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The effect of ovine placenta extract on mammogenesis, lactogenesis, and galactopoiesis in sheep

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Abstract: The aim of the present study was to evaluate the effect of ovine placenta extract, obtained by affinity chromatography on immobilized wheat germ lectin, on mammogenesis, lactogenesis, and galactopoiesis in pregnant and lactating sheep. Placental extract (100 µg/mL protein concentration) was subcutaneously injected to pregnant sheep in days 1, 4, 7, 10, and 13 of the experiment (totally 10 mL placenta extract/animal) alternatively on the right and left side of the neck. Placental extract induced a more intense development of udder parameters; udder circumference increase was of 16.73%, anteroposterior length increase was of 15.9%, and latero-lateral length of 11.1%. Placental extract administration resulted in 7.92% increase in milk yield. Lactating ewes were monitored for milk production for 6 days before the experiment, after placental extract administration from day 7 to day 14 of the experiment, twice a day, and they presented 14.14% higher milk yield in comparison with the control group, no significant differences in milk lactose and protein content, but an increase in milk fat (6.2%). It may be concluded that the glycoprotein or glycopeptide isolated from sheep placenta by affinity chromatography on wheat germ lectin increased mammogenesis, lactogenesis, and galactopoiesis in sheep.

Key words: Ovine placenta extract, mammogenesis, lactogenesis, galactopoiesis

Introduction

Placenta's endocrine function, during gestation, is one of the most important events that influence both the future of the newborn and the next lactation (1-4). Placental lactogen hormone, one of most important hormones, was identified in placentas of some domestic animals, such as cow and sheep, and also in human placenta (5,6).

Placental lactogen hormone was identified in the blood of pregnant females, during the second half of pregnancy; the climax of blood concentration being recorded simultaneously with the moment of maximum intensity of mammogenesis (7,8). In ewes, the placental lactogen hormone is represented by a single polypeptide chain containing 198 residues, generated from 236-amino acid precursor, and is a member of a family of related polypeptide hormones, which includes growth hormones, prolactins, and placental lactogen, and is produced by binucleate cells of the chorionic epithelium (9). It seems that, although this hormone is acting in normal conditions during the foregoing period of lactation, it is indispensable for normal development and function of the mammary gland (1,3). The placental lactogen is known to be involved in the process of maintaining the milk yield in pregnant ruminants. For this purpose, placental lactogen hormone substitutes the activity of the anterior pituitary LTH, whose secretion is progressive, inhibited along the

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pregnancy, under the influence of the placental progesterone (1,3,7,8). Besides that, the placental lactogen hormone stimulates the body growth of the fetus and also stimulates the development of the mammary epithelium in late pregnancy (1,3,7,8).

There have been several attempts of isolating and purifying this hormone (in cows, sheep, goats, etc.) with the purpose of modulating the milk yield in these species. Different methods, with different efficiency, were used for this purpose (5,6,10). The present approach assumes that, like other peptide hormones, it may be glycosylated, and wheat germ lectin was selected to obtain a preparation that had been subcutaneously administered to both pregnant and lactating sheep. Effects of this preparation were evaluated regarding its influence on mammogenesis, lactogenesis and leucopoiesis in sheep.

Materials and methods

Placental crude extract preparation: Fresh placentas, obtained from ewes at parturition and preserved by freezing, were homogenized with phosphate buffer saline, pH 7.2, (PBS), 1:5 W:V, overnight. Supernatant obtained after centrifugation at 10,000 rpm, was used for the next purification steps.

Lectin preparation: Wheat germs were extracted in acetate buffer pH 4.8, 1:5 W/V, overnight. Supernatant obtained after centrifugation, 5000 rpm, was suspended in chitin. After 3 washes with acetate buffer, elution was performed with 0.2 M acetic acid. The eluted lectin was immobilized with glutaraldehyde, 25%.

Placental extract preparation: Supernatant obtained after crude extract of placentas was homogenized with the immobilized wheat germ lectin overnight at 4 °C. After 3 washings with PBS, elution was performed with 0.2 M acetic acid. After centrifugation, the preparation was dialyzed against PBS, and ultrafiltrated to eliminate any supramolecular interference.

Animal experiments

Pregnant ewes were divided into 2 groups, balanced for age and anterior lactation milk yield. The ewes from the first group (control) (n = 5) were injected subcutaneously with 2 mL of 0.9% normal saline solution on days 1, 4, 7, 10, and 13 of the experiment. Those from the second group (n = 5) were injected subcutaneously with 2 mL of ovine placenta extract (protein concentration of 100 μg/mL) on days 1, 4, 7, 10, and 13 of the experiment (in total 10 mL placenta extract per animal). This pattern of extract application was adopted arbitrarily due to the fact that the tested placenta extract composition has not been described yet. The injections were made alternatively in the right and left side of the neck and they were administrated daily at 1000. On the parturition day, biometric measurements were taken regarding udder dimension parameters. Due to administrative and financial reasons, we used this doubtful method to evaluate the impact of our product on the mammary tissue epithelium. These parameters were: circumference of the udder at its basis, anteroposterior length of the udder, and latero-lateral length of the udder. The measurements were made using a compass and a tape measure (see the Figure). Starting with the first day of weaning, at 4-day intervals, the milk production level was determined by measuring its volume using a graded cylinder. This last procedure was repeated 11 times.

Lactating ewes were divided into 2 groups balanced for age and anterior lactation milk yield. The ewes from the first group (n = 6) were injected subcutaneously, daily, twice a day, with 1 mL 0.9 % normal saline solution between days 7 and 14 of the experiment. The ewes from the second group (n = 6) were injected subcutaneously, daily, twice a day, with 1 mL of ovine placenta extract (protein concentration of 100 μg/mL) between days 7 and 14 of the experiment (in total 16 mL placenta extract per animal).

Pattern of extract application was adopted arbitrarily due to the fact that the tested placenta extract composition has not been described yet. In this case we injected a larger quantity of placenta extract in a shorter period than in the first experiment to emphasize the possible effect of this extract on milk yield. The injections were made alternatively on the right and left side of the neck and they were administrated daily at 1000 and 1800 hours. This experiment was performed over 21 days. During the entire period of the experiment the milk yield was monitored daily, from a quantitative point of view,

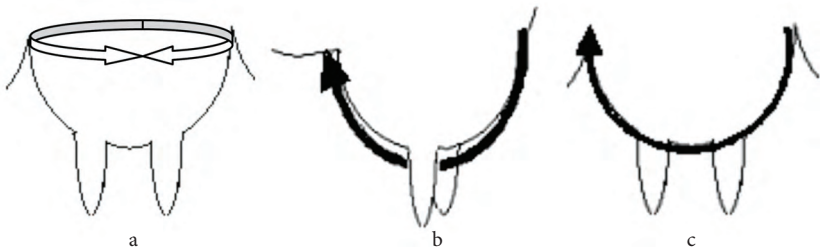

Figure. The manner of the measurement of the udder biometric parameters: a-circumference at udder basis, b-anteroposterior length of the udder and c-latero-lateral length of the udder.

using a graded cylinder. In days 7, 14, and 21, the milk yield was evaluated from a quantitative point of view and we measured lipid, total protein and lactose contents. These parameters were determined using a Lactoscan 60 LCD Milk Analyzer.

All the ewes used in our experiments were fed on a diet of fresh lucerne and concentrate for ad libitum intake throughout the whole experiment. Water was also available for ad libitum intake.

Mean, standard deviation, and standard error were also reported. We performed a comparison between treatments, saline and ovine placenta extract, by Mann-Whitney U test.

Results

Ovine placenta extract effects on mammogenesis are presented in Table 1. Sheep treated with ovine placenta extract of ovine origin presented:

- an udder circumference at the basis of 8.6 cm (16.73%) more than the sheep in the control group, a significant difference ($U = 3$, $P < 0.05$);
- an anteroposterior length of 5.6 cm (15.9%) more than the sheep treated with 0.9% normal saline solution, the difference being significant ($U = 4$, $P < 0.05$);
- a latero-lateral length of 3 cm (11.1%) more than the sheep treated with 0.9% normal saline solution, the difference being significant ($U = 4$, $P < 0.05$).

Results of the effect of ovine placenta extract on milk yield in sheep treated ante partum are presented in Table 1. Administration of ovine placenta extract in this study showed an increase in milk production with 15.91 mL (7.92%) compared to the control group, the difference being significant ($U = 4$, $P < 0.05$).

Results of the effect of ovine placenta extract on milk yield in lactating sheep are presented in Table 2. Milk yield recorded in the experimental group was higher with 14.14% in comparison to the milk production recorded for the control group, the difference being significant ($U = 6$, $P < 0.05$). This comparison was made for the values obtained between days 7 and 21 of the experiment.

Fat, protein, and lactose content of the milk determined from the experimental group are presented in Table 2. Fat concentration of the milk from sheep within the experimental group was 6.2% higher (significant from statistical point of view) than the one recorded in milk obtained from sheep within the control group ($U = 5$, $P < 0.05$) during the period when the ovine placenta extract was administrated, but also during the following observation period. Protein concentration in milk obtained from the sheep within the experimental group was increased by 6% in comparison to the one recorded within sheep of the control group, the difference being non-significant. During the following period of observation, there were no differences for this parameter. Lactose concentration in milk obtained from the sheep within the experimental group was increased by 12% in comparison to the one recorded in the milk of the sheep within the control group during the period of administration of the ovine placenta extract, the difference being non-significant. During the following period of observation, there were no differences for this parameter.

Table 1. Effects of saline and ovine placenta extract, administered ante partum in ewes, on milk yield and udder dimension parameters.

Parameter	Saline (n = 5) (Mean ± Standard error)	Ovine placenta extract (n = 5) (Mean ± Standard error)	P
Udder circumference (cm)	51.4 ± 0.5	60 ± 1.09	P < 0.05
Anteroposterior length (cm)	35.2 ± 1.06	40.8 ± 0.58	P < 0.05
Latero-lateral length (cm)	27 ± 0.31	30 ± 0.31	P < 0.05
Daily milk yield/sheep (mL)	200.7 ± 0.77	216.61 ± 1.38	P < 0.05

Table 2. Effects of saline and ovine placenta extract, administered in lactating ewes, on milk yield and major milk constituents.

Parameter		Saline (n = 6)	Ovine placental extract (n = 6)	P
Daily milk yield (ml)	-	248.8 ± 4.720	284 ± 3.510	P < 0.05
Fat (%)	Day 7	6.72	6.9	-
	Day 14	6.43	6.88	P < 0.05
	Day 21	6.75	7.2	P < 0.05
Protein (%)	Day 7	5.21	7.2	-
	Day 14	5.01	5.31	-
	Day 21	5.41	5.46	-
Lactose (%)	Day 7	4.39	3.9	-
	Day 14	3.7	4.21	-
	Day 21	4.81	4.56	-

The administration of the ovine placenta extract to the sheep involved in the experiment was made without any adverse reactions or secondary effects, and no risk towards the animal's health.

Discussion

The obtained results suggest a positive effect on mammogenesis in pregnant sheep treated with the ovine placenta extract. This possible positive effect was highlighted by measuring the udder. The ovine placenta extract contains specific hormonal entities, such as placental lactogen hormone, well known for its direct implications in mammogenesis in these species. It has been proven through in vivo and in vitro research that the placental lactogen hormone is involved in sheep mammogenesis. This hormone was noticed in Forsyth's in vitro research (11) and Leibovich's in vivo experiments (2) for its spectacular effects on mammary glands development in ewes.

Supplementation with this glycoprotein preparation yielded intensification of the development of the udder and, most probably, of the mammary secretor epithelium.

Furthermore, our results indicate a positive effect on the milk yield in sheep treated ante

partum with the tested ovine placenta extract. This phenomenon is determined, most probably, by stimulation of mammary gland growth by the hormonal components of the tested extract, such as placental lactogen hormone, well known for its direct implications in mammogenesis in these species. The results of this experiment indicate that udder development, observed as a consequence of the administration of the tested products, is produced by the growth of secretor tissue and not only of adjacent tissue that participates in the udder structure and that glycoproteic components of placenta may play important roles in mammogenesis and lactogenesis in sheep.

The results obtained through the present experiment confirm the conclusions drawn by Martal at al. (12), who observed that sheep giving birth to twins have up to 30% milk production increase compared to sheep giving birth to a single lamb. This phenomenon can be explained by the presence of 2 placentas inside the sheep's uterus, and a higher concentration of the placental lactogen hormone in the pregnant sheep's blood (1,3). This will lead to a more intense development of the mammary secretory structures in these sheep, and a higher level of milk yield during the next lactation.

This experiment, by administrating placenta extracts, simulates a twin gestation, by increasing the concentration of some pregnancy associated glycoproteins (placental lactogen hormone) within the sheep's blood. This fact proves the implication of the number of placentas and the concentration of placental lactogen hormone in the gestating sheep's blood in setting the volume of the milk yield for the next lactation, volume being correlated with the number of lambs being born, so the maternal organism can offer the necessary milk quantity for their feeding.

Moreover, our results showed that the placental extract used in our experiment yielded intensification of milk yield in lactating sheep.

This fact is obvious by analyzing the lactation curve of the 2 groups, an increase being remarked despite the period in which the experiment was conducted (the period of physiological decline of milk production, more obvious in the case of the sheep in the control group).

We consider that this product has a positive influence upon the milk yield in sheep, and has also a positive effect on the lactating mammary gland, most probably by stimulation of the function of the mammary gland by the hormonal components of the tested extracts, such as placental lactogen hormone, well known for its direct implications in galactopoiesis in this species (2).

In this experiment we reached the same results as Martal et al. (12); the fact that the lactogen placental hormone, obtained through a special technique, in the presence of insulin and corticosteroids, administrated in vitro, has yielded intensification in casein synthesis, and thus in milk production. We also reached the same result as Forsith et al. (11), Byatt et al. (13), and Leibovich et al. (14); the fact that bovine lactogen placental hormone increased milk yield during mid and late lactation in ruminants. It seems that in vivo, on the physiological background of the hormones involved in galactopoiesis (STH, prolactin, insulin, etc.) conditions met in the present experiment, administrating our product (some ovine pregnancy associated glycoproteins) improves the galactopoietic process in ovine species. However, our results are inconsistent with the results of a previous study (9), which reports the failure of the ovine placental lactogen galactopoietic effect. This inconsistency could be induced by the fact that in this research the authors administered the ovine placental lactogen for only 5 days. Furthermore, our product is not yet well characterized, containing some ovine pregnancy associated glycoproteins not only placental lactogen, a fact that could explain our positive results.

These results concerning the concentration of the milk fat are inconsistent with results of a previous report (9), which showed no effect of ovine placental lactogen upon this parameter. Our results concerning the concentration of milk protein and lactose are similar to those published by Min et al. (9).

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