THE EFFECT OF THE PERIPARTURIENT PERIOD ON THE PLASMA FLUORIDE CONCENTRATIONS IN GOATS *(CAPRA HIRCUS)* AND THEIR KIDS IN THE NEONATAL PERIOD

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ABSTRACT: The aim of this study was to examine the effect of the periparturient period on the plasma fluoride ion (F) concentrations in goats (C*apra hircus)*, from one month before to one month after parturition (at 4 weeks and 1 week before parturition and at 2 days, 10 days, and 4 weeks post-partum) and their kids in the neonatal period (before postnatal day 4, at postnatal days 10–30, and at postnatal days 30–50). The F concentrations measured in the peri-parturient period significantly differed from those observed in the non-pregnant goats. The periparturient period in goats results in an increase of the F concentration four weeks before and a decrease four weeks after parturition. The concentration of F in the plasma of the kids of the goats increased more than threefold in the postnatal days 30–50 period compared to the period before postnatal day 4.

Keywords: *Capra hircus*; Goats; Neonatal period; Periparturient period; Plasma fluoride.

INTRODUCTION

Pregnancy and the periparturient period are physiological states that are known to modify the metabolism in the maternal organism. In this period, many organs as well as the nervous and endocrine systems are highly mobilized in order to provide optimal conditions for embryonic development.[1-4] During pregnancy, maternal tissues are involved in providing energy for reproductive processes which have the potential to influence the concentrations of various metabolites in the blood. The reproductive processes may also themselves be affected by several other factors such as breed, age, energy balance, fetal growth, and season.[5]

During pregnancy, the progressive physiological changes fundamental to supporting the metabolic demands of the growing fetus increase the maternal requirement for micronutrients.[6] Micronutrient deficiencies have been associated with significantly higher reproductive risks, both in the conception period and throughout the course of gestation,[7] and may include anaemia, pregnancy-induced hypertension and preeclampsia, fetal growth restriction, and labour complications leading to increased maternal and fetal morbidity or mortality.[8]

The effects of the different phases of the reproductive cycle on blood metabolites and the concentrations of biogenic elements in sheep and goats have been studied, especially in relation to the oestrus cycle, pregnancy, and lactation.[3,9,10] Studies focusing on the periparturient and neonatal periods, especially in small ruminants including goats, are less common. Therefore, the aim of this study was to examine the effect of the periparturient period on the plasma F concentrations in goats (C*apra*

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hircus), from one month before to one month after parturition, and the kids of the goats, in the neonatal period, in comparison with non-pregnant animals.

MATERIALS AND METHODS

Animals: The research was carried out during spring on milk-goats living on an individual farm in standardized conditions. The research was conducted during the periparturient period on 12 clinically healthy pregnant goats (75% genotype of the Polish noble white aged between 2–3 years and weighing 45–55 kg). The parturitions were physiological, without any complications. All females delivered singletons. The clinical control group consisted of 12 non-pregnant goats of a similar age, 1–2 weeks before mating.

The goats were housed in boxes on rye straw bedding. The animals were fed in compliance with the expected nutritional requirements accounting for their physiological state. The feed ration value was estimated in accordance with the Polish feeding standards for ruminants.[11] The goats received good-quality grass hay (crude protein 9.5% and cellulose 34% of dry matter) as well as *ad libitum* water and salt licks (94% NaCl) enriched with minerals: Co: 30 ppm, Cu: 150 ppm, Zn: 1,300 ppm, I: 60 ppm, Mn: 1,400 ppm, Mg: 18,000 ppm, and Se: 25 ppm. Post-partum, the goats, who were each lactating about 2 L of milk per day, received an additional 600 g/day/goat of bruised barley grain and 400 g/day/goat of dry beet pulp resulting in a diet with an energy and protein content of 9247 kJ and 133 g, respectively. The non-pregnant goats received half of the ration of bruised barley grain and beet pulp resulting in a diet with an energy and protein content of 4908 kJ and 50 g, respectively. Before parturition, the goats received 400 g/day/goat bruised barley grain and 300 g/day/goat dry beet pulp resulting in a diet with an energy and protein content of 7183 kJ and 107 g, respectively. Representative feedstuff samples (hay, rye straw, bruised barley grain, and beet pulp) were sent to a local agricultural laboratory for analysis of the Zn, Cu, and Mn concentrations by hydride generation atomic absorption spectrometry. However, the F concentration in the feed was not measured.

The kids of the goats were fed, in all the neonatal periods by their mothers and had *ad libitum* access to grass hay, water, and salt licks.

Sample preparation: Whole blood was taken from the external jugular vein, per a heparinized test tube (250 IU heparin from Polfa, Poland), early in the morning before feeding. Blood was obtained once from the non-pregnant goats, twice from the goats before parturition (at 4 weeks and at 1 week before parturition) and three times after parturition at postnatal day (PND) 2, PND 10, and at 4 weeks post-partum). Blood from the kids of the goats was obtained the from animals before PND 4, at PND 10–30, and at PND 30–50.

The goats did not show any sign of any particular stress during the blood sampling. The blood was delivered to the laboratory in ice flasks and immediately analyzed. All the studies were approved by the Local Ethics Committee.

Measurement of the fluoride concentration in the plasma of the goats: The levels of F in the samples were determined using a potentiometric ion-selective electrode (Thermo Scientific Orion, USA). 0.5 mL of TISAB III buffer solution was added to a 0.5 mL plasma sample. After mixing, the potential difference of each sample was measured for 10 min: 5 min before the addition of the appropriate standard and 5 min afterwards. The F content in the plasma was calculated on the basis of the difference in the potentials measured in each sample and the concentration of the added standard. The electrode was calibrated using standard solutions.[12]

Statistical analysis: Statistical analysis was conducted using v.6.1 of Statistica software. The mean values and standard deviations (mean±SD) were calculated for each variable studied. The non-parametric Kruskal-Wallis ANOVA and the U Mann-Whitney test were used to assess the differences between the groups.

RESULTS

The F concentrations measured in the periparturient period differed significantly from those observed in the non-pregnant goats. The highest prenatal F concentration in the plasma was observed 4 weeks before parturition. This value was 44% (p=0.0044) higher than that observed in non-pregnant goats (Table).

Table. Mean values and standard deviations (mean±SD mg/L) of the plasma fluoride concentrations in non-pregnant goats (group A=non-pregnant goats) and in goats at different periparturient periods (group B=4 weeks before parturition, group C=1 week before parturition, group D=2 days post-partum, group E=10 days post-partum, and group F=4 weeks post-partum)

Parameter	Plasma fluoride in groups (mean±SD)					
	Group					
	A (non-pregnant goats) (n=10)	B (4 weeks before parturition) (n=12)	C (1 week before parturition) (n=12)	D (2 days post-partum) (n=12)	E (10 days post-partum) (n=12)	F (4 weeks post-partum) (n=12)
Plasma fluoride (mg/L)	0.055± 0.005	0.078± 0.018*	0.067± 0.059	0.085± 0.012†	0.086± 0.039	0.067± 0.028

*Compared to group A (non-pregnant goats) the significance of the difference on the Mann-Whitney test = 44% ↑

†Compared to group A (non-pregnant goats) the significance of the difference on the Mann-Whitney test = 56% ↑

At post-partum day (PPD) 2 there was a significant increase (by 56%, p=0.002) in the F concentration compared to the non-pregnant goats. This value remained stable until after PPD 10. The concentration measured after this period of time was close to that observed in the non-pregnant goats (Figure 1).

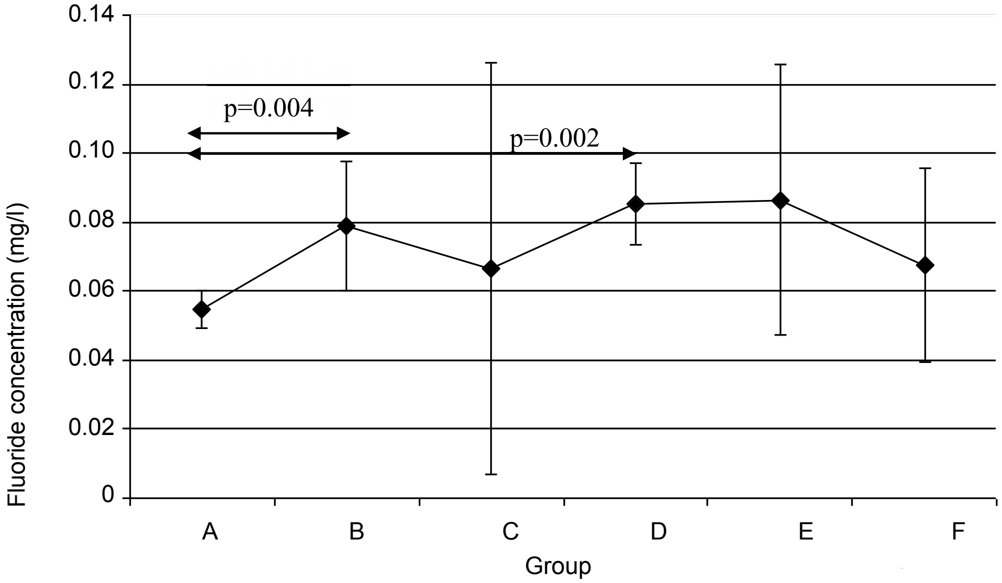

Figure 1. Fluoride concentrations in the plasma of the non-pregnant goats (group A) and the goats in the different periparturient periods (groups B, C, D, E, and F).

The periparturient period in goats results in an increase of F concentration 4 weeks before and a decrease 4 weeks after parturition. The concentration of F in the plasma of the kids of the goats in the neonatal period was significantly higher than in the mothers (p=0.002) and significantly increased on PND 30–50 in comparison to 4 PND<4 (p=0.002) (Figure 2).

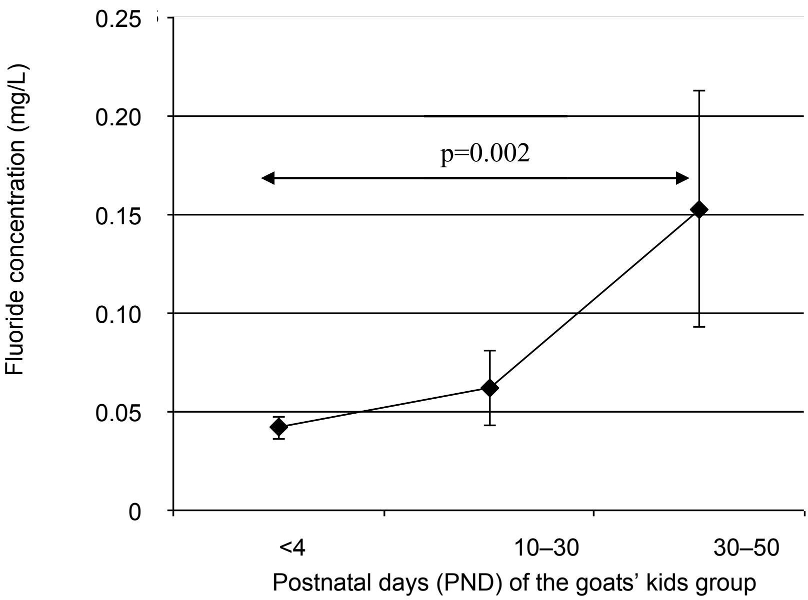

Figure 2. Fluoride concentrations in plasma of the goats' kids groups of less than 4, 10–30, and 30–50 postnatal days.

DISCUSSION

During pregnancy the increased metabolic demands on both macro- and micro-nutrition place a significant stress on the maternal physiological system. It is important that the pregnant female receive adequate micronutrition on a daily basis to reduce the risk of a potential adverse pregnancy and fetal outcome. Focusing on the essential trace elements (magnesium, copper, zinc, calcium, iodine, manganese, selenium, and iron) it is clear that all play an important role during pregnancy and that deficiencies prior to or during gestation may lead to adverse outcomes for both the mother and the fetus. Such complications include: anaemia, hypertension, fetal growth restriction, preeclampsia, labour complications, and even death.[13]

It is important to consider not only the role of trace elements in pregnancy but also the possible benefits of combining these elements with essential vitamins. Trace elements, such as selenium and zinc, in combination with vitamins A, B6, B12, C, D, E, and folate, have been found to improve immune function and reduce placental oxidative stress. They are considered to be important for supporting the maternal system to deal with the physiological stress of pregnancy[14,15] through modulation of maternal and fetal metabolism, reduction in inflammation, and support of placentation.[16] However, to the best of our knowledge, we present, for the first time, the results of a study of the effect of the periparturient period on the plasma F concentrations in goats and their kids in the neonatal period.

Administered F is mainly absorbed by the gastrointestinal tract and then distributed to soft and hard tissues and the various bodily fluids, such as saliva, tears, milk, cerebrospinal fluid, and plasma.[17] Over 86% of the F present in the human body is concentrated in calcified tissues.[18] There is a linear relationship between the F concentration present in drinking water and that in the hard tissues. A number of authors believe that F administration during pregnancy is the first step towards caries prevention[18,19] and proper bone mineralization.[20] However, the danger associated with such a procedure is the potential development of dental fluorosis.[21] Additionally, Leverett et al., in a randomized double-blind study showed, that prenatal fluoride supplementation did not have an increased caries-preventive effect.[22]

F interacts with the tooth structure (enamel) both during odontogenesis and after complete dental formation and eruption in the oral cavity. The systemic administration of F during odontogenesis allows the deposit of such mineral in the deepest strata of the forming enamel, and favours the creation of fluorapatite, that is less soluble than hydroxyapatite, more resistant to the acid attacks of the plaque, and more stable as it is formed by a smaller molecule.[17]

In our study, the concentration of F in the plasma of small goats increased more than three times on PND 30–50 in comparison with PND <4. Probably this was caused by the kids having, in addition to breast milk, access to F in the other parts of their diet, as well as the mothers also consuming F in their food and water. The basic source of fluorine in the diet of farm animals is drinking water. Under normal conditions, while maintaining the optimal nutritional principles, the fluorine content in the diet of ruminants (cereal products, i.e., bran, middlings, as well as leafy vegetables, green fodders, meadow hay, and licks) does not exceed several dozen micrograms/day. However, even such low amounts of this element, when consumed by a young animal, can negatively affect the developing organism, because immature animals have relatively greater sensitivity and susceptibility and less tolerance to F.[24] On the other hand, the results of many authors point to newborn ruminants (immature camel calves, goats' kids, and lambs) being protected naturally from F toxicity (dental or skeletal fluorosis) owing to the presence of ample amounts of calcium and vitamin C in their diet.[20]

Osteopenia and osteoporosis in goats' infants may be another explanation of the increased F concentration noted in plasma of goats' kids. This process has not been fully explained. Chronic deficiencies of minerals can decrease the formation of the osseous matrix and tissue mineralization. Ketosis, which can occur during pregnancy, is an additional factor that also intensifies the resorption of the osseous tissue through the activation of osteoclasts. This may lead to changes in the concentration of the biogenic elements in this tissue such as calcium and magnesium and also of fluorine. On the whole, the concentration of F in blood is low since it is excreted by the kidneys and is absorbed by the skeletal system. However, if the content of F in the urine and blood reaches twice the level present in control populations, the influence of F upon metalloenzymes becomes observable, especially with magnesium-dependent enzymes in both systemic fluids and tissues.[25,26]

Chronic exposure to F in drinking water causes fluorosis, not only in humans but also in domestic animals.[20,27] The primary manifestation of fluorosis is mottling of teeth (dental fluorosis) and osteosclerosis of the skeleton (skeletal fluorosis). Besides these, non-skeletal fluorosis or toxic effects of fluoride in soft tissues or organ systems have been reported in man[28] and animals, viz., gastro-intestinal disturbances, neurological disorders, reproductive dysfunctions, and teratogenic effects.[29] As well as the prevalence and severity of these chronic F effects being affected by the amount of F consumed, the duration of the F exposure, and the frequency of the F intake, several other determinants may also have an influence such as: age, nutrition, health, stress factors, differences in the biological response of individuals, local environmental temperature and humidity, and the presence of other dissolved salts in the drinking water.[29] Moreover, an inadequately balanced supply of F in the diet of pregnant females and newborns during the early stages of development may result in fertility disorders in both males and females in adulthood.[30]

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