Stimulation of the reproductive function of cows by kvatronan-Se preparation and complexes of nanocarboxylates

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There was researched the effect of the kvatronan-Se preparation and two complexes of nanocarboxylates on the reproductive function of cows, in particular their fertility, biochemical and hormonal status. For experimental research, there were formed four groups of cows – the control group included intact animals and three experimental ones. The cows of the 1st experimental group were subcutaneously injected with a complex of nanocarboxylates Se, Cu, Mn, Cr; of the 2nd group – with kvatronan-Se preparation; of the 3rd – with Ge, Cu, Mn, Cr. It has been found that kvatronan-Se injections (2nd experimental group) on the 10-12th day of the sexual cycle increases the fertility of cows by 27.3; (compared with the control); by 18.2 and 9.0%, respectively, compared to the 1st (complex of Se, Cu, Mn, Cr) and 2nd experimental group (Ge, Cu, Mn, Cr). It should be noted that the specified preparation and complexes of nanocarboxylates with microelements stimulate metabolic processes in the body of animals. For example, on the 13th day of sexual cycle the glucose content increased in experimental groups: in the 1st group by 7.6; 2nd – 14.2; 3rd – 4.5%; the leved increased cholesterol by 15.2; 15.6; 14.5 %, respectively. Concentration of total protein in the second group increased by 30.75, in the 3rd group – 2.60 %. In the group 1st they observed the tendency to a decrease by 2.1%. It has been established that kvatronan-Se preparation and the complex of Ge, Cu, Mn, Cr promote the synthesis of sex hormones, such as progesterone, which level in these groups increased by 20.3 and 16.9 % on the 13th day of the sexual cycle. Dynamics of estradiol and testosterone concentration in all experimental groups did not change significantly. Therefore, kvatronan-Se is the most effective preparation in stimulating the reproductive function of cows, and changes that occur in the body of animals are considered to be favorable for implantation of the embryo.

Key words: nanocarboxylates; reproductive function; cows; fertility; progesterone; testosterone; biochemical parameters; cholesterol; kvatronan-Se.

INTRODUCTION

The problem of the low level of reproduction of cows is always relevant. Therefore, the issue of the need for hormones, prostaglandins, and biologically active preparation to regulate and stimulate reproductive function of females remains open. The main purpose of stimulation of the reproductive function of females is to promote the normalization of sexual cycle, which, after insemination, should be completed by an egg fertilization and embryo implantation with the subsequent birth of offspring.

Today, many methods of stimulating the sexual function of animals are known [1]. One of them is the use of hormonal drugs for correction of the sexual cycle. Natural and synthetic hormones have long been used in the practice of biotechnology for the reproduction of animals [2]. The disadvantage of these drugs after stimulating the sexual function is that they can lead to a disruption of the hormonal status of animals, as well as to lower the quality of milk and meat. With regard to these issues, new methods of correction of reproductive function of cows and heifers, which ensure the high efficiency, safety and ecological purity of livestock products, are used [3]. The top priority are those methods that
are more natural and do not create a significant load on the body of animals in an intensive technology. These methods include the use of drugs that do not contain hormones for females in order to regulate the sexual function [4].

In modern veterinary medicine and biotechnology, there is a growing interest in the development of preparation, which include macro- and microelements, which play an important role in maintaining the body homeostasis. It has been found, that surplus, deficiency or imbalance of macro- and microelements leads to a decrease in productivity and reproductive ability, metabolic processes, and various diseases [5]. This is due to the fact that minerals are not only structural material in the body, they also affect and provide the necessary conditions for the functioning of vitamins, enzymes; are involved into the digestion, synthesis of substances; affect the blood elements; have the ability to increase the activity of hormones, closely related to the activity of the ovaries, thyroid gland and pituitary gland [6, 7]. In addition, microelements play an important role in the enzymatic and many metabolic processes that are crucial for the embryo development [7, 8]. According to this, the aim of the work was to study the effects of the kvatronan-Se preparation and complexes of nanocarboxylates of biogenic elements on the reproductive ability and related physiological changes in females.

**METHODS**

Nanocarboxylate complexes and the kvatronan-Se preparation, which includes germanium nanocarboxylates, selenium, copper, manganese, chromium, and distilled water, were used for the study. The biotechnological process of manufacturing the preparation was carried out in accordance with the scheme: 1 – water preparation unit, 2 – reservoir for the storage of water prepared, 3 – reactor for colloidal solutions, 4 – reservoir for the colloidal solutions accumulation, 5 – reactor for chemical transformations, 6 – reservoir for acid, 7 – automatic dispenser, 8 – mixing system of the components of the preparation, 9 – system of the preparation filtration, 10, 11, 12, 13 – chemical pumps of the blade type, 14, 15 – machines for the preparation of packaging material, 16 – dosing device, 17 – conveyor, 18 – packaging machine, 19 – system of thermoblocks.

The effect of substances under study on the reproductive function of females was examined in summer on cows of Ukrainian black-and-white milk breed at “Dolynivske” Ltd. in Zhytomyr region. One control and three experimental groups of cows (500-550 kg of body weight, with an average annual milk yield of 6000-6260 kg) were formed for the experiment. Animals showing signs of heat visually and rectally were further examined for the detection of mature follicles, and after the optimal time for insemination was determined. Cows of the control group were injected with isotonic sodium chloride solution; 1st group – with complex of Se, Cu, Mn, Cr; 2nd group – with kvatronan-Se preparation; in 3rd experimental group – with Ge, Cu, Mn та Cr. All preparations were injected subcutaneously on the 10-12th day of the sexual cycle at a dose of 0.02 ml/kg. Pregnancy was determined rectally in 3 months after the insemination, and additionally – for the confirmation of the results – ultrasound was performed.

To determine the biochemical and hormonal changes in animals after the use of kvatronan-Se preparation and complexes of nanocarboxylates, the following experiment was conducted. Each group included 5 cows by according to body weight, age, physiological state and productivity. Blood sample were taken from the caudal vein in the morning on the 9th day of the sexual cycle before the use of the preparation, and on the 13th day after it. Blood serum was obtained by sedimentation for 12 hours at room temperature. The formed supernatant was separated by centrifugation for 15 minutes. The serum obtained was frozen in a liquid nitrogen. The study on the biochemical composition of blood was carried out in the laboratory of the National Cancer Institute with the help of an automatic biochemical analyzer Vitros-250, USA using a
Concentration of hormones was determined in the medical laboratory “Analitika” (Kharkiv) by immunochemiluminescence (immuno-CL) method with the help of the automatic analyzer Bioscience AIA-600 of the closed type, TOSOH (Japan), using the original Japanese reagents according to the instructions. The statistical processing of the experimental results was performed using Microsoft Excel 2007 software, and the probability of the difference was determined using Student’s t criterion. Studies on animals were conducted in compliance with the principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986).

RESULTS AND THEIR DISCUSSION

The results of the studies on the effectiveness of stimulation of cows fertilization after the insemination with nanocarboxylate complexes and kvatronan-Se preparation showed that the highest level of fertilization was observed in the 2nd experimental group, where it significantly increased by 27.3 % (P<0.05), compared with the control group. In the 1st and 3rd experimental groups changes were statistics trialed insignificant it was 63.6 and 72.2 % (P>0.05), that is by 9.1(P>0.05) and 17.7 % (P>0.05) higher than control, and by 18.5 and 9.6 % (P>0.05) lower than that in the 2nd experimental group, respectively.

Biochemical analysis of the blood serum to determine the physiological mechanisms of regulating the reproductive ability of cows after the use of complexes of nanocarboxylates and the kvatronan-Se preparation (Table 1). The results of biochemical showed that the concentration of glucose, triglycerides, and creatinine in the blood of animals from the control group from the 9th to the 13th day of the sexual cycle changed only within 3 % (P>0.05). At the same day 13, compared to the second experimental group

| Indicator | Control | Experimental groups |
|-----------|---------|---------------------|
| Glucose, mmol/l | 3.06± 2.98± | 3.18± 3.44± |
|             | 0.141 0.150 | 0.170 0.140 |
| Urea, mmol/l | 3.74± 3.47± | 3.2± 3.78± |
|             | 0.160 0.120 | 0.19 0.371 |
| Creatinine, μmol/l | 76.6± 80.40± | 72.25± 73.2± |
|             | 3.231 2.910 | 2.861 2.94± ** |
| Uric acid, μmol/l | 146.8± 150.2± | 147.25± 148.2± |
|             | 10.29 5.67 | 7.390 3.20** |
| Cholesterol, mmol/l | 4.16± 4.40± | 4.66± 5.5± |
|             | 0.441 0.531 | 0.450 0.241 |
| Triglycerides, mmol/l | 0.28± 0.28± | 0.45± 0.32± |
|             | 0.071 0.021 | 0.071 0.051 |
| Total protein, g/l | 76.02± 78.36± | 78.94± 81.06± |
|             | 2.820 3.310 | 0.710 2.720 |

Note: *P<0.05 compared to the control group
**P<0.05 on the day 13, compared to the second experimental group
***P<0.05 compared to those indicators of the 9th day
time, the content of cholesterol and total protein increased on the 13\textsuperscript{th} day by 5.45 (P>0.05) and 7.70 (P>0.05), respectively; and the concentration of urea decreased by 7.20 % (P>0.05).

In group 1\textsuperscript{st} (complex of nanocarboxylates of Se, Cu, Mn and Cr), the concentration of uric acid, creatinine, and total protein did not change during the experiment. The level of glucose, urea and cholesterol up to the 13\textsuperscript{th} day had a tendency to increase by 7.5; 15.0, and 15.2 % (P>0.05), respectively. In the blood serum of cows in group 2\textsuperscript{nd} after three injections of kvatronan-Se, on the 10-12\textsuperscript{th} days after insemination, the concentration of glucose was 14.20 (P<0.05), creatinine – 4.95 (P>0.05); uric acid – 4.70 (P>0.05); cholesterol – 15.60 (P>0.05); total protein – 3.75 % (P>0.05); and the urea content decreased by 5.20 % (P>0.05). Analysis of the indicators of group 3\textsuperscript{rd} shows, that after the use of the Ge, Cu, Mn and Cr complex from the 9\textsuperscript{th} to the 13\textsuperscript{th} day, the content of urea, creatinine, uric acid, total protein and triglycerols in the blood of animals changed slightly (within 3 %); and the concentration of cholesterol and glucose increased (tendency) by 14.48 and 4.50 % (P>0.05), respectively.

Due to the fact that the highest fertility of cows was observed in the 2\textsuperscript{nd} group (kvatronan-Se), it was important to carry out a comparative analysis of biochemical changes in their blood with animals from the 1\textsuperscript{st} and the 3\textsuperscript{rd} experimental groups after the use of agents, and that is, on the 13\textsuperscript{th} day of the sexual cycle.

Early the embryo development and the hormone synthesis require a large amount of energy. Therefore, during the pregnancy, glucose levels increase due to the activation of energy metabolism [9]. The results of the analysis indicate that the 2\textsuperscript{nd} group had the highest level of glucose, which was by 1.7 and 4.8 % (P>0.05) higher than in those the 1\textsuperscript{st} and 3\textsuperscript{rd} groups. Due to the fact that the difference between the level of this metabolite in the 1\textsuperscript{st} and the 2\textsuperscript{nd} group was only 1.7 % (P>0.05), the can assume that the increase in glucose concentration was due to the presence of selenium in the applied preparation (group 2\textsuperscript{nd}) and in the complex (group 1\textsuperscript{st}). This may be explained by selenium participation in glucose metabolism through selenoproteins, having a redox potential. Se-dependent proteins are believed to influence the release and signaling of insulin, and they are regulated by the oxidative-reducing potential [10, 11]. Such changes can contribute to implantation and preservation of the embryo, as the synthesis of hormones and the development of the fetus require a large amount of energy.

Metabolites that represent protein metabolism, include creatinine, uric acid, urea, and total protein. Creatinine content in the blood was 84.8 μmol/l in the 2\textsuperscript{nd} group, and exceeded the index of the 1\textsuperscript{st} and the 3\textsuperscript{rd} groups by 13.7 and 7.3 % (P>0.05), respectively. An increase in the concentration of this metabolite may be caused by a more intense metabolism of creatine, which amount apparently increases with high use of ATP. In addition, in this group the increase in the content of uric acid was 12.5 and 14 % (P>0.05), respectively. At the same time, the level of urea, on the contrary, tended to decrease by 9.3 % (P>0.05) and 6.8 % (P>0.05). Significant changes in the dynamics of total protein concentration in cows’ blood samples in experimental groups after the use of kvatronan-Se and complexes of nanocarboxylates were not observed; the difference was within 1 %, which is within the norm.

Cholesterol is one of the most important metabolites that plays an important role in reproduction and is an indicator of lipid metabolism in the body. A comparative analysis showed that its level in the second group was 5.96 mmol/l, which was by 7.7 % (P>0.05) and 5.0 % (P>0.05) higher than in the first and third groups. This may indicate the stimulation of the synthesis of steroid hormones that contribute to the fertilization of animals. When analyzing the dynamics of changes in cholesterol levels during the experimental period (from the 9\textsuperscript{th} to the 13\textsuperscript{th} days of the sexual cycle), it is evident that in all experimental groups it tended to increase. Such changes can be caused by manganese, which
these complexes and the preparation contain. This microelement affects the processes of glucogenesis and is a coenzyme of dimethylphosphorus phosphate, which catalyzes the formation of farnesyl pyrophosphate by isopentenylpyrophosphate and geranyl pyrophosphate isomerization. This process is important in the biosynthesis of cholesterol [12].

The next step was to analyze the dynamics of aspartate aminotransferase (ASAT), alanine transaminase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in the serum of experimental cows on the 9th and 13th days of the sexual cycle, because Kvatonan-Se and the complexes of nanocarboxylates include microelements which are enzymes catalysts in this form (Table 2). In group 2nd, the activity of LP increased significantly by 1.9; ASAT – by 10.9; ALT – 7.6 and LDH – by 4.3 % (P>0.05). After analyzing the enzymatic changes that occurred in the blood samples of the 3rd group of animals, we found that ALAT activity increased by 5.7 (P>0.05); LF – by 3.6 (P<0.05), LDH – 5.4 % (P>0.05). At the same time, the activity of the ASAT tended to decrease by 15.9 % (P>0.05).

It is known that pregnancy period is characterized by the ratio of the concentration of steroid hormones, that affect the nature of parturition and postpartum period. The content and proportion of sex hormones in the blood varies depending on the physiological state [13]. The greatest influence on the course of pregnancy have progesterone and estradiol. The first is produced by the yellow body, which is formed after the ovulation at the place of the follicle. At this stage, it is called the yellow body of the sexual cycle, which in the beginning of the pregnancy is transformed into a yellow body of pregnancy. This hormone regulates the main mechanisms of the embryo implantation, as well as the early stages of zygote development. In case of violation of its formation, the death of the embryo occurs, which is not recorded by specialists in early terms. Lack of progesterone can also lead to abortions. Its concentration in the blood of animals from the fourth day begins to increase and reaches its maximum in 10-12 days [14, 15].

When analyzing the dynamics of progesterone concentration from the 9th to the 13th day after the insemination, it is evident that its content in the blood of the cows of all four groups increased (Table 3). In the control group, on the 13th day, the level of progesterone increased by 8.6 (P>0.05); in the 1st experimental – by 10.4 (P>0.05); in the 2nd and 3rd groups – by 20.3 (P<0.05) and 18.7 % (P<0.05), respectively. Therefore, the level of progesterone significantly increased in group 2nd, where kvatronan-

### Table 2. Enzymatic changes in serum of cows on the 9th and 13th day of the sexual cycle (M±m, n=5)

| Indicator                  | Control 9th day | Control 13th day | Experimental groups | Experimental groups |
|----------------------------|----------------|-----------------|---------------------|---------------------|
|                            |                |                 | kvatonan-Se         | kvatonan-Se         |
|                            |                |                 | Ge, Cu, Mn, Cr      | Ge, Cu, Mn, Cr      |
| Aspartate aminotransferase | 61.8± 57.6±    | 4.35 7.28       | 60.28± 57±          | 60.2± 67.6±         |
|                            | 60.2± 57±      | 1.910 3.19      | 3.84 3.28           | 2.82 3.0±           |
| Alanine aminotransferase   | 30± 32.2±      | 2.07 1.32       | 45.6± 36±           | 4.61* 2.91          |
|                            | 45.6± 36±      | 4.61* 2.91      | 3.86 41.8±          | 4.51 7.04           |
| Alkaline phosphatase       | 75.2± 69.6±    | 6.23 3.30       | 57.8± 67.8±         | 14.27 13.66         |
|                            | 67.8± 10±      | 14.27 13.66     | 3.58* 3.99*         | 3.99* 20.26         |
| Lactate dehydrogenase      | 1664.6± 1585.6± | 27.11 93.01     | 1670.8± 1673.6±     | 61.80 81.01         |
|                            | 1673.6±        | 81.01           | 38.63 89.65         | 38.63 52.71         |
|                            | 1672.8±        | 38.63           | 89.65 52.71         | 89.65 52.71         |
|                            | 1696±          | 89.65           | 52.71 1697±         | 52.71 1697.8±       |
|                            | 1605.8±        | 1697.8±         | 52.71 1697.8±       | 52.71 1697.8±       |

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Se was used. The largest number of calvers was found to be in this group as well. The progesterone precursor is cholesterol. According to the experimental studies presented above, it was found that the preparation contributes to increase in the content of this metabolite, from which pregnenolone is formed.

Basing on the said above, one can assume that the action of copper and chromium, which are components of the preparation, the activity of insulin increases, and this stimulates the activity of the pituitary gland; and, as a result, increases the level of luteinizing hormone (LH). Under the influence of LH, pregnenolone is transformed into 17-hydroxypregnenolon and progesterone, which are further transformed into estrogens and testosterone [16]. The main and most active estrogen is estradiol, and its intermediate product is testosterone [17]. The concentration of estradiol at the beginning and the middle of pregnancy is low, and it increases before parturition [13]. On the contrary, the dynamics of the concentration of estradiol, from the 9 th to the 13 th day tended to decrease: in the control group by 1.4; in 1 st, 2 nd and 3 rd experimental – by 2.5; 2.9 and 2.8 % (P>0.05), respectively. The next hormone, which content was determined in blood serum of heifers, was testosterone. In females, this hormone and its precursors are synthesized at significantly lower concentrations than in males. Testosterone is transformed into estrogen in ovaries [15]. Analysis of testosterone concentration in heifers’ blood shows, that in the control group, 1 st and 2 nd experimental ones, on the 13 th day after the insemination, there was a slight increase in the latter within 2 % (P>0.05). In the 2 nd group, this indicator did not.

For better analysis, we compared the index of hormones between groups on the 13 th day of the sexual cycle. The level of progesterone in the control group from the 9 th to the 13 th day increased by 8.6; in the 1 st experimental – by 10.4 (P>0.05); 2 nd – 20.3 (P<0.05), 3 rd – 16.9 % (P>0.05). The highest progesterone content was in the blood of cows of the 2 nd experimental group, which was 4.09 ng/ml; that was by 12.2 % (P>0.05) higher compared to the control group, and by 8.3% and 3.4 % (P>0.05) compared to the 1 st and 3 rd experimental groups, respectively. Due to the increase in the concentration of progesterone during this period, the myometrial excitability decreases and its contractile activity is suppressed. In addition, this hormone blocks the immune response of the mother organism to the embryo and thus, creates favorable conditions for its development. The fertility indicators in the second experimental group evidence this. Based on the data presented, the concentration of estradiol in the blood serum of test heifers in all four groups had a slight variation within 3 % (P>0.05).

| Indicator     | Control | Control | Experimental groups | Experimental groups |
|---------------|---------|---------|---------------------|---------------------|
|               | 9 th day | 13 th day | Se, Cu, Mn, Cr | kvatronan-Se |
| Progesterone, ng/ml | 3.28±0.381 | 3.59±0.275 | 3.36±0.256 | 3.75±0.254*** |
| Estradiol, pg/ml    | 17.08±1.659 | 16.84±1.303 | 16.71±1.237 | 16.28±1.353 |
| Testosterone, ng/ml | 3.16±0.318 | 3.21±0.441 | 3.13±0.301 | 3.20±0.693 |

Table 3. The content of hormones in the blood of test heifers (M±m, n=5)
CONCLUSIONS

The use of nanocarboxylates in livestock is a promising area of research. It has been found, that the highest level of fertilization is observed in the group of animals, where the kvatronan-Se preparation is used on the 10-12th days of the sexual cycle. The indicators here are higher by 20.3 % (P<0.05), compared to the control. In addition, the preparation and complexes of nanocarboxylates with microelements intensify protein, carbohydrate, and lipid metabolism in experimental animals. The results of the biochemical study indicate that glucose levels increases by 14.2 (P<0.05); cholesterol – by 5.2, and total protein – by 3.7 % (P>0.05) in the blood serum of cows after the use of kvatronan-Se preparation on the 13th day of the sexual cycle. The complex of nanocarboxylates containing Se, Cu, Mn, Cr contributes to an increase in the glucose and cholesterol concentration in the serum by 7.50 and 15.27% (P>0.05), respectively; while the complex of Ge, Cu, Mn, Cr increases their concentration by 4.5 and 14.48 % (P>0.05), respectively. Therefore, we consider that nanocarboxylates with microelements, in kvatronan-Se preparation and complexes that are involved in the synthesis of steroid hormones, lead in of to an increase in the level of progesterone by 10.4-20.3 % (P>0.05).

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of coauthors of the article.

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СТИМУЛЯЦІЯ РЕПРОДУКТИВНОЇ ФУНКЦІЇ КОРОВ ПРЕПАРАТОМ КВАТРОНАН-SE И КОМПЛЕКСАМИ НАНОКАРБОКСИЛАТОВ

Исследовано влияние препарата кватронан-Se и двух комплексов нанокарбоксилатов на репродуктивную функцию коров, в частности их оплодотворяемость, биохимический и гормональный статус. Для проведения экспериментальных исследований были сформированы четыре группы коров – контрольную, в которую вошли интактные животные, и три опытных. Коровам I опытной группы подкожно вводили комплекс нанокарбоксилатов Se, Cu, Mn, Cr; II опытной – препарат кватронан-Se; III – Ge, Cu, Mn и Cr. Установлено, что введение кватронану-Se на 10-12е сутки полового цикла повышает

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оплодотворяемость коров на 27,3, по сравнению с контролем, 18,2 и 9,0 %, соответственно, с I (Se, Cu, Mn, Cr) и III исследовательской группой (Ge, Cu, Mn, Cr). Также, следует отметить, что указанный препарат и комплексы нанокарбоксилатов микроэлементов стимулируют метаболические процессы в организме животных: в опытных группах отмечено на 13-е сутки полового цикла повышения содержания глюкозы на 7,6, 14,2, 4,5 % в I, II, и III группах соответственно; холестерина на 15,2, 15,6, 14,5 % соответственно. Концентрация общего белка во II группе выросла на 3,75, III – 2,60 %. В I группе наблюдалась тенденция к снижению показателя на 2,1 %. Установлено, что препарат кватронан-Se и комплексы Ge, Cu, Mn, Cr способствуют синтезу половых гормонов, а именно прогестерона, уровень которого на 13-е сутки полового цикла достоверно повысился в этих группах на 20,3 и 16,9%. Динамика концентрации эстрadiола и тестостерона во всех опытных группах достоверно не изменилась. Итак, препарат кватронан-Se является наиболее эффективным для стимуляции репродуктивной функции коров, а изменения, которые происходят в организме животных, благоприятные для имплантации эмбриона.

Ключевые слова: нанокарбоксилаты; воспроизводящая способность; коровы; оплодотворенность; прогестерон; тестостерон; биохимические показатели; холестерин; кватронан-Se.

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