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# The outcome of the first stages of pregnancy on mares' bloodstream thyroid hormones

E. Fazio*, P. Medica, C. Trifiletti, A. Ferlazzo

*Department of Veterinary Sciences, Polo Universitario Annunziata, University of Messina, 98168 Messina, Italy*

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ABSTRACT

The objective of this study was to compare in detail the total and free iodothyronines' pattern of mares from the first ovulation of the year over an extended period of 12 weeks. A total of 20 mares were used in the study. The mares were classified into two groups: mares mated at the ovulation (n = 10) used as observational group and mares unmated at the ovulation (n = 10) used as control group. Serum total and free triiodothyronine ($T_3$, $fT_3$) and thyroxine ($T_4$, $fT_4$) levels were measured in baseline conditions at the first ovulation of year and once a week until 12 weeks later. For the experimental group, the first week of postovulation mating was considered as the first week of gestation. One-way analysis of variance showed a significant effect of time over 12 weeks for $T_3$ ($F = 2.44$; $P = 0.007$) in pregnant mares, with the higher levels at the seventh and 12th weeks ($P < 0.05$) than baseline values, and for $fT_3$ ($F = 2.36$; $P = 0.009$), with the higher levels at the 11th week ($P < 0.05$) than baseline values. Two-way analysis of variance showed a significant pregnancy effect compared with nonpregnancy stage for $T_3$ ($F = 15.82$; $P = 0.009$), with the higher levels at the seventh and 12th weeks ($P < 0.05$) of pregnancy than that in nonpregnant values. Thus, it appears that, under similar environment, management and nutritional regime, the first trimester of pregnancy plays a dynamic role on the thyroid patterns by their anabolic activity; therefore, significant effects of time points on the $T_3$ and $fT_3$ concentrations probably may contribute to the control of early embryonic growth and development, before the onset of fetal thyroid activity. Considerable additional research, outwith the aim of this study, will be required to elucidate the mechanisms by which gestational age affects the physiological thyroid function in mares and/or fetus ratio in the first pregnancy stage.



## 1. Introduction

It is apparent in a number of species, including Equines, that gestational age dynamically affects the metabolic and endocrine mismatch between maternal and fetal interaction [1–3], with physiological requirements and essential changes. Thyroid hormones act directly through anabolic effects on fetal metabolism, and their bioavailability *in utero* depends on the development of the fetal hypothalamic-pituitary-thyroid gland axis [4] and on placental transfer from the mother in the fetal circulation [5]. What is more, maternal thyroid hormones may contribute to the control of early embryonic growth and development, before the onset of fetal thyroid hormone activity [1,6], showing that pregnancy, uteroplacental, and fetal development are highly dynamic processes [7]. Several studies suggest that, during early pregnancy, the fetus depends entirely or partially on maternal thyroid hormones that cross the placenta [4,8]; in this sense, the placenta may regulate the quantity and composition of different forms of transported thyroid hormones to ensure that requisite levels are present in the fetus for each stage of development [6]. Thyroid hormone activity varies with

* Corresponding author. Tel.: +39 90 3503583; fax: +39 90- 3503975.
*E-mail address:* fazio@unime.it (E. Fazio).

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species, type of placenta, and stage of fetal development. Human and rodent species have a hemochorial placenta that appears to be permeable to maternal thyroid hormones [9,10]; unlike humans and rodents, the structure of equine placenta is diffuse epitheliochorial, represented by six layers of tissue between maternal and fetal blood [11,12], with little placental permeability to maternal thyroid hormones [4,13].

A relationship between thyroid function and reproductive activities and the influence of pregnancy on diurnal and seasonal changes of $T_3$ and $T_4$ concentrations in the mare were also investigated [14–16], with a diurnal rhythm in $T_3$ concentrations in pregnant and nonpregnant mares [17]. However at the same time, no significant associations between fetal and maternal concentrations of TSH, thyroid binding globulin, or thyroid hormones were described [18]. Circulating thyroid hormones were significantly lower in anestrous mares compared to subjects with an estrous cycle during the anovulatory season [15,19], and no significant differences between estrous and luteal stage were revealed [20]. It is then reasonable to assume that $T_3$ and $T_4$ concentrations and reproductive physiology are regulated by similar hypothalamic control mechanisms [21].

The aim of this study was to evaluate in detail the total and free iodothyronines' pattern of mares over an extended period of 12 weeks, from the first ovulation of the year, by taking into account the comparative physiological state (pregnant vs. nonpregnant mares).

## 2. Materials and methods

### 2.1. Animals and management

A total of 20 multiparous broodmares with proven reproductive history and with the informed owner consent were used during the physiological breeding season (February–July, 2014). Mares belonged to a private stud farm in Messina (Italy), which is located in the Northern Hemisphere (38° 5′ 17.21″ N latitude and 15° 8′ 14.89″ E longitude). The mares of different breed (Italian Saddle, Sanfratellano, Thoroughbred), aged 5 to 6 years, were maintained in outside paddocks during the day and in 20.9 $m^2$ individual boxes at night, with interindividual visual contact; specimens were familiar with their group members.

At the start of the study, all mares had a body condition score of 6 point, and mean weight was 450 ± 35 kg. Ten mares became pregnant at the same time of the year (March–April) and were selected as the observational group; hence, the stage of pregnancy at the time of blood sampling was similar in all mares, and the data were defined on the basis of a pregnancy diagnosis test carried out 12 days and 35 days after breeding.

Ten nonpregnant mares were selected as the control group, by random selection. Mares were normally kept on green pasture, with good quality grass hay, from February to July, and they were also individually fed twice a day, with approximately 10 hours between feedings (at 7 AM and at 5 PM) with 2 kg of commercial feed and 2 kg of oats, and water were available ad libitum. From August to January, mares were individually fed twice a day (at 11 AM and at 4 PM) with 2 kg of commercial feed, 2 kg of oats, and 4 kg of hay, and water were available ad libitum. The composition and quantity of individual supplement was equal between pregnant and nonpregnant mares, to minimize the effect of different diets on the hormonal pattern.

All mares had normal estrous cycles and were free of uterine disease. Mares were considered to be in estrous on the basis of their behavior at teasing as determined once a day by experienced stud farm managers. During each teasing episode, a mature stallion was placed in an indoor teasing area, which allowed physical interaction between the mare being teased and the teaser. Follicular activity, uterine tone, and edema were monitored by rectal examination and ultrasonography.

Ovaries of each mare were daily assessed by ultrasonography on Day 0 (baseline) and every day, once mares were detected in estrous, using portable ultrasound scanner (Aquila-Esaote-PieMedical) equipped with endocavitary linear multisound (6–0, 8–0 MHz), and size and number of follicles were recorded for each ovary. When a preovulatory follicle (≥35 mm) was detected, mares were mated using stallions of proven fertility chosen by the owner. For the experimental group, the first week of postovulation mating was considered as the first week of gestation. Pregnancy diagnosis was carried out 12 days after ovulation by a transrectal palpation and ultrasonography examination using a linear 5-MHz Aloka transducer and confirmed at 35 days. Pregnancy rates were very similar in mated mares, with only about 2.5% of difference.

All methods and procedures used in this study were in compliance with the guidelines of the Italian law (D.L. 04/3/2014 n. 26) and EU directive (2010/63/EU) on the protection of animals used for scientific purposes.

### 2.2. Blood samples

Blood samples were taken from the jugular vein of both groups from the first ovulation of the year over an extended period of 12 weeks, once a week. To reduce circadian variations, all samples were collected between 7 AM and 8 AM, before animal fed and in quiet conditions by the same veterinarian. Blood samples were collected in evacuated tubes without anticoagulant (Venoject; Terumo, Belgium) and were allowed to clot for at least 1 hour at room temperature and then refrigerated at 4 °C until centrifuged. The harvested blood was centrifuged for 15 minutes at 3000 × *g*; after centrifugation, the serum was transferred to polystyrene tubes and stored at −20 °C until time of assay for determination of total ($T_3$, $T_4$) and free ($fT_3$, $fT_4$) iodothyronine concentrations.

### 2.3. Hormone analysis

Hormone assays were analyzed in duplicate using a commercially available immunoenzymatic kit and carried out according to the manufacturer's instructions (SEAC-RADIM; Pomezia, Rome). Limits of detection were 0.24 nmol/L for $T_3$, 5.79 nmol/L for $T_4$, 0.15 pmol/L for $fT_3$, and 1.3 pmol/L for $fT_4$. Intra-assay and interassay coefficients of variation were 7.3% and 11.4% for $T_3$, 2.3% and 5.7% for $T_4$, 4.2% and 11.9% for $fT_3$, and 6.6% and 9.6% for $fT_4$,