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Original article

Insulin and sugar concentration changes in mammary secretion in sheep during the periparturient period

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Summary — The radioimmunoassayable concentration of insulin in isolated whey fractions of mammary secretion of sheep (*n* = 28) was measured. The concentrations were high on the last 2 days of pregnancy (550 ± 80 and 580 ± 77 μU/ml, respectively), higher on the day of parturition (780 ± 71 μU/ml) and lower on the next day (290 ± 54 μU/ml). During the following 4 consecutive days the concentrations gradually decreased to an almost basal blood level (14 μU/ml). The high insulin concentration in the mammary secretion during the periparturient period might be the result of intensified transfer of insulin through the blood–mammary barrier and increasing needs for this hormone by developing mammocytes and probably for the newborn lambs (*n* = 42) adapting to extra-uterine life. Conversely to the consecutive decrease in insulin, progressively increasing concentrations of sugars in mammary secretions were observed (from 2.1 ± 0.22% on the second day prior to parturition to 5.5 ± 0.13% on the fifth day *post partum*).

insulin / sugars / mammary gland / sheep / periparturition

Résumé — Changements de concentrations d'insuline et de sucres dans la sécrétion mammaire chez des brebis péripartunentes. *Des concentrations d'insuline détectables par radioimmunoessai ont été mesurées dans les fractions «petit lait» de la sécrétion mammaire de brebis* (n = *28). Les concentrations sont élevées pendant les 2 derniers jours de gestation (550 ± 80 et 580 ± 77 μU/ml, respectivement), les plus importantes le jour de la mise bas (780 ± 71 μU/ml) et les plus faibles le jour suivant (290 ± 54 μU/ml). Pendant les 4 jours suivants, les concentrations décroissent graduellement à peu près jusqu'au niveau basal sanguin (14 μU/ml). La forte concentration d'insuline dans la sécrétion mammaire pendant la période de péri-implantation peut être le reflet d'un transfert accru à travers la barrière hémato-mammaire et d'une exigence croissante d'hormone des cellules grandulaires en développement et des agneaux nouveau-nés* (n = *42) s'adaptant à la vie extra-utérine. En relation inverse avec la décroissance ultérieure de la concentration en insuline, on observe une concentration croissante de sucres dans la sécrétion lactée (de 2,1 ± 0,22% le deuxième jour avant la parturition à 5,5 ± 0,13% le cinquième jour* post-partum*).*

insuline / sucres / glande mammaire / mouton / périparturition

INTRODUCTION

Mammary secretion (pre-milk, prebirth-milk, fore-milk, colostrum, mature milk, milk) possess antibodies, hormones, milk-born hormones, and other substances as growth-promoting factors, which fulfil the regulatory role and help the newborn to adapt to a new environment (according to review of Koldovský, 1989; Peaker, 1991; Pearlman, 1991). Research in the last few decades has provided information about the presence of insulin in colostrum (in women, Cevreska *et al,* 1975; Kulski and Hartmann, 1983; Read *et al,* 1984; in piglets, Asplund *et al,* 1962; in women, cows and sows, Nowak and Nowak, 1989; Nowak, 1990; and in sheep, Falkoner *et al,* 1984) in concentrations that even exceeded 40-fold that in serum. Studziński *et al* (1988) found that pathological and experimental hyperinsulinaemia, which occurs and is maintained during the fetal life of lambs, causes an enlargement of skeletal muscle mass and accumulation of fat. In contrast, the lack of insulin, caused by agenesis of the fetal pancreas, leads to a body mass decrease in lambs. The increase of body mass of newborn lambs depends on the volume and quantity of the colostrum and the individual ability to optimize food utilization (Studziński *et al,* 1988). According to Read *et al* (1984) a high concentration of insulin in the colostrum and in mature milk, is one of the most important growth-promoting factors for proliferation and differentiation of infant tissue.

The aim of this study was the determination of ovine colostrum insulin and lactose. The concentration of lactose was considered as a marker of maturity for mammary-gland secretion. The observations comprised the body weight gain of lambs during the 28 d after birth in relation to the age of ewes and number of lambs born to the same mother.

MATERIALS AND METHODS

In this study 28 pregnant ewes of different ages were used: 2 yr (n = 6); 3 yr (n = 5); 4 yr (n = 5); 5 yr (n = 7); and 7 yr (n = 5). The environmental conditions and feeding were equal for the all animals. The 28 ewes breed 42 lambs. Samples of mammary-gland secretion were taken on the second and the first days prior to parturition (pre-milk), on the parturition day, and during the 5 days following (colostrum and milk). The secreted samples were taken at 7.00 am (*ca* 10 ml each) into the tubes containing Aprotinin (Sigma) as an inhibitor of trypsin, chymotrypsin, kallikrein and plasmin (10 µl/ml sample). The samples were transported in a container with ice. High concentrations of insulin in the whey samples required dilution (1:20 v/v) with phosphate buffer (RIA–INS assay buffer 0.04 M, pH 7.4, containing 1.0 g bovine serum albumin (Sigma) and Thiomersal (BDH) per liter). After acidification of diluted milk secretion samples with acetic acid, under pH-meter control to pH 4.6 (casein isoelectric point), and centrifuged at 2 500 *g* for 15 min at 0°C; the fat layer and casein pellets were discarded and the whey supernatant was collected and kept either for immediate determination of insulin and sugars or stored at –20°C until use.

Insulin concentrations in the whey samples (0.2 ml each) were measured by the method of Yallow and Berson (1960) with the use of RIA–INS test kits (Swierk, Poland). Lactose concentrations (expressed as level of sugar complex) were determined using the method of Slopek (1970). The relationship between the body-weight gain and the age of the ewes, and the influence of the age of the ewes and litter size on the body weight gain of lambs during the 28 days *post partum* were estimated. Statistical calculations were conducted by Duncan's multiple new range test (Tashman and Lamborn, 1979).

RESULTS

The mean values of insulin concentrations in mammary secretion were on average 553.0 ± 80 and 581 ± 76.7 µU/ml on the second and first days before parturition, respectively, and 784 ± 71.3 µU/ml in colostrum whey on the day of parturition (P <

0.01). On the first day after, the insulin concentration decreased sharply to a mean value 289 ± 54.0 µU/ml and then successively decreased to the level of 34.0 ± 2.8 µU/ml observed on the fifth day *post partum* ($P < 0.01$) (fig 1). This level was almost equal to the basal insulin level (14–30 µU/ml).

The mean concentrations of sugars (lactose and probably free glucose and galactose) were measured by a method that was not specific for lactose in colostrum whey. They were calculated as the mean value for each day separately for all of the ewes studied and showed lowest concentration on the second and first prepartum days (2.9%), and then increased progressively to 5.47% on the fifth day after parturition (fig 2).

The mean body weight of the newborn lambs (n = 42) was 5.2 kg on the day of birth, and 11.9 kg on the 28th day; this increase was not related to the insulin content in the colostrum.

The body-weight gain was higher (275 g/d) in lambs born from the 7-yr-old ewes than in lambs from 4-yr-old ewes (196 g/d, fig 3). The final body weight was again greater in single offspring (8.0 kg), and smaller in twins (5.9 kg) and in triplets (4.9 kg) ($P < 0.01$, fig 4).

DISCUSSION

The mean insulin concentration in colostrum whey samples exceeded many times (over 28- to 40-fold) the basal level of this hormone in the ovine blood (34 µU/ml given by Blom *et al*, 1976). No symptoms of hypoglycaemia were found in lambs that had taken colostrum with high insulin concentrations. Similarly no state of hypoglycaemia was noted in piglets that had taken colostrum from sows receiving insulin (Nowak, 1989, 1990). It is known that insulin is absorbed from the digestive tract into blood in an intact form during the first 40 h

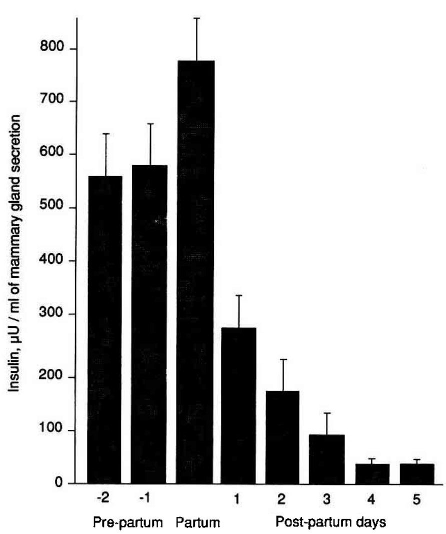

Fig 1. Insulin concentrations (mean values) in pre-milk, colostrum and milk (mammary-gland secretions) of 28 ewes.

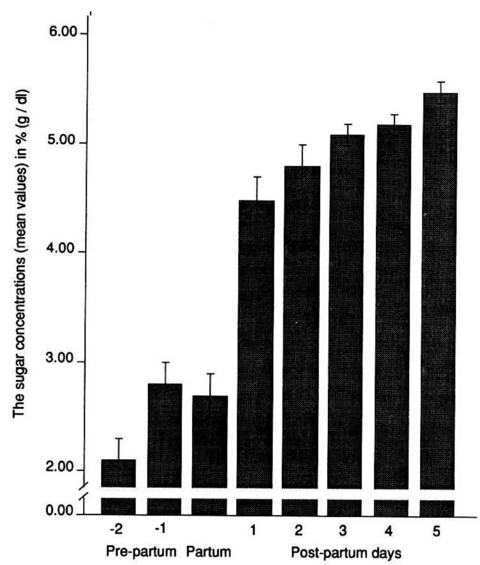

Fig 2. Sugar concentrations (mean values) in the mammary secretions of 28 ewes.

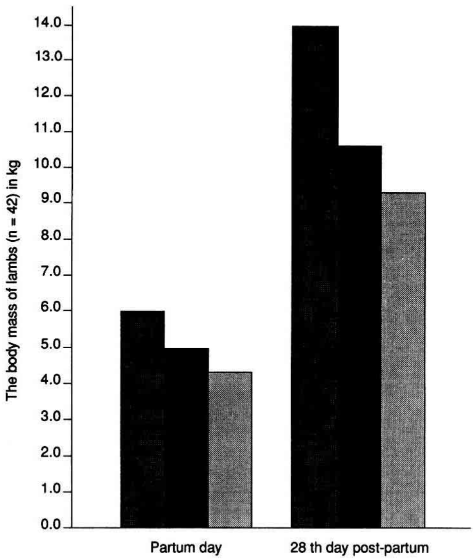

Fig 3. Mean values of body mass of newborn lambs (n = 42) on the day of birth and on the 28th day after birth (in single offspring ▒, twins ■, and triplets, ░).

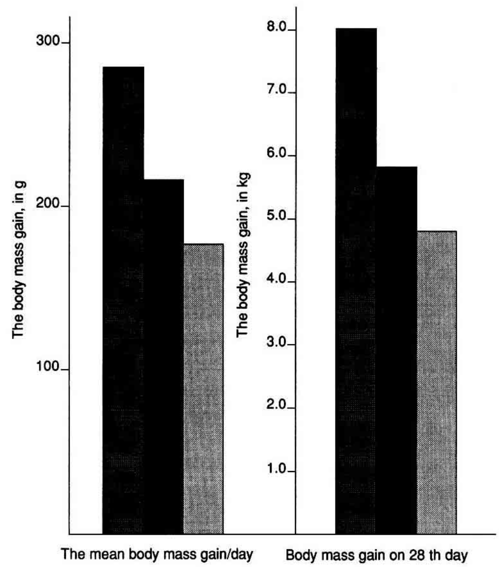

Fig 4. Mean values of body-weight gains observed at the age of 28 d of lambs (n = 42) born as single offspring ▒, twins ■, and triplets ░.

of life for newborn infants (Leissring *et al*, 1962; Koldovský, 1978; Read *et al*, 1984; Koldovský *et al*, 1991) and up to 25 h in piglets (Asplund *et al*, 1962; Nowak, 1989). The presence of a trypsin inhibitor secreted by the digestive tract (Nordbring and Olsson, 1958), which is found in the mammary gland during the preparturient period, seems to facilitate this absorption (Laskowski *et al*, 1957; Carlson *et al*, 1974). No signs of hypoglycaemia of colostral insulin intake could be related to efficient gluconeogenesis, which is already active within 30 min of birth (Tildon *et al*, 1971; Ballard, 1978). The process of gluconeogenesis is facilitated by the availability of glucose and glucose precursors (*eg*, arginine, leucine, alanine, glutaminic acid) and some free fatty acids derived from colostrum after its digestion and absorption in newborn mammals (Sperling, 1978).

On the other hand, newborn mammals often have insulin deficiency as a cause of backward postnatal adaptation. Glucose itself is a weak stimulator for secretion and the release of insulin from the B-cells of Langerhans immediately after birth (Ballard, 1978). However, after the first intake of colostrum, alanine, arginine and leucine are released from colostrum protein. Together with glucose, these are commonly considered as strong stimulators for insulin synthesis and release (Nowak, 1991). Therefore, the insulin in colostrum could supplement a transient endogenous insulin deficit during the first hours of the life of newborn mammals. Later, the *post partum* lowering of the insulin concentration in mammary secretion could favor an insulinaemia normalization in the newborn (Read *et al*, 1984; Nowak and Nowak, 1989).

It should be noted that the insulin concentration measured by RIA reveals the presence of an immunoreactive hormone in colostrum and milk, but does not determine the bioactivity of this hormone. It is assumed therefore that at least part of the insulin present in the mammary secretion, can supplement the insulin deficiency usually observed on the first day of life (Ballard, 1978; Sperling, 1978). Supplementing the endogenous hormone deficiency, *eg*, in neonates small for gestational age, with exogenous hormone seems to be possible since it is known that the gastrointestinal tract of newborn suckling infants (Koldovský, 1978; Read *et al,* 1984) and piglets (Nowak, 1989) absorbs intact and biologically active insulin in quantities that are sufficient to cause a biological response (reviewed by Koldovský, 1989; Koldovský *et al,* 1991; Pearlman, 1991). According to Read *et al* (1984) insulin, epithelial growth factor and insulin-like growth factor belong to the important group of growth-promoting factors that may play a role in the proliferation and maturation of the gut and other tissues, without necessarily entering the circulation. In support of this, these factors have been shown to regulate the development of intestinal mucosa of suckling mice and rats (reviewed by Read *et al,* 1984).

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