Comparative Analysis of Motility Characteristics and Kinematic Parameters of Fresh, Chilled and Sexed Ram Semen – Preliminary study

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Abstract. *The application of the bovine serum albumin (BSA) column method for sexing of spermatozoa is cost effective and appropriate for small ruminants. The current study compares motility characteristics and kinematic parameters of fresh, chilled and sexed ram semen with the aim to select the high-quality ejaculates for semen sexing. Fresh, chilled and sexed semen from 4 East Friesian rams was analysed by CASA, and immotile sperm cells, motile sperm cells, progressive motility, non-progressive motility, VCL, VAP, VSL, STR, LIN and WOB were determined. Semen sexing was carried out in bovine serum albumin columns and incubation for 45 min at a temperature of 25°C. The average values of all semen parameters and the change of the most important indices in individual rams were estimated. Significant differences ($P < 0.05$) were detected between immotile and motile sperm cells, progressive and non-progressive motility, VCL and WOB of fresh and chilled semen. All investigated parameters between fresh and isolated upper and bottom layer spermatozoa, excluding STR and WOB, differed significantly ($P < 0.05$). The same dependency ($P < 0.05$) was detected for motile sperm, progressive motility, VCL, STR and LIN between chilled and sexed spermatozoa. In conclusion, the average values for motile sperm, progressive motility, VCL, STR and LIN of chilled and sexed ram spermatozoa are significantly ($P < 0.05$) affected by chilling and sexing in BSA columns at 25°C for 45 min. CASA analysis of motility and kinematic parameters of chilled semen can provide a correct choice of ejaculates with high quality for sexing. The individual features of rams have influence on the semen characteristics and should be take into consideration in selection of the donors for a production of the sexed semen.*

Introduction

During different procedures as dilution, chilling or sexing alters the quality of the sperm cells, which can affect their fertilizing ability (Urry et al., 1983; Rodríguez-Martínez and Pena Vega, 2013; Acharya et al., 2020; Steele et al., 2020). The accurate determination of the different changes has a crucial role for a selection of the ejaculates for future handling. Motility characteristics and kinematic parameters determined by computer-assisted semen analysis (CASA) are used by many authors with an increasing trend worldwide in the last years, for exclusion of subjectivity in evaluation of the semen quality and for prediction of fertility of different type of semen (Robayo et al., 2007; Buchelly Imbachi et al., 2018). Additionally, CASA systems provide clear digital images of each spermatozoa track that allows for individual motion analysis and accurate assessment of important kinetic parameters (Verstegen et al., 2002, Wilson-Leedy and Ingermann, 2011; Amann and Waberski, 2014).

The introduction of sexed sperm is a new tool for improvement of the reproductive efficiency of small ruminants, allowing effective use of high producing animals and an optimal production of males and females in production systems (Hollinshead et al., 2002; Hamano, 2007; Ferreira-Silva et al., 2017; Gonzalez-Marín et al., 2021). The ram semen can be sexed by different sorting systems such as using an albumin gradient (Maxwell et al., 1984), flow-cytometry (Johnson, 2000) or a centrifugal counter with an aqueous two-phase system (Ollero et al., 2000). A few studies (Hadi and Al-Timimi, 2013; Solihati et al., 2019) report on the application of the bovine serum albumin (BSA) column for sexing of fresh ram semen. They describe this method as easier, cheaper and practically applicable, compared with others, but the obtained results are still debatable. Moreover, many experiments are conducted at different temperature and time for semen incubation with microscopic record of motility characteristics only, without determination of kinematic parameters. According to Solihati et al. (2019), the viability of X- and Y-sperm incubated from 45 min was better than obtained after incubation for 60 and 75 min, and the X-sperm has the highest viability. Agasi et al. (2020) separated bull sperm cells by the BSA column in a laminar cabinet at a room temperature of 27°C. They reported that motility, viability, and plasma membrane integrity of this semen could be maintained on the

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quality level after freezing and thawing. Sometimes after collection semen has to be preserved for an extended period before transporting it to the lab and submission of semen sexing procedure. It is associated with semen preservation at a low temperature (Maicas et al., 2020). There is a single report (Hollinshead et al., 2004) for sorting frozen semen in ruminants, but the information about CASA analysis of motility and kinematics of sexed spermatozoa of chilled ram semen was not available.

The aim of this study was to compare the motility characteristics and the kinematic parameters of fresh, chilled and sexed ram semen by CASA analysis in relation to the selection of high-quality ejaculates for semen sexing.

Material and methods

The study was carried out in 4 East Fresian rams at the age of 3.6 ± 0.4 years, body weight of 75 ± 8.5 kg, reared in a group box at a small ruminant farm, located at N 42.25 and E 25.38. The animals were housed in the uniform technology, and feeding included alfa-alfa and meadow straw, concentrate, vitamin and mineral premix and drinking of water *at libitum*. Investigation was performed during the breeding season after 20 days of sexual abstinence of all rams. The experiment was conducted according to the recommendations of the Local Animal Ethics Committee and regulations for human attitude and animal protection.

Semen collection, primary assessment, dilution and chilling

The semen was collected by the artificial vagina method in presence of a teaser sheep, between 8.00–9.00 am, transported to the laboratory and placed on a water bath at 37°C, and submitted to a primary assessment. The volume was measured on the graduated semen collection tube, the motility and sperm concentration ($\times 10^9$/mL) were evaluated by the CASA system. The percentage of abnormal sperms for fresh semen was recorded in stained slides by microscopic examination using of Motic Image Plus digital software system (Motic China Group Ltd, 2001–2004).

Only semen with normal color and transparency, volume > 1.5 mL, sperm concentration > 1.5×10^9/mL, motile sperm > 70% and abnormal sperms < 15% was used. After the primary assessment and on the basis of CASA results, each ejaculate was diluted with Tris-glucose-glycerol-based extender to a concentration of 400×10^6 sperm cells per mL and stored in a refrigerator at 5°C for 24 h.

Semen sexing method

Semen sexing was carried out by albumin gradient separation of the BSA column. Initially, BSA fraction V was placed in the Brackett and Oliphant (BO) medium in concentrations of 5% or 10% and stored in a refrigerator for dissolving. BSA columns were prepared in graduated glass tubes as the bottom layer contained 1 mL of 10% BSA and the upper layer contained 1 mL of 5% BSA. Chilled ram semen (1 mL) was added to each BSA column; it was preliminarily diluted with a BO medium until adjustment to a concentration of 200×10^6 cells per mL. The BSA columns were incubated at a temperature of 25°C for 45 min. After that, each layer was carefully separated in an individual tube and centrifuged at 1800 rpm for 10 min. The supernatant was discarded, and the semen was diluted with a semen extender and placed in a water bath at a temperature of 37°C until to a CASA analysis. The layers of the BSA column with a concentration of 5% (upper) and with a concentration of 10% (bottom) were accepted to contain spermatozoa bearing X and Y chromosome, respectively (Solihati et al., 2019).

Motility and kinematic parameters evaluation

The semen was evaluated immediately after collection (fresh), at 24 h after storage at 5°C and before dilution with the BO medium (chilled) and after processing by the BSA column method (sexed). Immediately before examination, the semen samples were gently mixed and a 5 μL drop was placed on a slide warmed at 37°C, and covered with a 20 mm × 20 mm cover slip. Computer-assisted semen analysis was carried out by qualified operator using the Sperm Class Analyzer software (SCA® 2002, Microptic, Barcelona, Spain). The measured motility characteristics and kinematic parameters included immotile sperm cells (%), motile sperm cells (%), progressive motility (%), non-progressive motility (%), curve linear velocity (VCL; μm/s), average path velocity (VAP; μm/s), straight-line velocity (VSL; μm/s), linearity (LIN; %), straightness (STR; %) and oscillation index (WOB; %). The software settings were adjusted to ram semen assessment according to manufacturer recommendations. A percentage of reduction for the different indices was calculated as the values before chilling and sexing were accepted as 100% and this information is presented in Figure 1.

Statistical analysis

The data were processed by statistical program Statistica version 7.0 (Stat-Soft., 1984–2000 Inc., Tulsa, OK, USA). The motility characteristics and kinematic parameters for each type of semen were given as mean ± standard deviation. Initially, the values were tested for normal distribution of variances by Kolmogorov-Smirnov and Lilliefors tests, then they were transformed logarithmically. The mean values of each index between fresh, chilled semen and spermatozoa originated from the upper and bottom layers were compared by non-parametric Mann-Whitney test. Statistical significance was considered at $P < 0.05$.

Results

During the primary assessment, considerable differences between the quality of ejaculates collected from different rams were not determined. The semen

was without impurity and with normal colour. The values for volume of ejaculate, sperm concentration, total motility, abnormal spermatozoa and pH varied between 1.5–2.4 mL, 2.5–3.2 x 10^9 per mL, 89.02%–98.5% and 8.4%–12.2%, 6.5–6.9, respectively. All parameters of fresh semen were in a normal range for small ruminants.

The average values for immotile and motile sperm cells, progressive and non-progressive motility, curve linear velocity and oscillation index (WOB) between fresh and chilled semen differed significantly ($P < 0.05$) while the rest parameters were identical (Table 1).

In rams 2 and 4, the progressive motility dropped with 46.4% and 61.4%, respectively, compared with the initial values (Fig. 1A). The same situation was recorded for curve linear velocity, but this process was strictly individual. The highest decrease (64.3%)

Table. 1 Motility characteristics and kinematics of fresh, chilled and sexed ram semen (Mean ± SD).

Parameter	Type of semen			
	Fresh (n = 4)	Chilled (n = 4)	Sexed (n = 4)	
			Upper layer (X)	Bottom layer (Y)
Immotile sperm (%)	6.23 ± 6.31^{a}	18.6 ± 6.97^{b}	32.4 ± 7.88^{bc}	44.27 ± 23.01^{bcd}
Motile sperm (%)	93.77 ± 6.31^{a}	85.15 ± 3.60^{b}	67.6 ± 7.88^{c}	63.23 ± 9.24^{cd}
Progressive motility (%)	70.27 ± 22.75^{a}	40.05 ± 5.25^{b}	15.05 ± 8.46^{c}	12.51 ± 7.70^{cd}
Non-progressive motility (%)	23.5 ± 16.48^{a}	45.1 ± 6.33^{b}	52.55 ± 0.49^{c}	50.72 ± 1.86^{bc}
VCL (µm/s)	149.44 ± 44.76^{a}	89.86 ± 22.46^{b}	51.53 ± 10.44^{c}	48.67 ± 10.22^{cd}
VAP (µm/s)	75.29 ± 22.25^{a}	47.94 ± 8.41^{ab}	35.34 ± 7.97^{bc}	32.64 ± 8.32^{bcd}
VSL (µm/s)	40.64 ± 12.00^{a}	27.39 ± 4.64^{ab}	25.29 ± 6.11^{abc}	23.16 ± 6.56^{abcd}
STR (%)	39.73 ± 16.06^{a}	55.7 ± 1.45^{ab}	63.34 ± 0.68^{c}	62.15 ± 2.30^{cd}
LIN (%)	38.68 ± 11.05^{a}	35.77 ± 2.28^{b}	47.01 ± 0.08^{ac}	45.01 ± 2.60^{cd}
WOB (%)	50.24 ± 1.18^{a}	59.21 ± 2.35^{b}	67.31 ± 0.55^{c}	65.18 ± 2.35^{abc}

Values with different superscript within a row differ each other at $P < 0.05$.

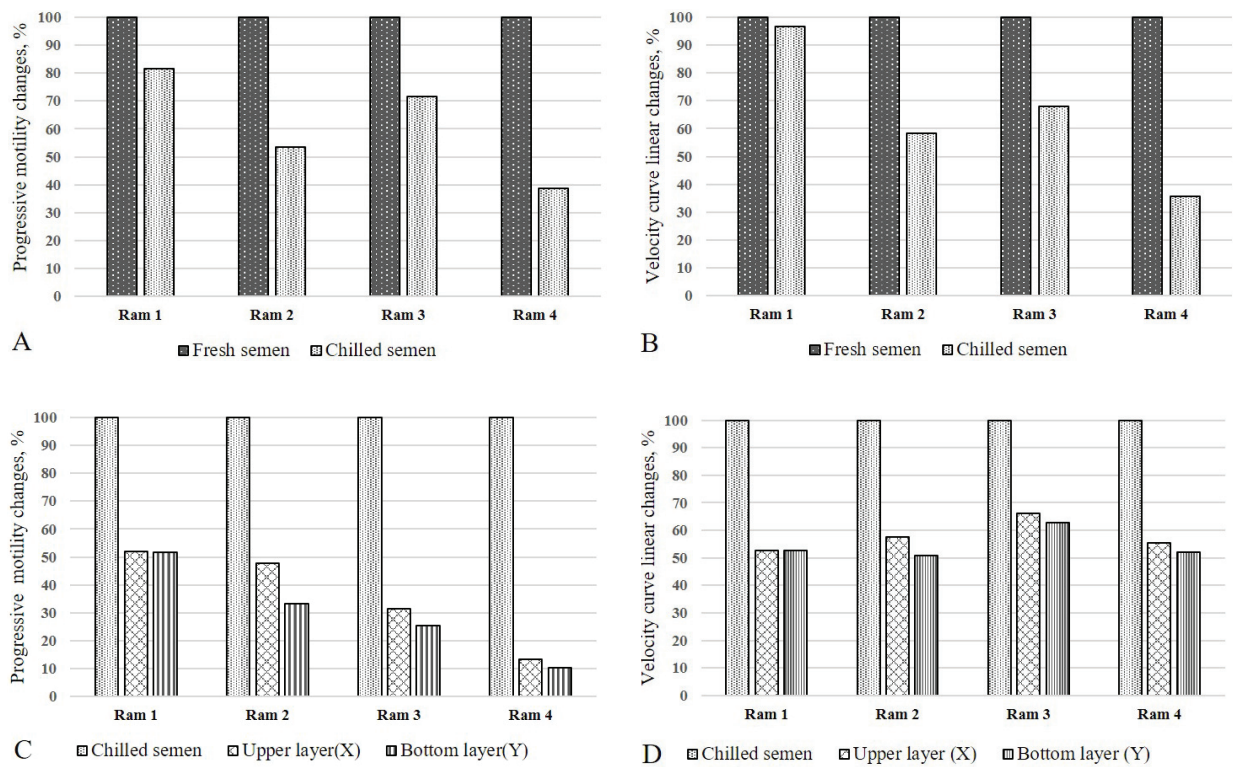

Figure 1. Changes of some motility characteristics and kinematic parameters of individual rams.
A – progressive motility of fresh semen vs. chilled semen; B – velocity curve linear of fresh semen vs. chilled semen; C – progressive motility of chilled semen vs. upper (X) and bottom (Y) layer; D – velocity curve linear of chilled semen vs. upper (X) and bottom (Y) layer.

was demonstrated in the semen of ram 4, followed by this of ram 2 (46.4%), while VCL in rams 1 and 3 was changed insignificantly (Fig. 1B).

After incubation of semen in the BSA column for 45 min, immotile sperm, non-progressive motility, STR, LIN and WOB were increased while motile sperm, progressive motility, VCL, VAP and VSL ($P < 0.05$) were decreased. All investigated parameters between fresh and isolated upper and bottom layer spermatozoa, excluding STR and WOB, differed significantly ($P < 0.05$). The same dependency ($P < 0.05$) was detected for motile sperm, progressive motility, VCL, STR and LIN between chilled and sexed spermatozoa. However, the greatest reduction of progressive motility (over 65%) was observed in samples from rams 3 and 4 for both types of sperm cells X and Y (Fig. 1C), while in ram 2, the same effect was registered only for spermatozoa with Y chromosome. The comparative analysis between the average values of all parameters for spermatozoa isolated from the upper and the bottom layer showed no significant differences (Table 1; $P > 0.05$). Nevertheless, there was a clear tendency ($P < 0.067$) to more motile sperm and a lower decrease of progressive motility for the spermatozoa from the upper layer, mainly in rams 1 and 3. The motility and progressive motility of sexed spermatozoa in rams 1 and 2 were the highest (> 65% and > 22%), whereas in ram 4, they were the lowest (< 60 % and < 10%). The sexing process resulted in an additional drop in VCL of the sorted semen with some differences between the individuals (Fig. 1D). Even in ram 1 showing the best semen quality, VSL was reduced with 47% for both types of sperm cells.

Discussion

The kinematic parameters, especially sperm motility, are the commonly used indicators in measurement of male fertility. The good motility is very important for sperm migration through the female genital tract and for gamete interaction at fertilization (Robayo et al., 2007). The current study indicated that chilling process significantly ($P < 0.05$) affects the average values for immotile and motile sperm cells, progressive and non-progressive motility, curve linear velocity and oscillation index with a slow effect on the other kinematic parameters. A similar decline of CASA recorded motility, progressive motility and VCL in Katahdin rams at 24 h after chilling was determined by Acharya et al. (2020). Regardless of this result, the additional analysis of the data showed high individual sensitivity of the spermatozoa from different samples to chilling process. It was clearly demonstrated by a considerable drop of the progressive motility in rams 2 and 4, compared with the obtained values for fresh semen. The same strict individuality was registered for curve linear velocity, presented with the highest decrease of this parameter in the semen of ram 4, followed by this of ram 2, ram 3 and ram 1. Rickard et al. (2016) reported also different semen resistance to a low temperature. They related the variation in freezing resilience of ram spermatozoa with the source and composition of the seminal plasma. Spermatozoa produced from low-resilience rams frozen with high-resilience seminal plasma exhibited higher motility than those from low-resilience rams frozen with low-resilience seminal plasma.

Different studies have reported using the BSA column method in fresh semen sexing, but information on sexing of chilled ram semen is too limited. The incubation of chilled semen in the BSA column for 45 min affected semen motility characteristics and kinematic parameters of the spermatozoa, irrespective of presence of X or Y chromosome. It was conducted to increase the average values of immotile sperm, non-progressive motility, STR, LIN and WOB and decrease motile sperm, progressive motility, VCL, VAP and VSL, compared with the obtained values immediately before sexing ($P < 0.05$). Solihati et al. (2019) determined that sexing of ram semen by the same method had a significant effect on motility, intact plasma membrane and intact acrosome cup, but did not significantly affect abnormalities. The incubation time of 45 min resulted in the same motility as the incubation time of 60 min, but at the incubation of 45 min, the highest percentage of spermatozoa were with intact plasma membrane and intact acrosome cup.

The chilling and sexing changed the motility and kinematics of the sorted spermatozoa, compared with the values for fresh and chilled semen. Evidence for that was significant differences ($P < 0.05$) in almost all investigated parameters between fresh and isolated upper and bottom layer spermatozoa and differences in motile sperm, progressive motility, VCL, STR and LIN between chilled and sexed sperm cells.

According to Agasi et al. (2020), the motility decrease during the separation process might be the result of reduced nutrition. In regard to sperm morphokinetics, sex sorting resulted in increased numbers of immotile sperm and decreased numbers of progressive and hyperactivated sperm (Steele et al., 2020). The highest reduction of progressive motility for sperm cells from upper (X) and bootom layers (Y), observed in samples of rams 3 and 4, and the same effect for spermatozoa with Y chromosome in ram 2 can be explained by an individual response of both types of gametes in different rams to separating process. Related to this, Burroughs (2011) reveals that in some animals BSA can bind sperm plasma membrane and adsorb cholesterol. It is a reason for damage of the plasma membrane causing loss of motility and fertilization of the sperm.

In spite of insignificant differences between the average values of all parameters for spermatozoa isolated from the upper and the bottom layer, there was a clear tendency ($P < 0.067$) to more motile sperm and a lower decrease of progressive motility for the spermatozoa from the upper layer, especially

in samples for rams 1 and 3. In support of the abovementioned are results obtained by Solihati et al. (2019) who also used the BSA column for ram semen sorting. They registered longer longevity for X sperm, compared with Y sperm after incubation time of 45, 60 and 75 min. The higher percentage of sperm with mitochondrial membrane potential and the percentage of live sperm with a reacted acrosome for X than Y group from 0 h to 4 h of incubation were observed by Carvalho et al. (2018). This preliminary study indicated that the chilled ram semen incubated at a temperature of 25°C for a period of 45 min can produce sexed sperm of good quality, but not from each ram. This was confirmed with the recorded highest motility and progressive motility of sexed spermatozoa in rams 1 and 2 and very low values in ram 4. The reduction in VCL of sorted semen, even in rams with the best semen quality, before sexing can be accepted as additional evidence for individual features of ejaculates in different animals. From the practical point of view, only sexed semen from rams 1 and 2 could be recommended for artificial insemination, because it had enough motile sperm cells. In agreement with this, Hollinshead et al. (2002) reported pregnancy after laparoscopic insemination with low numbers of sex-sorted frozen-thawed motile sperm per dose (2–4 x 10^6).

In the current study, we can speculate that high quality of motility and kinematic parameters of fresh ram semen not always is a guaranty for good results after chilling and sexing. The individual characteristics of different rams can affect significantly semen indices such as immotile sperm cells, motile sperm cells, progressive motility, non-progressive motility, velocity curve linear and oscillation index during the chilling and sorting. Sexing of chilled ram semen by the BSA column at a temperature of 25°C and incubation for 45 min can be used for separation of spermatozoa, bearing X or Y chromosome, but preliminary CASA analysis of the chilled semen is recommended. The determination of the kinematic parameters will be beneficial in selection of ejaculates for future semen sorting. Robayo et al. (2007) showed VCL, VAP, STR and LIN as highly significantly ($P < 0.01$) related with migration of sperm through the cervical mucus and suggested that specific kinematic parameters confer the ability of spermatozoa to colonize and migrate through epithelial mucus with different rheological properties. Future investigations with ejaculates from a large number of animals and use of sorted semen for artificial insemination will clarify additionally the questions about sexing of chilled ram semen.

In conclusion, the average values for motile sperm, progressive motility, VCL, STR and LIN of chilled and sexed ram spermatozoa are significantly ($P < 0.05$) affected by chilling process and incubation in the BSA column at 25°C for 45 min. CASA of motility and kinematic parameters of chilled semen provides a correct choice of ejaculates with high quality for sexing. The individual features of rams have influence on the semen characteristics and should be taken into consideration for a selection of the donors for production of sexed semen. The obtained information can be beneficial for optimization of the sheep reproduction.

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References

1. Acharya, M., Burke, J. M., Rorie, R. W. Effect of Semen Extender and Storage Temperature on Motility of Ram Spermatozoa. Advances in Reproductive Sciences. 2020. T. 8. P. 14–30.
2. Agasi F. A., Yusuf M., Said S., Toleng A. L. The quality of Sumba Ongole sperms after sexing using bovine serum albumin column in Bracket Oliphant extender at different temperatures. 2020. IOP Conference Series: Earth and EnvironmentalScience. T. 492. 012066. doi:10.1088/1755-1315/492/1/012066
3. Amann RP, Waberski D. Computer-assisted sperm analysis (CASA): capabilities and potential developments. Theriogenology. 2014. T. 81. P. 5–17.
4. Buchelly Imbachí F, Zalazar L, Pastore JI, Greco MB, Iniesta-Cuerda M, Garde JJ, Soler AJ, Ballarin V, Cesari A. Objective evaluation of ram and buck sperm motility by using a novel sperm tracker software. Reproduction. 2018. T. 156. P. 11–21.
5. Burroughs, C. A. Removing Seminal Plasma Improves Sex-Sorting of Bovine Sperm.Thesis. 2011. Department of Biomedical Sciences. Colorado State University. Colorado. P. 17.
6. Carvalho J. O., Sartori R., Rodello L., Mourão G. B., Bicudo S. D., Dode M. A. N. Flow cytometry sex sorting affects bull sperm longevity and compromises their capacity to bind to oviductal cells. Livestock Science. 2018. T. 207. P. 30–37.
7. Ferreira-Silva J.C., Moura M.T., Basto S.R.L., Oliveira L.R.S., Caldas E.L.C., Filho M.L.S., Oliveira M.A.L. Use of Percoll Density Centrifugation for Sperm Sexing in Small Ruminants. Global Journal of Science Frontier Research: D Agriculture and Veterinary. 2017. T. 17. P. 55–59.
8. Gonzalez-Marín C., Gongora C.E., Moreno J.F., Vishwanath R. Small ruminant SexedULTRA™ sperm sex-sorting: Status report and recent developments. Theriogenology. 2021. T. 162. P. 67–73.
9. Hadi S., Al-Timimi I.H. Separation of Y-chromosome Bearing Ram's Sperms using an Albumin Gradient Technique and Identification of Embryos by PCR. AL-Qadisiyah Journal of Veterinary Medicine Sciences. 2013. T. 12. P. 144–151.
10. Hamano K. Sex preselection in bovine by separation of X- and Y-chromosome bearing spermatozoa. The Journal of reproduction and development. 2007. T. 53. P. 27–38.
11. Hollinshead F.K., O'Brien J.K.,Maxwell W.M.C., Evans G. Production of lambs of predetermined sex after the inseminatin of ewes with low numbers of frozen-thawed sorted X- or Y-chromosome-bearing spermatozoa. Reproduction, Fertility and Development. 2002. T. 14. P. 503–508.
12. Hollinshead FK, Evans G, Evans KM, Catt SL, Maxwell WM, O'Brien JK. Birth of lambs of a pre-determined sex after in vitro production of embryos using frozen-thawed sex-sorted and re-frozen-thawed ram spermatozoa. Reproduction. 2004. T. 127. P. 557–68.
13. Johnson LA. Sexing mammalian sperm for production of offspring: the state of- the-art. Animal Reproduction Science .2000. T. 61. P. 93–107.

14. Maicas C, Holden SA, Drake E, Cromie AR, Lonergan P, Butler ST. Fertility of frozen sex-sorted sperm at 4 × 106 sperm per dose in lactating dairy cows in seasonal-calving pasture-based herds. Journal of Dairy Science. 2020. T. 103. P. 929–939.
15. Maxwell WMC, Meddoa G, Shite IG. Post thawing survival of motile ram sperm after isolation by layering on protein columns. Theriogenol. 1984. T. 21. P. 601–606.
16. Ollero M, Perez-Pe R, Gargallo I, Morlanes S, Osada J, MuinoBlanco T, Cebrian-Perez J (2000). Separation of ram spermatozoa bearing X and Y chromosome by centrifugal countercurrent distribution in an aqueous two phase system. 2000. Journal of Andrology. T. 21. P. 921–928.
17. Rickard J. P., Schmidt R. E., Maddison J. W., Bathgate R., Lynch G. W., Druart X., de Graaf S. P. Variation in seminal plasma alters the ability of ram spermatozoa to survive cryopreservation. Reproduction, Fertility and Development. 2014. T. 28. P. 516–523.
18. Robayo I, Montenegro V, Valdés C, Cox JF. CASA assessment of kinematic parameters of ram spermatozoa and their relationship to migration efficiency in ruminant cervical mucus. Reproduction Domestic Animmals. 2008. T. 43. P. 393–399. doi: 10.1111/j.1439-0531.2007.00920.x.
19. Rodríguez-Martínez H., Vega F.P. Semen technologies in domestic animal species. Animals Frontiers. 2013. T. 3. P. 26–33.
20. Solihati N, Rasad SD, Yusrina A, Winangun K, Toha. Quality and longevity of local ram's sexed sperm with albumin column. Jurnal Ilmu Ternak. 2019. T. 19. P. 113–121. DOI: 10.24198/jit.v19i2.25632
21. Steele H, Makri D, Maalouf WE, Reese S, Kolle S. Bovine sperm sexing alters sperm morphokinetics and subsequent early embryonic development. Scientific Reports. 2020. T. 10. P. 6255.
22. Verstegen J, Iguer-Ouada M, Onclin K. Computer assisted semen analyzers in andrology research and veterinary practice. Theriogenology. 2002. T. 57. P. 149–179.
23. Wilson-Leedy J., Ingermann R. 2011. Computer assisted sperm analysis using ImageJ; description of necessary components and use of free software. (available at: https://imagej.nih.gov/ij/plugins/docs/CASAinstructions.pdf)
24. Urry RL, Middleton RG, McNamara L, Vikari CA. The effect of single-density bovine serum albumin columns on sperm concentration, motility, and morphology. Fertility and Sterility. 1983. T. 40. 666–669.

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