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Original Article

The effects of Xylose monosaccharide on Water Buffalo (*Bubalus bubalis*) epididymal sperm kinetic parameters at 37 ˚C

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ABSTRACT

The objective of this study was to test xylose monosaccharide sugar on buffalo epididymal sperm kinetic parameters at 37 ˚C, of up to 24 h. Testes with epididymis were collected after death at Urmia industrial abattoir; they were recovered within 30 min after slaughter. Paired cauda epididymides from each mature buffalo bull were sliced and sperms transferred into tubes containing tissue culture medium (TCM, 199) with 10% bovine serum albumin at 37 ˚ C. Different levels of xylose (0,1, 3, 5, 10, 15 mM) were added into TCM-199 containing sperms (40-50×106 sperm/ml), with 10%, bovine serum albumin. Then samples were incubated at 37 ˚C. Thereafter, at 1, 3, 5, 7, 10, 12 and 24 hours incubation times, kinetics of at least 1 thousand sperms per samples were recorded with computer assisted sperm analysis (CASA). Results revealed that the motility parameters till 12 hrs. did not show any significant differences between groups, actually no significant difference was observed between control and xylose groups means. But at 24 h, after incubation the means of xylose levels were lower than that of the control. At 15 mM of xylose level the difference with control was significant ($p<0.05$). It is concluded that the xylose at high levels significantly reduces the kinetic parameters of buffalo bull epididymal sperm, especially at 24 h after incubation. Therefore, the usage of xylose sugar at the high levels is not recommended for the energy supply of Buffalo bull epididymal sperm at 37 ˚ C incubation in TCM 199.

اثرات مونوساکارید زایلوز بر الگو های حرکتی اسپرم اپیدیدیم گاومیش رودخانه ای در دمای ۳۷ درجهٔ سانتیگراد

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چکیده

هدف از این مطالعه آزمایش تاثیر قند مونو ساکارید زایلوز بر الگوی حرکتی اسپرم اپیدیدیمی گاومیش در محیط کشت سلول (TCM, 199) در دمای ۳۷ درجه سانتیگراد طی ۲۴ ساعت بود. نمونه های بیضه با اپیدیدیم از کشتارگاه صنعتی ارومیه و نهایتا تا نیم ساعت پس از کشتار دام جمع آوری گردید .اسپرم دم اپیدیدیم هر جفت بیضهٔ گاومیش نر بالغ پس از برش و استحصال، به میکرو تیوب های حاوی محیط کشت بافتی با ۱۰ درصد آلبومین سرم گاوی با دمای ۳۷ درجه سانتیگراد منتقل شد. سطوح مختلف قند زایلوز (۰، ۱، ۳، ۵، ۱۰ و ۱۵ میلی مول) به محیط کشت بافتی حاوی ۴۰ الی ۵۰ میلیون اسپرم و ۱۰ درصد آلبومین سرم گاوی اضافه گردید. سپس نمونه ها در انکوباتور ۳۷ درجه سانتیگراد قرار گرفت و در زمان های ۱، ۳، ۵، ۷، ۱۰، ۱۲ و ۲۴ ساعت، در هر نمونه الگوی حرکتی حداقل هزار اسپرم توسط نرم افزار و سخت افزار کامپیوتری (کاسا) ثبت گردید. نتایج نشان داد که تا ساعت ۱۲ بعد از انکوباسیون هیچ اختلاف معنی داری در میانگین شاخص های حرکتی اسپرم نبود در واقع بین میانگین های گروه شاهد و زایلوز تفاوت معنی دار مشاهده نگردید. اما در ساعت ۲۴ بعد از انکوباسیون میانگین های زایلوز بویژه در سطح ۱۵ میلی مول از شاهد کمتر و اختلاف معنی دار بود ($p<۰/۰۵$). چنین استنباط می شود که زایلوز شاخص های حرکتی اسپرم اپیدیدیم گاومیش را خصوصا" در سطوح بالا و ۲۴ ساعت پس از انکوباسیون معنی دار کاهش می دهد، بنابر این کاربرد سطوح بالای قند زایلوز به عنوان منبع انرژی اسپرم اپیدیدیم گاومیش نر در محیط کشت بافتی و دمای ۳۷ درجه سانتیگراد توصیه نمی شود.

واژه های کلیدی: زایلوز، کاسا، گاومیش، محیط کشت، اسپرم

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INTRODUCTION

The world buffalo population has reached more than 200 million heads in the last thirty years until 2017, and more than 97% of this amount is in developing countries. The adaptability of this species to the climate of tropical regions as well as its resistance to diseases has made buffalo husbandry very effective in the rural economy [1]; this amount of livestock population spreads across 42 countries, of which 96.4% are distributed in Asia, 2.9% in Africa, and rest in Europe and Latin America [2]. Despite the importance of milk, meat and leather production, no special attention has been paid to reforming its reproductive programs [3]. The results of several studies show that buffalo sperm can be easily preserved like bovine semen[4,5,6]. Reproductive technologies such as artificial insemination, in vitro fertilization, embryo transfer, gamete and embryo freezing are useful tools for preserving endangered species and wildlife. Performing these reproductive technologies allows genetic variations between different species without moving livestock reducing the problems related to behavioral and physiological inconsistencies that are barriers to natural mating [4,5] [7,8]. One of the important components of in vitro insemination or in vitro fertilization and embryo transfer is the presence of viable and active sperm cells. Due to the problems related to ejaculation sperm, epididymal sperm can be easily used, whether in live animals or after slaughter; epididymis can be obtained and used for reproductive programs [9-14]. If sperm collection is carried out at a different time and place, preparing female livestock, collecting oocytes and Increasing sperm lifespan are important for biotechnology programs [15]. One of the ways to increase sperm lifespan and actually increase semen quality and provide energy for sperm motility is to use nutrients. The most accessible source of energy for maintaining suitable motility for fertilization is adding sugars to the sperm culture medium or extenders. Sugar is divided into three categories: monosaccharides (glucose, fructose, galactose and xylose), disaccharides (maltose, lactose and trehalose) and polysaccharides (starch). Sugar is involved in contrasting the polar head of sperm membrane phospholipids, in dehydration and freezing by glass method (vitrification) and providing semen energy during incubation [16]. By adding monosaccharide, disaccharide or trisaccharide sugars to the culture medium or extenders, the stability and integrity of the sperm membrane is preserved and sperm energy is supplied. Sugar prevents the lipid peroxidation of cell membrane [17]. Xylose is a sugar from the family of monosaccharides, named for the first time due to its separation from wood [18]. Xylose monosaccharide sugar has several roles in the diluent or sperm culture medium: 1- Providing a source of energy for sperm cells during storage at 370. 2- Maintaining the osmotic pressure of the medium. 3- having an effective role as a cryoprotectant during the freezing and thawing process [19]. Xylose sugar increased the motility percentage of dog sperm during the equilibrium time compared to the control, and after freezing and thawing, this difference with the control was significant [19]. As well as, xylose sugar has been used in bovine sperm culture and useful results have been obtained [20]. Sugar provides energy for viability, cell membrane fluidity. It plays a role in preventing premature acrosomal reaction and persistence of sperm motility which is a prerequisite for successful fertility [21].

MATERIALS AND METHODS

In order to conduct the study, a total of 15 pairs of adult male buffalo bull testes (based on dental formula) were collected immediately after slaughter. Healthy and normal testis with a clear and prominent tail of epididymis were transferred within 30 min after slaughter to the laboratory in the presence of frozen plastic ice packs, without any contact and mixing with water, blood or discharge. Immediately after reaching samples to the laboratory, testicles were washed with normal saline (0.9% sodium chloride) and then dried carefully. After several incision to the tunica vaginalis and albuginea of the tail of epididymis, semen was transferred without being impregnated with blood to 5 ml microtubes, containing TCM 199 medium, with 10% bovine serum albumin (BSA). The range of sperm dilution was between 30 and 50 million sperm per ml. In fact, after obtaining the sperm, it was partially diluted with the cell culture medium so that the sperm head can be identified by the CASA system. The culture medium includes different levels of xylose sugar (0, 1, 3, 5, 10 and 15 mmol). To do the work, 1.5 ml microtubes with 800 µl culture medium and 10% bovine serum albumin containing dilution were separated from xylose and after adding 200 µl of sperm medium, our desired volume was 1 ml with an approximate concentration of 40-50 million sperms. It should be noted that the high concentration of 50 million sperm per milliliter cannot be evaluated by CASA. Sperm concentration can be determined by spectrophotometry method. After preparing the samples, culture was carried out for 24 hours in an incubator of 37 °C; sperm analysis was performed with the computer assisted sperm analysis system (HFTCASA, a trademark of Hooshmand Fanavar Tehran Co., Iran). Acquiring frames at 60 Hz. sample of sperm suspension was taken at 1, 3, 5, 7, 10, 12 and 24 hours and were loaded into a prewarmed disposable analysis Makler counting chamber with a depth of 10 µM. A minimum number of 1000 cells was counted for each assay. Main CASA parameters, i.e. progressive motility (Class A, Class B, Class A+B, %), motile sperm (Class A+B+C, %), average path velocity (VAP, µm/s), straight velocity (VSL, µm/s), curvilinear velocity (VCL, µm/s) were measured with the CASA [22-29].

Statistical analysis

The statistical analyses were performed using statistical software SPSS (version 24). One-way analysis of variance was used to analyse the effect of xylose on sperm motility. When ANOVA revealed a significant effect, the values were compared by Duncan's multiple range test and tukey. Differences between groups were considered statistically significant at $p< 0.05$.

and 0.1 mol citric acid at pH 6.0), was added, and finally, after 30 min, spermatozoa were subjected to flow cytometry. Green fluorescence of double-stranded DNA (intact DNA) and red fluorescence of single-stranded DNA (damaged DNA) were detected with FL1(500– 530 nm) and FL3 (620 nm) detectors respectively (Figure 1) [16].

Statistical analysis

Each treatment was replicated six times. For each replicate, four straws were thawed and pooled for evaluation of sperm parameters. One-way analysis of variance (ANOVA) along with Duncan's multiple range tests was used to evaluate differences between the groups. Results are presented as the mean percentage standard error of mean (SEM). The statistical analysis was carried out using SPSS computer program (Version 16.0; SPSS Co, Chicago, IL, USA). The value of $p<0.05$ was considered statistically significant.

RESULTS

The results of this study indicated that the mean percentage of fast-progressive motility (Class A, %), was not significantly different from the control until 12 hrs , but at 24 hrs the difference between the control and different levels of xylose was significant and the control results were better than xylose ($p<0.05$), (Table 1). The mean percentage of immotile sperm (Class D, %) till 12 hrs in treatment groups was not significantly different from control groups, but at 24 hrs the lowest level of Class D belonged to the control group (Table 2). The mean percentage of progressive motility (Class A+B, %), was higher than the control group at 10 hrs, in terms of numerical values in xylose 5, 10 and 15 mmol, but the difference was not significant. At 24 hrs, the mean of control was higher than all xylose levels and the control difference with the treatment group was significant ($p<0.05$), (Table 3). In the case of the percentage of motile sperms (Class A+B+C, %), the amount of this data between the groups was not significant until 12 hrs and the difference between the means was not clear, but at 24 hrs the mean of the control was higher than all levels of the xylose groups ($p<0.05$), (Table 4). The mean value of the curve line velocity (VCL, µm/s) and the amount of this variable did not show a significant difference between different groups till 12 hrs, but till 24 hrs, the results were in favor of the control group($p<0.05$), (Table 5). In relation to straight-line velocity (VSL, µm/s), the amount of this data did not show any specific change until 12 hrs; there was no significant difference between the control and the treatment group, however, at 10 hrs and in dilution 15 and at 12 hrs and in dilution 1 it had the highest numerical value, but at 24 hrs the mean difference was significant and the highest speed was related to the control ($p<0.05$), (Table 6). In connection with the mean sperm velocity average path (VAP , µm/s) the rate of this variable at 5 and 10 hrs was numerically lower than all xylose levels, but the difference was not significant. At 12 hrs the mean of xylose 5 and 10 was higher than the control, but the mean difference was not significant. At 24 hrs, sperm velocity at all levels of xylose was significantly lower than the control group ($p<0.05$), (Table 7).

Table 1. Mean (± SE) rapid progressive motility (Class A, %) and six xylose levels

Times	1 h	3 h	5 h	7 h	10 h	12 h	24 h
Groups							
Xylose 0 (Control)	70.44 ± 1.93	72.3 ± 2.17	64.41 ± 5.46	78.90 ± 2.03	68.33 ± 3.69	68.92 ± 3.30	36.92 ± 4.29 [a]
Xylose 1	72.91 ± 4.63	72.52 ± 4.32	72.41 ± 3.10	73.25 ± 2.50	69.03 ± 2.42	67.79 ± 2.32	28.78 ± 2.60
Xylose 3	63.58 ± 1.84	71.69 ± 4.7	79.28 ± 1.53	70.96 ± 2.65	68.61 ± 2.43	64.07 ± 3.48	28.47 ± 4.03
Xylose 5	67.38 ± 2.85	76.43 ± 2.62	75.93 ± 1.70	68.89 ± 1.00	72.57 ± 4.28	65.46 ± 7.64	29.03 ± 3.01
Xylose 10	67.07 ± 3.86	74.6 ± 5.184	73.55 ± 2.46	72.69 ± 1.85	67.84 ± 2.75	68.26 ± 4.69	27.10 ± 1.81
Xylose 15	71.04 ± 2.0	74.93 ± 2.16	68.36 ± 3.54	77.86 ± 1.619	75.35 ± 1.98	69.63 ± 3.03	16.66 ± 2.85 [b]

[a, b]: Within a column, for each subspecies, means without a common superscript differed ($p< 0.05$)

Table 2. Mean (± SE) immotile sperm (Class D, %) and xylose levels

Times	1 h	3 h	5 h	7 h	10 h	12 h	24 h
Groups							
Xylose 0 (Control)	9.95 ± 1.64	10.79 ± 2.17	12.55 ± 2.21	8.97 ± 1.69	15.81 ±. 4516	15.32 ± 2.99	37.39 ± 3.91 [a]
Xylose 1	8.11 ± 3.70	9.39 ± 5.47	10.30 ± 1.45	10.03 ± 2.63	14.29 ± 4.94	16.83 ± 3.08	55.02 ± 2.76
Xylose 3	14.89 ± 2.78	11.74 ± 3.72	5.94 ± 1.095	10.98 ± 1.68	15.88 ± 1.89	18.71 ± 1.27	60.89 ± 6.41[b]
Xylose 5	9.15 ± 3.27	8.66 ± 1.55	8.26 ± 1.60	12.64 ± 1.96	12.54 ± 4.17	21.38 ± 8.70	51.07 ± 4.64
Xylose 10	14.78 ± 5.57	7.68 ± 1.629	8.55 ± 2.14	8.54 ± 1.66	15.08 ± 2.66	17.89 ± 6.74	59.96 ± 1.56 [b]
Xylose 15	11.59 ± 2.67	8.036 ± 1.43	9.33 ± 2.18	6.136 ± 3.67	13.12 ± 2.20	12.79 ± 9.499	73.73 ± 6.07 [b]

[a, b]: Within a column, for each subspecies, means without a common superscript differed ($p < 0.05$)

Table 3. Mean (± SE) immotile sperm (Class D, %) and xylose levels

Times	1 h	3 h	5 h	7 h	10 h	12 h	24 h
Groups							
Xylose 0 (Control)	82.40 ± 1.86	82.69 ± 2.20	79.69 ± 3.39	87.28 ± 1.16	78.92 ± 1.02	78.72 ± 3.38	52.46 ± 6.75 [a]
Xylose 1	85.17 ± 4.16	84.22 ± 4.42	84.13 ± 1.85	83.46 ± 3.05	80.84 ± 4.15	78.81± 2.89	38.41 ± 4.76
Xylose3	76.01 ± 3.15	81.75 ± 3.92	89.37 ± 1.33	81.32 ± 1.84	78.67 ± 2.22	76.85 ± 2.05	35.06 ± 5.54
Xylose 5	82.10 ± 3.88	86.05 ± 1.54	85.24 ± 1.47	81.51 ± 1.89	84.13 ± 4.73	74.99 ± 8.13	43.12 ± 4.68
Xylose 10	77.69 ± 5.00	85.68 ± 4.149	85.84 ± 2.64	86.17 ± 1.51	79.86 ± 2.90	78.21 ± 6.32	35.32 ± 7.907
Xylose 15	81.90 ± 2.96	85.41 ± 1.51	82.76 ± 2.21	87.96 ± 4.239	83.0 ± 2.45	82.57 ±. 596	21.48 ± 4.85 [b]

[a, b]: Within a column, for each subspecies, means without a common superscript differed ($p < 0.05$)

Table 4. Mean (± SE) Motile (Live) sperm (Class A+B+C, %) and xylose levels

Times	1 h	3 h	5 h	7 h	10 h	12 h	24 h
Groups							
Xylose 0 (Control)	90.04 ± 1.64	89.20 ± 2.17	87.45 ± 2.21	91.02 ± 1.69	84.18 ± 4.516	84.67 ± 2.99	62.60 ± 3.91 [a]
Xylose 1	91.89 ± 3.70	90.60 ± 5.47	89.70 ± 1.452	89.97 ± 2.63	85.70 ± 4.94	83.16 ± 3.08	44.98 ± 2.76
Xylose 3	85.10 ± 2.78	88.25 ± 3.72	94.06 ± 1.095	89.02 ± 1.68	84.12 ± 1.89	81.29 ± 1.27	39.11 ± 6.41[b]
Xylose 5	90.84 ± 3.27	91.33 ± 1.55	91.74 ± 1.60	87.36 ± 1.96	87.45 ± 4.17	78.61 ± 8.70	48.92 ± 4.64
Xylose 10	85.22 ± 5.57	92.31± 1.629	91.44 ± 2.145	91.45 ± 1.66	84.92 ± 2.66	82.11 ± 6.74	40.04 ± 1.56[b]
Xylose 15	88.41 ± 2.67	91.96 ± 1.430	90.66 ± 2.18	93.86 ± 3.678	86.87 ± 2.20	87.21 ±9.499	26.27 ± 6.07 [b]

[a, b]: Within a column, for each subspecies, means without a common superscript differed ($p < 0.05$)

Table 5. Mean (± SE) Velocity curvilinear of sperm (VCL, µm/s) and xylose levels

Times / Groups	1 h	3 h	5 h	7 h	10 h	12 h	24 h
Xylose 0 (Control)	89.35 ± 3.26	84.56 ± 1.84	70.46 ± 6.29	86.72 ± 3.03	71.77 ± 3.67	70.77 ± 1.97	38.70 ± 2.57 [a]
Xylose1	89.99 ± 2.84	86.45 ± 3.01	83.64 ± 5.60	82.36 ± 1.63	74.08 ± 2.61	72.64 ± 2.82	27.84 ± 1.19
Xylose3	81.43 ± 1.55	85.94 ± 3.90	89.08 ± 1.31	82.97 ± 7.420	74.79 ± 1.85	67.46 ± 6.27	26.09 ± 4.29
Xylose5	85.67 ± 2.78	87.38 ± 3.90	87.69 ± 2.57	80.77 ± 2.11	77.36 ± 4.32	69.87 ± 8.82	26.80 ± 2.88
Xylose10	85.07 ± 6.21	89.11 ± 3.21	88.40 ± 4.03	79.35 ± 4.88	75.50 ± 4.50	69.14 ± 6.61	22.05 ± 5.975 [b]
Xylose15	85.88 ± 2.70	89.53 ± 3.00	82.25 ± 2.71	85.90 ± 3.93	81.73 ± 8.622	71.90 ± 3.14	14.73 ± 3.34 [b]

[a, b]: Within a column, for each subspecies, means without a common superscript differed ($p < 0.05$)

Table 6. Mean (± SE) Stright line velocity of sperm (VSL, µm/s) and xylose levels

Times / Groups	1 h	3 h	5 h	7 h	10 h	12 h	24 h
Xylose 0 (Control)	67.03 ± 3.63	63.84 ± 1.14	51.00 ± 5.92	69.99 ± 2.89	55.55 ± 4.16	55.31 ± 1.61	24.77 ± 2.56 [a]
Xylose1	67.41 ± 2.87	65.33 ± 3.82	64.40 ± 5.80	63.88 ± 1.15	57.81 ± 1.62	58.25 ± 2.11	19.64 ± 1.72
Xylose3	58.48 ± 4.878	64.38 ± 2.95	70.13 ± 9.341	63.62 ± 1.28	59.01 ± 1.31	52.44 ± 5.44	19.33 ± 3.09
Xylose5	60.58 ± 1.86	68.26 ± 2.68	67.59 ± 3.35	60.68 ± 1.45	61.32 ± 4.26	56.57 ± 7.17	18.43 ± 1.83
Xylose10	64.14 ± 5.35	68.11 ± 2.92	68.00 ± 4.31	60.36 ±6.636	59.55 ± 4.40	54.92 ± 5.68	15.66 ± 5.138
Xylose15	64.46 ± 2.75	66.96 ± 2.59	59.35 ± 2.92	65.96 ± 3.61	66.52 ± 9.931	57.45 ± 3.31	9.73 ± 1.89 [b]

[a, b]: Within a column, for each subspecies, means without a common superscript differed ($p < 0.05$)

Table 7. Mean ± SE Velocity Average Path (VAP, µm/s) and xylose levels

Times / Groups	1 h	3 h	5 h	7 h	10 h	12 h	24 h
Xylose 0 (Control)	75.03 ± 3.70	71.50 ± 1.27	57.50 ± 6.39	76.15 ± 3.04	61.41 ±4 .33	60.84 ± 1.97	25.99 ± 2.36 [a]
Xylose1	75.72 ± 3.43	72.70 ± 3.44	71.24 ± 5.51	69.97 ± 1.34	63.68 ± 1.79	63.74 ± 2.15	22.33 ± 1.69
Xylose3	66.34 ± 1.12	72.07 ± 3.61	77.08 ± 7.511	70.16 ± 8.635	64.38 ± 1.52	57.92 ± 5.80	21.82 ± 3.28
Xylose5	68.87 ± 1.82	75.76 ± 3.08	74.53 ± 3.03	67.37 ± 1.75	67.20 ± 4.26	61.56 ± 7.65	21.64 ± 2.03
Xylose10	71.96 ± 6.05	75.97 ± 2.87	74.93 ± 4.11	67.04 ± 5.424	64.98 ± 4.25	60.32 ± 6.32	18.01 ± 4.885 [b]
Xylose15	71.75 ± 2.78	75.40 ± 2.93	66.94 ± 3.03	72.78 ± 3.53	72.10 ± 1.02	62.87 ± 3.03	11.53 ± 2.31[b]

[a, b]: Within a column, for each subspecies, means without a common superscript differed ($p < 0.05$)

DISCUSSION

In the present study, xylose had no inhibitory effect on motility patterns of the treatment group until 12 hrs, and even at 15 mmol level, most of the motility indices had higher numerical values but there was not any significant difference compared to the control, at 24 hrs the difference between the averages was significant and the results were in favor of the control ($p<0.05$). Sugar has several functions in the culture medium during sperm incubation in the culture medium. They provide the necessary energy for sperm motility; they play a role in the protection of osmotic pressure of the extender; depending on the molecular weight of the sugar, the type of dilute buffer and the temperature of the culture medium,

they determine the protective effects against cold in the diluent [19]. In our study at 24 hrs, there was no sign of energy supply for sperm motility in the treatment group and the results of the control were significantly better than the treatment. In the Williams and Ford study in 2001, monosaccharide sugars were added to the culture medium containing human sperm, and after 18 hours of incubation of 37 degrees, the results showed that glucose or other sugars capable of cuffing or glycolysis increased ATP production, followed by increased sperm motility which is necessary for high sperm motility and sperm capacity. Other glucose metabolites are not necessary for capacity-capacity, but they may have a slightly incremental effect [30]. In the present study, although sometimes the numerical values of xylose group motility patterns were higher than the control, the difference was not significant at 24 hrs. Unlike Williams and Ford study, the significant control indices were better than the xylose group. In Sariozkan study, the effect of monosaccharide, disaccharide and trisaccharide sugars on sperm quality of epididymal sperm in rats were evaluated. No significantly differences were observed till first hour, but at 12 o'clock the motility pattern in the treatment containing sugar was higher than control [17]. However, in the present study, although there was no statistical difference between the control and treatment until 12 o'clock, but till 12 hours xylose group especially at 15 mmol level had better motility patterns than the control. In this regard, it can be stated that it is in line with the research of Sariozkan [17]. In Shanker study, using disaccharide (trehalose) on egg tris-yolk extender had beneficial effect on buffalo sperm quality ; the percentage of motility in disaccharide group was higher than control [31]. In our study, xylose monosaccharide sugar was considered and the results of 24 hours in the treatment group were significantly lower than the control group. In the study of Malo, disaccharide sugar (trehalose) in extender had better results than the monosaccharide sugar and the percentage of progressive sperm motility of the pig in the disaccharide group was higher [32]. Of course, there are similar results in rams sperm [33]. However, in the study of Fernandez-Santos, addition of sugar had no significant effect on epididymal sperm motility of Iberian red deer [34]. In Lapwood study on the effect of sugars on ram sperm motility, the results indicated that at 37°C, sperm motility with monosaccharide sugars in the extender was better than the control [35]. Of course, in the present study, monosaccharide xylose had higher numerical values than the control until 12 o'clock in some motility patterns, and until this hour, although the difference in averages was not significant, the 15 mmol level kinetic indices were better than the control ; it could be stated that it was consistent with Lapwood's study. In Lapwood study at 5°C, xylose had better result than control. In our study, the storage temperature was 37 degrees and the effect of 5 degrees temperature was not investigated. Also, in Lapwood study, the effect of osmotic pressure changes due to the addition of different concentrations of sugars on sperm motility was not reported, In the present study, sperm had an acceptable movement pattern until 12 o'clock and no effect was observed due to osmotic pressure that abnormalized sperm movement pattern, but at 24 hours the results of meaningful control were better than the whole treatment group. One of the effective factors of sugar on sperm is its molecular weight; sugars with low molecular weight easily pass through sperm membranes and provide the necessary energy for metabolic and physiological activities of sperm [36].

As for the effect of xylose sugar on sperm motility of dogs, the results showed that immediately after the addition of sugar, no significant difference was observed between different groups of sugar and control group in terms of motility. However, after the equilibrium time, the difference was significant; in xylose group the percentage of motility was higher than the rest of monosaccharide sugars, but the difference with the control was not significant [19]. In the present study, the effect of xylose glucose up to 12 o'clock, especially in dilution of 15 mmol was better than the control group in terms of both numerical values and the mean differences. The effect of monosaccharide or disaccharide sugars on sperm

varies in different animal species. In stallion sorbitol sugar, in extender compared to glucose and fructose, improves kinetics characteristics after thawing. However, its protective mechanism is not yet known [37]. On the other hand, in stallion epididymis, the activity of alpha-mannosidase enzyme has been reported; it can break down mannose sugar, which is actually epimer of glucose [38] .In dogs, adding monosaccharides to the extender, depending on the type, produces a different response in sperm motility, For example, fructose increases the straight line velocity (VSL). It increases or maintains the linearity of the movement path and decreases beat cross frequency (BCF) of the sperm head. However, glucose reduces the average path velocity (VAP) and the percentage of the linearity (LIN) of the path, but it maintains BCF [39]. In our study, up to the first 12 hours, there no significant and beneficial effect was reported on motility percentage indices and sperm velocity in the treatment group. Only numerical values of progressive-motility percentage parameter (class A), curvilinear velocity (VCL) , straight line velocity (VSL) and the average path velocity (VAP) at 15 mmol level were reported to be better than the control group.

CONCLUSION

It can be concluded that according to the results of this research, it seems that adding xylose monosaccharide sugar has no significant effect on improving the motility patterns of buffalo epididymal sperm. At 24 hrs and in higher dilutions, it is even harmful as one of the reasons for adding sugar to the diluent, other than energy supply, is to prevent the effects of cold shock and induce antioxidant effects. Thus, it is better to evaluate the effects of xylose sugar on equilibration time, freezing and thawing time of buffalo epididymal sperm with CASA.

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ETHICS

Approved.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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