Biotechnological Communication

Production, Evaluation, Cultivation and *In vitro* Fertilization of Cattle Oocytes: Recent Trends in Reproductive Biotechnologies in Animal Breeding : A Review

Vladimir Aleksandrovich Pleshkov,* Tatyana Vladimirovna Zubova, Oksana Vladimirovna Smolovskaya and Olga Borisovna Konstantinova

Kuzbass State Agricultural Academy, Kemerovo, Russian Federation Russia

ABSTRACT

The article presents the results of assessing reproductive biotechnology for cattle breeding. The issues of obtaining genetic material from bulls-producers and oocytes from donor cows, for their further cultivation and obtaining embryos, in order to replicate highly productive offspring from valuable animals, are considered. Oocyte production was analyzed in three different ways: puncture, section, and aspiration. A total number of 156 oocyte-cumulus complexes (OCCs) were collected out of 40 ovaries by puncture – 50 pcs, section - 47 pcs, and aspiration – 59 pcs. The results showed that puncture and section gave significantly higher total OCCs per an ovary (4.16 and 4.0, respectively) than aspiration (3.68), but a higher number of normal (grade A and B) OCCs per an ovary was observed with aspiration (2.5) than with puncture (1.82) and section (2.00). During aspiration, oocyte-cumulus complexes were collected from the surface of follicles with a diameter of 3 to 8 mm using a needle. During puncture, all surfaces were pierced with a hypodermic needle; during section, incisions were made along the entire ovarian surface with a scalpel, that is, all sizes of superficial follicles were collected. OCCs were divided into 4 classes based on cumulus and nucleus cells: grade A - oocytes completely surrounded by cumulus cells; Grade B - oocytes partially surrounded by cumulus cells; Grade C - oocytes not surrounded by cumulus cells; and grade D - degeneration observed in both oocyte and cumulus cells. Grades A and B were considered normal, while grades C and D were considered to be damaged. The obtained oocytes were cultured and fertilized *in vitro*, which means that their fertilization with sperm occurred under artificially maintained optimal conditions outside the body. Normal fertilization results are zygote formation with male and female pronuclei (PN). As a result of the conducted fertilization of 251 mature oocytes, 142 fertilizations were successful, which amounted to 56.57%.

KEY WORDS: BIOTECHNOLOGY, CRYOPRESERVATION, EMBRYO TRANSPLANTATION TECHNOLOGY IN VITRO, OOCYTE AND SPERM.

INTRODUCTION

An essential factor contributing to the successful development of the livestock industry is the good reproductive performance of heifers, cows and bulls. For a breeding bull, an effectiveness indicator of reproductive capabilities is its sperm ability to fertilize an oocyte. Sperm products are usually purchased at livestock breeding enterprises specializing in breeding servicing bulls. At such enterprises, breeders conduct a comprehensive assessment and work to improve the economically useful qualities of animals. Sperm products are obtained from bulls that have passed the assessment and received permission for use in accordance with the technologies provided by the enterprise (Luno et al. 2014; Morrell and Wallgren 2014; Iqbal et al. 2016; Alm-Kristiansen et al. 2018; Gorelik et al. 2021).

When obtaining semen, it is important to comply with all technological conditions, since the quality indicators are influenced by many factors, which include feeding and keeping bulls, hygiene of semen collection, composition of diluents, methods of freezing and thawing, and other factors. Sperm products obtained from breeding bulls are usually delivered to livestock enterprises frozen in liquid nitrogen (granules, pies). The use of artificial insemination and the development of cryopreservation technologies made it possible to obtain numerous offspring from a single sire (Luno et al. 2014; Morrell and Wallgren 2014; Iqbal et al. 2016). The biological characteristics of cows determine the

Article Information:*Corresponding Author: 6110699@mail.ru
Received 17/09/2021 Accepted after revision 20/12/2021
Published: 31st December 2021 Pp- 1452-1458

Available at: https://bbrc.in/ DOI: http://dx.doi.org/10.21786/bbrc/14.4.12

bearing period of one calf during the year, which, in contrast to bulls-producers, is a limiting factor for replicating highly productive heifers from highly productive cows. Most farms raise and evaluate cows and heifers independently at all technological stages (Alm-Kristiansen et al. 2018; Gorelik et al. 2021).

In this connection, they have the opportunity to form groups of animals according to the desired phenotype and genotype, timely conduct a comprehensive assessment of the animal, including its productive potential and reproductive abilities, and, based on the data obtained, make a decision on its further use (Gritsenko 2016; Mostek et al. 2017; Chalova et al. 2020; Gorelik et al. 2021). The development of reproductive biotechnology in animal husbandry has made it possible to obtain many offspring from one highly productive cow within one year. This became possible thanks to the use of such methods as *in vivo* and *in vitro* embryo transplantation technology, as well as cryopreservation of genetic material (sperm, oocytes, embryos).

Freezing and storage of genetic material in liquid nitrogen is the most important method of animal reproductive biotechnology, however, the quality indicators of frozen and then thawed biomaterials suffer greatly, which significantly reduces the successful fertilization rates. For example, frozen-thawed spermatozoa, due to poor quality characteristics, often remain incapable of fertilizing an ovum. Some oocytes and embryos die during freezing and thawing (Kumar et al. 2016; Naresh 2016; Murphy et al. 2018; Nongbua et al. 2018; Tkachev et al. 2020b Gorlik et al 2021).

For these reasons, research to improve the quality of cryopreserved genetic material is being carried out by scientists around the world. Despite the fact that many positive points have already been achieved, this factor of reproduction requires a study and a scientifically grounded proposal to livestock breeders to increase fruitful inseminations using various technologies (Kumar et al. 2016; Naresh 2016; Murphy et al. 2018; Nongbua et al. 2018). There are two main types of embryo cryopreservation: slow freezing and vitrification. Slow freezing is an older method. In this case, the embryo is placed in an environment with a cryoprotectant, glycerin, or ethylene glycol, packed in a plastic straw and cooled at a rate of 0.50 per minute to -70 (Tkachev et al. 2020b).

Then the straw is touched with tweezers soaked in liquid nitrogen - seeding is carried out (freezing water in the embryo), slowly cooled to -350, and then transferred into liquid nitrogen, and freezing is completed to -1960. The disadvantage of this method is the formation of ice crystals both in the embryo itself and in the environment around the embryo. Ice crystals damage the membranes and membranes of the embryo, causing its death both during freezing and thawing (Youngs 2011; Konc et al. 2014). Cryoprotectants used in variable speed freezing are designed to prevent intracellular crystallization by osmotic dehydration of frozen water and replacing intracellular water with a cryoprotective solution. The degree of dehydration depends on the duration of cooling or the temperature reached before the embryos are immersed in liquid nitrogen. The type of cryoprotectant and the cooling rate determine the way the embryos thaw (Niemann 1991; Asgari et al. 2012; Do et al. 2016; Tkachev et al. 2020a).

Vitrification is usually achieved by placing the embryos in a very high (5-6M) concentration of cryoprotectant solution, followed by rapid cooling (> 2000°C/min) in liquid nitrogen. Vitrification is based on the ability of highly concentrated aqueous solutions of cryoprotectants to be cooled to very low temperatures. At low enough temperatures, the solution becomes very viscous so that it solidifies into an amorphous glass without the formation of ice crystals. Vitrified embryos should be stored at a temperature not exceeding -130°C. Above this temperature, the solution may become crystalline, which can damage the embryos. To avoid this damage, vitrified embryos must always be stored at temperatures below –130°C, and thawing must be done very quickly to avoid crystallization (Díez et al. 2012; Do and Taylor-Robinson 2020).

Currently, much attention is focused on the study of genetic material and preparations for compliance with their quality standards, including the study of oocytes obtained by different methods. For the successful development of animal husbandry with the introduction of modern biotechnological methods of reproduction, it is important, along with the assessment of bulls by the quality of the offspring and the qualitative characteristics of sperm, to study the qualitative characteristics of oocytes obtained by different methods (Andrabi and Maxwell 2007; Borunova et al. 2017; Tkachev and Tkacheva 2017; Do and Taylor-Robinson 2020).

It has been proven that the cytophysical state of the gametal cells of the donor cow directly affects the state and development of embryos. Therefore, it becomes important to assess the biological characteristics of oocytes obtained both during aspiration from the follicles of donor cows and during removal from the ovaries of slaughter animals. With the development of modern reproductive biotechnology, it has become possible to evaluate oocytes that have been fertilized and cultured at different stages of development. According to the available data, there are significant morphological variations among oocytes, which can affect the ability to develop and the potential ability of the embryo to implant (Sirard and Blondin 1996; Andrabi and Maxwell 2007; Sprícigo et al. 2015; Iwasaki et al. 2018; Aguila et al. 2020).

Successful fertilization of oocytes and *in vitro* production of embryos depends on the quality and maturity degree of the oocytes upon receipt. The cumulus cells (CC) surrounding the oocyte play a key role in the maturation of oocytes and are known to supply nutrients, energy substrates, and / or messenger molecules for oocyte development (Vanderhyden et al. 1990; Prokofiev et al. 1992; Otoi et al. 1997). The CC concentration is highly dependent on the efficiency of oocyte collection. Several methods are used to collect oocytes from slaughterhouse ovaries of farm animals. A number of studies have been carried out to compare the effectiveness of methods for collecting oocytes from cattle

(Kątska 1984; Rizos et al. 2002; Bilodeau-Goeseels 2012; Do and Taylor-Robinson 2020).

MATERIAL AND METHODS

Under the research performed, the study used three methods for obtaining oocytes: puncture, section and aspiration for cultivation and *in vitro* fertilization of cow oocytes. The cow ovaries were collected from a local slaughter station and stored in a collection tube containing 0.9% saline in a thermos at 25-30°C. Each ovary was treated individually and the OCC was collected in one of the following three ways.

Puncture: the ovaries were placed in a Petri dish containing 5 ml of OCC's collection medium, held with forceps, and the entire surface of the ovary was punctured with an 18G hypodermic needle. Section: the ovaries were placed in a Petri dish containing 5 ml of OCC's collection medium, held with tweezers. The incisions were made over the entire surface of the ovary using a scalpel blade. Aspiration: visible follicles were aspirated using an 18G hypodermic needle attached to a sterile 5 ml disposable syringe containing 2 ml of collection medium. The medium, together with the collected OCCs, was then transferred to a 35 mm Petri dish. When collecting the OCC by puncture and cutting, the ovary was completely immersed in the medium. In all three methods, the Petri dishes were left at rest for 5 minutes, which allowed the OCC to settle. Excess medium was removed with a syringe without disturbing the oocytes at the bottom of the Petri dish.

Petri dishes were then examined under an inverted microscope and the total number of collected OCCs was counted. OCCs were divided into 4 classes based on cumulus and nucleus cells: grade A - oocytes completely surrounded by cumulus cells; Grade B - oocytes partially surrounded by cumulus cells; Grade C - oocytes not surrounded by cumulus cells; and grade D - degeneration observed in both oocyte and cumulus cells. Grades A and B were considered as normal, while grades C and D were considered to be damaged (Khandoker et al. 2001; Khandoker et al. 2005; Khandoker et al. 2012). Follicular fluid was collected from all categories of morphologically healthy superficial follicles by aspiration using a 10 ml syringe with a 19G needle. The following criteria were applied to assess the follicle quality: good - uniform bright appearance, extensive and very fine vascularization and no free-floating particles in the follicular fluid, and atresic - loss of translucency, slightly or dull grayish and/or opaque appearance and free-floating particles in the follicular fluid. At each collection, the liquid from each follicle was combined, centrifuged 2 times at 3000 rpm for 30 minutes. The supernatant was collected and filtered through a 45 mm millipore filter and heated at 65°C for 1 hour.

RESULTS AND DISCUSSION

The results of obtaining OCC by three different methods are presented in Table 1. A total number of 156 oocyte-cumulus complexes were collected out of 40 ovaries by puncture – 50 pcs, section – 47pcs, and aspiration – 59pcs (Table 1). The results showed that puncture and section gave significantly higher total OCCs per an ovary (4.16 and 4.0, respectively) than aspiration (3.68); but a higher number of normal (grade A and B) OCCs per an ovary was observed with aspiration (2.5) than with puncture (1.82) and sections (2.00) (Table 1). During aspiration, OCC was collected from the surface of follicles with a diameter of 3 to 8 mm using a needle. During puncture, all surfaces were pierced with a hypodermic needle; during section, incisions were made along the entire ovarian surface with a scalpel, that is, all sizes of superficial follicles were collected.

Table 1. Obtaining oocyte-cumulus complexes (OCC) by different methods

Method		Puncture	Section	Aspiration
Number of ovaries		12	12	16
Total number of OCCs	n	50	47	59
	n per an ovary	4.16	4.00	3.68
Grade A	n	17	16	32
	n per an ovary	1.41	1.33	2.0
Grade B	n	5	8	8
	n per an ovary	0.41	0.67	0.5
Grade C	n	8	6	5
	n per an ovary	0.67	0.50	0.31
Grade D	n	20	18	14
	n per an ovary	1.67	1.50	0.87

Thus, the smaller number of OCCs collected by the aspiration method in this experiment may be due to the fact that aspiration did not receive OCCs from small follicles and some follicles that are deeply embedded in the ovarian cortex. Puncture and section techniques generate more residuals, which can interfere with microscopic retrieval of oocytes and also require more washing than aspiration. As a result, the amount of OCC was removed from the cumulus cells due to repeated washing and, ultimately,

resulted in fewer normal OCCs compared to aspiration at the last observation (Chalova et al. 2020).

The result of the study is that aspiration of vesicular follicles with a diameter of 3 to 8 mm using an 18G hypodermic needle is the best method, a simple and effective way to extract morphologically normal oocyte-cumulus complexes from bovine ovaries. To obtain embryos, the method of oocyte aspiration from bovine ovaries with their subsequent cultivation, fertilization and cryopreservation was used. The ovaries were collected from local slaughterhouses and stored in a collection tube containing 0.9% saline in a thermos at 25-30°C. Each ovary was treated with three washes in Dulbecco's phosphate buffered saline (DPBS) and two washes in OCC collection medium (DPBS + 4 mg/ml of BSA + 50 IU/ml of penicillin). A total number of 134 ovaries from 67 cows were processed. The result of obtaining oocyte-cumulus complexes (OCC) is presented in Table. 2.

Table 2. The result of obtaining oocyte-cumulus complexes (OCC)

Method		Aspiration
Number of ovaries		134
Total number of OCCs	n	485
	n per an ovary	3.62
Grade A	n	268
	%	55.25
	n per an ovary	2.0
Grade B	n	76
	%	15.67
	n per an ovary	0.57
Grade C	n	51
	%	10.52
	n per an ovary	0.38
Grade D	n	90
	%	18.56
	n per an ovary	0.67

Table 3. Evaluation of oocyte maturation by expansion of cumulus cells

Indicator		Expansion degree of cumulus cells			
		Level 3	Level 2	Level 1	Total
Number of OCC	n	128	32	5	172
	%	74.42	18.61	6.97	100

As a result of the oocyte cultivation the stage of fertilization, 251 mature oocytes were obtained. Fertilization of prepared oocytes. *In vitro* fertilization means that the oocytes' fertilization with sperm has occurred under artificially maintained optimal conditions outside the uterus of the animal. For successful *in vitro* fertilization, bovine spermatozoa need to undergo a stage of capacitation in order to carry out an acrosome reaction to penetrate the oocyte

At present, there have not been developed the technologies for *in vitro* cultivation of bovine oocytes, which allow obtaining a sufficiently high yield of embryos. Oocyte maturation *in vitro* is subdivided into nuclear and cytoplasmic processes. Nuclear maturation includes resumption of meiosis and progression to the metaphase-II stage. Cytoplasmic maturation encompasses many cellular processes that must be completed in order for oocytes to be fertilized and develop into normal embryos and offspring. The rates of maturation and fertilization depend on the quality of oocytes, the sufficiency and efficiency of the environment, and the optimization of incubation conditions.

OCC of normal quality was cultured in TCM-199 medium (Sigma Chemicals, USA) supplemented with 2.5% BSA and 10% follicular fluid for 27 hours. After maturation, the degree of expansion of cumulus cells was determined under a microscope at 10-fold magnification as level-1: small expansion of the OCC; level-2: moderate expansion of the OCC, and level-3: significant expansion of the OCC. After that, half of the mature OCCs were taken from each drop, and oocytes were taken from the cumulus cells. Then the oocytes were placed on a glass slide covered with a cover slip (fixed with acetoethanol (acetic acid: ethanol, 1:3), stained with 1% acetoorcein) and examined under an inverted microscope at high magnification (100x) with immersion oil. The level of nuclear maturation was assessed. The assessment of oocyte maturation by the expansion of cumulus cells is presented in Table 3. As can be seen from the table, after 27 hours of cultivation, 74.42% of the oocytes matured to the stage of fertilization (Chalova et al. 2020).

The results of assessing the indicators of the oocyte nuclear maturation are presented in Table 4. After 27 hours of cultivation, 91.85% of oocytes-initiated meiosis, and 71.51% of oocytes reached the metaphase II stage. Thus, this method of OCC cultivation made it possible to achieve maturation of more than 70% of oocytes.

Table 4. Assessment of oocyte nuclear maturation

Indicator		Nuclear maturation of oocytes			Total
		Diplotene	Metaphase I	Metaphase II	
Number of OCC	n	14	35	123	172
	%	8.15	20.34	71.51	100

membrane and fusion with the oocyte plasma membrane. Normal fertilization results in the formation of zygotes with male and female pronuclei (PN). In order to fertilize the remaining matured OCCs, a fertilization medium was prepared and its pH was adjusted to 7.8 on the day of use. Sampels with frozen bullseed were thawed in a water bath at 37°C for 30-40 seconds. The concentration was approximately 250 µl thawed semen per 1 ml capacitation medium. Capacitation medium consisted of modified Tyrode's medium, without calcium ions. After incubation for

one hour, the upper layer of the medium with a volume of 0.5-0.8 ml, containing most of the motile spermatozoa, was removed from the tube and washed twice by centrifugation at 500 g for 7-10 minutes (Chalova et al. 2020).

After 15 min incubation with heparin (200 µg/ml), the suspension was diluted to a concentration of 50 million spermatozoa per ml. Then, droplets were prepared for insemination, covered with mineral oil and kept in a CO_2 incubator for 4-5 hours for pre-incubation. Then the remaining half of the matured OCC (the other half was used to assess the maturation of nuclei) was transferred into each of the sperm drops and incubated for 5 hours in an incubator at 38.5°C with 5% CO_2 in humidified air. After 5 h incubation, several OCCs from each drop were separated from cumulus cells by repeated pipetting and fixed on a glass slide with acetoethanol (acetic acid: ethanol, 1: 3 v/v) and stained with 1% acetoorcein (Gorelik et al. 2021).

After drying, the slides were examined at high magnification (100x) using immersion oil to observe the formation of pronuclei: the presence of a male (PN) and female pronucleus (PN) - normal fertilization; an oocyte with one PN, asynchronous PN development / parthenogenetic activation, or one PN was hidden by lipid droplets and an oocyte with more than two PNs - polyspermia. For fertilization, 251 oocytes were selected from experiments on methods of oocyte elution. The results of *in vitro* fertilization are presented in Table 5. As a result, 56.57% of mature oocytes were fertilized. 142 zygotes were sent for cryopreservation for further study (Gorelik et al. 2021).

Table 5. Results of *In vitro* fertilization

Indicator	Normal fertilization	1 pronucleus, lack of fertilization or parthenogenesis	Polyspermia (more than 3 pronuclei)	Total
n	142	85	24	251
%	56,57	33,86	9,57	100

Thus, the use of modern reproductive biotechnological methods in animal husbandry makes it possible to increase the offspring number produced by highly productive cows and outstanding bulls-producers. Our research on the reproductive biotechnology application to replicate highly productive animals and increase the number of cattle showed good results. It is important to note that oocytes can be obtained both by cows' aspiration using OPU technology and at slaughter stations after slaughtering highly productive cows culled for various reasons. In this regard, it becomes necessary to study the development of oocytes and embryos at all stages of growth and development (Chalova et al. 2020).

CONCLUSION

The findings of the present study highlight an urgent issue for future researchers is the development and creation of nutrient media and optimal cultivation conditions, close to natural, both for oocytes and for embryos, which will increase the percentage of oocyte and high quality embryo yield. In addition, further development, improvement and implementation of *in vitro* embryo transplantation technology will allow maintaining and increasing the number of animals used not only in the industry of the agro-industrial complex, but it will also be useful when working with rare and endangered animal species.

Data Availability Statement: The database generated and /or analysed during the current study are not publicly available due to privacy, but are available from the corresponding author on reasonable request.

Conflict of Interest: Authors declare no conflicts of interests to disclose.

REFERENCES

Aguila, L, Treulen, F, Therrien, J, et al. (2020). Oocyte selection for *in vitro* embryo production in bovine species: noninvasive approaches for new challenges of oocyte competence. Animals Vol 10 Article 2196. https://doi.org/10.3390/ani10122196

Alm-Kristiansen, AH, Gaustad, ER, Bai, G, et al. (2018). *In vitro* studies of Norwegian Red bovine semen immobilized and cryopreserved in alginate solid gel network. Reproduction of domestic animals Vol 53 No 2 Pages 365-370. https://doi.org/10.1111/rda.13115

Andrabi, SMH and Maxwell, WMC (2007). A review on reproductive biotechnologies for conservation of endangered mammalian species. Animal Reproduction Science Vol 99 No 3-4 Pages 223–243. https://doi.org/10.1016/j.anireprosci.2006.07.002

Asgari, V, Hosseini, SM, Forouzanfar, M, et al. (2012). Vitrification of *in vitro* produced bovine embryos: effect of embryonic block and developmental kinetics. Cryobiology Vol 65 No 3 Pages 278-283. http://dx.doi.org/10.1016/j.cryobiol.2012.08.002

Bilodeau-Goeseels, S (2012). Bovine oocyte meiotic inhibition before *in vitro* maturation and its value to *in vitro* embryo production: does it improve developmental competence? Reproduction in Domestic Animals Vol 47 No 4 Pages 687-693. http://dx.doi.org/10.1111/j.1439-0531.2011.01924.x

Borunova, SM, Iolchiev, BS, Abramov, PN, et al. (2017). An effective method for determining the integrity of the sperm acrosome in bull sperm. Veterinary Medicine, Animal Science and Biotechnology No 4 Pages 29-34.

Chalova, N, Zubova, T, Pleshkov, V, et al. (2020). Genotyping of the black-and-white breed embryos by markers of dairy productivity and for the presence of genetic defects. In Proceedings of XIX International Scientific and Practical Conference Current Trends of Agricultural Industry in Global Economy Kemerovo Russia December 8-9 2020 Pages 71-79. https://doi.org/10.32743/agri.gl.econ.2020.71-79

Díez, C, Muñoz, M, Caamaño, JN, et al. (2012). Cryopreservation of the bovine oocyte: current status and perspectives. Reproduction in Domestic Animals Vol 47 No S3 Pages 76-83. http://dx.doi.org/10.1111/j.1439-0531.2012.02029.x

Do, VH and Taylor-Robinson, AW (2020). Cryopreservation of *in vitro*-produced bovine embryos by vitrification: in pursuit of a simplified, standardized procedure that improves pregnancy rates to promote cattle industry use. Biotechnology in Animal Husbandry Vol 36 No 3 Pages 251-270. https://doi.org/10.2298/BAH2003251H

Do, VH, Walton, S, Catt, S, et al. (2016). Requirements for cryopreservation of *in vitro*-produced bovine embryos by a standard method of vitrification. Journal of Veterinary Science & Animal Husbandry Vol 4 No 1 Page 102. http://dx.doi.org/10.15744/2348-9790.4.102

Gorelik, OV, Brjanzev, AY, Safronov, SL, et al. (2021). Comparative assessment of cows by daughters from different breeding bulls in terms of milk production. IOP Conference Series: Earth and Environmental Science Vol 677 Article 022101. http://dx.doi.org/10.1088/1755-1315/677/2/022101

Gritsenko, SA (2016). Influence of reproductive ability on productivity indicators of black-and-white cows in the southern Urals zone. Scientific-methodical electronic journal Concept No 15 Pages 131-135.

Iqbal, S, Riaz, A, Andrabi, SM, et al. (2016). l-Cysteine improves antioxidant enzyme activity, post-thaw quality and fertility of Nili-Ravi buffalo (Bubalus bubalis) bull spermatozoa. Andrologia Vol 48 No 9 Pages 855-861. https://doi.org/10.1111/and.12520

Iwasaki, W, Yamanaka, K, Sugiyama, D, et al. (2018). Simple separation of good quality bovine oocytes using a microfluidic device. Scientific Reports Vol 8 Article 14273. https://doi.org/10.1038/s41598-018-32687-6

Kątska, L (1984). Comparison of two methods for recovery of ovarian oocytes from slaughter cattle. Animal Reproduction Science Vol 7 No 5 Pages 461-463. https://doi.org/10.1016/0378-4320(84)90049-6

Khandoker, M, Ali, MR, Islam, MR, et al. (2005). *In vitro* culture of mouse embryos. In Bangladesh Agricultural University Research Progress Mymensingh Bangladesh Pages 121-128.

Khandoker, M, Imai, K, Takahashi, T, et al. (2001). The role of gelatinase on follicular atresia in the bovine ovary. Biokogy of Reproduction Vol 65 No 3 Pages 726-732. https://doi.org/10.1095/biolreprod65.3.726

Khandoker, M, Jahan, N, Asad, L, et al. (2012). Qualitative and quantitative analysis of buffalo ovaries, follicles and oocytes in view of *in vitro* production of embryos. Bangladesh Journal of Animal Science Vol 40 No 1-2 Pages 23-27. http://dx.doi.org/10.3329/bjas.v40i1-2.10786

Konc, J, Kanyó, K, Kriston, R, et al. (2014). Cryopreservation of embryos and oocytes in human assisted reproduction. Biomed Research International Vol 2014 Article ID 307268. http://dx.doi.org/10.1155/2014/307268

Kumar, D, Kumar, P, Singh, P, et al. (2016). Assessment of sperm damage at different stages of cryopreservation in water buffaloes using fluorescent probes. Cytotechnology Vol 68 Pages 451-458. http://dx.doi.org/10.1007/s10616-014-9798-9

Luno, V, Gil, L, Olaciregui, M, et al. (2014). Rosmarinic acid improves the function and *in vitro* fertilization ability of boar sperm after cryopreservation. Cryobiology Vol 69 Pages 157-162. http://dx.doi.org/10.1016/j.cryobiol.2014.07.002

Morrell, JM and Wallgren, M (2014). Alternatives to antibiotics in semen extenders: a review. Pathogenic Vol 3 No 4 Pages 934-946.

Mostek, A, Dietrich, MA, Słowińska, M, et al. (2017). Cryopreservation of bull semen is associated with carbonylation of sperm proteins. Theriogenology Vol 92 Pages 95-102. http://dx.doi.org/10.1016/j.theriogenology.2017.01.011

Murphy, EM, Eivers, B, O'Meara, CM, et al. (2018). Effect of increasing equilibration time of diluted bull semen up to 72 h prior to freezing on sperm quality parameters and calving rate following artificial insemination. Theriogenology Vol 108 Pages 217-222. http://dx.doi.org/10.1016/j.theriogenology.2017.11.034

Naresh, S (2016). Effect of cooling (4°C) and cryopreservation on cytoskeleton actin and protein tyrosine phosphorylation in buffalo spermatozoa. Cryobiology Vol 72 No 1 Pages 7–13. http://dx.doi.org/10.1016/j.cryobiol.2015.12.004

Niemann, H (1991). Cryopreservation of ova and embryos from livestock: current status and research needs. Theriogenology Vol 35 No 1 Pages 109-124. https://doi.org/10.1016/0093-691X%2891%2990151-3

Nongbua, T, Al-Essawe, EM, Edman, A, et al. (2018). Adding bovine seminal plasma prior to freezing improves post-thaw bull sperm kinematics but decreases mitochondrial activity. Systems biology in reproductive

medicine Vol 64 No 3 Pages 183-190. http://dx.doi.org/10.1080/19396368.2018.1455245

Otoi, T, Yamamoto, K, Koyama, N, et al. (1997). Bovine oocyte diameter in relation to developmental competence. Theriogenology Vol 48 No 5 Pages 769-774. https://doi.org/10.1016/s0093-691x(97)00300-2

Prokofiev, MI, Ernst, LK, Suraeva, NM, et al. (1992). Bovine oocyte maturation, fertilization and further development *in vitro* and after transfer into recipients. Theriogenology Vol 38, No 3 Pages 461-469. https://doi.org/10.1016/0093-691x(92)90065-y

Rizos, D, Ward, F, Duffy, P, et al. (2002). Consequences of bovine oocyte maturation, fertilization or early embryo development *in vitro* versus in vivo: implications for blastocyst yield and blastocyst quality. Molecular Reproduction Development Vol 61 No 2 Pages 234-248. http://dx.doi.org/10.1002/mrd.1153

Sirard, MA and Blondin, P (1996). Oocyte maturation and IVF in cattle. Animal Reproduction Science Vol 42 No 1–4 Pages 417-426. https://doi.org/10.1016/0378-4320(96)01518-7

Sprícigo, JFW, Diógenes, MN, Leme, LO, et al. (2015). Effects of different maturation systems on bovine oocyte quality, plasma membrane phospholipid composition and resistance to vitrification and warming. PLoS ONE Vol 10 No 6, Article e0130164. http://dx.doi.org/10.1371/journal.pone.0130164

Tkachev, AV and Tkacheva, OL (2017). Comparison of the cytotoxic effect produced by zearalenone and t-2 toxin on the germ cells of horses and bulls *in vitro* before and after cryopreservation. Cytology Vol 59 No 1 Pages 45-52.

Tkachev, AV, Tkacheva, OL, Gazzavi-Rogozina, LV, et al. (2020a). Modern technology of poultry semen cryopreservation. In IV International Scientific and Practical Conference Modern S&T Equipments and Problems in Agriculture Kemerovo Russia June 25 2020 Pages 235-244. http://dx.doi.org/10.32743/kuz.mepa.2020

Tkachev, AV, Tkacheva, OL, Zubova, TV, et al. (2020b). Effect of mycotoxins on the spermatozoa and embryos of animals. Advances in Animal and Veterinary Sciences Vol 8 No s3 Pages 47-55. -http://dx.doi.org/10.17582/journal.aavs/2020/8.s3.47.55

Vanderhyden, BC, Caron, PJ, Buccione, R, et al. (1990). Developmental pattern of the secretion of cumulus expansion-enabling factor by mouse oocytes and the role of oocytes in promoting granulosa cell differentiation. Developmental Biology Vol 140 No 2 Pages 307-317. https://doi.org/10.1016/0012-1606(90)90081-s

Youngs, CR (2011). Cryopreservation of preimplantation embryos of cattle, sheep, and goats. Journal of visualized experiments Vol 54 e2764. http://dx.doi.org/10.3791/2764