Effect of hormonal synchronisation and/or short-term supplementation with maize on follicular dynamics and hormone profiles in goats during the non-breeding season

D.M. Nogueira [a, b*], J. Cavalieri [b], L.A. Fitzpatrick [b], B. Gummow [b, c], D. Blache [d], A.J. Parker [b]

[a] Embrapa Semiárido, BR 428, Km 152. Caixa Postal 23. CEP 56302-970, Petrolina, PE, Brazil.
[b] College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, QLD 4811, Australia.
[c] Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa.
[d] School of Animal Biology/Institute of Agriculture, University of Western Australia, Crawley, WA, Australia.
University of Western Australia, Perth, WA, Australia.

*Corresponding author. Email: daniel.nogueira@embrapa.br

Research highlights

- Nine days nutritional supplementation increased concentration of insulin, leptin and IGF-1

- Supplementation with maize in hormonally treated goats increased the ovulation rate by 43%

- Supplementation with maize could be reducing rate of atresia to influence ovulation rate

- Follicular development was not mediated by gonadotrophins secretion

Abstract. This study aimed to evaluate the reproductive response of anoestrous goats that were either hormonally treated and/or supplemented with maize for 9 days to determine which treatment combination was the most effective in enhancing follicular development and ovulation rate, and whether these responses were associated with increases in metabolic hormones. The experiment was carried out using 28 does, using a 2x2 factorial design with seven does in each group to test the effect of synchronisation of oestrus, supplementation with maize and their interactions. Synchronisation of oestrous cycles ($P < 0.001$) but not supplementation with maize or the interaction between the two ($P > 0.05$) increased the number of codominant follicles, the diameter of the largest follicle on Day 9 and growth rate of follicles during the period of supplementation. Compared with non-supplemented animals, supplementation with maize increased the total number of follicles observed between Days 7 and 9 ($P = 0.039$). In addition, nutritional supplementation with maize in combination with synchronisation of oestrus increased the ovulation rate by 43% ($P = 0.074$). Interactions between time and supplementation with maize showed that plasma concentrations of insulin, leptin and IGF-1 were greater in does supplemented with maize compared with non-supplemented does ($P < 0.001$). The findings show that hormonal synchronisation had the most influence on modifying follicular development and ovulation in anoestrous goats. Supplementation with maize increased the concentrations of

insulin, leptin and IGF-1, which could potentially modify the sensitivity of follicles to gonadotrophins and reduce rates of atresia.

Keywords: Anoestrus; breed; LH surge; progesterone; reproduction; seasonality.

1. Introduction

Photoperiod and the availability of nutrition limit times during the year when does ovulate and conceive. Management strategies that have been used to increase the duration of the breeding season or to induce ovulation during the non-breeding season and hence improve productivity in goats and sheep include synchronisation of oestrus using exogenous administration of progestins (Scaramuzzi and Martin, 1984), manipulating the duration of photoperiod that does are exposed to, administration of melatonin (Chemineau et al., 1992; Delgadillo et al., 2001), exposure to bucks (López-Sebastian et al., 2007; Delgadillo et al., 2011) and nutritional supplementation (Zarazaga et al., 2005; Duarte et al., 2008). These strategies are aimed at altering the hypothalamtic-pituitary-ovarian axis to increase the likelihood that ovulation will occur during the non-breeding season.

Data supporting an increased productivity in goats and sheep following nutritional supplementation in the non-breeding season are equivocal. In Payoya goats kept under a natural photoperiod (37º15' N), the duration of non-breeding season was 32 days shorter when does were fed 1.5 times maintenance compared with those that were fed a maintenance diet (Zarazaga et al., 2005). In contrast, reproductive seasonality in goats in a subtropical environment (26º23' N) persisted independently of food availability and it was concluded that photoperiod was the key factor regulating seasonality in subtropical latitudes (Duarte et al., 2008).

Supplementation with energy rich and/or protein rich diets exerts a significant influence on reproductive function in ruminants by affecting follicular development and ovulation rate (Scaramuzzi et al., 2006; Scaramuzzi and Martin, 2008). The action of nutrition on

3

folliculogenesis is thought to be mediated by different physiological pathways. The stimulatory effects of short-term supplementation on folliculogenesis are mediated by metabolites such as glucose and fatty acids and several metabolic hormones acting directly in the ovary (Meza-Herrera et al., 2008; Scaramuzzi et al., 2011). Changes in metabolites are thought to promote increased follicular steroidogenesis and an increase in ovulation rate, without changes in peripheral concentrations of FSH (Viñoles et al., 2005).

Exogenous administration of insulin has been shown to promote an increase in the number of small and large follicles in goats during the non-breeding season (Sarath et al., 2008). Leptin is a member of a cohort of factors, humoral and perhaps neural that influence the homeostasis of glucose in the body and GnRH-LH pulse secretion (Blache et al., 2000a; Zhang et al., 2004), thereby potentially influencing ovarian follicular development.

To the authors' knowledge, there are limited reports on the effect of a short-term supplementation with maize on follicular development, ovulation rate and metabolic hormones in anoestrous goats. Supplementation with maize is expected to increase the delivery of glucose to the small intestine (Landau et al., 1992) and increase circulating concentrations of glucose and IGF-1, which could in turn, modify the sensitivity of the ovary to gonadotrophins and increase ovulation rates. The aim of this study was to evaluate the reproductive response of seasonally anoestrous goats that were either hormonally treated and/or supplemented with maize to determine which treatment combination was the most effective in enhancing follicular development and ovulation rate and whether these responses were associated with increases in circulating concentrations of glucose, insulin, leptin and LH.

2. Material and Methods

2.1 Location, animals and evaluation period

The experiment was carried out at James Cook University, Townsville (19°19'30" S; 146°45'44" E), which is located in a tropical region of Queensland, Australia. The experiment

4

was conducted between October and November 2011, during the non-breeding season. A total of 28 nulliparous, anoestrous and non-pregnant female goats (20 rangeland and 8 Boer goats) were selected for this study. At the start of the study every goat was classified as being in anoestrus after i) no corpora lutea were observed in the ovaries during two examinations that were conducted 14 days apart using transrectal ultrasonography, and ii) oestrous behaviour was not observed in any goat following twice daily observations in the presence of two mature bucks over the same period. At the start of the experiment, the does were 1.5 ± 0.3 years old and had a live weight of 36.7 ± 0.7 kg (mean $\pm$ SEM). All experimental procedures for this study were approved by the Animal Ethics Committee of James Cook University (approval number: A1725).

2.2 Animal management and experimental design

Prior to commencement of the study, goats were adapted to housing for seven days (Days -7 to 0) by maintaining goats within single pens and supplementing them daily with a base ration consisting of lucerne pellets and lucerne hay, in order to provide nutritional requirements of 1.1 times maintenance (7.6 MJ ME/day) for a goat weighing 40 kg (NRC, 2007). Goats were then transferred to individual metabolic crates, allocated evenly to randomize the effect of breed in four groups of seven animals (**Table 1**) and supplemented over 9 days with the following diets: Control diet, goats were fed with a base ration of lucerne pellets and lucerne hay; Maize diet, fed with the same base ration plus supplementation with 220 g of cracked maize per day; Synch diet, fed the base ration plus treated with an intravaginal progesterone releasing device (CIDR, Eazi-Breed® CIDR®, Pfizer Australia, NSW) from Days 0 to 9 and eCG (250 IU IM; Folligon®, Intervet Australia, Victoria) administered on Day 7 (**Fig. 1**), and Synch x Maize diet, the same treatment as described for Synch group plus supplementation with 220 g of cracked maize per day.

From Days 0 to 9, maize and lucerne pellets were offered at 8 am. and lucerne hay was offered at 5 pm. Each day cracked maize was given about 15 minutes before the lucerne pellets

to help ensure that all of the maize was consumed. The feed intake of animals was monitored individually to confirm that the animals ate all the feed allocated each day. On Days 10 and 11, does were fed the same base ration of lucerne pellets and hay that was fed from Days -7 to 0.

On Days 10 and 11, does were tested twice daily (9 am. and 5 pm.) for behavioural signs of oestrus with two mature bucks for 30 min, starting from 16 h after removal of CIDR inserts and continued until 48 h after removal of inserts. Does which allowed any buck to mount were classified as being in oestrus. Intromission by the buck during mounting was prevented by manual withdrawal of the buck from the doe before mating occurred.

2.3 Blood samples and hormonal assays

Blood samples were collected from the jugular vein into 10 mL evacuated tubes containing heparin (BD Vacutainer®, Plymouth, UK) on Days 0, 3, 6 and 9. On Days 3, 6 and 9, samples were collected two hours after goats were fed the morning allocation of ration. On Day 0, samples were collected before insertion of CIDRs and, on Day 9, blood was collected nine hours after removal of inserts. On Day 7, indwelling intravenous catheters (14G x 5.25 in, BD Angiocath®, Oxford, UK) were inserted into the jugular vein of four goats from each treatment for intensive blood collection. Blood samples were collected every 15 minutes for five hours, starting at 0700 h and concluded before eCG was administered. In addition, blood samples were collected every four hours, from 12 to 56 hours after the end of supplementation on Day 9.

Blood samples were stored on ice then centrifuged (2500 g for 15 minutes) within two hours of collection. Plasma was then isolated and frozen (-20°C) until the time of assay. Plasma samples were analysed for concentrations of glucose, progesterone, LH, insulin, leptin and IGF-1. Plasma concentrations of glucose were measured using a commercial analyser (AU480 Beckman Coulter Australia Pty Ltd, Brisbane, Qld). For glucose assays, blood samples were collected with tubes containing sodium fluoride. The sensitivity of the assay was 0.04 mmol/L.

Plasma concentrations of progesterone were determined by radioimmunoassay (RIA) using anti-progesterone antibody-coated tubes (RIA Progesterone IM1188, Beckman Coulter Australia Pty Ltd, Yeerongpilly, Qld). The sensitivity of the assay was 0.05 ng/mL. The inter-assay coefficients of variation for low (0.80 ng/mL) and high (4.55 ng/mL) quality controls were 14.7% and 7.1%, respectively. The corresponding intra-assay coefficients of variation were 4.5% and 3.2%, respectively.

Plasma concentrations of insulin were measured by a double-antibody RIA that had been validated for ruminant blood samples (Miller et al., 1995). All samples were processed in a single assay and the limit of detection was 0.39 µU/mL. Six replicates of three control samples containing 2.74 µU/mL, 4.97 µU/mL and 10.75 µU/mL were included in the assay to estimate the intra-assay coefficients of variation of 7.4%, 1.7% and 3.8%, respectively.

Plasma concentrations of leptin were measured using a double-antibody RIA method previously described by Blache *et al.* (2000b). All samples were processed in a single assay and the limit of detection was 0.05 ng/mL. The assay included six replicates of three control samples containing 0.28 ng/mL, 0.62 ng/mL and 1.16 ng/mL, which were used to estimate the intra-assay coefficients of variation of 5.3%, 5.5% and 5.0%, respectively.

Plasma concentrations of IGF-1 were measured by double-antibody RIA method validated for ruminant samples (Breier et al., 1991). All samples were processed in a single assay and the limit of detection was 0.05 ng/mL. Four replicates of two control samples containing 0.21 ng/mL and 1.53 ng/mL were included in the assay to estimate the intra-assay coefficients of variation, which were 8.3% and 6.4%, respectively.

Plasma concentrations of LH were determined in samples collected during the intensive blood collection on Day 7 using a double-antibody RIA as described by Hötzel *et al.* (2003) which used ovine LH (NIDDK-oLH-I-4) for radio-iodination and AFP-8614B as a reference standard. All samples were processed in a single assay and the limit of detection was 0.18 ng/mL. The assay included six replicates of three control samples containing 0.5 ng/mL, 1.3

ng/mL and 2.0 ng/mL, which were used to estimate the intra-assay coefficients of variation of 7.2%, 5.8% and 5.0%, respectively.

Between Days 9 and 12, concentrations of LH were determined using a quantitative ELISA sandwich test (LH DETECT®, ReproPharm, Nouzilly, France). During this period, the LH surge was determined to occur when concentrations of LH first reached a concentration corresponding to three times the standard deviation above basal concentrations. The sensitivity of this assay was 0.1 ng/mL. The intra-assay coefficients of variation for low (0.5 ng/mL) and high (4 ng/mL) quality controls were 12.5% and 7.0%, respectively. The corresponding inter-assay coefficients of variation were 15.9% and 13.9%, respectively.

LH pulses in individual profiles on Day 7 were defined according to criteria described by Merriam and Wachter (1982). A pulse was defined when the concentration of LH exceeded the mean concentration (sum of LH/ number of blood samples) in a single point five times the standard deviation (SD), or in two consecutive points ≥ 3 times the SD or three consecutive points ≥ 2.5 times the SD.

2.4 Follicular dynamics

Transrectal ultrasound examinations of the ovaries were performed once daily from Days -5 to 11. Goats were monitored using a 6.6 MHz transrectal transducer (MyLab™ FiveVET, Medical Plus Australia Pty Ltd, Tullamarine, Victoria). Video recordings of each ultrasound examination were made. All follicles ≥ 2 mm in diameter and corpora lutea were measured using electronic callipers and ovarian maps were drawn. Follicles were classified according to their diameter into three categories: small (2 to 3 mm), medium (>3 and <5 mm) and large follicles (≥ 5 mm). Follicular growth rate (mm/day) was the time taken for a follicle to grow from the first time it was observed to its maximum diameter on Day 9. The day of emergence of follicles was identified as the day on which the dominant follicle within a given follicular wave was retrospectively first observed to be ≥ 3 mm in diameter (Nogueira et al., 2015). The day of maximum follicular diameter was the first day when a dominant follicles reached a maximum

diameter ≥ 5 mm. Follicles were classified as codominant when two or more follicles ≥ 5 mm were present within the same follicular wave. When codominant follicles were observed only data related to the largest follicle was used for the purpose of analyses related to the assessment of follicular waves. The day of ovulation was defined by the sudden loss of a follicle ≥ 5 mm in diameter followed by the development of a corpus luteum within same ovary. The total number of corpora lutea observed in the ovaries of each doe was recorded as the ovulation rate for each doe. During the present study, all ultrasound examinations were performed by the same operator.

2.5 Statistical analyses

Statistical analyses were conducted using the statistical software package IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp. Released 2013, Armonk, NY) using a 2x2 factorial design with seven replicates in each group. Analysis of variance (ANOVA) was used to compare the effects of treatments and breed (rangeland or Boer goats) for the variables: interval from device removal to onset of oestrus, maximum diameter of the largest follicle, growth rate of follicles, and number of small, medium and large follicles. Where mean differences between experimental groups were significant, Fisher's protected least significant difference (LSD) was used as a post-hoc multiple comparison test. Before ANOVA, data were tested for normal distribution using Q-Q plots and Levine's test was used to assess homogeneity of variance. Data which were not normally distributed (ovulation rate and the number of codominant follicles) were analysed using the Poisson regression (log-linear model). Repeated measures ANOVA was used to compare the effects of treatments (Maize, Synch or interaction between Maize x Synch), time and their interactions (Maize x Time, Synch x Time, Maize x Synch x Time) for the variables: the mean total number of small, medium and large follicles, plasma concentrations of progesterone, LH, insulin, leptin and IGF-1 between Days 0 and 9. If Mauchly's test indicated violation of the assumption of sphericity, probability values were obtained after degrees of freedom were adjusted using Greenhouse-Geisser statistic. The data expressed as percentages

were compared by Chi-square test. Results are presented as mean ± SEM and differences were considered significant when $P \leq 0.05$.

3. Results

For all analyses, effects of breed and the interaction of breed with treatment were not significant, so effects due to breed were removed from subsequent analyses and not reported. Behavioural signs of oestrus were detected in every doe that was subjected to the synchronisation treatments, but were not detected in any of the does in the non-synchronised treatments ($P < 0.001$; **Table 2**). Supplementation with maize also did not significantly affect the interval from removal of inserts to onset of oestrus in goats with synchronised oestrous cycles. The interval to onset of oestrus was 22.9 ± 3.2 h for both synchronised treatments.

There was a significant effect of synchronisation of oestrus on the ovulation rate, number of codominant follicles on Day 9, diameter of the largest follicles on Day 9 and growth rate of follicles, but there were no effect of the addition of maize in the diet or the interaction between synchronisation treatments and supplementation with maize on these variables (**Table 2**). Ovulation was observed in all of the does treated with a synchronisation treatment, but only one of the does subjected to a non-synchronised treatment ovulated. While the ovulation rate was increased by 43% in the Synch x Maize group compared with the Synch group, difference in the mean ovulation rate between these groups was not significant ($P = 0.074$; **Table 2**). The number of codominant follicles on Day 9, diameter of the largest follicles and growth rate between the follicular emergence and Day 9 were greater ($P < 0.001$) in the does with synchronised oestrous cycles compared with the non-synchronised groups (**Table 2**). However, for the same variables, there was no effect of supplementation with maize ($P > 0.05$) or interaction between synchronisation and supplementation with maize ($P > 0.05$).

For the each variable evaluated, no significant interactions were detected between Maize x Synch (P > 0.221) and Time x Maize x Synch (P > 0.069), so the results for each treatment over time were graphed and reported separately (**Figs. 2 and 3**).

The number of small and medium follicles from Days 7 to 9 of ultrasound evaluations were not significantly affected by the synchronisation of oestrus (P > 0. 237) or by supplementation with maize (P > 0.219). Significant interactions between time and synchronisation of oestrus (P = 0.008) were observed for the number of small follicles (**Fig. 2a**). Before Day 9, the number of small follicles were similar between treatments, but on Day 9, the number of small follicles was greater in the non-synchronised does than synchronised does.

The number of large follicles was significantly affected by the synchronisation of oestrus (P = 0.003) and by the interaction between time and synchronisation (P = 0.006), but were not affected by supplementation with maize (P = 0.219). On Days 8 and 9, the number of large follicles were greater (P < 0.001) in the synchronised groups compared with the non-synchronised groups (**Fig. 2c**). In addition, on Day 9 the number of large follicles tended to be greater (P = 0.078) in the does supplemented with maize than in the does not supplemented with maize (**Fig. 2c**).

The total number of follicles was affected by supplementation with maize (P = 0.039) and by interaction between time and synchronisation (P = 0.021), but were not affected by the synchronisation treatment (P = 0.782). The total number of follicles were greater in does supplemented with maize compared with those that were not supplemented with maize (**Fig. 2d**). Moreover, on Day 9 the total number of follicles was greater in the non-synchronised does than the synchronised does.

Overall, concentrations of glucose, insulin, leptin, IGF-1 group and LH were not affected by the synchronisation of oestrus (P > 0.142; data not shown). On Day 7, during intensive blood collection, concentrations of glucose were not affected by supplementation with maize (P = 0.423) and there were no interactions with time (P > 0.377), but there was an effect over time (P

< 0.001). Mean concentrations of glucose increased from 0700 h until the fifth hour after feeding in all experimental groups.

Plasma concentrations of progesterone were affected by the synchronisation treatment (P < 0.001), by time (P < 0.001) and an interaction between time and the synchronisation treatment was detected (P < 0.001). Concentrations of progesterone were not significantly affected by supplementation with maize (P = 0.247), and a significant interaction between time with maize (P = 0.373) was not detected. On Days 3 and 6, concentrations of progesterone were greater (P < 0.05) in the does with synchronised oestrous cycles compared with does in the non-synchronised groups (**Fig. 3a**).

Plasma concentrations of insulin were affected by supplementation with maize (P = 0.038), time (P = 0.001) and an interaction between supplementation and time with maize was detected (P = 0.042). Mean concentrations of insulin increased from Days 0 to 9 of treatment. On Days 6 and 9, concentrations of insulin were greater (P < 0.05) in does supplemented with maize compared with does that were not supplemented with maize (**Fig. 3b**). Concentrations of insulin were not affected by any interactions between the synchronisation treatment and time (P > 0.290).

Plasma concentrations of leptin tended to be affected by the supplementation with maize (P = 0.075). Concentrations of leptin were affected by time (P = 0.001) and an interaction between time and supplementation with maize was found (P < 0.001), but no other significant interactions were detected. Mean concentrations of leptin increased from Days 0 to 3 and then remained stable from Days 3 to 9 of the study. On Days 3, 6 and 9, concentrations of leptin were greater (P <0.05) in does supplemented with maize, compared with does that were not supplemented with maize (**Fig. 3c**).

Plasma concentrations of IGF-1 tended to be affected by supplementation with maize (P = 0.077), but concentrations of IGF-1 did not change significantly over time (P = 0.124). However, concentrations of IGF-1 were affected by the interactions between time and

supplementation with maize (P < 0.001) and time and synchronisation of oestrus (P = 0.002). On Day 6 and 9, concentrations of IGF-1 were greater (P < 0.05) in does with synchronised oestrous cycles compared with does in the non-synchronised groups. In addition, on Days 6 and 9, animals supplemented with maize had greater (P < 0.05) mean concentrations of IGF-1 than animals not supplemented with maize (**Fig. 3d**).

On Day 7, plasma concentrations of LH were not affected by supplementation with maize (P = 0.225), by time (P = 0.255) or any interactions with time (P = 0.166). The frequency of LH pulses was not affected by the synchronisation of oestrus (P = 0.295) and by supplementation with maize (P = 0.295).

The percentage of animals in which an LH surge was detected and the mean maximum concentrations of LH between 12 and 56 h after removal of inserts were affected by the synchronisation of oestrus (P < 0.001), but there was no effect of the addition of maize (P = 0.378) (**Table 3**). Compared with non-synchronised does, the percentage of animals in which an LH surge was detected and the mean maximum concentration of LH were greater in the synchronised groups. However, the mean interval between removal of inserts and the LH surge was not affected by supplementing synchronised goats with maize (P = 0.320; **Table 3**).

4. Discussion

This study illustrated that hormonal synchronisation was a highly effective method of inducing oestrus and ovulation in anoestrous goats during the non-breeding season. Nutritional supplementation with maize for nine days without administration of progesterone and eCG promoted changes in systemic concentrations of glucose, insulin and leptin over time, but did not induce oestrous behaviour or a greater ovulation rate compared with does that were treated with exogenous hormones. In does that were treated with progesterone and eCG, and supplementation with 220 g of maize during the 9-day treatment period there was a tendency for ovulation rate to increase compared with does that had only their oestrous cycles synchronised, with ovulation

13

rate increasing by 43% (**Table 2**). This demonstrated that supplementation with a relatively small quantity of maize has the potential to increase ovulation rate in anoestrous does when treated with exogenous hormones, but was ineffective in inducing ovulation in non-synchronised does.

In this study, the failure of supplementation with maize to significantly increase ovulation rate in does with synchronised oestrous cycles can be explained by the use of only seven goats in each experimental group and possibly the small difference in the amount of energy between our experimental groups (1.0 times maintenance vs 1.5 times maintenance). De Santiago-Miramontes *et al.* (2008) used two groups of 25 goats in postpartum anoestrous and reported that the number of females showing oestrous behaviour and the ovulation rate detected within 5 days of exposure of bucks was significantly greater in does supplemented with 950 g of lucerne hay, 290 g of maize and 140 g of soy bean compared with non-supplemented females. These authors fed diets to goats with an average of 13.9 MJ ME/day, which is equivalent to nutritional requirements of 2.0 times maintenance for a goat weighing 40 kg. Shiba goats supplemented with 2.5 times maintenance requirements had greater concentrations of glucose and insulin than compared with goats fed at maintenance (Haruna et al., 2009). In addition, greater concentrations of glucose, insulin and leptin were recorded in ewes supplemented with twice their maintenance requirements compared with ewes fed at maintenance (Viñoles et al., 2005; Viñoles et al., 2010). These studies also used a larger number of animals per group when compared with the present study.

The effects of short-term nutrient supplementation on follicular development and ovulation rates have been equivocal. This inconsistent effect could suggest that any influence of nutritional supplementation on ovulation rates may depend on whether short-term nutritional supplementation coincides with new wave emergence. To be more effective, if nutrient supplementation is to influence ovulation rate it needs to begin at the time of the emergence of the ovulatory wave (Viñoles et al., 2005; Viñoles et al., 2010). In our study, the nutritional

supplementation started between 4-5 days before the emergence of the largest follicles on Day 9, but the day of follicular emergence was not spread widely between experimental groups (**Table 2**). In addition, nutritional supplementation started from 4 to 8 days before ovulation in the ovulatory groups, as suggested by Viñoles *et al*. (2010). Therefore, the argument that nutritional supplementation started at a less than optimal time is not supported.

While significant effects of synchronisation of oestrus and interactions between treatment and time were detected for different classes of follicles between Days 7 and 9, there was no clear independent effects of supplementation with maize on enhancing the numbers of small, medium and large follicles. Greater follicular growth rates and diameters of the largest follicles on Day 9 were detected in does with synchronised oestrus cycles, but these variables were not significantly affected by supplementation with maize alone. This suggests that the synchronisation treatment was the main factor affecting these follicular characteristics by Day 9. On Day 9, the number of small follicles were greater in the non-synchronised groups compared with the synchronised groups of does (**Fig. 2a**). In the synchronised does, more of the smaller follicles were likely to have progressed into larger follicles which could have contributed to fewer smaller follicles being visualised in these does. However, the tendency for more larger follicles to be present in the ovary of does supplemented with maize on Day 9 (**Fig. 2c**) and a significantly greater total number of follicles throughout the study in does supplemented with maize (**Fig. 2d**) could suggest that supplementation with maize was exerting some influence on follicular development independent of the synchronisation treatment. In sheep, nutritional supplementation above maintenance requirements reduces atresia among large gonadotrophin-dependent follicles, increasing the number of ovulatory follicles (Viñoles et al., 2002; Viñoles et al., 2010).

Numerical differences in ovulation rates (+43%) and the number of codominant follicles detected on Day 9 (+43%) within the group of does treated with exogenous hormones plus supplementation with maize suggest that some growing follicles were prevented from atresia using this combination of treatments. During the latter stages of follicular development, the

reduction in circulating concentrations of FSH is thought to induce atresia of smaller follicles, while larger, dominant follicles avoid atresia by shifting their dependence from FSH to LH (Meza-Herrera et al., 2008; Scaramuzzi et al., 2011). According to the same authors, the number of large ovulatory follicles can be increased by decreasing the sensitivity of the hypothalamic-pituitary-ovarian axis to the negative effect of oestradiol to maintain the concentration of FSH above the threshold level for longer, thus allowing more time for follicles to pass through the so called, "widened gate". Perhaps supplementation with maize during the time of new wave emergence in does with synchronised oestrous cycles further reduces the sensitivity of the hypothalamus to oestradiol negative feedback and further stimulates gonadotrophin-dependent follicles to become more sensitive to FSH and hence develop precociously (Scaramuzzi et al., 2011) compared with does that are synchronised without supplementation.

Between Days 6 and 9 of this study, mean concentrations of insulin, leptin and IGF-1 were significantly greater in does supplemented with maize compared with the does that were not supplemented with maize (**Fig. 3**). These results support previous studies showing that nutritional supplementation increases plasma concentrations of glucose and insulin (Haruna et al., 2009; Zabuli et al., 2010), leptin (Viñoles et al., 2005; Maidin et al., 2014) and IGF-1 (Lucy, 2000; Webb et al., 2004). Supplementation with maize appeared to have selective effects on follicular development from Day 6 to Day 9 of this study, which coincides with the period of time when mean concentrations of insulin, leptin and IGF-1 were greater in the supplemented animals.

In this study, maize was selected due to its potential ability to modify concentrations of glucose and metabolic hormones, which could then influence follicular development. Maize has been reported to be partially degradable in the rumen of sheep (Nocek and Tamminga, 1991; Landau et al., 1995) and this allows undegradable starch to flow into the small intestine, increasing the entry rate of glucose and other energy-yielding substrates into the bloodstream for a greater period of time (Landau et al., 1997; Banchero et al., 2007). Consequently, the uptake of

glucose by the ovaries may be promoted, stimulating folliculogenesis via a direct effect of insulin or insulin-mediated glucose uptake by granulosa and theca cells (Scaramuzzi et al., 2010).

As expected, concentrations of progesterone were greater in does that were treated with exogenous progesterone, but concentrations of progesterone were not affected by supplementation with maize. Similarly, when Australian Cashmere goats were treated with exogenous progesterone and fed twice their maintenance requirement with 510 g of lupin grain per day, concentrations of progesterone were similar to goats fed at maintenance with only a base ration of 495 g of oaten chaff (Maidin et al., 2014). Parr *et al*. (1993), however, demonstrated that acute nutritional supplementation in sheep increases the metabolic clearance rate by the liver and a decrease in plasma concentrations of progesterone. The reason for differences in the effects of nutritional supplementation on circulating concentrations of progesterone between studies undertaken in sheep and goats is unclear, although it could be related to differences between species or perhaps differences in the level or type of supplementation provided in different studies.

The effects of nutritional supplementation on gonadotrophin secretion are contradictory. Some studies report that follicular development and ovulation rate are mediated by an increase in gonadotrophin secretion (Haruna et al., 2009; Seekallu et al., 2009). On the other hand, other studies suggest that the effect of nutrition on follicular development and ovulation rate does not involve enhanced secretion of GnRH, LH or FSH (Viñoles et al., 2005; Meza-Herrera et al., 2008). Thus, differences in the types and amount of supplements, breed, season or duration of blood sampling between these studies could explain the difference on gonadotrophin secretion. In this study, any potential dietary modulation of LH secretion may have been masked by the suppressive effect of exogenous treatment with progesterone in gonadotrophin secretion.

The duration of the intensive blood collection (5 hours) might have been too short to detect changes in the frequency of release of LH. Other studies reported significant differences in

LH pulse frequency when sheep blood samples were collected for at least 24 hours (Blache et al., 2003; Hötzel et al., 2003; Zhang et al., 2004), while other studies reported significant differences when blood samples were collected over only 6 hours (Haruna et al., 2009; Seekallu et al., 2009; Seekallu et al., 2010). The greater number of goats with a detectable LH surge and the greater mean maximum concentration of LH between 12 h and 56 h after removal of inserts in the synchronised groups compared with the non-synchronised does (**Table 3**) can be explained by the occurrence of ovulation in all does treated with a synchronisation treatment, but in only one doe supplemented with maize that was not treated with exogenous hormones.

5. Conclusion

Hormonal synchronisation had the most influence on modifying follicular development, inducing oestrus behaviour and ovulation in anoestrous goats. A short-term nutritional supplementation with maize increased the concentrations of insulin, leptin and IGF-1 and appeared to have some influence on follicular development, but these changes were not mediated by an increase of the mean concentrations of LH and frequency of LH pulses. Treatment with progesterone and eCG while supplementing with maize tended to increase ovulation rate compared with treatment with progesterone and eCG alone, with the combined treatment increasing ovulation rate by 43%. Nutritionally induced changes in metabolic hormones may have improved responsiveness of growing follicles to gonadotrophins and reduced the rate of atresia, thereby influencing ovulation rate. Supplementation with a relatively small quantity of maize has the potential to increase ovulation rate in anoestrous does when treated with exogenous hormones, although further investigation with larger groups of seasonally anoestrus goats is needed to verify this conclusion.

Acknowledgments

References

Banchero, G.E., Quintans, G., Vazquez, A., Gigena, F., La Manna, A., Lindsay, D.R., Milton, J.T.B., 2007. Effect of supplementation of ewes with barley or maize during the last week of pregnancy on colostrum production. The Animal Consortium 1, 625-630.

Blache, D., Chagas, L.M., Blackberry, M.A., Vercoe, P.E., Martin, G.B., 2000a. Metabolic factors affecting the reproductive axis in male sheep. Journal of Reproduction and Fertility 120, 1-11.

Blache, D., Tellam, R.L., Chagas, L.M., Blackberry, M.A., Vercoe, P.E., Martin, G.B., 2000b. Level of nutrition affects leptin concentrations in plasma and cerebrospinal fluid in sheep. Journal of Endocrinology 165, 625-637.

Blache, D., Zhang, S., Martin, G.B., 2003. Fertility in male sheep: modulators of the acute effects of nutrition on the reproductive axis of male sheep. Reproduction-Cambridge-Supplement, 387-402.

Breier, B.H., Gallaher, B.W., Gluckman, P.D., 1991. Radioimmunoassay for insulin-like growth factor-I: solutions to some potential problems and pitfalls. Journal of Endocrinology 128, 347-357.

Chemineau, P., Malpaux, B., Delgadillo, J.A., Guerin, Y., Ravault, J.P., Thimonier, J., Pelletier, J., 1992. Control of sheep and goat reproduction: use of light and melatonin. Anim Reprod Sci 30, 157-184.

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

Delgadillo, J.A., Carrillo, E., Morán, J., Duarte, G., Chemineau, P., Malpaux, B., 2001. Induction of sexual activity of male creole goats in subtropical northern Mexico using long days and melatonin. J Anim Sci 79, 2245-2252.

Delgadillo, J.A., Ungerfeld, R., Flores, J.A., Hernandez, H., Fitz-Rodríguez, G., 2011. The ovulatory response of anoestrous goats exposed to the male effect in the subtropics is unrelated to their follicular diameter at male exposure. Reproduction in Domestic Animals 46, 687-691.

Duarte, G., Flores, J.A., Malpaux, B., Delgadillo, J.A., 2008. Reproductive seasonality in female goats adapted to a subtropical environment persists independently of food availability. Domestic Animal Endocrinology 35, 362-370.

Haruna, S., Kuroiwa, T., Lu, W., Zabuli, J., Tanaka, T., Kamomae, H., 2009. The effects of short-term nutritional stimulus before and after the luteolysis on metabolic status, reproductive hormones and ovarian activity in goats. Journal of Reproduction and Development 55, 39-44.

Hötzel, M.J., Walkden-Brown, S.W., Fisher, J.S., Martin, G.B., 2003. Determinants of the annual pattern of reproduction in mature male Merino and Suffolk sheep: responses to a nutritional stimulus in the breeding and non-breeding seasons. Reproduction, Fertility and Development 15, 1-9.

Landau, S., Bor, A., Leibovich, H., Zoref, Z., Nitsan, Z., Madar, Z., 1995. The effect of ruminal starch degradability in the diet of Booroola crossbred ewes on induced ovulation rate and prolificacy. Anim Reprod Sci 38, 97-108.

Landau, S., Nitsan, Z., Zoref, Z., Madar, Z., 1992. The influence of processing corn grain on glucose metabolism in ewes. Reprod Nutr Dev 32, 231-240.

Landau, S., Zoref, Z., Nitsan, Z., Madar, Z., 1997. The influence of extruding corn grain in diets fed to Finn × Awassi crossbred ewes during late pregnancy on birth weight of lambs. Canadian Journal of Animal Science 77, 141-147.

López-Sebastian, A., González-Bulnes, A., Carrizosa, J.A., Urrutia, B., Díaz-Delfa, C., Santiago-Moreno, J., Gómez-Brunet, A., 2007. New estrus synchronization and artificial insemination protocol for goats based on male exposure, progesterone and cloprostenol during the non-breeding season. Theriogenology 68, 1081-1087.

Lucy, M.C., 2000. Regulation of Ovarian Follicular Growth by Somatotropin and Insulin-Like Growth Factors in Cattle. Journal of Dairy Science 83, 1635-1647.

Maidin, M.S., Blackberry, M.A., Milton, J.T.B., Hawken, P.A.R., Martin, G.B., 2014. Nutritional Supplements, Leptin, Insulin and Progesterone in Female Australian Cashmere Goats. APCBEE Procedia 8, 299-304.

Merriam, G.R., Wachter, K.W., 1982. Algorithms for the study of episodic hormone secretion. American Journal of Physiology - Endocrinology and Metabolism 243, E310-E318.

Meza-Herrera, C.A., Hallford, D.M., Ortiz, J.A., Cuevas, R.A., Sanchez, J.M., Salinas, H., Mellado, M., Gonzalez-Bulnes, A., 2008. Body condition and protein supplementation positively affect periovulatory ovarian activity by non LH-mediated pathways in goats. Anim Reprod Sci 106, 412-420.

Miller, D.W., Blache, D., Martin, G.B., 1995. The role of intracerebral insulin in the effect of nutrition on gonadotrophin secretion in mature male sheep. Journal of Endocrinology 147, 321-329.

Nocek, J.E., Tamminga, S., 1991. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. Journal of Dairy Science 74, 3598-3629.

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 non-breeding seasons in the tropics of Queensland, Australia. Small Ruminant Research 125, 93-100.

NRC, 2007. Nutrient Requirements of Small Ruminants: sheep, goats, cervids, and new world camelids., National Academy Press, Washington, DC, p. 362.

Parr, R.A., Davis, I.F., Miles, M.A., Squires, T.J., 1993. Feed intake affects metabolic clearance rate of progesterone in sheep. Research in Veterinary Science 55, 306-310.

Sarath, T., Mehrotra, S., Agarwal, S.K., Varshney, V.P., Hoque, M., Shankar, U., Singh, S.K., 2008. Effect of insulin administration on ovarian function and estrus induction in acyclic goats. Anim Reprod Sci 108, 216-225.

Scaramuzzi, R.J., Baird, D.T., Campbell, B.K., Driancourt, M.A., Dupont, J., Fortune, J.E., Gilchrist, R.B., Martin, G.B., McNatty, K.P., McNeilly, A.S., Monget, P., Monniaux, D., Viñoles, C., Webb, R., 2011. Regulation of folliculogenesis and the determination of ovulation rate in ruminants. Reproduction, Fertility and Development 23, 444-467.

Scaramuzzi, R.J., Brown, H.M., Dupont, J., 2010. Nutritional and metabolic mechanisms in the ovary and their role in mediating the effects of diet on folliculogenesis: A perspective. Reproduction in Domestic Animals 45, 32-41.

Scaramuzzi, R.J., Campbell, B.K., Downing, J.A., Kendall, N.R., Khalid, M., Muñoz-Gutiérrez, M., Somchit, A., 2006. A review of the effects of supplementary nutrition in the ewe on the concentrations of reproductive and metabolic hormones and the mechanisms that regulate folliculogenesis and ovulation rate. Reproduction Nutrition and Development 46, 339-354.

Scaramuzzi, R.J., Martin, G.B., 1984. Pharmacological agents for manipulating oestrus and ovulation in the ewe. Reproduction in sheep: Australian Wool Corporation technical publication/supervising editors, DR Lindsay and DT Pearce.

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. Reproduction in Domestic Animals 43, 129-136.

Seekallu, S.V., Barrett, D.M.W., Toosi, B.M., Clarke, K., Ewen, K.A., Duggavathi, R., Davies, K.L., Pattullo, K.M., Bagu, E.T., Rawlings, N.C., 2010. Pulsatile LH secretion and ovarian follicular wave emergence and growth in anestrous ewes. Theriogenology 74, 912-921.

Seekallu, S.V., Toosi, B.M., Rawlings, N.C., 2009. LH pulse frequency and the emergence and growth of ovarian antral follicular waves in the ewe during the luteal phase of the estrous cycle. Reproductive Biology and Endocrinology 7, 78.

Viñoles, C., Forsberg, M., Banchero, G., Rubianes, E., 2002. Ovarian follicular dynamics and endocrine profiles in Polwarth ewes with high and low body condition. Animal Science 74, 539-545.

Viñoles, C., Forsberg, M., Martin, G.B., Cajarville, C., Repetto, J., Meikle, A., 2005. Short-term nutritional supplementation of ewes in low body condition affects follicle development due to an increase in glucose and metabolic hormones. Reproduction 129, 299-309.

Viñoles, C., Paganoni, B., Glover, K.M.M., Milton, J.T.B., Blache, D., Blackberry, M.A., Martin, G.B., 2010. The use of a 'first-wave' model to study the effect of nutrition on ovarian follicular dynamics and ovulation rate in the sheep. Reproduction 140, 865-874.

Webb, R., Garnsworthy, P.C., Gong, J.G., Armstrong, D.G., 2004. Control of follicular growth: local interactions and nutritional influences. Journal of animal science. 82 E-Suppl, E63-74.

Zabuli, J., Tanaka, T., Lu, W., Kamomae, H., 2010. Intermittent nutritional stimulus by short-term treatment of high-energy diet promotes ovarian performance together with increases in blood levels of glucose and insulin in cycling goats. Anim Reprod Sci 122, 288-293.

Zarazaga, L.A., Guzmán, J.L., Domínguez, C., Pérez, M.C., Prieto, R., 2005. Effect of plane of nutrition on seasonality of reproduction in Spanish Payoya goats. Anim Reprod Sci 87, 253-267.

Zhang, S., Blache, D., Blackberry, M.A., Martin, G.B., 2004. Dynamics of the responses in secretion of luteinising hormone, leptin and insulin following an acute increase in nutrition in mature male sheep. Reproduction, Fertility and Development 16, 823-829.
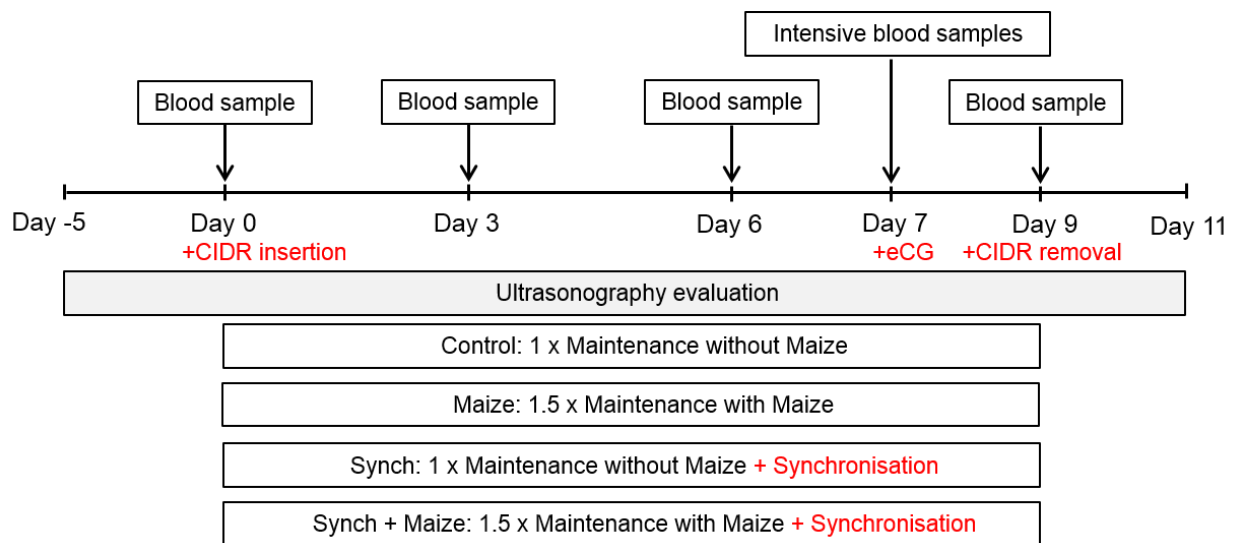
Fig. 1. Schematic representation of the experimental treatments, blood sampling, ultrasonography and synchronisation of oestrus. The synchronised groups received a progesterone releasing device (CIDR) and equine chorionic gonadotropin (eCG).
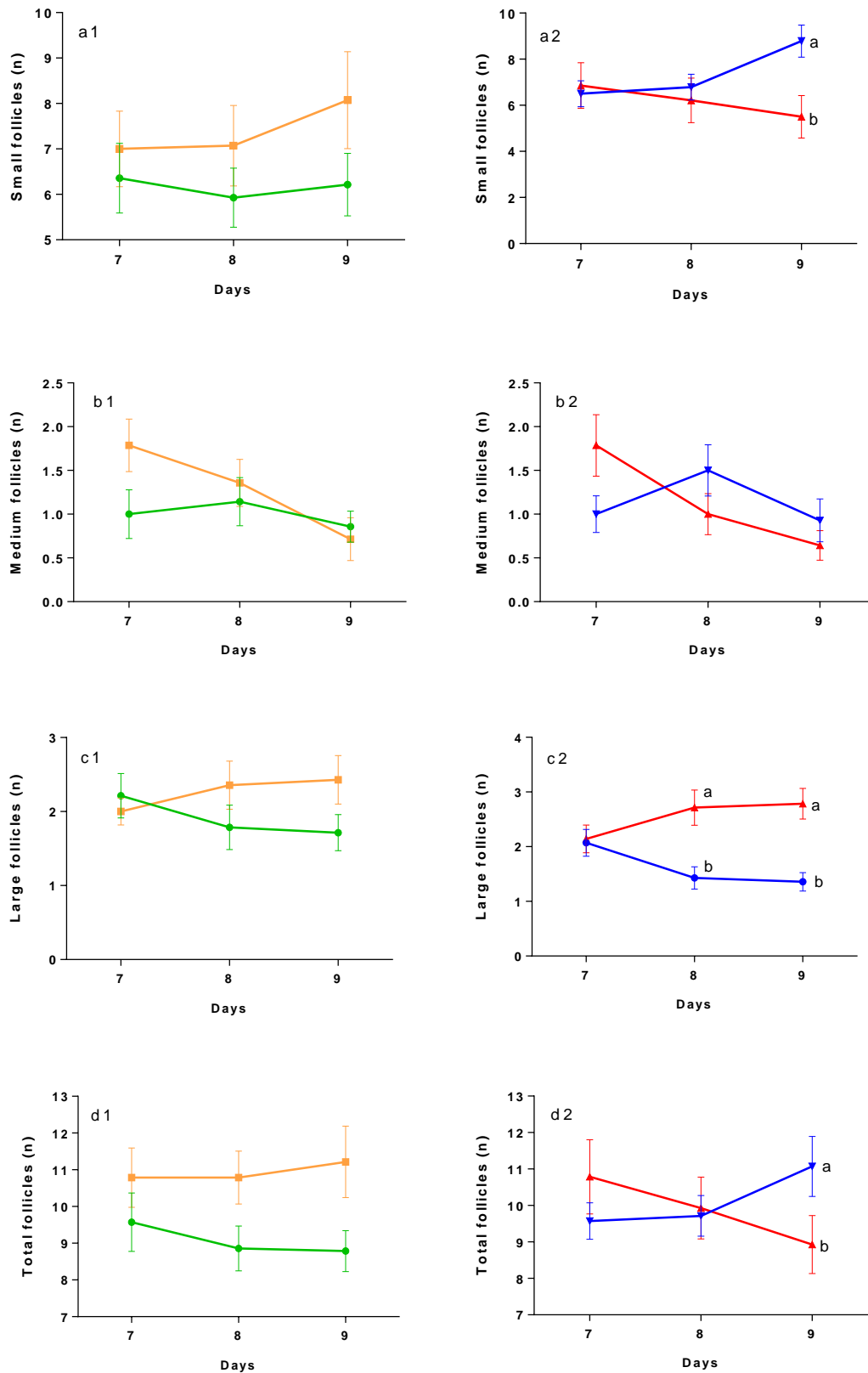
Fig 2. Mean (±SEM) number of (**a1** and **a2**) small (2 to 3 mm), (**b1** and **b2**) medium (>3 and <5 mm), (**c1** and **c2**) large (≥5mm), and (**d1** and **d2**) total number of follicles ≥ 3 mm during Days 7 to 9 of ultrasound evaluations in goats supplemented with maize (■) or not supplemented with maize (●), and goats subjected to synchronisation of oestrus (▲) or non-synchronised goats (▼) in the non-breeding season (different letters, within days indicate differences between groups; P < 0.05).
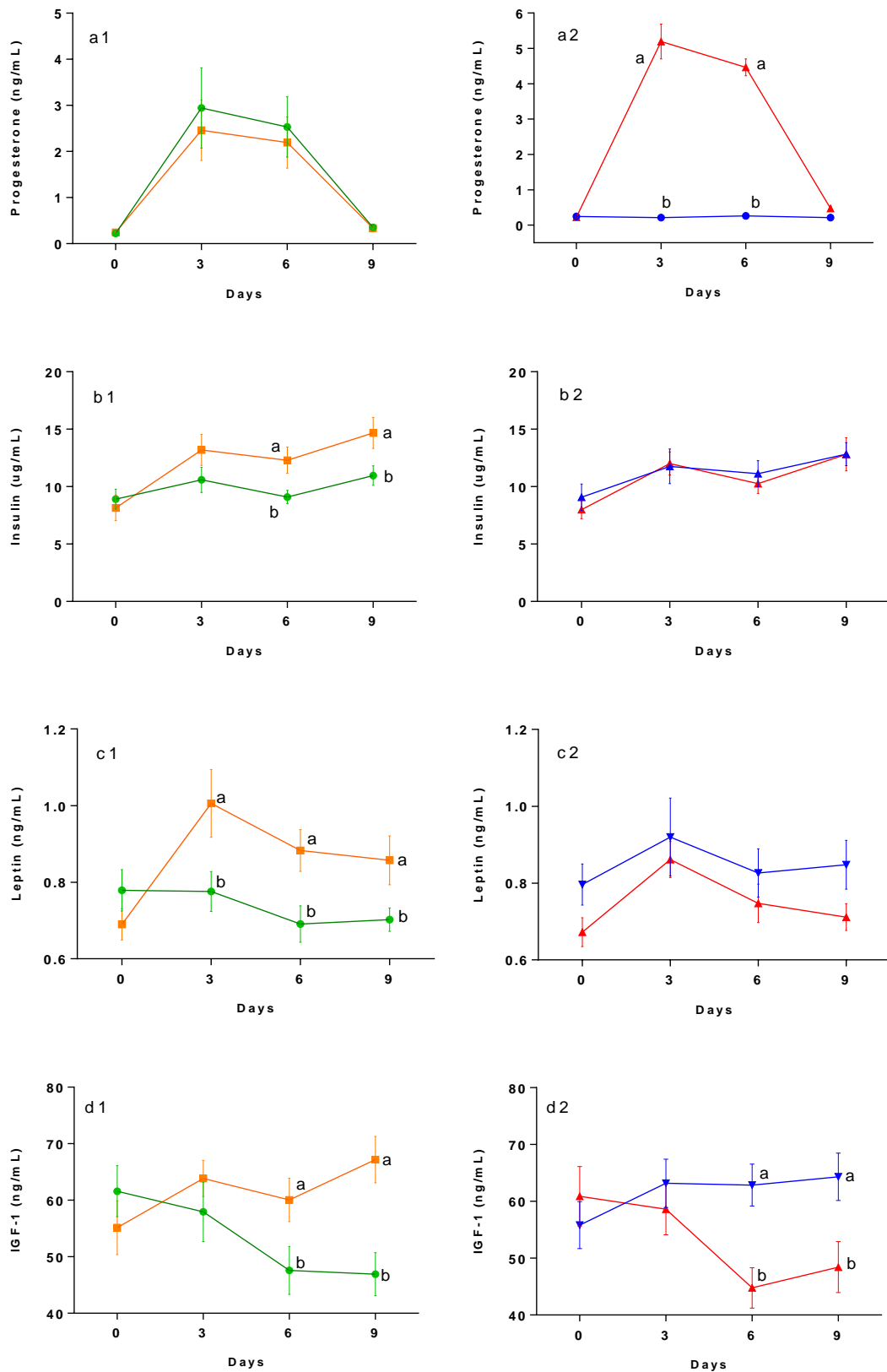
Fig. 3. Concentrations of progesterone (**a1** and **a2**), insulin (**b1** and **b2**), leptin (**c1** and **c2**) and IGF-1 (**d1** and **d2**) in goats supplemented with maize (■) or not supplemented with maize (●), and goats subjected to synchronisation of oestrus (▲) or non-synchronised goats (▼) the non-breeding season (different letters, within days indicate differences between groups; P < 0.05).

Table 1: Experimental groups and components of the ration and total energy (ME/day) administered from Days 0 to 9 for each group.

Variables	Experimental groups			
	Control	Maize	Synch	Synch x Maize
Animals (n)	7	7	7	7
Lucerne pellets (g/day)	820.0	820.0	820.0	820.0
Lucerne hay (g/day)	150.0	150.0	150.0	150.0
Cracked maize (g/day)	0	220.0	0	220.0
Total energy (MJ ME/day)	7.6	10.6	7.6	10.6

Table 2. Characteristics of oestrus, ovulation rate and follicular development in goats treated with a combination of either synchronisation of oestrus and/or maize supplementation.

Variables	Control	Maize	Synch	Synch x Maize	Probability		
					Maize	Synch	MxS[1]
Animals (n)	7	7	7	7	-	-	-
Oestrous detection (%)	0.0[b]	0.0[b]	100.0[a]	100.0[a]	1.000	<0.001	1.000
Ovulation rate/doe	0.0[b]	0.1±0.1[b]	2.3±0.3[a]	3.3±0.5[a]	0.074	<0.001	0.074
Codominant follicles on Day9 (n)	1.1±0.3[b]	1.3±0.3[b]	2.3±0.3[a]	3.3±0.3[a]	0.086	<0.001	0.192
Largest follicles on Day9 (mm)	5.8±0.3[b]	5.9±0.3[b]	6.9±0.3[a]	7.4±0.3[a]	0.428	<0.001	0.561
Emergence of largest follicles Day9 (day)[2]	4.3±0.6	5.2±0.6	5.0±0.6	4.8±0.6	0.472	0.810	0.339
Growth rate (mm/day)	0.6±0.1[b]	0.7±0.1[b]	0.9±0.1[a]	1.1±0.1[a]	0.309	0.004	0.761

[1]Interaction between maize and synch groups.

[2] Day of emergence of the largest follicles on Day 9 in relation to the beginning of nutrient supplementation. Values with different letters in the same row are significantly different ($P < 0.05$).

Table 3. Characteristics of LH secretion in does treated with a combination of either synchronisation of oestrus and/or maize supplementation 12 to 56 h after ending treatments.

Variables	Control	Maize[1]	Synch	Synch x Maize	Probability Maize	Synch	MxS[2]
Goat (n)	4	4	4	4	-	-	-
Goats with LH surge %(n)	0^b	$25(1)^b$	$100(4)^a$	$100(4)^a$	1.000	<0.001	1.000
Max concentration of LH (ng/mL)	0.5 ± 0.7^b	0.7 ± 0.7^b	3.6 ± 0.7^a	4.9 ± 0.7^a	0.378	<0.001	0.447
Interval to LH surge (h)	.	56	26 ± 1.9^a	23 ± 1.9^a	0.320	<0.001	0.320

[1]One doe in the maize group had an LH surge and a single ovulation. [2]Interaction between maize and synch groups. Values with different letters in the same row are significantly different (P < 0.05).