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Research on the Effects of Different Cryopreservated Mediums on Shanbei Cashmere Goat Semen Cryopreservation

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Abstract: Shanbei cashmere goat is one of the most famous native goat breeds with the advantages in velvet fineness and yield as well as many other characteristics in the Shanbei area in the Shannxi Province in China. The effects of different cryopreservated mediums (different glycerol and dosage yolk) on Shanbei cashmere goat semen cryopreservation are acrried out in this research in order to expand thoroughbred breeding using artificial insemination techniques. The results suggested as following; the PMS of thawed sperm motility in the group C (adding 6% glycerol) is up to 45.50% which is the best among different dosage adding glycerol (2, 4, 6, 8 and 10%) in the experiments affecting semen cryopreservation; the PMS of thawed sperm motility in group C (adding 13.5% yolk) is up to 44.06% which is the best among dosage adding yolk (11, 12, 13.5, 15 and 16%) in the experiments affecting semen cryopreservation. Therefore, this research found that the 6% added glycerol and 3.5% added yolk is the suitable adding ingredients in cryopreservation diluents for cryopreservating Shanbei cashmere goat' semen and lays a foundation for further research works.

Key words: Cryopreservation, dilutions, Shanbei cashmere goat, sperm quality, yolk

INTRODUCTION

In 1960s, the papers about research in goat artificial insemination using frozen semen gradually increased in the world. But the researches are gradually focused until 1980s and begin to apply this technology in production practice in china. According to the Chinese Academy of Agricultural Sciences reports: goat semen viability after thawing are not secure >50% in most trials (Yang, 2008). The rates of thawed semen of Liaoning cashmere goat is 52% reported by Lu *et al.* (2009). The rates of thawing sperm motility of Boer goat semen reached 42% and the rate of ewes not estrus reached 65.77% after carrying out artificial insemination using these cryopreservation semen reported by Mao Feng in 2003 (Jin *et al*., 2003). Among of the most research results about goat semen cryopreservation, the rate of semen viability is not >50% after thawing (Purdy, 2006). It is mainly due to breeding specification and limitations. Although, the disease control and semen cryopreservation technology has brought the introduction of improved varieties and cross-border breeding advantage, the technology is not well promoted in goats (Dorado *et al*., 2007). Currently, goats frozen semen research focuses on scientific research and little is done in productive application. With the research and technology to improve the rise of goat farming, goat frozen semen technology will be more widely used. With the number and economic value of farming Shanbei cashemre goats increasing in China, goat frozen semen technology will be more widely used for artificial insemination technology.

Therefore, the different cryopreservation solutions are compared to explore the suitable cryopreservation mediums for Shanbei cashmere goats frozen semen storage and pave a way for the further artificial insemination technology and building goats breeding sperm bank.

MATERIALS AND METHODS

Collection of semen: Semen was collected from fertile male goats (each male had produced offspring and be ahletic, sexually active), 3-4 years old from March to June with the Fake Vagina Collection Method. Semen was collected into glass containers with water coat at a temperature of 37°C. Following collection, the ejaculate volume was measured. The percentage of motile spermatozoa was determined in a light microscope with a thermostable table of 37, under x200 magnifications and spermatozoa concentration was calculated in the haemocytometer chamber. Ejaculates of a volume exceeding 0.5 mL, sperm motility >80% and sperm

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concentration >10×10^8 mL^{-1} were qualified for freezing. Five ejaculates from each male were thus qualified (Batista *et al*., 2009). Each ejaculate was divided in two equal parts and frozen according to the methods described.

Cryopreservation solution: Various chemical reagents were accurately weighed using an electronic balance and dissolved in ddH_2O into a 200 mL volumetric flask and then sterilized water bath 100°C 30 min, cooled to room temperature in the refrigerator at 4°C spare. The basic formula dilution is composited of Tris (6.0570 g), glucose (5.1346 g), Fructose (1.50 g), Sodium citrate (3.3186), Penicillin (0.25 mg) and Streptomycin (0.25 mg) dissolved in 200 mL ddH_2O. The cryopreservation solution I and cryopreservation solution II were made up of adding egg different dosage yolk or glycerol on the basis of above basic solution. The cryopreservation I solution: adding 5.4 mL fresh yolk into 14.6 mL basic formula dilution, magnetic stirrer stir, using now. The cryopreservation solution II: adding 1.2 mL glycerol into 8.8 mL cryopreservation solution I, magnetic stirrer stir using now.

Methods of semen freezing-thawing: Semen dilution is using one-step isothermal dilution method at the room temperature (23±2°C). Firstly, Semen is diluted in the I (Volume ratio 1:1) and pipette pipetting several times to mix evenly then immediately adds the cryopreservation solution II into that mediums (Volume ratio 1:1) and pipetting several times to mix evenly. Secondly, now immediately inhale 0.25 mL thin tube (sealed and label led) then placed in a refrigerator at 4°C balance least 3 h after wrapping a 12 layer gauze for further steps. The following step uses liquid nitrogen fumigation methods by thin tube (0.25 mL). Firstly, the bracket straw semen plane remains 2.0 cm above the liquid nitrogen surface through adjusting the freezing nitrogen tank and balanced straw semen is fumigation for 6 min collected from a refrigerator at 4°C. Secondly this balanced straw semen are quickly placed in liquid nitrogen tank preservation. The semen thawing method is the freezing semen in thin tube is quickly removed from the liquid nitrogen tank and quickly immersed in a water bath at 38-40°C for further experiments.

Measure of sperm motility: The one drop semen is placed in a haemocytometer and covered with glass to check the percentage of sperm motility after semen being right dilution. The number of motion sperm in sample of four grid and one central corners of the haemocytometer is counted under 200-400 magnification for recording the number (A) by using optical microscope through using the basis of dilution for maintaining the normal movement. Then, the haemocytometer is plated in 90°C incubator about 5 min in order to kill all the sperms and all the sperms are accounted under the microscope for recrding the number (B). Finally, the Percentage of Motile Sperm (PMS) is equal to A (the number of linear movement of sperm)/B (the number of total sperm count)×100% (Silva *et al*., 2012).

Experimental design: Sperm motility assessed mainly through measuring the sperm motility at least three times each sample and recording the mean for further analysis. The fresh sperm motility, equilibrium sperm motility and thawed sperm motility are separately measured according to the above methods and recorded in order to evaluate the effect of different cryopreservation solutions.

The experiment 1 is to research the effects of different dosage glycerol on semen cryopreservation of the Shanbei cashmere goats through adding five different Concentration (2, 4, 6, 8 and 10%) glycerol in the final cryopreservation solution to obtain five kinds of cryopreservation solution (A-E). The experiment 2 is to research the effects of different dosage yolk on semen cryopreservation of the Shanbei cashmere goats through adding five different concentration' (11, 12, 13.5, 15 and 16%) yolk in the final cryopreservation solution to obtain five kinds of cryopreservation solutions (A-E).

Analysis of experimental data: The results were presented as mean±standard deviation; statistical analysis was based on Student's t-test at the significance level of $p<0.05$ (Using Microsoft EXCEL 2007 Software to analyze the experimental data).

RESULTS

The effects of different dosage glycerol on semen cryopreservation: This experiment is comparing the five kinds of cryopreservation solutions in order to choose the suitable cryopreservation solutions for the Shanbei cashmere goats' semen cryopreservation (Table 1).

The experimental results showed the PMS (percentage of motile sperm) after balanced in the group C is highest (up to 73.64%) among these different diluents glycerol groups and there is no significant difference ($p<0.05$) between group A and E. The PMS in group C after thawing is the highest (up to 45.50%) and the PMS of between group B and D shows a significant difference ($p<0.05$) and there is not a significant differences with A, E group ($p>0.05$). Therefore, the optimal glycerol concentration of 6% (group C) is choose for subsequent tests according to these experimental results.

Table 1: Effects of different dosage glycerol on semen cryopreservation of Shanbei cashmere goats

Groups	Dosage of glycerol (%)	PMS of fresh semen (%)	PMS of balanced semen (%)	PMS of thawing semen (%)
A	2	77.06±1.29	70.85±0.88^{a}	36.46±1.29^{ac}
B	4	77.06±1.29	73.58±1.40^{a}	41.16±0.35^{b}
C	6	77.06±1.29	73.64±0.49^{b}	45.50±0.59^{b}
D	8	77.06±1.29	72.47±1.83^{ab}	40.75±0.30^{b}
E	10	77.06±1.29	71.06±1.10^{ab}	37.96±1.24^{c}

Table 2: Effects of different dosage yolk on semen cryopreservation of Shanbei cashmere goats

Groups	Dosage of yolk (%)	PMS of fresh semen (%)	PMS of balanced semen (%)	PMS of thawing semen (%)
A	11.0	76.77±1.10	70.85±1.13^{a}	36.54±1.00^{ac}
B	12.0	76.77±1.10	72.18±1.67^{a}	42.73±0.57^{b}
C	13.5	76.77±1.10	73.59±1.09^{a}	44.06±0.24^{b}
D	15.0	76.77±1.10	71.13±0.87^{a}	39.95±0.85^{c}
E	16.0	76.77±1.10	72.61±1.41^{a}	38.84±0.53^{c}

Percentage of Motile Sperm (PMS); marking different lowercase letters indicate significant differences ($p<0.05$), marking different uppercase letters indicate a significant difference ($p<0.01$)

The effects of different dosage yolk on semen cryopreservation: This test is carried out to compare the effects of different dosage yolk (group A:11%, group A:12%, group A:13.5%, group A:15%, group A:16%) on semen cryopreservation on the basis of the above expreimetal result in order to obtain the suitable yolk dosage for semen cryopreservation of the Shanbei cashmere goats (Table 2).

The experimental results showed the PMS (percentage of motile sperm) after balanced in the group C is highest (up to 73.59%) among these different diluents glycerol groups and there is no significant difference ($p<0.05$). The PMS in group C after thawing is the highest (up to 44.06%) and the PMS of between group A and B shows a significant difference ($p<0.05$) and there is not a significant differences with A, E group ($p>0.05$). Therefore, the optimal yolk concentration of 13.5% (group C) is choose for subsequent tests according to these experimental results.

DISCUSSION

The anti-freeze agents are usually added in cryopreservation diluents to reduce stress and permeability of the formation of ice crystals. The anti-freeze agents are divided into two categories according to their nature: permeable antifreeze agents (such as glycerol, DMSO, propylene glycol, etc.) and impermeable antifreeze agents (such as sucrose, sodium chloride solution and raffinose) (Purdy, 2006). It is known to us that glycerol is considered standard anti-freeze protection in most mammals frozen semen diluents but the adding dosage is depending on the rate of freezing and the different dosage of added glycerol would significantly affects the effects of semen cryopreservation (usually 2-10%; V:V). In this research, it is found that the PMS of the 6% (group C) added glycerol is the highest among these different dosage glycerol groups which is similar to the reported results in goat or sheep (Vasquez *et al*., 2013).

The reported results show that yolk also is the most effective composition in cryopreservation diluents because the yolks contain many lipids similar to the sperm membrane lipids for protecting sperm damage during the sperm cryopreservation process (Chauhan and Anand, 1990). The different dosage of added yolk would significantly affects the effects of semen cryopreservation (usually 5-30%; V:V). Therefore, it is very important to choose the suitable dosage yolk added in cryopreservation diluents in order to obtain the best effects of semen cryopreservation of Shanbei cashmere goat. In this research, it is found that the PMS of the 13.5% (group C) added yolk is the highest among these different dosage yolk groups which is similar to the reported results in other animals (Aboagla and Terada, 2004; Priyadharsini *et al*., 2011).

CONCLUSION

In a word, the study focus on research in choosing the suitable cryopreservation diluents for semen cryopreservation of Shanbei cashmere goat and the 6% added glycerol and 3.5%. Added yolk in cryopreservation diluents is the suitable adding ingredients for cryopreservation Shanbei cashmere goat' semen.

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