

THE EFFECT OF DEPOTOCIN INJ. SPOFA ON CONCENTRATIONS OF CHOLESTEROL, TOTAL LIPIDS, PROGESTERONE AND ON THE CONCEPTION RATE IN POSTPARTURIENT EWES

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Abstract

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The effect of Depotocin inj. Spofa on concentrations of cholesterol, total lipids and progesterone with subsequent synchronization effect on the conception of ewes was studied. A total of 19 ewes were included in the experiment; 10 animals served as a control, and 9 sheep were treated with Depotocin inj. Spofa 2 - 0 - methyltyrosin (deamino -1 - carba) oxytocinum at a dose of 2×0.07 mg, 24 and 72 h after lambing, respectively. Blood samples were obtained from v. jugularis at 36 h post partum, and on days 4, 7, 14, 17, 21, 25, 34, 42 and 51 after lambing. Agelin vaginal tampons were inserted to sheep of both groups after day 51 (20 mg of chlorsuperlutine). Ten days later, the tampons were removed and the animals were injected with 500 I. U. of PMSG. The expected date of parturition was calculated from the date of mating. Cholesterol and total lipids were determined using BIO-LA tests (LACHEMA, Brno) and the concentrations of progesterone were determined by the radioimmunological method, using kits from ÚRJVT, Košice. Cholesterol concentrations showed no significant differences between the control and experimental groups ($P > 0.05$). The concentrations of total lipids oscillated in the experimental group from day 25 post partum at non-significantly higher values in comparison with the control group by the end of the period studied (1.78 ± 0.48 to 2.45 ± 0.35 g · l⁻¹). In the experimental group, the levels of progesterone increased slowly on days 34 and 51 (0.5 ± 0.8 to 0.8 ± 1.3 nmol · l⁻¹). In other sampling days, its values were under the level of sensitivity of the trial. In conclusion, lambing after the oestrus induction on day 51 post partum showed that a 100% lambing with birth of 16 lambs was achieved in the experimental group whereas in the control group only 5 out of 10 mated animals lambed.

Puerperium, ewe, Depotocin, total lipids, cholesterol, progesterone

Puerperium length and course in ewes are extremely important from the point of view of lamb meat and lamb broilers production. In our previous studies (Krajníčáková 1990; Krajníčáková et al. 1991, 1992), the attention was paid to the physiological course of puerperium and medicamentous influencing of postpartal reparative processes on the reproductive apparatus of sheep following repeated administration of Depotocin inj. Spofa in early puerperium; the authors recorded a 64.3% decrease in the mass of uterine body and uterine horns as early as on day 7 of the period under study. The effect of repeated administration of Depotocin inj. Spofa on the dynamics of concentration changes of thyroxine, triiodothyronine and ovarian steroids was studied by Bekeová et al. (1992). The authors revealed that the administered carbetocin induces changes in the synthesis and production of both thyroid and steroid hormones through a direct or indirect effect.

Oxytocin, in addition to its uterotonic and luteolytic effect, acts as an ovarian factor in the local interfollicular regulation of steroidogenesis and as a modulator of uterine secretion of prostaglandins as well (Gregoraszczuk et al. 1988; Flint et al. 1990; Light et al. 1994).

The effect of steroid hormones on biosynthesis of prostaglandins can be manifested by the effect of their inhibitors or stimulators of synthetic enzymes. It is assumed that progesterone

inhibits prostaglandin release by induction of protein synthesis, which in turn inhibits the activity of phospholipase (Thorburn 1979). Stimulation of steroidogenesis by lipoproteins through endocytotic and lysosomal hydrolysis releases cholesterol, which can be the substrate for progesterone synthesis (Rajkumar 1985).

On the basis of the above mentioned studies as well as our previous investigations (Krajníčková et al. 1995), the present study was directed at the determination of the effect of double administration of Depotocin inj. SPOFA on the dynamics of concentration changes in cholesterol, total lipids and progesterone from 36 h to day 51 after parturition. The effect of Depotocin administration on the conception rate of ewes after the induction of oestrus on day 51 after lambing was studied as well.

Materials and Methods

Experimental animals

A total of 19 Slovak Merino ewes weighing 40 to 50 kg, 3 to 5-year-old, and after 2 to 3 births, that had lambed in the first half of February were included in the experiment. The animals were fed a diet consisting of silage, beet, hay and supplementation of the BAK feeding mixture. Water and salt were provided ad libitum.

Treatment

Nine sheep were included in the experimental group, and 10 sheep served as a control. The experimental ewes were subjected to treatment with carbetocin 2-0-methyltyroxin (deamino-1-carbo) oxytocinum at a dose of 2×0.07 mg per animal. The first dose was injected intramuscularly (i. m.) 24 h, the second one subcutaneously (s.c.) 72 h after parturition. On day 51 after parturition, after the weaning of lambs, oestrus was induced in ewes of both control and experimental groups by insertion of vaginal polyurethane tampons containing 20 mg of chlorsuperlutine (Agelin - vaginal tampons, Spofa). After 10 days the tampons were removed and the animals were administered 500 I. U. of PMSG. From the day of PMSG administration, sheep were kept together with rams for the following 6 days. The expected date of parturition was calculated according to the date of mating. According to the number of lambed animals, the conception rate in two observed groups was determined.

Sampling

Blood samples were obtained by puncture of the v. jugularis 36 h after lambing and on days 4, 7, 14, 17, 21, 25, 34, 42 and 51 post partum between 9.00 and 10.00 h. The days of sampling were determined according to the schedule of the experiment as well as the other indices, mainly functional-morphological ones, taking into account the termination of the involution process of endometrium and postparturient recovery of ovarian functions. The blood sera obtained by centrifugation were stored in polyurethane tubes at -18 °C until processing.

Analytical methods

Cholesterol, total lipids and progesterone were determined in the blood serum. Concentrations of cholesterol and total lipids were determined by means of BIO-La tests (Lachema Brno). The levels of progesterone were determined by RIA-test-Prog kits (URVIT Košice, Slovak Republic). The determinations were carried out directly, without extraction, in duplicates in 0.05 ml of serum. The declared test sensitivity was 0.5 nmol.l^{-1} . The intra-assay (five determinations for each of two pools containing 4.813 Z 0.66 and 44.17 Z 1.85 nmol.l⁻¹) was 13.71% and 4.18%, respectively. The inter-assay (ten and six determinations for each of two pools containing 4.81 Z 0.36 and 44.96 Z 1.92 nmol.l⁻¹) was 7.48% and 4.27%, respectively. The test recovery ranged from 90 to 110%.

Statistical methods

The significance of the parameters observed between 36 h and other days of the experiment, as well as the significance of differences between groups were determined by Student's t-test.

Results

In the control group, the concentrations of cholesterol (Table 1) ranged from 1.56 ± 0.27 to $2.10 \pm 0.53 \text{ mmol.l}^{-1}$.

In the experimental group (the administration of Depotocin inj. SPOFA) the levels of cholesterol were nearly the same up to day 7 after lambing (2.13 ± 0.18 - $2.08 \pm 0.38 \text{ mmol.l}^{-1}$). A significant decrease ($P < 0.01$) in its levels as compared to day 0 was recorded at

$1.68 \pm 0.21 \text{ mmol.l}^{-1}$) on days 14 and 21 as well as on days 17 and 42 of the experiment ($P<0.05$). Observation of the total lipids in the control group (Table 1) showed the significant increase ($P<0.01$) in their levels on days 4 and 7 in comparison with 36 h after lambing (day 0). Their slight decrease to $1.87 \pm 0.67 \text{ g.l}^{-1}$ on day 14 was replaced by significant increase ($P<0.001$), on days 17 and 21 of the period studied. A significant increase ($P<0.05$) was found also on days 25, 34 and 42 of the postpartal period.

Table 1
Concentrations of cholesterol, total lipids and progesterone in the puerperium of sheep after administration of Depotocin inj. SPOFA

Day post partum	Cholesterol (mmol.l ⁻¹)	Control group Total Lipids (g.l ⁻¹)	Progesterone (P ₄) (nmol.l ⁻¹)
36 h	1.92 ± 0.39	1.25 ± 0.39^b	—
d 4	2.10 ± 0.53	2.16 ± 0.57^{xx}	—
7	1.90 ± 0.32	2.15 ± 0.58^{xx}	—
14	1.68 ± 0.21	1.87 ± 0.67	—
17	1.74 ± 0.33	$2.42 \pm 0.41^{xxx\,a}$	—
21	1.86 ± 0.42	2.15 ± 0.22^{xx}	—
25	1.56 ± 0.27	1.84 ± 0.34^x	—
34	2.01 ± 0.42	2.01 ± 0.58^x	1.00 ± 2.80
42	1.74 ± 0.33	1.83 ± 0.46^x	—
51	1.74 ± 0.46	1.62 ± 0.37	—
Experimental group			
36 h	2.13 ± 0.18	$2.35 \pm 0.39^{b'}$	1.40 ± 0.90
d 4	2.08 ± 0.38	2.01 ± 0.29	—
7	2.08 ± 0.32	2.28 ± 0.58	—
14	1.68 ± 0.18^{xx}	1.70 ± 0.27^{xx}	—
17	1.77 ± 0.30^x	$1.78 \pm 0.48^{x\,a'}$	—
21	1.68 ± 0.21^{xx}	1.88 ± 0.49	—
25	1.89 ± 0.30	2.18 ± 0.48	—
34	1.79 ± 0.35	2.45 ± 0.35	0.50 ± 0.80
42	1.76 ± 0.27^x	2.13 ± 0.30	—
51	1.81 ± 0.34	2.07 ± 0.59	0.80 ± 1.30

The significance compared to 36 h post partum

x = $P<0.05$; xx = $P<0.01$; xxx = $P<0.001$.

The significance in intergroup differences a:a' = $P < 0.05$;

b:b' = $P < 0.01$

In the experimental group, the concentrations of total lipids from 36 h up to day 7 after lambing ranged from 2.01 ± 0.29 to $2.35 \pm 0.39 \text{ g.l}^{-1}$. The significant decrease in their concentrations with the lowest levels ($1.70 \pm 0.27 \text{ g.l}^{-1}$) was found on day 14 ($P<0.01$) and day 17 ($P<0.05$) p. p. From the 25th day to the end of the observed period, their concentrations ranged from 2.07 ± 0.59 to $2.45 \pm 0.35 \text{ g.l}^{-1}$ while their dynamics showed slightly decreasing tendency from day 34 to day 51 of the period studied.

The concentrations of progesterone (P₄) in the control group post partum (Table 1) ranged below the level of sensitivity except for day 34 after parturition, with widely dispersed individual values. In the experimental group of ewes (Table 1), its levels gradually increased on days 34 and 51.

Significant differences between groups in concentrations of total lipids were recorded at 36 h ($P<0.001$) and on d 17 ($P<0.05$) of the postpartal period. No significant differences in cholesterol levels were recorded between groups.

Table 2
Fertility parameters of sheep after the effect of Depotocin inj. SPOFA on their puerperium

Indicator	Ewes without treatment	Ewes treated with Depotocin at 24 and 72 h p. p.
Number of sheep prepared for mating	10	9
Number of lambed out of mated sheep	5	9
% of lambed sheep	50.00	100%
Numbers of born lambs	6	16
Lambs-Ewes	2	7
Lambs-Rams	4	9

Evaluation of the results of the lambing (Table 2) after induction of the oestrus on day 51 post partum showed that out of 10 animals, 5 ewes lambed giving birth to 6 lambs in the control group. In the experimental group, all 9 treated sheep lambed and gave birth to 16 lambs.

Discussion

Evaluation of the concentrations of cholesterol showed no significant differences between experimental and control groups. These data are comparable with the results obtained by Krajničáková et al. (1995) after the administration of gonadoliberin in the first phase of the postpartal period. In the experimental group, its significant decrease was recorded (administration of carbetocin, Depotocin inj. SPOFA) on days 14 and 21 ($P<0.001$) and on days 17 and 42 ($P<0.05$) of the period studied. From the literature data of Brown et al. (1981) it is evident that with the decreased cholesterol concentrations, a low-density lipoproteins decrease occurred. Metabolism of low-density lipoproteins is closely connected with the effect of estrogens and its ability to maintain the production of oxytocin receptors, with subsequent stimulatory effect on the system of prostaglandins through the increased activity of phospholipases (Thorburn 1979). Taking into account that the production of cholesterol that is the primary substrate of progesterone may depend also on LH as well as phospholipase regulation, then it is likely that the exogenous administration of carbetocin with its positive effect on prostaglandin synthesis did not influence the concentrations of cholesterol in the puerperium found by us.

In the experimental group, after a significant decrease ($P<0.01$) on days 14 and 17 ($P<0.05$) p. p. the concentrations of total lipids were insignificantly higher by the end of the period studied in comparison with the control group. In the metabolism of lipids, the effect of hormones can be manifested indirectly, too, i. e. by the subsequent action of endocrine lipolytic factors, which have either an inhibitory or stimulative effect on the lipase activity.

Repeated administration of Depotocin to the animals in our experiment presents one alternative to stimulate the synthesis of prostaglandins; the question, to what extent the fatty acids accumulating during the inhibitory phase of progesterone perform their function, just by means of prostaglandin synthesis, is still difficult to answer. The assumed increase in the concentration of prostaglandins after exogenous supply of Depotocin might lead also to the stimulation of other endocrine lipolytic factors which could consequently influence the dynamic changes of the lipid concentrations found in the experimental group in the period studied via the increased activity of enzymatic systems, in addition to the effect on the production of androgens and their aromatization (Dodson et Watson 1980).

In the control group, the levels of progesterone episodically increased on day 34 after parturition. In the experimental group, its levels close above the level of sensitivity of the trial were recorded on days 34 and 51. In next days, its average values were below 0.5 nmol.l^{-1} of the serum. It is in agreement with the findings of Bekeová et al. (1992);

Krajničáková et al. (1994) in sheep, and Kudláč and Píchová (1990); Dimmick et al. (1991), Zralý et al. (1989) by day 15 after the parturition of cows. According to Burd et al. (1976) the decrease in the levels of progesterone and oestradiol post partum to zero is connected with postparturient extinction of the fetoplacental unit and with response of adenohypophysis on hormonal treatment of ewes in the above mentioned period. From the experimental work of Schirar et al. (1989); Greylung and Van Niekerk (1991); Krajničáková et al. (1992) it is evident that the postparturient ovarian activity in sheep depends, to a great extent, on the uterine involution processes. The results obtained concerning the fertility in sheep observed in our experiment, presented in Table 2, are comparable to those of Ainsworth et al. (1982), Krajničáková et al. (1992) about the termination of involution processes, which have a favourable effect on the nidation of the ovum of mated sheep.

These results contribute to the knowledge about the puerperium of ewes and they point out to their functional importance in the utilization of biotechnical methods and techniques in the control of puerperium. The administration of an uterotonic preparation, in addition to its effect on reparation and involution processes of sexual apparatus, via enzymatic systems involved in the steroidogenesis influenced the protein and lipid metabolism with subsequent effect on the conception rates of ewes.

Vplyv Depotocinu inj. SPOFA na koncentrácie cholesterolu, celkových lipidov, progesteronu a na koncepciu bahníc po pôrode

V práci sme sa zamerali na sledovanie účinku Depotocinu inj. Spofa na koncentrácie cholesterolu, celkových lipidov a progesterónu s následným synchronizačným efektom na koncepciu bahníc. V experimente bolo zaradených 19 bahníc, z ktorých 10 slúžilo ako kontrola. Deviátim ovciam sme 24 a 72 h po vybahnení aplikovali 2×0.07 mg Depotocinu inj. Spofa 2-0-methyltyrosin (deamino-1-karba) oxytocinum. Vzorky krvi boli odobrané z v. jugularis v 36 h po pôrode, 4., 7., 14., 17., 21., 25., 34., 42. a 51. deň po vybahnení. Ovciam obidvoch skupín sme po 51. dni aplikovali Agelin pošvové tampóny (20 mg chlór-superlutínu). Po 10-tich dňoch sme tampóny odstránili a zvieratám aplikovali 500 m.j. PMSG. Očakávaný termín pôrodu sme určili od dátumu prípustenia. Cholesterol, celkové lipidy sme stanovili pomocou BIO-LACHEMA testov (LACHEMA, Brno) a koncentrácie progesterónu rádioimmunologicky, súpravami ÚRVJT, Košice. Pri hodnotení koncentrácií cholesterolu sme štatisticky významné rozdiely medzi kontrolou a pokusou skupinou nezaznamenali ($P > 0.05$). Koncentrácie celkových lipidov sa od 25. dňa po pôrode pohybovali na nesignifikantne vyšších hodnotách v pokusnej oproti kontrolnej skupine zvierat do konca sledovaného obdobia ($1.78 \pm 0.48 - 2.45 \pm 0.35$ g.l⁻¹). Pri koncentráciách progesterónu sme v pokusnej skupine zaznamenali v 34. a 51. dni pozvolný vzostup (0.5 ± 0.8 až 0.8 ± 1.3 nmol.l⁻¹). V ostatných dňoch sa pohybovali jeho hodnoty pod hladinou citlivosti skúšky. Pri hodnotení výsledkov bahnenia po navodení ruje v 51. dni po pôrode sa v kontrolnej skupine obahnilo 5 zvierat a v pokusnej skupine z 9 ošetrených sme dosiahli 100% bahnenie s uliahnutím 16 jahniat.

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