

## THE ADDITION OF STIMULATION SCHEME WITH ANTI-ESTERASE SUBSTANCES IMPROVES THE EWE LAMBING RATE

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*Most breeds of sheep exhibit seasonality of reproduction. To make it possible to inseminate outside the estrous season, ewes are subjected to hormonal stimulation. The basic scheme of stimulation includes the treatment with gonadotropins after preliminary gestagenization. The most suitable form of prolonged saturation of the body with gestagens is the vaginal sponge. However, the effectiveness of such a scheme is not always sufficient. It is suggested that one of the reasons may be irritation of the vaginal walls with the material of sponge, and one of the ways to reduce this is the treatment of animals with substances that affect the different parts of the nervous system. The purpose of the experiment was to compare the effectiveness of the basic scheme of sexual activity stimulation in sheep during anoestrous season with the scheme that was added by the use of a substance with anticholinesterase activity "Proserine". For this purpose 2 groups of 2-year-old ewes (control and experimental) were formed and kept together. Gestagenization of all animals was initiated on 11 April by the insertion of sponges with gestagen (Cronolone, 30 mg/sp., "Intervet"), which were removed on 23 April. To stimulate folliculogenesis, on April 22 all animals were injected with 500 IU of the "Sergon" (PMSG, Czech Republic) and 1 ml of 2.5 % oil solution of progesterone. In addition, all animals were twice treated with 2.5 ml of "SuperAmino-C" (South Korea), which is a mixture of essential amino acids and B vitamins, in the days of insertion and withdrawal of sponges. "Proserine" (0.05 % solution) was injected the experimental animals at a dose 1 ml per ewe per injection twice — in the days of insertion and withdrawal of sponges. All animals were artificially inseminated with freshly obtained semen one time per day during three consecutive days, starting at the next day after sponge withdrawal, without the previous heat testing. In the experimental group, 91.6 % of animals lambed, in the control one — 70.6 %. The prolificacy in the experimental group was  $1.18 \pm 0.19$  vs.  $1.68 \pm 0.34$  lambs/ewe in the control. The fecundity at lambing in the experimental group was 108.5 % vs. 118.5 % in the control, at weaning at 2-months old — 83.3 % vs. 74.1 %. The results of the experiment showed that addition to the basic scheme the 2-fold treatment with the antiesterase substance "Proserine" reduces prolificacy, but increases the efficiency of insemination and improves the survival of lambs to weaning that generally contributes to the increased efficiency of hormonal stimulation in sheep. It was made assumption that the positive effect of the neurotropic substance was able to manifest itself due to the simultaneous saturation of the animal organism with a mixture of essential amino acids and B vitamins.*

**Keywords:** EWE, HEAT, HORMONAL STIMULATION, PROSERINE

## ДОПОВНЕННЯ СХЕМИ СТИМУЛЯЦІЇ АНТИЕСТЕРАЗНОЮ РЕЧОВИНОЮ ПОЛІПШУЄ ПОКАЗНИКИ ЯГНІННЯ ОВЕЦЬ

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*Більшість порід овець виявляють сезонність репродукції. Для можливості осіменіння поза естральним сезоном вівцематки отримують гормональну стимуляцію. Базова схема стимуляції охоплює обробку гонадотропінами на фоні попередньої гестагенізації. Найзручнішою формою тривалого насичення організму гестагенами є вагінальний песарій. Проте ефективність такої схеми не завжди достатня. Припускається, що однією з причин може бути подразнення стінок піхви матеріалом песаріїв, а один зі способів зменшення цього — обробка тварин речовинами, які впливають на окремі ланки нервової системи. Метою досліджу було порівняти ефективність базової схеми стимуляції статеві активності овець в анестральний*

сезон зі схемою, доповненою застосуванням препарату з антихолінестеразною активністю «Прозерин». Для цього формували 2 групи (контрольну та дослідну) 2-річних вівцематок, яких утримували в одному загоні. Гестагенізацію усіх тварин започатковували 11 квітня встромлянням піхвових песаріїв з гестагеном («Кронолон», 30 мг/пес., «Intervet»), які вилучали 23 квітня. Для стимуляції фолікулогенезу 22 квітня усім тваринам ін'єктували по 500 ІО препарату «Сергон» (Чехія) і 1 мл 2,5 %-го масляного розчину прогестерону. Крім цього, усім тваринам у дні вставки та вилучення песаріїв ін'єктували по 2,5 мл препарату «СуперАміно-С» (Південна Корея), який містить суміш незамінних амінокислот та вітамінів групи В. Прозерин у дозі 1 мл на ін'єкцію на голову ін'єктували дослідним тваринам двічі — у дні вставки та вилучення песаріїв. Усіх тварин без перевірки на наявність статевої охоти штучно осіменяли свіжоотриманою спермою один раз у день три доби поспіль з початком на наступний день після вилучення песаріїв. В дослідній групі ягнились 91,6 % тварин, в контрольній — 70,6 %. Багатоплідність при ягнінні у дослідній групі становила  $1,18 \pm 0,19$  гол./гол. Порівняно з  $1,68 \pm 0,34$  гол./гол. у контрольній. Показник плодючості при ягнінні у дослідній групі становив 108,5 проти 118,5 % у контролі, при відлученні у 2-місячному віці — 83,3 проти 74,1 %. Результати досліді показали, що доповнення базової схеми 2-разовою обробкою препаратом з антиестеразною дією «Прозерин» зменшує багатоплідність, але збільшує результативність осіменіння та покращує виживаність ягнят до відлучення, що загалом сприяє збільшенню ефективності гормональної стимуляції статевої активності овець в анестральний сезон. Припущено, що позитивний вплив нейротропного препарату зміг проявитись завдяки одночасному насиченню організму тварин сумішшю незамінних амінокислот та вітамінів групи В.

**Ключові слова:** ВІВЦЯ, СТАТОВА ОХОТА, ГОРМОНАЛЬНА СТИМУЛЯЦІЯ, ПРОЗЕРИН

Artificial stimulation of sexual function is widely used in livestock breeding. The main purpose of its usage in sheep breeding is an initiation of out-of-season heat and subsequent increase in lambing rate over a certain period [8, 21]. Today, the main way of stimulation is the treatment of animals with hormones. Because of the high cost of hormones, an economic efficiency of its application will be the greater than the portion of lambed ewes will be the larger. The success of hormonal stimulation is influenced by many factors that are determined by animal physiology and conditions of ewe keeping. The study of nature of these factors and the development of ways to increase in the number of animals reacted positively will contribute to improving of the efficiency of hormones usage.

At the basic scheme of heat hormonal stimulation the gonadotropic and gestagen hormones are used [3, 15, 19]. Prior treatment with gestagens is necessary to reduce the androgenisation [2] and improve blood supply [6] of antral follicles, to provoke the manifestation of oestrus in animals [9]. Saturation of sheep organism with gestagen is carried out by intramuscular injection [7], through feeding [11], by subcutaneous implants [16], by insertion of vaginal sponges [3] or CIDR (*controlled internal drug release device*) [17, 25]. From the listed the most practical and convenient form of animal gestagenization is the usage of vaginal inserts. However, some studies have shown that

these devices may in themselves impair fertility and lambing rates in sheep [12]. One of the reasons for this may be the provoking of the development of opportunistic bacteria that is not overcome even with the addition of antibiotics [13]. Without denying the importance of microbial component, we assume that not less negative aspect of the action of the vaginal insert may be its ability to irritate the walls of the vagina and initiate a stress reaction. It is known that the long action of stressful factors negatively affects the functionality of the hypothalamic-pituitary-gonadal axis. In particular, it has been shown that physiological stress in sheep is able to reduce the amplitude of excretion of gonadotropin-releasing hormone (Gn-RH) [22], to impair pituitary sensitivity to Gn-RH [4, 5] and to reduce the frequency of gonadotropin excretion [1]. Considered proven that the thyroid hormones of stress mediate seasonal decrease in sexual activity in sheep [10, 23].

Since the stress is closely related to the activity of certain part of nervous system, it is fully justified to study the expediency of use of the substance that has neurotropic property, in particular, may enhance or weak the activity of certain links of the nervous system. Drugs from the group of esterase-inhibitors belong to these substances. It is known a method for treating sexual dysfunction with using of cholinesterase inhibitors [26]. The purpose of the experiment was to compare the effectiveness of the basic scheme of stimulation

of sexual activity in sheep in anoestrous season with the scheme, which was supplemented by the use of a substance that has neurotropic property. In the present research the “Proserine”, which is a 0.05 % solution of a synthetic cholinesterase-blocker with reverse action, was used.

### Materials and methods

**Animals and keeping conditions.** In the study 39 2-year-old ewes of the Ascanian Merino breed were used. Animals of this breed are characterized by a seasonal type of reproduction with the beginning of a natural manifestation of heat in the third decade of August—the first half of September and the disappearance of ovulation cases in February–March. Ewes belonged to and kept in research farm “Ascania-Nova” of the Institute of Animal Husbandry “Ascania-Nova”. The farm is located at 46°27' north latitude.

All the animals were kept together for all the time and formed a group that in the previous year was used to research the efficiency of scheme “three lambings in two years”. In the framework of this scheme, in August of year that preceded the experimental, ewes were kept with rams and in January 30 of them lambed. Lambing occurred from January 11 to February 22. Lambs were weaned in March 20. From weaning to the beginning of stimulation sheep were fed 2.5 kg of hay and 600 g of whole barley per head. Access to water was free. During both the preparatory period and next pregnancy in a favourable weather animals had a daily motion in the adjacent area for a period of at least 1 hour. The fatness of ewes at the beginning of stimulation ranged from 3 to 3.5 points. The pen, where animals were kept, had a shed for protection from the sun. In May, animals were sheared. Lambing of stimulated sheep took place in September. At all period animals were provided with skilled veterinary care.

**Manipulations.** At the beginning of stimulation, ewes were divided into two groups — experimental (EG) and control (CG), but continued to be kept together in one pen. The experimental group included 12, the control — 27 ewes.

Stimulation was started on April 11 by inserting to all the animals the vaginal sponge with Cronolone (30 mg, “Intravet”, Holland) and injecting

a mixture of vitamins and amino acids (2.5 ml/ewe, “SuperAmino-C”, Korea). Animals of the experimental group were additionally injected 1 ml of “Proserine”. On April 18, in 4 experimental and 6 control animals the state of genital organs was determined using an ultrasound device. On April 22, all the animals were treated with 500 IU/ewe (pregnant mare serum gonadotropin, “Sergon”, Czech Republic) and 0.5 ml of 2.5 % progesterone solution. On April 23, sponges were pulled out and all the animals were injected 2.5 ml of “SuperAmino-C”. Ewes of the experimental group were additionally injected 1 ml of “Proserine”. Active substance of “Proserine” is neostigmine methylsulphate (0.5 mg/ml).

The heat hadn't been being tested, and all the animals were subjected to “forced” artificial insemination.

The artificial insemination of the ewes was started in the next day after sponge withdrawal and repeated in the second and third day. Insemination was carried out only in the morning without repeating in the evening. For insemination fresh semen of three rams of the Ascanian Merino breed, tested for quality of sperm production, was used. Fresh undiluted semen was put in the cervix under visual control as deep as may be and in a volume not less than 0.1 ml. On the third day of insemination, 3 hours after its finishing, two rams of the Texel and Dorper breed were added in pen, in which ewes were kept, and left with females till the next morning.

**Registration the results of stimulation.** Effect of stimulation and insemination (F%, Fm%, and Pf) was determined after autumn lambing (at September). Fertility (F%) was determined by the arithmetic division of the amount of lambed ewes by the total amount of all ewes and followed by multiplication by 100. Fecundity (Fm%) was determined by dividing the amount of all received lambs by the total amount of ewes and followed by multiplication by 100. Prolificacy (Pf) at lambing was determined by dividing the total amount of all received lambs by the amount of lambed ewes. To calculate the fecundity at weaning only lambs, which continued to live with ewes, were taken into account.

**Assessment of the state of genital organs.** The state of genital organs was assessed by ultrasound scans using the indicators of genital functionality, which were developed by authors:

- degree of uterine friability (UF, from 0 to 3 points),
- degree of ovarian proliferation (OP, from 0 to 3 points),
- degree of folliculogenesis (FG, from 0 to 3 points).

Scanning was carried out at a frequency of 5 MHz.

All indicators were determined subjectively. Value of degree of uterus friability depended on a gradient of echogenicity of uterine horns. The UF indicator had been receiving a greater the value, if the difference in echogenicity of different parts of a horn was smaller. If the parts with high (light) and low (pale gray) echogenicity had been being revealed simultaneously, UF was estimated lower. Degree of ovarian proliferation (OP) was evaluated according to the number of observed follicles and the ratio of their diameters. The OP indicator received the smaller the value, if the number of follicles was less and the ratio of the diameters of small and large follicles didn't strongly differed from 1:1. Degree of folliculogenesis (FG) was evaluated according to the diameter of the group of the largest observed follicles (0, 0–1, 1–3 and >3 mm). The preovulatory follicles were not taken into account.

**Statistical analysis.** The statistical calculation of the data was carried out using generally accepted ANOVA algorithms with the mathematical tool of the *Excel* program of the *Microsoft Office* packet. Significance of differences (p) in parameters was determined by Student's criterion ( $t_d$ ).

## Results and discussion

Supplementation of the basic scheme of hormonal stimulation of heat at anoestrous season with the injection of substance with anti-esterase activity "Proserine" increased in fertility of animals, but reduced prolificacy (table 1). Difference between groups was not significant.

One ewe of the control group gave birth to 7 live lambs, one of which died at the first hour. Among the 6 remained, 1 died on the third day because of overfeeding, 4 were donated to bringing up, 1 remained with mother till weaning. Sheep of this breed are not multifetous and birth of such a number of lambs by one ewe is registered for the first time. Also, 2 ewes of control group gave

birth to lambs with external signs of rams that were used for "clean-up". Thus, adequate heat in these animals took place 72 hours after the withdrawal of vaginal sponges.

Among animals of the experimental group, 1 ewe gave birth to a lamb with a limb defect. Character of defect let to assume that a cause may be insufficient amount of amniotic fluid and restriction of foetus movement. All lambs in this group were characterized by features of Ascanian Merino breed, and, consequently, manifestation of heat in experimental animals was more rapid and grouped than in control ones.

Comparison of indicators, which characterized functionality of genital organs, showed a tendency towards higher values of UF and FG indicators and a lower OP in ewes of the experimental group versus the control (table 2). In the ultrasound-tested ewes the difference in fertility and prolificacy between experimental and control animals have similarity with data of all the stimulated animals.

It is known that echogenicity of tissues is determined by their ability to reflect ultrasound waves. High echogenicity is demonstrated by bones and connective tissue. Consequently, increased echogenicity can indicate a predominance of connective tissue, in particular, consolidation of tissue of the uterus, and the low — presence of a significant number of glandular cells, that is, about the well-posed proliferation of tissues of the organ. Thus, the ewes, which additionally were injected "Proserine", had been demonstrating a greater degree of uterine friability, a decrease in the number of follicles in ovaries and a larger follicle diameter on the 7<sup>th</sup> day of the sponge action that coincided with reduced prolificacy and improved fertility.

For further reveal of the positive effect of "Proserine", the lambing parameters of ewe, which were inseminated with semen of different rams, were analysed. After use of sperm of ram 74162, the rate of lambing of experimental ewes reached 100 %, control ones — 75 %. For ram 07800 the similar values were 100 and 50 %, for sire 53501 — 80 and 85.7 %. Wherein, the average index of activity of semen, which was received during 3 days of insemination, in ram 74162 was  $8.1 \pm 0.1$ , in sire 07800 —  $8.4 \pm 0.4$ , in ram 53501 —  $9.2 \pm 0.1$  points. Thus, use of semen with high activity (ram 53501)

showed almost identical efficiency for insemination of both the experimental and control animals. When using sperm with a worse quality, the increase in fertility was achieved only for ewes that were additionally treated with “Proserine”. These results suggest that injection of neurotropic substances promoted the creation of conditions for better movement of sperm to top of oviduct. Taking into account the data of genital functionality (table 2), it can make assumption that “Proserine” had been improving the proliferation of cells of the epithelial layer of the uterus that positively acted on insemination results.

As shown in table 1, the supplementation of the scheme of heat stimulation with double injections of “Proserine” caused a decrease in prolificacy at lambing in ewes of the experimental group compared with the control by 10 %. However, after taking into account the lambs that died or were donated at the period from birth to weaning, the fecundity at weaning at 2 months of age was 83.3 % in the experimental group and only 74.1 % in control one. Consequently, injections of “Proserine” simultaneously with the reduction of prolificacy at lambing promoted the improvement of viability of born lambs. According to data of table 2, it can be assumed that under the influence of “Proserine” the development of additional follicles in ovaries had been inhibited, but growth of existing follicles had been improved. Also, one should not reject the assumption of a better readiness of uterus to support foetus development. Together this led to formation of more viable foetuses.

Table 1  
Parameters of lambing in stimulated ewes

Ewe group	n	Parameters*		
		F%, %	Fm%, %	Pf, lamb/ewe
EG	12	91.6	108.3	1.18±0.19
CG	27	70.3	118.5	1.68±0.34

Note: here and further \* — the difference between values of groups is not significant ( $P>0.05$ ).

Table 2  
Indicators of genital organs and reproductive traits functionality in stimulated sheep

Ewe group	n	Indicators and traits*				
		UF, point	OP, point	FG, point	F%, %	Pf, lamb/ewe
EG	4	1.63±0.28	1.00±0.47	1.25±0.29	100.0	1.00±0.00
CG	6	0.92±0.30	1.17±0.44	0.83±0.34	66.7	1.25±0.29

To define the profitability of “Proserine” usage the difference in the money spent on the purchase of drug and those that could potentially be obtained after sale of lambs, which were presented at weaning, was calculated. Since “Proserine” was injected simultaneously with the inserting-withdrawal of sponges, the procedure of stimulation of experimental animals did not lead to increasing of labour costs. The calculations were based on the prices that were relevant on the beginning of stimulation: the cost of 1 ml “Proserine” was 2.5 UAH, the average realizable value of lambs at weaning was 35 UAH per 1 kg of live weight, the average weight of lambs at weaning was 18 kg. The profitability (Pa) per 1 stimulated ewe was calculated with the next formula:

$$Pa = \frac{Fm\%e - Fm\%c}{100 \times AWLw \times RVL} - CPr, (1)$$

where Fm% — fecundity at weaning of the experimental (e) and the control (c) groups;

AWLw — the average weight of lambs at weaning (kg);

RVL — realizable value of lambs (UAH per 1 kg of live weight);

CPr — the cost of “Proserine” used per 1 ewe.

Calculation showed that complement of the traditional scheme of heat stimulation with 2-time injections of “Proserine” promoted getting additionally 53 UAH per 1 stimulated ewe. Wherein, the total cost of hormonal stimulation in control group was 234 UAH, experimental — 239 UAH.

The nature of the positive effect of “Proserine” is due to its ability to suppress the activity of cholinesterase. One aspect of cholinesterase activity is deactivation of acetylcholine, and, accordingly, inhibition of the enzyme activity contributes to an increase in the amount of this transmitter. It is known that in the body there are two sites of acetylcholine synthesis. The first and main place is the nerve cells, in particular those that are part of the vagotropic chain of the autonomic nervous system. When artificially suppressed cholinesterase, the amount of acetylcholine in the nerve endings increases that forced action of this transmitter on subordinate tissues. The strengthening of innervation from the vagotropic chain counteracts the influence

of the sympathetic chain of the autonomic nervous system, the main transmitters of which are adrenaline and norepinephrine. It is the increase in the latter that occurs with prolonged irritation of the walls of the vagina with the material of sponges. Consequently, the counteraction to the stressful effects of sponge on the tissues of the vagina may be one of the aspects of the positive effect of "Proserine". The second site for the synthesis of acetylcholine is cells, which are not part of the nervous system. This is, so-called, the outside nervous acetylcholine [24]. It has been established that the source of its synthesis are granulosa cells in the ovaries [14] and the epithelium in oviducts [18]. It has been shown that the increase in the number of intraovarian acetylcholine correlates with increased follicular development and improved fertility in rats [20]. Therefore, "Proserine", due to its anticholinesterase activity, have ability directly stimulate folliculogenesis in the ovaries and affect the epithelium of the genital organs.

Without denying the role of nervous component, we think that an additional positive factor of both the experimental and control schemes was the 2-time treatment of animals with "SuperAmino-C". The latter is a mixture of essential amino acids and vitamins of group B and its composition is close to ones in medium that can support the cell growth *in vitro*. Let us note that usage only of 2-time treatment with "SuperAmino-C" in stimulation scheme (the control group) caused the lambing rate at the level, which is similar to ones that were received earlier in ewes of given flock after the use of the schemes supplemented with fat-soluble vitamins and 1-time treatment with "Proserine" (at the time of sponge inserting) (64–72 %). So, we can assume that the saturation of the organism of sheep with a mixture of amino acids and vitamins has created conditions for realization of stimulating effect of anticholinesterase substance.

## Conclusion

Results of the present study had showed that the addition of the traditional scheme of heat stimulation in sheep at anoestrous season by two injections of a substance with antiesterase activity may increase the fertility rate, improves lamb survival and ultimately contributes to additional profits.

**Perspectives of future investigations.** The results of the present research showed that the potential effectiveness of hormonal stimulation may reach the highest value. However, because the neurotropic drugs can affect activity of other compartments of the body, to prevent a possible negative effects it is necessary should clearly determine the doses, time and conditions of use of these substances in schemes to stimulate sexual activity. Possible positive synergism of the procedure of saturation of the body with a mixture of amino acids and vitamins of group B and treatment with substances of neurotropic action also needs further study.

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