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Effect of foetal number and sex on maternal thyroid and lipidic profiles in dairy goats

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ABSTRACT

The study aimed to evaluate the effects of the kids' number and sex on the thyroid and lipidic patterns of 30 dairy goats in the advanced mid-gestation and from 30 to 105 days of lactation.

Blood samples were monthly collected to measure circulating thyroid stimulating hormone (TSH), total and free triiodothyronines (T_3, fT_3) and thyroxines (T_4, fT_4); triglyceride (TG), total cholesterol (tCho), and very-low-density lipoprotein cholesterol (VLDL Cho).

Dams carrying a single foetus showed higher T_4 concentrations ($P < 0.04$) at >130–150 days than those carrying twins. Dams carrying one/two males had the highest fT_3 ($P < 0.001$) and fT_4 ($P < 0.03$) concentrations at 95–115 days and the highest fT_3 ($P < 0.0001$) and TSH ($P < 0.002$) at >115–130 days. Dams kidding male and female twins showed the highest TG and VLDL Cho concentrations ($P < 0.04$) at >70–105 days of lactation.

Single or twin births influenced the thyroid activity of pregnant goats, with a specific effect of foetal number on T_4 and of foetal sex on fT_3 and fT_4 concentrations. Kids' number and sex affected TG and VLDL Cho in lactating dams. This suggests that foetal number and sex are not a random chance event but an interactive process between maternal and foetal endocrine and metabolic processes.

1. Introduction

The growth of foetus and the development of mammary gland during the gestation and lactation periods are characterized by dynamic and consistent biochemical and hormonal changes of the dam (-Krajničáková et al., 2003; Skotnicka, 2003; Iriadam, 2007; Liotta et al., 2021). Thyroid hormones (THs) play an essential role in guaranteeing a number of the energy balance, metabolic homeostasis, and productive performances, assuming a pivotal role in the development and functionality of the foetus (Fowden et al., 2001) and the dam (Ramos et al., 2000). Specifically, thyroid activity increases in pregnant small ruminants (Todini, 2007; Sharma et al., 2015), showing the peculiar role of total thyroxine (T_4) secretion in metabolic adaptation to pregnancy, lactation, and energy balance in goats (Riis and Madsen, 1985). It is quite known that the valuable studies carried out in the eighties and nineties used the ovine foetuses or new born lambs as experimental model for thyroid function (Polk et al., 1986; Polk et al., 1988; Polk et al., 1994; Ferreiro et al., 1987; Ferreiro et al., 1988; Hetzel and Stanbury, 1994), including a recent and precious study of Steinhauser et al. (Steinhauser et al., 2021).

With regards to the lipidic profile, it is well known that the serum cholesterol concentration generally varies inversely with thyroid activity, as recently observed also in Nicastrese goats, in which free thyroxine (fT_4) negatively correlated with total cholesterol (tCho), triglyceride (TG) and very low density lipoprotein cholesterol (VLDL Cho); neverthless, in pregnant goats (Ismail et al., 2008), as well as in clinically healthy sheep (Nazifi et al., 2003), no correlations were found. The effects of pregnancy and post-kidding stages on lipidic profile and milk fat accumulation in lactating goats mammary gland represent a complex genetic and molecular mechanisms (Lin et al., 2013). Specifically, in goats, circulating tCho and TG showed lower concentrations in postpartum than before, approaching the lactation start and the increase of body energy consumption (Skotnicka et al., 2011; Cepeda-Palacios et al., 2018).

According to what would be expected, the number of foetuses *in utero* can or cannot cause significant differences in the haematochem-

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ical and hormonal parameters in goats, with a special emphasis for thyroid and lipidic patterns (Cepeda-Palacios et al., 2018; Khan and Ludri, 2002; Hefnawy et al., 2011; de Souza Castagnino et al., 2015).

In pregnant goats, little information is available regarding thyroid stimulating hormone (TSH) and THs when single or twin births are considered. Specifically, total triiodothyronine (T_3) values were significantly higher during all the periods of sampling in single compared to twin bearing goats, while T_4 was significantly lower in twin compared to single bearing goats (Khan and Ludri, 2002; Khan and Ludri, 2002). What is more, some researches focused their efforts on how the mother could influence the foetal sex before and after conception (Hardy, 1997; Grant et al., 2008; Grant and Chamley, 2010) in mammalian species and humans, but not *vice versa*. The importance of considering the foetal sex as a factor that influences the dynamic physiological state of the mother was demonstrated by the finding of a gender and genetic specific foeto-maternal dialogue (Anand-Ivell et al., 2011), when the evolutionary differentiation of placentae is considered (Klisch and Mess, 2007). Foetal sex plays an important role in modifying the course and complications related to pregnancy and may also have an impact on maternal health and well-being during and after pregnancy both in humans (Al-Qaraghouli and Fang, 2017) and in animal species (Nazifi et al., 2002; Probo et al., 2011; Tóthová et al., 2014; Osman and Omer, 2016).

Nowadays, the knowledge about whether there are differences in thyroid and lipidic profiles of pregnant and lactating goats according to the foetal number and sex are limited. Hence, it is possible to presume that a crosstalk between offspring and mother occurs, when the different foetal number and sex are considered. The aim of the present study was to evaluate the effects of single or twin births and related kids' sex on the thyroid and lipidic patterns of dairy goats in the advanced mid-gestation (>95–150 days), and from 30 to 105 days of lactation.

In particular, in this study we considered the Nicastrese goat. This breed, together with the Aspromontana and the Rustica di Calabria, represents one of the native goat populations of Calabria, a southern Italian region characterized by small- to large-goat farms, extending from the free-ranging to the semi-sedentary and natural pasture-based management (Usai et al., 2006). These goats have a black coat with white abdomen, limbs, and part of the head. Their head shows a straight frontal-nasal profile and characteristic lyre-shaped horns both in males and females. The average adult body weight is 78 kg for males and 46 kg for females. The primiparas' lactation lasts about 180 days, with a milk production of 150 kg; multiparous goats, instead, produce about 210 kg of milk during a 220- to 260-day lactation. Milk has an average fat content of 4.30%, protein of 3.50%, and lactose of 4.70% (Pino et al., 2021). The natural grazing land of Calabria region is represented by distinctive and unique aromatic Mediterranean plant biodiversity, conferring the typical physico-chemical, microbiological, and sensorial characteristics to the milk and its product, the Nicastrese cheese (Randazzo et al., 2014; Nicoloso et al., 2015; Pino et al., 2018; De Nardo, 2014).

2. Materials and methods

The experimental protocol was approved by the Ethical committee of the Department of Veterinary Science of the University of Messina, Italy (code 046/2020). The research complied with guidelines of Good Clinical Practices (EMEA, 2000). This study was performed according to the Italian and European regulations on animal welfare (Directive 2010/63/EU).

2.1. Animals

In the present study blood samples and body condition score (BCS) data were obtained from 30 multiparous Nicastrese goats during the transition period. Animals had an age comprised between 3 and 4 years and were sampled from a flock of 400 individuals, randomly selecting dams showing physical signs of pregnancy. The pregnancy diagnosis was mainly based on abdominal inspection, transabdominal palpation, and increased live weight, which are reliable only after the second half of gestation. The flock was reared in a semi-extensive farm, located in Catanzaro (39.048°N; 16.5653°E, 1215 m above the sea level). All animals were subjected to the same treatments of feeding and management: they all grazed in the same area, consisting of natural woodland pasture, from 8:00 a.m. to 4:00 p.m., and then they were confined in a stable covered with straw bedding area during the night. In this indoor area, all the goats, independently from their physiological status, were fed with meadow hay (on average 1 kg/head/day; CP: 110.9 g kg^{-1} of DM; EE: 25.0 g kg^{-1} of DM; NDF: 521.9 g kg^{-1} of DM; NEL 0.65 UFL kg^{-1} of DM) and concentrate (on average 0.7 kg/head/day; CP: 155.1 g kg^{-1} of DM; EE: 41.2 g kg^{-1} of DM; NDF: 196.1 g kg^{-1} of DM; NEL 1.08 UFL kg^{-1} of DM); water was available *ad libitum*.

The inclusion criteria of goats were the following: 1) reproductive history: the presence of normal cyclicity during the previous breeding seasons, absence of reproductive pathologies, like endometritis, pyometra or other processes related to the loss of fertility or physiological deliveries of viable kids; 2) absence of inflammatory and infectious processes occurred and treated with antibiotics or anti-inflammatory within a month before the start of blood samples. All the included animals got pregnant between the second half of June and the beginning of July; the first week after the seasonal mating was considered as the first week of gestation. All goats had a physiological mean gestational length of 150 days (145–155 days) and spontaneous delivery. All kids were alive, healthy, and diagnosed as clinically normal at the postpartum clinical examination. The kids were fed on their dam's milk until weaning.

2.2. Samples and analyses

All the animals were examined six times from September 2020 to January 2021 along the transitional period, from the advanced mid-gestation (at 95–115, >115–130, >130–150 days), and the first lactating period (at 30–50, >50–70, >70–105 days). The sampling was always performed between 7:00 and 9:00 a.m. to minimize the circadian rhythms' effect on endocrine parameters, in a quiet condition by the same operator. The goats' BCS was assessed according to Santucci et al. (Santucci et al., 1991) with a score ranging from 1 (thin) to 5 (fat). Blood sampling was carried out collecting approximately 5 mL of blood from the external jugular venepuncture into tubes containing clot activator and separating gel (Terumo Corporation, Tokyo, Japan) and placed in a sterile glass tube; serum was centrifugated within 60 min from the collection and refrigerated at 4 °C for hormonal analyses, performed within one week at the Veterinary Diagnostic Center BIOGENE (Catania, Italy).

Serum thyroid stimulating hormone (TSH), total and free triiodothyronine (T_3, fT_3) and thyroxine (T_4, fT_4) concentrations were assessed using a human homologous solid-phase, two-site chemiluminescent immunometric assay (Immulite 2000, Siemens Medical Solutions-Diagnostics-USA), according to the manufacturer's instructions. All assays were validated for linearity using goat serum prior to use.

The intra- and inter-assay coefficients of variation (CVs) were the following: for TSH, 5.5% and 9.5% at TSH concentrations of 0.2 and 2.35 ng/mL; for T_3, 12% and 5.5% at T_3 concentrations of 73 ng/dL and 171 ng/dL; for fT_3, 9.1% and 5.4% at fT_3 concentrations of 3.2 pg/dL and 13 pg/dL; for T_4, 11.1% and 5.6% at T_4 concentrations of 1.8 μg/dL and 16 μg/dL; and for fT_4, 3.0% and 10.2% at fT_4 concentrations of 4.82 ng/dL and 0.51 ng/dL.

The sensitivity of the assay was 0.01 ng/mL for TSH, 19 ng/dL for T_3, 1.0 pg/mL for fT_3, 0.3 μg/dL for T_4, and 0.11 ng/dL for fT_4.

Lipid profiles (triglyceride [TG] and total cholesterol [tCho]) were analysed by enzymatic colorimetric method, using BT3500 Biotechnic Instruments; very-low-density lipoprotein cholesterol (VLDL Cho) content was calculated as triglycerides divided by 5.

2.3. Statistical analyses

The statistical analyses were carried out using JMP® 15 (SAS Institute Inc., Cary, NC, USA). The parameters and results were described as mean ± standard deviation of the mean. Before being analysed, all the parameters were assessed graphically for normality and those that were not normally distributed were transformed as appropriate. The main focus of this work was to compare all the examined parameters for single *vs* twin pregnancy and for the kids' sex (male, female, male plus female) within different phases of lactation and pregnancy; therefore, we performed, an ANOVA that included the single animals as random effect; in this way, it also accounted for the correlation of the repeated measurement within each subject (Detry and Ma, 2016). Moreover, in order to assess the effect of both the period of lactation/pregnancy and the number/sex of the kids, we added both of them, as well as their interaction and the random effect of the single animals, in a second model. Tukey's HSD post-hoc test discriminated the differences between the groups. The conventional threshold of 0.05 was used to determine the significance of the results.

Results

Of the 30 sampled goats, 13 delivered a single kid and 17 goats delivered twin kids; 13 goats delivered one or two female kids, 17 one or two male kids, and four a male and a female.

Maternal thyroid and lipidic profiles in Nicastrese goats (*Capra hircus*) during the second half of pregnancy and the first 80 postpartum days abundances for this study were recently published elsewhere (Liotta et al., 2021).

The mean ± SD and the range of circulating TSH, total and free iodothyronines, triglyceride, total and VLDL cholesterol concentrations, and BCS measured during the transition period, from the advanced mid-gestation (at 95–115, >115–130, >130–150 days), and the first lactating period (at 30–50, >50–70, >70–105 days) are shown in Figs. 1–3 and Tables 1–4. The following parameters were not normally distributed: BCS, tCho, TG, and TSH.

The evaluation of the effect of single or twin foetuses along the second half of pregnancy showed that dam carrying single foetus had higher T_4 concentrations during all the second half of pregnancy, with significant higher values at >135–150 ($P < 0.04$) days of pregnancy compared to those carrying twin foetuses (Fig. 1). At the same time, the number of the foetuses in mothers did not cause significant differences in TSH, T_3, fT_3, fT_4 (Table 1), TG, tCho, and VLDL Cho concentrations, as well as BCS (Table 2).

A second model was performed, which included the number of kids, the gestation phase and their interaction. In this case, the period of gestation significantly influenced TG concentration, which was higher at >130–150 than at 95–115 days. The other factors were not significant for all the rest of the parameters.

Regarding the foetal sex, at 95–115 days, dams carrying male single or twin foetuses showed the highest fT_3 ($P < 0.001$) (Fig. 2) and fT_4 ($P < 0.03$) (Fig. 3) concentrations at >95–115 days. Moreover, at >115–135 days, dams carrying male kid(s) had higher fT_3 ($P < 0.0001$) and dams carrying only male or female kid(s) had higher TSH ($P < 0.002$) (Fig. 4) concentrations than those carrying a male and a female.

In the alternative model, we found a significant effect of the sex of the kid(s) for fT_3 ($P < 0.008$), with the highest concentrations in dams carrying male kid(s) and the lowest in those carrying female kid(s). Moreover, the gestation phase was associated with TG concentration ($P < 0.002$), with significantly lowest values at 95–115 days.

The evaluation of the effect of single or twin foetuses along the first lactating period showed that dams, independently from the number of foetuses, presented superimposable TSH and total and free iodothyronine concentrations, lipidic profile, and BCS (Table 3).

Applying the model also including the lactation phase and its interaction with the number of kids, the lactation phase was significant for tCho ($P < 0.04$), with higher concentrations at >50–70 than at 30–50 days, TG ($P < 0.01$), with higher values at 30–50 than at >50–70 days, and for T_4 ($P < 0.01$), which was higher at 70–105 than at 50–70 days. Moreover, TG were also affected by the number of kids ($P < 0.006$), they being higher in dams with a single foetus, and its interaction with the lactation phase ($P < 0.02$), with the highest con-

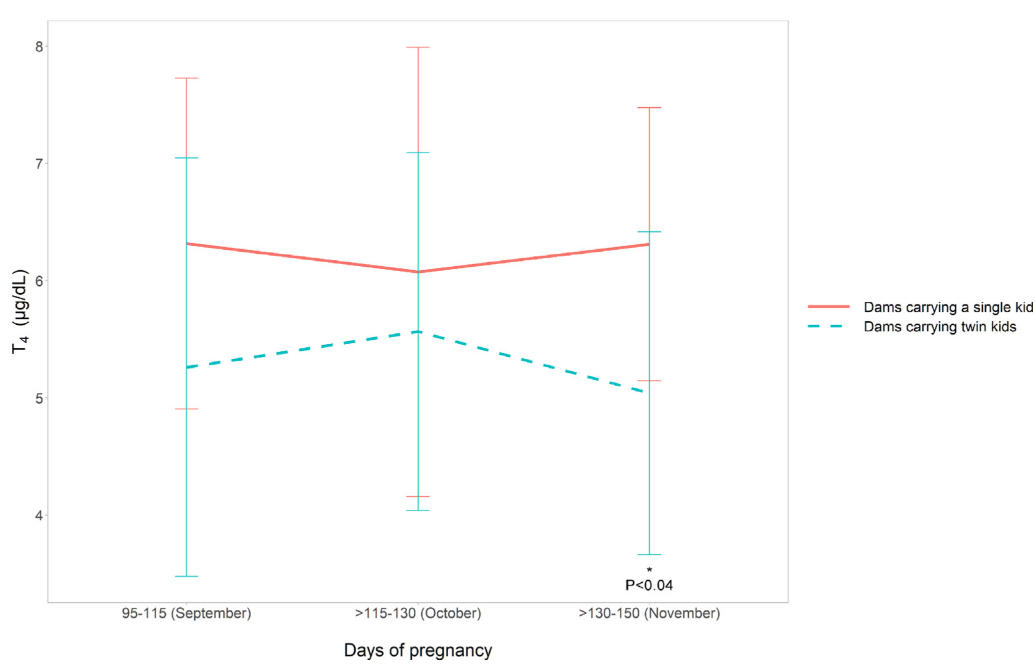

Fig. 1. Circulating total thyroxine (T_4) concentrations (mean ± SD) in 30 pregnant Nicastrese goats carrying single or twin kids.

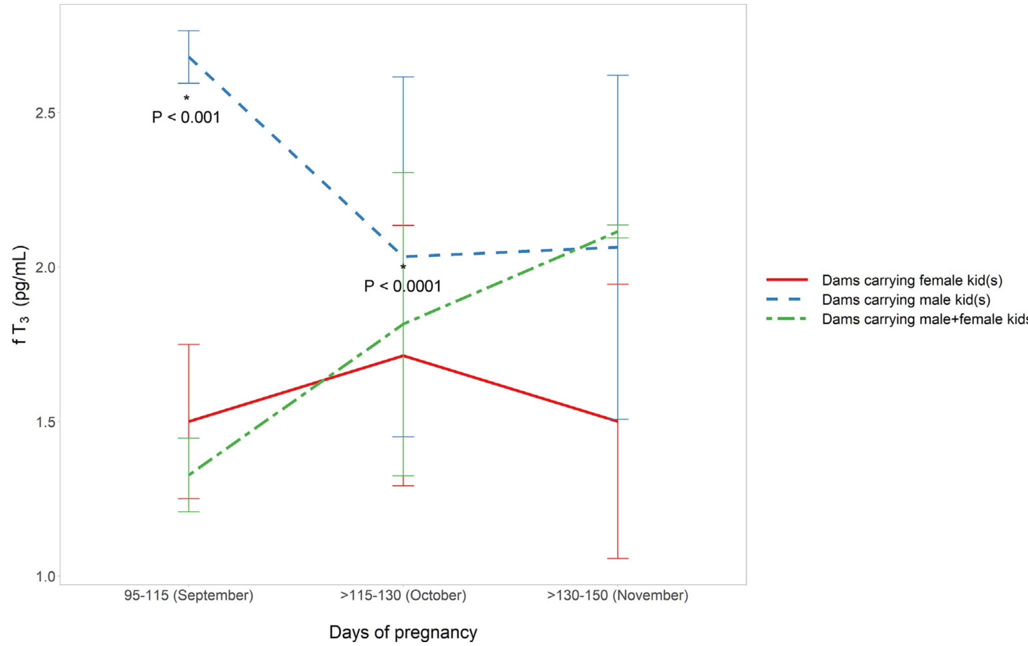

Fig. 2. Circulating free triiodothyronine (fT_3) concentrations (mean ± SD) in 30 pregnant Nicastrese goats according to the sex of their kids.

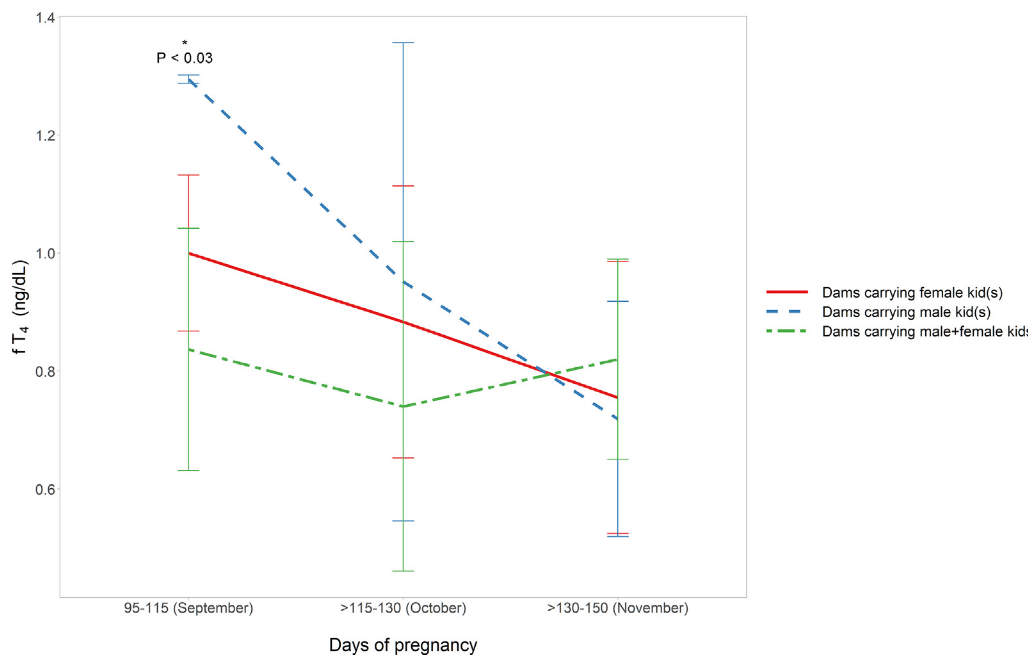

Fig. 3. Circulating free thyroxine (fT_4) concentrations (mean ± SD) in 30 pregnant Nicastrese goats according to the sex of their kids.

centrations in mothers of a single foetus at 30–50 days and the lowest in those of twins at 30–50 and > 50–70 days.

Lactating dams that gave birth to male and female twin foetuses showed the highest TG and VLDL Cholesterol concentrations ($P < 0.04$) at >70–105 days of lactation (Table 4).

When the second model was performed, neither the sex of the kids nor the lactation phase or their interaction had a significant effect on the analysed parameters.

4. Discussion

The overall mean TSH, T_3, fT_3, T_4, and fT_4 concentrations recorded in this study are in accordance with physiological ranges described for clinically healthy pregnant and postpartum goats, as reported by different Authors (Todini, 2007; Hefnawy et al., 2011; McDonald et al., 1988; Celi et al., 2008); and in pregnant and postpartum sheep for TSH (Sharma et al., 2015). Moreover, data obtained exclude the effect of circadian rhythms because the blood samples were always carried out at the same time point of the day, between 7:00 and 9:00 a.m., both in pregnant and postpartum periods.

In the present study, the highest values of T_4 concentrations observed along all the late mid-gestation period in dams carrying single foetus confirmed data previously described by Khan and Ludri (2002), that showed that the lower concentration of T_4 in goats carrying twins compared to single kid may be due to decrease secretion rate and not due to transplacental transfer as per McDonald et al. (1988).

Table 1
Circulating thyroid stimulating hormone (TSH), total and free triiodothyronine (T_3, fT_3), and total and free thyroxine (T_4, fT_4) concentrations (mean ± SD) of 30 Nicastrese goats in different pregnancy phases. During each phase, concentrations of goats carrying single or two fetuses were compared and significant differences are indicated in bold and with different superimposed letters ($P < 0.04$).

Days of pregnancy	95–115 d		>115–130 d		>130–150 d	
Number of kids	Single	Twin	Single	Twin	Single	Twin
TSH (ng/mL)	0.19 ± 0.06^{A}	0.16 ± 0.08^{A}	0.19 ± 0.08^{A}	0.15 ± 0.06^{A}	0.20 ± 0.06^{A}	0.16 ± 0.05^{A}
T_3 (ng/dL)	84.27 ± 5.94^{A}	93.54 ± 33.42^{A}	96.09 ± 24.77^{A}	92.24 ± 28.62^{A}	88.21 ± 13.18^{A}	90.38 ± 22.41^{A}
fT_3 (pg/mL)	1.94 ± 0.63^{A}	1.57 ± 0.54^{A}	1.98 ± 0.58^{A}	1.75 ± 0.42^{A}	1.78 ± 0.48^{A}	1.83 ± 0.63^{A}
T_4 (μg/dL)	6.32 ± 1.41^{A}	5.26 ± 1.78^{A}	6.08 ± 1.92^{A}	5.57 ± 1.53^{A}	**6.31 ± 1.16^{A}**	**5.04 ± 1.38^{B}**
fT_4 (ng/dL)	1.06 ± 0.24^{A}	0.99 ± 0.22^{A}	1.01 ± 0.35^{A}	0.79 ± 0.25^{A}	0.72 ± 0.23^{A}	0.77 ± 0.19^{A}

When results are in bold, they are significantly different ($p < 0.05$).

Table 2
Body condition score (BCS) and lipidic panel, including circulating cholesterol, triglyceride, and very-low-density lipoprotein cholesterol (VLDL Cho) concentrations (mean ± SD) of 30 Nicastrese goats in different pregnancy phases. Whitin each phase, no statistically differences associated with the sex of the kids were found.

Days of pregnancy	95–115 d		>115–130 d		>130–150 d	
Number of kids	Single	Twin	Single	Twin	Single	Twin
BCS (score 1–5)	2.75 ± 0.00	2.79 ± 0.17	2.78 ± 0.21	2.69 ± 0.29	2.55 ± 0.31	2.68 ± 0.28
Cholesterol (mg/dL)	85.33 ± 12.34	84.86 ± 31.84	101.88 ± 30.80	103.31 ± 29.24	107.27 ± 23.02	93.73 ± 18.48
Triglyceride (mg/dL)	23.00 ± 5.20	39.00 ± 36.78	58.00 ± 38.34	71.77 ± 37.08	72.91 ± 27.50	59.18 ± 23.90
VLDL Cho (mg/dL)	4.60 ± 1.04	7.80 ± 7.36	11.6 ± 4.67	14.35 ± 7.42	14.58 ± 5.50	11.84 ± 4.78

Table 3
Circulating thyroid stimulating hormone (TSH), total and free triiodothyronine (T_3, fT_3), total and free thyroxine (T_4, fT_4) concentrations (mean ± SD) of 30 Nicastrese goats in different lactation phases. Whitin each phase, no statistically differences associated with the sex of the kids were found.

Days of lactation	30–50 d			>50–70 d			>70–105 d		
Sex of kids	♀	♂	♂+♀	♀	♂	♂+♀	♀	♂	♂+♀
TSH (ng/mL)	0.14 ± 0.06	0.12 ± 0.04	0.11 ± 0.01	0.14 ± 0.02	0.14 ± 0.03	0.15 ± 0.04	0.11 ± 0.02	0.14 ± 0.03	0.11 ± 0.01
T_3 (ng/dL)	66.36 ± 11.41	91.30 ± 12.24	76.40 ± 5.37	77.26 ± 14.89	77.22 ± 15.75	62.25 ± 7.42	84.74 ± 20.01	80.60 ± 13.18	72.70 ± 0.14
fT_3 (pg/mL)	1.43 ± 0.36	1.75 ± 0.28	1.74 ± 0.28	1.65 ± 0.52	1.80 ± 0.22	1.97 ± 0.32	1.63 ± 0.45	2.05 ± 0.36	1.65 ± 0.16
T_4 (μg/dL)	5.62 ± 0.57	6.44 ± 0.63	5.38 ± 1.32	5.56 ± 0.67	4.76 ± 0.76	4.62 ± 2.23	6.30 ± 1.40	5.23 ± 1.22	5.10 ± 0.93
fT_4 (ng/dL)	1.02 ± 0.23	1.27 ± 0.19	1.01 ± 0.00	1.12 ± 0.13	1.10 ± 0.23	0.97 ± 0.04	1.08 ± 0.26	1.13 ± 0.28	0.82 ± 0.28

Table 4
Body condition score (BCS) and lipidic panel, including circulating cholesterol, triglyceride, and very-low-density lipoprotein cholesterol (VLDL Cho) concentrations (mean ± SD) of 30 Nicastrese goats in different lactation phases. During each phase, concentrations of goats that delivered male kid(s) (♀), female kid(s) (♂), and male and female kids (♂+♀) were compared and significant differences are indicated in bold and with different superimposed letters ($P < 0.04$).

Days of lactation	30–50 d			>50–70 d			>70–105 d		
Sex of kids	♀	♂	♂+♀	♀	♂	♂+♀	♀	♂	♂+♀
BCS (score 1–5)	2.90 ± 0.22^{A}	3.00 ± 0.22^{A}	2.63 ± 0.18^{A}	2.93 ± 0.12^{A}	2.81 ± 0.24^{A}	2.88 ± 0.18^{A}	2.85 ± 0.22^{A}	2.90 ± 0.14^{A}	2.63 ± 0.18^{A}
Cholesterol (mg/dL)	80.80 ± 4.76^{A}	80.00 ± 6.48^{A}	75.00 ± 15.56^{A}	83.29 ± 12.88^{A}	75.44 ± 10.75^{A}	87.50 ± 21.92^{A}	85.20 ± 7.82^{A}	73.20 ± 10.99^{A}	88.00 ± 15.56^{A}
Triglyceride (mg/dL)	29.80 ± 7.19^{A}	22.00 ± 6.85^{A}	25.50 ± 6.36^{A}	25.00 ± 3.00^{A}	25.22 ± 2.99^{A}	22.50 ± 3.54^{A}	**25.80 ± 1.30^{B}**	**26.80 ± 2.49^{AB}**	**31.00 ± 2.83^{A}**
VLDL Cho (mg/dL)	5.96 ± 1.44^{A}	4.40 ± 1.37^{A}	5.10 ± 1.27^{A}	5.00 ± 0.60^{A}	5.04 ± 0.60^{A}	4.50 ± 0.71^{A}	**5.16 ± 0.26^{B}**	**5.36 ± 0.50^{AB}**	**6.20 ± 0.57^{A}**

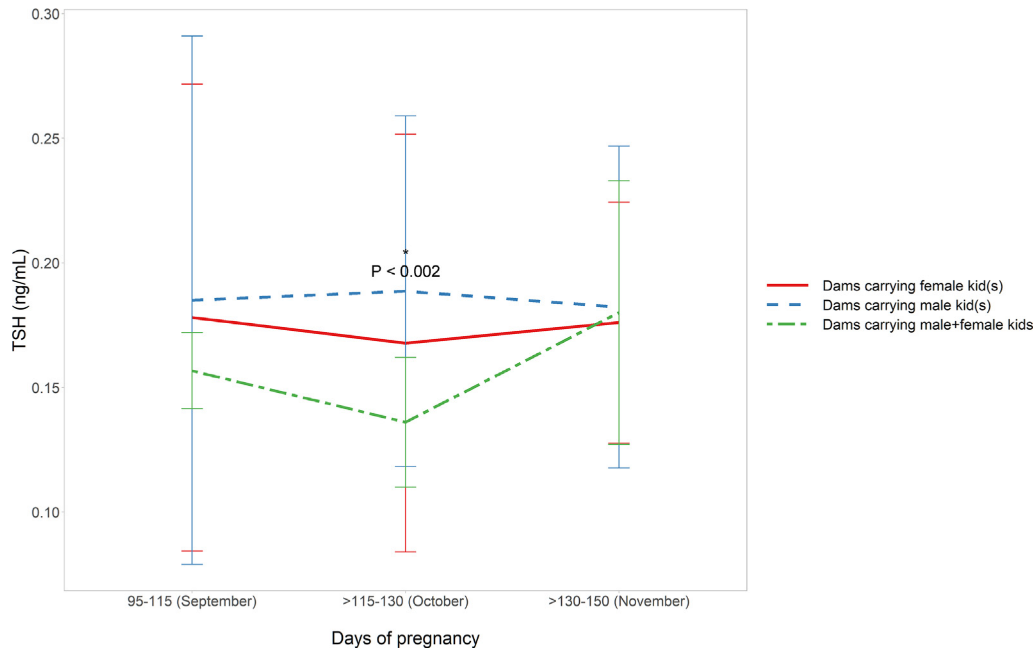

Fig. 4. Circulating thyroid stimulating hormone (TSH) concentrations (mean ± SD) in 30 pregnant Nicastrese goats according to the sex of their kids.

This decrease in T_4 concentrations could favour the mammary gland in the partitioning of nutrients between mammary and non-mammary tissue, as previously recorded by Riis and Madsen (1985), decreasing the adverse effect of nutrients deficiency in body tissue at the onset of lactation. This result corroborates the peculiar role of T_4 secretion in metabolic adaptation to pregnancy and energy balance in goats (Riis and Madsen, 1985).

In the same way, this result was consistent with the knowledge that the ovine foetus synthetizes endogenous THs during the second half of pregnancy and that localized placental transport and regulation of THs originating from the dam potentially play a role in placental development and function, influencing the foetal growth, throughout the pregnancy (Forhead and Fowden, 2014; Chen et al., 2005; Lanham et al., 2011; Segar et al., 2013). Moreover, maternal and foetal hormonal thyroid synthesis and release may substantially enhance the T_4 values over the remainder of pregnancy, according to data that the majority of T_4 in the ovine foetal circulation at day 135 of gestation is likely produced endogenously by the foetal thyroid (Steinhauser et al., 2021). On these bases, it is possible to presume that the significantly higher T_4 values at >130–150 days of pregnancy in dams carrying single foetus compared to those carrying twin foetuses could be a consequence of the start in foetus's thyroid activity; in this sense a probable superimposed negative feedback induced by twin foetuses' synthesis on the maternal total iodothyronines homeostasis occurs, indicating an activation of potential more intense compensatory mechanism of dams carrying twins. Hence, while there was little evidence that ovine maternal THs crossed the placenta into the foetal circulation, at least in the second half of pregnancy (Hopkins and Thorburn, 1971; Dussault et al., 1972), a new scientific segment was recently added. In fact, a study of Steinhauser et al. (2021) showed that a variety of transporters is present in the ovine placenta for THs due to the necessity of moving them across membrane, concentration gradients, and transporting different forms of THs (Steinhauser et al., 2021); in addition, placental deiodinases regulate TH availability to placental and foetal cells (Steinhauser et al., 2021). Whether there is a similar mechanism in goats, whereby the placenta regulates maternal thyroid hormone concentrations, is unknown but it is presumable that similar events may be occurring.

The presence of the highest fT_3 and fT_4 concentrations in pregnant goats from the advanced mid-gestation confirmed the active metabolic role of free iodothyronines also in this species, as previous recorded in both pregnant mares (Fazio et al., 2016; Fazio et al., 2016) and donkeys (Fazio et al., 2012). On these bases it is possible to presume that the highest free iodothyronines observed around the late mid-gestation of dam carrying single or twin male foetuses could be due the activation of the placental permeability with special emphasis for free iodothyronines and/or the ratio between linked and free hormones. However, there is limited information available in the current literature discussing the comparative role of THs in reproduction and reproductive tract development among species (Choksi et al., 2003; Rijntjes et al., 2017), with especial emphasis for rat and mice models. It is generally accepted that rat's postnatal development of the testis is highly dependent on the coordinated growth and differentiation of both somatic (Leydig and Sertoli) cells and germ cells (Picut et al., 2015). On the other hand, studies indicate that THs have little to no effect on female reproductive tract development. Besides, the gonadotropic hormones and THs have been implicated to play an important role in testicular, and more particularly in Leydig and Sertoli cell development (Gao et al., 2014). Indications that effects of THs on testicular development are at least partially mediated by thyroid hormone receptor α1 (TRA1) come from studies in *Tra1* knockout mice (Holsberger et al., 2005). This available evidence suggests a consistent sex role on the thyroid homeostasis of dams carrying single or twin male foetuses.

It should also be considered that during lactation the thyroid patterns may also be affected by the haemodilution resulting from a physiological increasing water metabolism to mammary gland trough the vascular system, as previous observed for haematological and biochemical compounds in lactating ewes (Brito et al., 2006). Hence, it is possible to explain the absence of significant differences in total and free iodothyronines along this functional period. However, it is plausible that there was a large amount of individual variation in the animals along different physiological period, which could be one additional reason why no significant differences were found.

Regarding the effect of single or twin foetuses on the lipidic endpoint of lactating mothers, the highest TG and VLDL Cho concentrations observed in goats carrying twin male and female foetuses at >70–150 days may also reflect the difference in nutrient availability and its utilization in catabolic or anabolic manner according to the lactogenesis phase. These results may be explained according to researches of Forsyth et al. (1985) and Kornalijnslijper et al. (1997) that reported higher growth hormone (GH) concentrations in twin foetus bearing compared to single foetus bearing goats during last week of pregnancy and the highest levels on the day of parturition. In addition, it is well known the lipolytic action of GH enabling depot fat to be mobilized for energy for the parturition and initiation of lactation by increasing the availability of milk precursor to meet the increased demand of energy for the initiation of milk secretion (Khan and Ludri, 2002). Taken together, these findings explained the highest total and VLDL Cho in twin bearing goats. Moreover, the evidence that the gestation period was associated with TG concentration, with significantly highest values in the last phases, confirms the lipidic trend previously recorded during the last gestational phase in small ruminants (Liotta et al., 2021; Waziri et al., 2010), which was attributed to an increased hepatic TG synthesis (Sharma et al., 2015; Waziri et al., 2010; Okonkwo et al., 2010; Tharwat et al., 2013). This effect was probably maintained also in early lactation, as shown by the higher TG concentrations at 30–50 than at >50–70 days when the number of kids was accounted in the model. Also, in the model that included the effect of the number of kids, the lactation phase was significant for tCho, with higher concentrations at >50–70 than at 30–50 days, and T_4, which was higher at 70–105 than at 50–70 days. This confirms the potential role of THs on milk production, with an inverse relationship between milk yield and thyroid activity. In addition, it is plausible that the highest T_4 values at the end of lactation could be due the presumed reduced local conversion of T_4 to T_3 through 5′-MD activity in the mammary gland during early and late lactation, compared to the mid-lactation phase (Slebodziński and Twardon, 2004); this changes in maternal hormonal pattern reflect T_4 and T_3 milk concentrations, suggesting that the quantity of THs available to newborn kids in milk may have a physiological role during the early postnatal life of suckling goats (Slebodziński and Twardon, 2004).

Results of the present study identified differences in THs and lipidic dynamics in maternal blood according to the different physiological periods, encouraging kindly additional research in this mean topic. This will help to create awareness that foetal number and sex are not just a random chance event but an interactive process between endocrine and metabolic processes of mother and foetus.

Data obtained in this study showed an endocrine and metabolic crosstalk between dam and foetus, according to the number and sex of the kids, along the advanced mid-gestation and approaching the lactation start. These results also indicate that thyroid hormones are required for foetal and maternal metabolic processes to ensure a normal dynamic planning in pregnant and lactating dams. These data contribute to the specific knowledge on the physiological thyroid and lipidic patterns also of Nicastrese goats, mainly intended to milk production for local cheeses manufacturing. An additional scientific segment could extend knowledge about this subject also in the first stage of pregnancy and the rest of lactation. Consideration of such information is important in designing, conducting, and interpreting studies to assess the potential effects of thyroid hormones on reproduction and development. It is possible to presume that the maternal–placental–foetal interaction has significant biological implications of which the mechanisms may be genetic, epigenetic, or endocrine. Determination of foetal sex may therefore be an important assessment in management of pregnant and lactating goat, and related kids.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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