GEORG-AUGUST-UNIVERSITÄT
GÖTTINGEN

Control of estrous cycle and superovulation in goats

Department of Animal Science

Georg-August University, Goettingen, Germany

Miguel Camacho

Control of estrous cycle and superovulation in goats

Dissertation
to obtain the Ph. D. degree
in the International Ph. D. Program for Agricultural Sciences in Goettingen (IPAG) at the Faculty of Agricultural Sciences,
Georg-August-University Göttingen, Germany

presented by

Miguel Angel Camacho Aranda

born in Monterrey, México

Göttingen, May 2020

D7

1. Name of supervisor: Prof.Dr.Dr. Matthias Gauly

2. Name of co-supervisor: Prof.Dr. Wolfgang Holtz

Date of dissertation: 12th May, 2016

Dedicated To

My beloved wife and sons

Acknowledgments

I would like to thank the Consejo Nacional de Ciencia y Tecnologia (CONACYT) and the German Academic Exchange Service (DAAD) for their financial support during my studies.

I would like to express my most sincere gratitude to my mentor Prof. Dr. Wolfgang Holtz for providing me with guidance and knowledge throughout my studies, and for his friendship.

I would also like to thank Prof. Dr. Dr. Matthias Gauly, for giving me the opportunity to be a part of his work group, and Prof. Dr. Christoph Knorr, who kindly accepted to be my third examiner.

I am thankful to my friends and senior colleagues Dr. Gürbuz Das, Dr. Morten Friedrich and Dr. Moataz El-Gayar for their advices, invaluable help and friendship.

Extra thanks to Dieter, Jürgen, Christina, Ricarda and Louisa for their priceless assistance with animal handling.

My most heartwarming appreciation goes to Elli, not only for her assistance with hormone analyses, but also for her incomparable friendship.

Thanks to all my friends, Dr. Daisy, Dr. Daniela, Daniel, Dr. Mazhar, Dr. Shayan, Dr. Abdussamad, Dr. Kalyakorn, Rania, Dr. Katrin, Dr. Kerstin, Dr. Sabrina, Dr. Vu, Dr. Alex, Eugenia, Lupita, Jorge, Pablo, Monica, Ari, Mary, Ramon, Valeria, Jose, Anika, Juan Ra, Ikal and Surem, for the family- like environment they created and for the great moments together, which I will always cherish.

Last but not least, thanks to my favorite all-time life companion and colleague, my wife, for her support, love that she has always given me, and for her calming words in my most stressful moments, such as “don’t worry, next time you will get more embryos”

To all whose names are inadvertently left out, please accept my sincere gratitude.

Table of contents

Chapter 3

Chapter 4

Chapter 5

List of Tables

Chapter 2

Chapter 3

Chapter 4

List of Figures

Chapter 2

Chapter 3

Chapter 4

List of Abbreviations

°C	Celcius
µg/ml	Micrograms per milliliter
ANOVA	Analysis of variance
AU	Armour units
BSA	Bovine serum albumin
CIDR	Controlled internal drug release
cm	Centimeters
eCG	Equine chorionic gonadotropin
ELISA	Enzyme-linked immunoassay
FGA	Fluorogestone acetate
FSH	Follicle stimulating hormone
g	Force of acceleration
GnRH	Gonadotropin releasing hormone
i.m.	Intramuscular
ID	Inner diameter
IU	International units
IU/ml	International units per milliliter
kg	Kilogram
LH	Luteinizing hormone
MAP	Medroxyprogesterone acetate
MHz	Megahertz
min	Minute
ml	Milliliter
mm	Millimeter
n	Number of animals
ng/ml	Nanogram per milliliter
OD	Outer diameter
Ovsynch	Ovulation synchronization
P	Probability
PE	Polyethylene
pFSH	Porcine follicle stimulating hormone
$PGF_{2\alpha}$	Prostaglanfin $F_{2\alpha}$
PVP	Polyvinilpyrrolidone
r	Correlation coefficient
SE	Standard error
vs.	versus

Summary

Estrous cycle control is a widely used reproductive biotechnology for controlled breeding of small ruminants. Besides serving the purpose of synchronizing estrus, so that breeding takes place at a particular time, it serves as basis for associated biotechnologies such as fixed-time artificial insemination, superovulation and embryo transfer.

The first of three studies addresses a comparison of various estrous cycle control protocols and the effect of season in the northern temperate zone on the superovulatory response in Boer goats. Forty-eight pluriparous Boer goat does from our own breeding flock were used in this study. Four groups of 12 does each were treated in February, May, August and November, respectively. Estrus was synchronized by means of progestagen impregnated vaginal pessaries. Half of the does of each group received sponges impregnated with 20 mg fluorogestone acetate (FGA, Cronolone), while the other half received Eazy-Breed CIDRs containing 0.3 mg progesterone. Intravaginal pessaries remained in place for 7 days. Within each subgroup half the does were treated with PGF2α ("Dinoprost") at pessary insertion, the other half at pessary withdrawal. Beginning 48 h before withdrawal, does were superovulated with six s.c. injection of 4, 4, 2, 2, 2 and 2 Armour Units (AU) of pFSH, supplemented with 40% pLH administered at 12 h intervals. Estrus detection was conducted at 8 h intervalas with the aid of an aproned adult buck and ovarian activity was monitored daily by ultrasonography. Does were mated and 7 days later non-surgical embryo collection was conducted. The type of intravaginal pessary had no effect on the time passing between pessary withdrawal and onset of estrus (sponge: 40.7 h vs. CIDR: 35.2 h), number of ovulations/doe assessed by echographic counting of collapsed large follicles (7.2 vs. 7.6) or corpora lutea (7.1 vs. 9.8), embryo recovery rate (39 % vs. 41 %) and proportion of transferable embryos (66 % vs. 65 %). The effect of PGF2α administration at insertion or at removal of intravaginal pessaries and the effect of season were not significant, except for a shorter interval between pessary removal and onset of estrus in August in comparison to the other months (29.3 vs. 39.4, 40.6 and 42.1 h; $P < 0.05$). From these results it can be concluded that a short (7 day) treatment with either Cronolone sponges or CIDRs as a part of a superovulation protocol is equally effective in synchronizing estrus. The luteolytic PGF2α treatment may be administered at the beginning or at the end of the progestogen

treatment; the latter having the advantage that any corpora lutea present will be receptive. Thus, superovulation and embryo collection may be performed throughout the year, i.e, during and out of the breeding season.

In the second study seasonality in Boer goats in Northern Europe and attempts to overcome it is addressed in three experiments. In the first experiment, estrus activity and serum progesterone concentrations were monitored on sexually matured nulliparous Boer goat crosses. Results indicated that ovarian activity ceased in February/March and was resumed from August onward. In 63.7% of 22 does the first estrus of the season was preceded by one or two ovulations, indicated by an increase in progesterone; in 22.7% first estrus and first ovulation coincided and in the remaining 13.6% the first estrus was not succeeded by formation of a functional corpus luteum. In the second experiment, 31 pluriparous Boer does that weaned between July and September, were randomly allocated to three treatment groups. Does were treated with intravaginal sponges impregnated with 20 mg of the synthetic progestagen fluorogestone acetate (FGA, Intervet, France), either one (Group 1; n=11), seven (Group 2; n=10) or fourteen days after weaning (Group 3; n=10). Sponges were withdrawn after 7 days and simultaneously 250 IU equine chorionic gonadotropin (eCG, Intergonan, Intervet, Unterschleissheim, Germany) was administered i.m. Does were tested for estrus with the aid of an aproned adult buck at 8 h intervals, and on each day of standing estrus they were naturally mated. Pregnancy was diagnosed by transrectal ultrasonography (ALOKA-SSD 500, equipped with transrectal 7.5 MHz linear array transducer) 30 and 45 days after mating. The proportion of does exhibiting estrus was 91%, 100% and 90% for Groups 1, 2 and 3, respectively. The overall average interval from sponge withdrawal to onset of estrus was 33.0 h, ranging from 16 to 72 h, with no significant difference among treatment groups ($P>0.05$). The overall proportion of pregnant does at 45days after mating was 42%. All of these carried to term (36% treated immediately after weaning, 40% treated one week later and 50% treated two weeks later) and gave birth to, on average, 2.1 kids. In the third experiment, 18 nulliparous does were subjected to the same sponge-eCG protocol described in the second experiment out-of-season between April and mid-June. Does were tested for estrus with the aid of an aproned adult buck at 8 h intervals, and on each day of standing estrus they were

naturally mated. Pregnancy was diagnosed 35 days after mating. Serum was analyzed for progesterone content by ELISA. Of 18 does treated, 15 (83%) exhibited estrous symptoms. The mean interval from sponge withdrawal to onset of estrus was of 44.3 (range 16 to 66) hours. One doe showed estrus but would not permit intromission. Of the 14 goats mated, only 6 (43%) were diagnosed pregnant and none carried to term. Serum progesterone concentration at insertion and withdrawal of sponges was less than 0.5 ng/ml. Thereafter, progesterone concentration increased to 5.0 ng/ml and 5.4 ng/ml one week after sponge withdrawal in pregnant and non-pregnant does, respectively ($P>0.05$). Maximum progesterone levels were reached two weeks after sponge withdrawal in both pregnant and non-pregnant does (14.0 and 9.3 respectively; $P>0.05$). A significant difference in progesterone concentration between pregnant and non-pregnant does was recorded three weeks after sponge withdrawal (13.0 vs. 4.8; $P<0.05$). From the fourth week after sponge withdrawal onward, in non-pregnant goats mean progesterone levels had declined to basal level (less than 0.5 ng/ml); whilst in pregnant goats only a slight, non-significant decline to 8.0 (SE 2.0) ng/ml was recorded by four weeks and 7.4 (SE 2.0) ng/ml by five weeks after sponge withdrawal. From the present study it may be concluded that in northern Europe Boer goats, albeit to a lesser degree than most dairy breeds, undergo a period of ovarian inactivity, extending approximately from April to August. The stage of ovarian quiescence may be overcome by progestogen-eCG treatment. When mated at induced estrus, close to 50% of weaned does produced, on average, 2.1 kids, whereas none of the young nulliparous does carried to term. To what extent unfavorable environmental factors may have played a role is open to conjunction.

The purpose of the third study was to determine temporal relationships between estrus, preovulatory LH surge and ovulation in Boer goat does subjected to different regimes for controlling estrus and ovulation. The study was conducted during the breeding season (late August to December) on 28 pluriparous does. Does were randomly allocated to three treatment groups. In Group 1 (n=8), blood samples were drawn daily from day 5 to day 12 of the estrous cycle to be analyzed for progesterone content. As soon as serum progesterone exceeded 5 ng/ml, two injections of dinoprost were administered at 12 h interval. Does were tested for estrus with the aid of an

aproned adult buck at 8 h intervals. Ovarian activity was monitored ultrasonographically 24, 72 and 96 hours after the first dinoprost injection. After the first dinoprost injection, blood samples were drawn every 3 hours until 30 hours after the onset of behavioural estrus. Does of Group 2 (n=10) were provided with intravaginal sponges impregnated with 20 mg of the synthetic progestagen flurogestone acetate for a period of 11 days. Forty-eight hours before withdrawal, does received two i.m. injection of 5 mg dinoprost at 7 hour interval. Concurrent with the first dinoprost injection, 200 IU of eCG was administered i.m. Does were tested for estrus with the aid of an aproned adult buck at 8 h intervals. Ovarian activity was monitored 24 and 48 hours after sponge withdrawal. Does of Group 3 (n=10) were subjected to the same treatment as does of Group 2, with the only difference that, 30 h after sponge withdrawal, they received an i.m. injection of 0.004 mg of the GnRH-analog buserelin. In Group 3 ovarian activity in the latter group was not monitored. Blood plasma collected from does in the three treatment groups was analyzed for luteinizing hormome (LH) content by ELISA. Ovulation was assumed to have taken place halfway between the ultrasound measurements before and after collapse of large follicles (Suyadi and Holtz, 2012). All does presented an estrous response irrespective of the treatments applied. Estrus lasted 37.5, 40.8 and 44.8 hours in Groups 1, 2 and 3, respectively ($P>0.05$). The interval to onset of estrus was significantly longer in Group 1 in comparison to in Groups 2 and 3 (40.3 vs. 23.4 and 26.4; $P<0.05$). In Group 1, the interval from the first dinoprost injection to the LH peak was significantly longer than the interval observed from sponge withdrawal in Groups 2 and 3 (44.7 vs. 34.5 and 32.0 h; $P<0.05$). The interval from the onset of estrus to the LH peak was 7.2, 12.8 and 5.6 h for Groups 1, 2 and 3, respectively, being only significantly different between Groups 2 and 3 ($P<0.05$). Does in Group 3 presented a tightly synchronized LH peak 32.0 h after sponge withdrawal, and 2 h after the administration of buserelin. In Groups 1 and 2, this peak was less synchronous, ranging from 24 to 57 h in Group 1 (after the first dinoprost injection) and 27 to 45 h in Group 2 (after sponge withdrawal). The magnitude of the LH peak was significantly lower in Group 1 as compared to Groups 2 and 3 (46.5, range 19.0-70.0 ng/ml vs. 59.6, range 32.0-96.0 ng/ml and 82.3, range 50-94.0 ng/ml; $P<0.05$). Based on the echographic monitoring, ovulation occurred 47.9 and 37.5 h after the occurrence of the recorded LH peak in Groups 1 and 2 ($P>0.05$). From the results obtained in the present study, iy may

be concluded that a prostaglandin regime designed to synchronize estrus during the breeding season is equally effective as a progestagen regime. Incorporating GnRH into the sponge-eCG treatment will induce a highly synchronized preovultory LH peak. This can be particularly useful when practicing fixed-time artificial insemination.

Chapter 1

General introduction and literature review

1.1 General introduction

Controlling the estrous cycle serves the purpose of synchronizing estrus in groups of females. Various estrus synchronization protocols have been suggested over the years, involving the use of natural or synthetic progestagens and their combination with other hormones such as eCG and prostaglandins. Moreover, estrus synchronization serves as basis for other associated biotechnologies such as artificial insemination, superovulation and embryo transfer. Traditionally, in goats progestagen treatment extended over a period of 18 days. However, studies have shown that short progestagen exposure is sufficient to induce a satisfactory estrus response. In contrast to the prolonged progestagen treatment, in which the corpus luteum undergoes natural atresia, in short progestagen treatments the administration of PGF2α to induce luteolysis is crucial for the treatment to be effective. In some treatment protocols PGF2α is administered at the beginning of the progesterone treatment (Martemucci and D´Alessandro, 2011b; Inya and Sumretprason, 2013) in others at the end (Beck et al., 1993; Romano, 1996; Lehloenya and Greyling, 2010). The objective of the first study of the dissertation was to assess the superovulatory response in goats subjected to a short-term progestagen treatment with one of two different progestagen releasing pessaries, and prostaglandin administration at the beginning or at the end of progestagen treatment.

Ovarian activity in goats commences as daylight decreases during autumn and winter and tends to cease when daylight increases (Shelton, 1978). There are reports indicating that estrus and ovulation can be induced outside the breeding season with the aid of progestagen-containing intravaginal pessaries or ear implants (Holtz and Sohnrey, 1992). The ability of the progestagen treatment to induce and synchronize estrus during this period permits year-round availability of goat products, including the increase of offspring/doe/year. At the end of progestagen treatment generally equine chorionic gonadotropin (eCG) is administered to support resumption of ovarian activity. The second study was an attempt to characterize the resumption of cyclicity with decreasing daylight in autumn and attempts to overcome seasonal acyclicity by estrus induction and insemination out-of-season.

One of the most studied parameters in estrus-controlled females has been the relative time of ovulation after synchronization treatment (Ritar et al., 1984; Holtz et al., 2008; Martemucci and D´Alessandro, 2011; Cox et al., 2012). Knowing the exact time of ovulation and other preovulatory events after estrus synchronization may improve the conception rates especially if artificial insemination at a predetermined time isi intended. Progestagen treatments in combination with GnRH analogs are normally used to achieve synchrony in the occurrence of ovulation in estrus controlled females. In order to pinpoint the exact time of ovulation several attempts have been made, such as repeated laparoscopies (Chemineau, 1983 and Baril and Vallet, 1990), ultrasonic monitoring of ovaries (Castro et al., 1999; Simões et al., 2006; Zongo et al., 2015) and determination of the preovulatory LH surge (Greyling and Van Nierkerk, 1991). In the third study of this dissertation it was attempted to determine the temporal relationships between behavioral estrus, preovulatory LH surge and ovulation in Boer goat does subjected to various regimes for controlling estrus and ovulation.

1.2. Literature review

1.2.1. Estrus synchronization

The control of the estrous cycle serves the purpose of synchronizing estrus in groups of females, so that breeding takes place at a predetermined time. Serving as basis for other associated biotechnologies such as, artificial insemination, superovulation and embryo transfer, estrus synchronization has become an important element in controlled breeding of small ruminants.

1.2.1.1. The use of prostaglandin $F_{2\alpha}$

Due to its luteolytic action a single prostaglandin $F_{2\alpha}$ injection at an appropriate dose will induce luteolysis followed by estrus (Ogunbiyi et al., 1979; Bretzlaff et al., 1983; Greyling and Van Niekerk, 1986). However, if groups of animals at different stages of the estrous cycle are to be synchronized, a second injection administered 10 or 11 days apart is required (Ogunbiyi et al., 1980; Nandy et al., 1990; Beck et al., 1993; Kusina et al., 2000; Menchaca and Rubianes, 2004; Fonseca et al., 2012). To be effective prostaglandin treatment requires the presence of functional corpora lutea; therefore, in periods of ovarian quiescence, characteristic of seasonal breeders, the treatment is of no avail.

1.2.1.2. The use of exogenous progestagens

Estrus synchronization can be accomplished with the use of natural or synthetic progestagens administered either as intravaginal pessaries (Dewesee et al., 1970; Baril et al., 1993; Wildeus, 2000; Holtz, 2005; Rahman et al., 2008) or as subcutaneous implants (Holtz and Sohnrey, 1992; Yuswiati and Holtz, 1996). The use of synthetic progestagens such as FGA (fluorogestone acetate) or MAP (medroxyprogesterone acetate) to synchronize estrus has been extensively reviewed in sheep (Dewesee et al., 1970; Colas et al., 1973; Quirke, 1977; Thompson et al., 1990; Luther et al., 2005) and goats (Puls-Kleingeld et al., 1991; Holtz and Sohnrey, 1992; Romano, 1994; Baril et al., 1993; Greyling and van der Nest, 2000; Motlomelo et al., 2002; Holtz et al., 2008). Steffan et al. (1983) and Motlomelo et al. (2002) report no differences in the effectiveness between FGA and MAP sponges apart from the interval from withdrawal

to estrus reported by Romano, 1996 and Ungerfeld and Rubianes, 2002.

The CIDR, a Y-shaped silicone-coated intravaginal device impregnated with natural progesterone, has been described as an alternative to the sponge treatment. Studies comparing the effectiveness of intravaginal sponges and CIDR devices, agree that both devices are equally effective in synchronizing estrus (Rhodes and Nathanielsz, 1988; Motlomelo et al., 2002; Ungerfeld and Rubianes, 2002; Bitaraf et al., 2007). Traditionally, the progestagen treatment extended over a period of 18 days, a period long enough for the corpora lutea to undergo natural luteolysis (Hotlz, 2005). It was suggested, however, that a short-term treatment of 5 to 7 days is enough to induce an estrous response, provided existing corpora lutea are induced to regress. This is most effectively accomplished by prostaglandin$F_{2\alpha}$ treatment (Christenson, 1976; Iglesias et al., 1996). Equine chorionic gonadotropin (eCG) is normally administered upon withdrawal or up to 48 hours before the end of the progestagen treatment (Cline et al., 2001; Maurel et al., 2003; Holtz, 2005). The progestagen-eCG treatment has proved to be effective in cycling as well as non-cycling animals (Leboeuf et al., 2003; Martemucci and D´Alessandro, 2011) although, due to an immunological response to the heterologous gonadotropin (Roy et al., 1999), the repeated use of eCG may result in impaired fertility (Baril et al., 1992; Drion et al., 2001).

1.2.2. Induction of ovulation

In estrus controlled females the time of ovulation may be programmed by administering a synthetic GnRH analog (Rubianes et al., 1997; Pierson et al., 2003). The "Ovsynch" protocol, introduced for cows by Pursley et al. (1995), combines the action of $PGF_{2\alpha}$ with the ovulation inducing capacity of GnRH, thus allowing for fixed-time insemination. This approach has been proven to be effective during the breeding season in goats as well (Holtz et al., 2008). To pinpoint the time of ovulation, Chemineau (1983) and Baril and Vallet (1990) conducted repeated laparoscopies. More recent approaches to that end are the ultrasonic monitoring of ovarian functions (de Castro et al., 1999; Simões et al., 2006; Zongo et al., 2015) and determination of the preovulatory LH surge (Greyling and Van Nierkerk, 1991; Holtz et al., 2008). According to Pierson et al. (2003), an LH surge is observed at approximately 2 hours after GnRH

administration. Based on several observations, ovulation is known to occur at approximately 18-24 hours after the preovulatory LH peak (Baril and Saumande, 2000; Hotlz, 2005; Martinez-Alvarez et al., 2007; Simões et al., 2008a).

1.2.3. Superovulation and embryo collection

Superovulation is generally accomplished by a combination of estrus cycle control with an elevated dose of gonadotropic hormones. Different regimens to induce superovulation have been proposed by several authors, including different FSH/LH ratios (Nowshari et al., 1995; D´Alessandro et al., 1997), the injection of a single FSH dose combined with a single dose of eCG (PMSG) (Batt et al., 1993; Baldassarre et al., 2002; Forcada et al., 2011), the injection of a single FSH dose dissolved in different vehicles or adjuvants such as aluminium hydroxide gel (Kimura et al., 2007) and polyvinylpyrrolidone (PVP) (D´Alessandro et al., 2001) or the stimulation of superovulation through immunization against inhibin (Padilla et al., 2008; Wang et al., 2009). The variability in ovarian response leading to unpredictable yields of viable embryos (Holtz, 2005), and the incidence of premature luteal regression with the consequence of unsatisfactory embryo recovery are major drawbacks associated with superovulation (Armstrong et al., 1982; Stubbings et al., 1986; Battye et al., 1988; Saharrea et al., 1998., Forcada et al., 2011). Different studies have suggested that manipulation of the reproductive tract (Riesenberg et al. 2001), undernutrition, breed (Forcada et al., 2011) and follicular status at the onset of superstimulatory treatment (Cognie, 1999; Menchaca et al., 2002; Cognie et al., 2003; Menchaca et al., 2007b) are possible reasons for this phenomenon.

The collection of embryos from superovulated animals and the subsequent embryo transfer permits the dissemination of genetic traits from genetically superior animals. The earlier techniques to collect embryos involved surgical interventions (Moore, 1974; Armstrong et al., 1983). However, it presents several drawbacks, such as the anesthetic and surgery related stress, post-operative adhesions and the high expenses of the procedure itself (Holtz, 1996). The laparoscopic approach resulted in fewer adhesions (Baril et al., 1989; Flores-Foxworth et al., 1992), but still, being a semi-invasive procedure, requires the need of special equipment and trained personnel. The

transcervical embryo collection technique, developed by Pereira et al. (1998), proved to be an efficient and feasible technique to conduct.

1.2.2. References

Armstrong, D.T., Pfitzner, A.P., Porter, K.J., Warnes, G.M., Janson, P.O., Seamark, R.F., 1982. Ovarian responses of anoestrous goats to stimulation with pregnant mare serum gonadotrophin. Anim. Reprod. Sci. 5, 15-23.

Armstrong, D.T., Pfitzner, A.P., Warnes, G.M., Seamark, R.F., 1983. Superovulation treatments and embryo transfer in Angora goats. J. Reprod. Fertil. 67, 403-410.

Baldassarre, H., Wang, B., Kafidi, N., Keefer, C., Lazaris, A., Karatzas, C.N., 2002. Advances in the production and propagation of transgenic goats using laparoscopic ovum pick-up and in vitro embryo production technologies. Theriogenology 57, 275–284.

Baril, B., Casamitjana, P., Perrin, J., Vallet, J. c., 1989. Embryo Production, Freezing and Transfer in Angora, Alpine and Saanen Goats. Reprod. Domest. Anim. 24, 101-115.

Baril, G., Leboeuf, B., Saumande, J., 1993. Synchronization of estrus in goats: the relationship between time of occurrence of estrus and fertility following artificial insemination. Theriogenology 40, 621-628.

Baril, G., Remy, B., Vallet, J. c., Beckers, J. f., 1992. Effect of Repeated Use of Progestagen- PMSG Treatment for Estrus Control in Dairy Goats out of Breeding Season. Reprod. Domest. Anim. 27, 161-168.

Baril, G., Vallet, J.C., 1990. Time of ovulations in dairy goats induced to superovulate with porcine follicle stimulating hormone during and out of the breeding season. Theriogenology 34, 303-311.

Batt, P.A., Killeen, I.D., Cameron, A.W., 1993. Use of single or multiple injections of FSH in embryo collection programmes in goats. Reprod. Fertil. Dev. 5, 49-56.

Battye, K.M., Fairclough, R.J., Cameron, A.W., Trounson, A.O., 1988. Evidence for prostaglandin involvement in early luteal regression of the superovulated nanny goat (Capra hircus). J. Reprod. Fertil. 84, 425-430.

Beck, N.F.G., Davies, B., Williams, S.P., 1993. Oestrous synchronization in ewes: the

effect of combining a prostaglandin analogue with a 5-day progestagen treatment. Anim. Sci. 56, 207-210.

Bitaraf, A., Zamiri, M.J., Kafi, M., Izadifard, J., 2007. Efficacy of CIDR, fluogestone acetate sponges and cloprostenol for estrous synchronization of Nadooshani goats during the breeding season. Iran. J. Vet. Res. 8, 218-224.

Bretzlaff, K.N., Hill, A., Ott, R.S., 1983. Induction of luteolysis in goats with prostaglandin F2 alpha. Am. J. Vet. Res. 44, 1162-1164.

Chemineau, P., 1983. Effect on oestrus and ovulation of exposing creole goats to the male at three times of the year. J. Reprod. Fertil. 67, 65-72.

Christenson, R.K., 1976. Effect of short-term progestogen treatment on induction of estrus and lambing in anestrous ewes. J. Anim. Science 43, 795-801.

Cline, M.A., Ralston, J.N., Seals, R.C., Lewis, G.S., 2001. Intervals from norgestomet withdrawal and injection of equine chorionic gonadotropin or P.G. 600 to estrus and ovulation in ewes. J. Anim. Sci. 79, 589-594.

Cognie, Y., 1999. State of the art in sheep-goat embryo transfer. Theriogenology 51, 105-116.

Cognié, Y., Baril, G., Poulin, N., Mermillod, P., 2003. Current status of embryo technologies in sheep and goat. Theriogenology, Proceedings of the annual Conference of the Internation Embryo transfer Society 59, 171-188.

Colas, G., Thimonier, J., Courot, M., Ortavant, R., Cornu, C., GuéRin, Y., 1973. Fertilité, prolificité et fécondité pendant la saison sexuelle des brebis inséminées artificiellement après traitement a l'acétate de fluorogestone. Ann. Zootech. 22, 441-451.

D'Alessandro, A., Martemucci, G., Colonna, M.A., Cafueri, C., Toteda, F., 1997. Some effects of adding p-LH in defined amounts to purified p-FSH to modify FSH/LH ratios during the superovulatory treatment of anestrous ewes. Anim. Reprod. Sci. 47, 91-98.

D'Alessandro, A.G., Martemucci, G., Colonna, M.A., Borghese, A., Terzano, M.G., Bellitti, A., 2001. Superovulation in ewes by a single injection of pFSH dissolved in polyvinylpyrrolidone (PVP): effects of PVP molecular weight, concentration and schedule of treatment. Anim. Reprod. Sci. 65, 255.264.

de Castro, T., Rubianes, E., Menchaca, A., Rivero, A., 1999. Ovarian dynamics, serum

estradiol and progesterone concentrations during the interovulatory interval in goats. Theriogenology 52, 399-411.

Deweese, W.P., Glimp, H.A., Dutt, R.H., 1970. Comparison of medroxyprogesterone acetate orally and in vaginal sponges for synchronizing estrus in ewes. J. Anim. Sci. 31, 394-397.

Drion, P.V., Furtoss, V., Baril, G., Manfredi, E., Bouvier, F., Pougnard, J.L., Bernelas, D., Caugnon, P., McNamara, E.M., Remy, B., Sulon, J., Beckers, J.F., Bodin, L., Lebceuf, B., 2001. Four years of induction/synchronization of estrus in dairy goats: effect on the evolution of eCG binding rate in relation with the parameters of reproduction. Reprod. Nutr. Dev. 41, 401-412.

Flores-Foxworth, G., McBride, B.M., Kraemer, D.C., Nuti, L.C., 1992. A comparison between laparoscopic and transcervical embryo collection and transfer in goats. Theriogenology, Proceedings Annual Conference of the International Embryo Transfer Society 37, 213.

Fonseca, J.F., Maffili, V.V., Santos, A.D.F., Fürst, R., Prosperi, C.P., Rovay, H., Souza, J.M.G., Torres, C. a. A., 2012. Effects of prostaglandin administration 10 days apart on reproductive parameters of cyclic dairy nulliparous goats. Arq. Bras. Med. Vet. Zootec. 64, 349-358.

Forcada, F., Ait Amer-Meziane, M., Abecia, J.A., Maurel, M.C., Cebrián-Pérez, J.A., Muiño- Blanco, T., Asenjo, B., Vázquez, M.I., Casao, A., 2011. Repeated superovulation using a simplified FSH/eCG treatment for in vivo embryo production in sheep. Theriogenology 75, 769-776.

Gérard, B., 2000. Baril G., Saumande J. 2000. Hormonal treatments to control time of ovulation and fertility of goats. 7th International Conference on Goats, 15-21 mai 2000, Tours, France Vol.1, p:400-405.

Greyling, J.P.C., and Van Niekerk, C.H., 1986. Synchronization of oestrus in the Boer goat doe: Dose effect of prostaglandin in the double injection regime. S. Afr. J. Anim. Sci. 16, 146- 150.

Greyling, J.P.C., van der Nest, M., 2000. Synchronization of oestrus in goats: dose effect of progestagen. Small Ruminant Res. 36, 201-207.

Greyling, J.P.C., Van Niekerk, C.H., 1991. Different synchronization techniques in

Boer goat does outside the normal breeding season. Small Ruminant Res. 5, 233-243.

Holtz, W., 1996. Embryotransfer bei der Ziege-eine Übersicht. Dtsch. Tierärztl. Wochenschr.103, 293-297.

Holtz, W., 2005. Recent developments in assisted reproduction in goats. Small Ruminant Research, Special Issue: Plenary papers of the 8th International Conference on Goats 60,

Holtz, W., and Sohnrey, B., 1992. Oestrus induction during the anoestrus season in goats by menas of intravaginal pessaries or subcutaneous implants. In: Lokeshwar, R.R. (Ed.), recent advances in Goat Production. Nutan printers, New Delhi, pp. 1284-1289

Holtz, W., Sohnrey, B., Gerland, M., Driancourt, M.-A., 2008. Ovsynch synchronization and fixed-time insemination in goats. Theriogenology 69, 785-792.

Iglesias, R.M.R., Ciccioli, N.H., Irazoqui, H., Giglioli, C., 1996. Ovulation rate in ewes after single oral glucogenic dosage during a ram-induced follicular phase. Anim. Reprod. Sci. 44, 211–221.

Kimura, K., Hirako, M., Iwata, H., Aoki, M., Kawaguchi, M., Seki, M., 2007. Successful superovulation of cattle by a single administration of FSH in aluminum hydroxide gel. Theriogenology 68, 633-639.

Kusina, N.T., Tarwirei., Hamudikuwanda, H., Agumba, G., Mukwena, J., 2000. A comparison of the effects of progesterone sponges and ear implants, PGF2alpha, and their combination on efficacy of estrus synchronization and fertility of Mashona goat does. Theriogenology 53, 1567-1580.

Leboeuf, B., Forgerit, Y., Bernelas, D., Pougnard, J.L., Senty, E., Driancourt, M.A., 2003. Efficacy of two types of vaginal sponges to control onset of oestrus, time of preovulatory LH peak and kidding rate in goats inseminated with variable numbers of spermatozoa. Theriogenology 60, 1371-1378.

Luther, J.S., Redmer, D.A., Reynolds, L.P., Choi, J.T., Pant, D., Navanukraw, C., Arnold, D.R., Scheaffer, A.N., Borowicz, P., Kirsch, J.D., Weigl, R.M., Kraft, K.C., Grazul-Bilska, A.T., 2005. Ovarian follicular development and oocyte

quality in anestrous ewes treated with melatonin, a controlled internal drug release (CIDR) device and follicle stimulating hormone. Theriogenology 63, 2136-2146.

Martemucci, G., D'Alessandro, A.G., 2011. Induction/synchronization of oestrus and ovulation in dairy goats with different short term treatments and fixed time intrauterine or exocervical insemination system. Anim. Reprod. Sci. 126, 187-194.

Martínez-Álvarez, L.E., Hernández-Cerón, J., González-Padilla, E., Perera-Marín, G., Valencia, J., 2007. Serum LH peak and ovulation following synchronized estrus in goats. Small Ruminant Res. 69, 124–128.

Maurel, M.-C., Roy, F., Hervé, V., Bertin, J., Vaiman, D., Cribiu, E., Manfredi, E., Bouvier, F., Lantier, I., Boue, P., Guillou, F., 2003. Réponse immunitaire à la eCG utilisée dans le traitement de l'induction d'ovulation chez la chèvre et la brebis. Gynécologie Obstétrique & Fertilité 31, 766-769.

Menchaca, A., Pinczak, A., Rubianes, E., 2002. . Follicular recruitment and ovulatory response to FSH treatment initiated on day 0 or day 3 postovulation in goats. Theriogenology 58, 1713-1721.

Menchaca, A., Rubianes, E., 2004. New treatments associated with timed artificial insemination in small ruminants. Reprod. Fertil. Dev. 16, 403-413.

Menchaca, A., Vilariño, M., Crispo, M., Pinczak, A., Rubianes, E., 2007b. Day 0 Protocol: Superstimulatory treatment initiated in the absence of a large follicle improves ovarian response and embryo yield in goats. Theriogenology 68, 1111-1117.

Moore, N.W., 1974. Multiple ovulation and ovum transfer in the goat. Prco. Aust. Soc. Anim.Prod. 10, 246.

Motlomelo, K.C., Greyling, J.P.C., Schwalbach, L.M.J., 2002. Synchronisation of oestrus in goats: the use of different progestagen treatments. Small Ruminant Res. 45, 45-49.

Nandy, D.K., Agarwal, K.P., Bhattacharya, N.K., 1990. Ovarian response to superovulation in nondescript does. Indian J. Anim. Sci. 60, 972-973.

Nowshari, M.A., Backers, J.F., Holtz, W., 1995. Superovulation of goats with purified

pFSH supplemented with defined amounts of pLH. Theriogenology 43, 797-802.

Ogunbiyi, P.O., Molokwu, E.C., Sooriyamoorthy, T., 1980. Estrus synchronization and controlled breeding in goats using prostaglandin F(2)alpha. Theriogenology 13, 257-261.

Padilla, G., Knight, P.G., Holtz, W., 2008. Superovulation and embryo collection in nulliparous Boer goat does immunized against a recombinant ovine α-subunit inhibin. Small Ruminant Res. 74, 159-164.

Pereira, R.J., Sohnrey, B., Holtz, W., 1998. Nonsurgical embryo collection in goats treated with prostaglandin F2alpha and oxytocin. J. Anim. Sci. 76, 360-363.

Pierson, J.T., Baldassarre, H., Keefer, C.L., Downey, B.R., 2003. Influence of GnRH administration on timing of the LH surge and ovulation in dwarf goats. Theriogenology 60, 397-406.

Puls-Kleingeld, E., Yuswiati, E., Nowshari, M.A., Beckers, J.F., Holtz, W., 1991. Effect of FSH/LH ratio and treatment schedule on superovulatory response in Boer goats. J. Reprod. Ferti. 43, 308.

Pursley, J.R., Mee, M.O., Wiltbank, M.C., 1995. Synchronization of ovulation in dairy cows using PGF2alpha and GnRH. Theriogenology 44, 915-923.

Quirke, J.F., 1979. Control of reproduction in adult ewes and ewe lambs, and estimation of reproductive wastage in ewe lambs following treatment with progestagen impregnated sponges and PMSG. Livest. Prod. Sci. 6, 295-305.

Rahman, A.N.M.A., Abdullah, R.B., Wan-Khadijah, W.E., 2008. Estrus Synchronization and Superovulation in Goats: A Review. J. Biol. Sci. 8, 1129-1137.

Rhodes, L., Nathanielsz, P.W., 1988. Comparison of a controlled internal drug release device containing progesterone with intravaginal medroxyprogesterone sponges for estrus synchronization in ewes. Theriogenology 30, 831-836.

Riesenberg, S., Meinecke-Tillmann, S., Meinecke, B., 2001. Ultrasonic survey of follicular development following superovulation with a single application of pFSH, eCG or hMG in goats. Small Ruminant Res. 40, 83–93.

Romano, J.E., 1994. Effect of service number on estrus duration in dairy goats.

Theriogenology 41, 1273-1277.

Romano, J.E., 1996. Comparison of fluorgestone and medroxyprogesterone intravaginal pessaries for estrus synchronization in dairy goats. Small Ruminant Res. 22, 219-223.

Roy, F., Maurel, M.C., Combes, B., Vaiman, D., Cribiu, E.P., Lantier, I., Pobel, T., Delétang, F., Combarnous, Y., Guillou, F., 1999. The negative effect of repeated equine chorionic gonadotropin treatment on subsequent fertility in Alpine goats is due to a humoral immune response involving the major histocompatibility complex. Biol. Reprod. 60, 805- 813.

Rubianes, E., Beard, A., Dierschke, D.J., Bartlewski, P., Adams, G.P., Rawlings, N.C., 1997. Endocrine and ultrasound evaluation of the response to PGF 2alpha and GnRH given at different stages of the luteal phase in cyclic ewes. Theriogenology 48, 1093-1104.

Saharrea, A., Valencia, J., Balcázar, A., Mejía, O., Cerbón, J.L., Caballero, V., Zarco, L., 1998. Premature luteal regression in goats superovulated with PMSG: effect of hCG or GnRH administration during the early luteal phase. Theriogenology 50, 1039-1052.

Simões, J., Almeida, J.C., Valentim, R., Baril, G., Azevedo, J., Fontes, P., Mascarenhas, R., 2006. Follicular dynamics in Serrana goats. Anim. Reprod. Sci. 95, 16-26.

Simões, J., Baril, G., Almeida, J.C., Azevedo, J., Fontes, P., Mascarenhas, R., 2008. Time of ovulation in nulliparous and multiparous goats. Animal 2, 761-768.

Steffan, J., Poissonnet, P., Thibier, M., 1983. Control of oestrus in ewe lambs and yearling ewes with medroxyprogesterone acetate and fluorogestone acetate. Anim. Reprod. Sci. 5, 191- 198.

Stubbings, R.B., Bosu, W.T., Barker, C.A., King, G.J., 1986. Serum progesterone concentrations associated with superovulation and premature corpus luteum failure in dairy goats. Can. J. Vet. Res. 50, 369-373.

Thompson, J.G.E., Simpson, A.C., James, R.W., Tervit, H.R., 1990. The application of progesterone-containing CIDR^{TM} devices to superovulated ewes. Theriogenology 33, 1297-1304. Ungerfeld, R., Rubianes, E., 2002. Short term

primings with different progestogen intravaginal devices (MAP, FGA and CIDR) for eCG-estrous induction in anestrus ewes. Small Ruminant Res. 46, 63-66.

Wang, X.L., El-Gayar, M., Knight, P.G., Holtz, W., 2009. The long-term effect of active immunization against inhibin in goats. Theriogenology 71, 318-322.

Wildeus, S., 1999. Current concepts in synchronization of estrus: Sheep and goats. J. Anim. Sci. 77, 1-14

Yuswiati, E., and Holtz, W., 1996. Superovulation with different exogenous gonadotropins in peripubertal goats. Indian J. Anim. Sci. 66, 131-133.

Zongo, M., Traor, B., Ababneh, M.M., Hanzen, C., Sawadogo, L., 2015. Ultrasonographic assessment of uterine involution and ovarian activity in West Africa Sahelian goats. J. Vet. Med. Anim. Health 7, 71-76.

Chapter 2

Superovulation of Boer goats with different synchronization regimes at different times of the year in the northern temperate zone

M. Camacho, D. Garza, M. Gauly, W. Holtz

Small Ruminant Research 177 (2019) 106-110

Abstract

The present study addresses a comparison of various estrous cycle control protocols and the effect of season in the northern temperate zone on the superovulatory response in Boer goats. Four groups of 12 does each were treated in February, May, August and November, respectively. Does were provided with progestogen containing intravaginal pessaries to synchronize estrus. Half the does of each group received sponges impregnated with 20 mg fluorogestone acetate ("Cronolone"), the other half "Eazy-Breed" CIDRs containing 0.3 mg progesterone. Pessaries remained in place for 7 days. Within each subgroup half the does were treated with PGF2α ("Dinoprost") at pessary insertion, the other half at pessary withdrawal. Beginning 48 h before pessaries were removed does received i.m. injections of 4, 4, 2, 2, 2 and 2 Armour Units (AU) FSH, supplemented with 40% LH, at 12 h intervals. Estrus detection was conducted at 8 h intervals and ovarian activity was monitored by daily transrectal ultrasonography. Does were mated and 7 days later non-surgical embryo collection was conducted. It transpired that the type of intravaginal pessary had no effect on the time passing between pessary withdrawal and onset of estrus (sponge: 40.7 h vs. CIDR: 35.2 h), number of ovulations/doe assessed by echographic counting of collapsed large follicles (7.2 vs. 7.6) or corpora lutea (7.1 vs. 9.8), embryo recovery rate (39% vs. 41%) and proportion of transferable embryos (66% vs. 65%). The number of collapsed large follicles was significantly higher than that of corpora lutea on the day before embryo collection (7.4 vs. 8.5; $P < 0.05$), the correlation between the two being $r=0.55$ ($P < 0.05$). The effect of PGF2α administration at the onset or at the end of progestogen treatment and the effect of season were not significant, except for a shorter interval between pessary removal and onset of estrus in August in comparison to the other months (29.3 vs. 39.4, 40.6 and 42.1 h; $P < 0.05$). In conclusion, Boer goats, although being seasonal breeders in the northern temperate zone, will respond to superovulatory treatment and produce embryos of high quality year round regardless of type of pessary and time of PGF2α administration.

Keywords: Goats, Synchronization, Superovulation, Intravaginal pessaries, Seasonality

2.1 Introduction

Estrous cycle control in goats serves as the basis for biotechnological interventions such as artificial insemination, superovulation and embryo transfer (Baril and Vallet, 1990; Baril et al., 1993; Baldassarre and Karatzas, 2004). During the breeding season estrus may be induced by administration of prostaglandin F2α (PGF2α) in the presence of a functional corpus luteum (Bretzlaff et al., 1983; Greyling and van Niekerk, 1986). When intending to synchronize estrus in groups of goats two PGF2α injections at 10–12 d interval are required (Beck et al., 1993; Kusina et al., 2000). The ovsynch protocol, combining the action of PGF2α and of ovulation inducing GnRH, originally introduced for cows by Pursley et al. (1995), has been shown to be effective for implementing fixed-time insemination in goats (Holtz et al., 2008). The most widely applied method of controlling the estrous cycle in small ruminants is by administration of progesterone or a synthetic progestogen via intravaginal pessaries (Wildeus, 2000; Holtz, 2005) or, occasionally, via subcutaneous implant (Bretzlaff and Madrid, 1985; East and Rowe, 1989; Holtz and Sohnrey, 1992). The use of progestogenimpregnated intravaginal pessaries is well documented for sheep but to a lesser degree for goats. Originally the recommendation was to leave the pessaries in position for 18 days to allow all corpora lutea to regress, regardless at what stage of the cycle treatment began (Holtz, 2005). This long-term administration involves the risk of subluteal progestogen levels toward the end of treatment, resulting in abnormalities in follicular development, ovulation time and luteal function, as well as poor quality oocytes (Menchaca and Rubianes, 2004). In small ruminants, just as in cows, follicles grow in waves recurring every 5–7 days (Evans, 2003; Rubianes and Menchaca, 2003). Therefore it is permissible to reduce the length of progestogen treatment to 5–7 days, provided existing corpora lutea are regressed by administration of PGF2α (Karaca et al., 2009). Whereas some researchers favor administration of PGF2α at the time of pessary insertion (Menchaca et al., 2007a; Martemucci and D´Alessandro, 2011b), others prefer treatment at the time of pessary removal (Beck et al., 1993; Menchaca and Rubianes, 2004; Lehloenya and Greyling, 2010; Martemucci and D´Alessandro, 2011a). Superovulation is most commonly accomplished by administration of high doses of gonadotropic hormone in association with a means of estrus cycle control. Over the years various superstimulatory regimens

have been proposed, including administration of equine Chorionic Gonadotropin (eCG) (Holtz, 1996; Pintado et al., 1998; Saharrea et al., 1998), FSH with different admixtures of LH (Nowshari et al., 1995), FSH followed by a single high dose of LH (Baril et al., 1996; Suyadi, Beckers and Holtz, 1999), FSH dissolved in various vehicles or adjuvants such as aluminum hydroxide gel (Kimura et al., 2007) or polyvinylpyrrolidone (PVP) (D'Alessandro et al., 2001), FSH combined with eCG in a single dose (Batt et al., 1993; Forcada et al., 2011) or active immunization against inhibin (Padilla et al., 2008; Holtz et al., 2012). Most commonly FSH with an admixture of LH is administered by means of 6–10 i.m. injections at 12 h intervals. This treatment, though being labor intensive, has been shown to be most suitable (Nowshari et al., 1992; Mahmood et al., 1991; Pendleton et al., 1992). An unsolved major obstacle hampering a reliable supply of embryos for transfer purposes in goats is the unpredictability of the ovarian response to superstimulatory treatment (Holtz, 2005). Factors suspected to be involved are age, season, health and physiological and nutritional state (Cognié, 1999; Holtz, 2005). A frequent complication with the superovulation of goats is the high incidence of premature luteal regression (Armstrong et al., 1982, 1983; Chemineau et al., 1986; Stubbings et al., 1986; Battye et al., 1988; Saharrea et al., 1998; Forcada et al., 2011). To date no plausible explanation for this impediment has been found. The present study addresses the superovulatory response to FSH administration of Boer goats in the northern temperate zone treated at various times of the year in association with synchronization by two types of progestogen releasing intravaginal pessaries and administration of PGF2α at pessary insertion or withdrawal.

2.2 Materials and methods

The project was approved by "Niedersaechsisches Landesamt fuer Verbraucherschutz und Lebensmittelsicherheit "(33.14-42502-04-14/ 1519). A total of 48 pluriparous Boer goat does from the breeding flock of Goettingen University in Germany (9° 41´E, 51° 46´N), on average 3.7 (SD 0.3, range 2–7) years of age and weighing 60 (SD 4.5, range 46–79) kg were group-housed in open barns with straw-covered floor and outdoor concrete runs. Does were fed a daily ration of 600 g concentrate consisting of

equal parts of oats, dried sugar beet pulp and a pelleted lactation diet for breeding ewes supplemented with selenium, zinc and iodine, and had free access to wheat or barley straw, salt lick and water. Conditions were kept constant throughout the year. Does were allocated at random with reference to age to four groups of twelve that were subjected to superovulatory treatment in February, May, August and November, respectively. Each group was subdivided into two subgroups; one provided with intravaginal polyurethane sponges impregnated with 20 mg fluorogestone acetate ("Cronolone", Intervet, Igoville, France), the other two with "controlled internal drug release"- devices impregnated with 0.3 g progesterone (CIDR, "Eazy-Breed", Zoetis, Berlin, Germany). Pessaries were removed 7 days after insertion and, beginning 48 h before removal, porcine FSH, supplemented with 40% porcine LH (Nowshari et al., 1995), was administered by six i.m. injections of 4, 4, 2, 2, 2 and 2 Armour Units (AU) at 12 h intervals. One half of the goats of each subgroup received two i.m. injections of 5 mg dinoprost (1 mL Dinolytic, Pfizer, Berlin, Germany), administered at 12 h interval on the day of pessary insertion; in the other half on the day of pessary withdrawal. Does were presented to an aproned adult buck at 8 h intervals and naturally bred once daily when displaying standing estrus. Ovarian activity was monitored by daily transrectal ultrasonography (ALOKA-SSD 500 equipped with a transrectal 7.5 MHz linear array transducer). Antral follicles were classified as small (3.0–3.9 mm), medium (4.0–4.9 mm) and large (more than 4.9 mm). Ovulation was assumed to have taken place halfway between the time when collapsed large follicles were first observed and the previous measurement, as has been described by Suyadi and Holtz (2012). Corpora lutea, visualized by ultrasound, were counted one day before embryo collection. Seven days after the last mating, non-surgical embryo collection was conducted, preceded by 16 h by an i.m. administration of 5 mg Dinoprost. The procedure of embryo collection has been described in detail by Pereira et al. (1998) and Suyadi Sohnrey and Holtz (2000). Briefly, animals were fixed on a restraining device in upright position. With the aid of a duck-bill speculum and pen light the lip of the external cervical os was grasped with a long sharp-pointed tenaculum forceps and gently pulled toward the vulvar orifice. A catheter with a pliable stylet inserted was passaged through the cervical canal and, after removal of the stylet, further advanced into one uterine horn directed by a finger in the vaginal fornix. Eight flushes with 20 ml

Dulbecco´s medium containing 0.06% bovine serum albumin (BSA; A9647-50 G, Sigma-Aldrich, Steinheim, Germany), 100 IU/ml penicillin and 100 µg/ml streptomycin (PAA P11-010, Darmstadt, Germany) were conducted. Thereafter the catheter was partially withdrawn and advanced into the other uterine horn which was flushed in the same way. The reflux of the flushings was collected via embryo filter (75 µm Em Con Embryofilter, Albrecht, Aulendorf, Germany) into a graduated 1000 mL cylinder. The flushing procedure was terminated once all medium had been recovered. The filter was thoroughly rinsed with Dulbecco´s medium and the eggs and embryos recovered under a stereoscope at X20 to 40 were transferred to fresh Dulbecco´s medium supplemented with 0.06% bovine serum albumin (BSA; A9647-50 G, Sigma-Aldrich, Steinheim, Germany). They were counted under a stereoscope at X20 to 40 and classified from grade 1 to grade 4 depending on developmental stage and morphological appearance in accordance with the guidelines of the International Embryo Transfer Society (Stringfellow and Givens, 2010). Data were analyzed by three-way analysis of variance considering the effects “type of intravaginal device” (sponge or CIDR), “time of dinoprost administration” (at pessary insertion or at pessary withdrawal) and “season” (February, May, August or November) and the respective interactions. A paired samples t-test was conducted comparing the number of ovulations assessed either by number of collapsed follicles or number of corpora lutea counted one day before embryo collection.

2.3 Results

As shown in Table 1, 46 of 48 treated does (96%) came in estrus; one doe in each of the two progestogen-groups did not respond. The interval between pessary withdrawal and onset of estrus was numerically slightly longer and more variable after cronolone treatment than after CIDR treatment (40.7 ± 2.6 vs. 35.2 ± 1.9 h; $P > 0.05$). With regard to ovulation rate, recovery rate and proportion of transferable embryos there was no difference between the two types of pessaries used. The number of ovulations deduced from the number of collapsed large follicles viewed echographically was significantly lower than the number of corpora lutea viewed one day before embryo collection (7.4 vs. 8.5; $P < 0.05$); the correlation between the two being $r=0.55$ ($P < 0.05$). Whereas there was perfect agreement between the two parameters in 11 does (23%), in 12 does (25%)

the number of collapsed follicles exceeded that of corpora lutea and in the remaining 25 does (52%) it was less. No significant difference existed between does in which prostaglandin was administered at the beginning or at the end of the progestogen treatment.

The number of small, medium and large follicles discernible at different stages of treatment is depicted in Fig. 1. At pessary insertion (Day 0) small follicles dominated, especially during the months when does were not cycling (February and May). At the time of pessary removal, i.e. after four of six FSH injections (Day 7), there were significantly fewer small follicles ($P < 0.05$), whereas the number of large follicles dominated. This tendency persisted until one day after pessary removal (Day 8). The number of medium-size follicles remained fairly constant throughout the year. In May and August at the time of pessary withdrawal (Day 7) significantly more large follicles were present than in February and November (10.8 and 11.5 vs. 6.4 and 4.6; $P < 0.05$), a (non-significant) trend that persisted until the following day (Day 8). No effect of season on superovulatory response, embryo recovery rate and embryo quality was detected with the exception of a shorter interval between pessary withdrawal and onset of estrus in August as compared to February, May and November (29.3 ± 2.1 vs. 39.4 ± 3.0, 40.6 ± 3.4 and 42.1 ± 3.6, $P < 0.05$).

Table 1: Effect of type of pessary (Cronolone sponge vs. Eazy-Breed CIDR) and time of prostaglandin administration relative to progestagen treatment on ovulatory response and embryo yield in superstimulated Boer goat does.

Variable	Sponge			CIDR			Overall
	Insertion	Removal	Total	Insertion	Removal	Total	
Does treated	12	12	24	12	12	24	48
Does in estrus	11	12	23	11	12	23	46
Pessary withdrawal to estrus (h)							
Mean	41.7	39.7	40.7	35.5	34.9	35.2	37.9
SEM	3.7	3.9	2.6	3.1	2.4	1.9	1.7
Range	27-60	24-60	24-60	26-58	26-51	26-58	24-60
Ovulations/doe (collapsed large follicles)							
Mean	6.3	8.1	7.2	7.4	7.8	7.6	7.4[a]
SEM	0.9	0.9	0.7	0.7	0.6	0.4	0.4
Range	1-12	1-12	1-12	4-11	5-11	4-11	1-12
Ovulations/doe (corpus luteum count)							
Mean	6.8	7.5	7.1	9.7	10.0	9.8	8.5[b]
SEM	0.9	1.0	0.7	1.1	0.7	0.6	0.5
Range	1-11	2-12	1-12	4-17	6-14	4-17	1-17
Ova and embryos /donor							
Mean	2.8	3.6	3.2	2.9	3.5	3.2	3.2
SEM	0.8	1.1	0.7	0.7	0.7	0.5	1.0
Range	0-7	0-10	0-10	0-8	0-7	0-8	0-10
Recovery rate (%)[c]	42	37	39	36	46	41	40
Transferable embryos (%)	71	63	66	74	57	65	66

[a,b] Means with different superscripts differ ($P<0.05$)

[c] Ova and embryos/number of ovulations (collapsed follicles)

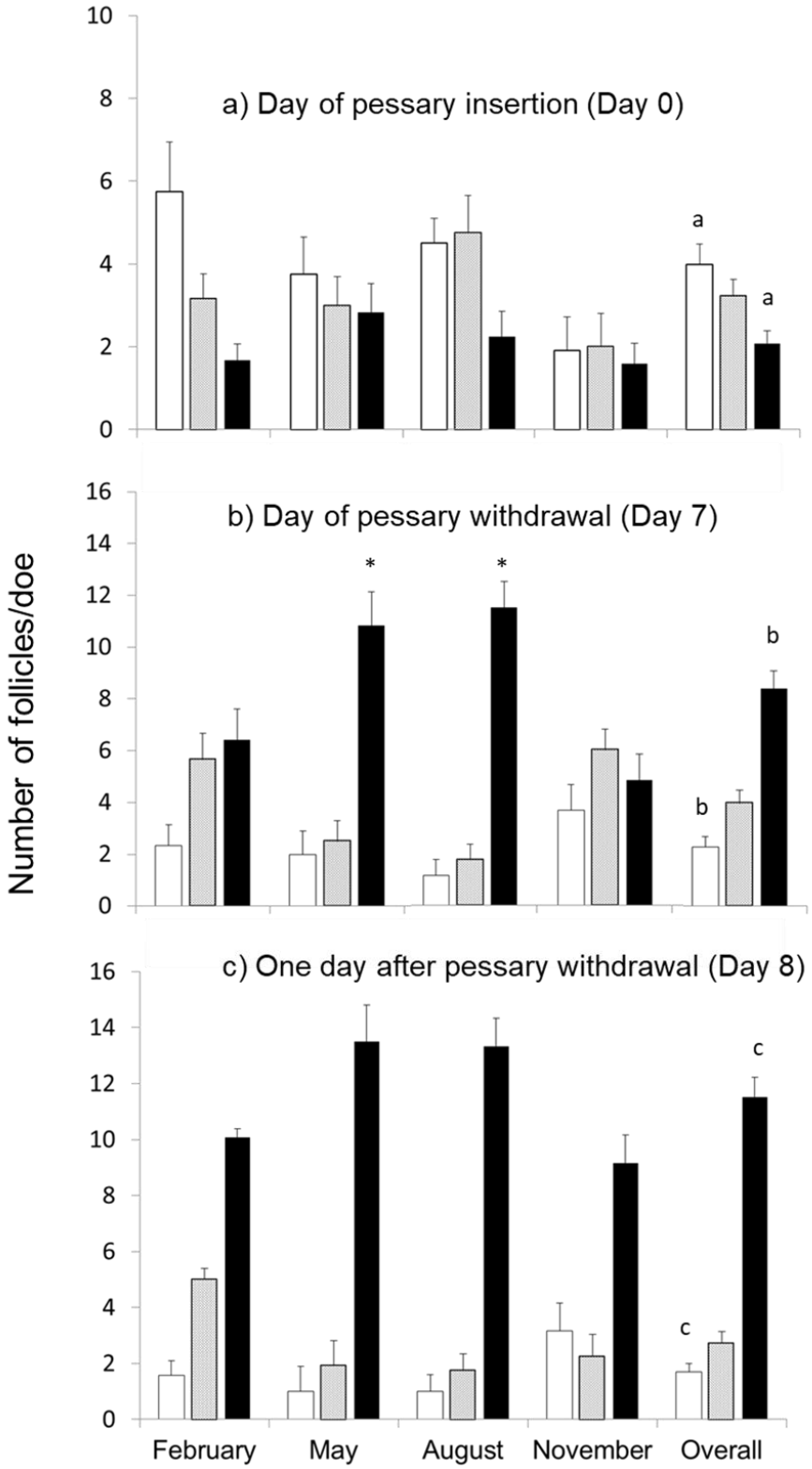

Fig. 1. Mean (±SEM) number of small (3.0–3.9 mm, light bars), medium (4.0–4.9 mm, shaded bars) and large follicles/doe (> 4.9 mm, dark bars) at different times of the year. * denotes significant differences within days ($P < 0.05$); a,b,c denotes significant differences between days ($P < 0.05$).

2.4 Discussion

The fact that 96% of the females had responded to superstimulatory treatment is proof that the 7 day progestogen treatment with sponges containing as little as 20 mg FGA is adequate, confirming the experience of Knights et al. (2001), Leboeuf et al. (2003) and Karaca et al. (2009). The traditional long-term progestogen treatment (Motlomelo et al., 2002; Karaca et al., 2009) is, thus, oblivious. According to Menchaca and Rubianes (2001) the advantage of the short-term treatment is attributable to the high progestogen concentration at the time of pessary removal, creating optimal conditions for follicular turnover. With a sudden drop in progestogen concentration the amplitude of FSH and LH episodes is known to increase, bringing about a "rebound effect" (Ireland and Roche, 1982). Sponges and CIDRs proved to be equally effective in synchronizing estrus. A drawback of the sponge treatment was that in several does adhesions formed between sponge material and vaginal mucosa, leading to distress upon sponge removal. This adverse effect, as well as the efflux of foul-smelling vaginal discharge accompanying sponge withdrawal (Holtz and Sohnrey, 1992; Wheaton et al., 1993), impairs the acceptance of the sponge treatment; although Branscheid et al. (1985) showed in sheep that mucosal lesions and bacterial contamination associated with sponge treatment were fully remedied by the time of mating or insemination 3–4 days later.

Administration of PGF2α at pessary insertion will lead to regression of existing corpora lutea. The expectation is that the ovarian status of all females is standardized before the superovulatory treatment. In the two non-responding goats of that treatment group, in all likelihood, corpora lutea were at an early stage of development when they are known to be non-receptive to PGF2α (Acritopoulou and Haresign, 1980; Wiltbank and Niswender, 1992). When administering PGF2α at pessary removal, corpora lutea of all animals are bound to be at an advanced stage of development, therefore all animals are expected to respond, which was the case in the present study. As a rule a single PGF2α injection will suffice but in the present study two injections were administered at 12 h interval. The reason is that in cows occasionally a single injection does not bring about complete luteolysis (Stevenson et al., 1987; Martins et al., 2011). Therefore in our

group the double injection regimen was adopted as a routine (Suyadi Sohnrey and Holtz, 2000). Premature luteal regression, a problem frequently encountered in superovulated goats (Armstrong et al., 1982; Stubbings et al., 1986; Battye et al., 1988; Saharrea et al., 1998; Espinosa-Márquez et al., 2004; Al Yacoub et al., 2011; Saleh, 2011) was, inexplicably, not an issue in the present study.

The difference in number of ovulations deduced from collapsed large follicles vs. corpus luteum count 5 d after ovulation may be explained by the difficulty to echographically distinguish individual corpora lutea in superovulated does. Suyadi and Holtz (2012) found that the number of collapsed large follicles and laparoscopic ovulation count were highly correlated (r=0.82). The number of collapsed large follicles was, therefore, considered to be the more reliable parameter.

Most goat breeds originating in the northern temperate zone are seasonal breeders (Chemineau, 1992). As shown by Camacho et al. (2017) this does also apply to Boer goats of our own flock, though not to the extent of most dairy goat breeds. Almost complete arrest of ovarian activity was observed from April to August. This state of ovarian dormancy may be overcome by progestogen/eCG treatment (Baril and Vallet, 1990; Lehloenya et al., 2008) although pregnancies are usually not attained. In the present study it was possible to elicit an adequate superovulatory response at any time of the year. During the months with little or erratic estrous cyclicity (February, May, August) ovaries exhibited primarily small and medium sized follicles prior to hormonal stimulation (Day 0); an observation corroborating findings by Nogueira et al. (2015) in goats and others (Hutchinson and Robertson, 1966; Smeaton and Robertson, 1971; Noel et al., 1993) in sheep. During seasonal anestrus ovarian activity does not altogether cease, but follicles emerge and undergo atresia without ovulating owing to inadequate LH pulsatility. After 4 of the 6 FSH injections on Day 7 a significant increase in number of large follicles was observed, obviously a response to the superovulatory stimulation. This was particularly evident during the off-season in May and August. The slightly lower numbers of large follicles recorded during February and November can be explained by the occurrence of ovulations since, during these months, does still presented sexual behavior. Moreover, the echographically detected presence of corpora lutea at pessary

insertion in the majority of does treated during these months indicate that, in fact, ovulation had occurred before pessary insertion.

The low embryo recovery rate of merely 40% was traced back to the defective mesh of the embryo filter as discovered under the stereoscope. With the same procedure and similar equipment Pereira et al. (1998) and Suyadi Sohnrey and Holtz (2000) achieved recovery rates of 78% and 62%, respectively. With few exceptions the recovered blastocysts were morphologically intact; when transferred after several months of storage in a vitrified state, a pregnancy rate of 55% was achieved (Garza et al., 2018).

2.5 Conclusion

To carry out a short (7 day) progestogen treatment as part of a superovulation protocol, Eazy-Breed CIDRs and Cronolone sponges impregnated with 20 mg FGA proved to be equally effective. From a practical and hygienic viewpoint the CIDR was found to be preferable. The luteolytic PGF2α treatment may be administered at the beginning or at the end of the progestogen treatment; the latter having the advantage that any corpora lutea present will be receptive. Boer goats, although being seasonal breeders in the northern temperate zone, may serve as embryo donors year round.

2.6 Acknowledgements

The authors thank J.-F. Beckers (Faculty of Veterinary Medicine, State University of Liège, Belgium) for donating FSH and LH and D. Daniel and the barn staff for assisting with the handling of the goats. The senior author was supported by a fellowship of the Consejo Nacional de Ciencia y Tecnología (CONACYT)-German Academic Exchange Service (DAAD), D. Garza by a fellowship of the German Academic Exchange Service (DAAD).

2.7 References

Acritopoulou, S., Haresign, W., 1980. Response of ewes to a single injection of an analogue of PGF2α given at different stages of the oestrous cycle. J. Reprod. Fertil. 58, 219–223. https://doi.org/10.1530/jrf.0.0580219.

Al Yacoub, A.N., Gauly, M., Sohnrey, B., Holtz, W., 2011. Fixed-time deep uterine insemination in PGF2α-synchronized goats. Theriogenology 76, 1730–1735. https:// doi.org/10.1016/j.theriogenology.2011.07.005.

Armstrong, D.T., Pfitzner, A.P., Porter, K.J., Warnes, G.M., Janson, P.O., Seamark, R.F., 1982. Ovarian responses of anoestrous goats to stimulation with pregnant mare serum gonadotrophin. Anim. Reprod. Sci. 5, 15–23. https://doi.org/10.1016/0378-4320(82)90033-1.

Armstrong, D.T., Pfitzner, A.P., Warnes, G.M., Seamark, R.F., 1983. Superovulation treatments and embryo transfer in Angora goats. J. Reprod. Fertil. 67, 403–410.

Baldassarre, H., Karatzas, C., 2004. Advanced assisted reproduction technologies (ART) in goats. Anim. Reprod. Sci. 82–83, 255–266. https://doi.org/10.1016/j.anireprosci. 2004.04.027.

Baril, G., Pougnard, J.L., Freitas, V.J.F., Lebouf, B., Soumande, J., 1996. A new method for controlling the precise time of occurrence of the preovulatory gonadotropin surge in superovulated goats. Theriogenology 45, 697–706.

Baril, G., Vallet, J.C., 1990. Time of ovulation in dairy goats induced to superovulate with porcine follicle stimulating hormone during and out of the breeding season. Theriogenology 34, 303–311. https://doi.org/10.1016/0093-691X(90)90523-V.

Baril, G., Leboeuf, B., Saumande, J., 1993. Synchronization of estrus in goats: the relationship between time of occurrence of estrus and fertility following artificial insemination. Theriogenology 40, 621–628. https://doi.org/10.1016/0093-691X(93) 90414-Z.

Batt, P.A., Killeen, I.D., Cameron, A.W., 1993. Use of single or multiple injections of FSH in embryo collection programmes in goats. Reprod. Fertil. Dev. 5, 49–56. https://doi. org/10.1071/RD9930049.

Battye, K.M., Fairclough, R.J., Cameron, A.W.N., Trounson, A.O., 1988. Evidence for prostaglandin involvement in early luteal regression of the superovulated nanny goat (Capra hircus). J. Reprod. Fertil. 84, 425–430. https://doi.org/10.1530/jrf.0. 0840425.

Beck, N.F.G., Davies, B., Williams, S.P., 1993. Oestrous synchronization in ewes: the effect of combining a prostaglandin analogue with a 5-day progestagen treatment. Anim. Sci. 56, 207–210. https://doi.org/10.1017/S0003356100021279.

Branscheid, W., Libal, U., Holtz, W., 1985. Effect of two types of intravaginal implants on the vagina of ewes. Zuchthygiene 20, 87–99.

Bretzlaff, K.N., Madrid, N., 1985. Synchronization of estrus and fertility in goats with norgestomet ear implants. Theriogenology 24, 351–357. https://doi.org/10.1016/ 0093-691X(85)90227-4.

Bretzlaff, K.N., Hill, A., Ott, R.S., 1983. Induction of luteolysis in goats with prostaglandin F2 alpha. Am. J. Vet. Res. 44, 1162–1164.

Camacho, M., Stiehl, J., Garza, D., Gauly, M., Holtz, W., 2017. Seasonality of Boer goats in northern Europe and induction of estrus out of season. Small Rumin. Res. 151, 26–31. https://doi.org/10.1016/j.smallrumres.2017.04.008.

Chemineau, P., 1992. Seasonality and photoperiodic influence in the female goat reproduction. In: Lokeshwar, R.R. (Ed.), Recent Advances in Goat Production. Nutan printers, New Delhi, pp. 355–368.

Chemineau, P., Procureur, R., Cognie, Y., Lefevre, P.C., Locatelli, A., Chupin, D., 1986. Production, freezing and transfer of embryos from a blue tongue-infected goat herd without blue tongue transmission. Theriogenology 26, 279–290. https://doi.org/10. 1016/0093-691X(86)90147- 0.

Cognié, Y., 1999. State of the art in sheep-goat embryo transfer. Theriogenology 51, 105–116. https://doi.org/10.1016/S0093-691X(98)00235-0.

D'Alessandro, A.G., Martemucci, G., Colonna, M.A., Borghese, A., Terzano, M.G., Bellitti, A., 2001. Superovulation in ewes by a single injection of pFSH dissolved in polyvinylpyrrolidone (PVP): effects of PVP molecular weight, concentration and schedule of treatment. Anim. Reprod. Sci. 65, 255–264. https://doi.org/10.1016/S0378- 4320(01)00080-X.

East, N.E., Rowe, J.D., 1989. Subcutaneous progestin implants versus intravaginal sponges for dairy goat estrus synchronization during the transitional period. Theriogenology 32, 921–928. https://doi.org/10.1016/0093-691X(89)90501-3.

Espinosa-Márquez, M.C., Valencia, J., Zarco, L., Escobar-Medina, F.J., Colina-Flores, F., Arechiga-Flores, C.F., 2004. Effect of fluorogestone acetate on embryo recovery and quality in eCG-superovulated goats with premature luteal regression. Theriogenology 62, 624–630. https://doi.org/10.1016/j.theriogenology.2004.01.001.

Evans, A.C.O., 2003. Characteristics of ovarian follicle development in domestic animals. Reprod. Domest. Anim. 38, 240–246. https://doi.org/10.1046/j.1439-0531.2003. 00439.x.

Forcada, F., Ait Amer-Meziane, M., Abecia, J.A., Maurel, M.C., Cebrián-Pérez, J.A., Muiño-Blanco, T., Asenjo, B., Vázquez, M.I., Casao, A., 2011. Repeated superovulation using a simplified FSH/eCG treatment for in vivo embryo production in sheep. Theriogenology 75, 769–776. https://doi.org/10.1016/j.theriogenology. 2010.10.019.

Garza, D., Camacho, M., Gauly, M., Holtz, W., 2018. Technical Note: transfer of caprine blastocysts vitrified by the open pulled straw (OPS) or the solid surface procedure and warmed in sucrose-free medium. Small Rumin. Res. 165, 111–114. https://doi.org/ 10.1016/j.smallrumres.2018.04.004.

Greyling, J.P.C., van Niekerk, C.H., 1986. Synchronization of oestrus in the Boer goat doe: dose effect of prostaglandin in the double injection regime. S. Afr. J. Anim. Sci. 16, 146–150.

Holtz, W., 1996. Embryo transfer in goats – a review (in German). Dtsch. Tieraerztl. Wochenschrift 103, 293–297.

Holtz, W., 2005. Recent developments in assisted reproduction in goats. Small Rumin. Res. 60, 95–110. https://doi.org/10.1016/j.smallrumres.2005.06.032. Special Issue: Plenary papers of the 8th International Conference on Goats.

Holtz, W., Sohnrey, B., 1992. Oestrus induction during the anoestrus season in goats by means of intravaginal pessaries or subcutaneous implants. In: Lokeshwar, R.R. (Ed.), Recent Advances in Goat Production. Nutan printers, New Delhi, pp. 1284–1289.

Holtz, W., Sohnrey, B., Gerland, M., Driancourt, M.-A., 2008. Ovsynch synchronization and fixed-time insemination in goats. Theriogenology 69, 785–792. https://doi.org/10.1016/j.theriogenology.2007.10.004.

Holtz, W., Wang, X., El-Gayar, M., Knight, P.G., 2012. The effect of exogenous gonadotropins on ovarian function in goats actively immunized against inhibin. Theriogenology 77, 253–259. https://doi.org/10.1016/j.theriogenology.2011.07.037.

Hutchinson, J.S., Robertson, H.A., 1966. The growth of the follicle and corpus luteum in the ovary of the sheep. Res. Vet. Sci. 7, 17–24.

Ireland, J.J., Roche, J.F., 1982. Effect of progesterone on basal LH and episodic LH and FSH secretion in heifers. J. Reprod. Fertil. 64, 295–302. https://doi.org/10.1530/jrf. 0.0640295.

Karaca, F., Ataman, M.B., Çoyan, K., 2009. Synchronization of estrus with short- and longterm progestagen treatments and the use of GnRH prior to short-term progestagen treatment in ewes. Small Rumin. Res. 81, 185–188. https://doi.org/10.1016/j.smallrumres.2008.12.002.

Kimura, K., Hirako, M., Iwata, H., Aoki, M., Kawaguchi, M., Seki, M., 2007. Successful superovulation of cattle by a single administration of FSH in aluminum hydroxide gel. Theriogenology 68, 633–639. https://doi.org/10.1016/j.theriogenology.2007. 02.016.

Knights, M., Maze, T.D., Bridges, P.J., Lewis, P.E., Inskeep, E.K., 2001. Short-term treatment with a controlled internal drug releasing (CIDR) device and FSH to induce fertile estrus and increase prolificacy in anestrous ewes. Theriogenology 55, 1181–1191. https://doi.org/10.1016/S0093-691X(01)00476-9.

Kusina, N.T., Tarwirei Hamudikuwanda, H., Agumba, G., Mukwena, J., 2000. A comparison of the effects of progesterone sponges and ear implants, PGF2alpha, and their combination on efficacy of estrus synchronization and fertility of Mashona goat does. Theriogenology 53, 1567–1580. https://doi.org/10.1016/S0093-691X(00)00298-3.

Leboeuf, B., Forgerit, Y., Bernelas, D., Pougnard, J.L., Senty, E., Driancourt, M.A., 2003. Efficacy of two types of vaginal sponges to control onset of oestrus, time of preovulatory LH peak and kidding rate in goats inseminated with variable numbers of spermatozoa. Theriogenology 60, 1371–1378. https://doi.org/10.1016/S0093-691X (03)00149-3.

Lehloenya, K.C., Greyling, J.P.C., 2010. The ovarian response and embryo recovery rate in Boer goat does following different superovulation protocols, during the breeding season. Small Rumin. Res. 88, 38–43. https://doi.org/10.1016/j.smallrumres.2009. 11.007.

Lehloenya, K.C., Greyling, J.P.C., Grobler, S., 2008. Effect of season on the superovulatory response in Boer goat does. Small Rumin. Res. 78, 74–79. https://doi.org/10.1016/j. smallrumres.2008.05.003.

Mahmood, S., Koul, G.W., Biswas, J.C., 1991. Comparative efficacy of FSH-P and PMSG on superovulation in Pashmina goat. Theriogenology 35, 1191–1196.

Martemucci, G., D´Alessandro, A.G., 2011a. Induction/synchronization of oestrus and ovulation in dairy goats with different short term treatments and fixed time

intrauterine or exocervical insemination system. Anim. Reprod. Sci. 126, 187–194. https://doi.org/10.1016/j.anireprosci.2011.05.011.

Martemucci, G., D´Alessandro, A.G., 2011b. Synchronization of oestrus and ovulation by short time combined FGA, PGF2α, GnRH, eCG treatments for natural service or AI fixed time. Anim. Reprod. Sci. 123, 32–39. https://doi.org/10.1016/j.anireprosci. 2010.11.007.

Martins, J.P.N., Policelli, R.K., Neuder, L.M., Raphael, W., Pursley, J.R., 2011. Effects of cloprostenol sodium at final prostaglandin F2α of Ovsynch on complete luteolysis and pregnancy per artificial insemination in lactating dairy cows. J. Dairy Sci. 94, 2815–2824. https://doi.org/10.3168/jds.2010-3652.

Menchaca, A., Rubianes, E., 2004. New treatments associated with timed artificial insemination in small ruminants. Reprod. Fertil. Dev. 16, 403–413. https://doi.org/10. 10371/RD04037.

Menchaca, A., Rubianes, E., 2001. Effect of high progesterone concentrations during the early luteal phase on the length of the ovulatory cycle of goats. Anim. Reprod. Sci. 31, 69–76. https://doi.org/10.1016/S0378-4320(01)00139-7.

Menchaca, A., Miller, V., Salveraglio, V., Rubianes, E., 2007a. Endocrine, luteal and follicular responses after the use of the Short-Term Protocol to synchronize ovulation in goats. Anim. Reprod. Sci. 102, 76–87. https://doi.org/10.1016/j.anireprosci.2006. 10.001.

Motlomelo, K.C., Greyling, J.P.C., Schwalbach, L.M.J., 2002. Synchronization of oestrus in goats: the use of different progestagen treatments. Small Rumin. Res. 45, 45–49. https://doi.org/10.1016/S0921-4488(02)00113-X.

Noel, B., Bister, J.L., Paquay, R., 1993. Ovarian follicular dynamics in Suffolk ewes at different periods of the year. J. Reprod. Fertil. 99, 695–700. https://doi.org/10. 1530/jrf.0.0990695.

Nogueira, D.M., Cavalieri, J., Gummow, B., Parker, A.J., 2015. Comparison of follicular dynamics and hormone profiles in Boer goats examined during the breeding and

nonbreeding season in the tropics of Queensland. Australia. Small Rumin. Res. 125, 93–100. https://doi.org/10.1016/j.smallrumres.2015.02.014.

Nowshari, M.A., Yuswiati, E., Puls-Kleingeld, M., Holtz, W., 1992. Superovulation in peripubertal and adult goats treated with PMSG or pFSH. In: In: Lokeshwar, R.R. (Ed.), Recent Advances in Goat Production 1992. Nutan Printers, New Delhi, India, pp. 1358–1363.

Nowshari, M.A., Beckers, J.F., Holtz, W., 1995. Superovulation of goats with purified pFSH supplemented with defined amounts of pLH. Theriogenology 43, 797–802. https://doi.org/10.1016/0093-691X(95)00022-Z.

Padilla, G., Knight, P.G., Holtz, W., 2008. Superovulation and embryo collection in nulliparous Boer goat does immunized against a recombinant ovine α-subunit inhibin. Small Rumin. Res. 74, 159–164. https://doi.org/10.1016/j.smallrumres.2007.05. 006.

Pendleton, R.J., Youngs, C.R., Rorie, R.W., Pool, S.H., Memon, M.A., Godke, R.A., 1992. Follicle stimulating hormone versus pregnant mare serum gonadotropin for superovulation of dairy goats. Small Rumin. Res. 8, 217–224. https://doi.org/10.1016/ 0921-4488(92)90042-3.

Pereira, R.J., Sohnrey, B., Holtz, W., 1998. Nonsurgical embryo collection in goats treated with prostaglandin F2α and oxytocin. J. Anim. Sci. 76, 360–363. https://doi.org/10. 2527/1998.762360x.

Pintado, B., Gutiérrez-Adán, A., Pérez Llano, B., 1998. Superovulatory response of Murciana goats to treatments based on PMSG/anti-PMSG or combined FSH/PMSG administration. Theriogenology 50, 357–364. https://doi.org/10.1016/S0093-691X (98)00145-9.

Pursley, J.R., Mee, M.O., Wiltbank, M.C., 1995. Synchronization of ovulation in dairy cows using PGF2alpha and GnRH. Theriogenology 44, 915–923. https://doi.org/10. 1016/0093-691X(95)00279-H.

Rubianes, E., Menchaca, A., 2003. The pattern and manipulation of ovarian follicular growth in goats. Anim. Reprod. Sci. 78, 271–287. https://doi.org/10.1016/S0378-4320(03)00095-2.

Saharrea, A., Valencia, J., Balcázar, A., Mejía, O., Cerbón, J.L., Caballero, V., Zarco, L., 1998. Premature luteal regression in goats superovulated with PMSG: effect of hCG or GnRH administration during the early luteal phase. Theriogenology 50, 1039–1052. https://doi.org/10.1016/S0093-691X(98)00206-4.

Saleh, M., 2011. Synchronization and superovulation of boer goats with PGF2α and GnRH or hCG and parentage analysis using microsatellite markers. Diss. Goettingen 1–93.

Smeaton, T.C., Robertson, H.A., 1971. Studies on the growth and atresia of graafian follicles in the ovary of the sheep. J. Reprod. Fertil. 25, 243–252. https://doi.org/10. 1530/jrf.0.0250243.

Stevenson, J.S., Lucy, M.C., Call, E.P., 1987. Failure of timed inseminations and associated luteal function in dairy cattle after two injections of prostaglandin F2-alpha. Theriogenology 28, 937–946. https://doi.org/10.1016/0093-691X(87)90044-6.

Stringfellow, D.A., Givens, M.D., 2010. Manual of the International Embryo Transfer Society (IETS), 4th ed. IETS., Champaign, IL. Stubbings, R.B., Bosu, W.T., Barker, C.A., King, G.J., 1986. Serum progesterone concentrations associated with superovulation and premature corpus luteum failure in dairy goats. Can. J. Vet. Res. 50, 369–373.

Suyadi, Holtz, W., 2012. Ultrasound vs. laparoscopic assessment of the number of ovulations in superovulated goats. Book of Abstracts. XI Internat. Conf. Goats R-43, 409.

Suyadi Beckers, J.F., Holtz, W., 1999. Ovarian response, embryo yield and plasma LH in FSH or FSH/LH-treated goats with or without ovulation-inducing LH injection. Reprod. Domest. Anim. 34, 32 (abstr.).

Suyadi Sohnrey, B., Holtz, W., 2000. Transcervical embryo collection in Boer goats. Small Rumin. Res. 36, 195–200. https://doi.org/10.1016/S0921-4488(99)00164-9.

Wheaton, J.E., Carlson, K.M., Windels, H.F., Johnson, L.J., 1993. CIDR: a new progesterone- releasing intravaginal device for induction of estrus and cycle control in sheep and goats. Anim. Reprod. Sci. 33, 127–141. https://doi.org/10.1016/0378-4320(93) 90111-4.

Wildeus, S., 2000. Current concepts in synchronization of estrus: sheep and goats. J. Anim. Sci. 77, 1–14. https://doi.org/10.2527/jas2000.00218812007700ES0040x.

Wiltbank, M.C., Niswender, G.D., 1992. Functional aspects of differentiation and degeneration of the steroidogenic cells of the corpus luteum in domestic animals. Anim. Reprod. Sci. 28, 103–110.https://doi.org/10.1016/0378-4320(92)90097-W.

Chapter 3

Seasonality of Boer goats in northern Europe and induction of estrus out of season

Miguel Camacho, Jennifer Stiehl, Denisse Garza, Matthias Gauly, Wolfgang Holtz

Small Ruminant Research 151 (2017) 26-314

Abstract

Seasonality in Boer goats in Northern Europe and attempts to overcome it is addressed in three studies. In Experiment 1, monitoring of estrus and serum progesterone concentration in sexually mature nulliparous Boer goat crosses indicated that ovarian activity ceased in February/March and was resumed from August onward. In 63.7% of 22 does the first estrus of the season was preceded by one or two ovulations, indicated by an increase in progesterone; in 22.7% first estrus and first ovulation coincided and in the remaining 13.6% the first estrus was not succeeded by formation of a functional corpus luteum. In Experiment 2, 31 Boer goats, weaned between July and September, were treated with progestogen-containing intravaginal sponges and 250 IU eCG either one (n =11), seven (n =10) or fourteen (n =10) days after weaning. All but two does showed estrous symptoms and were naturally mated. Transrectal ultrasonography 45 days after mating revealed a pregnancy rate of 42%. All of these carried to term (36% treated immediately after weaning, 40% treated one week later and 50% treated two weeks later) and gave birth to, on average, 2.1 kids. In Experiment 3, of 18 nulliparous does, 9–12 months of age, subjected to progestogen-eCG treatment out-of-season, 15 (83%) responded by showing estrous symptoms; 14 were naturally mated and six were diagnosed as pregnant five weeks later. One of the pregnant does aborted two weeks before parturition was due; in the others pregnancy had ceased at an earlier stage without visible symptoms. In conclusion, in northern Europe, Boer goats undergo a period of reduced ovarian activity, although to a lesser degree than dairy breeds. In most cases acyclicity was overcome by hormonal stimulation and, if mated, part of the weaned does carried to term, whereas in young nulliparous does pregnancy was not maintained.

Keywords: Seasonality, Goats, Estrus synchronization, Sponge treatment, Progesterone.

3.1 Introduction

Boer goats were first brought to Germany in 1977 (Holtz, 2002). At present, the breed constitutes approximately 25% of the German goat population and has gained foothold in various other European countries. They are primarily kept for brush and weed control, especially in the vicinity of cities and in regions where farmland has fallen fallow. There is, also, an increasing demand for meat of young goats as a delicacy, thus an incentive to obtain more offspring per doe by increasing the number of kiddings per year and having young females kid at an early age. Whereas in South-Africa Boer goats are considered "seasonally polyestrous with an extended breeding season" (Greyling, 2000), in our experience under the conditions prevailing in Germany there is a distinct period of acyclicity. Kids are generally born in spring and weaned in summer. Weaning thus occurs at the most inappropriate time for re-breeding. The same applies to young females achieving sexual competence at the age of approximately one year. To breed does out-of-season, most commonly progestogen-impregnated intravaginal pessaries (Baril et al., 1993; Greyling and Van Niekerk, 1991) or ear implants (Bretzlaff et al., 1992; Holtz and Sohnrey, 1992; Freitas et al., 1997; Avendaño et al., 2003) are administered, usually in conjunction with the i.m. administration of the gonadotropic agent eCG (Corteel et al., 1988; Baril et al., 1993; Leboeuf et al., 2003; Holtz, 2005). Alternative approaches to overcome seasonality, such as the administration of exogenous melatonin or of photoperiod programs are out of the question because the former is not freely available and the latter is not practicable under extensive husbandry conditions typical for meat goats. The present investigation, consisting of three experiments, addresses seasonality in Boer goats under conditions in northern Europe and attempts to overcome it in freshly weaned and nulliparous does.

3.2 Materials and methods

Three experiments were conducted in the Boer goat breeding flock of the Department of Animal Science at Goettingen University, Germany (9° 41′E, 51° 46′N). Animals of Experiment 1 were crossbred 75%–87.5% Boer goats; in Experiments 2 and

3 purebred Boer goats were available. Does were group-housed in an open barn with strawcovered floor and an outdoor concrete run. They were fed a daily ration of about 600 g concentrate, consisting of equal parts of a pelleted lactation diet for breeding ewes, supplemented with selenium, zinc and iodine, oats and dried sugar beet pulp, and had free access to wheat or barley straw, salt lick and water.

3.2.1. Experiment 1. Onset of seasonal cyclicity

To determine the end of the breeding season, weekly blood samples were collected by jugular venipuncture from 11 cycling does, 9–25 mo of age and weighing 60 (46–79) kg, throughout February and March. Beginning in April, six of these goats (five were assigned to another study) plus another 16 (in total 22 does), 9–41 mo of age and weighing 45–79 kg, were sampled at weekly intervals, and from mid-August to December, twice weekly. Blood was centrifuged for 15 min at 3000 x g after being kept at 4 °C for 24 h. Serum was stored at −20 °C and analyzed for progesterone content by ELISA (Van de Wiel and Koops, 1986, modified by Moeller, 1991). Assay sensitivity was 0.4 pg; interand intraassay variance, averaged from determinations of serum with progesterone concentrations 1.0, 5.0 and 25.0 ng/mL, were 12.0% and 16.1%, respectively. Throughout the sampling period does were tested for estrus twice daily with an aproned adult buck. When in estrus, they were naturally mated to fertility proven bucks once per day as long as they would posture.

3.2.2. Experiment 2. Post-weaning induction of estrus out-of-season

Pluriparous does (n = 31), on average 3.7 (range 2–7) years of age and weighing 60 (46–79) kg, were weaned between July and September, a time when does are generally not cycling. Does were provided with intravaginal polyurethane sponges of 40 mm diameter, impregnated with 20 mg of the synthetic progestogen fluorogestone acetate (Cronolone®, Intervet, France), either one day (Group 1; n = 11), seven days (Group 2; n = 10) or fourteen days after weaning (Group 3; n =10). Sponges were withdrawn after 7 days and, concurrently, an i.m. injection of 250 IU equine chorionic gonadotropin (eCG, Intergonan®, Intervet, Unterschleissheim, Germany) was

administered. Does were tested for estrous symptoms with an aproned adult male at 8 h intervals. When displaying standing estrus they were naturally mated daily to one of two fertility-proven breeding bucks. Pregnancy diagnosis was conducted 30 and 45 days after mating by transrectal ultrasonography (ALOKA-SSD 500, equipped with transrectal 7.5 MHz linear array transducer) as described by Padilla-Rivas et al. (2005). The number of goats exhibiting estrous symptoms as well as pregnancy and kidding rate were analyzed by Chi square test. Interval from sponge withdrawal to estrus as well as litter size was analyzed by one-way analysis of variance with "time of treatment after weaning" as main effect.

3.2.3. Experiment 3. Induction of estrus in nulliparous does out-of-season

During the non-breeding season between April and mid-June, 18 nulliparous Boer goat does, 11.0 (range 9–12) mo of age and weighing 55 (45–65) kg, received the similar sponge/eCG treatment as the weaned does of Experiment 2. They were tested for estrus with an aproned adult buck at 8 h intervals and naturally mated on each day of standing estrus. Pregnancy was confirmed by transrectal ultrasonography 35 d after mating. From sponge insertion until 35 d after mating weekly blood samples were drawn by jugular venipuncture. Serum progesterone concentration was determined by ELISA as described in Experiment 1. Differences in progesterone concentration between pregnant and non-pregnant goats were subject to analysis of variance, followed by post hoc assessment of differences at various weeks by the non-parametric (small number of degrees of freedom did not permit assumption of normal distribution) Wilcoxon's signed rank test.

3.3. Results

3.3.1. Experiment 1

By monitoring progesterone profiles of 11 does to establish when cyclicity ceases, it was found that estrus occurred last in one doe in February and in the remaining 10 does in March. Monitoring of 22 does from April onward revealed that neither behavioral estrus nor a progesterone increase had occurred until the end of July. Incidence and cumulative frequency of first standing estrus of the new breeding season are presented in Fig. 1. A single doe (4.5%) exhibited standing estrus in August, seven does (31.8%) in September, nine does (40.9%) in October and the remaining five does (22.7%) in November. Once cyclicity had resumed, mean interestrus intervals were 24.0 (SE 2.0) days, ranging from 8 to 48 days. Does kidded between March and April, indicating that none had conceived before October.

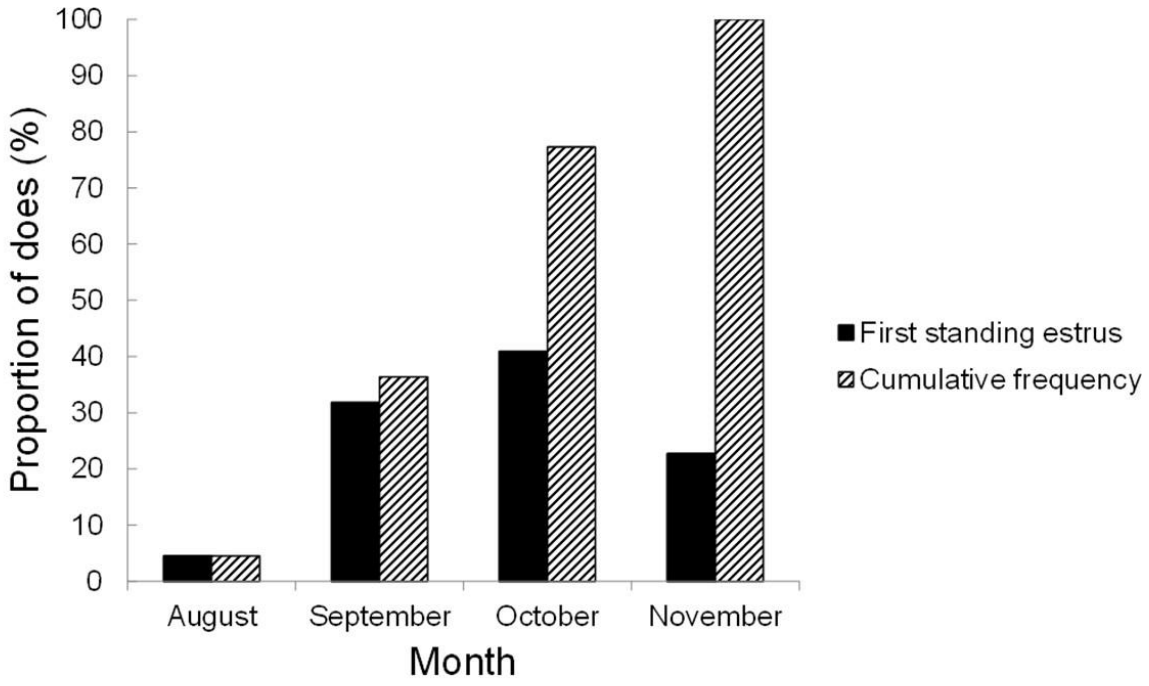

Figure 1. Incidence of first standing estrus at the onset of the breeding season in 22 does of a Boer goat (75% to 87.5%) cross (Experiment 1).

At the beginning of the mating season four characteristic progesterone patterns were observed (Fig. 2). In 45.5% of the does the first behavioral estrus was preceded by one ovulation, indicated by a subsequent increase in progesterone (Fig. 2a); in another

18.2% it was preceded by two ovulations (Fig. 2b). In 22.7% of the does the first standing estrus coincided with the first ovulation (Fig. 2c) and in the remaining 13.6% it occurred after two previous behavioral estruses (Fig. 2d). Considering a concentration of 3 ng/mL as progesterone baseline, the corpus luteum phase in cycling does lasted, on average, 12.0 d (SE 1.0), ranging from 4 to 28 d. Maximum progesterone levels of, on average, 10.0 ng/mL (SE 0.4) were reached on d 10 of the cycle with range 5–19 (d 1 being the first day of standing estrus).

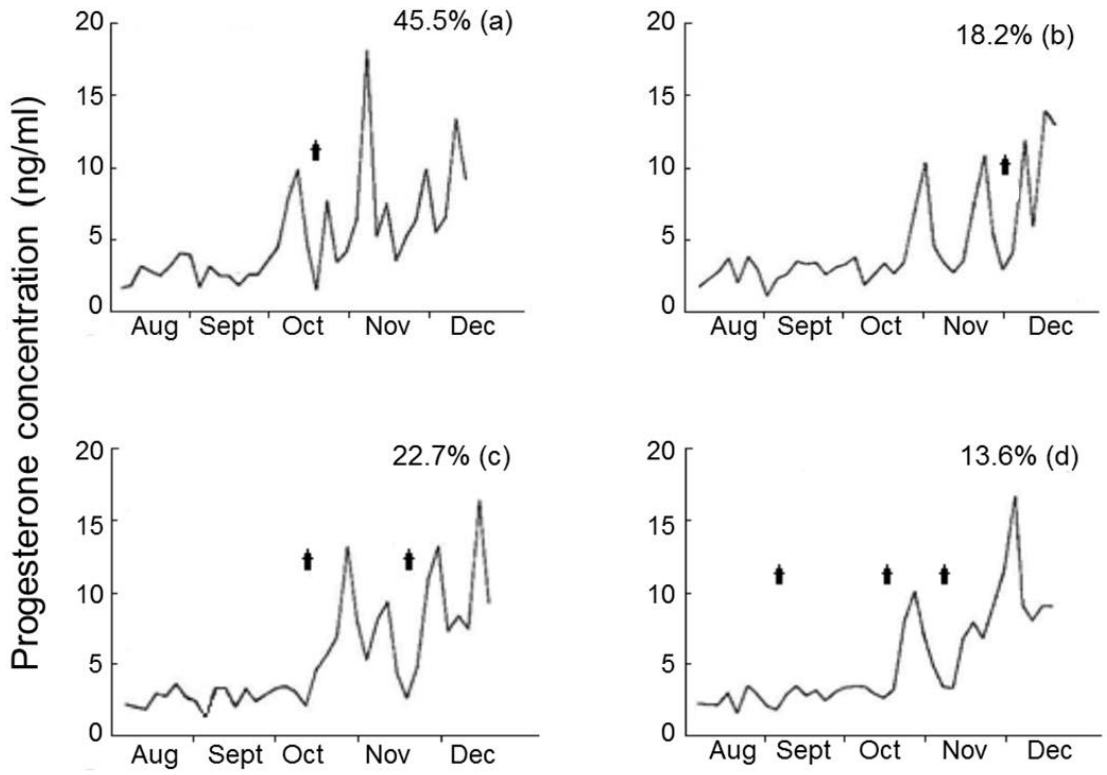

Figure 2. Characteristic progesterone patterns observed during the course of the experiment. The percentage indicates the number of animals belonging to each characteristic pattern. Arrows indicate standing estrus (Experiment 1).

3.3.2. Experiment 2

As shown in Table 1, 91%, 100% and 90% of the does treated one, seven and fourteen days after weaning, respectively, exhibited estrus. The overall average interval from sponge withdrawal to onset of estrus was 33.0 h (SE 2.2, range 16–72) with no significant difference among the three groups. Pregnancy rate 30 days after mating, across groups, was 58%. By 45 days it had decreased to 42%; all of these does kidded with an average litter size of 2.1 kids. Kidding rates in does treated one, seven or fourteen days after weaning, were 36%, 40% and 50%, respectively, the differences not being statistically significant. All but four does (two had singlets, one each a triplet and a quadruped) gave birth to twins. No effect of age or parity on pregnancy rate or litter size was detected

Table 1: Estrous-, pregnancy- and kidding rates in Boer goat does estrus induced during the non-breeding season by sponge-eCG treatment conducted one (Group 1), seven (Group 2) or fourteen days after weaning (Group 3) (Experiment 2).

Variable	Group 1	Group 2	Group 3	Overall
Does treated	11	10	10	31
Does in estrus	10	10	9	29
Sponge withdrawal to estrus (h)				
Mean	37.3	31.0	30.0	33.0
SE	5.0	3.0	4.0	2.2
Range	24-72	24-41	16-48	16-72
Pregnancy rate at 30 days (%)	55	60	60	58
Pregnancy rate at 45 days (%)	36	40	50	42
Does kidding (%)	36	40	50	42
Litter size				
Mean	2.0	2.0	2.4	2.1
SE	0.2	0.4	0.4	0.2

No significant differences between groups were recorded

3.3.3. Experiment 3

Of 18 nulliparous goats subjected to sponge-eCG treatment out-of-season, 15 (83%) displayed estrus 44.3 (SE 3.0, range 16–66) h after sponge withdrawal. One doe did not permit intromission. Six of 14 mated does (43%) were diagnosed as pregnant by ultrasonography 35 days after mating. Eventually, with the exception of one doe that aborted twins two weeks before the expected date of parturition, pregnancy was terminated in all does; apparently at an early stage, because no signs of abortion were

noticed. Serum progesterone concentration at insertion and withdrawal of sponges was less than 0.5 ng/mL (Fig. 3). One wk after withdrawal it had increased to 5.0 (SE 1.4) ng/mL in pregnant and 5.2 (SE 2.1) ng/mL in non-pregnant goats. A maximum of 14 (SE 1.3) ng/mL in pregnant and 9.3 (SE 4.0) ng/mL in non-pregnant does was reached two wk after sponge withdrawal ($P > 0.05$). In non-pregnant does progesterone concentration had returned to base level by 4 weeks after sponge withdrawal, whereas, in pregnant does it remained high. Differences between pregnant and non-pregnant does were significant from the third wk after sponge withdrawal onward ($P > 0.05$). No blood samples were drawn at a later stage.

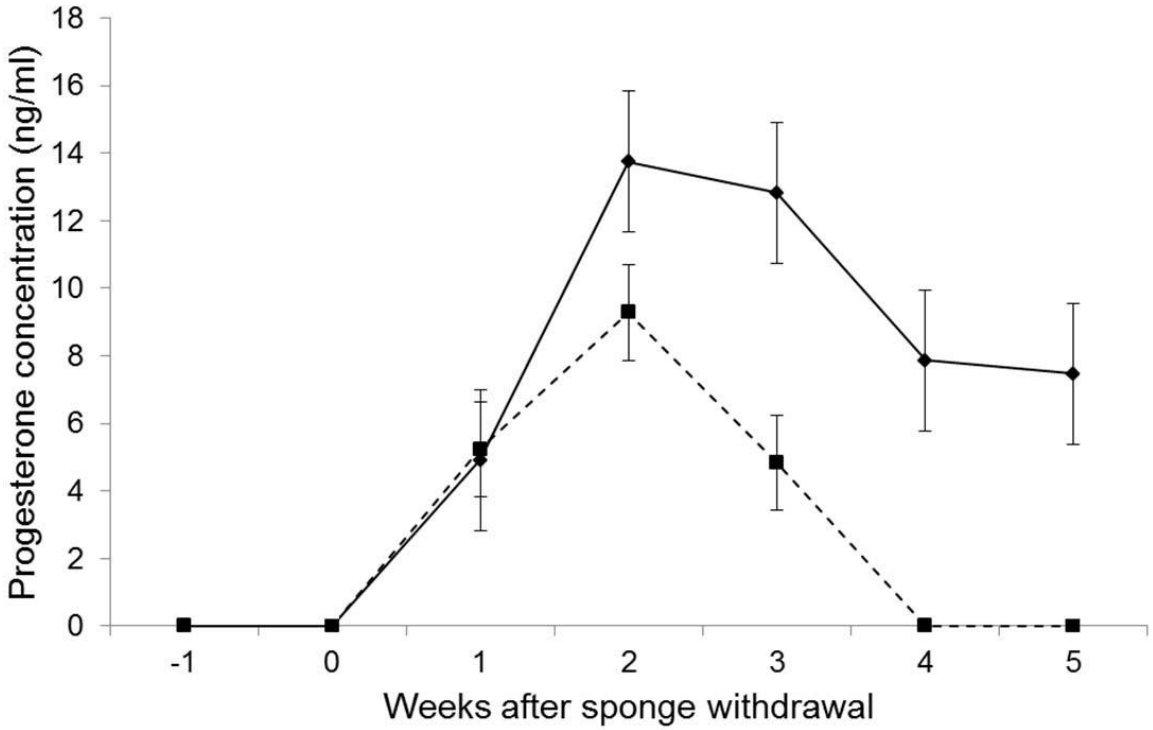

Figure 3. Serum progesterone levels (mean ± SE) in does pregnant (solid line) or not (dotted line) up to 35 days after sponge withdrawal (Experiment 3).

3.4. Discussion

The results of Experiment 1 clearly indicate that under conditions prevailing in northern Europe reproductive activity of Boer goats follows a seasonal pattern. No cyclicity was observed between March (day length 11.8 h) and July (16.2 h). With decreasing daylight hours in August (14.8 h) one out of 22 does started cycling; in September (12.8 h) 31.8% of the does had resumed cyclicity and by November (day length 8.5 h) all does had displayed at least one standing estrus. In accord with findings in other breeds (Chemineau et al., 1992; Delgadillo et al., 2007; Fatet et al., 2011), reproductive activity culminated in autumn and winter, the season being slightly longer than in French Alpine goats in which a complete lack of sexual activity prevails from March to September (Chemineau et al., 1992), and substantially longer than in breeds with extremely short breeding season such as Australian Cashmere goats (Restall, 1992), Syrian Damascus goats (Zarkawi et al., 1999) and South African Angora goats (Shelton, 1978). According to Chemineau et al. (1992), goats originating from temperate regions remain seasonal even when brought to the tropics. Goats from tropical or subtropical regions, moved to temperate latitudes, are less seasonal than local breeds (Santa Maria et al., 1990) or even lack seasonality (Chemineau, 1986). Boer goats, originally brought to Germany in 1977, originate from latitudes in South-Africa south of 30°S where estrous activity peaks in autumn but, according to Greyling (2000), never ceases completely. Apparently the trend toward seasonality displayed by Boer goats under South African conditions is maintained and expressed more distinctly in northern Europe. Even in Boer goats kept at a more southerly latitude in Croatia, under husbandry conditions resembling those of the present study, 76% of the kiddings occurred in winter and spring and breeding activity was not resumed before August (Duričić et al., 2012). Very few published investigations are available dealing with an interaction between nutrition and photoperiodism in goats but, according to Scaramuzzi and Martin (2008) and Estrada-Cortés et al. (2009), a causal relationship between nutritional state and duration of the anovulatory period appears likely. Feed analyses we conducted in an attempt to explain the extended period of acyclicity in our flock did not reveal qualitative deficiencies. Serum progesterone concentration in weekly blood samples collected from twelve adult

Boer goats, randomly chosen from a flock spending the summer months (May to August) on pasture in the absence of males, remained constantly low. Thus, despite lush pasturage there was a complete lack in cyclic activity. Nonetheless, in does running with a male during summer occasionally pregnancies occur, presumably ascribable to the stimulatory buck effect (Chemineau, 1987; Claus et al., 1990; Mellado et al., 2000; De Santiago-Miramontes et al., 2008; Véliz et al., 2009). The occurrence of one or two ovarian cycles preceding the first behavioral estrus of the season in nearly two thirds of 22 does monitored in Experiment 1 corroborates observations by Thorburn and Schneider (1972) and Kakusya (1980) who state that, at the onset of the breeding season, silent estruses are the rule. The effect of estrogens on brain regions responsible for the expression of estrous behavior is modulated by progesterone (Barraclough et al., 1986; Brown and MacLusky, 1994; Blaustein, 2004); consequently the first estrous symptoms of the season have to be preceded by a functional corpus luteum. The few cases in which no rise in progesterone occurred after a behavioral estrus conceivably result from failure of follicles to ovulate or formation of functional corpora lutea. The latter is not uncommon in goats emerging from seasonal anestrus (Chemineau et al., 1987; Agwu, 1988; Baird, 1992; Camp et al., 1983).

The objective of Experiment 2 was to attempt to overcome seasonal acyclicity in freshly weaned does by employing the worldwide commonly used sponge/eCG treatment, expecting to exploit the rebound phenomenon following termination of progestogen treatment. In accord with various recent studies (Rubianes and Menchaca, 2003; Karaca et al., 2010; Martemucci and D'Alessandro, 2011; Pietroski et al., 2013) the duration of sponge residence was only seven days. Accordingly (Chronogest®) sponges containing 20 mg of progestogen were used instead of 45 mg as used to be the case in the past (Vinoles et al., 2001; Leboeuf et al., 2003). In cows (Smith et al., 1981; Edwards, 1985) and ewes (Mandiki et al., 1990) it was found that pulsatile LH secretion, inhibited during the suckling period, was immediately resumed upon weaning. Apparently this applies to goats as well, inasmuch as virtually all does weaned responded to treatment and could be mated. The numerically slightly lower pregnancy rate recorded in does that were treated immediately after weaning might be explained by emotional

and/or physiological stress associated with separation from the kids and drying off and involution of the mammary gland. In conclusion, as in many other breeds (Allen and Lamming, 1960; Robinson, 1971; Thimonier, 1981; Carnevali et al., 1997), in Boer goats out-of-season breeding is feasible, even in freshly weaned does, though with suboptimal kidding rates. There is, thus, a perspective for flock owners to achieve more than one litter per year in at least part of their breeding does.

In Experiment 3, out-of-season breeding of nulliparous young Boer goat does was attempted. Animals had reached 60–75% of their mature body mass, considered a prerequisite (Smith et al., 1981). In accordance with findings by Fonseca and Torres (2005) and Pietroski et al. (2013), almost all does treated showed estrous symptoms. The time lapse between sponge withdrawal and onset of estrus agrees with findings by Simões et al. (2008), but was slightly less than reported by Souza- Fabjan et al. (2013). Does in which serum progesterone concentration had returned to basal level within 3–4 weeks, apparently failed to conceive or had suffered early embryonic death. The initial increase in serum progesterone followed the typical pattern (Braun et al., 1988; Bauernfeind and Holtz, 1991) and did not resemble the weak transitory progesterone rise described by Yuswiati (1991) and Al Yacoub et al. (2011) typical for corpus luteum insufficiency occasionally observed after hormonal intervention (Armstrong et al., 1982; Battye et al., 1988; Pintado et al., 1998; Espinosa-Márquez et al., 2004; Chao et al., 2008). Nonetheless, except one doe that aborted two weeks before the expected date of parturition, in all does positively diagnosed 35 days after mating pregnancy was interrupted; presumably at an early stage because no abortion or bleeding was noticed although the animals were under permanent close surveillance. Maternal recognition of pregnancy occurs around days 15–17 (Gnatek et al., 1989) and the process of implantation lasts until about seven weeks after conception. The findings of Experiments 2 and 3 suggest that embryonic loss may occur at some time during that critical early stage of pregnancy. Pathological reasons may be ruled out because the flock was under permanent veterinary control. A negative seasonal effect on the side of the males appears unlikely because a study on Boer goat bucks from the Departmental flock showed that during the non-breeding season only a moderate reduction in semen volume, proportion

of live spermatozoa and progressive motility (Tuli and Holtz, 1992) as well as in freezability (Tuli and Holtz, 1995) is observed. French studies (Corteel, 1975; Corteel et al., 1987) show that out-of-season fertilizability of spermatozoa is not jeopardized. Libido in males is maintained throughout the year as long as they are regularly collected. Findings by Mani et al. (1992, 1994) indicate that underfeeding of pregnant females may result in embryonic loss, usually preimplantatory. In the Departmental flock feed supply was quantitatively modest. It may be surmised that, in view of group housing, young does have suffered from competition for feed and also social stress exerted by domineering older goats, which might have contributed to the poor results.

3.5 . Conclusion

From the present study it may be concluded that in northern Europe Boer goats, albeit to a lesser degree than most dairy breeds, undergo a period of ovarian inactivity, extending approximately from April to August. The stage of ovarian quiescence may be overcome by progestogen- eCG treatment. When mated at induced estrus, close to 50% of weaned does produced, on average, 2.1 kids, whereas none of the young nulliparous does carried to term. To what extent unfavorable environmental factors may have played a role is open to conjunction.

Conflict of interest

None of the authors have a conflict of interest to declare.

3.6 Acknowledgments

The authors owe thanks to E. Stuewe for assisting with hormone analyses, G. Das for helping with statistical analyses and D. Daniel and the barn staff for assisting with the handling of the animals. Of the authors M.C. was supported by a fellowship of the Consejo Nacional de Ciencia y Tecnología (CONACYT)-German Academic Exchange Service (DAAD) and D.G. by the German Academic Exchange Service (DAAD).

3.7 References

Agwu, J.O., 1988. Progesterone and Luteinizing Hormone during breeding, non-breeding season and after oestrus induction in goats. Diss. Goettingen 1–133.

Al Yacoub, A.N., Gauly, M., Sohnrey, B., Holtz, W., 2011. Fixed-time deep uterine insemination in PGF2a-synchronized goats. Theriogenology 76, 1730–1735.

Allen, D.M., Lamming, G.E., 1960. The induction of breeding activity in lactating ewes during anoestrus. J. Reprod. Fertil. 1, 213–222.

Armstrong, D.T., Pfitzner, A.P., Porter, K.J., Warnes, G.M., Janson, P.O., Seamark, R.F., 1982. Ovarian responses of anoestrous goats to stimulation with pregnant mare serum gonadotrophin. Anim. Reprod. Sci. 5, 15–23.

Avendaño, L., Álvarez, D., Correa, A., 2003. Induction of estrus and fertility using subcutaneous implants in anestrous dairy goats. Interciencia 28, 225–228.

Baird, D.T., 1992. Luteotrophic control of the corpus luteum. Anim. Reprod. Sci. 28, 95–102.

Baril, G., Leboeuf, B., Saumande, J., 1993. Synchronization of estrus in goats: the relationship between time of occurrence of estrus and fertility following artificial insemination. Theriogenology 40, 621–628.

Barraclough, C.A., Camp, P., Weiland, N., Akabori, A., 1986. Stimulatory versus inhibitory effects of progesterone on estrogen-induced phasic LH and prolactin secretion correlated with estrogen nuclear and progestin cytosol receptor concentrations in brain and pituitary gland. Neuroendocrinology 42, 6–14.

Battye, K.M., Fairclough, R.J., Cameron, A.W., Trounson, A.Q., 1988. Evidence for prostaglandin involvement in early luteal regression of the superovulated nanny goat (Capra hircus). J. Reprod. Fertil. 84, 425–430.

Bauernfeind, M., Holtz, W., 1991. Progesterone and estrogen levels in serum of cycling goats measured by enzyme immunoassay. Small Rumin. Res. 6, 95–102.

Blaustein, J.D., 2004. Minireview: neuronal steroid hormone receptors: they're not just for hormones anymore. Endocrinology 145, 1075–1081.

Braun, F.W., Solorzano, N.M., Bierschwal, C.J., 1988. Characterization of the caprine estrous cycle using enzyme immunoassay for the determination of whole blood progesterone concentration. Theriogenology 29, 1155–1166.

Bretzlaff, K.N., Nuti, L.C., Elmore, R.G., Meyers, S.A., Rugila, J.N., Brinsko, S.P., Blanchard, T.L., Weston, P.G., 1992. Synchronization of estrus in dairy goats given norgestomet and estradiol valerate at various stages of the estrous cycle. Am. J. Vet. Res. 53, 930–934.

Brown, T.J., MacLusky, N.J., 1994. Progesterone modulation of estrogen receptors in microdissected regions of the rat hypothalamus. Mol. Cell. Neurosci. 5, 283–290.

Camp, J.C., Wildt, D.E., Howard, P.K., Stuart, L.D., Chakraborty, P.K., 1983. Ovarian activity during normal and abnormal length estrous cycles in the goat. Biol. Reprod. 28, 673–681.

Carnevali, F., Schino, G., Diverio, S., Misiti, S., 1997. Oestrus induction and synchronization during treatment in cashmere goats using hormonal treatment in association with male effect. Eur. Fine Fibre Netw. 6, 55–63.

Chao, L.M., Takayama, K., Nakanishi, Y., Hamana, K., Takagi, M., Kubota, C., 2008. Luteal lifespan and fertility after estrus synchronization in goats. J. Vet. Sci. 9, 95–101.

Chemineau, P., Martin, G.B., Saumande, J., Esperon, F.M., 1987. Seasonal and hormonal control of LH secretion in the French dairy goat. In: Brasilia, Brazil. Odon, P.S., Allomar, G., Foote, W.C. (Eds.), Proc. 4th Intern. Conf. on Goats, March 8–13, 1987, vol. II 271 (abstr.).

Chemineau, P., Daveau, A., Maurice, F., Delgadillo, J.A., 1992. Seasonality of estrus and ovulation is not modified by subjecting female Alpine goats to a tropical photoperiod. Small Rumin. Res. 8, 299–312.

Chemineau, P., 1986. Sexual behaviour and gonadal activity during the year in the tropical Creole meat goat. I Female oestrous behaviour and ovarian activity. Reprod. Nutr. Dev. 26, 441–452.

Chemineau, P., 1987. Possibilities for using bucks to stimulate ovarian and estrous cycles in anovulatory goats – a review. Livest. Prod. Sci. 17, 135–147.

Claus, R., Over, R., Dehnhard, M., 1990. Effect of male odour on LH secretion and induction of ovulation in seasonally anestrous goats. Anim. Reprod. Sci. 22, 27–38.

Corteel, J.M., Baril, G., Leboeuf, B., 1987. Out of season breeding and AI with frozen thawed spermatozoa. Proc. 4th Internat. Conf. on Goats, March 8–13, 1987, Brasilia, Brazil. Symposium on Reproduction.

Corteel, J.M., Leboeuf, B., Baril, G., 1988. Artificial breeding of adult goats and kids induced with hormones to ovulate outside the breeding season. Small Rumin. Res. 1, 19–35.

Corteel, J.M., 1975. Production du sperme chez la bouc: variation saisonniére de la quantité et de la qualité du sperme récolté selon l'age des animaux. 1ères Journées de la Recherche Ovine et Caprine, Paris, December 2–3 1975. In: I.N.R.A.-I.T.O.V.I.C. (Ed.), 1, 28–47.

De Santiago-Miramontes, M.A., Rivas-Munoz, R., Munoz-Gutiérres, M., Malpaux, B., Scaramuzzi, R.J., Delgadillo, J.A., 2008. Ovulation rate in female anoestrous goats exposed to the male effect is increased by nutritional supplementation under grazing conditions. Anim. Reprod. Sci. 105, 409–416.

Delgadillo, J.A., De Santiago-Miramontes, M.A., Carrillo, E., 2007. Season of birth modifies puberty in female and male goats raised under subtropical conditions. Animal 1, 858–864.

Duričić, D., Grizelj, J., Dobranić, T., Harapin, I., Vince, S., Kočila, P., Folnožić, I., Lipar, M., Gregurić, G., Samardžija, M., 2012. Reproductive performance of boer goats in a moderate climate zone. Veterinarski Archiv 82, 351–358.

Edwards, S., 1985. The effects of short term calf removal on pulsatile LH secretion in the postpartum beef cow. Theriogenology 23, 777–785.

Espinosa-Márquez, M.C., Valencia, J., Zarco, L., Escobar-Medina, F.J., Colina-Flores, F., Arechiga-Flores, C.F., 2004. Effect of fluorogestone acetate on embryo recovery and quality in eCG-superovulated goats with premature luteal regression. Theriogenology 62, 624–630.

Estrada-Cortés, E., Vera-Avila, H.R., Urrutia-Morales, J., Villagómez-Amezcua, E., Jiménez-Severiano, H., Mejía-Guadarrama, C.A., Rivera-Lozano, M.T., Gámez-Vázquez, H.G., 2009. Nutritional status influences reproductive seasonality in Creole goats: 1: Ovarian activity during seasonal reproductive transitions. Anim. Reprod. Sci. 116, 282–290.

Fatet, A., Pellicer-Rubio, M.T., Leboeuf, B., 2011. Reproductive cycle of goats. Anim. Reprod. Sci. 124, 211–219.

Fonseca, J., Torres, C., 2005. Administration of hCG 5 days after breeding and reproductive performance in nulliparous dairy goats. Reprod. Dom. Anim. 40, 495–499.

Freitas, V.J., Baril, G., Saumande, J., 1997. Estrus synchronization in dairy goats: use of fluorogestone acetate vaginal sponges or Norgestomet ear implants. Anim. Reprod. Sci. 46, 237–244.

Gnatek, G.G., Smith, L.D., Duby, R.T., Godkin, J.D., 1989. Maternal recognition of pregnancy in the goat: effects of conceptus removal on interestrus intervals and characterization of conceptus protein production during early pregnancy. Biol. Reprod. 41, 655–663.

Greyling, J.P.C., Van Niekerk, C.H., 1991. Different synchronization techniques in Boer goat does outside the normal breeding season. Small Rumin. Res. 5, 233–243.

Greyling, J.P.C., 2000. Reproduction traits in the Boer goat doe. Small Rumin. Res. 36, 171–177

Holtz, W., Sohnrey, B., 1992. Oestrus induction during the anoestrus season in goats by means of intravaginal pessaries or subcutaneous implants. Recent Advances in Goat Production. In: Lokeshwar, R.R. (Ed.), Recent Advantages in Goat Production. Nutan Printers, pp. 1284–1289.

Holtz, W., 2002. Boer goats in Germany and the Goettingen breeding program (in German). Der Ziegenzuechter 18 (4), 2–7.

Holtz, W., 2005. Recent developments in assisted reproduction in goats. small ruminant research, special issue: plenary papers of the 8th int. conf. Goats 60, 95–110 Special Issue.

Kakusya, G.R.E., 1980. Reproductive hormone patterns in female pygmy goats. Diss. Abstr. Internat. B, 40, 3504. Animal Breeding Abstracts 1980, vol. 48. Manitoba University, Canada, pp. 806 (abstr.).

Karaca, F., Dogruer, G., Saribay, M.K., Ates, C.T., 2010. Oestrus synchronization with short-term and long-term progestogen treatments in goats: the use of GnRH prior to short-term progestogen treatment. Ital. J. Anim. Sci. 9, e22. http://dx.doi.org/10.4081/ijas.2010.e22.

Leboeuf, B., Forgerit, Y., Bernelas, D., Pougnard, J.L., Senty, E., Driancourt, M.A., 2003. Efficacy of two types of vaginal sponges to control onset of oestrus, time of preovulatory LH peak and kidding rate in goats inseminated with variable numbers of spermatozoa. Theriogenology 60, 1371–1378.

Mandiki, S.N., Bister, J.L., Paquay, R., 1990. Effects of suckling mode on endocrine control of reproductive activity resumption in Texel ewes lambing in July or November. Theriogenology 33, 397–413.

Mani, A.U., McKelvey, W.A.C., Watson, E.D., 1992. The effects of low level of feeding on response to synchronization of estrus, ovulation rate and embryo loss in goats. Theriogenology 38, 1013–1022.

Mani, A.U., Watson, E.D., McKelvey, W.A.C., 1994. The effects of subnutrition before or after embryo transfer on pregnancy rate and embryo survival in does. Theriogenology 41, 1673–1678.

Martemucci, G., D'Alessandro, A.G., 2011. Induction/synchronization of oestrus and ovulation in dairy goats with different short term treatments and fixed time intrauterine or exocervical insemination system. Anim. Reprod. Sci. 126, 187–194.

Mellado, M., Olivas, R., Ruiz, F., 2000. Effect of buck stimulus on mature and prepubertal norgestomet-treated goats. Small Rumin. Res. 36, 269–274.

Moeller, R., 1991. Enzymimmunoassays mit zweitem Antikoerper fuer Progesteron und porcines LH (Enzymeimmunoassays with second antibody for progesterone and porcine LH). Diss Goettingen 1–125.

Padilla-Rivas, G.R., Sohnrey, B., Holtz, W., 2005. Early pregnancy detection by real-time ultrasonography in Boer goats. Small Rumin. Res. 58, 87–92.

Pietroski, A.C.C.A., Brandão, F.Z., Souza, J.M.G., Fonseca, J.F., 2013. Short, medium or long-term hormonal treatments for induction of synchronized estrus and ovulation in Saanen goats during the nonbreeding season. R. Bras. Zootec. 42, 168–173.

Pintado, B., Gutierrez-Adan, A., Perez-Llano, B., 1998. Superovulatory response of Murciana goats to treatments based on PMSG/Anti-PMSG or combined FSH/PMSG administration. Theriogenology 50, 357–364.

Restall, B.J., 1992. Seasonal variation in reproductive activity in Australian goats. Anim. Reprod. Sci. 27, 305–318.

Robinson, T.J., 1971. The seasonal nature of reproductive phenomena in sheep. II. Variation in fertility following synchronization of oestrus. J. Reprod. Fertil. 21, 19–27.

Rubianes, E., Menchaca, A., 2003. The pattern and manipulation of ovarian follicular growth in goats. Anim. Reprod. Sci. 78, 271–287.

Santa Maria, A., Cox, J., Munoz, E., Rodriguez, R., Caldera, L., 1990. Study of sexual cycle, reproductive seasonality and control of oestrus in Criollo goats in Chile.

Livestock reproduction in Latin America. Proceedings of the Final Research Coordination Meeting, Bogota,S Eptember 19–23, 1988. Organized by the Joint FAO/ IAEA Division of Nuclear Techniques in Food and Agriculture 363–385.

Scaramuzzi, R.J., Martin, G.B., 2008. The importance of interactions among nutrition, seasonality and socio-sexual factors in the development of hormone-free methods for controlling fertility. Reprod. Domest. Anim. 43, 129–136.

Shelton, M., 1978. Reproduction and breeding of goats. J. Dairy Sci. 61, 994–1010.

Simões, J., Baril, G., Almeida, J.C., Azevedo, J., Fontes, P., Mascarenhas, R., 2008. Time of ovulation in nulliparous and multiparous goats. Animal 2, 761–768.

Smith, J.F., Payne, E., Tervid, H.R., McGowan, L.H., Fairclough, R., Kilgour, R., Goold, P.G., 1981. The effect of suckling upon the endocrine changes associated with anoestrus in identical twin dairy cows. J. Reprod. Fertil. Suppl. 30, 241–249.

Souza-Fabjan, J.M.G., Pereira, A.F., Melo, C.H.S., Sanchez, D.J.D., Oba, E., Mermillod, P., Melo, L.M., Teixeira, D.I.A., Freitas, V.J.F., 2013. Assessment of the reproductive parameters, laparoscopic oocyte recovery and the first embryos produced in vitro from endangered Canindé goats (Capra hircus). Reprod. Biol. 13, 325–332.

Thimonier, J., 1981. Control of seasonal reproduction in sheep and goats by light and hormones. J. Reprod. Fertil. 30, 33–45.

Thorburn, G.D., Schneider, W., 1972. The progesterone concentration in the plasma of the goat during the estrous cycle and pregnancy. J. Endocr. 52, 23–36.

Tuli, R.K., Holtz, W., 1992. The effect of season on seminal characters in Boer goat bucks in the northern temperate zone. In: Lokeshwar, R.R. (Ed.), Recent Advances in Goat Production. Nutan Printers, New Delhi, India, pp. 1195–1200.

Tuli, R.K., Holtz, W., 1995. Effect of season on the freezeability of Boer goat semen in the northern temperate zone. Theriogenology 43, 1359–1363.

Véliz, F.G., Meza-Herrera, C.A., De Santiago-Miramontes, M.A., Arellano-Rodriguez, G., Leyva, C., Rivas-Muñoz, R., Mellado, M., 2009. Effect of parity and progesterone priming on induction of reproductive function in Saanen goats by buck exposure. Livest. Sci. 125, 261–265.

Van de Wiel, D.F.M., Koops, W., 1986. Development and validation of an enzyme immunoassay for progesterone in bovine milk or blood plasma. Anim. Reprod. Sci. 10, 201–213.

Vinoles, C., Forsberg, M., Canchero, G., Rubianes, E., 2001. Effect of long-term and short term progestogen treatment on follicular development and pregnancy rate in cyclic ewes. Theriogenology 55, 993–1004.

Yuswiati, E., 1991. Cited in Holtz, W., 1996. Embryo transfer in the goat: a review (in German). Dtsch. Tieraerztl. Wschr. 103, 293–297.

Zarkawi, M., Al-Merestani, M.R., Wardeh, M.F., 1999. Induction of synchronized oestrous in indigenous Damascus goats outside the breeding season. Small Rumin. Res. 33, 193–197.

Chapter 4

Temporal relationship between estrus, LH surge and ovulation in estrus induced Boer goat does

Abstract

The present study was conducted to determine the temporal relationships between behavioral estrus, preovulatory LH surge and ovulation in Boer goats following different estrus synchronization regimes. Twenty-eight Boer goat does were allocated to three treatment groups.Does of Group 1 (n=8) received two i.m. injections of 5 mg dinoprost (1 mL Dinolytic, Zoetis, Berlin, Germany) at 12 hour interval provided serum progesterone concentration exceeding 5 ng/mL. Does of Group 2 (n=10) were provided with intravaginal sponges impregnated with 20 mg of flurogestone acetate that were left in place for 11 days. Forty-eight h after sponge withdrawal two i.m. injections at 7 h interval of 5 mg dinoprost each were administered. Concurrent with the first injection an i.m. injection of 200 IU eCG (Intergonan, Intervet, Unterschleissheim, Germany) was administered. In Group 3 (n=10) does were treated similar to Group 2 with the only difference that 30 h after sponge withdrawal an i.m. injection of 0.004 mg of the GnRH-analog buserelin (1 mL Receptal, Intervet, Unterschleissheim, Germany) was administered. In all treatment groups all does showed an estrous response. In Group 1 the average interval from the first dinoprost injection to the onset of estrus was 40.2 h. Significantly shorter intervals of 23.4 and 26.4 hours after sponge withdrawal were recorded in Groups 2 and 3 ($P<0.05$). The duration of estrus in Groups 1, 2, and 3 was 37.5, 40.8 and 44.8 h ($P>0.05$), respectively. In Group 1 the LH peak ocurred44.7 h after the first dinoprost injection; in Groups 2 and 3, 34.5 and 32.0 h after sponge withdrawal. The magnitude of the LH peak was significantly lower in Group 1 than in Groups 2 and 3 (46.5 vs. 59.6 and 82.3 ng/ml; $P<0.05$). The interval between onset of estrus and LH peak for Groups 1, 2 and 3 was 7.2 h, 12.8 h and 5.6 h, respectively. The only significant difference was between Groups 2 and 3 ($P<0.05$). Ovulation occurred 53.5 h and 48. 6 h after the onset of estrus in Groups 1 and 2, respectively 47.9 h and 37.5 h after the LH peak ($P>0.05$). The results indicate that for the synchronization of estrus during the breeding season the dinoprost treatment was equally effective as the progestagen treatment. Additional

buserelin treatment will induce synchronicity of the preovulatory events facilitating fixed-time artificial insemination programs.

Keywords: Estrus synchronization, breeding season, ovulation, LH, Boer goats

4.1 Introduction

Controlling the estrous cycle by means of exogenous hormones serves the purpose of breeding animals at a predetermined time. In small ruminants this is most commonly accomplished by administering progesterone or a synthetic progestagen (Wildeus, 2000; Holtz, 2005), combined with the administration of equine chorionic gonadotropin (eCG) that augments the ovarian response following progestagen withdrawal (Cline et al., 2001; Maurel et al., 2003; Holtz, 2005). The progestagen-eCG treatment proved to be effective in cycling as well as non-cycling animals (Leboeuf et al., 2003; Martemucci and D´Alessandro, 2011) although, due to an immunological response to the heterologous gonadotropin (Roy et al., 1999), the repeated use of eCG may result in an impairment of fertility (Baril et al., 1992; Drion et al., 2001). An alternative to the progestagen-eCG treatment is the administration of prostaglandin $F_{2\alpha}$ or one of its synthetic analogs (Beck et al., 1993; Kusina et al., 2000; Khanum et al., 2006; Fonseca et al., 2012), often administered as two injections at 10 to 11 day interval, to achieve complete synchronization in groups of animals (Beck et al., 1993; Kusina et al., 2000). However, prostaglandin $F_{2\alpha}$ treatment will only be effective during the breeding season and if functional corpora lutea are present.

In estrus controlled females the time of ovulation may be terminated by administration a synthetic GnRH analog (Rubianes et al., 1997; Pierson et al., 2003). This measure facilitates the implementation of fixed-time artificial insemination and may improve fertilization rates when using semen compromised by manipulations such as cryopreservation or sex sorting. In an attempt to pinpoint ovulation time in goats, Chemineau (1983) and Baril and Vallet (1990) conducted repeated laparoscopies, a semi-invasive intervention requiring anesthesia and the availability of sophisticated equipment as well as trained personnel. More recent approaches to that end are ultrasonic monitoring of ovarian function (de Castro et al., 1999; Simões et al., 2006; Suyadi and Holtz, 2012; Zongo et al., 2015) and determination of the preovulatory LH surge (Greyling and Van Nierkerk, 1991; Holtz et al., 2008).

The present investigation is an attempt to determine temporal relationships between behavioral estrus, preovulatory LH surge and ovulation in Boer goat does subjected to various estrus and ovulation controlling regimes.

4.2 Materials and Methods

The experiment was conducted during the breeding season between late August and December in Goettingen, Germany (9° 41´E, 51° 46´N) on 28 pluriparous Boer goat does from our own breeding flock, on average, 3.7 (2 to 7) years of age and weighing 60 (46 to 79) kg. Does were group-housed in open barns with straw-covered floor and outdoor concrete runs and fed a daily ration of about 600 g concentrate, consisting of equal parts of a pelleted lactation diet for breeding ewes, oats and dried sugar beet pulp and had free access to wheat or barley straw, salt lick and water.

All animals were fitted with permanently indwelling jugular catheters consisting of polyethylene (PE) tubing, 0.86 mm ID and 1.52 mm OD (Portex Hythe Telex, Kent, England) as follows. The insertion site halfway between mandible and sternum was shaved and disinfected. A jugular vein was manually occluded and punctured ventrad with a Strauss cannula (2 mm OD; 43 mm in length). Polyethylene tubing, 15 cm in length, was threaded through the Strauss cannula and advanced about 10 cm. Once blood could be aspirated, the Strauss cannula was withdrawn and a short (1cm), blunted 20 gauge hypodermic needle was inserted into the free end of the polyethylene tubing. After clearing the catheter of blood by infusing a small amount of sterile physiologic saline the hub of the needle was stoppered with a closing cone (Braun, Melsungen, D-34209, Germany). A narrow strip of leucoplast was wrapped around the free end of the catheter close to the insertion site, forming a little “flag”. Two 10 cm strips of leucoplast, 5 cm wide, were taped above and below the insertion site; a third strip was taped across them covering the insertion site and fixing the protruding catheter in position, leaving a few cm leeway to enable drawing of blood samples. Finally, leucoplast was taped around the neck of the animal to secure and protect the catheter. To draw blood samples, after removal of the stopper a small amount was aspirated to

remove saline from the catheter before drawing 5 mL with another syringe, followed by infusion of sterile physiologic saline containing 100µg/mL of the anticoagulant heparin to clear the catheter of remaining blood before re-stoppering. On each occasion fingertips, free end of the catheter and stopper were thoroughly wiped with 70% ethanol. Blood samples were transferred to test tubes containing 4 drops of 38% sodium citrate to prevent clotting. After 8 h at 4°C they were centrifuged at 3000 X g for 15 min. Plasma samples were stored at -20°C and analyzed for progesterone content by ELISA as described by van de Wiel and Koops (1986), modified by Moeller (1991) and for LH content as described by Moeller (1991).

Catheterized does were randomly allocated to three treatment groups. In does of Group 1 (n=8) daily blood samples were drawn beginning on d 5 of the estrous cycle (d 1 being the first day of standing estrus). Once serum progesterone concentration exceeded 5 ng/mL, does were administered two i.m. injections of 5 mg dinoprost (1 ml Dinolytic, Zoetis, Berlin, Germany) at 12 h interval and sampling frequency was increased to once every 3 h until 30 h after the onset of behavioral estrus,. Estrus was detected by introducing an aproned adult buck at 8 h intervals. Ovarian activity was monitored by transrectal ultrasonography (ALOKA-SSD 500, equipped with a 7.5 MHz transrectal linear array transducer) conducted 24, 72 and 96 hours after the first dinoprost injection.

The does of Group 2 (n=10) were provided with intravaginal polyurethane sponges impregnated with 20 mg of the synthetic progestagen flurogestone acetate (FGA; Cronolone, Intervet, France). Sponges were left in plaace for 11 days and 48 hours before withdrawal two i.m. injections of 5 mg dinoprost at 7 hour interval were administered. Concurrent with the first dinoprost administration an i.m injection of 200 IU eCG (Intergonan, Intervet, Unterschleissheim, Germany) was administered. Estrus detection with an aproned adult buck was carried out at 8 hour intervals and ovarian activity was monitored 24, 48 and 72 h after sponge withdrawal. Blood was sampled daily from 2 d before to 1 d after sponge withdrawal, and at 3 h intervals during the

subsequent 48 h. Thereafter, until d 21 of the estrous cycle, sampling frequency was reduced to twice daily. Does of Group 3 (n=10) received the identical treatment with the only difference that 0.004 mg of the GnRH-analog buserelin (1 mL Receptal, Intervet, Unterschleissheim, Germany) was administered 30 h after sponge removal.

Normally distributed data were analyzed by one-way analysis of variance (ANOVA) with the respective treatments as main effects. Not normally distributed data were analyzed by the non-parametric Kruskal-Wallis test.

4.3 Results

In Table 1 the temporal relationships between the onset of treatment (dinoprost administration in Group 1 and sponge withdrawal in Groups 2 and 3), onset of estrus, emergence of preovulatory LH surge and ovulation is shown. All does, irrespective of treatment, showed an estrous response. The interval from the first dinoprost injection to the onset of estrus in Group 1 was significantly longer than that between sponge withdrawal and estrus in Groups 2 and 3 (40.2 vs. 23.4 and 26.4 h, $P<0.05$). The duration of estrus did not differ among groups (37.5, 40.8 and 44.8 h, $P>0.05$). The preovulatory LH surge emerged 40.3 h after dinoprost treatment in Group 1, as compared to 31.6 h and 31.7 h after sponge withdrawal, respectively, in Groups 2 and 3.

The corresponding intervals to the LH peak were 44.7, 34.5 and 32.0 h, being significantly longer in Group 1 than in Groups 2 and 3 ($P<0.05$). The LH peaks in Group 1 ranged from 24 to 57 h after treatment; in Group 2 from 27 to 45 h; in Group 3 they were tightly synchronized at 32.0 h after sponge withdrawal, respectively 2 h after buserelin administration (Figure 1). The amplitude of the LH peaks was significantly lower in Group 1 than in Groups 2 and 3 (46.5 ng/mL vs. 59.6 ng/mL and 82.3 ng/mL), the difference between Groups 1 and 3 being significant ($P<0.05$). The interval from onset of estrus to the peak of the LH surge was 7.2 h, 12.8 h and 5.6 h for Groups 1, 2

and 3, respectively; the difference between Groups 2 and 3 being significant ($P<0.05$). Ultrasonic monitoring of ovarian activity indicated that ovulation in Groups 1 and 2 occurred, on average, 53.5 hours and 48.6 h after the onset of estrus, respectively 47.9 and 37.5 h after emergence of the LH peak ($P>0.05$).

Table 1. Temporal relationship between end of treatment and onset of estrus to preovulatory LH surge and ovulation in Boer goats treated with dinoprost (group 1) or with the sponge-eCG regime without (group 2) or with buserelin (group 3)

Parameter	Group 1	Group 2	Group 3
Number of does treated	8	10	10
Number of does in estrus	8	10	10
End of treatment to estrus (h)*			
Mean	40.2[a]	23.4[b]	26.4[b]
SE	4.7	2.6	1.2
Range	20-57	17-33	24-32
Duration of estrus (h)			
Mean	37.5	40.8	44.8
SE	3.2	2.9	2.9
Range	24-48	24-56	24-56
End of treatment to emergence of preovulatory LH surge (h)*			
Mean	40.3	31.6	31.7
SE	5.8	1.7	0.3
Range	24-51	27-42	29-32
End of treatment to LH peak (h)*			
Mean	44.7[a]	34.5[b]	32.0[b]
SE	5.4	1.9	0.0
Range	24-57	27-45	32**

Continued Table 1

Peak LH concentration (ng\ml)			
Mean	46.5[a]	59.6[ab]	82.3[b]
SE	8.2	8.3	4.9
Range	19-70	32-96	50-94
Onset of estrus to LH peak (h)			
Mean	7.16[ab]	12.8[a]	5.6[b]
SE	1.5	2.3	1.2
Range	4-13	3-30	0-8
Onset of estrus to ovulation (h)			
Mean	53.5	48.6	-
SE	6.8	2.6	-
Range	27-76	39-55	-
LH peak to ovulation (h)			
Mean	47.9	37.5	-
SE	6.7	1.9	-
Range	23-72	27-42	-

[a b] Within rows data with different superscript differed ($P<0.05$)
*Interval considered from the first dinoprost injection (Group 1), respectively sponge withdrawal (Groups 2 and 3)
** All does presented the LH peak at 32 hours after sponge withdrawal

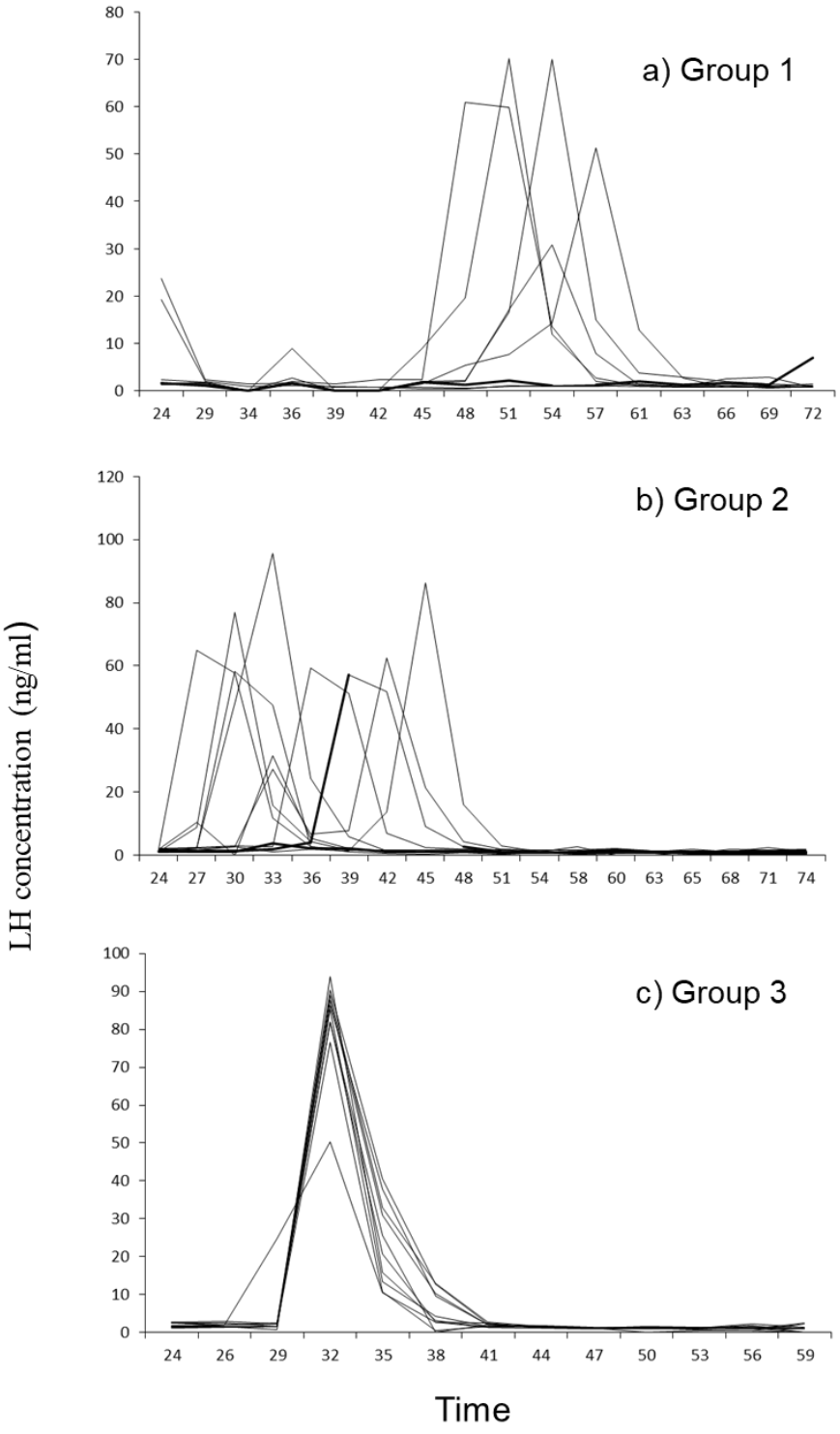

Figure 1. The preovulatory LH surges of individual goats treated with dinoprost (Group 1) and with the sponge-eCG regime without (Group 2) or with buserelin (Group 3). "Time" refers to hours after dinoprost treatment (Group 1) and sponge withdrawal (Groups 2 and 3).

4.4 Discussion

The temporal relationships between estrus, associated preovulatory events and ovulation were determined in does subjected to dinoprost treatment and a sponge-eCG regime with or without administration of the ovulation inducing agent buserelin. Estrous response was observed in all does regardless of treatment. Normally, to achieve complete synchronization of estrus in groups of females by way of prostaglandin treatment, due to the difference in ovarian status within the group two injections, administered 10 to 11 days apart, are required. The proportion of animals responding in the dinoprost group (Group 1) indicate that, once the corpus luteum has attained full functionality (high plasma progesterone level), a single injection of prostaglandin or one of its analogs will suffice to induce luteolysis followed by estrus. This confirms findings by Greyling and Van Niekerk (1986) and Fonseca et al. (2012). In the present study a second dinoprost injection was administered 12 hours after the first to ensure the occurrence luteolysis (Suyadi, Sohnrey ahd Holtz, 2000). The estrous response obtained with the sponge-eCG regime (Groups 2 and 3) confirms findings by Motlomelo et al. (2002) and Martemucci and D'Alessandro (2011). The advantage of the sponge treatment is that, contrary to the prostaglandin treatment, it can be applied throughout the year by being able to overcome the seasonality factor (Camacho et al., 2019). The interval from the end of treatment to the onset of estrus in the present study was longer in does treated with dinoprost alone than in does treated according to the sponge-eCG regime (40.2 vs 23.4 and 26.4 hours), consistent with studies by Romano (1998) and Amarinditis et al. (2004). These differences might be due to the exogenous progestagen itself in combination with eCG, which induce and augment the so-called "rebound effect", bringing about a faster estrous response.

As shown in Table 1 the emergence of the preovulatory LH surge following the end of the respective treatment presents a high degree of variability in does of Groups 1 (22-51 h) and 2 (27-42 h) in comparison to does belonging to Group 3 (29-32 h). Nevertheless, the means of the groups did not differ (40.3 h; 31.6 h; 31.7 h; $P>0.05$). The peak of the LH surge occurred significantly earlier in sponge/eCG treated does

than in dinoprost treated does (34.5 h and 31.7 vs. 44.7 h). The difference in emergence of the preovulatory surge and the LH peak appears to be associated with the earlier occurrence of estrus recorded after administration of exogenous progestagen, reducing the interval from the end of treatment to the preovulatory LH events. In does of Group 3 emergence and peak of the preovulatory LH surge following the administration of ovulation inducing buserelin were highly synchronized. The LH surge emerged as soon as 2 h after buserelin administration. Concurring results have been reported by Pierson et al. (2003). The magnitude of the LH peaks recorded in Groups 1 and 2 were numerically, though not statistically different (46.5 and 59.6 ng/mL), were in accord with the range of 40 to 60 ng/mL encountered by other researchers (Bono et al., 1983; Ritar et al., 1984; Martinez- Alvarez et al; 2007; Simões et al., 2008a; Simões et al., 2008b. The magnitude of the LH peak folowing ovulation induction with buserelin (82.3 ng/mL) resembles findings in the context of an ovsynch protocol by Holtz et al. (2008)**.** Apparently the dose of buserelin administered was sufficient to induce a normal LH surge. The prolonged interval from onset of estrus to LH peak in does of Group 2 (sponge/eCG), compared to that of does of Groups 1 (dinoprost) and 3 (sponge/eCG-buserelin), and the variability of the interval from estrus and LH peak to ovulation (Groups 1 and 2), are aspects that must be considered when conducting fixed-time artificial insemination.

4.5 Conclusion

From the results of the present study it can be concluded that, for synchronizing estrus during the breeding season, dinoprost treatment is equally effective as a progestagen/eCG regime. Incorporating a GnRH-analog with the sponge/eCG treatment will synchronize the emergence of the preovularory LH surge as well as ovulation. This creates favorable conditions for practicing fixed-time artificial insemination. Further studies need to be conducted to determine the temporal relationship between buserelin administration and the exact time of ovulation.

4.6 References

Amarantidis, I., Karagiannidis, A., Saratsis, P., Brikas, P., 2004. Efficiency of methods used for estrous synchronization in indigenous Greek goats. Small Ruminant Res. 52, 247–252.

Baril, G., and Saumande, J., 2000. Hormonal treatments to control time of ovulation and fertility of goats. 7th International Conference on Goats, 15-21 mai 2000, Tours, France Vol.1, p:400-405.

Baril, G., and Vallet, J.C., 1990. Time of ovulations in dairy goats induced to superovulate with porcine follicle stimulating hormone during and out of the breeding season. Theriogenology 34, 303–311.

Baril, G., Remy, B., Vallet, J. C., Beckers, J. F., 1992. Effect of repeated use of progestagen- PMSG treatment for estrus control in dairy goats out of breeding season. Reprod. Domest. Anim. 27, 161–168.

Beck, N.F.G., Davies, B., Williams, S.P., 1993. Oestrous synchronization in ewes: the effect of combining a prostaglandin analogue with a 5-day progestagen treatment. Anim. Sci. 56, 207–210.

Bono, G., Cairoli, F., Tamanini, C., Abrate, L., 1983. Progesterone, estrogen, LH, FSH and PRL concentrations in plasma during the estrous cycle in goat. Reprod. Nutr. Dev. 23, 217–222.

Camacho, M., Garza, D., Gauly, M., Holtz, W., in preparation. Superovulation of Boer goats does subjected to different estrus synchronization regimes. Small Rum. Res. 151, 26-31

Chemineau, P., 1983. Effect on oestrus and ovulation of exposing creole goats to the male at three times of the year. J. Reprod. Fertil. 67, 65–72.

Cline, M.A., Ralston, J.N., Seals, R.C., Lewis, G.S., 2001. Intervals from norgestomet withdrawal and injection of equine chorionic gonadotropin or P.G. 600 to estrus and ovulation in ewes. J. Anim. Sci. 79, 589–594.

de Castro, T., Rubianes, E., Menchaca, A., Rivero, A., 1999. Ovarian dynamics, serum estradiol and progesterone concentrations during the interovulatory interval in goats. Theriogenology 52, 399–411.

Drion, P.V., Furtoss, V., Baril, G., Manfredi, E., Bouvier, F., Pougnard, J.L., Bernelas, D., Caugnon, P., McNamara, E.M., Remy, B., Sulon, J., Beckers, J.F., Bodin, L., Leboeuf, B., 2001. Four years of induction/synchronization of estrus in dairy goats: effect on the evolution of eCG binding rate in relation with the parameters of reproduction. Reprod. Nutr. Dev. 41, 401–412.

Fonseca, J.F., Maffili, V.V., Santos, A.D.F., Fürst, R., Prosperi, C.P., Rovay, H., Souza, J.M.G., Torres, C. A. A., 2012. Effects of prostaglandin administration 10 days apart on reproductive parameters of cyclic dairy nulliparous goats. Arq. Bras. Med. Vet. Zootec. 64, 349–358.

Greyling, J.P.C., and Van Niekerk, C.H., 1986. Synchronization of oestrus in the Boer goat doe: Dose effect of prostaglandin in the double injection regime. S. Afr. J. Anim. Sci. 16, 146– 150.

Greyling, J.P.C., and Van Niekerk, C.H., 1991. Different synchronization techniques in Boer goat does outside the normal breeding season. Small Ruminant Res. 5, 233–243.

Holtz, W., 2005. Recent developments in assisted reproduction in goats. Small Ruminant Research, Special Issue: Plenary papers of the 8th International Conference on Goats 60, 95–110.

Holtz, W., Sohnrey, B., Gerland, M., Driancourt, M.A., 2008. Ovsynch synchronization and fixed-time insemination in goats. Theriogenology 69, 785–792.

Khanum, S.A., Hussain, M., Kausar, R., 2006. Manipulation of estrous cycle in dwarf goat (Capra hircus) using estrumate under different management conditions. Anim. Reprod. Sci. 92, 97–106.

Kusina, N.T., Tarwire., Hamudikuwanda, H., Agumba, G., Mukwena, J., 2000. A comparison of the effects of progesterone sponges and ear implants, PGF2alpha, and their combination on efficacy of estrus synchronization and fertility of Mashona goat does. Theriogenology 53, 1567–1580.

Leboeuf, B., Forgerit, Y., Bernelas, D., Pougnard, J.L., Senty, E., Driancourt, M.A., 2003. Efficacy of two types of vaginal sponges to control onset of oestrus, time of preovulatory LH peak and kidding rate in goats inseminated with variable numbers of spermatozoa. Theriogenology 60, 1371–1378.

Martemucci, G., and D'Alessandro, A.G., 2011. Synchronization of oestrus and ovulation by short time combined FGA, PGF(2α), GnRH, eCG treatments for natural service or AI fixed-time. Anim. Reprod. Sci. 123, 32–39.

Martínez-Álvarez, L.E., Hernández-Cerón, J., González-Padilla, E., Perera-Marín, G., Valencia, J., 2007. Serum LH peak and ovulation following synchronized estrus in goats. Small Ruminant Res. 69, 124–128.

Maurel, M.C., Roy, F., Hervé, V., Bertin, J., Vaiman, D., Cribiu, E., Manfredi, E., Bouvier, F., Lantier, I., Boue, P., Guillou, F., 2003. Réponse immunitaire à la eCG utilisée dans le traitement de l'induction d'ovulation chez la chèvre et la brebis. Gynécologie Obstétrique & Fertilité 31, 766–769.

Moeller, R., 1991. Enzymimmunoassays mit zweitem Antikoerper fuer Progesteron und porcines LH (Enzymeimmunoassays with second antibody for progesterone and porcine LH). Diss. Goettingen 1–125.

Motlomelo, K.C., Greyling, J.P.C., Schwalbach, L.M.J., 2002. Synchronisation of oestrus in goats: the use of different progestagen treatments. Small Ruminant Res. 45, 45–49.

Pierson, J.T., Baldassarre, H., Keefer, C.L., Downey, B.R., 2003. Influence of GnRH administration on timing of the LH surge and ovulation in dwarf goats. Theriogenology 60, 397–406.

Ritar, A.J., Maxwell, W.M., Salamon, S., 1984. Ovulation and LH secretion in the goat after intravaginal progestagen sponge-PMSG treatment. J. Reprod. Fertil. 72, 559–563.

Romano, J.E., 1998. The effect of continuous presence of bucks on hastening the onset of estrus in synchronized does during the breeding season. Small Ruminant Res. 30, 99–103.

Roy, F., Maurel, M.C., Combes, B., Vaiman, D., Cribiu, E.P., Lantier, I., Pobel, T., Delétang, F., Combarnous, Y., Guillou, F., 1999. The negative effect of repeated equine chorionic gonadotropin treatment on subsequent fertility in Alpine goats is due to a humoral immune response involving the major histocompatibility complex. Biol. Reprod. 60, 805–813.

Rubianes, E., Beard, A., Dierschke, D.J., Bartlewski, P., Adams, G.P., Rawlings, N.C., 1997. Endocrine and ultrasound evaluation of the response to PGF 2alpha and GnRH given at different stages of the luteal phase in cyclic ewes. Theriogenology 48, 1093–1104.

Simões, J., Almeida, J.C., Valentim, R., Baril, G., Azevedo, J., Fontes, P., Mascarenhas, R., 2006. Follicular dynamics in Serrana goats. Anim. Reprod. Sci. 95, 16–26.

Simões, J., Baril, G., Almeida, J.C., Azevedo, J., Fontes, P., Mascarenhas, R., 2008a. Time of ovulation in nulliparous and multiparous goats. Animal 2, 761–768.

Simões, J., Baril, G., Cunha, T., Azevedo, J., Mascarenhas, R., 2008b. Preovulatory LH peak and time of ovulation in nulliparous and primiparous goats after male effect. XII Annual ESDAR Conference. 20-23 November 2008, Utrecht, Netherlands.

Suyadi and Holtz, W., 2012. Ultrasound vs. Laparoscopic assessment of the number of ovulations in superovulated goats. Poster session presented at: XI International Conference on Goats, IGA; 2012 September 23-27: Gran Canaria, Spain.

Van de Wiel D.F.M., and Koops W., 1986. Development and validation of an enzyme immunoassay for progesterone in bovine milk or blood plasma. Anim. Reprod. Sci. 10, 201- 13.

Wildeus, S., 2000. Current concepts in synchronization of estrus: Sheep and goats. J. Anim. Sci.77, 1-14.

Zongo, M., Traor, B., Ababneh, M.M., Hanzen, C., Sawadogo, L., 2015. Ultrasonographic assessment of uterine involution and ovarian activity in West Africa Sahelian goats. J. Vet. Med. Anim. Health 7, 71-76.

Chapter 5

General discussion

5.1 General discussion

The dissertation includes three studies addressing the control of the estrous cycle in Boer goats.

In Study 1, two progestagen impregnated pessaries, Cronolone sponges and CIDRs, were equally effective in synchronizing estrus. A functional difference, however, was encountered between the two pessaries. Whereas the removal of CIDRs was easy, causing less distress to the animals, in the case of Cronolone sponges it was frequently associated with distress and foul-smelling vaginal discharge. Dinoprost administration at the beginning or at the end of the progestagen treatment was equally effective. It is to be expected, however, that treatment at the end would be more effective because at an early stage of development corpora lutea are less responsive to prostaglandin treatment as encountered in two does failing to show estrus in Chapter 2. Furthermore, our results indicate that, apart from a successful estrous response, superovulatory treatment can be induced at any time of the year, i.e. during and out of the breeding season. This suggests that the number of embryos and ova recovered from superovulated goats is independent of the season. The reason for the low recovery, however, is not clear. As mentioned in several studies (Battye et al., 1988; Saharrea et al., 1998), the incidence of early luteal regression in superovulated goats is a common phenomenon, however, inexplicably it was not an issue in the present study. The assessment of the ovulatory response by counting the number of corpora lutea in superovulated goats proved to be difficult, therefore assessment on the basis of the number of collapsing follicles between two successive ultrasound measurements proved to be more reliable.According to this study a 7 day-progestagen treatment proved to be an effective alternative to traditional long-term progestagen treatments. It does, however, imply administration of. prostaglandin $F_{2\alpha}$, preferably at the end of the protocol. Seasonality did affect neither the estrous response of the does nor the superovulatory response, leading to the conclusion that embryo collection out of season may beconsidered a viable option.

Study 2 consists of three experiments aimed at determining the degree of seasonality in Boer goats in the northern temperate zone, assessing ovarian response and conception rate after induced estrus in does weaned out of season and producing offspring from nulliparous does estrus-induced out of season. The results of Experiment 1 indicate that under the prevailing conditions Boer goat crosses display a seasonal pattern being based on daylight length; with increasing daylight sexual activity in does ceased, whereas reduction of daylight led to resumption of sexual activity. This confirms findings by Chemineau et al. (1992) and Fatet et al. (2011) showing that reproductive activity of goats in temperate regions is restricted to the autumn and winter months. However, variation within geographic location and also within breed, may be encountered (Chemineau et al., 1992; Restall, 1992). In Experiments 2 and 3 purebred Boer goats exhibited a seasonal pattern resembling that of the crossbreds in Experiment 1. In the region of origin, estrous activity in Boer goats peaks in autumn but, unlike the Boer crosses in the present study, complete acyclicity does not occur (Greyling, 2000). This demonstrates that goats from our own flock have adapted to the changing climatic conditions, although originating from the opposite hemisphere. In Experiment 2 it was found that intervals between weaning and synchronization treatment of zero, one or two weeks had no significant effect on estrous response, with virtually all does responding. However, slightly better pregnancy rates appear to be achievable when a resting period between weaning and insemination of one or two weeks is allowed. This indicates the feasibility of inducing estrus and pregnancy in weaned Boer goats out-of-season which might be relevant when attempting to increase productivity of a flock by having does kid more than once every twelve months. In Experiment 3 it was attempted to increase the productivity of the flock by subjecting nulliparous goats to estrus inducing treatment and mating during the non-breeding season. Results demonstrated that, although an estrous response was successfully induced in a large proportion of young females, pregnancy was not maintained. Further studies should be conducted to explain the failure to maintain pregnancy. From these three experiments it may be concluded that, under the prevailing conditions, Boer

goats, although less seasonal than dairy breeds, undergo a period of ovarian inactivity. From an economic point of view it could be desirable to negotiate this drawback. By employing the commonly applied 7 day-sponge-eCG treatment it was possible to overcome seasonality in does weaned in summer, with little effect of the post-weaning interval. In nulliparous does, even if these were old enough to be bred, estrus could be induced, but pregnancies were not maintained.

Study 3 was an attempt to determine temporal relationships between behavioral estrus, preovulatory LH surge and ovulation in Boer goat does subjected to various regimes for controlling estrus and ovulation. All does presented estrous symptoms regardless of treatment. Considering animals only treated with dinoprost it was found that, once corpora lutea have attained full functionality, a single injection will suffice to induce luteolysis followed by estrus. The reduced interval from the end of treatment to preovulatory events in Groups 2 and 3 appeared to be associated with an earlier estrous response. While a considerable variability was recorded regarding the occurrence of the preovulatory surge in Groups 1 and 2, in Group 3, on account of treatment with the GnRH analog buserelin, LH surges were highly synchronized. This is the reason why GnRH-analogs are implemented in fixed-timed artificial insemination programs. Based on the highest LH concentration recorded in does of Groups 1 and 2, it may be concluded that the dose of buserelin administered in the present study was sufficient to induce a suitable LH surge. The longer interval from the onset of estrus to the LH peak in does of Group 2 (sponge/eCG), compared to that of does of Groups 1 (dinoprost) and 3 (sponge/eCG-buserelin), and the variability of the interval from estrus and LH peak to ovulation (Groups 1 and 2), are aspects that must be considered when conducting fixed-timed artificial insemination. From the results of the present study it can be concluded that, for synchronizing estrus during the breeding season, dinoprost treatment in the presence of functional corpora lutea is equally effective as a progestagen regime. Incorporating a GnRH-analog with the sponge/eCG treatment will synchronize the emergence of the preovularory LH surge, followed by ovulation. This creates favorable conditions when intending to practice fixed-time artificial

insemination. Further studies should be conducted to determine the temporal relationship between buserelin administration and the exact time of ovulation.

5.1 References

Battye, K.M., Fairclough, R.J., Cameron, A.W., Trounson, A.O., 1988. Evidence for prostaglandin involvement in early luteal regression of the superovulated nanny goat (Capra hircus). J. Reprod. Fertil. 84, 425-430.

Chemineau, P., Daveau, A., Maurice, F., Delgadillo, J.A., 1992. Seasonality of estrus and ovulation is not modified by subjecting female Alpine goats to a tropical photoperiod. Small Ruminant Research 8, 299-312.

Fatet, A., Pellicer-Rubio, M.-T., Leboeuf, B., 2011. Reproductive cycle of goats. Anim. Reprod.Sci. 124, 211-219.

Greyling, J.P., 2000. Reproduction traits in the Boer goat doe. Small Rumin. Res. 36, 171-177. Restall, B.J., 1992. Seasonal variation in reproductive activity in Australian goats. Animal Reproduction Science 27, 305-318.

Saharrea, A., Valencia, J., Balcázar, A., Mejía, O., Cerbón, J.L., Caballero, V., Zarco, L., 1998. Premature luteal regression in goats superovulated with PMSG: effect of hCG or GnRH administration during the early luteal phase. Theriogenology 50, 1039-1052.

Curriculum Vitae

Personal details

Name: Miguel Angel Camacho Aranda

Date of birth: 13th March 1984

Pace of birth: Monterrey, Nuevo León, México

E-mail address: mvzmcamacho@hotmail.com

Education

2000-2002: High school certificate, Monterrey, Nuevo Leon, Mexico.

2002-2007: Bachelor in Veterinary Medicine and Zootechny, Faculty of Veterinary Medicine and Zootechnics, Autonomous University of Nuevo Leon, Mexico.

2007-2009: Master in Veterinary Science, Faculty of Veterinary Medicine and Zootechnics, Autonomous University of Nuevo Leon, Mexico.

2011-present: Postgraduate studies at the Department of Animal Science, Georg-August-University Goettingen, Goettingen, Germany.