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Original Article

The effect of maltose on post-mortem epididymal sperm kinetics in water buffalo (*Bubalus bubalis*)

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ABSTRACT

The maltose disaccharide is easily converted into two glucose molecules during cellular glycolysis and is an energy source for sperm motility. To evaluate the effect of maltose on the kinetics of water buffalo epididymal sperm, 10 pairs of buffalo bull testicles, after the usual industrial slaughter, were transported to the laboratory of the Faculty of Veterinary Medicine in a polystyrene box at a temperature of 5o to 8o Celsius. In the laboratory, after fixing the tail of the epididymis with two fingers, several incisions were made in noncapillary zones. After the milky liquid containing concentrated spermatozoa was extracted, this liquid was transferred to an Eppendorf cell culture medium containing 10% bovine serum albumin (BSA). Five levels of maltose sugar (1, 3, 5, 10, 15 mM) were added to the Eppendorf containing 1 ml culture medium and 30-40 million sperm with 10% BSA and incubated at 37o C for 24 hours. Sperm kinetics were assessed at 1, 6, 12 and 24 hours by computer-assisted sperm analysis (CASA). Statistical analysis showed that CASA data such as rapid progressive motility (class A, %), progressive motility (class B, %), viability (class A+B+C, %), straight-line velocity (VSL, µm/s), curvilinear velocity (VCL, µm/s), average path velocity (VAP, µm/s) and lateral head amplitude (ALH, µm) at 24 hrs were higher in the maltose group than in the control group ($p<0.05$). In conclusion, this study suggests that maltose has a desirable effect on buffalo epididymal spermatozoa and maltose may act as an energy substrate for sperm motility.

تاثیر قند مالتوز برالگوی حرکتی اسپرم اپیدیدیمی گاومیش رودخانه ای پس از مرگ

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چکیده

قند دی ساکارید مالتوز طی فرایند قندکافت به دو مولکول گلوکز تبدیل و انرژی لازم برای تحرک اسپرم را فراهم می سازد. برای ارزیابی تاثیر مالتوز بر الگوی حرکتی اسپرم اپیدیدیم گاومیش، پس از کشتار صنعتی مرسوم، ده جفت بیضه گاومیش نر بالغ داخل یونولیت با دمای ۵ الی ۸ درجه سلسیوس به آزمایشگاه منتقل گردید. پس از تثبیت دم اپیدیدیم بین دو انگشت، در مناطق فاقد مویرگ خونی، چندین برش انجام شد. پس از استخراج مایع شیری رنگ غنی از اسپرم، این مایع به اپندورف های حاوی محیط کشت سلول با ده درصد آلبومین سرم گاوی منتقل گردید. پنج سطح قند مالتوز (۱، ۳، ۵، ۱۰ و ۱۵ میلی مول) به یک میلی لیتر محیط کشت با ۳۰ الی ۴۰ میلیون اسپرم و ۱۰ درصد آلبومین سرم گاوی منتقل و در دمای ۳۷ درجه سلسیوس به مدت ۲۴ ساعت انکوبه گردید. الگوی حرکتی اسپرم در زمان های ۱، ۶، ۱۲ و ۲۴ ساعت توسط سیستم آنالیز رایانه ای (کاسا)، ارزیابی شد. آنالیز آماری نشان داد که داده های کاسا مثل درصد حرکت سریع پیش رونده، حرکت پیش رونده، زنده مانی، شاخص های سرعت بر حسب میکرو متر در ثانیه مثل سرعت خط مستقیم، سرعت مسیر منحنی، متوسط سرعت مسیر حرکت ، دامنهٔ حرکت اسپرم (بر حسب میکرومتر) در ساعت ۲۴ و در گروه مالتوز از شاهد بیشتر بود ($p<۰/۰۵$). در بیان نتیجه گیری کلی مالتوز اثر مفید بر تحرک اسپرم اپیدیدیم گاومیش رودخانه ای دارد و ممکن است به عنوان منبع انرژی برای تحرک اسپرم باشد.

واژه های کلیدی: اپیدیدیم، مالتوز، اسپرم، گاومیش

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INTRODUCTION

It has been 346 years since Antonius Leeuwenhoek observed the movement of human sperm in semen with his microscope in 1677. The excitement of watching sperm move has a long history. From the late 19th to the early 20th century, most studies focused on invertebrate sperm, in vitro fertilization, where a mass of eggs outside the body is fertilized with a mass of sperm in seawater or a river. Lillie in 1919 in her classic book, Problems of Fertilization, expressed the requirements for sperm motility as follows: "Sperm cells, after being fully differentiated, may not be able to obtain nutrients outside the testis, especially in cases where insemination takes place outside the body ،Sperm do not have a chance to absorb nutrient substances. Two decades after the publication of Lillie's book, in 1933, Redenz made his first findings that bovine sperm needed nutrients outside the body and in the absence of oxygen, and it was found that sperm could maintain motility in the presence of oxygen even when washed and free of seminal plasma. Of course, washed sperm need glucose or other sugars that can be fermented into lactate [1]. Mammalian sperm need energy for various functions, the most important of which is motility, which is very important for fertilization. Glucose is essential to support hyperactivated motility. To maintain optimal ATP concentration and support optimal sperm motility, we need to glycolyze sugars, even if there is sufficient pyruvate and lactate in the sperm [2]. Sugars have several different functions in an extender or culture medium; during incubation they provide the energy needed by the sperm cells, maintain the osmotic pressure in the culture medium or extender and act as a cryoprotectant [3]. Maltose sugar is easily split into two glucose molecules and is provided to the sperm as an energy source in the glycolysis process [4]. Due to climate change and excessive human interference, animal genetic resources are under threat; in this situation, the only available source of animal sperm after slaughter, extinction and destruction is the tail epididymis, and it can be used in assisted reproductive technology with a high success rate [5]. In the river buffalo (*Bubalus bubalis*), epididymal sperm from the tail is easily collected after the death of the animal and can be used in reproductive programs as a liquid, frozen and thawed for artificial insemination and ultimately fertilization and pregnancy of the buffalo [5]. During the incubation of sperm in the culture medium or diluent, sugars play a role in the stability of the membrane by interacting with the polar part of the phospholipids of the sperm membrane and, on the other hand, by supplying energy to the sperm metabolism [6].

The aim of the present study was to test the efficacy of different levels of maltose on sperm motility characteristics in TCM199 using Computer Assisted Sperm Analysis (CASA).

MATERIALS AND METHODS

Regents

All media, chemicals and reagents used in this study were purchased from Sigma-Aldrich Chemical Co. Ltd (through Barnard company, Urmia, Iran).

Sperm sampling

Immediately after the animals were slaughtered, the testicles of adult male buffaloes with prominent epididymides and without any defects or adhesions were collected in a number of 10 pairs. The testicles

were placed in a polystyrene box at a temperature between 5o and 8o C and transported to the reference laboratory of the Faculty of Veterinary Medicine, located at km 20 of the "Sento" highway. In the laboratory [7], after drying the testicle samples from secretions, debris and blood, the covering of the testicle "Tunica Vaginalis" was cut, then the tail of the epididymis was fixed between two fingers, and in areas with fewer blood vessels, several incisions were made with a scalpel to obtain sperm, then with Eppendorf containing 4 ml of cell culture medium and 10% bovine serum albumin, sperm droplets were collected from the tail of the epididymis [5, 8-10]. After diluting the sperm to below 50 million sperm per milliliter of culture medium (approximately between 30 and 40 million sperm), five levels of maltose sugar (1, 3, 5, 10, 15 millimoles) were added to the Eppendorf containing 1 ml of culture medium and 30-40 million sperm (a dilution above 50 million sperm cannot be assessed by CASA) with 10% BSA and incubated for 24 hours at 37o Celsius. Sperm was diluted to a concentration of less than 50 million spermatozoa/mL and a 5 mL aliquot was placed in a Maker counting chamber at 37o C. Sperm kinetics were assessed at 1, 6, 12 and 24 hours by CASA (HFT CASA, a trademark of Hooshmand Fanavar Tehran Co., Iran ver 1.2). CASA is a minimum error method to check sperm motility pattern and its effectiveness in controlling the quality of sperm kinetics has been proven in all animal species [9, 11-15]. However, in the present study, for a more detailed investigation, five randomly selected microscopic fields were scanned at 40X magnification. The following parameters were evaluated: rapid progressive motility (class A, %), progressive motility (class B, %), viability (class A+B+C, %), straight line velocity (VSL, µm/s), curved line velocity (VCL, µm/s), average path velocity (VAP, µm/s) and head amplitude (ALH, µm).

Statistical analyses

In this research, after repeating the experiment three times, the Statistical Package for Social Sciences (SPSS version 26) software was used for statistical analysis. After the data were examined for the significance of the difference in variances, to perform multiple range tests or after the experiment, when the equality of the variances of the groups was satisfied, Tukey's test was used, and when the inequality of the variances of the groups was assumed, Tamaneh test was used at the 95% confidence level (statistical significance was set at a p value ≤ 0.05). Results are presented in Tables 1-7 as Mean ± SEM.

RESULTS

Table 1: Effect of different maltose levels on Mean ± SEM rapid progressive motility (Class A, %)

Treatment	Time (hrs.)			
	1	6	12	24
Maltose 0	70.44±1.93	64.41±5.46	68.92±3.30	27.43±5.48 [b]
Maltose 1	74.17±2.96	78.64±3.95	72.46±2.35	42.28±5.24
Maltose 3	73.31±3.06	79.37±1.87	72.41±1.52	55.06±4.03 [a]
Maltose 5	68.45±3.16	78.28±1.22	69.11±0.73	40.67±6.49
Maltose 10	72.61±3.16	77.69±2.25	70.79±2.55	51.03±2.71[a]
Maltose 15	67.93±2.70	75.42±0.58	69.38±1.87	41.24±6.83

In each column values with different superscripts are significantly different ($p < 0.05$).

The results of the effect of maltose sugar on motility patterns such as: rapid progressive motility (class A, %), progressive motility (class B, %), motile spermatozoa (class A+B+C, %) of buffalo epididymal spermatozoa, showed that maltose causes an increase in the motility patterns of spermatozoa during storage at 5O Celsius, and at all storage times (1 to 24 hours), the average numerical values of maltose are higher than the control (maltose 0), and this increase at 24 hours and at dilutions of 3 and 10 mmol was significant (Table 1-3). In relation to the average sperm velocity indicators, such as curvilinear velocity (VCL, µm s-1), straight line velocity (VSL, µm s-1), average path velocity (VAP, µm s-1), the average of these velocity data was higher in the maltose group than in the control at different times, although a significant difference with the control was observed at 24 hours and at the level of 3 and 10 mmol of maltose (Table 4-6). According to the amplitude of the head (ALH, µm), the average of these data at different times was mostly higher than the control, although a significant difference with the control was observed at 24 hours and at the level of 3 and 10 mmol (Table 7).

DISCUSSION

The observations of this research suggest that maltose disaccharide sugar is effective in improving and preserving sperm quantitative indicators during storage in cell culture medium (TCM 199). Mammalian sperm have been found to have the ability to use glucose, fructose, mannose, maltose and glycogen for metabolism and maintenance of motility [16]. Mammalian sperm are the only cells outside the body that require strong and forward motility to reach the oocyte; in fact, sperm swimming in the culture medium or in the secretions of the female genital tract is necessary for oocyte fertilisation. The energy generator for sperm motility is present in both

Table 2: Effect of different maltose levels on Mean ± SEM sperm progressive motility (Class B, %)

Treatment	Time (hrs.)			
	1	6	12	24
Maltose 0	82.40±1.86	79.69±3.39	78.72±3.38	37±6.94 [b]
Maltose 1	87.20±1.71	87.26±2.71	82.36±1.06	51.47±4.60
Maltose 3	84.63±2.23	88.45±2.77	81.28±1.63	65.56±2.0 [a]
Maltose 5	81.27±2.67	88.13±0.66	79.32±0.73	49.59±5.73
Maltose 10	83.41±2.63	86.83±1.99	79.37±1.72	62.23±2.98[a]
Maltose 15	80.18±3.75	83.71±0.63	78.98±2.52	48.86±7.36

In each column values with different superscripts are significantly different ($p < 0.05$).

Table 3: Effect of different maltose levels on Mean ±S EM motile or live sperm (Class A+B+C, %)

Treatment	Time (hrs.)			
	1	6	12	24
Maltose 0	90.04±1.64	87.45±2.21	84.67±2.99	42.39±7.20 [b]
Maltose 1	94.99±1.27	92.27±1.66	86.50±0.73	57±4.26
Maltose 3	92.21±2.31	92.05±2.80	85.57±1.00	70.71±1.03 [a]
Maltose 5	89.30±3.42	94.22±0.23	85.05±0.58	55.37±6.0
Maltose 10	92.51±1.31	92.36±1.52	84.61±1.65	67.53±1.61[a]
Maltose 15	88.51±3.10	88.85±1.90	84.13±1.51	53.35±7.29

In each column values with different superscripts are significantly different ($p < 0.05$).

Table 4: Effect of different maltose levels on Mean ± SEM curvilinear velocity VCL (µm s^{-1})

Treatment	Time (hrs.)			
	1	6	12	24
Maltose 0	89.35±3.26	70.46±6.29	70.77±1.97	22.88±4.15 [b]
Maltose 1	90.31±4.06	90.38±3.43	73.06±3.96	36.50±3.62
Maltose 3	90.36±1.34	88.06±2.34	77.32±3.0	47.60±2.83 [a]
Maltose 5	84.77±3.72	92.16±1.25	74.64±0.39	41.43±6.20
Maltose 10	89.20±1.60	89.91±3.78	73.97±2.28	47.54±0.34 [a]
Maltose 15	83.89±1.34	85.24±2.15	74.70±0.67	36.11±5.25

In each column values with different superscripts are significantly different ($p < 0.05$).

Table 5: Effect of different maltose levels on Mean ± SEM straight-line velocity VSL (µm s^{-1})

Treatment	Time (hrs.)			
	1	6	12	24
Maltose 0	67.03±3.63	51±5.92	55.31±1.61	15.45±3.04 [b]
Maltose 1	66.54±5.08	71.82±4.59	57.60±3.56	27.67±3.31
Maltose 3	67.82±2	68.36±1.89	62.65±2.27	36.77±3.08 [a]
Maltose 5	61.84±2.84	71.09±0.54	58.18±0.80	31.50±4.69
Maltose 10	64.36±1.85	71.28±3.90	58.90±2.16	37.13±0.44 [a]
Maltose 15	60.30±0.61	66.62±1.14	60.12±0.82	27.50±4.19

In each column values with different superscripts are significantly different ($p < 0.05$).

Table 6: Effect of different maltose levels on Mean ± SEM average path velocity VAP (µm s^{-1})

Treatment	Time (hrs.)			
	1	6	12	24
Maltose 0	75.03±3.70	57.50±6.39	60.84±1.97	18.11±3.51 [b]
Maltose 1	75.60±4.73	78.67±4.26	63.34±3.36	30.93±3.42
Maltose 3	76.25±1.28	75.32±2.28	68.12±2.3	40.68±3.03 [a]
Maltose 5	70.13±3.37	78.47±0.31	63.98±0.58	34.81±5.09
Maltose 10	72.52±1.90	77.60±3.77	64±2.20	40.81±0.53 [a]
Maltose 15	68.14±0.86	73.06±1.36	65.31±0.71	30.85±4.63

In each column values with different superscripts are significantly different ($p < 0.05$).

Table 7: Effect of different maltose levels on Mean ± SEM amplitude lateral head displacement (ALH,µm)

Treatment	Time (hrs.)			
	1	6	12	24
Maltose 0	3.03±0.03	2.74±0.10	2.57±0.08	1.12±0.19 [b]
Maltose 1	3.21±0.01	2.95±0.06	2.57±0.09	1.51±0.14
Maltose 3	3.06±0.10	3±0.10	2.57±0.06	1.96±0.08 [a]
Maltose 5	3±0.17	3.11±0.09	2.62±0.05	1.59±0.23
Maltose 10	3.18±0.03	3.03±0.09	2.59±0.08	1.85±0.02 [a]
Maltose 15	3.08±0.09	3±0.05	2.55±0.03	1.46±0.23

In each column values with different superscripts are significantly different ($p < 0.05$).

and the main part (mitochondrial sheath) of the sperm flagellum for the Krebs cycle [17]. The plasma membrane of mammalian spermatozoa and mitochondria has the ability to transport all types of sugars, pyruvate, ketone bodies, lactate, citrate and carnitine; in fact, membrane transporters specific for hexoses, monocarboxylates, citrate and carnitine have been detected in mammalian spermatozoa [17]. In our study, buffalo sperm had longer and better motility in the presence of maltose sugar compared to the control group, indicating the metabolism of maltose by the flagellum of buffalo sperm epididymis. Monosaccharide, disaccharide and polysaccharide sugars are the energy source for sperm and act as extracellular cryoprotectants during freezing. Several types of sugars that are commonly added to the extender include: glucose in ram and pig semen, raffinose in goat semen, trehalose in bull semen, lactose in goat and ram semen, and maltose, which is added to ram semen during the freezing process. Maltose has the ability to protect sperm as a cryoprotectant and also acts as an energy source for sperm[18]. In the study of the effect of maltose sugar on the quality of epididymal sperm from cows kept at 5 degrees, the percentage of motile sperm, the percentage of live sperm and the percentage of healthy and intact acrosomes were higher in the maltose groups than in the control [18]. In the current study, the data obtained from the effect of maltose sugar on the motility pattern of epididymal spermatozoa was higher than the control group and is in agreement with the Wattimena study. In addition to being a source of energy, sugars maintain the permeability and integrity of phospholipids during freezing and dehydration and prevent the binding of phosphatidylcholine bilayer vesicles during dehydration. It is maintained by the fluidity of the sperm membrane [19]. In the study by Yildiz et al, monosaccharide and disaccharide sugars, including maltose, were added to Tris-citric acid diluent and the motility of canine sperm was assessed after culture, during equilibration and after freezing. Their results showed that immediately after sugar addition, no significant difference in motility was observed between the different sugar groups and the control group [3]. In our study, at the time of 1 and 12 hours after the addition of maltose sugar to the sperm culture medium, no significant effect was observed in improving sperm motility patterns, but at 24 hours after culture, the maltose results were better than the control and the difference between the means was significant. Although the study by Yildiz et al. was conducted on canine sperm, similar results would have been obtained if more time had been allowed for the interaction between sperm and maltose sugar. Maltose, as a disaccharide sugar, is used as an energy source through glycolysis, but before glycolysis can take place, the disaccharide must be broken down by the enzyme disaccharidase. In the case of maltose, this enzyme is called maltase and it splits maltose into two glucose molecules. Sperm break down glucose more easily than the natural sugar found in seminal plasma, fructose [18] .The presence of sugar in the diluent causes the stability of the sperm membrane and changes the crystallisation pattern as well as the shape and width of the channels of the unfrozen solution and actually reduces the complications caused by rapid cooling [18]. In Lapwood and Martin's study on the effect of sugars on ram sperm motility, the results showed that at 37 0C the percentage of sperm motility with the presence of glucose-mannose-fructose and sucrose sugars in the extender was better than the control [20]. In the present study, the beneficial effect of sugars in improving sperm motility after culture at 37 degrees Celsius was suggested.

CONCLUSION

In conclusion, the results of this investigation have shown that the addition of maltose sugar in low dilutions can provide the energy required by buffalo epididymal spermatozoa, probably maltase enzyme is active in buffalo tail epididymal spermatozoa and can be effective in providing energy to spermatozoa by converting maltose to glucose.

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ETHICS

In this study, all samples were collected immediately after the routine slaughter of animals according to Islamic Sharia standards at the industrial slaughterhouse in Urmia.

CONFLICT OF INTEREST

None.

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