Praca oryginalna *Original paper*

Selected electrolytes and metabolites in the puerperal ewes with twins and singleton*)

IGOR VALOCKÝ, ŠTEFAN MOZEŠ*, ĽUDOVÍT LENHARDT, JURAJ KAČMÁRIK

Clinic of Animal Obstetrics, Gynaecology and Andrology, University of Veterinary Medicine, Komenského 73, 041 81 Košice, Slovak Republic
*Institute of Animal Physiology Slovak Academy of Sciences, Šoltesovej 4-6, 040 01 Košice, Slovak Republic

Valocký I., Mozeš Š., Lenhardt Ľ., Kačmárik J.

Selected electrolytes and metabolites in puerperal ewes with twins and single lambs

Summary

The object of the experiment was to determine the extent to which selected electrolytes (Na, K, Ca, P) and metabolites (cholesterol, total lipids, and total proteins) changed in the blood serum during the postpartum period in ewes and their correlation to litter size. Blood was collected from the jugularis v. of 149 Slovak merino breed ewes on 1, 4, 7, 14, 17, 21, 25 and 34 postpartum days.

Lower Na and K levels were recorded in ewes with twins compared to ewes with a single lamb, with the exception of day 34.When evaluating Ca levels, it was found that the group of ewes with a single lamb exhibited a range from 2.38 ± 0.16 to 2.54 ± 0.26 mmol.l^{-1} and the group with twins a range from 2.39 ± 0.15 to 2.51 ± 0.19 mmol.l^{-1} and there was no significant difference. Phosphorus levels in the group of ewes with twins were lower on all observed days than those in the group of ewes with single lambs. Lower cholesterol levels were observed from day 7 of the observed period in ewes with twins compared to ewes with a single lamb. By day 21 of the observed period total lipids exhibited lower values in ewes with twins and the total serum protein levels in ewes with single lambs tended to slightly rise during this time. From day 14 postpartum there was a mild decrease in the total protein levels in the group of ewes with twins. The discussion focused on the effect of litter size on selected biochemical parameters during the postpartum period.

Keywords: ewe, litter size, electrolites

The enhancement of gene pool with fertility gene lines FecB in Merino breed necessitates the study of compensating possibilities of the ewe organism in the puerperal period. Metabolism of mineral substances which make up the basic constituents of the inner environment plays an important role in the regulation of physiological functions in the puerperal period. Their concentrations in the blood represent homeostatic mechanisms which are closely related to neurohumoral regulation. Changes in the concentrations of mineral substances affect metabolism of myometrial and endometrial cells (3). Differences in Na and K functions during early and late pregnancy in relation to season were observed in Marwari sheep (23) and in Bahri and Rahmani sheep (24). Kudláč (21) recorded the dynamics of changes in Ca and P ration in connection to cow fertility. Ca and P concentrations in pregnant women blood serum were observed by Brommage and Baxter (4). Jelínek et al. (12) monitored these parameters in fattening merino sheep throughout the year. Selected parameters of mineral profile in Slovak merino sheep in individual phases of their reproductive cycle were evaluated by Krajničáková et al. (16, 17) with a significant increase in values found in the period from insemination to the third month of pregnancy. The puerperal period is characteristic not only of involution processes in the reproductive tract of the ewes but also of metabolic and hormonal changes which are influenced by a decrease in systemic concentration of ovarian steroids. Cholesterol, which is the primary substrate of steroid synthesis, arises when of 3-hydroxy-3-methylglutaryl of coenzyme A reductase increases (26). It is produced either by the ovaries or the ovaries acquire it from plasmatic fractions of low – density (LDL) and high – density lipoproteins (HDL) (25). Postpartum increase in cholesterol levels in the peripheral blood of cows to day 45 postpartum was reported by Doležel (6) and to day 56 postpartum by Bekeová (2). A decrease in cholesterol concentrations initiates a decline in low – density proteins, metabolism of which is closely connected with the effect of estrogens (5). The influence of estradiol on lipid and lipoprotein concentrations was investigated by Fahraeus (7). The dependence of some biochemical parameters in the blood serum of goats on the phase of the reproductive cycle was noted by Aldasy et al. (1), Krokavec et al. (20) and on supplementary protein diet in Angora goats by Gregoire et al. (8).

The aim of this study was to determine the extent of changes of selected biochemical parameters in the blood serum during the postpartum period of ewes in relation to litter size.

*) These studies were founded by Ministry of Education of Slovak Republic, VEGA project 1/0565/03.

Tab. 1. Mean (± S.E.M.) concentrations of selected mineral substances in the puerperal ewes according to litter size

Day *pp*	Na (mmol.l^{-1})		K (mmol.l^{-1})		Ca (mmol.l^{-1})		P (mmol.l^{-1})	
	EwS	EwT	Single	EwT	Single	EwT	Single	EwT
1	160,48 ± 7,56	154,20 ± 5,98	5,27 ± 0,46	4,87 ± 0,24	2,38 ± 0,16	2,39 ± 0,15	2,38 ± 0,19	1,81 ± 0,22
4	158,07 ± 2,72	153,27 ± 5,72	5,41 ± 0,39	4,94 ± 0,41	2,41 ± 0,11	2,40 ± 0,15	2,33 ± 0,25	1,90 ± 0,14
7	160,02 ± 5,42	152,10 ± 5,07	5,39 ± 0,33	5,00 ± 0,19	2,49 ± 0,09	2,48 ± 0,16	2,31 ± 0,33	1,94 ± 0,21
14	158,50 ± 2,15	154,75 ± 4,99	5,06 ± 0,17	4,84 ± 0,29	2,44 ± 0,13	2,51 ± 0,19	2,27 ± 0,24	2,14 ± 0,22
17	160,98 ± 2,33	154,88 ± 5,81	5,33 ± 0,63	4,68 ± 0,50	2,54 ± 0,26	2,46 ± 0,17	2,48 ± 0,29	2,08 ± 0,19
21	159,53 ± 1,48	154,14 ± 5,22	5,06 ± 0,22	4,89 ± 0,45	2,43 ± 0,21	2,43 ± 0,24	2,14 ± 0,03	1,99 ± 0,22
25	159,60 ± 1,66	153,50 ± 3,73	5,21 ± 0,20	5,02 ± 0,17	2,44 ± 0,09	2,45 ± 0,13	2,33 ± 0,22	2,05 ± 0,10
34	*155,3 ± 2,19	148,33 ± 2,49	*4,59 ± 0,36	4,83 ± 0,12	2,38 ± 0,13	2,47 ± 0,08	2,17 ± 0,04	2,02 ± 0,08

Explanation: pp – postpartum, *P < 0,05 – statistical significance compared to day 1 postpartum, EwS – ewes with singleton, EwT – ewes with twins

Material and methods

Experimental animals. Slovak merino ewes (n = 149) with mean body weight 39.4 ± 1.87 kg aged 2-3 years were included in the experiment. The animals were divided into two groups a group of ewes with a single lamb (n = 106) and a group of ewes with twins (n = 43). During lambing season (February, March) the ewes were raised in standard conditions on a commercial farm in a submontane region. Feed ration per animal/day was composed of meadow hay, fodder concentrate, straw and root, crops. A mineral supplement and water were given *ad libitum* (27).

Blood samples were collected from *v. jugularis* on days 1, 4, 7, 14, 17, 21, 25 and 34 postpartum. Blood sera were stored in single test tubes after centrifugation at a temperature of –18°C until analysed.

Laboratory analysis. Na, K and Ca concentrations were determined in the blood serum by flame spectrophotometry (acetylene, air) using Analyst 100, Perkin Elmer Co. The automatic biochemical analyser Alyze, Lisa-Bio Co; test Bio Mérieux was used for the photometric determination of P concentrations. Analyses of total protein, total lipids and total cholesterol were carried out by Bio-Lachema Tests, Lachema, Inc. Brno, and read spectrophotometrically at the determined wavelength.

Statistical evaluation. The statistical analysis of the above parameters was carried out by one-way analysis of variance (ANOVA). Statistical significance compared to day 1 postpartum was done by Tukey test. For data analysis, the Sigma Stat statistical software (Jandel Scientific® 2000) was used.

Results and discussion

Balanced dynamics of Na concentration in the group of ewes with singleton was notedto 25 day postpartum. A statistically significant decrease in its level (P < 0.05) occurred on day 34 (155.33 ± 2.19 mmol.l^{-1}). However, a declining trend was note in ewes with twins from day 1 (154.2 ± 5.98 mmol.l^{-1})to day 7 (152.1 ± 5.07 mmol.l^{-1}). Gradual normalization occurred on day 14 (154.75 ± 4.99 mmol.l^{-1}). The results obtained in this experiment are comparable with the data on sheep fertilized in winter season (24) and on sheep after parturition (12). A slight declining tendency of Na level to day 7 in the group of ewes with twins may be linked with increased Na transport to milk and changes of its contents in the uterine wall (15, 18, 21, 29).

The concentration of K (tab. 1) in the group of ewes with a singleton exhibited higher concentrations by day 25 than in the group of ewes with twins. This difference is probably due to higher energetic load of mothers with more lambs. A statistically significant decrease (P < 0.05) was observed in the group of ewes with single lamb on day 34 (4.59 ± 0.36 mmol.l^{-1}) compared to day 1 (5.27± 0.46 mmol.l^{-1}). Similar K values in the puerperal period, however, irrespective of the number of lambs were found by Okab (24).

Mean Ca values are set out tab. 1. They ranged from 2.38 ± 0.16 mmol.l^{-1} to 2.54 ± 0.26 mmol.l^{-1} in the group of ewes with a singleton and from 2.39 ± 0.15 mmol.l^{-1} to 2.51 ± 0.19 mmol.l^{-1} in the group of ewes with twins. Despite lower Ca concentrations, which are characteristic of the puerperal period, reported by Haraszti et al. (10), Ivanov et al. (11) Kudláč et al. (21) in cows and by Krajničáková et al. (19) in goats, no significant difference was noted between the groups of individual days in this experiment. Higher Ca levels in the blood serum of sheep during synchronization of the estrous cycle ranging form 2.98 to 2.96 mmol.l^{-1} were reported by Krajničáková et al. (17).

The concentration of inorganic P in the blood serum of ewes with a singleton (from 2.14 ± 0.03 mmol.l^{-1} to 2.48 ± 0.29 mmol.l^{-1}) was higher on all observed days than in the group of ewes with twins (from 1.81 ± 0.22 to 2.14 ± 0.22 mmol.l^{-1}).

The authors infer that a decrease in P concentration in the observed period in the group of ewes with twins is related to lactation. The content of P in the diet affects its level in blood plasma a while the content of Ca in the diet does not immediately affect the level of P (13). The phosphorus values obtained by the authors in the group of ewes with twins approach the observations made by Lippmann and During (22) during the winter and vernal seasons.

Mean values of total cholesterol concentrations are summarised in tab. 2. Ewes with a singleton exhibited a decline form day 4 postpartum (2.06 ± 0.24 mmol.l^{-1}) to day 14 (1.75 ± 0.24 mmol.l^{-1}). From day 17 elevation to a level of 2.03 ± 0.39 mmol.l^{-1} occurred. Total cholesterol levels of the blood serum of ewes with twins ranged at a lower level except for days 7 and 14 compared to ewes with a singleton.

Higher total lipid concentrations (tab. 2) were observed in ewes with a single lamb than in ewes with twins except for day 25. Postpartum enrichment of lipoprotein concentrations independent of lactation my point to esterification of free fatty acids mobilized form regressive maternal fat deposits and other tissues (14). A postpartum decline in

total lipid concentrations in ewes with twins is related to depletion of nutrients towards the end of pregnancy via continual breakdown and synthesis of proteins and fats. Lower levels can be related to the inhibition of synthesis of apoproteins and the number of their receptors which are important to very low-density lipoprotein (VLDL) production as started by Grummer and Carroll (9).

Ewes with a singleton exhibited an moderate increase in total protein value from day 1 postpartum to day 34 of the observation period 65.79 ± 4.53-69.2 ± 0.98 $g.l^{-1}$ (tab. 2).Total protein increase in the first observed days after parturition correlated with concentration of 17-β estradiol Surani (28) in ewes with singleton and twins. A decline in total protein level was noted in ewes with twins on day 14 from 66.21 ± 7.03 to 63.7 ± 1.51 $g.l^{-1}$. A decrease in total proteins in the first month postpartum i.e. at the time of maximal lactation load was reported also by Jelínek et al. (12).

Tab. 2. Mean (± S.E.M.) concentrations of total proteins, cholesterol and lipids in the puerperal period of ewes according to litter size

Day pp	TP ($g.l^{-1}$)		Chol ($mmol.l^{-1}$)		TL ($g.l^{-1}$)	
	EwS	EwT	EwS	EwT	EwS	EwT
1	65,79 ± 4,53	66,21 ± 7,03	1,95 ± 0,32	2,07 ± 0,29	2,20 ± 0,30	1,97 ± 0,32
4	66,60 ± 4,66	69,85 ± 5,18	2,06 ± 0,24	2,12 ± 0,23	2,44 ± 0,25	2,00 ± 0,29
7	66,97 ± 6,40	67,50 ± 4,63	1,95 ± 0,18	1,82 ± 0,25	2,37 ± 0,31	2,01 ± 0,22
14	66,08 ± 8,29	63,70 ± 1,51	1,75 ± 0,24	1,73 ± 0,16	2,13 ± 0,35	1,86 ± 0,26
17	66,53 ± 7,39	62,23 ± 4,96	2,03 ± 0,39	1,63 ± 0,17	2,33 ± 0,33	1,66 ± 0,34
21	66,03 ± 3,99	64,19 ± 2,74	2,06 ± 0,12	1,80 ± 0,24	2,18 ± 0,48	2,00 ± 0,23
25	66,54 ± 5,72	60,32 ± 1,36	1,93 ± 0,27	1,77 ± 0,18	1,96 ± 0,44	1,98 ± 0,14
34	69,20 ± 0,98	65,73 ± 3,95	1,90 ± 0,16	1,68 ± 0,12	1,70 ± 0,16	1,63 ± 0,25

Explanation: TP – total proteins, Chol – cholesterol, TL – total lipids, pp – postpartum, EwS – ewes with singleton, EwT – ewes with twins

The load in the observed period is represented by the onset and culmination of lactation, especially in ewes with twins as well as by regressive and regenerative processes leading to the restoration of the surface epithelium of the uterine mucous membrane. The authors infer from the evaluation of the changes of selected metabolic parameters and mineral profile that during the puerperal period there is increased stress on the regulatory mechanisms involved in the involution in the reproductive tract tissues mainly in ewes with more lambs. The evaluated parameters are in close mutual relation with in homeostatic and metabolic adaptation of the organism.

References

1. *Aldasy P., Bajáky P., Eerrus T.*: Vér alapertek tejhasznu kecske lomanyban. Magy. Állatorv. Lap. 1981, 36, 536-538.
2. *Bekeová E., Elečko J., Hendrichovský V., Choma J., Krajničáková M.*: The effect of beta-carotene on the changes in T_4 and cholesterol concentrations in calving heifers before and after parturition. Vet. Med. (Praha) 1987, 32, 459-468.
3. *Bitman J., Hawk W., Cecil C., Sykes J. F.*: Water and electrolyte composition of the bovine uterus in pregnancy. Amer. J. Physiol. 1961, 4, 827-834.
4. *Brommage R., Baxter D. C.*: Vitamin D-dependent stimulation of intestinal calcium and phosphorus absorption during reproduction. J. Bone Min. Res. 1988, 3, 156-158.
5. *Brown M. S., Kovanen P. T., Goldstein J. L.*: Regulation of plasma cholesterol by lipoprotein receptors. Science 1981, 212, 628-635.
6. *Doležel R., Kudláč E., Studenčík B., Balaštík J.*: Biochemické zmeny v periférní krvi krav v průběhu 45 dnu po porodu. Veter. Med. (Praha) 1991, 36, 256-271.
7. *Fahraeus L.*: The effects of estradiol in blood lipids and lipoproteins in postmenopausal women. Obstet. Gynec. 1988, 72, 188-220.
8. *Gregoire R. J., Fahmy M. H., Boucher J. M., Tremblay A., Mercier J.*: Effect of four protein supplements on growth feed conversion, mohair production, fibre characteristic and blood parameters of Angora goats. Small Ruminant Res. 1996, 19, 121-130.
9. *Grummer R. B., Caroll D. J.*: A review of lipoprotein cholesterol metabolism importance to ovarian function. J. Anim. Sci. 1998, 66, 3160-3173.
10. *Haraszti J., Huszenica G., Molnar L., Solti L., Fekete S., Ekes K., Yard A. C.*: Some metabolic characteristic of dairy cows with different postpartum ovarian function. J. Vet. Med. 1988, 35, 506-515.
11. *Ivanov I., Rajič I., Jovanovič M. J., Lalic M.*: Concentration of calcium in the blood serum in high-pregnant and lactating cows in intensive breeding. Vet. Glasnik 1990, 44, 359-364.
12. *Jelínek P., Illek J., Frais Z., Jurajdová J., Helanová I.*: The annual dynamics of the biochemical blood parameters in ewes. Živ. Výr. 1985, 30, 556-564.
13. *Kincaid L. R., Hiller K. J., Cronrath D. J.*: Calcium and phosphorus supplementation ratio for lactating cows. J. Dairy Sci. 1981, 64, 754-758.
14. *Knopp R. H., Warth M. R., Carroll C. J.*: Lipid metabolism in pregnancy. I. Changes in lipoprotein triglyceride and cholesterol in normal pregnancy and the effects of diabetes mellitus. J. Reprod. Metab. 1985, 10, 95-107.
15. *Krajničáková M., Bekeová E., Maraček I., Hendrichovský V.*: The dynamics of some haematological and biochemical parameters in the puerperal period of sheep. Živ. Výroba (Praha) 1991, 36, 885-893.
16. *Krajničáková M., Elečko J., Bekeová E., Maraček I., Hendrichovský V.*: Some biochemical parameters in the blood serum of sheep in the puerperal period. Scientific Papers UEVM 1992, 8, 39-48.
17. *Krajničáková M., Bekeová E., Maraček I., Hendrichovský V.*: Selected parameters of the mineral profile of the blood serum of sheep in individual phases of the reproduction cycle. Živ. Výroba (Praha) 1993, 38, 717-724.
18. *Krajničáková M., Bekeová E., Maraček I., Hendrichovský V.*: Concentrations of sodium, potassium and their relationship to the ovarian hormones at the time of synchronization of estrus and ewe pregnancy. Vet. Med. (Praha) 1994, 39, 541-550.
19. *Krajničáková M., Kováč G., Kostecký M., Valocký I., Maraček I., Šutiaková I., Lenhardt Ľ.*: Selected clinico-biochemical parameters in the puerperal period of goats. Bull. Vet. Inst. Pulawy 2003, 47, 177-182.
20. *Krokavec M., Šimo K., Martinko A., Krokavcová A.*: A study of the values of metabolic profile parameters in the large-scale breeding of goats throughout the seasons of the year. Vet. Med. (Praha) 1992, 37, 113-118.
21. *Kudláč E., Sakour M., Čanderle J.*: Metabolic profile in the puerperal period in cows without retention and with retention of secundines. Vet. Med. (Praha) 1995, 40, 201-207.
22. *Lippmann P., During B.*: Untersuchungen zum Gehalt Kalzium, anorganischen Phosphat und Magnesium im Blutserum von Mutterschafen. Mh. Vet. Med. 1973, 28, 106-109.
23. *Mali C. P., Patnayak B. C., Ghosal A. K.*: Levels of certain blood nutrients in grazing non-pregnant, pregnant and lactating Marwari ewes. Annals Arid. Zone 1994, 33, 319-323.
24. *Okab A. B., Mekkawy M. Y., Elbanna I. M., Hassan G. A., El-Nouty F. D., Salem M. H.*: Seasonal changes in plasma volume, adrenocortical hormones, osmolality and electrolytes during pregnancy and at parturition in Bahri and Rahmani ewes. Indian. J. Anim. Sci. 1992, 62, 302-306.
25. *Paavola L. G., Strauss J. F., Boyd CH. O., Nestler J. E.*: Uptake of gland- and (3H) cholesteryl linoleate-labeled human low density lipoprotein by cultured rat granulosa cells. Cellular mechanism involved in lipoprotein metabolism and their importance to steroidogenesis. J. Cell Biol. 1985, 100, 1235-1247.
26. *Parinaud L. G., Peret B., Ribees H., Chap H., Pontonnier G., Douste-Blazy L.*: High density lipoprotein and low density lipoprotein utilization by human granulosa cells for progesterone synthesis in serum-free culture: Respective contributions of free and esterified cholesterol. J. Clin. Endocrinol. Metab. 1987, 64, 409-417.
27. *Sommer A., Geršáková Z., Frydrych Z., Králik O.*: The nutrient requirements and nutrient value of feeds for cattle, sheep and goats. In Scientific Studies, 1st ed., Research Institute of Animal Husbandry Nitra 1994, 71-73.
28. *Surani M. A.*: Hormonal regulation of proteins in the uterine secretion of ovariectomized rats and the implications for implantation and embryonic diapause J. Reprod. Fertil. 1975, 43, 411-417.
29. *Webb G. D., Ashmed G. B., Al-Mahdi S., Auletta F. J., Mc Laughlings M. K.*: Changes in sodium transport during the human menstrual cycle and pregnancy. Clin. Sci. 1993, 84, 401-405.

Author's address: Igor Valocky, DVM, PhD, Clinic of Animal Obstetrics, Gynaecology and Andrology University of Veterinary Medicine, Komenského 73, 041 81 Košice, Slovakia