

Melatonin, cortisol and progesterone in alpine goat's superstimulateds with FSH-OV and artificial photoperiod

Abstract

Background: It is already well known the importance of hormones such as progesterone, cortisol and melatonin on the reproduction of females of various species. However, as regards the goat species, there is still a lack of data, especially in animals used in oocyte donation programs for subsequent in vitro production of embryos. The objective of this study was to verify the profile of progesterone, cortisol and melatonin during the hormonal treatment of ovarian stimulation in the anestrus, transition and reproductive season in Alpine goats using artificial and natural photoperiod. Seventeen female Alpine goats were reared at latitude 21°15'22"S. The animals were treated with 10mg oFSH (Ovagen, ICP, New Zealand) in decreasing doses for four consecutive days at the end of a progestin treatment with CIDR® (Intervet, New Zealand) at the reproductive season (G1,n=7) and anestrus with artificial photoperiod (G2,n=5) or natural (G3,n=5). Blood samples were taken before, during and after the period of ovarian stimulation and during oocyte harvest by laparotomy. Blood collection for melatonin was performed overnight, for progesterone and cortisol were performed for 16days in the morning. The values were presented as mean, standard deviation and median for the groups and compared by non-parametric analysis (Kruskal-Wallis, analysis of variance and Friedman). With regard to progesterone, there was a difference ($P<0.05$) between the three groups studied and in the two periods (reproductive season/anestrus), with higher values in the reproductive season. Statistical differences ($P>0.05$) were not observed for cortisol between groups, however, after laparotomy there was a significant increase ($P<0.05$) in the operated goats (G1:40.94±18.53nmol/L) compared to those not operated at the reproductive season (G2:17.86±14.90nmol/L and G3:16.00±16.51nmol/L). Serum cortisol values were lower than those of the reproductive season for all groups, G1(13.18±9.43nmol/L, natural photoperiod, non-laparotomized goats), G2(26.70±15.39nmol/L, artificial photoperiod, laparotomized goats) and G3 (35.35±17.10nmol/L, natural photoperiod, laparotomized goats). However, the surgical group (G2 in artificial photoperiod) presented serum cortisol levels between the non-surgical group (G1 in the natural photoperiod) and the surgical group (G3 in the natural photoperiod), leading to the belief that melatonin might have interfered with the values Cortisol of these animals in anestrus. During the experimental period, melatonin values were always high in all groups in the anestrus period (G1:68.19pg/mL, G2: 45.53pg/mL and G3:58.28pg/mL).

Conclusion: Under the conditions of this study, it is possible to verify an influence of the season (reproductive or anestrus), and possibly melatonin, on the secretion of cortisol in these animals.

Keywords: caprines, melatonin, hormones

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Introduction

Cortisol secreted by the adrenal cortex is an important hormone related to inflammation and stress. Cortisol levels increase in goats after 8 h of surgical procedure, being significant ($p<0.05$) and after 72h, the level decreases drastically.¹ Cortisol levels in goats ranges 14-18ng/mL.² The type of anesthetic block may also influence the amount of blood cortisol in animals,³ as well as temperature and estrous in goats, interfering directly with exogenous melatonin.⁴ The endocrine response is one of the physiological mechanisms to respond to stress in the temperature levels and is a mechanism of homeostasis defense, increases with body temperature, however melatonin regulates the level of cortisol. In addition, melatonin regulates cortisol and TSH levels in goats and the findings reveal that there is an interaction of melatonin with cortisol in goats,⁴ melatonin directly inhibits the adrenal gland for ACTH.⁵ The stimulatory effect

of light through the eye on the expression of the adrenal gland gene causing plasma corticosterone and release without activating the hypothalamic-pituitary-adrenal axis,⁶ and it may be speculated that melatonin interferes in the gene by reducing the level cortisol secretory. In humans melatonin and cortisol oppose themselves during 24h, while cortisol is low early in the morning, melatonin peaks at this time, with a circadian rhythm of reproducibility for six weeks.⁷ Blind individuals there are no melatonin profile and the cortisol does not appear in saliva and can be altered by stress or sleep disorders. Its concentration is present upon awakening (8-10h.a.m.).⁸ Goats show period of reproductive and anestrus seasonality in different latitudes, north and south, and depends when they are distanced from the equator line, and males can prevent this seasonal anestrus. The reduction of the light period is detected by the retina, the suprachiasmatic nucleus, by sympathetic neurons, via the pineal gland, producing melatonin and neurotransmitters (dopamine, serotonin and other amino acids),

and stimulated by melatonin modulating GnRH secretion).⁹

The variation in animal behavior has been reported in consistent individual differences, however they receive less attention.¹⁰ Recent research has focused on the observation of this profile of the production animals, in the individual characteristic. Social groups have been studied to define new strategies in social behavior and morphological adaptations.¹¹ Animals with high capacity and cognitive ability should be able to produce better and better to change and adapt to the environment in which they are inserted, thus avoiding stress and suffering.¹² There is a high correlation between plasma progesterone with a large number of follicles, and there is a high individual variation of plasma progesterone in goats. In addition, the duration of progestin use time may determine a better response for prolonged use of more than seven days.¹³ During the reproductive season on goats can be synchronized/superovaluated without exogenous progesterone supplementation, only with FSH use for recruitment of the follicles, six to nine days after estrus.¹⁴ The aim of the present study was to observe the profile of melatonin, cortisol and progesterone in alpine goats overestimated in natural/artificial photoperiod in the period of estrous and seasonal anestrous in latitude 21°15'22"S and longitude 48°18'58" for the surgical collection of oocytes.

Methods

Experimental animals and facilities

The work was carried out in the Department of Goat Breeding and in the Department of Preventive Veterinary Medicine and Animal Reproduction of the Unesp-Jaboticabal (latitude 21°15'22"S and longitude 48°18'58" and altitude of 595m). We used 17 alpine goats, nulliparous and multiparous, during estrous and seasonal anestrous (feb-oct). The females were previously examined for the general clinical condition and considered suitable for sanitary and reproductive status. The animals were kept in boxes of three m² under natural light until the beginning of the artificial photoperiod for G2. The sanitary management of all parasites and the clinical condition of the animals were weekly evaluated.

Period of adaptation

The animals used underwent a pre-experimental period of one month, for adaptation to feeding and shed, being weighed and distributed in the experimental groups.

Food

Feeding consisted of feed (14% crude protein and 5% crude fiber) and *Cynodon dactylon* hay ("Coast cross") (4.98% crude protein and 35.88% crude fiber in the dry matter). The animals received 0.5kg/animal/day of concentrated feed and 2kg/animal/day of hay, with ad libitum availability of mineral mixture and water.

Experimental design

Three groups were formed and studied during the following periods:

Seasonal Anestrous period and reproductive season

G1- Control group with natural photoperiod, n=7

G2- group using artificial photoperiod, n=5

G3- group with natural photoperiod, n=5

The laparotomy for oocyte collection was performed in the seasonal anestrous in groups G2 and G3 and in the reproductive season only the G1 group.

Ovarian stimulation-FSH-ov (all groups)

Ovarian stimulation was performed in the animals to increase follicular recruitment and consequently exogenous progesterone (CIDR®-0.3g-Intervet, New Zealand) was used for 12days (withdrawn prior to surgery), ovarian overstimulation with FSH-ov-10mg (Ovagen, ICP Biolumino-chemical Ltd, New Zealand, item 2182) in decreasing doses on days D9,10 and 11, divided into 12h intervals in volumes of (3,2,1,1.1ml), respectively. On day D 10, 125µg of cloprostenol (Cooper do Brasil, item 005/00) was applied.

Blood collection for analysis of melatonin, cortisol and progesterone

The melatonin collection was carry out in all animals, before the beginning of the artificial photoperiod (august/08/2002). During the artificial photoperiod (july/13/2002), after the use of the same (august/17 /2002), during the hormonal stimulation of anestrous, in the period CIDR-G (august/28/2002) and CIDR-G+FSH-ov (03/03/2002), at the end of the year, in the transition from anestrous and reproductive season (december/18/2001). Melatonin collection, every two hours, starting at 5:00 p.m. and ending at 9:00 p.m. the following day. In order to illuminate the place during darkness without interfering with the concentration of melatonin, a red light (<0.2 lux) filter 25A4, 58 M, coupled to a common torch was used, totaling 8 collections, with 117 samples each at 9 hours, totaling 936 samples.

Blood samples for melatonin dosing were centrifuged for plasma at 1500G for ten minutes. The aliquots were identified, stored in ependorfs and then frozen at -18°C until to be analyzed in São Carlos city, in the Physiology laboratory. The ovine antibody G/S704-6483 or G/S/704-8483 (Stockgrand Ltda, England) was used for the dosage of melatonin, being 100% specificity for goats, already diluted (twoml) and fractionated in 500 ependorf µL, by dosing method.¹⁵ For the dosage of cortisol (endogenous) and progesterone (endogenous and exogenous) daily blood samples were collected in the morning for 16days (12days of ovarian overestimation and four days after CIDR-G withdrawal) (D0-D16) . Averages of every three and four days, respectively, were used in order to minimize the variation of the absolute hormonal value and were called moments (M) for progesterone M1, 2 and 3 and cortisol M1,2,3,4 and 5. Were evaluated by the radioimmunoassay technique at the FCAV-Unesp-Jaboticabal Laboratory of Physiology, with commercial kits (Coat-A-Count, total Progesterone and Cortisol, DPC).

Artificial Photoperiod (G2)

The light treatment used only in the G2 group during 45 days in the period of seasonal anestrous between June 15 and July 2001. The animals were kept in an appropriate shed in the FCAV-Unesp-Jaboticabal Caprinocultura, in joint bays with three m²/ animal. In the shed, the five Alpine goats received 120 lux light average supplementation composed of four 40w fluorescent lamps, between them one meter and a half and two meters high. Group G2 (artificial photoperiod) received the luminous treatment 600 meters away from the other groups (G1 and G3). The light period was 20h daily (natural-11.26h+artificial -8.74h), reaching 20h, according by the specific technique.¹⁶

Statistical analysis

To characterize group data at each time point, means and standard deviations (\pm S) and median (Md) were used.

For the comparisons between the groups at each time point, depending on the type of the variable, the non-parametric Kruskal-wallis test or analysis of variance was used. In both situations the levels of significance (p-value) were explained. For the comparisons between moments within each group, the non-parametric Friedman test was used. All analyzes were considered significant when $p < 0.05$. In cases where $0.05 < p < 0.10$ a tendency to significance was reported (p is the probability of erroneously concluding for significance) and for melatonin only descriptive analysis was performed.¹⁷

Results

Progesterone

Mean progesterone plasma concentrations (ng/mL) were elevated in absolute values in the reproductive season on the same day in all groups. The overstimulated group (G1) presented high mean levels in the D0 of endogenous progesterone, G1 (53.0 ng/mL), G2 (33.0 ng/mL) and G3 (27.2 ng/mL). The other G2 and G3 groups also showed a reduction in plasma progesterone levels, even without CIDR-G. However, for better visualization and statistical work, we condense by the means at moments (M1, M2 and M3) the 16 days of samples always adding three consecutive days and obtaining their respective mean to minimize the variable effect of progesterone values on each day, on samples days. The results of the statistical analysis are shown in Figure 1 (averages and medians). In the period of the season there was a reduction in the plasma progesterone level from M1 to M2 and all were different ($p < 0.05$) for all groups. At the moment M1 there was no statistical difference and the groups were equal in values, as in M3. However, in M2 there was a tendency of $G1 \geq (G2 = G3)$. Comparing moments in each group, it is observed that in G1, moments $M1 > M2 > M3$ were identical in G2, whereas in G3 $M1 > (M2 = M3)$, all $p < 0.05$.

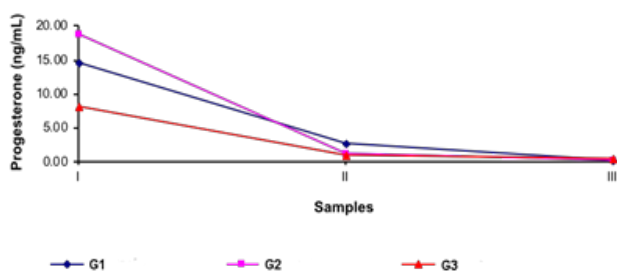


Figure 1 Mean plasma concentrations of progesterone (ng/mL) in goats submitted or not to the artificial photoperiod during ovarian overstimulation of the G1 group at the reproductive season, Jaboticabal, 2002.

In the period of seasonal anestrus, the mean absolute value of plasma progesterone was low (D0) in all groups, as opposed to the season, showing clear ovarian inactivity. Group G1 presented endogenous progesterone value of 0.05 ng/mL, 0.02 ng/mL G2 and 0.06 ng/mL G3. Ovarian synchronization and overstimulation in this period showed an increase due to the CIDR-G device in the G2 and G3 groups, but G1 also had a high concentration of endogenous plasma

progesterone. The time in this period was different ($p < 0.05$) for the groups, and G1 presented ($M1 = M2$) $> M3$, in G2 and G3 $M1 > M3$ and M2 intermediate, coinciding with the same hormonal treatment. G1 $< G3 < G2$ and for moments M1 and M2, $G1 > (G2 = G3)$ were also different ($p < 0.05$). Figure 2 shows graphically the values, being low for G1 at the beginning of the experiment, reducing in M2 and M3.

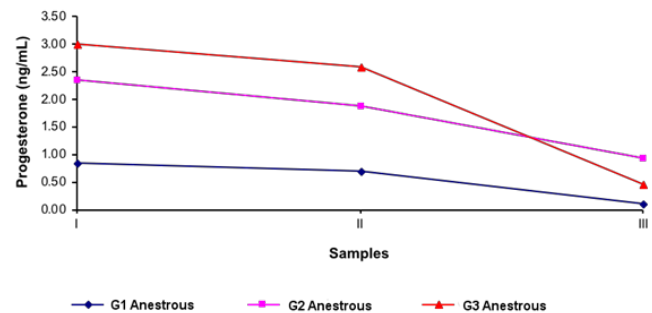


Figure 2 Mean plasma concentrations of progesterone (ng/mL) in goats submitted or not to artificial photoperiod during ovarian overstimulation of G2 and G3, in seasonal anestrus, Jaboticabal, 2002.

Cortisol

The mean cortisol concentrations (nmol/L) of G1 goats on day D12 were 48 nmol/L, G2 30.70 nmol/L and G3 37.20 nmol/L, for the period of the breeding season. On day D13, they changed their values for G1 to 70.60 nmol/L, G2 25.80 nmol/L and G3 10.20 nmol/L, exactly 24h after performing the surgical procedure. G1 (70.60), a group that underwent laparotomy, presented higher values than those of G2 (25.80 nmol/L) and G3 (10.20 nmol/L). G1 also showed an increase in D15 (72h) after surgery (45.50 nmol/L), but not in amounts higher than D13. At the beginning of the collection (D0), the animals did not present high levels of cortisol at the reproductive season, varying over time, but not exceeding values higher than 30 nmol/L. After D12 (surgical procedure), there was an increase in cortisol levels (Figure 3). In the studied at moments, there was a difference ($p < 0.05$) for G1, with $M1 < (M4 = M5)$ and $M2 = M3$ intermediates. As for G2 ($p > 0.10$) $M1 = M2 = M3 = M4 = M5$ and G3 $0.05 < p < 0.10$, with trend $M2 \geq (M3 = M5)$.

The comparison between groups at each moment varied from the M2 moment, being in M4 and M5 ($p < 0.05$), tending to $G1 \geq G2$ and G3 intermediate and M5 $G1 > (G2 = G3)$. In which only G1, with surgical intervention, altered the cortisol value in nmol/L. The cortisol values (nmol/mL) were elevated (higher than 20 nmol/mL) at the beginning of the collection, whereas at the reproductive season, the values were between 10 and 20 nmol/mL, G2 (25.21 nmol/mL) and G3 (31.78 nmol/mL). After this period of D0, the values continued to oscillate, but remained between 45 and 9 nmol/mL until D12 (surgical act for G2 and G3). In D13, G1 (11.79 nmol/mL), G2 (13.56 nmol/mL) and G3 (25.84 nmol/mL) were G1 (22.24 nmol/mL), G2 (49.85 nmol/mL) and G3 (70.19 nmol/mL). After this period the values remained between 9 and 30 nmol/mL (Figure 3).

In all studied moments, at G1 group there was no statistical difference between moments ($p > 0.10$), $M1 = M2 = M3 = M4 = M5$, while in G2 there was a trend ($0.05 < p < 0.10$) and there was no statistical difference ($p > 0.10$) ($M1 = M5$) $\geq M2$. In M5, $G1 < G3$, with G2 intermediate. Elucidating better, figure 4 can collaborate in the statistical understanding of the moments or complement the

comments. It was observed in G2, in the M5, after the surgical procedure, it did not follow the G3 values in which it underwent the same intervention, being intermediate and ($0.05 < p < 0.10$). Note that endogenous melatonin probably interfered with these values to balance the hormones, improving the process of surgical stress. The artificial photoperiod stimulated in this group G2 may have influenced the result, moved by the endogenous melatonin produced by these animals. The cortisol values (nmol/L) were elevated (higher than 20nmol/L) at the beginning of the harvest, whereas at the reproductive season, the values were between 10 and 20nmol/L, mainly in G1 (45.24nmol/L), comparing the others: G2 (25.21nmol/L) and G3 (31.78nmol/L). After this period of D0, the values remained between 9 and 30 nmol/L (Figure 4).

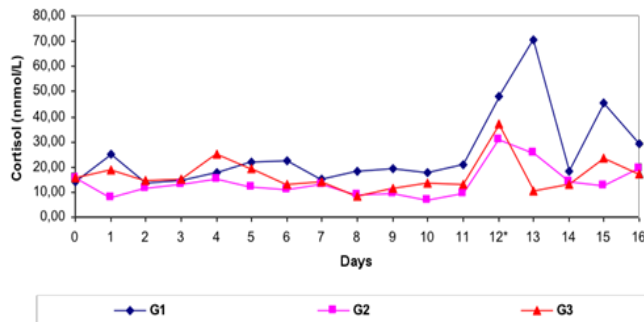


Figure 3 Mean plasmatic concentrations of cortisol (nmol/L) in goats submitted or not to the artificial photoperiod, during ovarian overstimulation of G1, at the reproductive season, Jaboticabal, 2002.

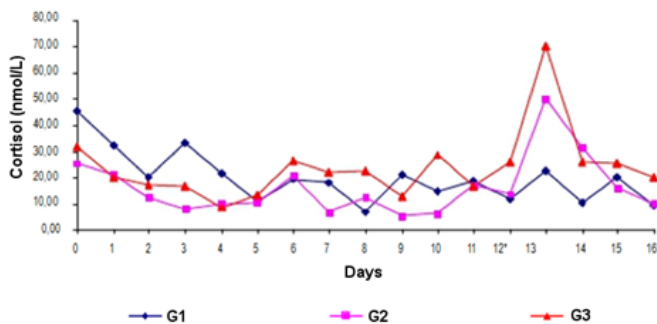


Figure 4 Mean plasma concentrations of cortisol (nmol/L) in goats submitted or not to the artificial photoperiod, during ovarian overstimulation of G2 and G3, in the period of seasonal anestrus, Jaboticabal, 2002.

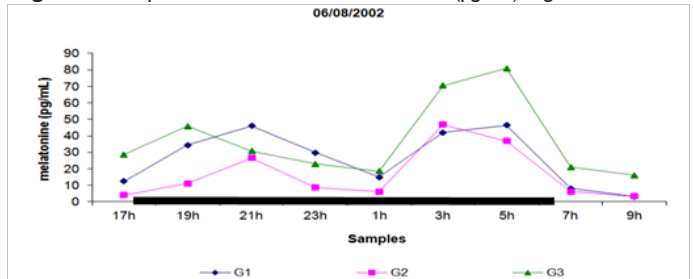
At all times studied for G1, there were no differences ($p > 0.10$) ($M1=M2=M3=M4=M5$); in G2 there was a tendency ($0.05 < p < 0.10$) and ($M1=M5$) \geq M2. In M5, $G1 \leq G3$, with G2 intermediate. It was observed in the G2, in the M5, after the surgical intervention, it did not follow the G3 values in which it underwent the same intervention, being intermediate and ($0, 05 < p < 0.10$).

Melatonin

The melatonin dosage of august/06/2002 (Figure 5), previous to light supplementation, increased from 17h (first harvest of the series). The dark period for the date is between 5:35p.m. up to 06:47p.m. on the following day and specified on all figures with continuous dark dash, calculated for all dates. For groups G1 and G2, the mean dosage was increasing until 21h and for G3 at 19h. It declined for the three groups at 1am, rising to 3h, but more for G3 than G1 and G2. At 09h

on 09/06/2002, the mean dosages of the three groups were low.

Figure 5 Mean plasma concentrations of melatonin (pg/mL) in goats submitted



or not to the artificial photoperiod prior to the artificial photoperiod, in the seasonal anestrus, Jaboticabal, 2002.

During light supplementation, on July/13/2002, groups G1 and G3 exhibited melatonin levels higher than G2. The lights were on in the period between 17h and 00h and between 04h and 07h, coinciding with low melatonin averages (Figure 6). The local dark period was from 17:42min until 06:55 the following day (dark dash), influencing the G1 and G3 groups and the light supplementation between 17:00h and 04h and 07h for the G2 group (dashed dotted lines). Group G1 presented mean dosage for melatonin higher at 23 h intermediate for G3 group and lower on G2 group. At 09 h the three groups presented average melatonin levels that were still high for the time. After 45 days of light supplementation, on August/17/2002, already in natural photoperiod, the groups G1, G2 and G3 presented mean values of melatonin closer to each other. The highest peak occurred at 19 h for G3, while for G1 at 05h. At 9h the groups still had increasing amounts of melatonin (Figure 7). The dark period is between 5:45p.m. until 6p.m. 43min.

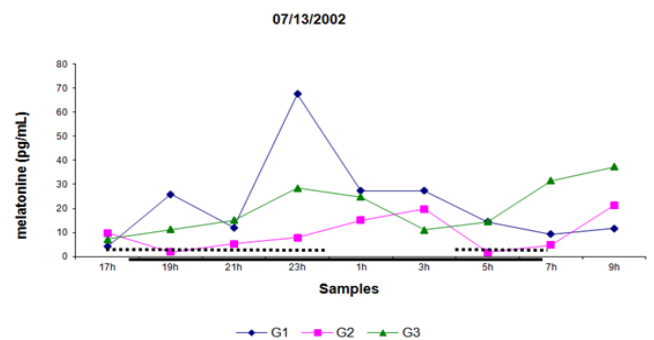


Figure 6 Mean plasma concentrations of melatonin (pg/mL) in goats submitted or not to the artificial photoperiod during light supplementation, in the seasonal anestrus, Jaboticabal, 2002.

In the CIDR-G period, at the time of seasonal anestrus, during ovarian overstimulation, the mean dosage for melatonin in all three groups was irregular throughout the harvest (Figure 8). Increasing and decreasing peaks during all schedules were observed, and at 9o'clock the mean dosage was lower throughout the analysis period. The dark period of the time is between 17:57min and 06:36min. On August 31, 2002, during the CIDR-G+FSH-ov period, during ovarian overstimulation of G2 and G3, melatonin was more uniform among the studied groups G1, G2 and G3 (Figure 9). It increased from 5pm, reducing itself to the time of 23h, rising again at 01h. It is also observed that in G1, at 09h, was low, intermediate for G3 and high for G2. The dark period is between 18 h and 06:27min. This collection

occurred three days after the previous one, reducing the dark period in practically 1h.

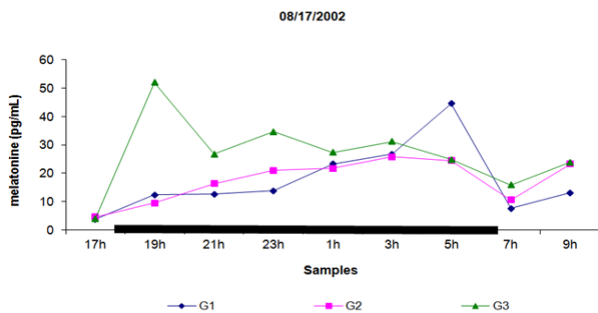


Figure 7 Mean plasma concentrations of melatonin (pg/mL) in goats submitted or not to the artificial photoperiod following light supplementation, in the seasonal anestrus. Jaboticabal, 2002.

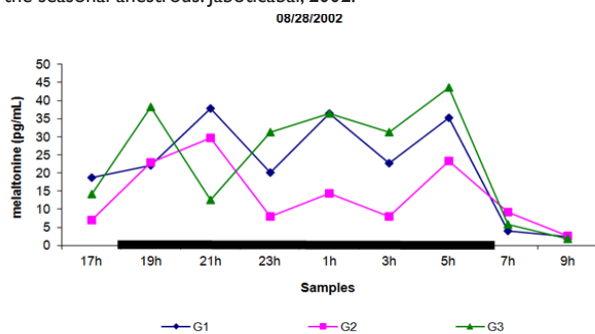


Figure 8 Mean plasma concentrations of melatonin (pg/mL) in goats submitted or not to the artificial photoperiod during the CIDR-G period of ovarian overstimulation of G2 and G3 groups in seasonal anestrus. Jaboticabal, 2002.

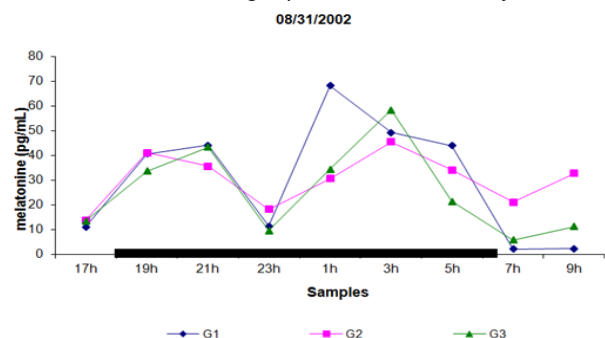


Figure 9 Mean plasma concentrations of melatonin (pg/mL) in goats submitted or not to the artificial photoperiod during the CIDR-G+FSH-ov period of the ovarian overstimulation of the G2 and G3 groups, in the seasonal anestrus. Jaboticabal, 2002.

At the end of the year, three days before the start of the official summer entry on Dec/18/2002, the mean of samples for melatonin in the three groups G1, G2 and G3 were low at 17h. After that time, they rose, arriving at maximum average values at 23h and reducing at 09h for the three groups. At that time, the dark period is between the aurora boreal and austral from 18h 44min until 05h 28min (Figure 10). For the CIDR-G period, in the ovarian overstimulation of G1 during the breeding season, on february/25/2002, the mean melatonin dosages of goats were increasing in the three groups until 23h, except for G2, which presented low. Already at 01h, G1 was lower, G2 intermediate and G3 slightly higher than this (Figure 11). At 09 a.m., the three groups equalled their values, being low, as observed the previous

day, at 5:00p.m. The local dark period is between 18:44min and 06:10min. The plasma levels of melatonin on march/03/2002 (Figure 12), whose collection was performed six days after the previous one, during ovarian super-stimulation, during the reproductive season, with application of FSH-ov, presented increasing after at 5:00p.m. It was reduced at 23h for groups G1 and G2 and elevated for group G3, which increased until 05:00h, equating to group G2. Mean melatonin dosages for group G1 did not increase throughout the process until 9h. At that time, the groups G1, G2 and G3 presented mean values of melatonin high in the clear period. The local dark period is between 18h 38min and 06h 13min (04/03/02), making 12 h 25 min of darkness. The dark variation of the whole local annual period is small (02h 28min) when compared to the other regions of Brazil.

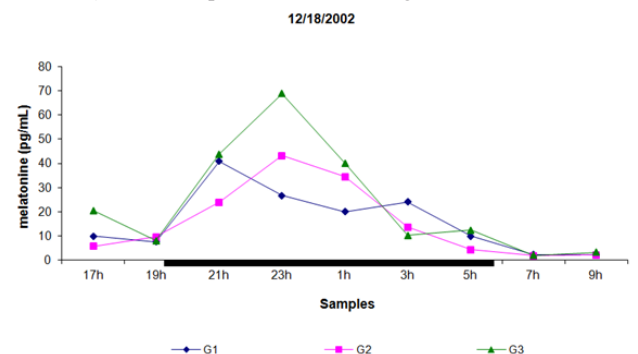


Figure 10 Mean plasma concentrations of melatonin (pg/mL) in goats submitted or not to the artificial photoperiod at the anestrus / season transition. Jaboticabal, 2002.

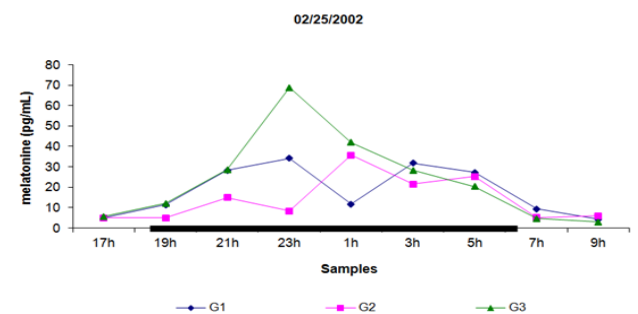


Figure 11 Mean plasma concentrations of melatonin (pg/mL) in goats submitted or not to the artificial photoperiod, in the CIDR-G period of ovarian overstimulation of G1, at the reproductive season. Jaboticabal, 2002.

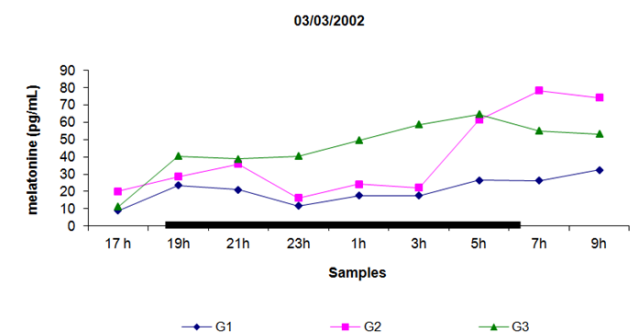


Figure 12 Mean plasma concentrations of melatonin (pg/mL) in goats submitted or not to the artificial photoperiod in the CIDR-G+FSH-ov period of ovarian overstimulation of G1, at the reproductive season. Jaboticabal, 2002.

Discussion

The improvement of the reproductive response in goats needs techniques, besides nutrition and sanitary management. The use of substances such as progestins, prostaglandins and male effects are used to improve the reproductive response in goats in Nigeria. However, results on farms are still not encouraging, especially when studying specific breed or crosses for estrus synchronization. Use of appropriate techniques with good nutritional management.¹⁸ More studies are needed to decrease the amount of hormones to improve response in ovarian overstimulation protocols, since the interaction between the hormones progesterone, cortisol and melatonin is evident and may interfere with the results observed to date. In the period of the reproductive season there is a tendency of regulation of endogenous hormones when exogenous hormones are used in this period. Photoperiod is one of the major factors influencing activity in small ruminants. In subtropical regions and high latitudes, there are periods of season and against a well-defined breeding season and they have a significant impact on the reproduction and production of these animals.¹⁹ The number of luxes for the use of the artificial photoperiod is also important, because 98 luxes during 60days were enough to synchronize goats, with success and prolificacy of 1.6.²⁰ To produce oocytes, the artificial photoperiod was used for 45 days in alpine goats, with production by in vitro fertilization of the first goat product from frozen embryo in Brazil and from the group stimulated by artificial photoperiod.²¹ Exogenous progesterone (CIDR® Controlled Internal drug release) is not maintained at constant values during synchronization and even being restored within 6h promotes synchronization.²²

The non-use of progestogens to oocytes collections is an important procedure for superovulation. Follicular waves are determined by different progesterone values. The presence of corpora lutea and dominant follicles are determinant factors in the impact of superovulation, and the time of FSH administration in the corpus luteum is a decisive factor. During the reproductive season goats may be superovulated without exogenous progesterone using FSH to recruit follicles of the second follicular wave (6-9days after clinical estrus),¹⁴ opposing the others²³ that recommend the use of exogenous progesterone to improve the results in Boer goats. More studies to progesterone and cortisol are necessities, because in women under stress, it rises in relation to several factors. It is not recommended to evaluate progesterone in the menstrual phase, since cortisol and progesterone are elevated.²⁴ High levels of cortisol and low progesterone levels appears in early aborted goats.²⁵ The use of hormones in the period of seasonal anestrus is more efficient than the period of the reproductive season, since the exogenous hormonal control produces a better and faster answer, in addition, more studies are also important in relation to these hormones in goats in the sense of clarifying their probable interactions.

Aggressive goats have low levels of cortisol and high glucose, while those with fearful behavior have high levels of cortisol and glucose. The animals of passive behavior have intermediate physiological levels of cortisol.²⁶ The animal's social behavior may determine and interfere with the results of biotech material such as semen and embryos for small ruminants and interfere with the results accordingly. Cortisol is known to be a hormone detected in the blood and confirms the presence of stress in animals and humans.^{2,27} The presence of human manipulation in animals does not cause stress. The level of cortisol increased significantly in short period in production animals

handled for vaccination and dosing procedures. Other stressors such as estrus, deprivation of food and water were not significant when compared to baseline cortisol level.²⁸ Goats in dehorn and with local anesthesia with 2% lidocaine and epinephrine in the infratrochlear and lacrimal nerve did not block the production of cortisol and pain response in these animals.²⁹ In humans, cortisol was elevated after 24 and 48 hours after abdominal surgery, independent of circadian rhythm.³⁰ Cortisol increased after 24 h of the surgical procedure, a fact that confirms the occurrence of surgical stress. In medical students in France they presented levels of cortisol and melatonin in opposite circadian rhythm at night, and several factors can alter these values and their respective rhythmicity.⁷

Melatonin acts directly on the adrenal gland, inhibiting the synthesis of glucocorticoids in response to ACTH in several species.³¹⁻³⁴ In addition, it exerts an inhibitory effect on the secretion of ACTH in the anterior pituitary and production of adrenal cortisol by different mechanisms.³⁵ It reduces the production of catecholamines via cAMP, in addition to lowering nocturnal blood pressure.³⁶ Melatonin has been used in humans undergoing bariatric surgery, increasing sleep and reducing postoperative pain, promoting patients' quality of life.³⁷ In goats, there was alteration of cortisol after surgery and anesthesia in all groups.³ Goats are very sensitive to manipulation and anesthesia, animals that do not like routine changes. In thermal stress, they also presented lower cortisol changes for those who underwent exogenous melatonin.⁴

The melatonin profile in blind humans, without any presence of light, does not present the hormone, however the cortisol rises during the day. The two hormones are similar in the same range in the 24-hour cycle for some patients and over a longer period. In saliva cortisol was not detected during this period⁸. In ovine fetuses, exogenous melatonin was able to lower blood pressure observed in specific recipients MT1 and MT2, and may collaborate in future research to regulate blood pressure in hypertensive patients or with pregnant women in developing uterus.³⁸

Conclusion

Plasma concentrations of progesterone during the season and seasonal anestrous varied in ovarian overstimulation ($p < 0.05$), follicular aspiration and four days after the surgical procedure in the groups studied. Plasma concentrations of cortisol during the reproductive season and seasonal anestrous varied in ovarian overstimulation, follicular aspiration and four days after the surgical procedure. G2 (artificial photoperiod) presented plasma cortisol concentrations between G1 (control) and the G3 (natural photoperiod). Plasma concentrations of melatonin were altered during ovarian overstimulation (CIDR-G and CIDR-G+FSH-ov), being more visible in the seasonal anestrous than in the period of the reproductive season. There is a relationship between the hormones melatonin and cortisol in alpine goats overestimated with natural/artificial photoperiod, and cortisol was inferior to the surgical group of the artificial photoperiod. Endogenous progesterone was present at different levels in the season and anestrous at the latitude inserted in this experiment. More biochemical studies are needed to evaluate the relationship between them and positive or negative reproductive consequences.

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Conflict of interests

The author declares no conflict of interest.

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