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Development Methods to Reduce Agglutination of Sperm Cells in The Preparation of Freshly Sperm to The Process for Production Embryos In Vitro.

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ABSTRACT

Goal of research work is search the new methods of reducing agglutination of sperm cells in preparation of freshly sperm to the process for production of embryos in vitro. As a result of researches it is established that after the swim up procedure agglutination in a new environment 15 (!) times less than when placing the sperm directly into the environment, SOF w.

Keywords: sperm agglutination, breeding ground, swim-up

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INTRODUCTION

The introduction of new reproductive technologies associated with the use of the process of in vitro fertilization (IVF) and obtaining embryos in vitro is an important area in modern animal husbandry and is actively developing all over the world (Joyce Harper1, M. Cristina Magli, Kersti Lundin, Christopher L. R. Barratt, and Daniel Brison., 2012). However, at the present time in Russia this method in sheep is almost never used in connection with its complexity and the lack of serious scientific researches of domestic scientists in this field. A paper on embryo transfer in sheep (V.I. Trukhachev, 2007).

One of the problems arising in the implementation of in vitro fertilization is the quality of the sperm used for this process. If fertilization inside the body of the animal requires the presence of a single sperm in the oviduct, while performing IVF in contact with the egg must join thousands of sperm (L.P. Dyakonov, 2009). In some cases, the reason for the low fertility of the sperm cells during IVF is increased their agglutination when included in the culture medium (Maxwell, 1996). Sperm immediately before fertilization needs at least 6 hours to be in the abdominal part of alcaravea. During this time they "ripen" and become capable of fertilization. This phenomenon is called capacitating (incubation of sperm. Thus, fertilization can occur only when a sufficient concentration of sperm. (A.P. Studentsov, 1986). Usually for the preparation of sperm and the induction of capacitated sperm is used making freshly sperm in a solution of wash SOF (Synthetic Oviduct Fluid; Cognie Y., Baril G., Poulin N., Mermillod P., 2003). However, in experiments we often observed an intense agglutination after exposure of sperm cells to the environment, leading to very low yield of zygotes in the IVF process. In the literature available to us there is little data on the prevention of agglutination of sperm during the procedure of IVF using freshly sperm, as most authors prefer to use fresh frozen material (Morrier et al., 2002).

The aim of our work was the search for new methods of reducing the agglutination of sperm cells in the preparation of freshly sperm to the process of production of embryos in vitro.

MATERIALS AND METHODS

The studies were conducting at the laboratory of assisted reproductive technologies Scientific – diagnostic and therapeutic veterinary center FGBOU VPO Stavropol state agrarian University in the period from January to may 2014.

The research objects were the sheep of the North Caucasian breed at the age of 2 – 3 years by 10 goals. The sperm carried out of each animal twice a week with an interval of 2 days. There were performed 10 experiments. Sperm received in the arena of artificial insemination urethral method, using an artificial vagina. Microscopic evaluation of sperm quality at various stages of investigations were carried out by means of a microscope "Mikmed-2 (LOMO, Russia) according to methodical recommendations (I. S. Novopashina, 2013).

To preparation sperm for fertilization we had used a developed combine methodology according which pre-cum introduced in a glucose-citrate – yolk diluent (HCG), prepared according to GOST 14746 – 69 (glucose medical anhydrous – 30,0, sodium citrate translesanas, patent – 14.0 g, egg yolk – 200.0 ml., spermcan 3 – 750 - 900 thousand units, distilled water – 1000,0 ml). Then transferred to SOF medium wash, were prepared without glucose and glutamine (Cognie Y., Baril G., Poulin N., Mermillod P., 2003) with the addition of 6 mg/ml bovine serum albumin, 0.2 mg/ml of caffeine and heparin 50 µg/ml.

We have compared the sperm preparation by two methods: 1) using GCJ environment before amending the SOF w; 2) without the use of HCG environment before amending the SOF w.

The technique by HCG environment perform as follows: Into a test tube type, Eppendorf (2 ml) was introduced GCJ 450 µl medium and 50 µl freshly sperm had thoroughly mixed. Then centrifuged for 3 minutes at 200 G, the supernatant was removed and 200 µl resuspendable fresh GCG environment. The vial was placed in a thermostat for 15 minutes (T = 38.5 C.; 5% CO2) for the procedure swim-up. Technology swim-up necessary to remove seminal plasma and obtaining the maximum a full-fledged faction of progressive sperm motility (Irvine et al, 2000 ; Zini et al, 1993 , 2009). Dead and agglutinative sperm settle to the bottom of the tube and are active in the upper layer of supernatant liquid (Henkel et al., 2003). At the same time is the capacitation, that is, the acquisition of sperm fertilizing capacity (L. V. holubec et al., 2010). After incubation,

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the supernatant, containing live sperm, had selecting and transferred to a tube with 200 µl of SOF medium w. The vial was placing in a thermostat for 15 minutes to repeat the procedure of swim up. Then we assessed the quality of sperm.

Technique without to use of GCG environment was performed as follows: 50 µl of freshly sperm immediately introduced into a tube containing 450 µl of SOF medium was described in (Henkel et al., 2003). Centrifuged 3 minutes at 200 G. Then the supernatant was removed and resuspendable in 200 µl of SOF medium w. The vial have placed in a thermostat for 15 minutes (swim up). Then we assessed the quality of sperm.

Statistical processing of results was performed using student's t-test in the program "Primer of Biostatistic 3. 01 for Windows for IBM compatible PC. The differences were considered significant at $p<0.05$.

RESULTS

The results of the evaluation of sperm parameters immediately after receipt and under different conditions of preparation for IVF are show in table 1.

Table 1: Comparative characteristics of the functional parameters of semen of sheep when using different environments

Comparison options		Undiluted	SOF w	SOF w + GCJ medium
Sperm activity points, M ± m	Freshly sperm	8.77 ± 0.14	-	
	Prior to swim up procedure (n = 10)	-	5.50 ± 0.22	8.90 ± 0.17*
	After the swim up procedure (n = 10)	-	6.30 ± 0.15#	8.70± 0.15*
Agglutination of spermatozoa in%, M ± m	Freshly sperm	4.00 ± 0.47	-	
	Prior to swim up procedure (n = 10)	-	61.00 ± 2.05	1.60± 0.40*
	After the swim up procedure (n = 10)	-	45.50 ± 1.35#	3.00 ± 0.33*#

Note: * - differences with sperm introduced directly into the SOF w significant at $p< 0,05$; # - the difference with sperm before the procedure swim-up significant at $p< 0.05$.

In our experiment, the activity of the sperm cells (not subjected to agglutination) prior to the procedure swim up when placed in SOF w + GCJ environment more by 3.4 points, compared with the activity in the environment of SOF w.

After the procedure swim-up activity of the sperm cells when placed in SOF w + GCJ environment significantly higher by 2.4 points, compared to the activity in the environment of SOF w.

Also noted that after the procedure swim up in the medium SOF w activity of sperm not subjected to agglutination increased by 0.8 points. In SOF w + GCJ environment activity was not significantly changed and remained high.

When comparing the degree of agglutination is established that when placing the sperm directly into the environment, SOF w agglutination by 59.4% more than the placement of sperm in pre-GCI environment. Noted, however, that after the procedure, swim up in the medium SOF w agglutination is had reduced by 15.5 % and in the SOF w + GCJ environment increases by 1.4 %. However, the analysis of the absolute values of the rate of agglutination shows that after the swim up procedure agglutination in the medium SOF w + GCG 15 (!) times less than when placing the sperm directly into the environment, SOF w.

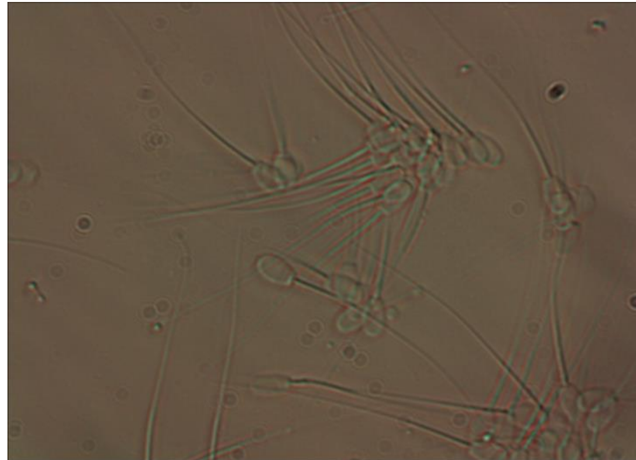

Figure 1: Sperm agglutination

DISCUSSION

Unfortunately, there are almost no sources in the literature, which describe the study of agglutination in the preparation of sperm for in vitro fertilization. Usually sperm preparation is hadperformed in two ways: using the "swim-up" and using separation by density gradient percoll (holubec L. et al., 2010). Most often in the articles mentioned using the frozen – melted sperm, where it is already in any of the diluents. The analysis of the efficiency of this sperm showed that fertilization of oocytes in vitro the degree of fertilization is had reduced compared to freshly sperm by 30 – 40 % (Maxwell and Watson, 1996).

In this regard, perhaps the authors and have not been given such attention agglutination, as they used the sperm is frozen, that is, the last stage of preparation in the diluent. We believe, however, as well as a number of foreign researchers that it is preferable to work with fresh sperm because the number of active sperm cells is much higher than in frozen semen (L. O Hara., J et al., 2009). Sperm in the semen freshly have a greater fertilizing capacity than in the frozen – melted (Watson, 2000;Niu et al., 2006). The viability of sperm after cryopreservation and thawing is had reduced from 85.6 to 34.3 % (Hiemstra et al., 2005; Marco – Jimenez et al., 2006). Moreover, after cryopreservation can be changed the mechanism of the process is capacitated, which significantly affects the fertility of spermatozoa (Maxwell and Watson, 1996; Morrier et al., 2002).

To eliminate agglutination of sperm cells at the stage of preparation of the sperm we used GCG environment, has resulted in a significant (almost 15 times!) reduce the number of associated spermatozoa. In our opinion, the decrease in agglutination in GCI buffer associated with the specific effect of its constituent components on the sperm.

Sodium citrate, the main component GZZ – provides buffer capacity of the medium, to neutralize the products of vital activity of the sperm cells, binding of calcium ions and heavy metals. Glucose serves as energy source for sperm, in addition, involved in maintaining osmotic pressure, reduces the conductivity of the environment, and protects sperm from the loss of electric charge. Egg yolk contains lecithin and lipoproteins, which create on the surface of sperm adsorption layer that protects it from cold shock (G. D. Nekrasov, I. A. Cumanova, 2007).

CONCLUSION

Thus, we proposed a method of preparing freshly sperm for IVF allows you to achieve sharp decline in agglutination of spermatozoa, which would increase the impregnation capacity of oocytes in the process of obtaining embryos of sheep in vitro.

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