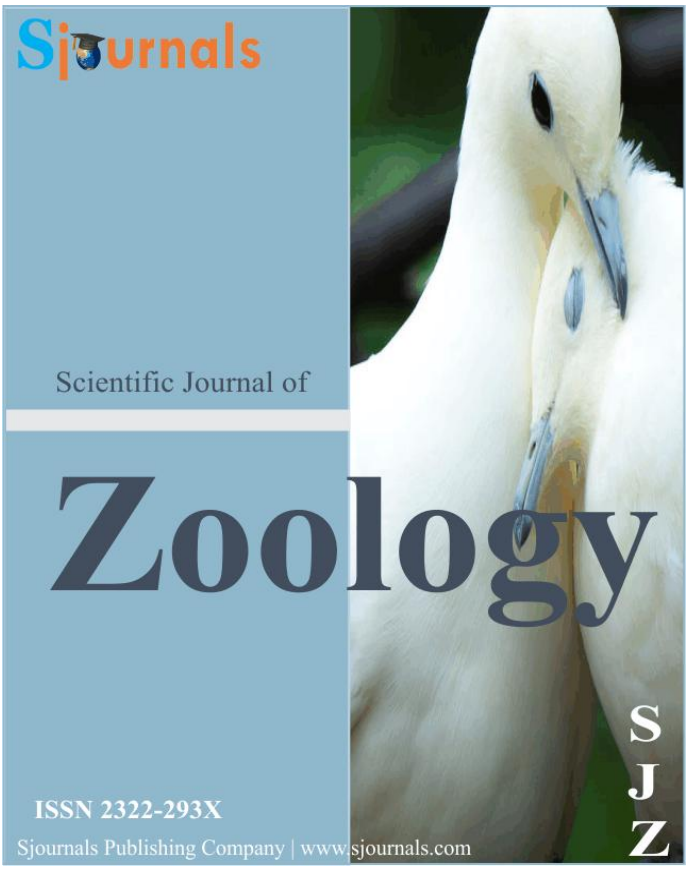

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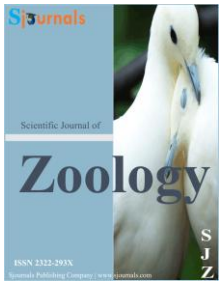

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Original article

Increase in the efficiency impregnating sperm serie's capabilities out of the organism

Alzhan Shamshidin[a], Orinbasar Alshinbaev[b], Aidos Mussabekov[c,*]
[a]*All-Russian Research Institute of Animal Husbandry, LK Ernst, Moscow, Russian Federation.*
[b]*Auezov South Kazakhstan State University, South-Kazakhstan Region, Kazakhstan.*
[c]*Saken Seifullin Kazakh Agro Technical University, Astana, Akmola Region, Kazakhstan.*

*Corresponding author: doc-1982@mail.ru

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ABSTRACT

There is a number of works in which it is reported about positive impact of physical factors on maturing of ova. The positive effect of stimulation of development of cages by the pulse of electric field, the helium neon laser is noted. Earlier positive influence of constant magnetic field on quality indicators of sperm at artificial insemination has been proved. Technology in vitro is most often used for thawing of cattle sperm. The freezing and subsequent thawing of spermatozoa induce adverse development of intracellular changes (increase in the level of reactive oxygen species, damage to membrane structures), reduces the survival and functional activity of cells. The plasma membrane of sperm contains a sufficiently large amount of unsaturated fatty acids undergoing peroxidation, which causes its destabilization of ionic homeostasis violation cells, and decreases the potential of the plasma membrane of mitochondria, DNA fragmentation, and without falling lower antioxidant protection. The aim of this work was to increase the efficiency of fertilizing capacity of sperm outside the body with the use of hormonal and biophysical methods of influence, establishing criteria for the metabolic viability of spermatozoa.

1. Introduction

Active introduction of cellular reproductive technologies in cattle breeding is a priority in the economic policy of many countries. The sharp decline in reproductive function of high producing dairy cows is becoming a worldwide problem, the solution of which is the use of the latest achievements of biotechnology reproduction, to which the technology of production and pre-implantation embryos from in vitro matured oocytes. Currently, in many laboratories around the world developed a system maturation of oocytes from the ovaries of cows slaughtered in a meat factory (Abeydeera, 1996; Fujita et al., 2006; Karashev, 1987; Kirkwood, 2007).

Successful fertilization of an ovum, both in vivo, and in vitro, happens in case of accomplishment of two conditions: The ovum shall ripen, and sperm to have training for fertilization. Under natural conditions spermatozoa in a genital tract of a female within several hours undergo the essential changes necessary for acquisition of the impregnating capability by them. They consist in transformation of a structure of cellular membranes on a head sperm, merge of a plasmatic membrane with ovolem (acrosome reaction), and also in providing initially motionless gametes with necessary substances for giving of swimming activity to them. This process under natural conditions takes several hours. In artificial conditions, preparation of sperm for fertilization (capacitation) is much quicker. The final stage of transformation of spermatozoa in case of statement of experiences of in vitro can be considered acquisition of the impregnating capability by them (Kolesnikova and Shagimaga, 2006; Kozumplik and Martinek, 1986).

Numerous studies have shown that sperm capacitation outside the body contributes to the factors destabilizing the state of their membranes, inducing their acrosome reaction, increasing the fertilizing capacity. Many scientists in their works have shown high efficiency of influence of caffeine on sperm capacitation. The presence of prostaglandin in the body of the female has a positive effect on sperm viability. However, the literature on the effects of prostaglandin on sperm in vitro in a contradictory: It inhibits or more, has no effect on the morphology and motility of the sperm acrosome (Mussabekov et al., 2016; Mussabekov, 2016; Mussabekov et al., 2016).

2. Materials and methods

Studies were conducted in the laboratory of genetics of Republican Center of livestock breeding JSC "ASIL TYLIK." Akmola Province, Kazakhstan.

Served as an object of researches it is refrigerated the thawed sperm of cattle which after defrosting was placed in a test tube with 1 ml of nutrient medium and put in the thermostat for 1 hour with the 9th purpose of separation of mobile and motionless (dead) the sperm. As the main nutrient medium for a capacitation used Tirode's circle. For the purpose of increase in efficiency of maturing sperms added synthetic analogs of F2α prostaglandin to the main nutrient medium estrofan and timestrofan in number of 25, 50 and 100 mkg/ml. As the capacitation agent used heparin (150 pieces/ml) and caffeine in various doses (2, 4, 8 mg/ml of solution). Further manipulations with sperm were carried out according to the commonly accepted techniques: methods of dilution, centrifugation, a resuspention and incubation in various environments. Then the sperm in an amount of 1×106 spermatozoa per 1 ml was added to the matured oocytes at this time to determine its fertilizing capacity. Oocyte-cumulus complexes (OCC) were isolated by dissection of ovarian tissue obtained at the factory after slaughter. Maturing oocytes was performed according to our scheme for 24 hours in a CO_2 incubator in a humid environment at a temperature of 39°C and 5% CO_2 in air. The joint incubation of sperm and oocytes proceeded 18-20 hours at a temperature 39°C in the atmosphere from 5% of CO_2 and the maximum humidity. For the purpose of increase in viability of sperm influence of laser radiation and the directional polarized light on viability and the impregnating capability of spermatozoa by impact of these physical factors on freshly thawed sperm and after its capacitation was studied. For work used the magneto-laser device "Vector - 03" and a lamp of the polarized Bioptron light. Influenced freshly thawed sperm a laser beam with a frequency of 5 and 10 Hz during 10 and 20 sec. The polarized light influenced on filched, right after carrying out swim-up of the procedure, or after process of a capacitation during 10 sec. Efficiency of a capacitation was determined by the level of crushing and an exit of viable germs.

Comparative characteristics of the functional state of frozen-thawed sperm producers of bulls was carried out after the capacitation for 2 hours by determining the intensity of lipid peroxidation, intracellular ATP (adenosine threephosphate), respiration rate and membrane potential.

All manipulations with the eggs, sperm activity evaluation, stages of development and the quality of early embryos in cattle was performed under a microscope MBS-10 at a magnification of 56 times.

3. Results and discussion

The influence synthetic analogues of prostaglandin F2α (estrofane, timestrofane) on the fertilizing capacity the sperm of bovine cattle in the preparation of embryos outside the body (Table 1). The studies found that the addition of a medium for capacitation estrofane and timestrofane dose of 50 ug / ml led to an increase in the level of fragmentation as compared to control at 3.1-2.3% respectively. Reducing the prostaglandin concentration to 25 ug / ml reduced the level of fragmentation as compared to the control at 10.4 - 8.8% and with said group on 11.9-12.5% respectively. Estrofan increased concentrations up to 100 ug / ml led to a significant reduction in the level of fragmentation by 13.5% as compared with the control. However, it had the highest yield of preimplantation embryos at developmental stages in the group with 16.6%, higher compared with the groups capacitation in medium supplemented with 25 and 50 ug on estrofana 9.1-5.5% and timestrofana 7.8-5.7%, respectively.

Table 1
Effect of estrofane and timestrofane on the fertilizing capacity the sperm.

Treatments	Concentration, ug / ml	Number of oocytes, n	Crushing level, n-%	Out morul-blastocysts, n-%
Control	-	46	19-41.3	7-15.2
Estrofan in an environment for capacitation	25	40	13	3-7.5
	50	27	12	3-11.1
	100	54	15	9-16.6
Estrofan in an environment for fertilization	25	54	16-29.6	10-18.5
Timestrofan in an environment for capacitation	25	68	21-30.9	6-8.8
	50	46	20-43.4	5-10.9

When adding estrofane Wednesday to fertilization, cleavage rate was 29.6%, but the embryos were received at the stage of morula, blastocyst, which accounted for 18.5% of the number of oocytes, put to maturation. In preparing the sperm for fertilization outside the body as capacitation agent heparin is widely used. However, there are works, which indicate that the use of caffeine contributes to the successful passage of the capacitation process. Our studies have shown that caffeine use did not contribute to increase the fertilizing capacity of sperm (Table 2). The level of cleavage compared to a control group was lower and ranged from 15.6 (with caffeine concentration of 4 mg / ml) to 25.9% (with caffeine concentration of 8mg / ml). The yield morula-blastocysts using caffeine at a concentration of 2 mg / ml was 7.5%, using a concentration of 8 mg / ml with 14.8%, which is lower compared to the control at 9.2-1.9%. When caffeine concentration of 4 mg / ml of preimplantation embryos received.

Table 2
The effectiveness of caffeine as capacitation agent.

The concentration of caffeine, mg / ml	Total fertilized cell, n	Crushing level, n-%	Out morul-blastocysts, n-%
2	40	8-20.0	3-7.5
4	32	5-15.6	-
8	27	7-25.9	4-14.8
Control	36	14-38.9	6-16.7

The impact of the laser beam with a frequency of 5 Hz for 10 sec on freshly thawed semen possible to obtain 44.1% cleaving the number of cells set by culturing (15 of 34), and 5 morula stage embryos, blastocyst, which is

14.7% of fertilized cell. Increasing exposure time to the laser beam for 20 sec not possible to obtain dividing cells. After exposure to the laser beam power of 10 Hz for 10 sec 8 cells were obtained on 8-16 cell stage, which amounted to 25.0% of the number assigned to the cultivation. Embryos at preimplantation stages received. Apparently, the effect of laser radiation on the cells immediately after thawing is ambiguous depth and requires further research. The effect of polarized light directed on physiological indicators and fertilizing capacity sperm of cattle in obtaining embryos outside the body (Table 3).

Table 3
Effect of polarized light directed on the efficiency of capacitation frozen-thawed semen in cattle.

Dates exposure polarized light	Total fertilized cell, n	Crushing level, n-%	Out morul-blastocysts, n-%
After a swim-up procedures	24	6-25.0	4-16.7
Before insemination	31	17-54.8	4-12.9

As a result of studies found that exposure to polarized light within 10 seconds of visible changes in indicators such as mobility and the level of aggregation, not cause. Meanwhile, when the processing of sperm after swim-up procedure cleaving embryo yield was 25.0% of the number assigned to the cultivation (6 of 24), 16.7% of them had developed to early morula. After exposure to polarized light on sperm after 17 dividing embryos were obtained her capacitation, which amounted to 54.8%. However, access to the embryos preimplantation stages in this experiment was lower and amounted to 12.9% of the number of fertilized.

The comparative characteristic of functional state of the frozen-thawed sperm depending on the individual characteristics of animals and sperm freezing method (Table 4). The semen of bulls and Bakan, Kyran was frozen in Payette and bull Balhash in the granules. We investigated such factors as the intensity of lipid peroxidation, intracellular ATP content, respiration rate and membrane potential.

Table 4
Comparative characteristics of the functional state of the frozen-thawed sperm capacitation after.

The process of sperm freezing, bulls	The intensity of lipid peroxidation cond. pcs./106 cells	Intracellular ATP content, nm / 106 cells	Respiration rate, tg α	The membrane potential, mV
Bakan	0.89	1.17	0.45	-35
Kyran	1.46	0.23	0.11	-18
Balhash	0.88	0.97	0.41	-33

As can be seen from the table, the level of lipid peroxidation was higher in Kyran as compared with the Bakan on 0.57-0.71 cond. u / 106 cells and sperm in the pellet at 0.58-0.76 cond. u / 106 cells respectively. At the same time, the intracellular concentration of ATP as an indicator of the vitality, the hero of sperm cells was 1.17-1.60 nm / 106 cl., which exceeds the same indicator of cell pellets frozen at 0.2-0.35 nm / 106 cells, and the cells Kyran at 0.94-1.24 nm / 106 cells. Respiration rate of the bull and the cells of Balhash are at the same level, and significantly higher than the respiration rate of Kyran cells. A similar relationship is observed on the membrane potential of -18 mV against -35-33mV. Out of embryos at fertilization of mature oocytes, sperm Bakan of 17.3%, the bull semen Balhash -16.4%, when using sperm Kyran preimplantation embryos have been received.

4. Conclusion

Adding on Wednesday for capacitation of 50 ug / ml prostaglandin increased the level of fragmentation in the 2.3-3.1%, but at the same time reduces the yield of embryos at preimplantation stages. The increase in estrofane concentration in the medium for the capacitation and 100 ug / ml, and adding it to the environment for fertilization has allowed to increase the yield of embryos at the morula stage, blastocyst to 16.6-18.5%. When used as capacitation an agent of caffeine in an amount of 8 mg / ml in the preparation of sperm for bovine in vitro

fertilized embryos yield was 16.7% at a 25.9% level of crushing. Impact directional polarized light on sperm after their maturation is more efficient compared to the impact on it immediately after swim-up procedure, the yield from preimplantation embryos was 16.7% versus 12.9%. Using laser yielded 14.7% morula-blastocysts. The intensity of respiration of spermatozoa 0.41-0.63 tgα, lipid peroxidation 0.88-0.97 cond. u / 106 cl. The intracellular ATP content 0.97-1.60 nm / 106 cells. and membrane potential -33-35mV let you receive during fertilization in vitro matured oocytes cattle 16.4-17.3% pre-implantation embryos split level 39.8-42.3%. Improving the efficiency of fertilizing capacity of sperm outside the body can be achieved by administration of 25 mg / ml estrofane Wednesday to fertilization with preimplantation embryos output will be 18.5%. Impact directional polarized light for 10 seconds with an intensity of 40 mW / cm2 increases the yield morula-blastocysts 3.8%.

References

Abeydeera, 1996. In vitro penetration of pig oocytes by commercially prepared frozen-thawed. Boar spermatozoa in a chemically semi-de-fined bicarbonate-free medium. Theriogenol., 45(1), 265.

Fujita, T., Umeki, H., Shimura, H., Kugumiya, K., Shiga, K., 2006. Effect of group culture and embryo-culture conditioned medium on development of bovine embryos. J. Reprod. Dev., 52(1), 137-142.

Karashev, M.F., 1987. Oocyte maturation and fertilization of eggs large of cattle in vitro in a medium with the biologically active substances: Abst. Diss. Cand. Biol. Sci. Kharkiv, 25.

Kirkwood, 2007. Effect cloprostenol treatment at artifical insemination on sov fertility. Reprod. Domest. Anim., 42(2), 26-28.

Kolesnikova, A.A., Shagimaga, V.A., 2006. Stimulation of mammalian oocytes in vitro. Recent advances in biotechnology and problems agricultural animals: Mes. Rep. 6th Intern. Conf. VIZH. Dubrovi-Tzu: 85-87.

Kozumplik, J., Martinek, J., 1986. The effect of Oestrphan Spofa (synthetic analog of prostaglandin F2 alpha) added to the insemination dose on pregnancy and fertility in sow. Vet. Med., 31(4), 227-232.

Mussabekov, A., Shamshidin, A., Alshinbaev, O., 2016. Influence of age on sperm production's indicators of bulls. Sci. J. Anim. Sci., 5(8), 339-341.

Mussabekov, A., Shamshidin, A., Alshinbaev, O., 2016. Research properties of the environment on basis of vegetable components from extract soy at the cryopreservation of sperm of bulls. Sci. J. Anim. Sci., 5(8), 342-345.

Mussabekov, A.T., 2016. Impact of bacterial and viral infections for barrennes of productive animals. Sci. J. Biol. Sci., 5(3), 155-157.

Mussabekov, A.T., Borovikov, S.N., Suranshiyev, Z.A., Shamshidin, A.S., 2016. Comparative analysis of Holstein, black-motley, Angler, Simmental bulls semen. Int. J. Anim. Health Livest. Prod. Res., Published by European Centre for Research Training and Development UK.

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