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Effect of peripartum selenium supplementation on plasma selenium and T3 levels relationships in hair ewes and their newborn lambs.

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Abstract

The effect of pre- and postpartum selenium (Se) supplementation in ewes on thyroid hormone (T3) levels were evaluated. The relationship between plasma and milk Se concentration and serum T3 levels in ewes and their newborn lambs were analyzed. Twenty 90 d of gestation primiparous crossbreed hair ewes were selected and distributed randomly into two groups. The first group received subcutaneous Se supplementation (0.1mg of sodium selenite/Kg BW) at the 8th, 5th prepartum and 1st postpartum wk while the other was control without Se injection. Plasma and serum maternal samples were collected weekly since 8th prepartum week to delivery. At delivery and weekly until 8th postpartum wk, plasma, serum, colostrum and milk samples were taken from ewes. Plasma and serum samples were collected at 48 hours, 1st, 2nd, 3rd, 5th and 8th, postpartum wk from newborn lambs. Muscular and thyroid histological changes were studied. Results demonstrated significant positive relationship between maternal plasma Se and serum T3 in supplemented and control ewes (r = 0.69 to 0.72, P < 0.05). Increase in T3 concentration in supplemented ewes and their lambs until the 8th postpartum week was demonstrated (P < 0.001).There was positive relationship between milk Se concentration and serum T3 in the newborn lambs of the supplemented group (r= 0.84, P < 0.01), while the relationship was negative in the control group (r = -0.89, P < 0.01). Muscular and thyroid pathological changes were described and were independents of Se-treatment suggesting a pre-experimental Se deficiency period.

Keywords: Selenium, thyroid, T3, sheep

Introduction

Recognized as an essential trace element in 1957 [1], selenium (Se) is a key component of the so-called selenoproteins and plays a critical role in various aspects of human and animal health [2,3]. In the thyroid gland, Se is associated with the activity of redox-protective peroxidases which prevent thyroid cell from oxidative damage during the process of hormonogenesis [4,5]. Furthermore, Se is also necessary in the synthesis of iodothyronine deiodinases (D), the family of selenoenzymes which are critical for the control of thyroid hormone (TH) action at the cellular level. Deiodinases type 1 and 2 (D1 and D2) catalyze the activation of tetraiodothyronine (T_4) to triiodothyronine (T_3) and D1 and D3 inactivate T_4 to reverse T_3 or T_3 to T_2 [2,6,7]. Se deficiency induces a significant reduction in T_3 with the corresponding increase in T_4 and a reduction in the activity of hepatic D1 [2,8,9,10,11,12,13]. On the other hand and as recently reviewed [3], several studies have shown that supplemental sodium selenite and sodium selenate by oral or parenteral administration forestall the clinical signs of Se deficiency and animal losses in ruminant and non-ruminant species [14,15]. Transfer of nutrients and Se from the dam to the offspring occurs via two pathways, placental transfer and colostrum-milk ingestion. The amount of nutrients transferred to offspring depends on the maternal nutrient status and the efficiency of the transplacental and mammary transport mechanism. Selenium is efficiently transferred via the placenta to the fetus, even in situations of low maternal concentration of the element [16,17].

This background and our previous studies document that as a consequence of its volcanic soils, México has serious deficiencies in almost all the country in plant and animal Se contents [18], led us to conduct the present study aimed to assess the effect of pre- and postpartum Se-supplementation on thyroid and Se homeostasis in ewes and their newborn lambs.

Materials and methods

At approximately 90 d of gestation and based on ultrasound examination, 20 primiparus one year old crossbreed hair ewes gestating twin fetuses were selected. Animals were supplemented at weaning, two month old, with a subcutaneously injection of selenium (0.1 mg Se/10Kg BW). Deficiency of selenium blood levels in experimental sheep were 250 ppb . Ewes had a high corporal condition (average body weight: 41.09 ± 0.8 Kg), and were divided into two groups (10 animals each). The supplemented group was injected subcutaneously with a solution of sodium selenite (0.1mg Se/Kg body weight) at the 8th and 5th prepartum wk and at the 1st postpartum wk. The control group did not receive Se. Both groups feed a diet elaborated with alfalfa, corn and soybean with a mineral salt containing 0.5 iodine mg/kg DM and without Se and water *ad libitum.* The Se concentration in the diet was 0.22 and 0.23 ppm during pregnancy and lactation respectively.

Blood samples from gestated ewes were collected weekly since 8th wk to delivery and until 8th postpartum wk. Postpartum samples from newborns were obtained at 48 h. and at the 1st, 2nd, 3rd, 5th, and 8th wk. In both, ewes and newborns, blood was collected in the morning (8:00 am) by jugular vein puncture using vacutainer tubes with and without anticoagulant (EDTA). Colostrum and milk (2 ml) were collected in the same schedule that blood samples. All the samples were kept frozen (-20 ◦C) until analyzed. As previously described, Se was measured in plasma, milk and dietary by atomic absorption spectrophotometry [17] and serum T_3 by radioimmunoassay [19]. At the end of the experiment ewes and lambs were sent to slaughterhouse and part of the thyroid gland, the diaphragmatic muscle and the myocardium were collected in 10% buffered formalin solution and processed for histopathological evaluation to obtain paraffin cuts as routine.

Statistical analysis

Means Pearson correlation coefficient and Analysis of Variance (ANOVA) were performed using the Microsoft Excel and Statgraphic plus v. 4.

Results

One ewe from the control group aborted and was eliminated. Se levels incremented the next week of application and maintained higher levels during experimental weeks in supplemented ewes ($P < 0.05$) and their lambs ($P < 0.01$) . Compared to control animals and during the entire study, circulating levels of T_3 were higher ($P < 0.001$) in Se-supplemented mothers. Moreover, these differences in serum hormone concentrations became more evident during lactation where T_3 mean values for supplemented and non-supplemented ewes were 200 ± 60 ng/dl and 150 ± 35 ng/dl, respectively. Similarly, serum T_3 in lambs born from Se-supplemented mothers was higher ($P < 0.01$) than in those born from control ewes . This difference between both groups of newborns was maintained up to the 8th wk of age. There was a positive correlation between maternal plasma Se concentration and serum T_3 in both, supplemented and non-supplemented ewes ($r = 0.69$ to 0.72, $P < 0.05$). In contrast, whereas serum T_3 and milk Se concentrations were positively correlated in the supplemented mothers ($r = 0.60$, $P < 0.05$), this correlation was negative in the control group ($r = -0.80$, $P < 0.05$). A similar pattern between serum T_3 and milk Se concentration was observed in newborns. The correlation was positive in lambs born from the Se-supplemented mothers ($P < 0.01$, $r = 0.84$), and negative in the newborns from the control group ($P < 0.01$, $r = -0.89$).

The diaphragm and myocardium histopathological studies revealed muscular nucleus proliferation and mononuclear macrophages infiltrate in association with swollen muscular fibers (Fig. 1). However, the most conspicuous alterations were observed in the thyroid gland of ewes. There were notorious differences in size, follicular content aspect, staining homogeneous or vacuolated, and empty follicles. Large and dilated follicles, macrofollicles, with flat epithelial cells (goiter-aspect) coexisted with much smaller follicles that presented columnar epithelium (embryonic-aspect). The most relevant changes included folded follicular epithelial structures, with villous aspect and irregular follicular lumen. Double epithelial layer were observed in some follicles coexisting with collapsed follicular structures observed as cords and nodular columnar epithelial structures. Pyknotic nucleuses were observed in epithelial and fusiform interstitial cells (Fig. 2). Lambs thyroid glands were considered normal, some differences on follicular size were observed only. Histological muscular and thyroid changes were independent of treatment.

Discussion

Besides agreeing with previous studies showing the important deficit in vegetal and animal selenium levels in most regions of México [18], present results add further support to the notion that Se-supplementation is critical in these conditions to sustain ruminants' production and thyroid function, similar to other animals and humans [2,4,5,6,7,8,13,18,20,21]. On the other hand, iodine deficiency and goitrogenic factors have been reported in humans in focalized areas of México only [22,23]. Animal deficiency has not been reported in México.

According with studies in bovines and ewes [8,12,13,21] present results demonstrate clearly the importance of Se-supplementation to maintain Se and T_3 homeostasis in both pregnant ewes and their offspring. Rock *et al.* [13] demonstrated this condition in pregnant ewes but T_3 only presented a tendency to have higher levels in their lambs. Differences between this work and our results may be due to original levels of Se in ewes, the source of Se supplementation, breed and age of experimental ewes and in the fact that they evaluated 12 h pospartum experimental lambs only. Rock *et al.*[13] used crossbreed wool sheep (Rambouillet x Polypay) in contrast in this work crossbreed hair ewes (Pelibuey x Katahdin) were used, this difference may affect Se and T_3 thermo metabolism requirements. Our data show that lambs that were born from Se-supplemented ewes and took naturally colostrum and milk from their dams had serum T_3 significantly higher than those from non-supplemented mothers.

Moreover, serum T_3, plasma and milk Se concentration were positively correlated in supplemented and non-supplemented ewes as well as in lambs that were born from supplemented mothers. In contrast, this correlation was negative in newborns from non-supplemented ewes. These data strongly suggest that non-supplemented mothers reduce Se-transfer to milk and to the offspring, diverting Se reserves primarily to maintain their own Se and thyroid homeostasis. These results agree with those of other researchers [8,11] and confirm the hierarchical importance of selenoenzyme synthesis in the maternal organism [5,24,25].

High mortality rates in newborns lambs from non-supplemented mothers than in those born from supplemented ewes have been reported [26]. In this context hypothermia is one of the major causes of newborn lamb mortality and that T_3 is essential to the synthesis of uncoupling protein necessary for the thermogenic activity of brown adipose tissue [27,28]. Nevertheless Rock *et al.* [13], did not demonstrate effects of Se supplementation on thermo metabolism of experimental lambs. This data support the proposal of maintaining ewes Se-supplementation throughout the lactation period.

Significant increase of T3 in ewes and lambs of Se supplemented group against control may be attributed to increase activity of Se dependent deiodinase enzymes [29].

The significant positive relationship between maternal plasma Se and serum T_3 concentrations and the significant decrease in T_3 levels towards parturition in the control group, indicates the importance of maternal Se-supplementation to maintain Se and T_3 homeostasis in pregnant ewes. These results agree with Rock *et al.*[13] and Rowntree *et al*.[21] observations in cows as well as with the well-known transplacental Se transference [17,30] and its beneficial effects throughout colostrum and milk [20,31].

According with previous studies in ewes and cows [12,13] lambs born from Se-supplemented ewes had serum T_3 significantly higher than those born from non-supplemented mothers. At the 8th postpartum wk, no significant differences between Se supplemented and control groups lambs were observed, probably associated with decrease milk Se concentration at this moment [31]. Head *et al*. [32] found that T3 in newborn lambs increased since the 4th day postpartum and then decreased on the 84th day and then began to rise. In this work T3 level began to decrease on the 5th postpartum wk and rose again on the 8th wk. This finding suggest that T3 levels were elevated during lambs active growth associated with high ewe milk production, then decreased and began to rise newly when lamb initiated forage intake. This enforces the importance of lambs Se supplementation before 8th wk old to maintain plasma Se level and consequently T3 activity.

Calves born from Se supplemented dams had higher levels of T3 than that born from non-supplemented [12]. These results agree with the ones of the present study. Nevertheless Rowntree *et al*. [21] found that maternal Se supplementation only until calving did not influence neonatal calf thyroid hormone concentrations.

No relationships between muscular and thyroid histopathological changes with Se supplementation were observed. Probably lesions occurred in a previous experimental Se deficiency period and persist as a scar, nevertheless ewes were supplemented. Early reproduction activity and twin gestation in hair ewes used for the experiment may cause Se deficiency condition and serious lesions described.

Short supplementation period before animals were sacrificed, probably was insufficient to lesions resolution. Observed lesions were similar to experimental animals previously described with Se and iodine deficiencies [5,33] and suggested a combination between degenerative necrotic lesions and hypertrophic compensatory changes. These severe thyroid pathological changes have not been previously described in ewes or other domestic animals. Human studies in México did not consider Se deficiency as a goitrogenic factor [22,23]. The usage of sheep as a model should be considered Se status of experimental animals to evaluate thyroid function [34,35].

Conclusion: Pre and pospartum Se supplementation is essential to maintain Se and T_3 homeostasis during late pregnancy and pospartum in ewes and their lambs. Furthermore, postpartum Se supplementation should continue until the 8^{th} wk of age.

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Fig 5. Muscular dystrophy. A: mononuclear infiltrated replaced a damage muscular fiber, HE 160x. B: Muscular nucleus proliferation in damage pale fibers. HE. 80x

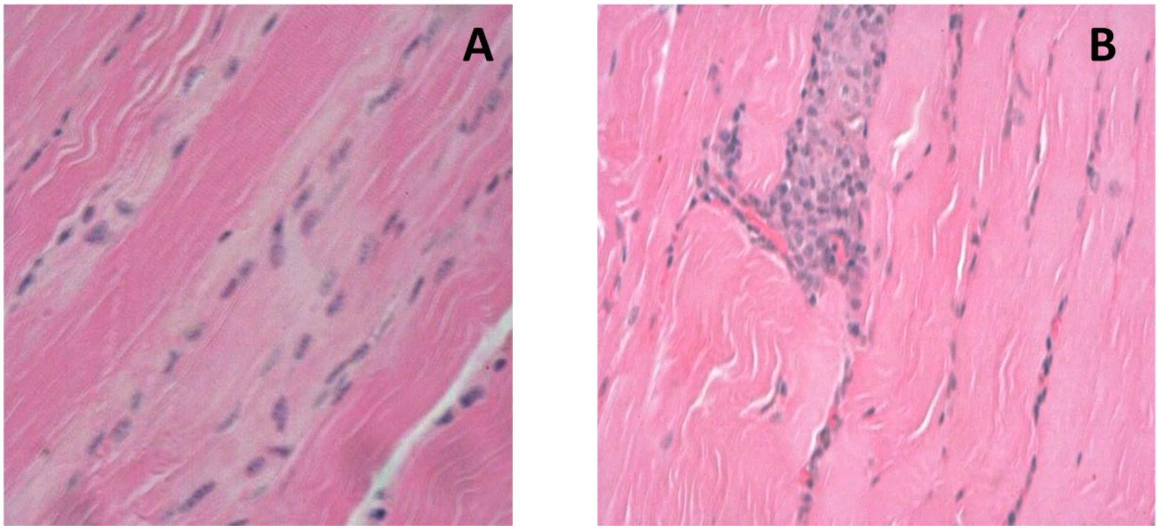

Fig. 6. Thyroid sections. **A-** A macrofollicular structure (right) contrast with collapsed and folded empty follicular structures. HE, 80x. **B-** Detail of collapsed and folded empty follicular structures, HE, 160x.