

Association between a single measurement of progesterone and cortisol blood concentrations at two to one week before parturition, and number of fetuses in the Teramana goat*

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Summary

The Teramana goat is an at-risk breed, needing population protection and programs to increase its numbers. The first step for a population increase is the best management of reproduction, leading to an as high as possible number of healthy and viable kids born. To this purpose, beside the optimization of mating, the best possible management of pregnancy and parturition is mandatory. The goat is a prolific farm animal in which single, double, or triple ovulations can occur, leading to singleton, twin or triple pregnancies, and the birth of multiple kids. Twins and triplets are associated to increased risk for perinatal mortality and need a special surveillance and possible assistance at birth. Knowledge of the number of fetuses that have to be delivered from each goat could be a practical tool for a better management of parturition. Among the methods to define the number of fetuses in the goat, the measurement of blood progesterone (P4) concentrations have provided inconsistent results. Therefore, the present study was aimed to assess the possible association between the maternal concentrations of plasma P4 and cortisol (C), two hormones possibly associated to the number of fetuses, measured only once at about two to one week before parturition in Teramana goats, and the number of fetuses. The results, obtained from 23 does, showed that both plasma P4 and C are higher in does bearing multiple fetuses than does with singleton pregnancies. However, the single measurement of plasma C, but not P4, two to one week before the expected parturition in the Teramana goat is useful to distinguish between does bearing singleton and triplet pregnancies for a better surveillance and assistance at delivery. Therefore, it could represent a tool for the best management of reproduction in a breed population at risk for extinction.

Keywords: goat, late pregnancy, cortisol, progesterone, number of fetuses

The Teramana goat breed is an ancient Italian breed typical of the Abruzzo region, and especially of the Appennini mountains region around the city of Teramo, obtained by crossbreeding with the Garganica breed, typical of the Gargano area in the near Puglia region. The Teramana breed is fully adapted to hostile environments and rugged or bushy pastures. Since 2002,

an official breed registry defined the typical breed characteristics: a medium body-size breed, its height is 60-85 cm, and body weight ranges between 45 and 80 kg. Although in origin this breed was a double-attitude breed for meat and milk production, at present its main attitude is the production of milk, with an average production of 250-400 L of milk in 180-240 days of lactation. Although in the past the Teramana breed had over 500 animals, since 2014 the national pastoral association (ASSONAPA) reported a total

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of 56 recorded does, reared in only two flocks in the L'Aquila area. The Teramana goat breed was considered among the endangered breeds, which needed population protection and programs to increase its numbers. The first step for a population increase is the best management of reproduction, leading to an as high as possible number of healthy and viable kids born. For this purpose, beside the optimization of mating, the best management of pregnancy and parturition is mandatory.

The goat is a prolific farm animal in which single, double, or triple ovulations can occur, leading to singleton, twin or triple pregnancies, and the birth of multiple kids. The number of ovulations and, in turn, the number of fetuses is influenced by multiple factors such as breed, age, parity, nutrition, season and bodyweight (16). Twin or triplets' pregnancies in goats were associated with increased risk of perinatal death (16). It was reported that in milk producing goats, perinatal mortality can amount to 0-17% in singleton (9, 18), 13-18% in twin, and 18-83% in \geq triplet (1, 12) pregnancies. Therefore, for a better management of parturition and to improve kids' survival the knowledge of the number of fetuses carried by each doe could be useful in a practical setting.

From a physiological perspective, pregnancy in does is maintained by the action of progesterone (P4) entirely produced by the corpora lutea, developed after single or multiple ovulations, and possibly associated to a similar number of developing embryos. This aspect led to a scientific investigation on the possible relationship between the number of corpora lutea, circulating P4 concentrations and number of fetuses. However, the studies on the association between blood P4 concentrations and number of fetuses provides inconsistent results. In 1991, Jarrel and Dziuk (8) reported that blood P4 was higher in goats with multiple corpora lutea from 7 to 30 days of pregnancy, while the number of corpora lutea or of fetuses did not show an effect on plasma P4 concentrations after 30 days of pregnancy. A recent study (17) reported that plasma P4 does not correlate with the number of fetuses during early pregnancy in goats with a relation between fetuses' number and P4 at 51 days of pregnancy. In mid pregnancy, the plasma P4 was greater in does with twins than those bearing singleton pregnancies (18.91 ± 0.67 vs 14.51 ± 0.47 ng/mL) (17).

Beside P4, in late pregnancy also cortisol (C) plays an important role, mainly for final fetal maturation processes and for triggering the parturition in several species. Studies in sheep have shown that cortisol produced by the fetal adrenal glands is pivotal to initiate the hormonal cascade leading to the parturition onset (10), and, similarly, increased C plasma concentrations were reported in goats during labor (7). The maternal circulating C concentrations could therefore sum up C produced by the fetus(es) and by the mother.

According to this, the measurement of maternal blood C concentrations could vary depending on the number of fetus(es) carried by the mothers. However, the body homeostasis could balance the overall maternal blood C concentrations, independently to the number of fetus(es).

Therefore, the aim of the present study was to assess the possible association between maternal P4 and C plasma concentrations and number of fetuses in the Teramana goat. The possible effect played by maternal age and parity on number of fetuses was also assessed, as well as the correlation among all the studied factors.

Material and methods

Ethics. The study was approved by the Comitato per il Benessere Animale Università di Bologna (Prot N 62126-2018).

Animals. The study was performed on a group of goats reared in the Abruzzo region under a program for population increase in the Teramana breed. All the Teramana goats were kept under natural photoperiod at a latitude of $42^{\circ}43'34.351''N$ and a longitude of $13^{\circ}46'21.539''E$, at about 270 m above sea level.

The flock consisted of 44 does and 2 bucks kept in open paddocks and fed hay *ad libitum* and 400 g/d of a commercially prepared food. Water was available *ad libitum*. At spontaneous estrus occurring during the breeding season, 30 does were submitted to natural mating. Pregnancy was detected by trans-rectal ultrasonography using a portable ultrasound machine (@Ge Logiq Book Xp, Best Medical srl, Santarcangelo di Romagna, RN, Italy) equipped with a linear 7.5 MHz probe during the 4th week after mating and confirmed by trans-abdominal ultrasonography at 35-40 days of pregnancy, using a convex 3.5 MHz probe. Twenty-four does, 1-6 years old, 1-4 parity (4 primiparous and 20 multiparous) were found pregnant and enrolled in the study. After a normal pregnancy course, at impending parturition the does were allowed to deliver spontaneously, but under kidding surveillance for a prompt obstetrical intervention in case of dystocia occurrence. At birth, the number of kids for each doe was recorded. The timing of placenta shedding was also considered normal when it was < 12 hours from birth (13). In the first week postpartum, clinical daily monitoring was also performed to assess the normal postpartum period in does and to assess the survival of kids.

Blood sampling. At about two to one week before the expected date of parturition, each doe was submitted to a single blood sampling. Blood samples were collected into heparinized vacuum tubes from the jugular vein. Samples were centrifuged (20 min at $1000 \times g$) within 30 min of collection, and plasma was stored at $-20^{\circ}C$ until analysis.

Hormonal analyses

Progesterone. Plasma progesterone concentrations were determined by radioimmunoassay (RIA) as described and validated in goats by Bono et al. (2). The antiserum, raised in a rabbit to 11α -hydroxyprogesterone-hemisuccinate-BSA, was used at a dilution at 1:12000. The cross-reactions of other steroids were: 11α -hydroxyprogesterone 83.3%; 11β -hydroxyprogesterone 15.7%; 21-hydroxy-

progesterone 4.0%; 17 α -hydroxyprogesterone 1.7%; 20 α -dihydroprogesterone <0.1%. The assay sensitivity was 21.9 \pm 0.74 pg/tube.

Cortisol. Plasma cortisol concentrations were determined as reported by Tamanini et al. (19) using a RIA method. The sensitivity (90% B/B0) of the cortisol antibody was 4.9 ng/mL, Intra-assay and the cross-reactivities were as follows: 20.4% with cortisone, 74.6% with deoxycortisol-11, 1.1% with corticosterone and 0% with progesterone and oestrogens.

Grouping of animals. Does were retrospectively grouped on the basis of number of fetuses: i.e. single, twin or triplet pregnancy.

Statistical analysis. Data about the possible association between the number of fetus(es) and: 1) the maternal blood P4 and C concentrations one week before parturition; 2) the maternal age and parity were assessed through ANOVA with the number of fetus(es) as fixed factor, and plasma P4, C, maternal age and parity as random factors, followed by a post-hoc test. The Pearson's correlation test was used to assess possible correlations among all the studied parameters, while the Spearman's correlation test was used to assess possible correlations between the number of fetus(es) and the plasma P4 and C, and maternal age and parity (Jamovi ver. 1.6.23 for Windows operating system). Significance was set with $p < 0.05$.

Results and discussion

Clinical findings. Out of the 30 mated does, 24 (80%) resulted in pregnancies. However, one primiparous doe was found dead before parturition and its samplings were excluded from further analysis. In the other 23 does, kidding occurred at term, with pregnancy lasting 149 \pm 5 (143-157) days from mating. In twenty-one (91.3%) does, kidding occurred spontaneously without the need for obstetrical assistance, while 2 goats (8.7%) needed manual obstetrical assistance. Three (13%) does delivered single kids, 16 (69.6%) twins, and 4 (17.4%) triplets. Therefore, a total of 47 kids were born, with the mean of 2.0 kids/goat. One kid was stillborn (2.1%), while all the other kids were healthy, viable and survived the first postpartum week. The one stillbirth was delivered by a twin pregnant doe. Two (8.7%) of the does showed placental retention with spontaneous expulsion between 12 and 24 hours after parturition but did not develop any postpartum disturbance.

Results from ANOVA test. The ANOVA showed a significant ($p < 0.05$) association between the number of fetuses and plasma C concentrations, with higher C concentrations in does bearing triple fetuses, and with a trend ($p = 0.057$) for higher plasma C concentrations in does with two fetuses in comparison to does bearing only one kid. No significant associations were found between maternal age and parity on number of fetus(es). On the contrary, P4 concentrations did not show statistically significant association with the number of fetus(es).

Tab. 1. Data about the maternal plasma P4 and C concentrations in the 23 does grouped according to the number of fetus(es) (mean \pm SD)

Hormonal concentrations	Number of fetus(es)		
	1 (n = 3)	2 (n = 16)	3 (n = 4)
P4 (ng/mL)	8.9 \pm 3.98 ^a	15.5 \pm 5.26 ^a	19.1 \pm 9.95 ^a
C (ng/mL)	15.7 \pm 2.91 ^a	23.0 \pm 10.42 ^{ab}	29.1 \pm 9.66 ^b

Explanation: a, b – superscripts in rows indicate significance with $p < 0.05$

Data (mean \pm SD) about the maternal plasma P4 and C concentrations in does grouped according to the number of fetus(es) are reported in Table 1.

Results from Pearson's correlation test. The Pearson's correlation test showed a significant positive correlation between maternal age and parity ($r = 0.97$, $p < 0.001$); between maternal plasma P4 concentrations and the number of fetus(es) ($r = 0.39$, $p < 0.05$); between maternal plasma C concentrations and the number of fetus(es) ($r = 0.36$, $p < 0.05$); and between maternal plasma P4 and C concentrations ($r = 0.40$, $p < 0.05$).

Results from Spearman's correlation test. When the correlation among all the other parameters (maternal age and parity, plasma P4 and C concentrations) was assessed in relation to the number of fetuses, age and parity were significantly positively correlated within pregnancies with one ($r = 1.0$, $p < 0.001$), two ($r = 0.99$, $p < 0.001$) or three ($r = 1.0$, $p < 0.001$) fetuses.

In order to increase the knowledge about reproduction in the at risk for extinction of the Teramana goat breed, the aim of the present study was to assess the possible relationship between the number of fetuses and maternal plasma P4 and C concentrations measured one week before the expected date of parturition, and maternal age and parity.

From a clinical standpoint, the 80% pregnancy rate is very similar to the 83-85% reported by Jarrell and Dziuk (8) and by Singh et al. (17). However, a recent review by Robertson et al. (16), reported that conception rates in goats depends on multiple factors and could range widely between 60 and >93%. Pregnancy length was 149 \pm 5 days, which is in agreement with the 146-151 day range reported by Hydbring et al. (7), and with the 150 (145-155 days) reported by Probo et al. (14) for other goat breeds.

The does gave birth to 47 kids, with only one kid loss (stillborn) (2.1%) from a twin pregnant doe. This stillborn rate is very low when compared to the 4.2% reported by Mellado et al. (11) for dairy goats. Similar to other reports (16), this stillborn kid in our study was not caused by dystocia. At parturition, the 8.7% of dystocia was also the 8.7% placental retention (however, not occurring in the same does with dystocia), which is a bit higher than the 3-5% rate reported by Braun (3).

According to the number of fetuses, the distribution found in the present study agrees with data previously reported for other breeds, in which most goats give birth to twins (8, 14, 16, 17). This result was also confirmed by the 2.0 mean number of kids/goat, in agreement with previous reports in other goat breeds (14, 16). One week before the expected date of parturition, the mean maternal plasma P4 concentrations were lower in does bearing singleton pregnancies than those bearing twins, while does bearing triple pregnancies showed the highest concentrations (8.9; 15.5 and 19.1 ng/mL, respectively). Although there was an apparent difference among the mean plasma concentrations in relation to the number of fetuses, the absence of statistically significant differences prevents the use of plasma P4 concentrations measurement to predict the number of fetuses bearing by the goat.

This result agrees with a previous study (8) in which plasma P4 concentrations were correlated to number of fetuses only in early pregnancy and converged toward the concentration of about 5 ng/mL by 35 days of pregnancy, irrespective by the number of fetuses. A more recent study showed that plasma P4 concentrations in does during mid-pregnancy was affected by fetal number, with higher concentrations in does carrying twins than singleton fetuses (18.91 ± 0.67 vs 14.51 ± 0.47 ng/mL). A study from Haldar et al. (6) found a significant association between the number of fetuses and plasma P4 concentrations with higher concentrations in does bearing triplets than twins or singleton pregnancy, between 84 and 21 days before parturition.

It should be noted that the plasma P4 concentrations observed in the present study were higher than the study from Jarrell and Dziuk (5-10 ng/mL at 35 days of pregnancy) (8), but comparable to those reported by Probo et al. (12.9 ng/mL) (14) at a similar timing of sampling. In contrast, the plasma P4 concentrations found in the present study were lower than those reported by Singh et al. (17) in mid-pregnancy does with singleton fetuses (14.5 ng/mL), but very similar when twin pregnancies were concerned (18.9 ng/mL). In the present study plasma P4 concentrations were also lower than those reported by Gaafar et al. (24.5 ng/mL) (5). However, as stated by Singh et al. (17), the different values reported by the different studies could be attributable to multiple factors, such as the breed, age, breeding system and especially by the method of P4 analysis.

The mean plasma C concentrations, ranging between 15.7 to 29.1 ng/mL, were markedly higher than the concentrations reported by Probo et al. (5 ng/mL) (14). The mean plasma C concentrations showed a similar trend of increase in dependence of the number of fetuses, but, differently to plasma P4 concentrations, the plasma concentrations of C were significantly higher in goats bearing triplets than singleton pregnancies. This result suggests that the measurement of plasma

C on a single blood sample collected two to one week before the expected day of parturition could be a useful tool to estimate in advance the number of fetuses that each goat will deliver. From a practical standpoint, this is important for parturition surveillance and assistance, allowing a more focused attention to the goats at the time of delivery and a better assistance to the newborn kids.

The increasing plasma C concentrations according to the number of fetuses found two to one week before parturition could be reasonably addressed to the cumulative secretion of cortisol by multiple fetuses at the end of pregnancy. In goats, in fact, the direct effect of the fetus in the process of triggering parturition through the activation of placental C21-steroid 17 α hydroxylase was demonstrated (4).

The association between increasing plasma P4 and C concentrations and increasing number of fetuses were also confirmed by the significant positive correlation found for both hormones.

The similar trend of higher plasma P4 and C concentrations with increasing number of fetuses were also corroborated by the significant correlation found between the two hormones. If higher plasma C concentrations could be attributable to the increased number of fetuses, the higher plasma P4 concentrations found in does bearing multiple fetuses could be the result of the cumulative activity of multiple corpora lutea, even if to the authors knowledge this aspect at the end of pregnancy was not investigated in the goats.

Although the significant positive correlation between maternal age and parity is not surprising, age and parity resulted positively correlated to the number of fetuses bearing by the does. It was reported that the ovulation rate in goats is influenced by many factors, among them age and parity have a significant effect, with a lower rate in primiparous goats (16).

In conclusion, although both plasma P4 and C were higher in does bearing multiple fetuses than does with singleton pregnancies, the results from the present study showed that the single measurement of plasma C, but not of P4, two to one week before the expected parturition in the Teramana goat might be useful to distinguish between does bearing singleton and triplet pregnancies and for a better surveillance and assistance at delivery. Therefore, it could represent a tool for the best management of reproduction in an at risk for extinction breed population.

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