Total and free iodothyronines profile in the donkey (*Equus asinus*) over a 12-month period

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Received July 26, 2010
Accepted May 16, 2012

Abstract

Several studies show the correlation between thyroid function and reproductive activity in horses, but no data are available in donkeys. The aim of this study was to determine physiological thyroid changes occurring in 10 pregnant and 14 barren donkeys over a period of 12 months. Blood samples were collected monthly from the jugular vein of pregnant and barren donkeys from June 2008 to June 2009. No significant differences ($P < 0.05$) in iodothyronine concentrations were observed between pregnant and barren donkeys in the same month. Seasonal total thyroxine (T_4) and free triiodothyronine (fT_3) patterns remained unmodified, regardless of physiological state, with the lowest T_4 levels at September and the highest at February, and the lowest fT_3 levels at June and the highest at July, in both pregnant and barren donkeys. Ranges of total triiodothyronine (T_3) concentrations in pregnant donkeys over a 12 months period ranged from 1.57 to 2.90 nmol/l, T_4 from 31.01 to 63.67 nmol/l, fT_3 from 3.15 to 15.52 pmol/l and free thyroxine (fT_4) from 22.47 to 33.69 pmol/l. Mean T_3 concentrations in barren donkeys over a 12 months period ranged from 1.64 to 3.37 nmol/l, T_4 from 42.95 nmol/l and 61.90 nmol/l, fT_3 from 3.15 to 11.19 pmol/l and fT_4 from 25.46 to 35.06 pmol/l. This is the first study that provided a seasonal thyroid hormonal profile in healthy barren and pregnant donkeys.

Thyroid gland, reproduction, seasonality

The thyroid system is involved during physical and psychological challenges in domestic animals through the release of iodothyronines (Anderson et al. 1988; Hegstad-Davies 2006; Fazio et al. 2007). A relationship between thyroid function and seasonal reproductive activity was investigated in mares (Huszenicza et al. 2000) and significant differences in plasma thyroxine concentrations were observed between cyclic and anoestrous mares during anovulatory season (Fitzgerald and Davison 1998).

Many reproductive aspects in donkey show close similarity with the mare (Ginther et al. 1987; Lemma et al. 2006; Carluccio et al. 2008). Only few studies have been carried out on the physiology of reproduction in donkeys (Henry et al. 1998; Pugh 2002; Carluccio et al. 2007; Taberner et al. 2008) but no data are available on the thyroid function in this species.

The current study aimed to investigate the seasonal profile of thyroid hormones in pregnant and barren donkeys over the period of 12 months.

Materials and Methods

Experimental animals

The study was carried out on a total of 24 clinically healthy Ragusana donkeys, aged 4–13 years, living on a farm located in Southern Sicily at Agrigento (37° 19' 19" N latitude; 13° 35' 23" E longitude). The breeding season of donkeys in Sicily occurs from February to July. The study took place from June 2008 to June 2009. The animals were divided into two groups according to the physiological state (Group 1 with 10 pregnant donkeys; Group 2 with 14 barren donkeys). The animals were kept in paddocks during the day and in 20.9 m^2 individual boxes at night, with inter-individual visual contact. They were familiar with their group members.

Both barren and pregnant donkeys had free access to pasture during the day and they were also individually fed with 2 kg of a grain supplement, straw and vetch hay twice a day. The composition and quantity of individual

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supplement was equal between groups 1 and 2 in order to minimize the effect of different diets on the variables studied.

Donkeys of group 1 became pregnant at the same time of the year (April-June) and the pregnancy was nearly of the same duration in all animals studied. The stage of pregnancy at the time of blood sampling was different in all animals studied, and the data were normalized in relation to medical history and confirmed by the timing of parturition that occurred 5 to 10 days after the final blood sampling. Consequently, at the beginning of the first month of sampling (June), pregnant donkeys were not at the same month of pregnancy. They showed normal pregnancy and foaled spontaneously, eutocic delivery, after 372.3 ± 5.48 days of pregnancy. All foals were healthy and viable. Donkeys of Group 2 were selected as control.

Blood sampling

Blood samples were taken from the jugular vein once monthly over the 12 months. All samples were taken between 7.00 and 9.00 in the morning to minimize the effect of the circadian rhythm on hormone measurements, in quiet conditions by the same operator. Blood samples were collected in evacuated tubes (Venoject, Terumo®; Belgium) without anticoagulant and were, immediately after withdrawal, refrigerated at 4 °C and were subsequently (within 1 h) centrifuged for 15 min at 1500 × *g*. Serum was stored in polystyrene tubes at -20 °C and assayed for triiodothyronine (T_3), thyroxine (T_4), free triiodothyronine (fT_3) and free thyroxine (fT_4).

Variables and methods

Serum total (T_3, T_4) and free (fT_3, fT_4) iodothyronine concentrations were analysed in duplicate using a commercial EIA Kits (RADIM, Pomezia, Roma, Italy). Sensitivities of the assays were as follows: 0.24 nmol/l for T_3, 29.63 nmol/l for T_4, 0.15 pmol/l for fT_3 and < 12.87 pmol/l for fT_4. Precision was evaluated determining the repeatability and reproducibility of the assay (intra- and interassay variability, CVs), on 3 different sera at different total and free iodothyronine concentrations. The respective intra- and interassay CVs were as follows: 7.3% and 11.4% for T_3, 2.3% and 5.7% for T_4, 4.2% and 11.9% for fT_3, 6.6% and 9.6% for fT_4, respectively.

Statistical analysis

Data are presented as means ± standard deviation (SD). Statistical analysis was done by one way repeated measures analysis of variance (RM-ANOVA). Significant differences of all the months versus each other and between pregnant and barren donkeys were established using Bonferroni's multiple comparison test. The level of significance was set at $P < 0.05$, $P < 0.02$ and $P < 0.001$. All calculations were performed using the PRISM package (GraphPad Software Inc., San Diego, CA). The relation between thyroid hormone concentrations was evaluated by correlation and linear regression.

Results

Mean total and free iodothyronine concentrations of pregnant and barren donkeys over a period of 12 months ± standard deviation are reported in Figs 1 and 2, respectively. Compared to the previous month, significantly higher concentrations of fT_3 were observed in pregnant donkeys in September ($P < 0.01$) and fT_4 in December ($P < 0.02$). Pregnant donkeys showed the lowest concentrations of T_3 in March, T_4 in September, fT_3 in June and fT_4 in February, and the highest concentrations of T_3 in the successive June, T_4 in February, fT_3 in July and fT_4 in December.

Compared to the previous month, significantly lower concentrations of T_3 in July ($P < 0.02$) and higher in the successive June ($P < 0.001$), lower concentrations of T_4 in September ($P < 0.05$) and higher in August ($P < 0.05$) and in February ($P < 0.05$), lower fT_3 concentrations in September ($P < 0.01$) were observed in barren donkeys.

Barren donkeys showed the lowest concentrations of T_3 in May, T_4 in September, fT_3 in May and fT_4 in March, and the highest concentrations of T_3 in June, T_4 in February, fT_3 and fT_4 in July.

No significant differences of total and free iodothyronine concentrations between pregnant and barren donkeys were observed. Triiodothyronine (T_3) concentrations in pregnant donkeys over a 12-month period ranged from 1.57 to 2.90 nmol/l, T_4 ranged from 31.01 to 63.67 nmol/l, fT_3 ranged from 3.15 and 15.52 pmol/l and free thyroxine (fT_4) from 22.47 and 33.69 pmol/l.

However, a significant effect of season on T_3 ($P < 0.001$) and fT_3 ($P < 0.001$) changes in both pregnant and barren donkeys was observed. Significantly positive correlations between T_3 : T_4 ($r = 0.9832$; $P < 0.05$), T_3 : fT_3 ($r = 0.9911$; $P < 0.01$) and T_4 : fT_4 ($r = 0.9949$; $P < 0.01$) were observed in pregnant donkeys, and significantly positive correlations between fT_3 : fT_4

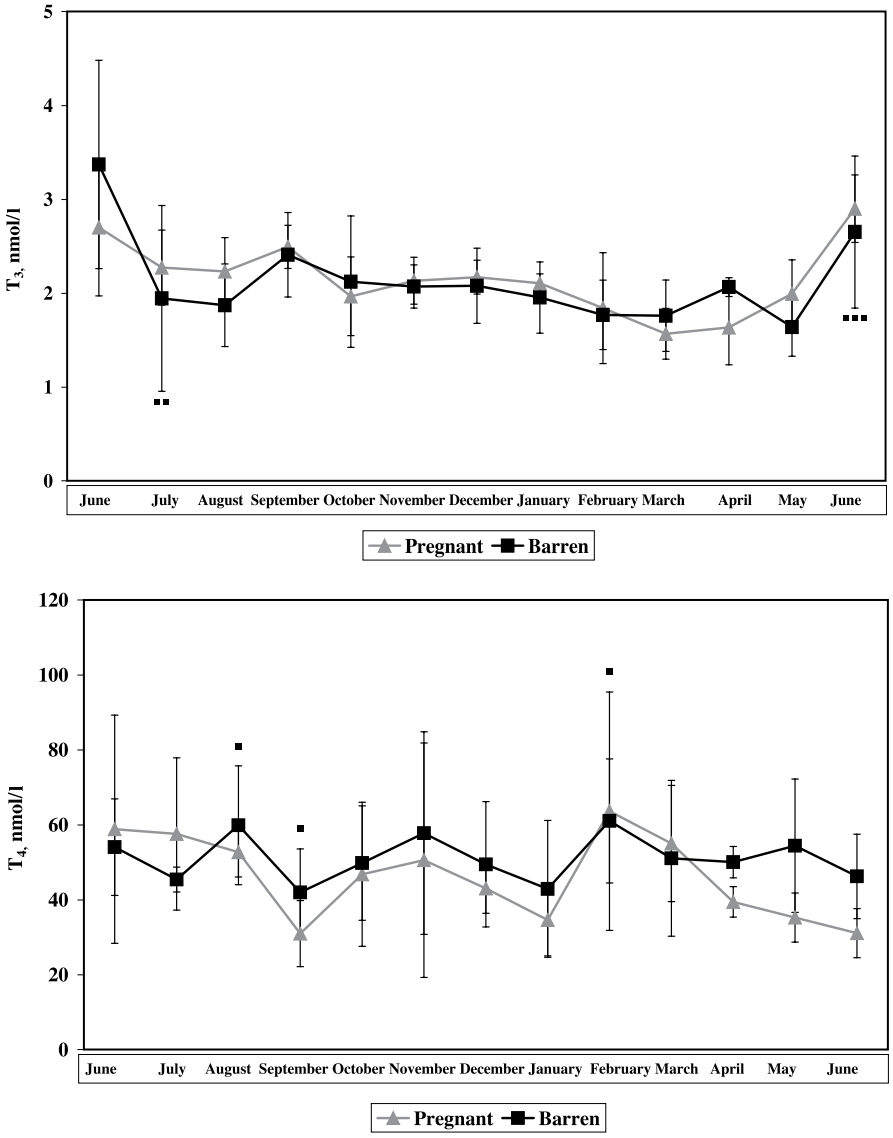

Fig. 1. Seasonal serum total iodothyronine concentrations (T_3, T_4) of pregnant and barren donkeys (Mean ± SD) over a 12 months period from June to the next June

Symbols indicate significant (▪$P < 0.05$; ▪▪$P < 0.02$; ▪▪▪$P < 0.001$) differences in average hormone compared to previous month

($r = 0.9977$; $P < 0.01$), T_3 : fT_3 ($r = 0.9966$; $P < 0.01$) and T_4 : fT_4 ($r = 0.9768$; $P < 0.05$) were observed in barren donkeys.

Discussion

Comparison of results obtained in donkeys with published data reported for horses reveals no discrepancies for circulating total and free iodothyronine concentrations

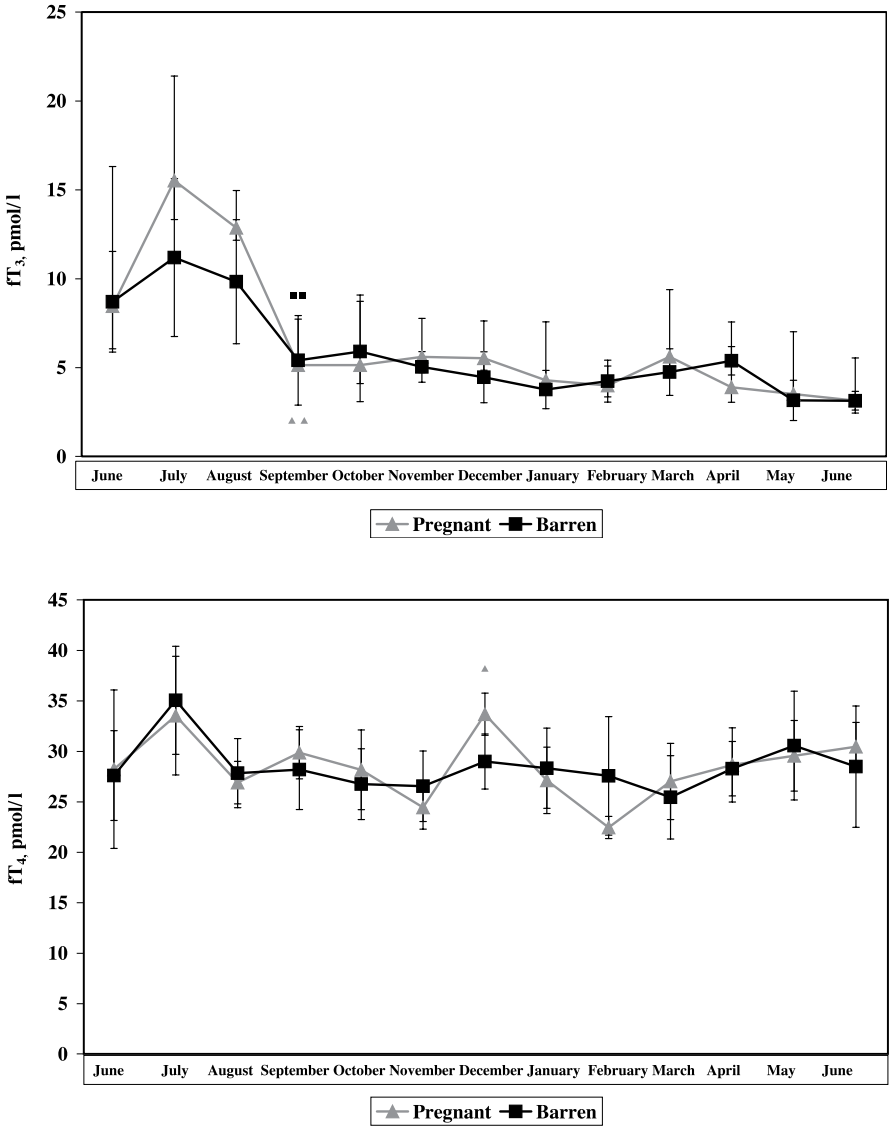

Fig. 2. Seasonal serum free iodothyronine concentrations (fT_3, fT_4) of pregnant and barren donkeys (Mean ± SD) over a 12 months period from June to the next June

Symbols indicate significant ($^{\blacktriangle}P < 0.05$; $^{\blacktriangle\blacktriangle}P < 0.01$) differences in average hormone compared to previous month

(Anderson et al. 1988; Kaneko 1989; Flisińska-Bojanowska et al. 1991), but some variation might be due to differences in the techniques used. Some differences could be explained by the influence of results with physiological or geographic factors and different management.

The results did not exclude the fact that some stress conditions such as stabling or

management (Hoffman et al. 1995; Malinowski et al. 1996; Christensen et al. 1997; Moons et al. 2005) and diet (Messer et al. 1995; Sticker et al. 1995; Powell et al. 2000) may determine higher T_4, fT_3 and fT_4 concentrations in both barren and pregnant donkeys than data reported in horses. Stress conditions may be induced by blood drawing which was always performed in donkeys that were out to pasture. Moreover, the data obtained exclude possible influence of thyroid rhythms because blood sampling was always performed at the same time.

Data obtained in donkeys confirm previous data reported in horses, that showed a similar pattern of thyroid hormones, with higher T_3 and T_4 concentrations in barren than in pregnant mares (Flisińska-Bojanowska et al. 1991). In addition, the absence of significant differences of T_3 and T_4 concentrations between barren and pregnant donkeys in the same month of sampling confirms previous data observed in barren and pregnant broodmares (Meredith and Dobrinski 2004).

Although lower T_3 and T_4 concentrations in pregnant compared to barren donkeys are not significant, this trend could be indicative of their high utilization in general metabolism during pregnancy, as indicated by the positive significant correlation between T_3 and T_4 described only in pregnant donkeys. It is possible that these results could be due to high thyroidal activity of the foetus with a probable negative feed-back on the maternal total iodothyronine concentrations. However, this hypothesis will require more information related to placental permeability to thyroid hormones in donkeys.

In addition, observation of unmodified seasonal T_4 and fT_3 patterns in both pregnant and barren donkeys with the lowest T_4 concentrations in September and the highest in February, and the lowest fT_3 concentrations in June and the highest in July, showed the probable existence of intrinsic seasonal changes of these hormones, irrespective of the physiological condition, indicating that thyroid hormone concentrations and pregnancy are not related in donkeys. The acrophase of T_4 observed in barren donkeys in February confirms previous data reported for T_4 in mares (Flisińska-Bojanowska et al. 1991). On the other hand, the acrophase of T_3 observed in both barren and pregnant donkeys in June does not confirm previous data reported for T_3 in mares, with acrophase occurring in July (Flisińska-Bojanowska et al. 1991).

The different seasonal pattern of total and free iodothyronines between pregnant and barren donkeys can be interpreted in terms of peripheral metabolism of thyroid hormones, although positive and significant correlations were observed between $T_3 : fT_3$ and $T_4 : fT_4$ in both pregnant and barren donkeys. Hence, maternal and foetal thyroid synthesis could be recognised as hormones that may enhance substantially the conversion of T_4 to T_3 in peripheral tissues, as demonstrated by the positive significant correlation between T_3 and T_4 observed only in pregnant donkeys. These changes may result subsequently in a temporary decrease in thyroid activity of pregnant females with no significant lower T_3, T_4 and fT_4 concentrations compared to barren females, and confirm that the utilization rates of thyroid hormones in mammals change unpredictably during pregnancy (Tomasi 1991).

On the other hand, the significant positive correlation observed between fT_3 and fT_4 only in barren donkeys could be due to preferential conversion of fT_4 to fT_3 that represents the active form of free iodothyronines.

In conclusion, although no significant differences between pregnant and barren donkeys were observed, the results showed a significant effect of season on T_3 and fT_3 concentrations in both pregnant and barren donkeys. These results confirm the active metabolic role of these iodothyronines in donkeys, as reported in previous studies in horses (Fazio et al. 2007). Data reported in this study are the first seasonal thyroid hormonal profile of barren and pregnant donkeys over a 12 months period.

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