Influence of silicon dioxide nanoparticles on the fertility of heifers in frontal insemination

P I Khristianovskii1,2, S A Platonov2, V A Gontiurev1

1Federal Research Centre of Biological Systems and Agro-Technologies of the Russian Academy of Sciences, 29, 9 Yanvarya st., Orenburg, 460000, Russia
2Orenburg State Agrarian University, 18, Cheliuskimstev st., Orenburg, 460014, Russia

E-mail: Paor1953@bk.ru

Abstract. Investigation of the effect of silicon dioxide nanoparticles on the processes of reproduction in cattle was performed on heifers of the breeding age of red steppe breed. Synchronization of the sexual activity by double use of the estrophan, prostaglandin drug, with addition of a releasing hormone (surfagon) to the scheme before insemination was performed in the control and experimental groups. Heifers were simultaneously injected with a solution of silicon dioxide nanoparticles with estrophan injection intramuscularly in the experimental group. All animals were inseminated in frontal, rectocervical way, with deep frozen sperm, twice. Heifers were taken the blood at estrophan injections and before insemination to study the dynamics of progesterone levels. As a result, 50.0% of the heifers were fertilized from frontal insemination in the control group, in the experimental one - 70.0%, that is, the increase in fertility with the use of silicon dioxide nanoparticles was 20.0%. Progesterone level in blood serum of the heifers on the 11th day of the experiment exceeded the initial by 23.5–69.2%, and before insemination it dropped by 38.1 to 50.0% compared with the maximum value. These changes were more significant in animals of the experimental group. Presumably, the use of silicon dioxide nanoparticles increases the hormonal activity of heifers’ ovaries, which contributes to their higher fertility during insemination.

1. Introduction
The use of artificial regulation methods of the sexual cycle of females is important in the organization of the reproduction system of cattle, including synchronization of the sexual activity with subsequent frontal insemination. This makes it possible to plan offspring in the optimal period [1, 2]. Various schemes and preparations are used to synchronize sexual activity, but the fertility of cows and heifers from frontal insemination is low and averages 40-50% [3]. Increasing the fertility of animals in frontal insemination is a very important task. The task can be solved by using micronutrient preparations, including the form of nanoform. [4]. The high effectiveness of nanocrystalline forms of metals in comparison with inorganic salts and other sources is confirmed by a number of studies [5-10]. Studies on the effect of nanoforms on metabolism, biochemical parameters, elemental composition of body tissues [11, 12], features of structural and functional reorganization of target organs were carried out [13, 14]. However, despite the special attention to the problem of biological effects of substances in nanoform, there is a serious lack of information regarding their use in livestock husbandry [15]. This determines the need for research on the problem, including with the aim of creating new microelements based on ultradispersed forms.
The active biological effects of silicon in the ionic form on various functions of animal organisms, including on the processes of reproduction, have been repeatedly noted [16, 17]. In the female genitals, periodic growth and regeneration of tissues regulated by hormones are carried out. The hormonal regulation system is extremely sensitive to various factors [18]. The effect of silicon compounds in the form of nanoparticles on the hormonal regulation of the reproductive function of cattle is not studied well [19-22].

The purpose of the study is to study the effect of double application of silicon dioxide nanoparticles on the fertility of heifers in frontal insemination after synchronization of sexual activity with the use of prostaglandin preparations. Presumably, silicon dioxide particles promote a more intense interaction of ovarian receptors with prostaglandins.

2. Methods and materials
The experiment was conducted in ZAO Niva in Orenburg region Oktyabrsky district on heifers of a red steppe rock in December 2019 to study the effect of silicon dioxide nanoparticles on the fertility of heifers. According to the principle of group analogs, two groups of heifers with 10 heads in each - the control and experimental were selected. The age of animals is 18-20 months, live weight is 320-350 kg.

Before the experiment, a gynecological examination of animals was carried out. The heifers were selected with a normal condition of genitals. During a rectal examination, attention was paid to the size of the uterus and ovaries, rigidity of the uterus, and the presence of follicles and corpus luteum in ovaries. During vaginal examination, the color and condition of the vulvovaginal mucous membrane, the nature of mucus, the absence of pathological overlays was noted.

All animals of the formed groups were synchronized the sexual activity according to the following scheme: 1st day - Tetravit s/c at a dose of 6 ml and estrophan at a dose of 2.5 ml i/m, 11th day - estrophan at a dose of 2.5 ml. Experimental animals were simultaneously injected suspension of silicon dioxide nanoparticles with estrophan injections at a dose of 10 μg/kg i/m. Before injection, a portion of the dry matter of silicon dioxide nanoparticles was dispersed in sterile isotonic sodium chloride solution in the mode of f=35 kHz, N=300 W, A=10 μA for 30 minutes. Silicon dioxide nanoparticles with a diameter of 89.6±16.6 nm, Z- potential -31±0.5 mV, with a specific surface of 55.4 S/m²/g, were obtained by plasma-chemical synthesis.

Material science certification of nanoparticles was carried out using electron scanning, transmission, atomic force microscopy with LEX T OLS4100, JSM 7401F, JEM-2000FX (“JEOL”, Japan). The particle size distribution and Z-potential were studied on a nanoparticle analyze of Brookhaven 90Plus /BIMAS ZetaPALS and Photocor Compact (“Photocor”, Russia).

Frontal insemination of heifers was performed in both groups in 72 and 96 hours after the second injection of estrophan. They were inseminated with deep-frozen sperm in straws by rectocervical method. All animals were injected with a dose of 2 ml i/m simultaneously with the first insemination to synchronize ovulation. Animals were bled before the experiment start in order to study the dynamics of sex hormones, during the second estrophan injection and during the first insemination. The resulting blood serum was frozen and stored in the freezer. The progesterone concentration was determined in blood serum by enzyme multiplied immunoassay (ELISA) in the laboratory of FSBSI “Federal Scientific Center for Biosystems and Agrotechnologies of the Russian Academy of Sciences” (FSBSI FSC RAS). The heifers were monitored after insemination. The cases of rebooting were recorded in the register. The experiment results were processed biometrically by small sample method using Statistica 10 program.

3. Research results
The examination of heifers was carried out on 04/10/2019 at ZAO Niva. The pregnant heifers were revealed in the control group (4 months) in the amount of 5 heads of livestock, nonpregnant – 5 heads. The pregnant heifers were revealed in the experimental group (4 months) in the amount of 7 heads of livestock, nonpregnant – 3 heads. Thus, 50% of the animals were fertilized from frontal insemination in the control group, in the experimental - 70% (Table 1).
Table 1. Control study results of heifers for pregnancy

| Animal No. | Study result | Animal No. | Study result |
|------------|--------------|------------|--------------|
| 61126      | Pregnant     | 62136      | Pregnant (4 months) |
| 72012      | Nonpregnant (4 months) | 72014      | Nonpregnant |
| 71012      | Nonpregnant  | 72006      | Nonpregnant  |
| 71028      | Pregnant (4 months) | 72034      | Pregnant (4 months) |
| 72020      | Nonpregnant  | 72058      | Pregnant (4 months) |
| 72018      | Pregnant (4 months) | 61076      | Pregnant (4 months) |
| 72024      | Nonpregnant  | 72036      | Pregnant (4 months) |
| 62176      | Pregnant (4 months) | 71008      | Pregnant (4 months) |
| 62210      | Nonpregnant  | 61094      | Nonpregnant  |
| 71030      | Nonpregnant  | 62200      | Pregnant (4 months) |

Number of pregnant 5
Number of pregnant 7
Pregnancy % 50
Pregnancy % 70

Insemination results are consistent with data on the dynamics of progesterone in the blood of experimental animals. The level of luteal hormone (progesterone) is a very characteristic indicator of the animal readiness for ovulation and fertilization. In our experiment, progesterone was determined in the blood serum of heifers at the first and second injections of the drugs, and then before frontal insemination. The results of hormone determination are presented in Table 2.

Table 2. Changes in progesterone level in the blood serum of experimental heifers (average for groups)

| Number of animals (n) | Progesterone level (ng/ml) Before first injection of drugs (1st day of experiment) | Number of animals (n) | Progesterone level (ng/ml) |
|-----------------------|-----------------------------------------------------------------|-----------------------|-----------------------------|
| n = 10                | \[ \sum = 17.114 \] M = m\( \pm \) 1.71 ± 0.401                | n = 10                | \[ \sum = 13.000 \] M = m\( \pm \) 1.30 ± 0.091 |
| n = 10                | \[ \sum = 21.091 \] M = m\( \pm \) 2.11 ± 0.245                | n = 10                | \[ \sum = 22.231 \] M = m\( \pm \) 2.22 ± 0.246 |
| n = 10                | \[ \sum = 13.058 \] M = m\( \pm \) 1.31 ± 0.091                | n = 10                | \[ \sum = 11.187 \] M = m\( \pm \) 1.12 ± 0.081 |

Analysis of table 2 shows that in animals of the control group, the level of progesterone in the blood at the time of the second injection of estrophan increased by 0.4 ng/ml (23.5%) compared to the initial one, and before insemination it decreased to 0.8 ng/ml (38.1%) compared to previous value (p<0.05).

The heifers of the experimental group, in the same periods, have an increase in progesterone level in the blood by 0.9 ng/ml (69.2%), and then a decrease by 1.1 ng/ml (50.0 %). The changes are veracious (p<0.05) and more significant than in the control group. Dynamics of progesterone level in the blood serum of heifers are presented as a graph in Figure 1.
Fig 1. Progesterone level in the blood serum of experimental heifers

The fertility control of animals was carried out in April 2019 by rectal examination. The established period of pregnancy (4 months) allows us to conclude that fertilization in animals of both groups occurred precisely as a result of frontal insemination, after preliminary synchronization of sexual activity. The fertility of the experimental group heifers exceeded the control by 20.0%. Presumably, the use of silicon dioxide nanoparticles in experimental animals was a factor in increasing the fertility of heifers.

Analysis of changes in progesterone level in the blood of heifers confirms our assumptions. The second injection of prostaglandins is performed on the 11th day after the first one, that is, during the period of maximal activity of the corpus luteum, while the progesterone level was also at the maximum. Immediately before insemination, the content of progesterone decreased to a level less than the initial, therefore, the animals were in a preovulatory condition. The dynamics of progesterone level in the control and experimental groups is similar; however, in the animals of the experimental group these changes have reached higher values. It is possible that the mechanism of silicon dioxide nanoparticles impact on the fertility of heifers is manifested in this.

4. Discussion
First of all, the current level of livestock production requires the use of intensive systems of herd reproduction. Frequently, the synchronization of sexual activity of cows and heifers is applied with subsequent frontal insemination. However, world practice shows that in case of frontal insemination, the fertility of females is low (40–50 %) [3]. Increasing the fertility of cows and heifers in frontal insemination is a relevant task.

The positive effect of microelements in nanoform on the reproduction processes in animals is known [4]. Silicon dioxide in the form of nanoparticles is safe to use in reproduction [23]. Studies have shown that silicon nanoparticles are a powerful and safe delivery tool, which makes them an excellent candidate for use in reproduction studies [24].

As a result of use, the highest fertility is marked in animals of the experimental group compared with the control one. When studying changes in progesterone content in the blood of heifers, as one of the main hormones regulating the sexual cycle, a general pattern of its dynamics in both groups was found, but in the animals of the experimental group the changes in the hormone were more significant, which was graphically represented.
Presumably, silicon dioxide in a state of nanoparticles has an impact on ovarian receptors, providing a closer relationship with their sex hormones.

5. Conclusion
The double use of silicon dioxide nanoparticles simultaneously with estraphan injections increased the fertility of heifers during frontal insemination by 20.0% compared with the control group. The dynamics of progesterone level in the blood serum of heifers during the synchronization of sexual activity was similar in both groups; however, the changes in progesterone level were more significant in the experimental group than in control one. The study was conducted under the theme “Development of new approaches to the creation of promising breeds and crosses of farm animals based on knowledge of genetically determined metal-dependent functions affecting productivity parameters”.

6. Acknowledgments
The studies were carried out in accordance with the research plan for 2019–2020 of the Federal Research Center for Biological Systems and Agrotechnology of the Russian Academy of Sciences No 0761-2019-0006.

References
[1] Patel G, Haque N, Madhavat M, Chaudhari A K, Patel D, Bhalayka N, Jamnesh A, Patel P and Kumar R 2017 Artificial insemination: A tool to improve livestock productivity J. of Pharmacognosy and Phytochem. 1 307–13
[2] MacGregor R G and Case N H 2000 The effects of maternal calving date and calving interval on growth performance of beef calves South African J. of Animal Sci. 30 70–6
[3] Tada O, Masamha B and Gadzirayi C T 2010 Efficacy of crestar (progesterone analogue) and proisolvin (prostaglandin analogue) in heat synchronization of indigenous smallholder dairy and commercial beef cows Electronic J. of Environmental, Agricultural and Food Chem. 9(2) 385–94
[4] Safa S, Moghaddam G, Jozani R J, Daghigh Kia H and Jammohammadi H 2016 Effect of vitamin E and selenium nanoparticles on post-thaw variables and oxidative status of rooster semen Anim. Reprod Sci. 174 100–6 doi: 10.1016/j.anireprosci.2016.09.011. Epub 2016 Sep 15
[5] Miroshnikova S A, Yausheva E V, Sizova E A, Miroshnikova E P and Levahin V I 2015 Comparative assessment of effect of copper nano- and microparticles in Chicken Oriental J. of Chem. 31(4) 2327–36
[6] Miroshnikova E, Arinzhano A, Kilyakova Y, Sizova E and Miroshnikov S 2015 Antagonist metal alloy nanoparticles of iron and cobalt: impact on trace element metabolism in carp and chicken Human and Veterinary Medicine 7(4) 253–9
[7] Yausheva E V, Miroshnikov S A, Kosyan D B and Sizova E A 2016 Nanoparticles in combination with amino acids change productive and immunological indicators of broiler chicken Agricultural Biology 51(6) 912–20
[8] Sizova E A, Kornev V L, Makave Sh A, Miroshnikova E P and Shakhov V A 2016 Morphological and biochemical blood parameters in broilers at correction with dietary copper salts and nanoparticles Agricultural Biology 51(6) 903–11
[9] Yausheva E, Miroshnikov S and Sizova E 2018 Intestinal microbiome of broiler chickens after use of nanoparticles and metal salts Environ. Sci. Pollut. Res. Int. 25(18) 18109–20
[10] Sizova E, Miroshnikov S, Lebedev S, Kudasheva A and Ryabov N 2016 To the development of innovative mineral additives based on alloy of Fe and Co antagonists as an example Agricultural Biology 51(4) 553–62
[11] Sizova E, Miroshnikov S, Yausheva E and Polyakova V 2015 Assessment of morphological and functional changes in organs of rats after intramuscular introduction of iron nanoparticles and their agglomerates Bio. Med. Res. International 243173
[12] Sizova E, Glushchenko N, Miroshnikov S and Skalny A 2011 Influence of Cu10x copper nanoparticles intramuscular injection on mineral composition of rat spleen J. of Trace Elements in Medicine and Biol. 25(SUPPL. 1) S84–9

[13] Polyakova V S, Sizova E A, Miroshnikov S A, Notova S V and Zavaleyeva S M 2015 Morpho-functional characteristic of the thyroid gland after administration of copper nanoparticles Morphologia 148(6) 54–8

[14] Sizova E A, Yausheva E V, Miroshnikov S A, Lebedev S V and Duskaev G K 2015 Element status in rats at intramuscular injection of iron nanoparticles Biosci. Biotechnol. Res. Asia 12 119–27

[15] Fisinin V I, Miroshnikov S A, Sizova E A, Ushakov A S and Miroshnikova E P 2018 Metal particles as trace-element sources: current state and future prospects World's Poultry Sci. J. 74(3) 523–40

[16] Wa R, Song B, Wu Ju, Zhang Ya, Chen A and Shao L 2018 Int. J. Nanomedicine 13 8487–506

[17] Medina C, Santos-Martinez M J, Radomski A, Corrigan O I and Radomski M W 2009 Nanoparticles: Pharmacological and toxicological significance Br. J. Pharmacol. 150 552–8

[18] Vranic S, Shimada Y, Ichihara S, Kimata M, Wu W, Tanaka T, Boland S, Tran L and Ichihara G 2019 Toxicological Evaluation of SiO2 Nanoparticles by Zebrafish Embryo Toxicity Test Int. J. Mol. Sci. 20(4) 882

[19] Chen L, Liu J, Zhang Y, Zhang G, Kang Y and Chen A 2018 The toxicity of silica nanoparticles to the immune system Nanomedicine 13(15) 1939–62

[20] Zoroddu M A, Medicì S, Ledda A, Nurchi V M, Lachowicz J I and Peana M 2014 Toxicity of nanoparticles Curr. Med. Chem. 21 3837–53

[21] Brohi R D, Wang L, Talpur H S, Wu D, Khan F A, Bhattarai D, Rehman Z U, Farmanullah F and Huo L J 2017 Toxicity of nanoparticles on the reproductive system in animal models: a review Front. Pharmacol. 8 606

[22] Almansour M, Alarifi S and Jarrar B 2018 In vivo investigation on the chronic hepatotoxicity induced by intraperitoneal administration of 10-nm silicon dioxide nanoparticles Int. J. Nanomedicine. 13 2685–96

[23] Leclerc L, Klein J P, Forest V, Boudard D, Martini M, Pourchez J, Blanchin M G and Cottier M 2015 Testicular biodistribution of silica-gold nanoparticles after intramuscular injection in mice Biomed. Microdevices 17(4) 66 doi: 10.1007/s10544-015-9968-3

[24] Barkalina N, Jones C, Kashir J, Coote S, Huang X, Morrison R, Townley H and Coward K 2014 Effects of mesoporous silica nanoparticles upon the function of mammalian sperm in vitro Nanomedicine 10(4) 859–70 doi: 10.1016/j.nano.2013.10.011. Epub 2013 Nov 4