONISED TO BE SERVICED. HOWEVER, THE FERTILITY RATE FOLLOWING LONGER OESTRUS DURATION NEEDS MORE INVESTIGATION AS COULD IMPLY CHANGES IN THE TIME OF THE PRE-OVULATORY LH PEAK AND OVULATION.

6.4.7. Pregnancy rate

MORE DOES FROM THE TREATMENT GROUP A AND B DEMONSTRATED NO CL'S, WHILE ONLY ONE DOE FROM GROUP C HAD NO CL DURING OVARIAN INSPECTION PRIOR TO EMBRYO TRANSFER. THIS OBSERVATION GAVE THE GROUP C SYNCHRONISATION REGIME AN ADVANTAGE OVER THE OTHER TWO GROUPS. IN GROUP C (CHRONOGEST AS ECG SOURCE), THIS GONADOTROPHIN LED TO THE

DEVELOPMENT OF MORE CL'S, WHICH COULD SUGGEST MORE PREGNANCIES. ALTHOUGH THERE WAS NO SIGNIFICANT DIFFERENCE REGARDING THE PREGNANCY RATE BETWEEN THE THREE GROUPS, GROUP C DOES RECORDED A HIGHER PREGNANCY RATE. THE PREGNANCY RATES OBTAINED IN THIS TRIAL FOLLOWING FRESH AND FROZEN-THAWED TRANSFERRED GOAT BLASTOCYSTS WERE LOWER THAN E.G. THE 60.4% AND 61.0% RECORDED FOR FRESH AND FROZEN EMBRYOS, RESPECTIVELY IN CASHMERE/ANGORA CROSS GOATS (LI ET AL., 1990). THE LOWER PREGNANCY RATES RECORDED IN THIS STUDY MAY BE ATTRIBUTED TO THE AGE OF THE RECIPIENTS USED IN THE CURRENT TRIAL - WITH MORE MAIDEN DOES BEING USED. THIS IS FURTHER EMPHASIZED BY THE HIGHER PREGNANCY RATE OF 85.7% AND 50.0% OBTAINED FOLLOWING THE TRANSFER OF FRESH AND FROZEN-THAWED EMBRYOS, IN THE PREVIOUS TRIALS WITH MULTIPAROUS BOER GOAT DOES BEING UTILISED AS RECIPIENTS (TABLE 4.3). IN SHEEP HOWEVER, PARITY AND AGE OF THE RECIPIENT DID NOT INFLUENCE THE SURVIVAL RATE OF THE EMBRYOS (BARI ET AL., 2003). THE PREGNANCY RATE OF A 44.4% RECORDED IN THE CURRENT STUDY FOLLOWING THE TRANSFER OF FRESH EMBRYOS IS SIMILAR TO 40% PREGNANCY RATE RECORDED IN SAANEN AND NUBIAN GOATS FOLLOWING THE TRANSFER OF MICROINJECTED EMBRYOS (GOOTWINE ET AL., 1997). THE UNSATISFACTORY PREGNANCY RATES OBSERVED IN THIS STUDY ARE NOT DUE TO THE SERUM PROGESTERONE CONCENTRATIONS DETERMINED IN THE RECIPIENTS. IN ALL GROUPS THE PROGESTERONE WAS HIGH DURING EMBRYO FLUSHING INDICATING EXISTENCE OF ACTIVE CL'S.

6.5. Conclusions

THE INCLUSION OF A PGF2A TREATMENT IN A SUPEROVULATORY PROTOCOL USING A LONG PROGESTAGEN SYNCHRONISATION TREATMENT HAD NO EFFECT ON THE OCCURRENCE OF OESTRUS, TIME FROM CIDR REMOVAL TO ONSET OF OESTRUS AND DURATION OF THE INDUCED OESTROUS PERIOD. THE DAY 0 PROTOCOL ENGAGED IN THIS TRIAL RESULTED IN A POOR SUPEROVULATORY RESPONSE (BASED ON THE OVULATION RATE, TOTAL NUMBER OF STRUCTURES AND EMBRYOS RECOVERED), COMPARED TO THE OTHER SUPEROVULATORY TREATMENTS. CONVERSELY, THE TOTAL NUMBER OF UNFERTILISED OVA AND DEGENERATE EMBRYOS PER DONOR WERE NOT SIGNIFICANTLY DIFFERENT BETWEEN TREATMENT GROUPS. THE FERTILISATION RATE AND TOTAL NUMBER OF TRANSFERABLE EMBRYOS BEING SIMILAR FOR GROUP 2 AND 3. IT COULD ALSO BE CONCLUDED THAT THE POOR OVARIAN RESPONSE TO THE SUPEROVULATORY TREATMENT IN GROUP 1 (DAY 0) WARRANTS FURTHER RESEARCH, WHICH WILL HAVE TO FOCUS ON THE SYNCHRONISATION OF OVULATION AND THE APPROPRIATE TIME FOR INITIATING A SUPEROVULATORY TREATMENT IN THIS PROTOCOL. THE ADDITION OF A PGF2A TREATMENT IN THE SUPEROVULATORY PROTOCOL FOLLOWING A LONG PROGESTAGEN SYNCHRONISATION TREATMENT IN BOER GOAT DOES ALSO SHOWED NO REAL ADVANTAGE REGARDING THE PARAMETERS MEASURED - AS TREATMENT WITH OR WITHOUT PGF2A, DID NOT ALTER THE RESPONSE. THE TIME OF PROSTAGLANDIN ADMINISTRATION AND THE DOSAGE USED COULD HAVE CONTRIBUTED TO THE OBSERVED RESULTS.

THE AGE OF THE DONOR DOE HAS BEEN SHOWN TO HAVE A BIG EFFECT ON THE OVARIAN ACTIVITY

FOLLOWING SUPEROVULATION TREATMENT. YOUNG DOES GENERALLY TAKE A LONGER TIME TO EXHIBIT OVERT SIGNS OF OESTRUS FOLLOWING THE TERMINATION OF A SYNCHRONISATION TREATMENT, COMPARED TO OLDER MULTIPAROUS DOES. HOWEVER, THE DURATION OF THE INDUCED OESTROUS PERIOD WAS NOT INFLUENCED BY AGE. MATURE DOES ALSO PRODUCED A HIGHER NUMBER OF CL'S, STRUCTURES AND EMBRYOS RECOVERED, COMPARED TO THE YOUNGER DOES. THE LONGER REACTION TIME TAKEN TO THE ONSET OF OESTRUS DID NO INFLUENCE THE FERTILISATION RATE IN YOUNG DOES - AS THE FERTILISATION RATE WAS 100%. ALTHOUGH THE YOUNG DOES RECORDED AN ACCEPTABLE FERTILISATION RATE, THE NUMBER OF TRANSFERABLE EMBRYOS WAS HOWEVER LOWER. THIS COULD BE ASCRIBED TO A LOWER TOTAL NUMBER OF EMBRYOS PRODUCED BY THE YOUNG DOES. THEREFORE, IF A LARGE NUMBER OF EMBRYOS (THE MAXIMUM) ARE TO BE EXPECTED IN A MOET PROGRAMME, IT IS MORE APPROPRIATE TO SUPEROVULATE MULTIPAROUS MATURE GOATS. THIS WILL LEAD TO THE PRODUCTION OF MORE TRANSFERABLE EMBRYOS. HOWEVER, YOUNG DOES CAN STILL BE UTILISED IN A MOET PROGRAMME AS THEY GENERALLY RESPOND WELL TO SUPEROVULATION TREATMENT AND COULD STILL LEAD TO A REASONABLY HIGH NUMBER OF TRANSFERABLE EMBRYOS BEING OBTAINED.

THE NUMBER OF TIMES THAT A DONOR CAN BE UTILISED IN A GOAT MOET PROGRAMME IS LIMITED, AS EMBRYOS ARE ALWAYS FLUSHED SURGICALLY WITH THE FORMATION OF ADHESIONS. THE EFFECT OF REPEATED SUPEROVULATION TREATMENT AND THE IMMUNITY BUILD-UP IN THE CURRENT TRIAL ON OVULATION RATE WAS NOT OBSERVED AND WAS PROBABLY OBSCURED BY AN AGE EFFECT. HOWEVER

THE 4TH SUPEROVULATORY TREATMENT IN THE DOES RESULTED IN A LOWER NUMBER OF STRUCTURES AND EMBRYOS BEING RECOVERED. MOREOVER, THE NUMBER OF UNFERTILISED OVA INCREASED FOLLOWING REPEATED SUPEROVULATION, HENCE REDUCING THE FERTILISATION RATE AND EVENTUALLY THE NUMBER OF TRANSFERABLE EMBRYOS BEING REDUCED. IT WOULD SEEM AS IF THE NUMBER OF TIMES THAT BOER GOAT DOES CAN BE UTILISED AS DONORS IS LIMITED TO THREE TIMES.

THE SYNCHRONISATION OF THE RECIPIENTS AND EMBRYO MANIPULATION ALSO PLAY AN IMPORTANT ROLE IN A MOET PROGRAMME. THE THREE PROTOCOLS USED FOR OESTROUS SYNCHRONISATION IN RECIPIENTS WERE EFFICIENT IN SYNCHRONISING OESTRUS. THE OESTROUS RESPONSE DID SHOW A CLEAR TREND AS TO WHICH PROTOCOL PERFORMS BETTER, AS ALL TREATMENTS WERE SIMILAR. THE PREGNANCY RATE OBTAINED WAS GENERALLY LOW IN ALL THE TREATMENT GROUPS AND THIS ALSO COMPLICATES THE MAKING OF ANY RECOMMENDATIONS. IT IS GENERALLY ACCEPTED THAT THE OESTROUS SYNCHRONISATION TECHNIQUE WOULD NOT HAVE A SIGNIFICANT EFFECT ON AN ACCEPTABLE PREGNANCY RATE. THERE ARE MANY ENDOGENOUS FACTORS THAT COULD AFFECT IMPLANTATION AND PREGNANCY DURING THE FIRST 40 DAYS OF GESTATION.

CHAPTER 7

GENERAL CONCLUSIONS AND RECOMMENDATIONS

IN THE FIRST EXPERIMENT THE PRE-TREATMENT WITH A GNRH AGONIST IN A FSH SUPEROVULATION PROTOCOL SHOWED NO REAL BENEFICIAL EFFECT ON THE OVARIAN RESPONSE AND OESTROUS SYNCHRONIZATION. A HIGHER INCIDENCE OF ABNORMAL CL'S (INDICATION OF PREMATURE LUTEAL REGRESSION) DURING THE NATURAL BREEDING SEASON, COMPARED TO OUTSIDE THE BREEDING SEASON IN DONOR DOES WAS RECORDED. THIS INCIDENCE REDUCED THE NUMBER OF DOES EVENTUALLY FLUSHED DURING THE NATURAL BREEDING SEASON AND THUS LIMITED THE EFFECTS OF THE PRE-TREATMENT WITH A GNRH AGONIST DURING THE NATURAL BREEDING SEASON. THE TENDENCIES HOWEVER, INDICATED THE PRE-TREATMENT WITH A GNRH AGONIST IN A SUPEROVULATION PROTOCOL TO INCREASE THE RECOVERY OF DEGENERATED EMBRYOS AND HENCE REDUCE THE NUMBER OF TRANSFERABLE EMBRYOS. IN SPRING (OUTSIDE THE NATURAL BREEDING SEASON) WHEN THIS TRIAL WAS REPLICATED, THE PRE-TREATMENT WITH A GNRH AGONIST DID NOT HAVE ANY EFFECT ON THE RESPONSE TO SYNCHRONIZATION AND THE OVULATION RATE. DETRIMENTAL EFFECTS OF A GNRH AGONIST PRE-TREATMENT IN A SUPEROVULATION REGIME WERE HOWEVER OBSERVED. THIS PROTOCOL LED TO A REDUCED NUMBER OF STRUCTURES RECOVERED (RECOVERY RATE), A LOWER EMBRYO YIELD AND FERTILISATION RATE BEING RECORDED. THE

NUMBER OF DEGENERATE EMBRYOS WERE INCREASED BY THE USE OF THE PRE-TREATMENT, HENCE REDUCING THE NUMBER OF TRANSFERABLE (VIABLE) EMBRYOS RECOVERED. THE PRE-TREATMENT WITH A GNRH AGONIST IN THE FSH SUPEROVULATION PROTOCOL, WAS THEREFORE NOT WARRANTED IN A BOER GOAT MOET PROGRAMME DURING OR OUTSIDE THE NATURAL BREEDING SEASON – ESPECIALLY IF THE ADDITIONAL COST FACTOR IS ALSO TAKEN INTO ACCOUNT.

DUE TO THE BETTER OVARIAN RESPONSE TO SUPEROVULATION OBTAINED WHEN PFSH WAS USED WITHOUT A PRE-TREATMENT WITH A GNRH AGONIST, THIS PROTOCOL WAS FURTHER REFINED BY EVALUATING THE ROUTE OF ADMINISTRATION OF THE SUPEROVULATORY AGENT. THE INTRAMUSCULAR AND SUBCUTANEOUS ROUTES OF ADMINISTRATION OF THE GONADOTROPHINS HAD NO EFFECT ON THE RESPONSE TO OESTROUS SYNCHRONISATION AND SUPEROVULATION. THESE TWO ROUTES RESULTED IN A SIMILAR OVULATION RATE, EMBRYO RECOVERY RATE AND EMBRYO YIELD. HOWEVER, THE SUBCUTANEOUS ROUTE LED TO A LOWER NUMBER OF UNFERTILISED OVA BEING PRODUCED, BUT THIS ADVANTAGE WAS NEGATED BY THE PRODUCTION OF A HIGHER NUMBER OF DEGENERATE EMBRYOS. EVENTUALLY BOTH ROUTES OF GONADOTROPHIN ADMINISTRATION RESULTED IN A SIMILAR NUMBER OF TRANSFERABLE EMBRYOS BEING PRODUCED. THUS, IT WOULD SEEM AS IF BOTH ROUTES COULD BE USED IN THE ADMINISTRATION OF EXOGENOUS GONADOTROPHINS DURING THE BREEDING SEASON IN BOER GOATS, AS PART OF THE SUPEROVULATION REGIME.

AS THE ROUTE OF FSH SUPEROVULATORY TREATMENT ADMINISTRATION (SUBCUTANEOUS OR

INTRAMUSCULAR) PERFORMED SIMILAR, THE ADDITION OF A PROSTAGLANDIN-F2A (PGF2A) TREATMENT IN THE FSH SUPEROVULATORY REGIME WAS EVALUATED, AND COMPARED TO THE DAY 0 PROTOCOL. THE DAY 0 PROTOCOL IMPLEMENTED IN THIS TRIAL RESULTED IN A POOR OVARIAN RESPONSE TO SUPERSTIMULATION. FURTHER RESEARCH IS HOWEVER NEEDED FOR THIS PROTOCOL IF IT IS TO BE UTILISED WITHOUT OVARIAN INSPECTION WITH THE AID OF A SONOGRAPHY. FOR SYNCHRONISATION OF OVULATION, THE EXACT PREDICTION OF OVULATION IN DIFFERENT BREEDS AND THE APPROPRIATE TIME FOR INITIATING THE SUPEROVULATORY TREATMENT IS NEEDED. THE INCLUSION OF A PGF2A TREATMENT IN A SUPEROVULATION PROGRAMME WITH A LONG PROGESTAGEN SYNCHRONISATION TREATMENT SHOWED NO BENEFICIAL EFFECT IN TERMS OF THE OESTROUS AND OVARIAN RESPONSE TO SUPERSTIMULATION. BASED ON THE RESULTS FROM THE THREE TRIALS REGARDING SUPEROVULATION TECHNIQUES, THE USE OF A LONG SYNCHRONISATION PROGESTAGEN TREATMENT WITH CIDR’S AND SUPEROVULATION UTILISING PFSH REMAINED THE PREFERRED PROTOCOL IN BOER GOAT DOES.

REGARDING OTHER FACTORS THAT COULD INFLUENCE THE OVARIAN RESPONSE TO SUPEROVULATION, SEASON, AGE OF THE DONOR AND REPEATED SUPEROVULATION WERE EVALUATED. THE TIME INTERVAL FROM INTRAVAGINAL PROGESTAGEN WITHDRAWAL TO THE ONSET OF OESTRUS WAS SHORTER DURING THE NATURAL BREEDING SEASON, COMPARED TO OUTSIDE THE BREEDING SEASON – BECAUSE OF ACTIVE AND FUNCTIONAL OVARIES. THE DURATION OF THE INDUCED OESTROUS PERIOD WAS LONGER DURING

THE BREEDING SEASON THAN OUTSIDE THE BREEDING SEASON. THIS COULD BE ATTRIBUTED TO HIGHER CIRCULATING GONADOTROPHIN (LEADING TO HIGH LEVELS OF OESTROGEN) LEVELS BEING PRESENT DURING THE ACTIVE, NATURAL BREEDING SEASON. THEREFORE, A FIXED-TIME AI DURING THE NATURAL BREEDING SEASON SHOULD BE PERFORMED EARLIER THAN OUTSIDE THE BREEDING SEASON. ALSO WHEN TWO INSEMINATIONS ARE PERFORMED, THE TIME INTERVAL BETWEEN INSEMINATIONS SHOULD BE REDUCED ACCORDINGLY TO THE BREEDING SEASON - DUE TO THE SHORTER DURATION OF THE INDUCED OESTROUS PERIOD OUTSIDE THE BREEDING SEASON. THE NUMBER OF UNFERTILISED OVA WAS ALSO HIGHER OUTSIDE THE BREEDING SEASON, WHILE ALL OTHER PARAMETERS FOLLOWING SUPEROVULATION WERE NOT AFFECTED BY SEASON. IT COULD THEREFORE BE CONCLUDED THAT SUPEROVULATION IN BOER GOAT DOES IS WARRANTED DURING AND OUTSIDE THE BREEDING SEASON. ONLY THE TIMING OF THE FIXED-TIME AI SHOULD BE ADJUSTED ACCORDING TO THE DIFFERENT SEASONS.

WITH RESPECT TO THE AGE OF THE DONOR, IT WAS FOUND THAT YOUNG DOES DEMONSTRATED A LONGER TIME INTERVAL (REACTION TIME) FROM CIDR WITHDRAWAL TO THE ONSET OF OESTRUS. THIS HAD NO EFFECT ON THE FERTILISATION RATE, AS THE DURATION OF THE INDUCED OESTROUS PERIOD AND POSSIBLE TIME OF OVULATION WAS NOT AFFECTED BY THE AGE OF THE DOE. ADULT MULTIPAROUS DOES PRODUCED A HIGHER OVULATION RATE, STRUCTURES AND EMBRYO RECOVERY RATES AND THE NUMBER OF TRANSFERABLE EMBRYOS, COMPARED TO YOUNG DOES. REPEATED (4TH TIME) HORMONAL SUPEROVULATION TREATMENT REDUCED THE

NUMBER OF STRUCTURES AND EMBRYOS COLLECTED AND INCREASED THE NUMBER OF UNFERTILISED OVA RECOVERED PER DOE. IT WOULD THEREFORE SEEM AS IF A DOE CAN BE SUPEROVULATED AND FLUSHED AT LEAST THREE TIMES, BEFORE OVARIAN RESPONSE IS PARTIALLY IMPAIRED.

THE BLOOD PROGESTERONE LEVELS IN THE WHOLE PERIOD OF A MOET PROGRAMME ALSO PLAYS A MAJOR ROLE. THE SERUM PROGESTERONE CONCENTRATION DURING OESTRUS, IN THE EARLY LUTEAL PHASE AND AROUND EMBRYO FLUSHING TIME INFLUENCED THE QUALITY AND YIELD OF EMBRYOS RECOVERED. THE HIGH PROGESTERONE CONCENTRATION DURING THE EARLY LUTEAL PHASE AND AT THE TIME OF EMBRYO FLUSHING WAS INDICATIVE OF ACTIVE CL'S AND LED TO HIGH TOTAL NUMBER AND GOOD QUALITY (TRANSFERABLE EMBRYOS) EMBRYOS AT FLUSHING.

THE PHYSICAL TRANSFER OF THE EMBRYO AS THE LAST PHASE OF A MOET PROGRAMME WAS ALSO EVALUATED. THE 3 OESTROUS SYNCHRONISATION PROTOCOLS USED WERE ALL EFFICIENT IN SYNCHRONISING OESTRUS, ALTHOUGH THE PREGNANCY RATE FOLLOWING FRESH AND FROZEN-THAWED GOAT EMBRYOS WAS GENERALLY LOW. IN ANOTHER TRIAL WHEN FRESH, FROZEN AND VITRIFIED EMBRYOS WERE TRANSFERRED, THE PREGNANCY RATE FOLLOWING TRANSFER OF FRESH GOAT EMBRYOS WAS SATISFACTORY - BUT NOT ALL DOES CONFIRMED PREGNANT KIDDED - HENCE REDUCING THE NUMBER OF RECIPIENTS KIDDING. THE PREGNANCY RATE FOLLOWING TRANSFER OF CRYOPRESERVED (CONVENTIONAL SLOW FROZEN OR VITRIFIED EMBRYOS) GOAT EMBRYOS WAS GENERALLY LOW. THE EMBRYO SURVIVAL RATE FOLLOWING THE TRANSFER FRESH AND

CRYOPRESERVED EMBRYOS WAS ALSO LOW AND UNSATISFACTORY. FURTHER RESEARCH IS WARRANTED IN IMPROVING THE CRYOPRESERVATION TECHNIQUES AND THUS THE EMBRYO SURVIVAL RATE OF GOAT EMBRYOS.

ABSTRACT

MULTIPLE OVULATION AND EMBRYO TRANSFER IN GOATS

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FOUR TRIALS WERE CONDUCTED AT THE UFS'S EXPERIMENTAL FARM TO EVALUATE, AND DEVELOP A MORE EFFICIENT PROTOCOL FOR SUPEROVULATION AND EMBRYO CRYOPRESERVATION AND TRANSFER METHODS IN BOER GOATS. EACH TRIAL WAS CONDUCTED WITH SPECIFIC OBJECTIVES. THE FIRST TWO TRIALS WERE CONDUCTED DURING THE AUTUMN AND SPRING SEASONS OF 2005, TO EVALUATE THE OVARIAN RESPONSE OF BOER GOAT DOES TO SUPEROVULATION AND EMBRYO RECOVERY PROCEDURES FOLLOWING A PRE-TREATMENT WITH A GNRH AGONIST. FURTHER ALSO TO EVALUATE THE EFFECT OF SEASON ON THE OVARIAN RESPONSE TO SUPEROVULATION. TWENTY-ONE MULTIPAROUS MATURE BOER GOAT DOES WERE USED AS DONORS DURING THE NATURAL BREEDING SEASON (AUTUMN). IN ALL DOES, THE ONSET OF THE OESTRUS WAS SYNCHRONISED WITH CIDR'S INSERTED INTRAVAGINALLY FOR A PERIOD OF 17 DAYS. DOES WERE ALL SUPEROVULATED WITH 200

MG PFSH/DOE ADMINISTERED I.M. IN 7 DOSAGES, AT 12H INTERVALS, STARTING 48H PRIOR TO CIDR REMOVAL. THE TREATMENT GROUP RECEIVED A GNRH AGONIST (GNRHA) (40µG/DAY/DOE) TREATMENT, ADMINISTERED AS 2 INJECTIONS PER DAY FOR 7 DAYS, STARTING ON DAY 8 OF CIDR INSERTION. THE CONTROL DOES WERE SYNCHRONISED AND SUPEROVULATED, BUT RECEIVED NO GNRHA.

OESTROUS DETECTION WAS PERFORMED TWICE DAILY PRIOR TO PROGESTAGEN TREATMENT (CIDR APPLICATION) AND AT 8H INTERVALS FOLLOWING CIDR REMOVAL FOR A PERIOD OF 72H. FIXED-TIME LAPAROSCOPIC AI WITH DILUTED BOER GOAT SEMEN WAS PERFORMED 36 AND 48H FOLLOWING CIDR REMOVAL. BLOOD SAMPLES WERE ALSO COLLECTED FROM 5 ANIMALS IN EACH GROUP AT 4 DAY INTERVALS DURING PROGESTAGEN TREATMENT AND AT DAY 14 OF PROGESTAGEN TREATMENT TO EVALUATE THE HORMONAL EFFECT OF GNRHA TREATMENT. FROM SUPEROVULATION TREATMENT UNTIL CIDR REMOVAL, BLOOD SAMPLES WERE COLLECTED TWICE DAILY AND THEN AT 8H INTERVALS FROM CIDR REMOVAL FOR A PERIOD OF 3 DAYS. THE BLOOD SAMPLES WERE ALSO TAKEN AT 24 H INTERVALS FOLLOWING THE SECOND AI UNTIL EMBRYO FLUSHING (DAY 6 FOLLOWING AI). SERUM PROGESTERONE AND OESTROGEN CONCENTRATIONS WERE DETERMINED VIA RADIOIMMUNOASSAY. EMBRYOS WERE THEN FINALLY SURGICALLY RECOVERED (DAY 6) UNDER GENERAL ANAESTHESIA. THE OESTROUS SYNCHRONISATION RESPONSE WAS EVALUATED BASED ON THE OCCURRENCE OF OESTRUS, TIME TO ONSET OF OESTROUS AND DURATION OF THE INDUCED OESTROUS PERIOD. THE EMBRYO YIELD AND QUALITY WAS ALSO EVALUATED.

ONLY 81% OF THE DOES EXHIBITED OVERT SIGNS OF OESTRUS PRIOR TO SYNCHRONISATION TREATMENT APPLICATION (INDICATION OF CYCLIC ACTIVITY). THE MEAN DURATION OF THESE NATURAL OESTROUS CYCLES WAS RECORDED AS 20.1±3.5D, WHILE THE NATURAL OESTROUS PERIOD LASTED FOR 37.7±11.9H. FOLLOWING SYNCHRONISATION AND SUPEROVULATION TREATMENT, 100% AND 80% OF THE DOES IN THE FSH/GNRHA AND FSH-TREATED DOES SHOWED SIGNS OF OESTRUS, RESPECTIVELY. THE TIME INTERVAL FROM CIDR REMOVAL TO ONSET OF OESTRUS AND DURATION OF THE INDUCED OESTROUS PERIOD DID NOT DIFFER SIGNIFICANTLY, ALTHOUGH THE DURATION OF THE INDUCED OESTROUS PERIOD IN THE FSH/GNRHA GROUP (19.6±5.5H) TENDED TO BE SHORTER, COMPARED TO THE FSH-TREATED DOES (25.0±7.4H). THE OCCURRENCE OF ABNORMAL CL'S WAS 38.1% IN TOTAL, WHILE 23.8% OF THE DOES DID NOT HAVE ANY CL'S. THERE WERE NO SIGNIFICANT DIFFERENCES IN EMBRYO YIELD AND QUALITY PARAMETERS MEASURED. IN DOES PRE-TREATED WITH GNRHA HOWEVER, THE NUMBER OF DEGENERATE EMBRYOS TENDED TO BE HIGHER, WHILE THE NUMBER OF TRANSFERABLE EMBRYOS TENDED TO BE LOWER, COMPARED TO THE CONTROL (FSH) DOES (6.6±4.2 VS. 1.7±1.5 AND 3.4±2.7 VS. 9.3±6.1, RESPECTIVELY).

THE SECOND TRIAL REPEATING ALL THE PROCEDURES PERFORMED IN THE FIRST TRIAL WAS CONDUCTED IN THE SPRING OF 2005 (OUTSIDE THE BREEDING SEASON), USING 22 MULTIPAROUS BOER GOAT DOES AS RECIPIENTS. ONLY 45.5% OF THE DOES SHOWED OVERT SIGNS OF OESTRUS PRIOR TO THE APPLICATION OF OESTROUS SYNCHRONISATION AND SUPEROVULATION TREATMENT. GNRHA TREATMENT HAD NO BENEFICIAL EFFECT IN RESPONSE TO

OESTROUS SYNCHRONISATION AND OVULATION RATE. THE TOTAL MEAN STRUCTURES FLUSHED WAS SIGNIFICANTLY ($P<0.05$) LOWER IN THE DOES PRE-TREATED WITH GNRHA (12.6±6.0 PER DOE, COMPARED TO THE CONTROL DOES (17.6±4.9 PER DOE). THE MEAN TOTAL NUMBER OF EMBRYOS COLLECTED PER DONOR AND THE FERTILISATION RATE WERE SIGNIFICANTLY ($P<0.05$) LOWER IN DOES PRE-TREATED WITH GNRHA THAN IN THE FSH-TREATED DOES (11.5.±5.3 VS. 16.5±6.1 AND 81.6±32.2% VS. 92.6±19.5%, RESPECTIVELY). GNRHA TREATMENT HAD NO EFFECT ON THE MEAN NUMBER OF UNFERTILISED OVA PER DONOR. PRE-TREATMENT OF DOES WITH GNRHA HOWEVER RESULTED IN A SIGNIFICANTLY ($P<0.01$) HIGHER MEAN NUMBER OF DEGENERATIVE EMBRYOS WHEN COMPARED TO THE CONTROL DOES (6.9±4.5 VS. 3.2±4.2 PER DOE). SUBSEQUENTLY, THE NUMBER OF TRANSFERABLE EMBRYOS AND TRANSFERABLE RATE (4.3±4.0 AND 32.7±26.9%) WAS LOWER IN DOES PRE-TREATED WITH GNRHA, COMPARED TO THE CONTROL DOES (13.1±5.3 AND 75.2±26.8%). THE MEAN TIME TO ONSET OF OESTRUS WAS SIGNIFICANTLY ($P<0.05$) EARLIER DURING THE NATURAL BREEDING SEASON (24.9±4.8H), COMPARED TO OUTSIDE THE BREEDING SEASON IN DOES (30.5±9.1H). SIMILARLY, THE MEAN DURATION OF THE INDUCED OESTRUS WAS SIGNIFICANTLY ($P<0.05$) LONGER DURING THE NATURAL BREEDING SEASON (24.0±5.7H) - THAN OUTSIDE THE BREEDING SEASON (18.2±3.7H). SEASON DID NOT HAVE ANY SIGNIFICANT EFFECT ON THE TOTAL NUMBER OF CL'S INDUCED, ALTHOUGH THE NUMBER OF CL'S ON THE RIGHT OVARY WAS SIGNIFICANTLY ($P<0.05$) LOWER DURING THE NATURAL BREEDING SEASON. SEASON DID NOT HAVE EFFECT ON THE NUMBER OF STRUCTURES RECOVERED, EMBRYOS, DEGENERATIVE AND TRANSFERABLE EMBRYOS PER DONOR. THE MEAN

NUMBER OF UNFERTILISED OVA PER DONOR (3.3±2.8) WAS SIGNIFICANTLY ($P<0.05$) HIGHER IN GOATS TREATED OUTSIDE THE NATURAL BREEDING SEASON THAN DURING THE NATURAL BREEDING SEASON (0.9±2.4).

IT COULD BE CONCLUDED THAT PRE-TREATMENT WITH GNRHA DOES NOT HAVE ANY BENEFICIAL EFFECT ON OESTROUS RESPONSE, TIME TO ONSET AND DURATION OF THE INDUCED OESTROUS PERIOD. THE ADDITION OF THE GNRHA INTO THE FSH GOAT SUPEROVULATION PROTOCOL REDUCED THE NUMBER OF STRUCTURES RECOVERED, EMBRYO YIELD, AND THE FERTILISATION RATE (THE NUMBER OF EMBRYOS PER STRUCTURES ON OVARIES). THE PRE-TREATMENT WITH GNRHA ALSO INCREASED THE NUMBER OF DEGENERATE EMBRYOS - WHICH ULTIMATELY REDUCED THE TOTAL NUMBER OF TRANSFERABLE EMBRYOS. IT COULD THUS BE RECOMMENDED THAT A PRE-TREATMENT WITH A GNRH AGONIST IN A BOER GOAT MOET PROGRAMME IS NOT WARRANTED. THE SERUM PROGESTERONE CONCENTRATIONS DID NOT DIFFER BETWEEN THE TREATMENT GROUPS, THROUGHOUT THE OESTROUS SYNCHRONISATION PERIOD. AT 48H FOLLOWING THE SECOND AI (96H AFTER CIDR REMOVAL), THE CONTROL DOES RECORDED A SIGNIFICANTLY ($P<0.05$) HIGHER MEAN SERUM PROGESTERONE CONCENTRATION (11.3±4.9 NG/ML), THAN IN THE FSH/GNRHA-TREATED DOES (4.3±2.1 NG/ML). THE SERUM PROGESTERONE CONCENTRATIONS INCREASED FOLLOWING THE SECOND AI AND WERE HIGHEST ON THE DAY OF EMBRYO FLUSHING IN BOTH GROUPS – WITHOUT ANY SIGNIFICANT DIFFERENCES. THE MEAN SERUM OESTROGEN CONCENTRATION WAS SIGNIFICANTLY ($P<0.05$) HIGHER ON DAY 12 OF SYNCHRONISATION (5^{TH} DAY OF GNRHA TREATMENT) AND AT THE 4^{TH} PFSH

(SUPEROVULATION) INJECTION IN THE FSH/GNRHA TREATED GROUP COMPARED TO THE CONTROL DOES. AT THE TIME OF THE FIRST AI (36H FOLLOWING CIDR REMOVAL) THE MEAN SERUM OESTROGEN CONCENTRATION WAS SIGNIFICANTLY ($P<0.05$) HIGHER (58.3±26.9 PG/ML) IN THE CONTROL (FSH-STIMULATED) DOES, COMPARED TO THE FSH/GNRHA-TREATED DOES (15.7±17.4 PG/ML). THE BLOOD HORMONAL LEVELS INDUCED DURING SUPEROVULATION WERE DEPENDENT ON VARIOUS OVARIAN FACTORS WHICH ULTIMATELY DETERMINE THE COMPETENCE OF THE OOCYTES AND EVENTUALLY VIABILITY OF THE EMBRYOS.

A THIRD TRIAL WAS CONDUCTED DURING THE NATURAL BREEDING SEASON (AUTUMN, 2006). SEVENTEEN MULTIPAROUS MATURE BOER GOAT DOES WERE USED AS DONORS IN THIS TRIAL TO EVALUATE AND REFINE THE FSH SUPEROVULATORY TREATMENT BY COMPARING TWO ROUTES OF GONADOTROPHIN (PFSH) ADMINISTRATION. HERE 27 RECIPIENTS WERE USED TO EVALUATE THE SURVIVAL RATE OF THE GOAT EMBRYOS FOLLOWING CRYOPRESERVATION USING THE CONVENTIONAL SLOW FREEZING OR VITRIFICATION METHOD. DOES RECEIVED A PFSH SUPEROVULATION TREATMENT INTRAMUSCULAR OR SUBCUTANEOUS, WHILE RECIPIENTS RECEIVED FRESH, SLOW FROZEN-THAWED OR VITRIFIED-THAWED EMBRYOS. THE ROUTE OF ADMINISTERING GONADOTROPHIN TREATMENT DID NOT AFFECT THE OESTROUS RESPONSE, THE TIME FROM CIDR REMOVAL TO THE ONSET OF OESTRUS AND DURATION OF THE INDUCED OESTROUS PERIOD. THE ROUTE OF GONADOTROPHIN ADMINISTRATION DID NOT AFFECT THE OVULATION RATE, THE TOTAL NUMBER OF STRUCTURES, NUMBER OF EMBRYOS AND TRANSFERABLE EMBRYOS COLLECTED PER DONOR. THE MEAN

NUMBER OF UNFERTILISED OVA IN THE I.M. GROUP (3.3±4.8) WAS SIGNIFICANTLY (P<0.05) HIGHER, THAN IN THE S.C. GROUP (0.3±0.8). SIMILARLY, THE MEAN NUMBER OF DEGENERATED EMBRYOS PER DONOR IN THE DOES ADMINISTERED THE FSH SUBCUTANEOUSLY (5.9±4.5) WAS SIGNIFICANTLY (P<0.05) HIGHER, WHEN COMPARED TO THE INTRAMUSCULAR GROUP (2.6±2.3). A TOTAL OF 88.9% RECIPIENTS DEMONSTRATED SIGNS OF OESTRUS FOLLOWING CIDR REMOVAL. A PREGNANCY RATE OF 85.7%, 50.0% AND 37.5% WAS RECORDED FOLLOWING THE TRANSFER OF FRESH, SLOW-FROZEN AND VITRIFIED EMBRYOS, RESPECTIVELY. EMBRYO SURVIVAL RATES OF 35.7%, 25.0% AND 31.3% WERE THEN EVENTUALLY RECORDED FOLLOWING FRESH, SLOW-FROZEN AND VITRIFIED EMBRYO TRANSFER. IT COULD THUS BE CONCLUDED THAT AN ADVANTAGE OF THE SUBCUTANEOUS ROUTE OF ADMINISTRATION RESULTED IN A LOWER NUMBER OF UNFERTILISED OVA RECOVERED, WHICH WAS HOWEVER NEGATED BY A HIGHER NUMBER OF DEGENERATE EMBRYOS RECORDED. THEREFORE BOTH ROUTES CAN BE USED FOR THE ADMINISTRATION OF PFSH SUPEROVULATION TREATMENT IN BOER GOAT DOES DURING THE BREEDING SEASON. A RELATIVELY HIGH PREGNANCY RATE WAS OBTAINED FOLLOWING THE TRANSFER OF FRESH EMBRYOS. HOWEVER, THE SURVIVAL RATE OF EMBRYOS FOLLOWING EITHER FRESH, SLOW FROZEN-THAWED OR VITRIFIED-THAWED EMBRYOS WAS UNSATISFACTORY. MORE RESEARCH IS THUS WARRANTED, WITH A HIGHER NUMBER OF ANIMALS, DIRECTED AT IMPROVING THE SURVIVABILITY OF EMBRYOS FOLLOWING FRESH AND CRYOPRESERVED GOAT EMBRYO TRANSFER. THE FOURTH TRIAL WAS CONDUCTED DURING THE NATURAL BREEDING (AUTUMN, 2007). DONOR DOES WERE SUPEROVULATED FOLLOWING A LONG (17

DAY) PROGESTAGEN SYNCHRONISATION PROTOCOL, WITH OR WITHOUT PROSTAGLANDIN-F2A OR SUPEROVULATED FOLLOWING A PREDETERMINED TIME OF OVULATION (DAY 0 PROTOCOL). RECIPIENT DOES RECEIVED FROZEN-THAWED OR FRESH BOER GOAT EMBRYOS FOLLOWING OESTROUS SYNCHRONISATION USING DIFFERENT PROTOCOLS. DURING THE SYNCHRONISATION TREATMENT PRIOR TO SUPEROVULATION ONLY 71.4% OF THE DOES IN THE DAY 0 PROTOCOL EXHIBITED SIGNS OF OESTRUS, WHILE ONLY ONE DOE EXHIBITED SIGNS OF OESTRUS FOLLOWING SUPEROVULATION. ALL DOES SHOWED OVERT SIGNS OF OESTRUS FOLLOWING LONG PROGESTAGEN TREATMENT WITH OR WITHOUT ADMINISTRATION OF PROSTAGLANDIN-F2A. ALL DOES ALSO DEMONSTRATED A SIMILAR RESPONSE TIME FROM CIDR REMOVAL TO THE ONSET OF OESTRUS AND THE DURATION OF THE INDUCED OESTROUS PERIOD. THE DAY 0 PROTOCOL RESULTED INTO A SIGNIFICANTLY ($P<0.01$) LOWER TOTAL NUMBER OF CL'S (4.0 ± 3.5) PER DOE - COMPARED TO GROUP 2 (17-DAY PROGESTAGEN TREATMENT PLUS PGF2A) (14.5 ± 4.6) AND GROUP 3 (17-DAY PROGESTAGEN TREATMENT) (16.5 ± 5.9). THE MEAN NUMBER OF STRUCTURES RECOVERED FROM GROUP 1 (1.4 ± 0.5) WAS SIGNIFICANTLY ($P<0.05$) LOWER, COMPARED TO GROUP 3 (11.4 ± 7.3). THIS MEAN IN THE DAY 0 GROUP HOWEVER DID NOT DIFFER SIGNIFICANTLY, WHEN COMPARED TO GROUP 2 (8.4 ± 7.7). THE VARIATION RECORDED BETWEEN ANIMALS IN THE GROUP 2 WAS VERY HIGH. THE DAY 0 PROTOCOL RESULTED IN A SIGNIFICANTLY ($P<0.01$) LOWER MEAN NUMBER OF EMBRYOS BEING RECORDED. HOWEVER, THE MEAN NUMBER OF UNFERTILISED OVA AND DEGENERATING EMBRYOS WAS SIMILAR FOR ALL THE TREATMENT GROUPS. THE DAY 0 (GROUP 1) GROUP RESULTED IN THE

COLLECTION OF ONLY ONE EMBRYO, WHICH WAS ALSO TRANSFERABLE, BUT DUE TO THE SINGLE VALUE THIS DATA WAS NOT ANALYSED. THE ADDITION OF PROSTAGLANDIN-F2A HAD NO EFFECT ON THE FERTILISATION RATE AND THE MEAN NUMBER OF TRANSFERABLE EMBRYOS RECORDED BETWEEN GROUP 2 AND 3.

THE TIME INTERVAL FROM CIDR REMOVAL TO THE ONSET OF OESTRUS IN THE ADULT DOES WAS SIGNIFICANTLY (P<0.01) SHORTER THAN THAT RECORDED IN THE YOUNG DOES. HOWEVER, AGE DID NOT HAVE ANY SIGNIFICANT EFFECT ON THE DURATION OF THE INDUCED OESTROUS PERIOD. THE MEAN NUMBER OF CL'S, STRUCTURES AND EMBRYOS RECOVERED WERE HOWEVER SIGNIFICANTLY (P<0.01) HIGHER IN THE ADULT DOES. NO RECOVERY OF UNFERTILISED OVA WAS RECORDED IN THE YOUNG DOES AND THE FERTILISATION RATE AND MEAN NUMBER OF UNFERTILISED OVA DID NOT DIFFER BETWEEN THE YOUNG AND ADULT DOES. SIMILARLY THE AGE OF THE DOE HAD NO EFFECT ON THE MEAN NUMBER OF DEGENERATE EMBRYOS PER DONOR RECORDED. THE MEAN NUMBER OF TRANSFERABLE EMBRYOS PRODUCED IN THE ADULT DOES (15.8±6.4) WAS SIGNIFICANTLY (P<0.01) HIGHER, THAN IN THE YOUNG DOES (9.5±3.7).

THE TIME FROM CIDR REMOVAL TO THE ONSET OF OESTRUS WAS NOT AFFECTED BY REPEATED SUPEROVULATION TREATMENT. DOES SUPEROVULATED FOR THE FIRST TIME SHOWED A SIGNIFICANTLY (P<0.05) SHORTER MEAN DURATION OF OESTRUS (20.8±10.1H), WHEN COMPARED TO THOSE REPEATEDLY SUPEROVULATED (30.4±6.7 H). REPEATED SUPEROVULATION DID NOT SHOW AN EFFECT ON THE TOTAL NUMBER OF CL'S PRODUCED PER DOE. HOWEVER, THE MEAN NUMBER OF STRUCTURES RECOVERED WAS SIGNIFICANTLY

(P<0.05) LOWER IN THE REPEAT-TREATED DOES (6.0±8.7), COMPARED TO DOES SUPEROVULATED FOR THE FIRST TIME (11.7±5.0). SIMILARLY, THE MEAN NUMBER OF EMBRYOS RECOVERED PER DOE WAS SIGNIFICANTLY (P<0.05) HIGHER IN DOES SUPEROVULATED FOR THE FIRST TIME. THE MEAN NUMBER OF UNFERTILISED OVA PER DONOR WAS ALSO SIGNIFICANTLY (P<0.05) HIGHER IN THE REPEATEDLY-TREATED DOES (5.5±7.8), COMPARED TO THE 0.1±0.3 FOR DOES SUPEROVULATED FOR THE FIRST TIME. THIS RESULTED IN A SIGNIFICANTLY (P<0.05) LOWER FERTILISATION RATE BEING OBTAINED IN THE REPEATEDLY-TREATED DOES (50.0±70.7%), COMPARED TO DOES SUPEROVULATED FOR THE FIRST TIME (99.4±1.9%). THE MEAN NUMBER OF DEGENERATE EMBRYOS DID NOT DIFFER SIGNIFICANTLY BETWEEN THESE GROUPS. THE NUMBER OF TRANSFERABLE EMBRYOS RECORDED WAS SIGNIFICANTLY (P<0.05) LOWER IN THE REPEATEDLY TREATED DOES (3.8±8.5), COMPARED TO THEIR COUNTERPARTS (10.7±4.0).

IN GROUP A (CIDR + PGF2A + ECG; CHRONOGEST) 88.9% OF THE DOES RESPONDED TO OESTROUS SYNCHRONISATION. ALL DOES IN GROUP B (CIDR +ECG; FOLLIGON) AND GROUP C (CIDR + ECG; CHRONOGEST) EXHIBITED SIGNS OF OESTRUS FOLLOWING OESTROUS SYNCHRONISATION. DOES FROM GROUP A (42.0±3.7 H) EXHIBITED A LONGER (P<0.05) TIME INTERVAL FROM CIDR WITHDRAWAL TO THE ONSET OF OESTRUS, COMPARED TO GROUP C DOES (32.0±8.6H). THERE WAS HOWEVER, NO SIGNIFICANT DIFFERENCE WITH RESPECT TO THE TIME INTERVAL FROM CIDR REMOVAL TO THE ONSET OF OESTRUS BETWEEN GROUP A AND B. GROUP B AND C DOES ALSO RECORDED A SIMILAR RESPONSE TIME FROM CIDR WITHDRAWAL TO THE ONSET OF OESTRUS. THE DURATION OF THE INDUCED

OESTROUS PERIOD BEING SIGNIFICANTLY ($P<0.05$) SHORTER IN GROUP B (19.0±13.5H), WHEN COMPARED TO GROUP A (39.0±15.1H). NO SIGNIFICANT DIFFERENCE WAS RECORDED BETWEEN GROUP B AND C REGARDING THE DURATION OF THE INDUCED OESTROUS PERIOD. IT COULD THUS BE CONCLUDED THAT THE POOR OVARIAN RESPONSE TO THE SUPEROVULATORY TREATMENT IN THE GROUP 1 (DAY 0 PROTOCOL) WARRANTS FURTHER RESEARCH WHICH WILL HAVE TO FOCUS ON THE SYNCHRONISATION OF OVULATION AND AN APPROPRIATE TIME FOR INITIATING A SUPEROVULATORY TREATMENT IN THIS PROTOCOL. THE ADDITION OF A PROSTAGLANDIN-F2A TREATMENT IN THE SUPEROVULATORY PROTOCOL FOLLOWING A LONG PROGESTAGEN SYNCHRONISATION TREATMENT IN BOER GOAT DOES ALSO HAS NO REAL ADVANTAGE. THE TIME OF PROSTAGLANDIN ADMINISTRATION AND THE DOSAGE USED COULD HAVE CONTRIBUTED TO THE OBSERVED RESULTS.

THE AGE OF THE DONOR DOE HAS BEEN SHOWN TO HAVE A MAJOR EFFECT ON THE OVARIAN ACTIVITY FOLLOWING SUPEROVULATION TREATMENT. ALTHOUGH THE YOUNG DOES RECORDED AN ACCEPTABLE FERTILISATION RATE, THE NUMBER OF TRANSFERABLE EMBRYOS WAS HOWEVER LOWER. THEREFORE, IF A LARGE NUMBER OF ACCEPTABLE EMBRYOS (THE MAXIMUM) ARE REQUIRED IN A MOET PROGRAMME, IT IS MORE APPROPRIATE TO SUPEROVULATE MULTIPAROUS MATURE GOATS. THIS WILL LEAD TO THE PRODUCTION OF MORE TRANSFERABLE EMBRYOS. THE NUMBER OF TIMES THAT A DONOR CAN BE UTILISED IN A BOER GOAT MOET PROGRAMME SEEM IS TO BE LIMITED TO THREE TIMES DUE TO REDUCTION IN THE NUMBER OF STRUCTURES AND EMBRYOS BEING RECOVERED

FROM THE 4^{TH} TIME REPEATEDLY TREATED DOES. MOREOVER, THE NUMBER OF UNFERTILISED OVA INCREASED FOLLOWING REPEATED SUPEROVULATION, HENCE REDUCING THE FERTILISATION RATE AND EVENTUALLY THE NUMBER OF TRANSFERABLE EMBRYOS.

THE THREE PROTOCOLS USED FOR OESTROUS SYNCHRONISATION IN RECIPIENTS WERE EFFICIENT IN SYNCHRONISING OESTRUS. THE PREGNANCY RATE OBTAINED WAS GENERALLY LOW IN ALL THE TREATMENT GROUPS AND POSSIBLE INTERACTIONS THUS COMPLICATE THE MAKING OF ANY DEFINITE RECOMMENDATIONS.

OPSOMMING

SUPEROVULASIE EN EMBRIO-OORPLASING IN BOERBOKKE

DEUR
KHOBOSO CHRISTINA LEHLOENYA

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VIER PROEWE IS OP DIE UV SE PROEFPLAAS UITGEVOER OM 'N MEER DOELTREFFENDE PROTOKOL VIR SUPEROVULASIE EN EMBRIOBEVRIESING- EN OORPLASINGSTEGNIEKE IN BOERBOKKE TE ONTWIKKEL. DIE EERSTE 2 PROEWE IS UITGEVOER TYDENS DIE HERFS EN LENTE VAN 2005 OM DIE OVARIALE RESPONS TE EVALUEER VAN BOERBOKOOIE T.O.V. SUPEROVULASIE EN EMBRIO-HERWINNINGSPROSEDURES NA BEHANDELING MET 'N GNRH AGONIS EN OM DIE EFFEK VAN DIE SEISOEN OP OVARIALE RESPONS OP SUPEROVULASIE TE EVALUEER. EEN EN TWINTIG VEELBARENDE VOLWASSE BOERBOKOOIE IS GEBRUIK AS DONORS TYDENS DIE NATUURLIKE TEELSEISOEN (HERFS). IN ALLE OOIE WAS DIE VOORKOMS VAN OESTRUS GESINKRONISEERD M.B.V. CIDR'S WAT INTRAVAGINAAL TOEGEDIEN IS VIR 'N PERIODE VAN 17 DAE. OOIE IS ALMAL GESUPEROVULEER MET 200 MG PFSH/OOI. FSH IS I.M. TOEGEDIEN IN 7 STAPPE TEEN 12H INTERVALLE - BEGINNENDE 48H VOOR CIDR VERWYDERING. DIE BEHANDELDE GROEP HET

'N GNRH AGONIS (GNRHA) BEHANDELING (40µG/DAG/OOI) ONTVANG. BEHANDELINGS IS TOEGEDIEN AS 2 INSPUITINGS PER DAG VIR 7 DAE – BEGINNENDE OP DAG 8 VAN CIDR TOEDIENING. DIE KONTROLE OOIE IS GESINKRONISEER EN GESUPEROVULEER SONDER GNRHA TOEDIENING. OESTRUSWAARNEMINGS IS 2 MAAL PER DAG UITGEVOER VOOR CIDR TOEDIENING EN WEER MET 8H INTERVALLE NA CIDR VERWYDERING VIR 'N PERIODE VAN 72H. VASGESTELDE LAPAROSKOPIESE KI MET VERDUNDE BOERBOK SEMEN IS UITGEVOER 36 EN 48H NA CIDR ONTTREKKING. BLOEDMONSTERS IS OOK GEKOLLEKTEER VAN 5 DIERE PER GROEP MET 4 DAE INTERVALLE TYDENS PROGESTAGEENBEHANDELING EN OP DAG 14 (ONTTREKKING) VAN BEHANDELING OM DIE HORMONALE EFFEK VAN GNRHA BEHANDELING TE EVALUEER. VANAF SUPEROVULASIEBEHANDELING TOT CIDR ONTTREKKING IS BLOEDMONSTERS 2 MAAL PER DAG GENEEM – DAARNA MET 8H INTERVALLE VANAF CIDR ONTTREKKING VIR 'N PERIODE VAN 3 DAE. BLOEDMONSTERS IS OOK GENEEM MET 24H INTERVALLE NA DIE TWEEDE KI TOT EMBRIOSPOELING (DAG 6 NA KI). DIE SERUM-PROGESTEROONKONSENTRASIES IS BEPAAL D.M.V. RIA. EMBRIOS IS EINDELIK CHIRURGIES GESPOEL (DAG 6) NA ALGEHELE VERDOWING. DIE OESTRUS RESPONS IS GEËVALUEER, GEBASEER OP DIE VOORKOMS VAN OESTRUS, TYD VAN ONTTREKKING TOT OESTRUS EN DIE TYDSDUUR VAN DIE GEÏNDUSEERDE OESTRUSPERIODE. EMBRIOPRODUKSIE EN -KWALITEIT IS OOK BEPAAL. SLEGS 81% VAN DIE OOIE HET TEKENS VAN OESTRUS GETOON VOOR HORMOONBEHANDELING (INDIKASIE VAN SIKLIESE AKTIWITEIT). DIE GEMIDDELDE DUUR VAN DIE OESTRUSSIKLUS WAS GEMEET AS 20.1 ± 3.5D, MET 'N OESTRUSPERIODE VAN 37.7 ± 11.9H. NA

SINKRONISASIE EN SUPEROVULASIE HET 100% EN 80% VAN DIE OOIE IN DIE FSH/GNRHA EN FSH-BEHANDELDE OOIE RESPEKTIEWELIK OESTRUS GETOON. DIE TYDSDUUR VANAF CIDR ONTTREKKING TOT DIE AANVANG VAN ESTRUS EN DIE TYDSDUUR VAN DIE GEÏNDUSEERDE OESTRUSPERIODE HET NIE BETEKENISVOL VERSKIL NIE, ALHOEWEL DIE LENGTE VAN DIE GEÏNDUSEERDE OESTRUSPERIODE GENEIG HET OM KORTER TE WEES IN DIE FSH/GNRHA GROEP (19.6 ± 55H), VERGELEKE MET DIE FSH-BEHANDELDE OOIE (25.0 ± 7.4H). DIE VOORKOMS VAN ABNORMALE CL'S WAS 38.1% IN TOTAAL, TERWYL 23.8% VAN DIE OOIE GEEN CL'S GEHAD HET NIE. GEEN BETEKENISVOLLE VERSKIL IS GEVIND IN ALLE EMBRIOPRODUKSIE EN KWALITEITSPARAMETERS GEMEET. IN ALLE BEHANDELINGS MET GNRHA HET DIE AANTAL DEGENEREREND EMBRIOS GENEIG OM HOËR TE WEES, TERWYL DIE AANTAL OORPLAASBARE EMBRIOS WEER GENEIG HET OM LAER TE WEES – WANNEER VERGELYK WORD MET DIE KONTROLE (FSH) OOIE (6.6 ± 4.2 VS 1.7 ± 1.5 EN 3.4 ± 2.7 VS 9.3 ± 6.1 RESPEKTIEWELIK).

IN DIE TWEEDE PROEF IS 'N HERHALING VAN DIE PROEFPROSEDURES GEDOEN, HIERDIE KEER IN DIE LENTE (BUITE DIE NATUURLIKE TEELSEISOEN) – DEUR ADDISIONEEL GEBRUIK TE MAAK VAN 22 VOLWASSE BOERBOKOOIE AS ONTVANGERS. SLEGS 45.5% VAN DIE OOIE HET TEKENS VAN OESTRUS GETOON VOOR SINKRONISASIE EN SUPEROVULASIE-BEHANDELING. GNRHA BEHANDELING HET GEEN BETEKENISVOLLE EFFEK GEHAD OP DIE SINKRONISASIE EN OVULASIE TEMPO. DIE TOTALE AANTAL STRUKTURE GESPOEL WAS BETEKENISVOL ($P<0.05$) MINDER IN DIE GNRHA-BEHANDELDE OOIE (12.6 ± 6.0 PER OOI), VERGELEKE MET DIE KONTROLE OOIE (17.6 ± 4.9 PER OOI). DIE GEMIDDELDE TOTALE

AANTAL EMBRIOS GEKOLLEKTEER PER DONOR EN DIE BEVRUGTINGSTEMPO WAS BETEKENISVOL LAER IN OOIE BEHANDEL MET GNRHA, VERGELEKE MET DIE FSH-BEHANDELDE OOIE (11.5 ± 5.3 VS 16.5 ± 6.1 EN 81.6 ± 32.2% VS 92.6 ± 19.5% RESPEKTIEWELIK). GNRHA BEHANDELING HET GEEN EFFEK GEHAD OP DIE AANTAL ONBEVRUGTE OVA PER DONOR. BEHANDELING MET GNRHA HET EGTER 'N BETEKENISVOLLE (P<0.01) VOORKOMS VAN DEGENERATIEWE EMBRIOS TOT GEVOLG GEHAD, WANNEER VERGELYK MET DIE KONTROLE OOIE (6.9 ± 4.5 VS 3.2 ± 4.2 PER OOI). GEVOLGLIK WAS DIE AANTAL OORPLAASBARE EMBRIOS PER DONOR EN OORPLASINGSTEMPO (4.3 ± 4.0 EN 32.7 ± 26.9%) LAER IN DIERE BEHANDEL MET GNRHA, VERGELEKE MET DIE KONTROLE OOIE (13.1 ± 5.3 EN 75.2 ± 26.8%). DIE GEMIDDELDE TYD VAN DIE VOORKOMS VAN OESTRUS WAS BETEKENISVOL (P<0.05) VROEËR TYDENS DIE NATUURLIKE TEELSEISOEN (24.9 ± 4.8H), VERGELEKE MET BUITE DIE TEELSEISOEN (30.5 ± 9.1H). SOORTGELYK WAS DIE TYDSDUUR VAN DIE GEÏNDUSEERDE OESTRUS BETEKENISVOL (P<0.05) LANGER TYDENS DIE NATUURLIKE TEELSEISOEN (24.0 ± 5.7H) – VERGELEKE MET BUITE DIE TEELSEISOEN (18.2 ± 3.7H). SEISOEN HET GEEN BETEKENISVOLLE EFFEK GEHAD OP DIE TOTALE AANTAL CL'S, ALHOEWEL DIE AANTAL CL'S OP DIE REGTER OVARIUM BETEKENISVOL (P<0.05) MINDER WAS TYDENS DIE TEELSEISOEN. SEISOEN HET GEEN EFFEK GEHAD OP DIE AANTAL STRUKTURE HERWIN, EMBRIOS, DEGENERATIEWE EN OORPLAASBARE EMBRIOS PER DONOR NIE. DIE GEMIDDELDE AANTAL ONBEVRUGTE OVA PER DONOR (3.3 ± 2.8) WAS BETEKENISVOL (P<0.05) HOËR IN OOIE BEHANDEL BUITE DIE NATUURLIKE TEELSEISOEN – VERGELEKE MET 0.9 ± 2.4 TYDENS DIE TEELSEISOEN. DIT KON AFGELEI WORD DAT DIE BEHANDELING MET GNRHA

GEEN VOORDELIGE EFFEK HET OP OESTRUSRESPONS, TYD TOT OESTRUS EN TYDSDUUR VAN DIE GEÏNDUSEERDE OESTRUSPERIODE NIE. DIE AANVULLING VAN GNRHA IN DIE FSH BOK SUPEROVULASIE PROTOKOL HET DIE AANTAL STRUKTURE HERWIN, EMBRIOPRODUKSIE EN BEVRUGTINGSTEMPO (AANTAL EMBRIOS PER STRUKTURE OP OVARIA) VERLAAG. DIE BEHANDELING MET GNRHA HET OOK DIE AANTAL DEGENERATIEWE EMBRIOS VERMEERDER – WAT EINDELIK DIE TOTALE AANTAL OORPLAASBARE EMBRIOS VERMINDER HET. DIT WORD DUS AANBEVEEL DAT 'N VOOR-BEHANDELING MET 'N GNRHA AGONIS IN 'N BOERBOK SUPEROVULASIE EN OORPLASINGSPROGRAM NIE GEREGVERDIG IS NIE. DIE SERUM-PROGESTEROONKONSENTRASIES HET GEEN VERSKILLE GETOON TUSSEN BEHANDELINGSGROEPE TYDENS DIE OESTRUS SINKRONISASIEPERIODE NIE. BY 48H NA DIE TWEEDE KI (96H NA CIDR ONTTREKKING) HET DIE KONTROLE OOIE 'N BETEKENISVOLE ($P<0.05$) HOËR GEMIDDELDE SERUM-PROGESTEROONKONSENTRASIE (11.3 ± 4.9 NG/ML) GEMEET, VERGELEKE MET DIE FSH/GNRHA-BEHANDELDE OOIE (4.3 ± 2.1 NG/ML). DIE SERUM-PROGESTEROONKONSENTRASIE HET GESTYG NA DIE TWEEDE KI EN WAS DIE HOOGSTE OP DIE DAG VAN SPOELING IN BEIDE GROEPE – SONDER ENIGE BETEKENISVOLLE VERSKILLE. DIE GEMIDDELDE SERUM OESTROGEENKONSENTRASIE WAS BETEKENISVOL ($P<0.05$) HOER OP DAG 12 VAN SINKRONISASIE (5DE DAG VAN GNRHA BEHANDELING) EN BY DIE 4DE PFSH (SUPEROVULASIE) INSPUITING IN DIE FSH/GNRHA BEHANDELDE GROEP, VERGELEKE MET DIE KONTROLE OOIE. BY TYE VAN DIE EERSE KI (36H NA CIDR ONTTREKKING) WAS DIE GEMIDDELDE SERUM OESTROGEENKONSENTRASIE BETEKENISVOL ($P<0.05$)

HOËR (58.3 ± 26.9PG/MOL) IN DIE KONTROLE (FSH-BEHANDELING), VERGELEKE MET DIE FSH/GNRHA-BEHANDELDE OOIE (15.7 ± 17.4 PG/ML). DIE BLOED HORMOONVLAKKE GEÏNDUSEER TYDENS SUPEROVULASIE WAS AFHANKLIK VAN VERSKEIE OVARIALE FAKTORE EN DUS EINDELIK OP DIE LEWENSVATBAARHEID VAN DIE OÖSIETE EN DAN DIE EMBRIOS. 'N DERDE PROEF WAS UITGEVOER TYDENS DIE NATUURLIKE TEELSEISOEN (HERFS, 2006). SEWENTIEN VOLWASSE BOERBOKOOIE IS GEBRUIK AS DONORS OM DIE DOELTREFFENDHEID VAN FSH SUPEROVULASIEBEHANDELING TE VERFYN DEUR GEBRUIK TE MAAK VAN 2 ROETES VAN GONADOTROPIENTOEDIENING. HIER IS 27 ONTVANGERS GEBRUIK OM DIE OORLEWINGSTEMPO VAN DIE BOKEMBRIOS TE EVALUEER NA BEVRIESING DEUR GEBRUIK TE MAAK VAN DIE KONVENSIONELE STADIGE BEVRIESING – OF VITRIFIKASIEMETODE. OOIE HET DIE PFSH BEHANDELING OF INTRAMUSKULÊR OF ONDERHUIDS ONTVANG, TERWYL DIE ONTVANGERS EMBRIOS ONTVANG HET WAT VOLGENS DIE VERSKILLENDE METODES GEVRIES IS. DIE ROETE VAN HORMONALE TOEDIENING HET GEEN EFFEK GEHAD OP DIE OESTRUS RESPONS, TYDSDUUR VAN CIDR ONTTREKKING TOT DIE VOORKOMS VAN OESTRUS EN DIE TYDSDUUR VAN DIE GEÏNDUSEERDE OESTRUSPERIODE. DIE ROETE VAN GONADOTROPIEN TOEDIENING HET NIE DIE OVULASIETEMPO, DIE TOTALE AANTAL STRUKTURE, AANTAL EMBRIOS EN OORPLAASBARE EMBRIOS GEKOLLEKTEER PER DONOR, BEÏNVLOED NIE. DIE GEMIDDELDE AANTAL ONBEVRUGTE OVA IN DIE I.M. GROEP (3.3 ± 4.8 PER DONOR) WAS BETEKENISVOL ($P<0.05$) HOËR AS DIE S.C. (ONDERHUIDSE) GROEP (0.3 ± 0.8). SOORTGELYK WAS DIE GEMIDDELDE AANTAL GEDEGENEREERDE EMBRIOS PER DONOR IN DIE FSH ONDERHUIDSE

BEHANDELDE GROEP (5.9 ± 4.5) BETEKENISVOL ($P<0.05$) HOËR AS DIE INTRAMUSKULÊRE-BEHANDELDE GROEP (2.6 ± 2.3). ’N TOTAAL VAN 88.9% VAN DIE ONTVANGEROOIE HET TEKENS VAN OESTRUS GETOON NA CIDR ONTTREKKING. ’N BESETTINGSYFER VAN 85.7%, 50.0% EN 37.5% IS WAARGENEEM NA DIE OORPLAAS VAN VARS, STADIG-BEVRORE EN GEVITRIFIKEERDE EMBRIOS RESPEKTIEWELIK. EMBRIO-OORLEWINGSTEMPOS VAN 35.7%, 25.0% EN 31.3% IS DAN EINDELIK GEMEET NA VARS, STADIG-BEVRORE EN GEVITRIFIKEERDE EMBRIO-OORPLASINGS. DIT KAN DUS AANVAAR WORD DAT ’N VOORDEEL IN DIE ONDERHUIDSE TOEDIENING VAN HORMONE TOT GEVOLG GEHAD HET DAT ’N KLEINER AANTAL ONBEVRUGTE OVA HERWIN IS – WAT EGTER TEËGEWERK IS DEUR ’N HOËR AANTAL DEGENERATIEWE EMBRIOS GEMEET. DUS KAN BEIDE ROETES VAN PFSH TOEDIENING GEBRUIK WORD IN SUPEROVULASIE BEHANDELINGS BY BOERBOKOOIE TYDENS DIE TEELSEISOEN. ’N RELATIEWE HOËR BESETTINGSSYFER IS VERKRY NA OORPLASING VAN VARS EMBRIOS. DIE OORLEWINGSTEMPO VAN DIE EMBRIOS NA VARS, STADIG-BEVRORE EN GEVITRIFIKEERDE EMBRIOS WAS TELEURSTELLEND. MEER NAVORSING IS DUS NODIG MET ’N HOËR GETAL DIERE – GEMIK NA DIE VERBETERING VAN DIE OORLEEFBAARHEID VAN EMBRIOS MET VARS OF BEVRORE BOK EMBRIO-OORPLASINGS.

DIE VIERDE PROEF IS UITGEVOER TYDENS DIE NATUURLIKE TEELSEISOEN (HERFS, 2007). DONOROOIE IS GESUPEROVULEER NA ’N LANG PROGESTAGEEN (17 DAE) PROTOKOL, MET OF SONDER PROSTAGLANDIEN $F_{2\propto}$ OF GESUPEROVULEER BY ’N BERAAMDE TYD VAN OVULASIE (DAG 0 PROTOKOL). ONTVANGER BOKOOIE HET GEVRIES-ONTDOOIDE OF VARS BOERBOK EMBRIOS ONTVANG

NA OESTRUS SINKRONISASIE MET DIE GEBRUIK VAN DIE VERSKILLENDE METODES. MET DIE DAG 0 PROTOKOL VAN SINKRONISASIE BEHANDELING VOOR SUPEROVULASIE, HET SLEGS 71.4% VAN DIE OOIE OESTRUS GETOON, TERWYL SLEGS 1 OOI OESTRUS GETOON HET NA SUPEROVULASIE. ALLE OOIE HET OESTRUS GETOON NA DIE LANG PROGESTAGEEN BEHANDELING MET OF SONDER PROSTAGLANDIEN $F_{2\propto}$ BEHANDELING. ALLE OOIE HET OOK 'N SOORTGELYKE TYD VAN VOORKOMS VAN OESTRUS NA CIDR ONTTREKKING EN TYDSDUUR VAN DIE GEÏNDUSEERDE OESTRUSPERIODE GETOON. DIE DAG 0 PROTOKOL HET 'N BETEKENISVOLLE ($P<0.01$) LAER AANTAL CL'S (4.0 ± 3.5) PER DONOR TOT GEVOLG GEHAD – VERGELEKE MET GROEP 2 (17 DAE PROGESTAGEEN BEHANDELING PLUS $PGF_{2\propto}$ (14.5 ± 4.6) EN GROEP 3 (17 DAE PROGESTAGEEN BEHANDELING) (16.5 ± 5.9). DIE GEMIDDELDE AANTAL STRUKTURE VERKRY IN GROEP 1 (1.4 ± 0.5) WAS BETEKENISVOL LAER, VERGELEKE MET GROEP 3 (11.4 ± 7.3). HIERDIE GEMIDDELD IN DIE DAG 0 GROEP HET EGTER NIE BETEKENISVOL VERSKIL WANNEER VERGELYK MET GROEP 2 (8.4 ± 7.7). DIE VARIASIE GEMEET IN GROEP 2 WAS BAIE HOOG.

DIE DAG 0 PROTOKOL HET DUS 'N BETEKENISVOL ($P<0.01$) LAER GEMIDDELDE AANTAL EMBRIOS TOT GEVOLG GEHAD, WANNEER VERGELYK MET GROEP 2 EN 3. DIE GEMIDDELDE AANTAL ONBEVRUGTE OVA EN DEGENERATIEWE EMBRIOS WAS EGTER SOORTGELYK VIR ALLE BEHANDELINGSGROEPE. DIE DAG 0 (GROEP 1) GROEP HET SLEGS EEN EMBRIO GEREALISEER, WAT OORPLAASBAAR WAS – MAAR AS GEVOLG VAN DIE ENKELWAARDE IS DIE DATA NIE GEANALISEER NIE. DIE BYVOEGING VAN PROSTAGLANDIEN $F_{2\propto}$ HET GEEN EFFEK OP DIE BEVRUGTINGSYFER EN DIE GEMIDDELDE AANTAL OORPLAASBARE EMBRIOS IN GROEP 2 EN 3 GEHAD

NIE. DIE TYDSDUUR VAN CIDR ONTTREKKING TOT DIE AANVANG VAN OESTRUS IN VOLWASSE OOIE WAS BETEKENISVOL KORTER AS IN JONG OOITJIES. OUDERDOM HET EGTER NIE 'N EFFEK GEHAD OP DIE TYDSDUUR VAN DIE GEÏNDUSEERDE OESTRUSPERIODE NIE. DIE GEMIDDELDE AANTAL CL STRUKTURE EN EMBRIOS HERWIN WAS BETEKENISVOL ($P<0.01$) HOËR IN DIE VOLWASSE OOIE. GEEN HERWINNING VAN ONBEVRUGTE OVA IS AANGETEKEN IN DIE JONG OOIE NIE EN DIE BEVRUGTINGSTEMPO EN GEMIDDELDE AANTAL ONBEVRUGTE OVA HET NIE VERSKIL TUSSEN DIE JONG EN OUER OOIE NIE. SOORTGELYK HET DIE OUDERDOM VAN DIE OOI GEEN EFFEK GEHAD OP DIE AANTAL DEGENERATIEWE EMBRIOS PER DONOR NIE. DIE GEMIDDELDE AANTAL OORPLAASBARE EMBRIOS WAS EGTER BETEKENISVOL ($P<0.01$) HOËR IN DIE VOLWASSE OOIE (15.8 ± 6.4), VERGELEKE MET DIE JONGOOIE (9.5 ± 3.7).

DIE TYDSDUUR VAN CIDR ONTTREKKING TOT OESTRUS WAS NIE GEAFFEKTEER DEUR HERHAALDE SUPEROVULASIE BEHANDELINGS NIE. BOKOOIE WAT VIR DIE EERSTE KEER GESUPEROVULEER IS, HET 'N BETEKENISVOL ($P<0.05$) KORTER TYDSDUUR VAN OESTRUS (20.8 ± 10.1H) GETOON, VERGELEKE MET OOIE WAT HERHAALDELIK BEHANDEL IS (30.4 ± 6.7H). HERHAALDE BEHANDELING HET GEEN EFFEK OP DIE GEMIDDELDE AANTAL CL'S GEPRODUSEER PER OOI NIE. DIE GEMIDDELDE AANTAL STRUKTURE HERWIN WAS EGTER BETEKENISVOL ($P<0.05$) LAER IN DIE HERHAALDE BEHANDELDE GROEP (6.0 ± 8.7), VERGELEKE MET OOIE BEHANDEL VIR DIE EERSTE KEER (11.7 ± 5.0). SOORTGELYK WAS DIE GEMIDDELDE AANTAL EMBRIOS HERWIN PER OOI BETEKENISVOL ($P<0.05$) HOËR (12.9 ± 5.0) IN OOIE GESUPEROVULEER VIR DIE EERSTE KEER – VERGELYK MET DAARDIE OOIE WAT HERHAALDELIK

BEHANDEL IS (3.8 ± 8.4). DIE GEMIDDELDE AANTAL ONBEVRUGTE OVA PER DONOR WAS OOK BETEKENISVOL ($P<0.05$) HOËR IN DIE HERHAALDE BEHANDELINGSGROEP (5.5 ± 7.8), VERGELEKE MET DIE 0.1 ± 0.3 VIR OOIE BEHANDEL VIR DIE EERSTE KEER. DIT HET 'N BETEKENISVOL ($P<0.05$) LAER BEVRUGTINGSSYFER TOT GEVOLG GEHAD IN DIE GROEP WAT HERHAALDE BEHANDELINGS ONTVANG HET (50.0 ± 70.7%), VERGELEKE MET OOIE GESUPEROVULEER VIR DIE EERSTE KEER (99.4 ± 1.9%). DIE GEMIDDELDE AANTAL DEGENERATIEWE EMBRIOS HET NIE BETEKENISVOL VERSKIL TUSSEN DIE GROEPE NIE. DIE AANTAL OORPLAASBARE EMBRIOS WAS LAER ($P<0.05$) IN DIE HERHAALDE BEHANDELINGSGROEP (3.8 ± 8.5), VERGELEKE MET DIE ANDER (10.7 ± 4.0).

IN GROEP A (CIDR + $PGF_{2\propto}$ + ECG; CHRONOGEST) HET 88.9% OOIE REAGEER OP OESTRUS SINKRONISASIE. ALLE OOIE IN GROEP B (CIDR + ECG; FOLLIGON) EN GROEP C (CIDR + ECG; CHRONOGEST) HET TEKENS VAN OESTRUS GETOON NA SINKRONISASIE. OOIE VAN GROEP A (42.0 ± 3.7H) HET 'N LANG TYD ($P<0.05$) GETOON VANAF CIDR ONTTREKKING TOT OESTRUS, VERGELEKE MET GROEP C (32.0 ± 8.6H). DAAR WAS EGTER GEEN BETEKENISVOLLE VERSKIL TUSSEN GROEP A EN B NIE. GROEP B EN C HET OOK 'N SOORTGELYKE TYD GEMEET VANAF CIDR ONTTREKKING TOT DIE AANVANG VAN OESTRUS. DIE TYDSDUUR VAN DIE GEÏNDUSEERDE OESTRUSPERIODE WAS BETEKENISVOL ($P<0.05$) KORTER IN GROEP B (19.0 ± 13.5H), WANNEER VERGELYK MET GROEP A (39.0 ± 15.1H). GEEN BETEKENISVOLLE VERSKIL AANGAANDE DIE TYDSDUUR VAN DIE GEÏNDUSEERDE OESTRUSPERIODE WAS WAARGENEEM TUSSEN GROEP B EN C NIE. DIT KAN DUS AANVAAR WORD DAT DIE SWAK OVARIALE RESPONS TOT

SUPEROVULASIE BEHANDELING IN GROEP 1 (DAG 0 PROTOKOL) VERDERE NAVORSING REGVERDIG WAT MOET FOKUS OP DIE SINKRONISASIE VAN OVULASIE EN ’N TOEGEPASTE TYD VIR DIE INISIËRING VAN DIE SUPEROVULASIEBEHANDELING IN HIERDIE PROTOKOL. DIE BEHANDELING MET $PGF_{2\propto}$ IN DIE SUPEROVULASIE PROTOKOL NA ’N LANG PROGESTAGEEN SINKRONISASIE IN DIE BOERBOKOOI HET OOK GEEN VOORDEEL NIE. DIE TYD VAN PROSTAGLANDIENTOEDIENING EN DIE DOSIS GEBRUIK KON TOT BOGENOEMDE RESULTATE BYGEDRA HET.

DIE OUDERDOM VAN DIE DONOR BOKOOI IS BEWYS OM ’N GROOT ROL TE SPEEL OP DIE OVARIALE AKTIWITEIT NA SUPEROVULASIE. ALHOEWEL DIE JONGOOIE ’N AANVAARBARE BEVRUGTINGSYFER GETOON HET, WAS DIE AANTAL OORPLAASBARE EMBRIOS LAER. DUS AS ’N GROOT AANTAL EMBRIOS BENODIG WORD, IS DIT WENSLIK OM VOLWASSE OOIE TE SUPEROVULEER. DIT SAL LEI TOT DIE PRODUKSIE VAN MEER OORPLAASBARE EMBRIOS.

DIE AANTAL KERE WAT ’N DONOR GEBRUIK KAN WORD IN ’N BOERBOK EMBRIOPROGRAM, BLYK OM BEPERK TE WEES TOT 3 KEER – A.G.V. ’N AFNAME IN DIE AANTAL STRUKTURE EN EMBRIOS HERWIN – GEMEET AAN OOIE WAT ’N VIERDE KEER BEHANDEL IS. SOORTGELYK HET DIE AANTAL ONBEVRUGTE OVA VERMEERDER NA HERHAALDE BEHANDELING MET ’N GEVOLGLIKE AFNAME IN BEVRUGTINGSTEMPO EN EINDELIK GETAL EMBRIOS WAT OORPLAASBAAR IS.

DIE 3 OESTRUS SINKRONISASIE PROTOKOLS VIR ONTVANGEROOIE WAS EFFEKTIEF. DIE DRAGTIGHEIDSSYFER WAS EGTER LAAG IN AL DIE BEHANDELINGSGROEPE EN DIE INTERACTSIE KOMPLISEER DIE SINVOLLE MAAK VAN ENIGE AANBEVELINGS.

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